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A morphological study of the mouse subependymal layer from embryonic life to old age

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INTRODUCTION

The subependymal layer associated with the lateral ventricles was first observed by Allen (1912) to be a site of mitotic activity in adult rat brains, and this was confirmed by Bryans (1959). With the introduction of autoradiographic techniques this area became the subject of renewed interest. Smart (1961) examined this region in the mouse and concluded that cell production continued into adult life although the volume of the layer decreased. He also doubted whether the layer contributed greatly to the cell population of the adult brain. Lewis (1968) demonstrated that subendymal cells appeared to migrate into the corpus callosum and were distributed throughout the corpus callosum and forebrain grey matter excluding the cortex. Electron micrographic studies have confirmed the presence of undifferentiated cells, presumably glial precursors, in the adult rat subependymal layer (Blakemore, 1969; Privat & Leblond, 1972), and autoradiographic studies on semithin sections have confirmed the previous suggestions that migration of glial precursors continues from the subependymal layer in adult life (Paterson, Privat, Ling & Leblond, 1973). Although decrease in volume of the subependymal layer from birth has been commented on (Smart, 1961; Westergaard, 1970) no attempt appears to have been made to follow the natural history of the subependymal layer from fetal life.

In the mouse the subependymal layer is first found in the region of the ganglionic eminences or ventricular elevations (Smart, 1976). By 15 days postconception the number of subependymal mitotic figures exceeds that of the ependymal layer (Smart, 1976; Sturrock, 1979) which is the initial site of cell production in the central nervous system. The present study investigates changes in morphology of the subependymal layer from the time of its first appearance at 11 days postconception up to 22 months postnatum in ASH/TO mice. The number of mitotic figures in the subependymal layer from 15 days postconception up to 22 months in this strain has already been estimated (Sturrock, 1979) and the mitotic activity in the subependymal and ependymal layers from 11 days postconception up to birth has also been quantified (Smart, 1976).

MATERIALS AND METHODS

Mouse embryos were obtained at daily intervals from 11 to 19 days postconception from pregnant females anaesthetized by an intraperitoneal injection of Nembutal. The embryos were fixed by vascular perfusion with a solution of 2 % glutaraldehyde and 2 % paraformaldehyde in a 0·05 M-cacodylate buffer. Each 100 ml of fixative contained 1·2 ml dimethylsulphoxide and 0·3 ml 10 % calcium chloride. The method adopted to perfuse the embryos has been described previously (Sturrock, 1978*a*). The relatively low osmolarity of the fixative was chosen as it was noted by Privat &

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Leblond (1972) that solutions of low osmolarity gave better fixation of the ependymal and subependymal layers.

Newborn mice and postnatal mice aged 1, 2, 3, 4, 5, 10, 15, 25, 35 and 45 days and mice aged 3, 6, 9, 12, 15, 18 and 22 months were anaesthetized by an intraperitoneal injection of Nembutal and killed by perfusion fixation with the same fixative used for the embryos after the vascular system had been flushed out with physiological saline at 37 °C. After fixation, embryos and postnatal animals were placed in polythene bags and left at 4 °C overnight. The following morning the brains were removed from the skulls. In the case of the embryos the brains were bisected at the level of the optic chiasma in the coronal plane, while in the postnatal animals the brains were bisected in the sagittal plane and coronal sections 1–2 mm thick were cut from the rostral pole to the optic chiasma. The half brains or coronal slices were rinsed in 0·05 M-cacodylate buffer, post-fixed in 1 % osmium tetroxide in 0·1 M-phosphate buffer, dehydrated in graded alcohols and flat embedded in Spurr's resin.

Sections (1 μm) stained with toluidine blue were examined for orientation purposes, the blocks were trimmed and ultrathin sections stained with uranyl acetate and lead citrate were examined in an AEI 801 electron microscope.

A complete parallel series of 6 μm paraffin-embedded serial sections was available for comparison purposes (Sturrock, 1979).

RESULTS

The ventricular elevations first appear in the lateral wall of the cerebral vesicle at E11. From this age the ventricular layer can be divided into four major regions based on differences in morphology and future development. These regions are: (1) the ventricular roof, which initially lies suprolateral (Fig. 1) but gradually, due to growth of the cortex and development of the corpus callosum, comes to form the true superior roof of the lateral ventricle; (2) the medial roof, which lies opposite the medial extension of the cortical plate (Fig. 1), and eventually forms the medial border of the upper triangular part of the ventricle; (3) the ventricular elevations, medial and lateral, which eventually fuse to form the lateral wall of the ventricle; (4) the medial wall of the ventricle which lies opposite the ventricular elevations (Fig. 1) and eventually forms the medial wall of the narrow portion of the ventricle. The medial roof is not an ideal name as this part does not properly form a roof for the ventricle at any stage, but it is histologically and developmentally closely related to, but distinct from, the roof proper.

Description of ventricular layer development is complicated by the unique pattern of development of the neural epithelium. At E11 most of the cerebral vesicle is a true pseudostratified columnar epithelium with one surface, the external or pial one

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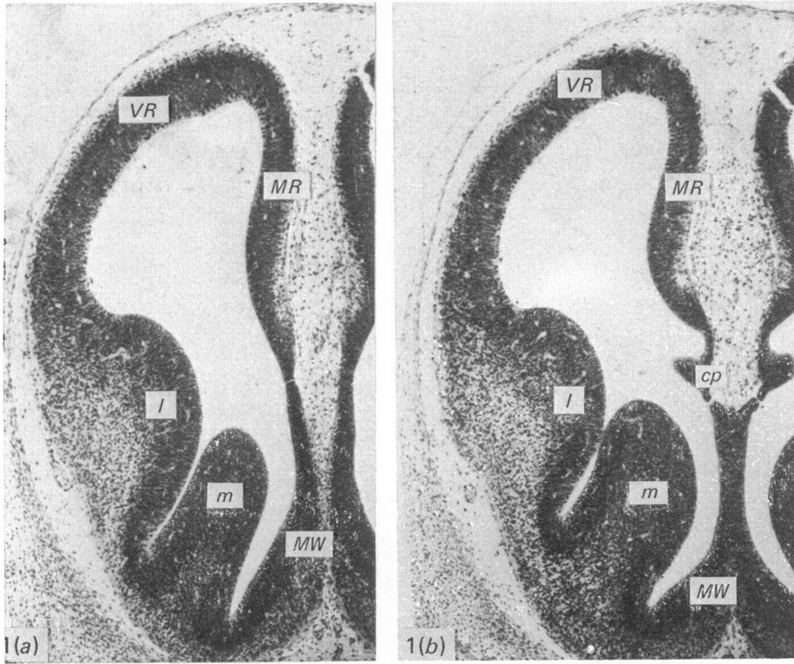
Fig. 1. Two 6 μm coronal sections through the brain of an E12 mouse. The medial (*m*) and lateral (*l*) ventricular elevations can be clearly seen. The ventricular roof (*VR*), medial roof (*MR*) and the medial wall (*MW*) are indicated in (*a*). (*b*) is just rostral to the interventricular foramen. The beginning of the choroid plexus (*cp*) is apparent just inferior to the medial roof. Even at this stage the difference in depth of the ventricular roof compared with the medial roof is evident in Fig. 1 (*a*). H & E. ×50.

Fig. 2. Demonstrates the pattern of ependymoglial processes. A large bunch of fibres can be seen extending laterally from the caudatopallial angle (*c*). Processes are absent from the neostriatum (NS) but can be seen at the inferior part of the ventricle (arrows). Golgi section, E17. ×50.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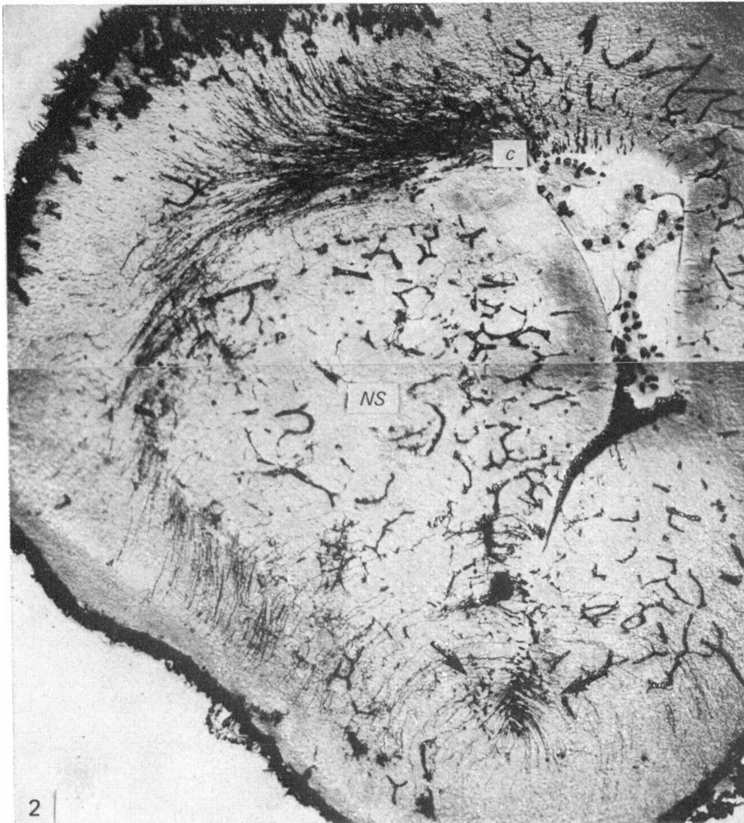
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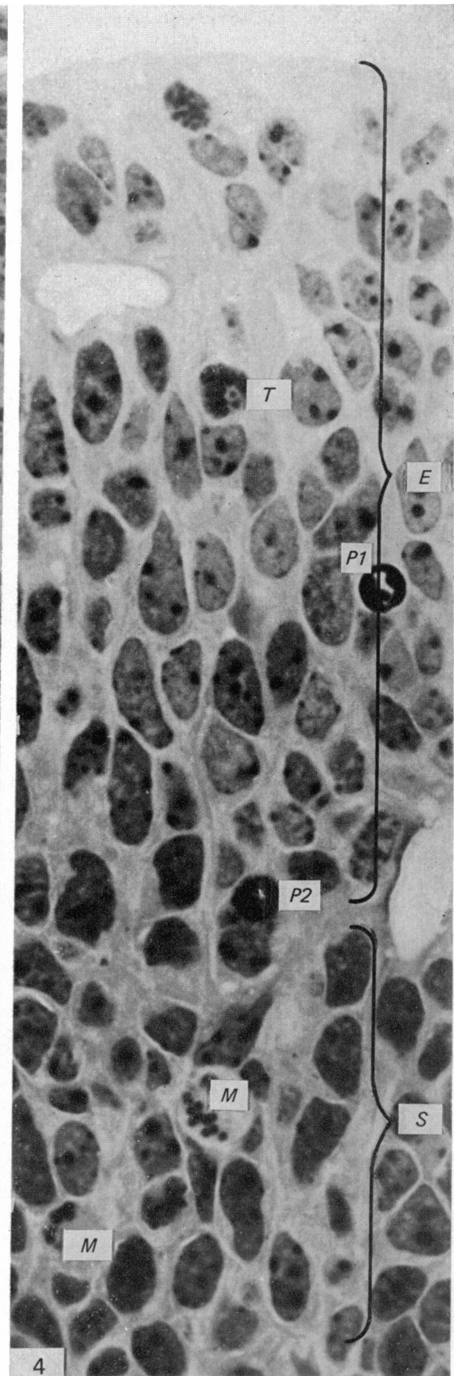

Fig. 3. Coronal section through the lateral ganglionic eminence at E12. The ependymal layer (*E*) is several nuclei thick, and pale-staining ependymoglial fibres can be clearly seen throughout the depth of the layer but absent from the subependymal layer (*S*). The lateral ventricle (*LV*) is on the left: 1 μm toluidine blue-stained section. $\times 600$.

