A CORRELATIVE DEVELOPMENTAL STUDY OF STRUCTURES INVOLVED IN THE PRODUCTION, CIRCULATION AND REMOVAL OF CEREBROSPINAL FLUID

By

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DEDICATED

to

My Parents

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ABSTRACT

The development of the choroid plexus, the IVth ventricular foramina and subarachnoid space has been studied by optical microscopy and morphometric analysis of semi-thin plastic sections and by TEM and SEM in a series of CBA mouse embryos from 11th day p.c. to newborn.

1. Choroid plexus of the IVth ventricle

At the 11th day p.c. the choroid epithelial cells were already recognisable in SEM by their bulging apices and relatively abundant microvilli; their lateral plasma membranes, as shown by TEM, were straight and simple. The tela choroidea was more vascular in relation to this specialised epithelium but invagination into the ventricle had not yet begun. By the 13th day p.c. important qualitative changes had taken place: The apical surfaces of choroid epithelial cells were densely covered by microvilli and their basilateral plasma membranes showed complex infoldings and interdigitations. These two features together with cytoplasmic organelles (mitochondria, rough endoplasmic reticulum, Golgi complex) were essentially similar between 13th day p.c. and birth. Although there was an apparent increase in the size of the choroid plexus from the time of its first appearance, no significant progressive changes were found in the volume densities of its components (epithelium, blood vessels and connective tissue) during prenatal development. The structural evidence is consistent with functional secretory

capacity of the plexus from the 13th day p.c.

SEM showed the IVth ventricular choroidal "capillaries" to be sinusoidal in nature, and clearly different from those of adjacent subependymal cerebral capillaries, and also surprisingly, those of the lateral ventricle choroid plexus. Although they remain sinusoidal until birth, their endothelial lining was shown by TEM to become progressively and uniformly thinner and to contain an increasing number of fenestrae as development progressed. The endothelial basement membrane was ill-defined and poorly developed throughout development. The choroidal capillaries also became more intimately related to the epithelium as development proceeded. All these features of endothelium would facilitate the movement of substances across the capillary wall.

2. Foramina of the IVth ventricle

Although the caudal part of the roof of the IVth ventricle remained extremely attenuated throughout development, no foramina or interependymal pores were found.

The ependymal lining of the lateral recesses started to attenuate by the 17th day p.c. but lateral foramina did not appear until birth. The lateral foramina seemed to arise as the result of an active developmental process and not merely through the rupture of the ependymal wall by the CSF pressure. The ventricular cavity was therefore an anatomically closed one until the appearance of the lateral foramina at birth when bulk flow of CSF from the

ventricular cavity into the subarachnoid space was first established.

3. The subarachnoid space

At the 11th day p.c., a vascular cellular mesenchyme occupied the entire interval between the neuroepithelium and the future epidermis. At the 13th day p.c., the small, spindle-shaped mesenchymal cells were becoming widely separated, in some symmetrically placed areas around the brain, by abundant intercellular matrix. Definite "spaces", presumably fluid-filled, were first seen at the 14th day p.c., particularly over the lateral, ventral and dorsolateral aspects of the hind brain, but not on the dorsal aspect, adjacent to the thin roof of the IVth ventricle. The appearance of the subarachnoid spaces coincided with the appearance of a large number of macrophages in these spaces. The significance of this association is discussed. Contrary to previously held views, it was concluded that bulk flow of CSF does not initiate the development of the subarachnoid space.

4. Arachnoid villi

Arachnoid villi were not present in the mouse, but the supporting dura was found to be a thin layer with loosely and randomly oriented fibres. This thin supporting dural layer is apparently adequate for the support of the small size mouse brain and at the same time allows CSF to percolate through from the subarachnoid space to reach the venous sinuses without the need for arachnoid villi.

INTRODUCTION

I. Aims and objectives

The objective of this study was to provide correlated morphological data on the development, in the mouse, of structures involved in the production, circulation and removal of cerebrospinal fluid (CSF):

1 - Development and histogenesis of the choroid plexus of the IVth ventricle, with particular reference to the onset of functional activity.

2 - Establishment of foramina in the roof of the IVth ventricle, with particular reference to the establishment of bulk flow of CSF from the IVth ventricle to the subarachnoid space.