Fig. 4. At E14 differences can be seen between the ependymal and subependymal nuclei. The ependymal nuclei tend to be larger and paler. In the ependymal layer a subsurface telophase (*T*) and pyknotic nuclei (*P1*) can be seen. A pyknotic nucleus (*P2*) is also apparent at the junction of the two layers. Supependymal mitotic figures (*M*) are also evident. 1 μm toluidine blue-stained section. $\times 1500$.

covered with a basement membrane. In the region of the developing ventricular elevations, however, the ventricular or ependymal layer remains as a pseudostratified columnar epithelium but the base or external surface is separated from its basement membrane by a mass of loosely packed cells; indeed this separation is continued upwards for a short distance by the beginning of the development of the cortical plate.

The next stage of development involves a change in the characteristics of the neural epithelium. Continuity between the ventricular surface of the epithelium and the external or pial surface is maintained by the presence of ependymoglial cells which extend from the ventricle to pia so that the pial surface maintains contact with the ventricular surface for a considerable period during development. The ventricular ependymal layer remains throughout prenatal development as a pseudostratified columnar epithelium, the thickness and histological character of which vary in different parts and also with the stage of development. The characteristics depend partly on the proportion of ependymoglial cells and whether or not the cells retain contact with the pia.

The major features of the four divisions of the lateral ventricular wall and its associated subependymal layer are as follows. The medial roof retains the characteristic appearance of a pseudostratified columnar epithelium 8–10 nuclei deep from 11 until 16 days postconception. At 17 and 18 days the depth of the epithelium is reduced to 3–4 cells thick and by 18 days cilia are a prominent feature of this region. The small dark cells characteristic of subependymal cells, particularly in the ventricular elevations, are absent. The morphology of this region has already been described in great detail by Shoukimas & Hinds (1978) and as the present findings were similar to those of that study the morphology of this region will not be examined further in the present study. The medial wall was similar in development to the roof except that the surface mitotic figures were much fewer in number. This area retained a 'pseudocolumnar' appearance due to ependymoglial fibres extending into the septum.

As both the ventricular roof region and the ventricular elevations show special features, development of these regions will be described in detail. The major differences in the features of the two regions are largely the result of the distribution of ependymoglial guide fibres, the pattern of which is well demonstrated in Golgi sections (Fig. 2) and has previously been described in some detail by Astrom (1967), Smart (1978) and Smart & Sturrock (1979). The presence of ependymoglial cells to some extent complicates the morphology of the ependymal and subependymal layers. At the roof of the ventricle the ependymal fibres run almost vertically upwards to the glia limitans and probably are initially responsible for the formation of this layer. Laterally towards the caudatopallial angle the ependymoglial fibres are gradually deflected in a more and more lateral direction around the neostriatal anlage (Fig. 2). The ependymoglial fibres of the ventricular elevations barely extend into the subependymal layer of the elevations and are absent from the neostriatal anlage. Ependymoglial fibres are present in the lowest part of the lateral ventricular wall and along the whole extent of the medial wall and medial roof. Where ependymoglial fibres extend through the subependymal layer they separate the subependymal layer into columns, making the distinction between the pseudostratified columnar epithelium of the ependymal layer and the 'pseudocolumnar' subependymal layer extremely difficult. It is very easy to detect the junction between the ependymal and subependymal layers of the ventricular elevations (Figs. 3, 4) because the ependymo-

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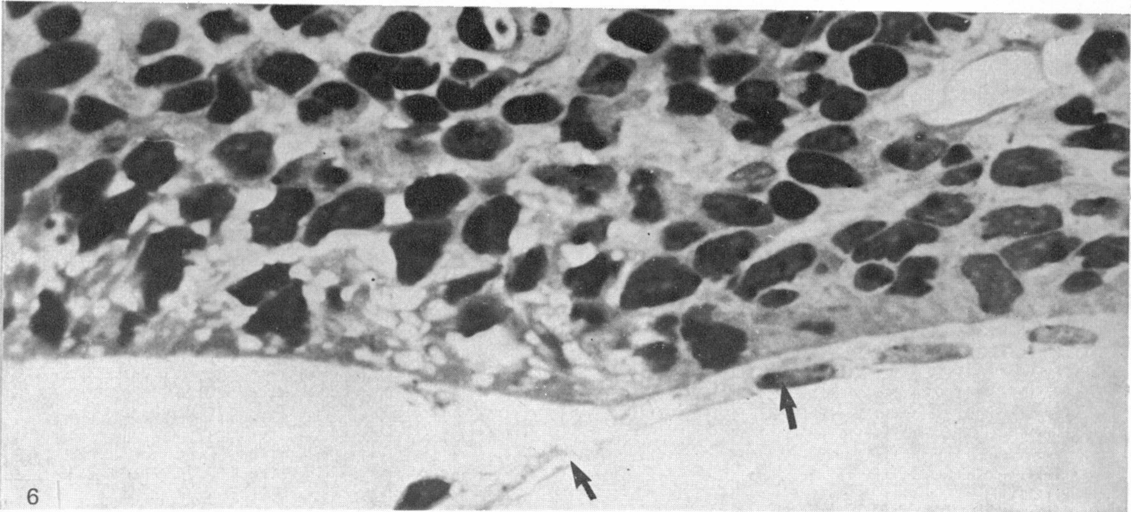

glial fibres do not extend for any distance into the subependymal layer. The distinction between the ependymal and subependymal layers of the ventricular roof, for example, is much more difficult.

The caudatopallial angle is the region where a large number of ependymoglial fibres come together before passing laterally around the neostriatum (Fig. 2). The boundary of this region is below the angle of the ventricle; it is evident in semithin sections as a strip of larger pale staining nuclei (Fig. 5) separating the subependymal region of the ventricular elevations, which is largely composed of small dark nuclei (Fig. 5), and the subependymal region of the ventricular roof, which is made up of small dark nuclei and some larger lighter nuclei (Fig. 5).

From 17 days postconception until a few days after birth a change occurs in the histology of the ventricular walls. Up to 15–16 days postconception, provided a low osmolarity fixative is used there is relatively little extracellular space (Figs. 3, 4, 15). At 17 days, however, there seems to be a sudden appearance of large extracellular clefts between the ependymal cells and in the subependymal region. This may be artifactual as these spaces are absent where the choroid plexus is firmly attached to the ependyma, but immediately the choroid plexus separates from the ependyma these spaces are present (Fig. 6).

With increasing age the fibre bundle of the caudatopallial angle shifts from below the upper lateral corner of the ventricle to just medial to the upper lateral corner on the ventricular roof (Fig. 7), but this may be due to a change in shape of the ventricle. As the subependymal layer decreases rapidly in size postnatally (Figs. 7–9) the ependymoglial fibres disappear entirely, usually by one week postnatum.

In the mature animal the subependymal layer is not restricted to the caudatopallial angle as is often assumed. The subependymal layer is most obvious at the caudatopallial angle and around the inferior horn of the ventricle (Fig. 9), but scattered subependymal cells can be found all around the lateral ventricle and are often aggregated at the superior part of ventricular coarctations (Fig. 9). In semithin sections the subependymal layer of the caudatopallial angle can be seen to contain a few neurons, astrocytes and subependymal cells (Figs. 10, 11). Mitotic figures are present throughout the region (Fig. 11) and even in areas of coarctation (Fig. 12). The caudatopallial subependymal layer also contains scattered myelinated axons apparently running in a rostrocaudal or caudorostral direction. In regions of ventricular coarctation multi-ciliated ventricular cells, neurons, astrocytes, microglia and subependymal cells are present, scattered along the site of fusion (Figs. 12, 13). Mitotic cells are present in the subependymal layer at all ages including 22 months (Fig. 14).

It is not intended to describe the ventricular or ependymal layer in any detail as this has been the subject of a number of ultrastructural studies (Hinds & Ruffet,

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Fig. 5. Shows the caudatopallial angle at E17. A band made up mainly of pale staining nuclei can be seen extending laterally from the ventricle between the dotted lines. This corresponds to the bunch of ependymoglial fibres. Above and medial to this the subependymal layer consists of a mixture of pale and dark nuclei with the dark nuclei tending to predominate at this age. Below the band of pale cells the majority of subependymal cells have small dark nuclei. 1 μm toluidine blue-stained section. × 750.

Fig. 6. Shows the lateral ventricular roof of a newborn mouse. The choroid plexus (arrows) can be seen. To the right of the Figure it is adherent to the ventricular roof and no extracellular space can be seen in contrast to the left where large extracellular spaces are present between ependymal cells. This suggests that these spaces are artefactual in origin. 1 μm toluidine blue-stained section. × 1200.

cc

7

cc

cp

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SEL

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1971; Seymour & Berry, 1975; Shoukimas & Hinds, 1978) and the findings of the present study were in agreement with earlier studies, except in the finding that the extracellular spaces described in the ependymal layer appear to be absent in young embryos fixed by perfusion (Fig. 15). The observation of Smart (1976) that the density of mitotic figures was much higher along the roof of the lateral ventricles and around the walls was substantiated. The depth of the pseudostratified columnar epithelium of the ependymal layer is difficult to determine for the reasons given above, but it appears to remain thicker over the ventricular elevations than along the roof, medial roof and medial wall. There is also an inverse correlation between the age of appearance of substantial numbers of ependymal cilia and the thickness of the ependyma. While an occasional cilium could be seen at all ages from 11 days postconception, ependymal cells containing numerous cilia were first observed at 18 days postconception, in the region of the medial roof (Fig. 16). Similar cells first appeared at birth along the ventricular roof and were not found along the lateral wall until 6 days postnatum, appearing first along the inferior parts of the lateral wall and extending superiorly. By 15 days postnatum ependymal cells in all parts of the ventricle appear to have developed cilia to a similar extent. Both light and dark ependymal cells are present by P15 (Fig. 17). Westergaard's observation (1970) that the adult ependyma varies from cuboidal to squamous, with squamous cells predominating in areas of white matter and cuboidal cells predominating in areas of grey matter, was confirmed.

Ependymoglial cells in the spinal cord of the embryonic mouse were described by Henrikson & Vaughn (1974). The characteristic feature of the cells was the pale cytoplasm largely devoid of organelles and containing scattered glycogen granules. A few processes resembling this description were found along the roof of the ventricles (Fig. 18). Similar processes were observed in sections cut at right angles to the fibre bundles streaming laterally from the caudatopallial angle (Fig. 19). These processes were quite distinct from the axons running in the same region. Large cells with a pale cytoplasm containing a few strands of rough endoplasmic reticulum and numerous rosettes of free ribosomes and also a few scattered glycogen granules were found in the ependymal layer and subependymal layer of the ventricular roof and in the developing corpus callosum (Fig. 20). The cytoplasm of these cells and their processes closely resembled the processes forming the pia-glial membrane (Fig. 21). It was concluded that these cells and their processes are the ependymoglia cells and in some cases extend from the ventricle to the pia-glial membrane, particularly in young embryos. The glycogen is sparsely scattered in small granules and differs from the coarser clumps usually found in immature astrocytes (Sturrock,

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Fig. 7. In this coronal section from a newborn mouse ependymoglial fibres can still be seen (arrows) but their site of origin appears to have moved medial to the caudatopallial angle (cf. Fig. 5). The characteristics of the subependymal layer above and below the fibres still differ, with the larger pale nuclei mainly restricted to the part superior to the fibres. At this age the ependyma of the ventricular roof is still separated from the corpus callosum (*cc*) by a substantial subendymal layer. 1 μm toluidine blue-stained section. × 1200.

Fig. 8. By P15 a few myelinated axons can be seen in the corpus callosum (*cc*) and, while scattered subependymal cells can still be found between the ependymal layer and the ventricular roof, the subependymal layer no longer forms a complete layer between the ependyma and corpus callosum. The subependymal layer (*SEL*) is thickest at the upper lateral border of the ventricle. By 15 days postnatum the ependymal layer (*E*) is ciliated throughout the lateral ventricle. 1 μm toluidine blue-stained section. × 1200.

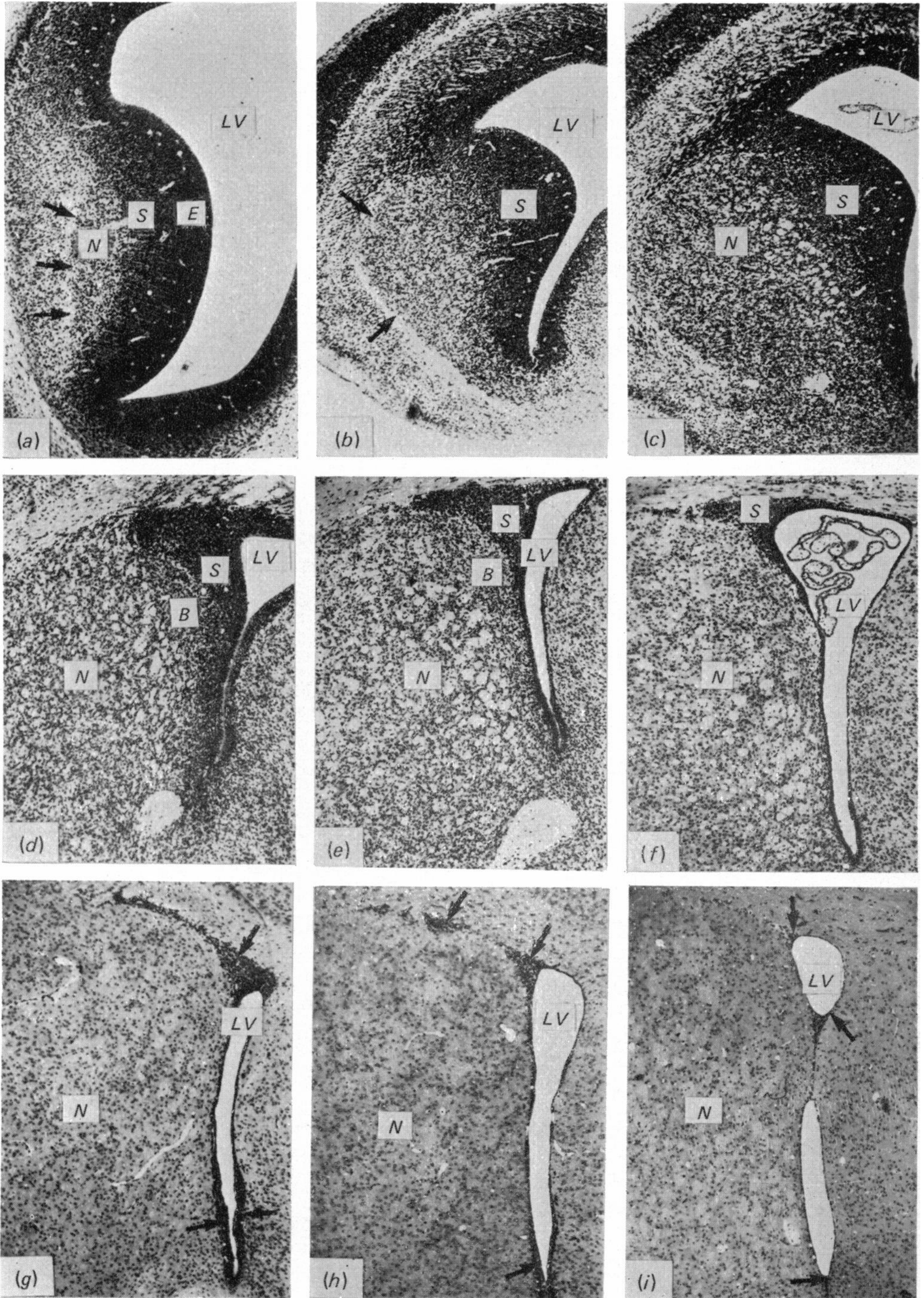

Fig. 9.

1974, 1976). These cells appear identical to the cells previously described as early glioblasts (Sturrock, 1974, 1976).

Cells of the subependymal layer of the ventricular elevations are mainly small cells with irregular nuclei and moderately densely staining cytoplasm (Figs. 22, 23). Most mitotic figures in the subependymal layer have a similar cytoplasmic density (Fig. 22). The cytoplasm is rich in free ribosomes and also contains scattered mitochondria (Figs. 22, 23). The features of these cells do not appear to change with age. Subependymal cells at 22 months (Fig. 30) are similar to those at 15 days postconception (Figs. 22, 23). Over the roof of the ventricle as well as the dark glioblasts larger cells with a lighter cytoplasm are found (Fig. 24). These cells differ from the ependymoglial cells in having more heterochromatin in their nuclei (Fig. 24) and more organelles including rough endoplasmic reticulum and mitochondria. Glycogen granules are absent. A few light cells are present in the caudatopallial subependymal layer up to 15 days postnatum (Fig. 26) although dark glioblasts (Fig. 25) predominate. Both light and dark cells are capable of mitosis at this age (Figs. 28, 29) although the majority of mitotic cells are dark ones. Astrocytes are found in the subependymal region after birth. The time of their appearance seems to depend on the age at which the ependymal cells differentiate into a single-layered epithelium. At first dark astrocytes (Fig. 27) predominate and are still the most common type of astrocyte at 15 days postnatum (Fig. 27) although light astrocytes are appearing at this age (Fig. 27). With increasing age the main features of subependymal astrocytes are the great increase in microfibrils and lipofuscin bodies (Fig. 31). Lipofuscin and large lipid droplets are present in ependymal cells from 3 months of age, and large masses of lipofuscin are also present in adjacent subependymal astrocytes.

DISCUSSION

As one might expect in areas where cells are being produced in large numbers as well as apparently distinct cell types, there are cells with intermediate features which cannot easily be placed into one category or another. It is also likely that different cells are produced over different periods of time. Prenatally there are four major

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Fig. 9. Demonstrates changes in extent of the subependymal layer (*S*) from E13 up to E90. Lateral ventricle (*LV*). H. & E.-stained coronal sections. × 30.

(*a*) E13. The neostriatal anlage (*N*) lies lateral to the subependymal layer (*S*) and ependymal layer (*E*). The lateral boundary is formed by the line of palely staining glial guide fibres (arrows). Note the relatively rich blood supply to the ependyma and subependymal layers.

(*b*) E15. The subependymal layer is most obvious on the lateral side of the ventricle. The pale guide fibres can clearly be seen extending from below the caudatopallial angle (arrows).

(*c*) E17. The subependymal layer on the lateral side of the ventricle is still thick (*S*).

(*d*) E19. The subependymal layer has decreased in thickness and the border area (*b*) between the subependymal layer and neostriatum (*N*) is more obvious than in earlier photographs.