3 - The development of the subarachnoid space.

4 - The development of the arachnoid villi, with reference to the uptake of CSF into the venous blood.

II. Survey of the previous literature:

Although there have been many studies of one or two of the topics listed, there have been none, since the work of Weed in 1917, to determine if there is a temporal relationship between the development of the choroid plexus, the appearance of foramina in the roof of the IVth ventricle, the development of the subarachnoid space and the drainage of cerebrospinal fluid into the venous system.

Since the significance of many of the observations on the developing CSF system depends on comparison with its structure and function in the adult, this survey begins with the adult situation.

A. ADULT CHOROID PLEXUS

i) Fine structure

The fine structure of the adult choroid plexus has been extensively studied in many species: in the cat, rabbit and rat by Maxwell and Pease (1956); in rabbit, monkey, rat, dog and woodchuck by Wislocki and Ladman (1958); and in the rabbit by Tennyson and Pappas (1968).

The fine structure of the choroid plexus of all the mammalian species so far examined was more or less similar.

The choroid plexus consists of a single layer of cuboidal epithelial cells resting on a basement membrane, and a core of vascular connective tissue. The blood vessels towards the free edge of the plexus are mainly capillaries whereas arterioles and small arteries predominate at the root of the plexus, where connective tissue elements are more abundant.

The apical surface of the choroid epithelial cells shows numerous microvilli and occasional cilia although microvilli are absent in the Gecko (Murakami, 1961). The microvilli vary in shape and size from slender projections to apical blebs; these variations provided the basis for a speculation about an apocrine mode of secretion made by Wislocki and Ladman (1958). Millen and Rogers (1956) maintained that these blebs were due to unfolding and distension of the microvilli. Santolaya and Echandia (1968) and Collins and Morriss (1975) found that there was a great increase in the number of apical blebs when cerebrospinal fluid production was stimulated by the

administration of pilocarpine, and a reduction when the production of cerebrospinal fluid was inhibited by the intraperitoneal administration of acetazolamide (Diamox). They considered this as evidence of apocrine secretion by the choroid plexus. Tennyson and Pappas (1964) on the other hand, considered these blebs as fixation artifacts. Davi$, Lloyd and Milhorat (1973) did not find apical blebs when studying the choroid plexus of immature pigs and they also considered their presence to be an artifact.

Clusters of cilia, 3 to 4 per cell, were observed protruding from the apical surface. Their fine structure was similar to that first described by Fawcett and Porter (1954), with nine pairs of peripheral, and two pairs of central, filaments.

Lateral cell membranes were closely approximated and terminal bars or junctions were noted near the ventricular surface. Brightman (1968) showed that the apical junctions in the choroid plexus of the mouse were a barrier to horseradish peroxidase injected into the cerebrospinal fluid. Near the base of the cells the lateral cell membranes were infolded. This feature was common to the epithelia of the submaxillary gland, ciliary body of the eye, avian salt gland and kidney tubules and has been linked by Pease (1956) with the function of water transport. Fawcett (1962) pointed out that basilateral plasmalemmal infoldings occur in cells engaged in active ion transport (e.g. the epithelium of the avian salt gland)

and suggested that the degree of the infolding might indicate the extent to which a cell can accomplish such function. The localisation of ATPase on the basilateral plasmalemmal infoldings of the epithelial cells of the avian salt gland was considered by Ernst (1972a,b) to be important for the function of active transport; Torack and Barnett (1964) demonstrated ATPase activity on the basilateral plasmalemmal infoldings of the epithelial cells of the rat choroid plexus.

The cells rest on a basement membrane which does not take part in the lateral plasma membrane infoldings. The basement membrane of adult choroidal epithelium has been considered a barrier to certain substances injected into the blood stream such as silver particles (Dempsey and Wislocki, 1955), thorium dioxide (Tennyson and Pappas, 1961), but not to horseradish peroxidase (Brightman, 1968).

The choroid epithelial cells have round, basally or centrally-located nuclei, with up to three nucleoli. Dohrmann and Herdsen (1969) described lobated nuclei in the choroid ependymal cells of young mice but not in ageing mice. They assumed a greater nucleo-cytoplasmic interaction in the choroid ependymal cells of young mice.