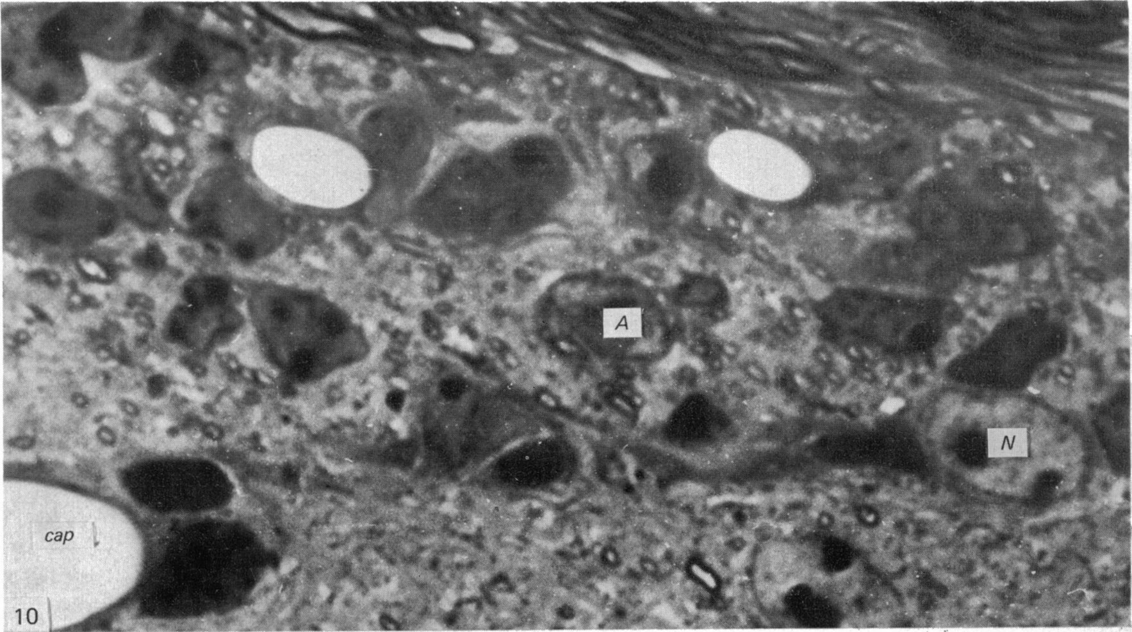
(*e*) P4. The subependymal layer is now most prominent at the caudatopallial angle and inferior tip of the ventricle.

(*f*) P8. The subependymal layer volume has declined further.

(*g*) P15. The subependymal layer (arrows) has continued to decline except at the caudatopallial angle and the inferior part of the ventricle.

(*h*) P25. Shows the continuing decrease in subependymal volume which is mainly restricted to the caudatopallial angle and inferior tip of the ventricle. Small scattered islands of subependymal cells can be seen laterally below the corpus callosum.

(*i*) P90. The subependymal layer is now restricted to a few cells at the caudatopallial angle and tip of the ventricle, although at higher power subependymal cells can be found scattered all round the ventricular wall. This lateral ventricle shows a degree of coarctation.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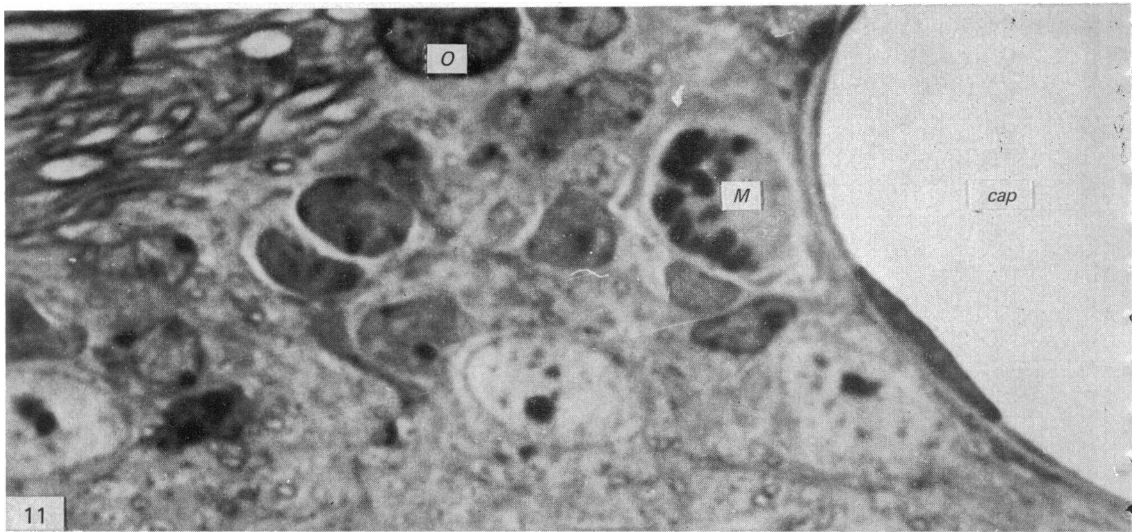
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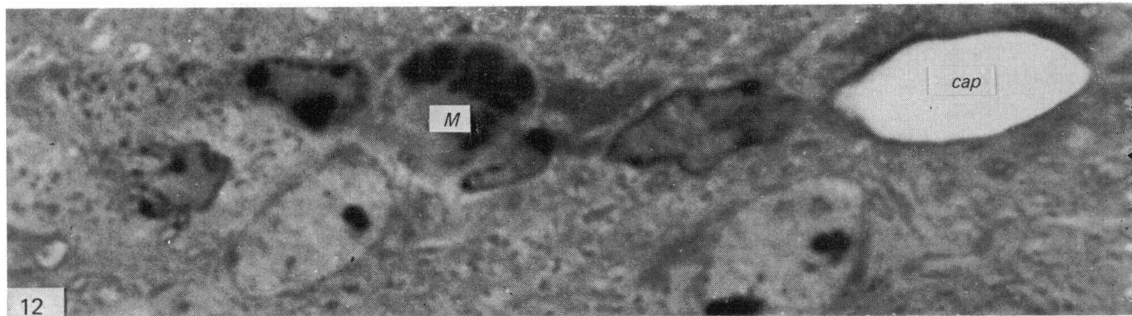
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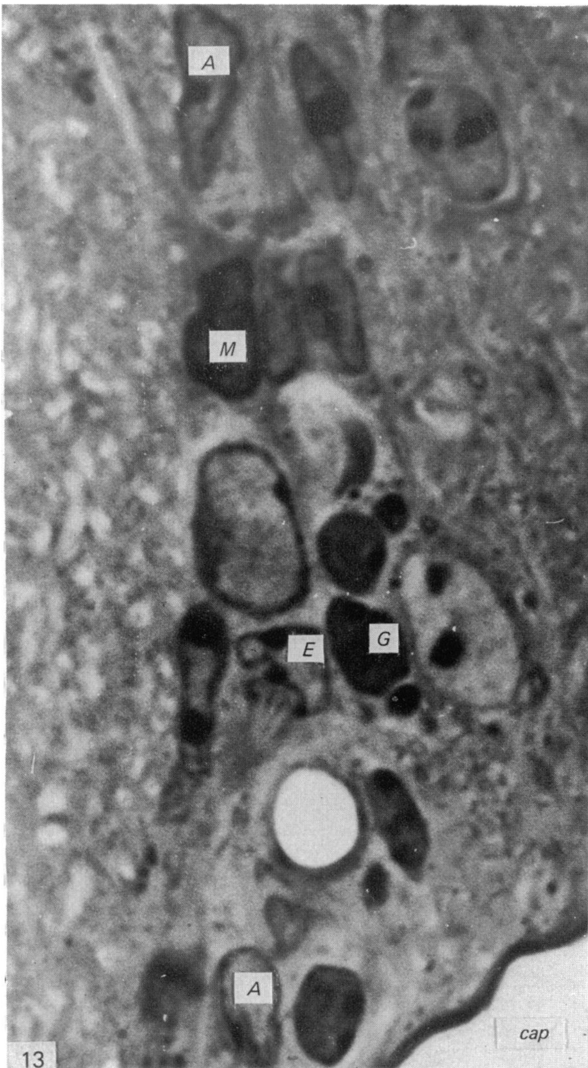


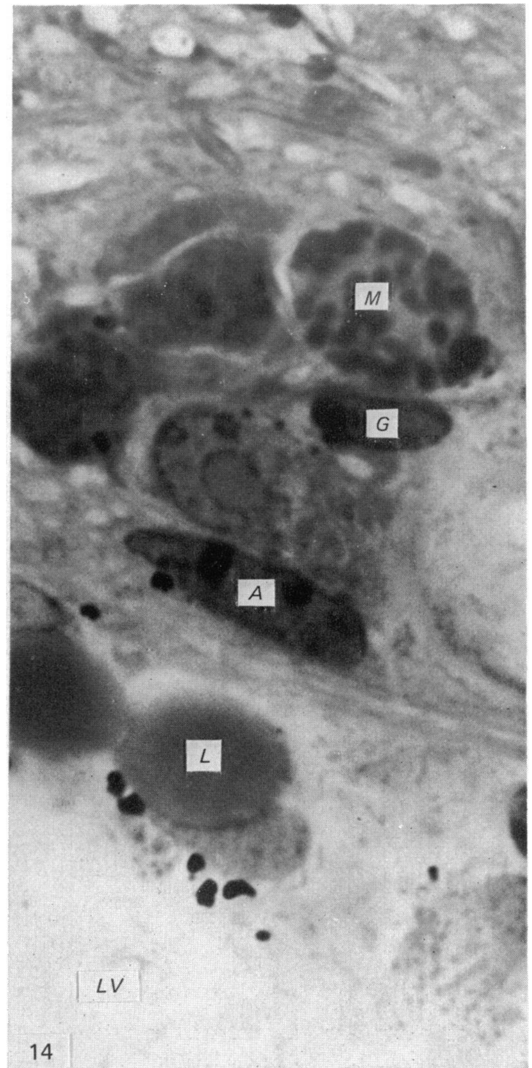


Fig. 13. Another example of subependymal cells in a ventricular coarctation at 3 months. Examples of astrocytes (*A*), microglia (*M*) and glioblasts (*G*) can be seen in this micrograph as well as a multi-ciliated ependymal cell (*E*).

Fig. 14. At 22 months mitotic cells (*M*) and glioblasts (*G*) can still be found in the subependymal layer. The astrocyte (*A*) contains lipofuscin body. At this age the ependyma contains masses of lipofuscin and large lipid droplets (*L*). 1 μm toluidine blue-stained section. ×2400.

Fig. 10. This shows the subependymal layer at the caudatopallial angle at 3 months of age. The majority of cells appear to be glioblasts, but astrocytes (*A*) and neurons (*N*) can be identified. Note the large number of myelinated axons scattered throughout the subependymal layer. *cap*, capillary. 1 μm toluidine blue-stained section. ×2400.