Mitochondria, having a typical fine structure of a double membrane with the inner one infolded to form cristae, were dispersed in large numbers throughout the cytoplasm. They could readily supply the energy needed for active transport (Tennyson and Pappas, 1968). Mitochondria, in many other situations, have been shown to

contain many of the cellular enzyme systems, and their number is generally thought to be related to the metabolic activity of the cell. A high rate of metabolism and abundant mitochondria might be confidently expected of the choroid plexus epithelium which is known to be engaged in active transport. The Golgi complex occupied a paranuclear position and consisted of vesicles, tubules and sacs lined by a smooth membrane. Its function in the choroid plexus is not known.

The endoplasmic reticulum was usually scattered randomly throughout the cytoplasm as rough-surfaced tubules and canaliculi although cisternal elements might be found (Tennyson and Pappas, 1968).

Capillaries with attenuated, fenestrated endothelium were present in the connective tissue core of the choroid plexus. These capillaries have a larger diameter than those in most parts of the body (Maxwell and Pease, 1956). It is interesting to note that capillaries in several different areas of active fluid transport have a similar structure. Thus the capillaries of intestinal villi (Palay and Karlin, 1959), renal proximal tubule (Pease, 1955), ciliary body of the eye (Pappas et al., 1959) and choroid plexus (Maxwell and Pease, 1956) are all structurally similar.

Myelinated and unmyelinated nerve fibres were found in association with choroidal blood vessels (Voetmann, 1949). She regarded the myelinated fibres as sensory and

the unmyelinated fibres as vasomotor. Clark (1928) and Tennyson and Pappas (1968) also demonstrated intraepithelial nerve fibres in the choroid plexus of neonatal kitten and human fetuses respectively.

ii) Functions

Many functions have been attributed to the choroid plexus, including secretion, dialysis, absorption, purification and endocrine activity.

(a) Secretion

The mechanism of production of cerebrospinal fluid by the choroid plexus has received a lot of attention in the literature, since Willis (1664) first put forward the hypothesis that the choroid plexus contained gland-like structures which produce cerebrospinal fluid. Luschka (1855) suggested that the whole choroid plexus had a secretory activity. Findlay (1899) and others observed secretory globules within the choroid ependymal cells and on the free surface of the epithelium and consequently considered that the choroid plexus had a secretory function. The concept of secretory globules came to be known as the "vesicular theory of secretion". Meek (1907) and others cast doubt on this theory by demonstrating that the number of "globules" increased with the time the choroid plexus remained unfixed and with mechanical injury to fresh choroid plexus. Meek (1907) investigated the effects of ether anaesthesia, pilocarpine, atropine and muscarine on the production of cerebrospinal fluid in dogs, rabbits, guinea pigs and rats. He found that the amount of

cerebrospinal fluid produced was increased by ether and pilocarpine and decreased by atropine and muscarine. He observed that the choroid epithelium of stimulated animals increased in height and that the cell cytoplasm was separated into a clear apical zone and a granular basal zone. Increased cerebrospinal fluid production in dogs was also noted following intravenous administration of extract of the posterior lobe of the pituitary (Weed and Cushing, 1915) and epinephrine (Becht, 1920). However, morphological studies were not done on the choroid plexus of these experimental animals.

Direct experimental evidence of involvement of choroid plexus in production of cerebrospinal fluid was produced by Dandy and Blackfan (1913). They caused hydrocephalus in dogs by obstructing the aqueduct of Sylvius and showed that, at least in this species, cerebrospinal fluid is predominantly produced in the ventricles. Later, Dandy (1914) thought that he had proved that all the cerebrospinal fluid is produced by the choroid plexus. He showed that when one foramen of Monro was occluded, an ipsilateral hydrocephalus was produced, but if the choroid plexus was first removed, occlusion resulted in collapse of the ipsilateral ventricle. Dandy's results have been questioned by Bering (1955) who attributed the ability of the plexus to produce hydrocephalus proximal to a block to pulsations of choroidal blood vessels rather than to its presumed capacity to produce cerebrospinal

fluid. His conclusion was based on results of experiments in which he removed the choroid plexus from one lateral ventricle and blocked the escape of cerebrospinal fluid from the IVth ventricle by kaolin injection. Although the ventricles communicated, symmetrical hydrocephalus did not occur. The lateral ventricle without the choroid plexus (vascular pulsations) remained small.