Fig. 11. Shows the lateral part of the subependymal layer at the caudatopallial angle at 3 months. The large capillary (*cap*) is a common landmark in coronal sections. An oligodendrocyte (*O*) can be seen adjacent to the corpus callosum and a mitotic cell (*M*) is present close to the capillary. 1μm toluidine blue-stained section. ×2400.

Fig. 12. This shows a mitotic subependymal cell (*M*) lying in part of the subependymal layer at a coarcted portion of the ventricle (see Fig. 9). *cap*, capillary. 1 μm toluidine blue-stained section. ×2400.

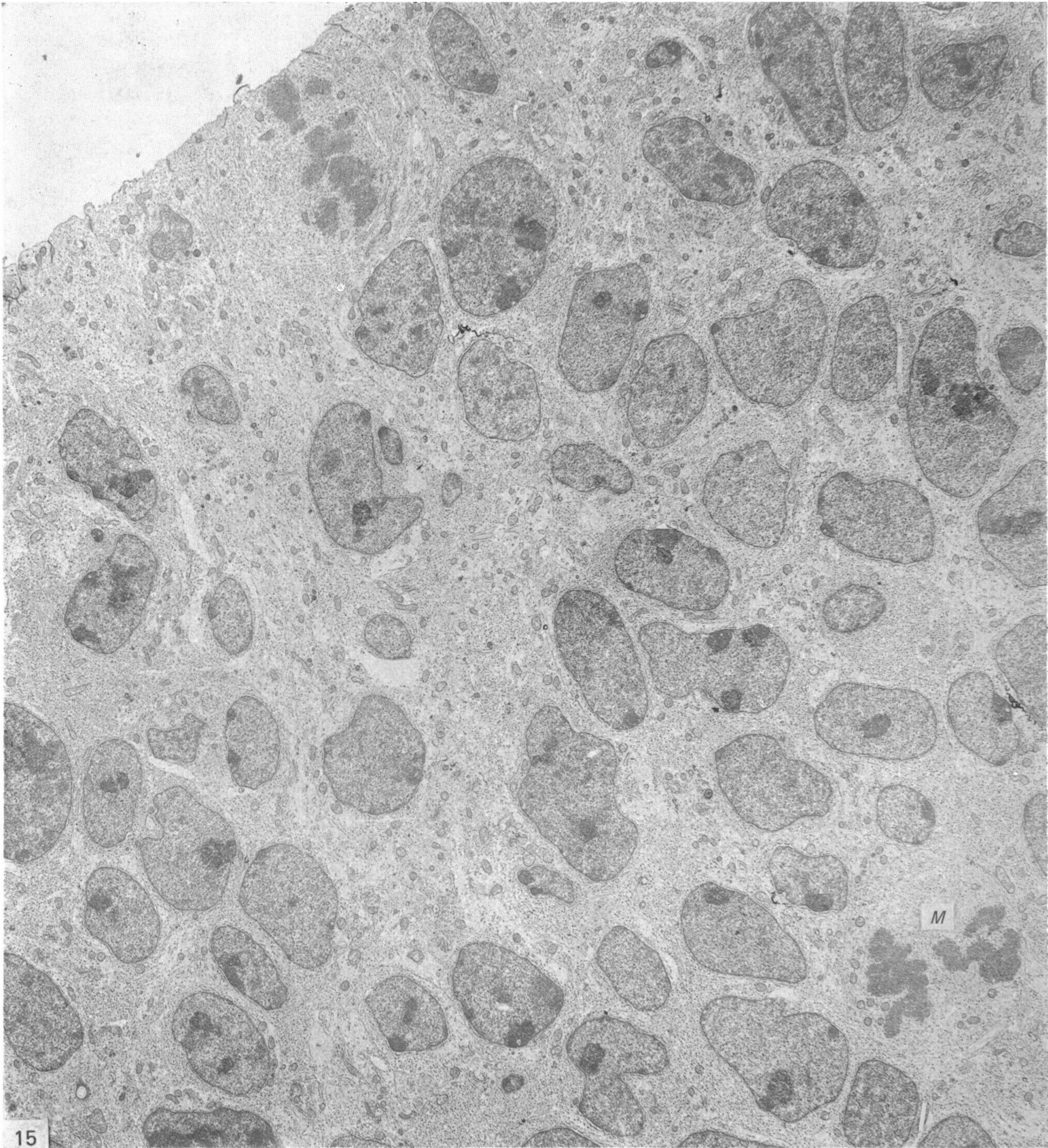
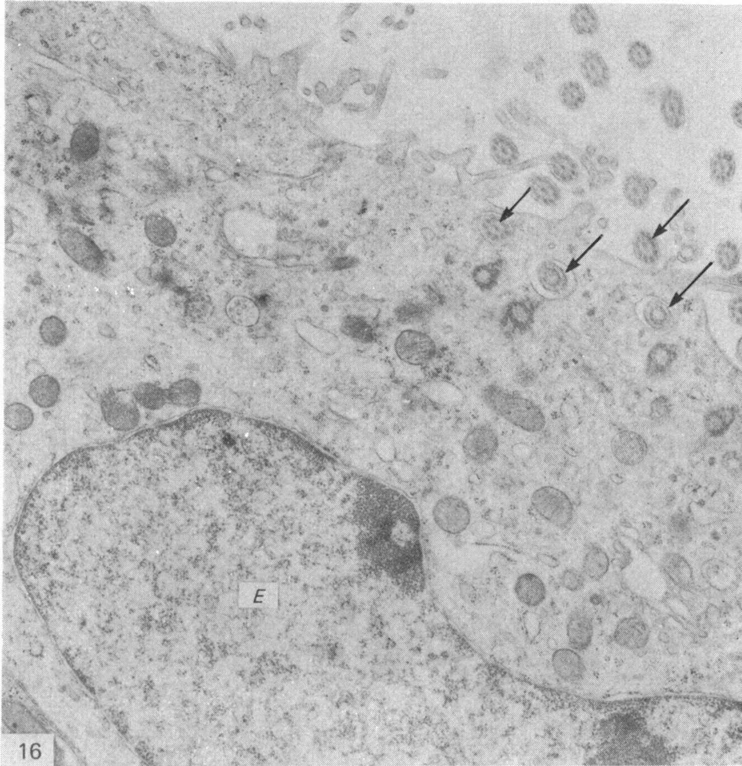


Fig. 15. Shows the ependymal layer at 14 days postconception over the lateral ganglionic eminence. At the bottom right hand edge the beginning of the subependymal layer, including a mitotic cell (*M*), is apparent. This seems to demonstrate that in this area at least there is no marked extracellular space. Electron micrograph. ×4000.



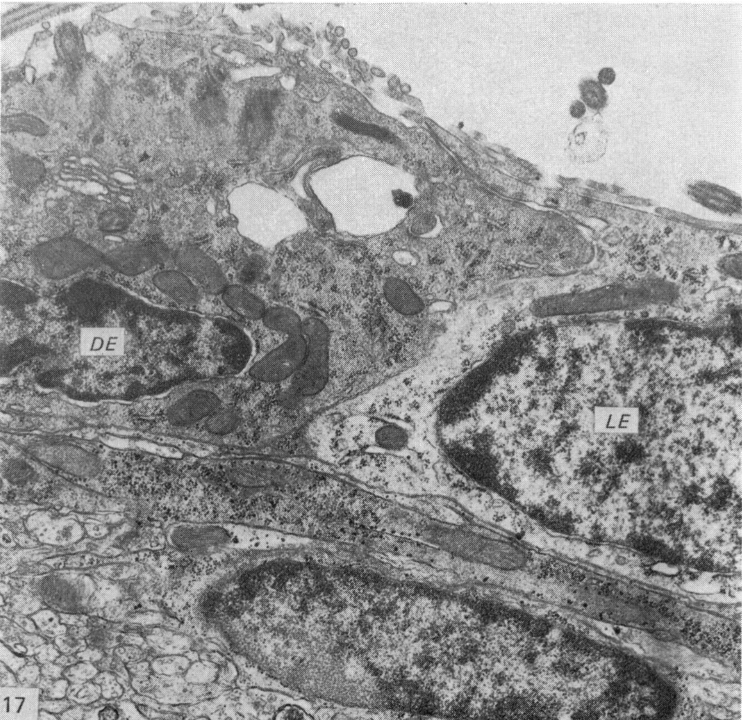
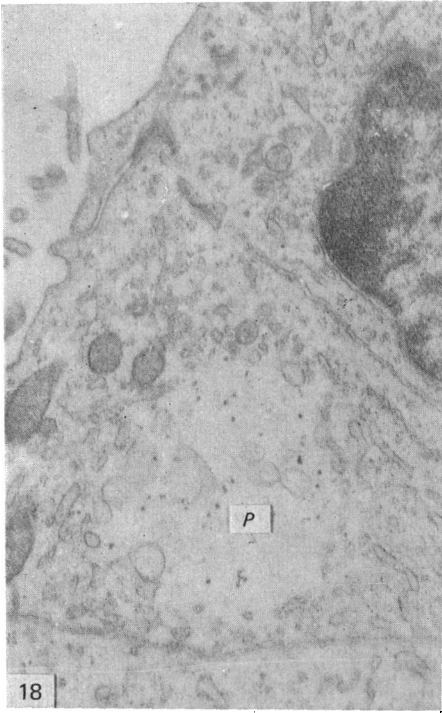
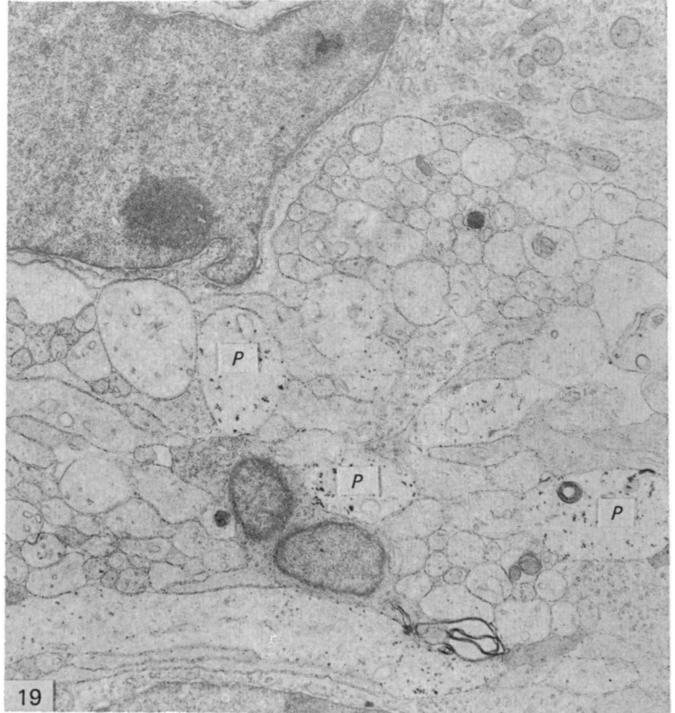
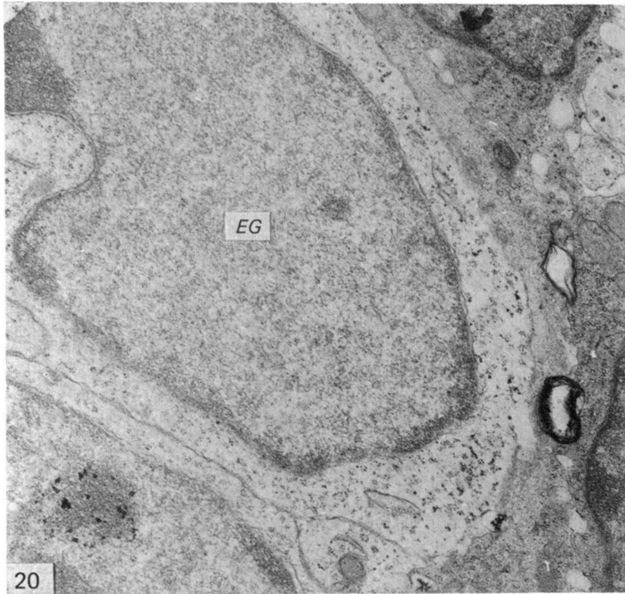


Fig. 16. This ependymal cell (*E*) has numerous cilia (arrows). Multi-ciliated ependymal cells first appear in the region of the medial roof followed by the ventricular roof. Electron micrograph. E.17. ×12600.

Fig. 17. By P15 two types of ependymal cell are present: one type has a light cytoplasm (*LE*) and the other has a dark cytoplasm (*DE*). Electron micrograph. P15. ×12600.







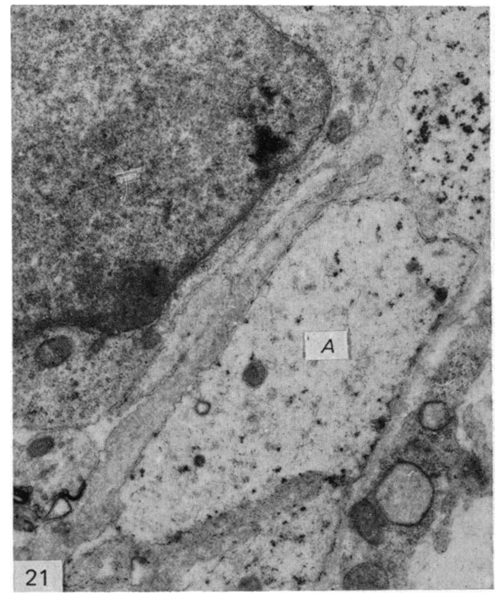
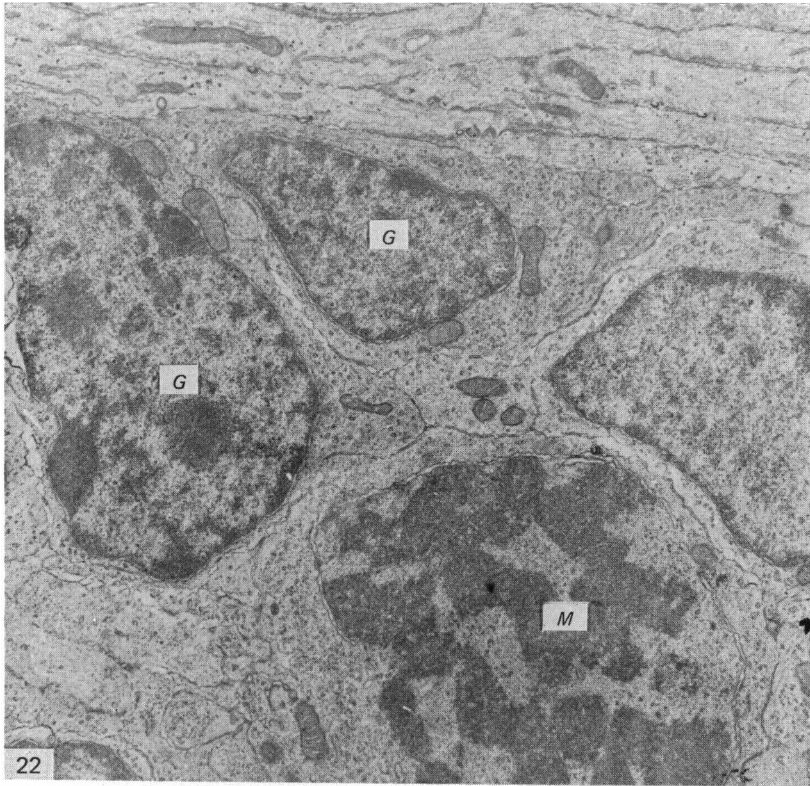


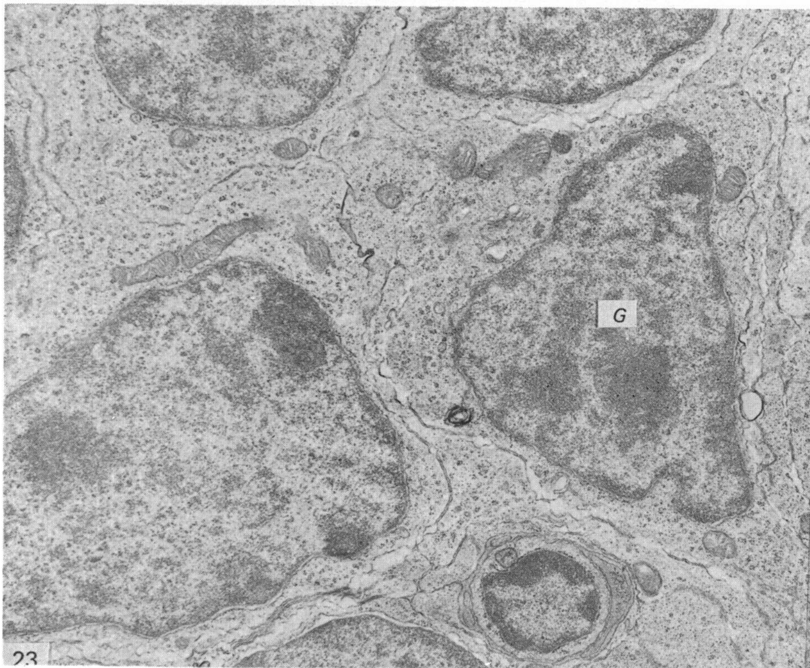
Fig. 18. This process (*P*) was observed in the ventricular roof at E17. It contains a large pale area containing a few scattered glycogen granules. Electron micrograph. ×20000.

Fig. 19. Tangential section through the junction of the developing corpus callosum and the leash of ependymoglial fibres at E15. Note the numerous pale fairly large diameter processes (*P*) containing scattered glycogen granules. Electron micrograph. ×12600.

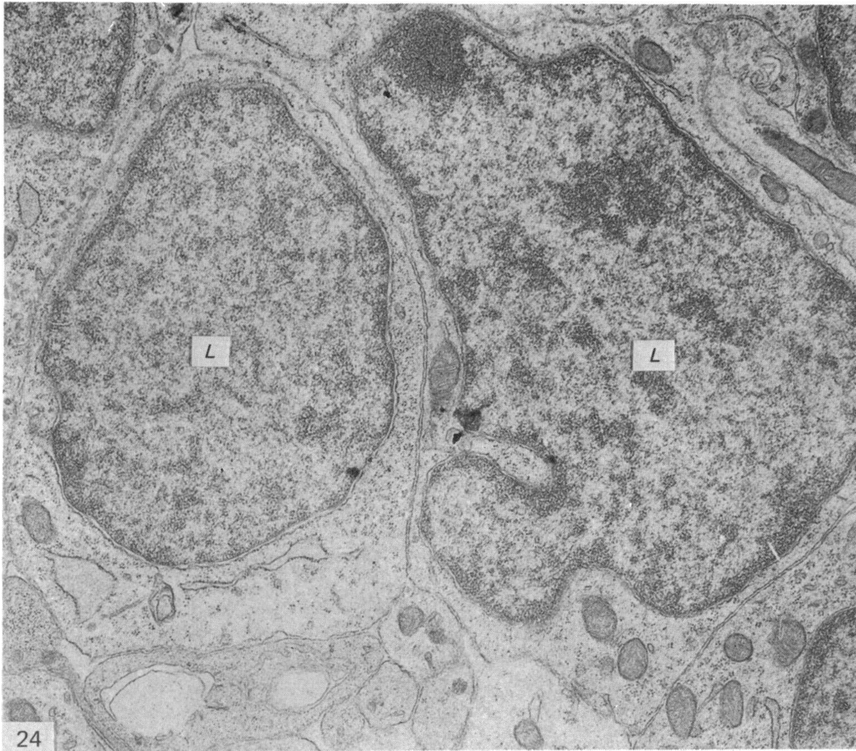
Fig. 20. Shows an 'early' glioblast (*EG*) in the developing cortical plate at E15. The cytoplasm contains few organelles except for rosettes of free ribosomes and a few strands of endoplasmic reticulum. A few scattered glycogen granules are present. Electron micrograph. ×12600.

Fig. 21. Glia limitans at E15. The processes forming the glia limitans are pale staining and contain few organelles, but do contain scattered glycogen granules. A comparison of Figs. 18–21 suggests that the processes in Figs. 18, 19 and 21 may well be related to cells similar to that shown in Fig. 21. Electron micrograph. ×12600.





Figs. 22–23. These micrographs show glioblasts (*G*) in the subependymal layer just inferior to the caudatopallial angle at E15. The glioblasts in Fig. 22 are lying adjacent to the fibre bundle which extends laterally from the angle. A mitotic glioblast is also present (*M*). Fig. 23 shows how closely glioblasts are packed together in this region. Electron micrographs. × 12600.