Cushing (1926) studied the human choroid plexus during brain operations and noticed droplets of serous fluid exuding from the epithelial cells into the ventricular cavity. After he obstructed the choroidal artery the exudation of fluid stopped. However, his observation has frequently been dismissed as unphysiologic since the plexus was exposed to air and the pressure differential across the choroid plexus epithelium was abnormal.

It was believed that papillary tumours of the choroid plexus may be associated with overproduction of cerebrospinal fluid. This belief depends on the coexistence of these tumours and hydrocephalus and the fact that the hydrocephalus improved following removal of these tumours (Matson and Crofton, 1960). However, choroid plexus papillomas have been encountered in patients without hydrocephalus (Milhorat, 1972). Of the many cases reported of cerebrospinal fluid overproduction by choroid plexus papillomas the most convincing was the case, reported by Milhorat (1976a), of a young hydrocephalic boy who was diagnosed as having a choroid plexus papilloma of the left

lateral ventricle. A five-fold decrease in the rate of cerebrospinal fluid formation, as determined by ventriculo-lumbar perfusion, was found after the extirpation of the tumour. This provided conclusive evidence that choroid plexus papillomas do indeed produce excess cerebrospinal fluid. The chemical composition of this fluid showed no differences from that of normal cerebrospinal fluid and the ultrastructure of the tumour was found to be similar to that of normal mammalian choroid plexus tissue.

Rougement, Ames, Nesbett and Hofmann (1960) provided what they regarded as definitive evidence that, in the cat, the choroid plexus does produce cerebrospinal fluid. They filled the cavity of the ventricle with oil and using a micropipette collected cerebrospinal fluid from the surface of the choroid plexus. Analysis of electrolyte composition of this fluid revealed significant differences from an ultrafiltrate of plasma. Similar analyses of choroidal fluid in species other than cat have not been made. Nevertheless, the basic morphological similarity among vertebrate choroid plexuses (Carpenter, 1966) would make it reasonable to assume that fluid secretion is a function common to all vertebrate plexuses. The oil used to fill the ventricle, however, might have had a toxic effect on the choroid plexus thereby affecting the electrolyte composition of the nascent fluid.

Flexner (1934) found that the composition of cerebrospinal fluid is different from that of an

ultrafiltrate of plasma; it contains higher concentrations of chloride and magnesium ions and lower concentrations of protein, glucose and calcium and potassium ions. He concluded that, on the basis of the evidence then available, the fluid should be considered a secretion, with the understanding that the term secretion means that cells must do work in formation of cerebrospinal fluid.

By comparing the cerebrospinal fluid and blood plasma sodium concentrations of chick embryos between stages 29 and 38 Longridge (1966) found that at about stage 35 the cerebrospinal fluid sodium concentration ceased to be significantly lower than the blood plasma sodium concentration and he considered this stage as the one at which the secretory activity of the choroid plexus started and cerebrospinal fluid became a secretion. At the same stage (Stage 35) the choroid plexus was found to have a well developed apical microvillous border in which adenosine triphosphatase activity was first observed. The stainability of the mitochondria of the choroid epithelial cells, as revealed by both Regaud's iron haematoxylin method and Baker's acid haematin test, increased considerably; this coincided with the time of onset of choroid plexus secretion.

A biochemical analysis of the choroid plexus was done by Fischer and Copenhaver (1959) who examined enzyme systems active in cellular metabolism, namely succinic dehydrogenase and cytochrome oxidase. The activity of the enzmes which might play a role in active transport was also

studied. They concluded that biochemical analysis of the choroid plexus indicates that general metabolic activity is one-third to one-half that of the kidney. This means that the choroid plexus is a very active tissue: Na-K activated adenosine triphosphatase, which is presumed to play a role in active cation transport, has been found in the choroid plexus by biochemical assay (Bonting et al., 1961).

Karr, Stary and Winternitz (1929) dialysed cerebrospinal fluid and serum from the same patient against each other using a collodion membrane. They noticed post-dialysis alterations which indicated that the cerebrospinal fluid is not produced as a dialysate. Welch and Sadler (1965) measured the electrical potential across the choroidal epithelium and found that sodium must ascend a steep electrochemical gradient to be secreted into the cerebrospinal fluid.