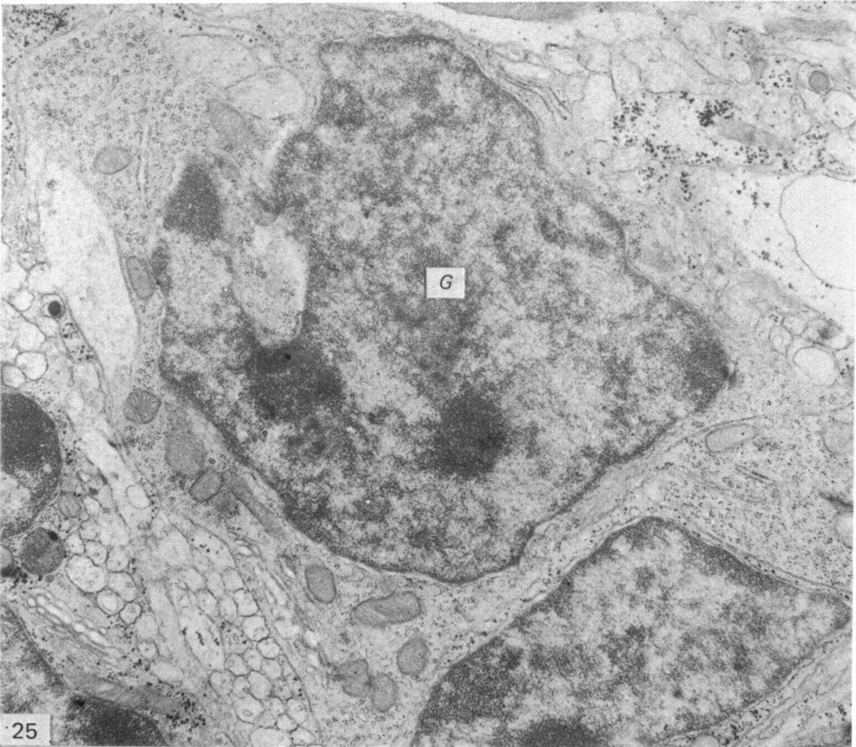
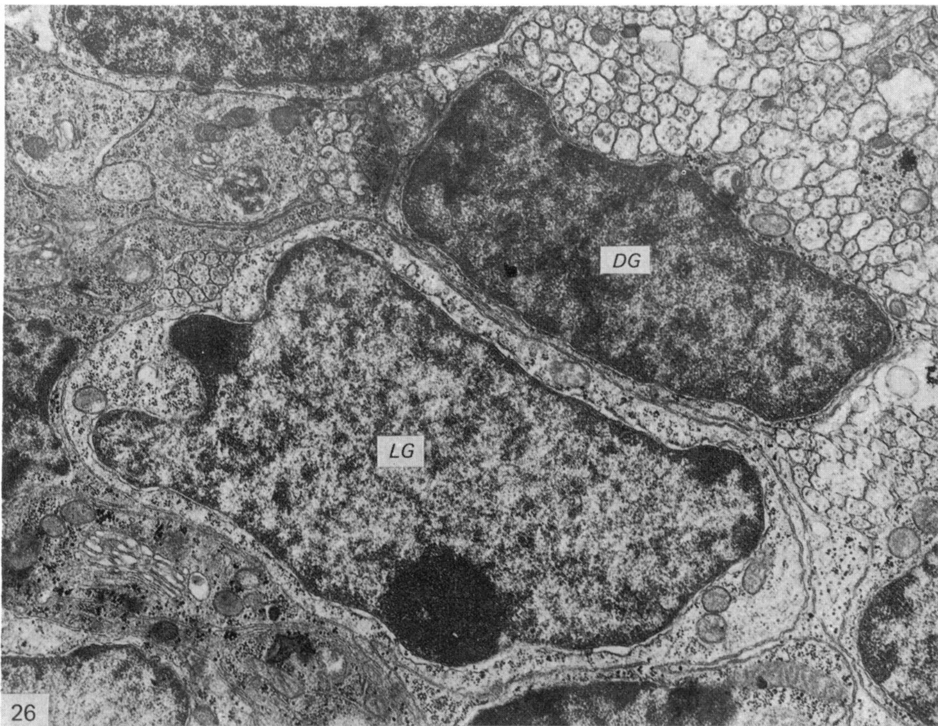


Fig. 24. Two light cells (*L*) in the subependymal region of the ventricular roof at E17. These cells are larger than glioblasts in the subependymal layer of the lateral wall (cf. Figs. 22–23). The cytoplasm is also lighter but mitochondria are present which helps to differentiate these cells from the 'early' or ependymoglioblasts (cf. Fig. 20). Electron micrograph. ×12600.

Fig. 25. Glioblast from the subependymal layer at the caudatopallial angle at P15. These cells are larger than glioblasts seen in prenatal animals but the nuclei are similar in appearance. The cytoplasm density is also similar. Electron micrograph. ×12600.



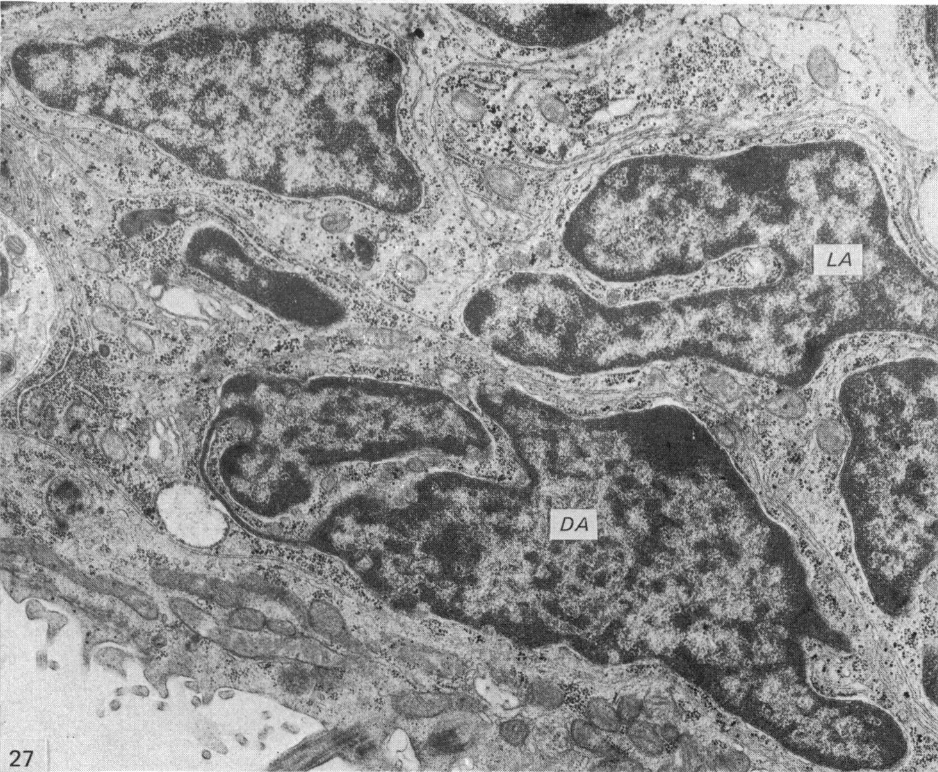
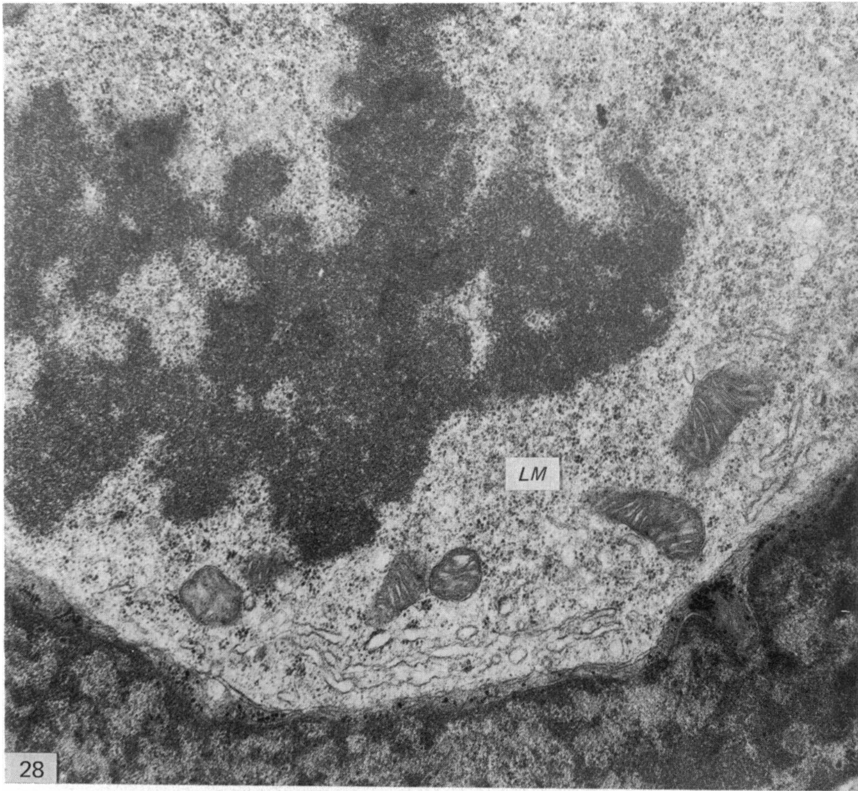
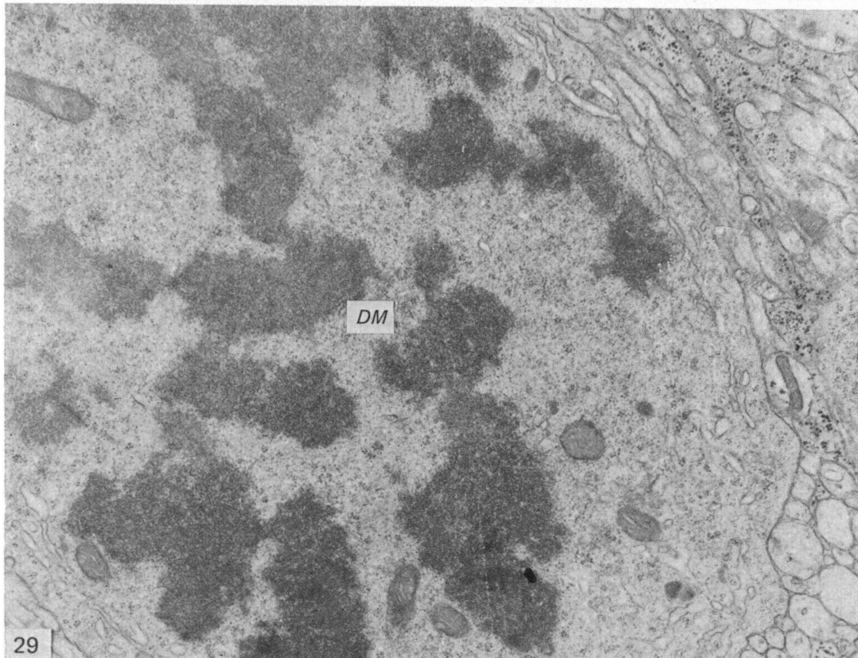


Fig. 26. This shows a dark glioblast (*DG*) and a light glioblast (*LG*) at P15. Light glioblasts are fewer in number and are not present prenatally. They are smaller than the light cells seen in the ventricular roof prenatally and the chromatin is usually more condensed in clumps. Electron micrograph. × 12600.

Fig. 27. At P15 both dark (*DA*) and light astrocytes (*LA*) are present in the subependymal layer. The main features of these cells are the extremely irregular nuclei and numerous glycogen granules. Electron micrograph. × 12600.





Figs. 28–29. At P15 both light (*LM*) and dark (*DM*) mitotic figures are present, suggesting that both light and dark cells undergo mitosis. This seems to disprove the suggestion sometimes made that dark and light cells represent different phases of the mitotic cycle of the same cell. Both these mitotic figures appear to be metaphases. Electron micrographs. × 20000.

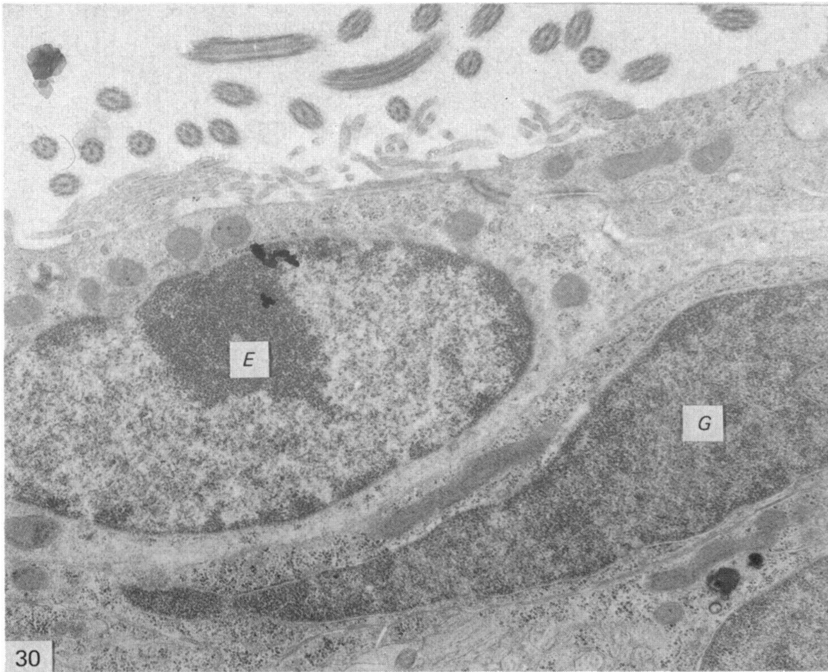


Fig. 30. At 22 months dark glioblasts (*G*) are still present in the subependymal layer. The ependymal cell (*E*) has both microvilli and cilia. Electron micrograph. × 12600.

Fig. 31. At 22 months subependymal astrocytes *A* contain large clumps of microfilaments (*mf*) and lipofuscin granules (*L*). Subependymal astrocytes (*A*) are also rich in glycogen. Electron micrograph. × 12600.

groups of cells in the ependymal and subependymal layers. The simple columnar ependymal or ventricular cell was described by Hinds & Ruffett (1971) and Shoukimas & Hinds (1978). This cell retains its deep columnar characteristics for the longest period of time in the medial roof and ventricular elevations. The ventricular roof becomes a highly mitotically active low columnar epithelium at an early stage of development. A characteristic feature of the neural epithelium is the elevator movement of the nucleus during mitosis (Sauer, 1935, 1936; Hinds & Ruffett, 1971; Seymour & Berry, 1975). The thinning of the roof epithelium may represent a mechanism for reducing the duration of the generation cycle of germinal cells by reducing the extent of the elevator movement. The guide fibre system seems to indicate that the roof of the ventricle is the site of production of most cortical neurons, and this is supported by radioautographic studies (Smart & Smart, 1977, and unpublished). The second type of cell is the ependymoglial, which is a large pale cell with very few organelles. Processes of these cells predominate in the fibre bundles extending from the caudatopallial angle until 15 days postconception, when axons forming the corpus callosum gradually take over. Cells similar to ependymoglial cells were present in very small numbers in the prenatal anterior commissure (Sturrock, 1974) and in the early postnatal corpus callosum (Sturrock, 1976). The nuclei of these cells are situated in the subependymal layer of the ventricular roof, in the fibre bundles extending from the caudatopallial angle and in the developing cortex. They are not present in the subependymal layer of the ventricular elevation. The third type of cell in the prenatal subependymal layer is the predominant type over the roof of the ventricle and is the large pale cell moderately well endowed with organelles and believed to be a neuroblast. These cells are present in the leash of fibres extending from the caudatopallial angle but are relatively infrequent in the subependymal layer of the ventricular elevations. The small dark cell predominates in the subependymal layer of the ventricular elevations, especially after 16 days postconception. This is the type of cell described as characteristic of the postnatal subependymal layer (Blakemore, 1969; Privat & Leblond, 1972).

In the postnatal subependymal layer another type of large pale cell appears around 5 days postnatum, and this is probably a light oligodendrocyte. These cells are absent after 45 days when the major types of cell in the subependymal layer are astrocytes and glioblasts. A few neurons are present, as are a few microglia, although it seems unlikely that they are more common here than elsewhere in the brain despite claims to the contrary, particularly in neonatal animals (Cammermeyer, 1965; Stensaas & Gilson, 1972). The relatively large number of microglia observed in this region in perinatal animals may be due to migration of intraventricular macrophages through the ventricular walls (Sturrock, 1978*b*). The hypothesis that microglia arise from intraventricular macrophages (Lewis, 1974; Imamoto & Leblond, 1978; Sturrock, 1978*b*) receives further indirect support from the observation that microglia are present in the neostriatum of mice as early as 13 days postconception (Sturrock, 1978*b*; Smart & Sturrock, 1979), whereas they appear to be absent from rat occipital cortex until 6 days after birth (Luder, Parnavelas & Lieberman, 1979). The difference in time of appearance could be explained in terms of the distance of the two structures from the lateral ventricle.

The scheme of differentiation proposed from the present study is as follows. The ependymal or ventricular layer of cells gives rise *in situ* to ependymoglial cells which initially retain contact with both ventricular and pial surfaces. These cells form the pia-glial membrane, the nucleus migrates to the pial surface, contact is lost with the

ventricle and they eventually differentiate into astrocytes of the glia limitans. The pattern of differentiation of these cells is different from that of astrocytes in other regions. The ependymal layer also produces neuroblasts, and this production is greatest over the roof of the ventricle where most of the cortical neurons are produced. Ependymal cells which lose contact with the surface give rise to subependymal cells. The mitotically active subependymal cell is probably the small dark cell which is a glial precursor, but the possibility that neuroblasts divide while migrating through the subependymal layer cannot be excluded. The major part of subependymal cell production occurs prenatally (Smart, 1976; Sturrock, 1979) and postnatally the major feature is the rapid decrease in volume of the subependymal layer which begins prenatally (Smart & Sturrock, 1979). Compared with other parts of the brain, however, cell division continues to be relatively frequent in the subependymal layer. Radioautographic studies (Paterson *et al.* 1973; Imamoto, Paterson & Leblond, 1978) suggest that the majoı function of the postnatal subependymal layer is the production of oligodendrocytes, but astrocytes may also be produced postnatally. The development of astrocytes from dark glioblasts has been described previously (Sturrock, 1974, 1976). It is worth noting that dark astrocytes appear to be present in the subependymal layer in large numbers at 15 days postnatum, by which age they have disappeared completely from the indusium griseum (Sturrock, 1978*c*) and largely from the white matter (Sturrock, 1974, 1976).

In effect, while the ependymal layer produces neuroblasts directly and glioblasts via the subependymal layer all round the ventricle, the ventricular roof appears to be the major site of neuron production and the subependymal layer of the ventricular elevations seems to be the major site of glial production. The astrocytes of the subependymal layer contain large amounts of lipofuscin at a relatively early age, as do the adjacent ependymal cells. Ependymal cells also contain large lipid droplets. It is not possible to determine whether this early build up of lipofuscin is due to early ageing or to extrusion of lipofuscin from neurons and glia of the adjacent septum and neostriatum into subependymal astrocytes and ependymal cells. Removal of lipofuscin from neurons by astrocytes and microglia has been proposed by Brizzee, Kaack & Klara (1975) and by Glees & Hasan (1976), but directly evidence for this is equivocal and there appears to be no difference in the build-up of lipofuscin and lipid between the parts of the ventricle adjacent to grey matter and the parts adjacent to white matter. At no age is lipofuscin found in the glioblasts of the subependymal plate.

SUMMARY

The natural history of the subependymal layer around the lateral ventricle of the mouse brain was studied from its appearance at E11 up to 22 months postnatum. In the young embryo four regions of the ventricle can be recognized by their histological characteristics: (1) the ventricular roof, (2) the medial roof, (3) the ventricular elevations and (4) the medial wall. The characteristics of the ventricular roof and ventriculaı elevations were examined in detail. The ventricular roof appeaıs to be the main site of production of cortical neurons while the subependymal layer of the ventricular elevations seems to be the main site of origin of foıebrain glia. The age of differentiation of the ependyma differs for each region, with the medial roof differentiating first, followed by the ventricular roof and medial wall, and ventricular elevations or lateral wall last. Diffeıentiation begins with a change from pseudostratified columnar epithelium to simple columnar epithelium and the appearance of cilia in large numbers.

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