The morphological similarity between the choroid plexus and other tissues known for their secretory activity strongly supports the idea that the choroid plexus itself is involved in active transport. Thus the kidney tubules, the ciliary body of the eye, the submaxillary salivary gland, the avian salt gland and the choroid plexus have essentially similar structure. The features in common included the infolding and interdigitation of the basilateral plasma membrane, well developed microvillous apical border and abundant mitochondria.

Scothorne (1956) examined the nasal glands of the

Aylesbury duck and found that the cells contained a large number of mitochondria, which were more abundant in the cells of the inner five-sixths of the secretory tubules (towards the centre of each lobule) where they occupied most of the available cytoplasmic space. These mitochondria were found by Scothorne to be arranged in "curious regular rows" separated from each other by "narrow unstained bands".

Scothorne (1956) found that the nasal glands of ducks were capable of actively producing a hypertonic saline secretion after oral administration of sodium chloride. This function of the nasal glands might be beneficial for marine birds which can drink sea water and excrete the excess salt, retaining the water.

Fawcett (1962), using the TEM, observed an extraordinarily elaborate compartmentation of the cells of the nasal salt gland of marine birds. The entire epithelium was divided into thin leaflike compartments extending nearly to the free surface of the cells. A large number of mitochondria, arranged in regular rows, was lodged in these slender compartments, and between them was an elaborate system of extracellular spaces. These extracellular spaces were formed by the extensive and deep infoldings of the basal cell membrane. So the "narrow unstained bands" described by Scothorne were actually extracellular spaces lying between rows of mitochondria.

Excess salt intake was also found to stimulate the nasal gland of penguins to secrete large quantities of

hypertonic sodium chloride solution into the nasal cavities (Schmidt-Nielsen and Sladen, 1958).

The degree of infolding of the basilateral plasma membranes varied in those organs engaged in active transport (avian salt gland, ciliary body of the eye, choroid plexus, kidney tubules, etc.) being shallow in the ciliary body and the choroid plexus and very deep in the avian salt gland (Fawcett, 1962). This difference in the degree of infolding might indicate the extent to which these organs were engaged in active transport.

(b) Dialysis

The theory of dialysis as the mechanism of cerebrospinal fluid formation by the choroid plexus, though unpopular, was favoured by some early investigators (Becht and Matill, 1920; Becht and Gunnar, 1921; Foley, 1923; Forbes, Fremont-Smith and Wolff, 1928). Becht and Matill (1920) and Becht and Gunnar (1921) found that a rise in either the arterial or venous pressure causes increased cerebrospinal fluid production; however, the increased production was correlated most closely with the increased venous pressure. Foley (1923) and Forbes, Fremont-Smith and Wolff (1928) demonstrated a reversal of flow through the choroid plexus by showing an uptake of fluid from the ventricles when the choroid plexus was infused in vivo with hypertonic saline. They injected a solution of potassium ferrocyanide and iron ammonium citrate into the ventricles. Then they removed the choroid plexus, fixed it in acid

medium and the uptake of the iron salt was indicated by its presence in the choroid ependymal cells as a prussian blue reaction. Fremont-Smith (1927) found that there is a close relationship between the concentration of plasma proteins and the distribution of the chloride ion; the greater the protein content of the plasma, the greater the chloride ion content of the cerebrospinal fluid. From these findings he concluded that the cerebrospinal fluid was a dialysate and that the Donnan equilibrium played an important role in the distribution of chloride ion across the choroid plexus and thereby is a significant factor in cerebrospinal fluid production.

It is now known that not all the cerebrospinal fluid is produced by the choroid plexus; extrachoroidal sites are also involved. Milhorat (1969) reported enlargement of both normal and plexectomised lateral ventricles in rhesus monkeys with unilateral plexectomy and with both foramina of Monro occluded. He attributed previous failures to obtain hydrocephalus in plexectomised ventricles to scar tissue formation within the ventricular cavity rather than either to a reduction in fluid production rate (Dandy, 1919) or to the loss of choroidal pulsations (Bering, 1955). This might be regarded as a direct experimental evidence for an extrachoroidal origin of cerebrospinal fluid - in this case the ventricular ependyma. Other experimental evidence for origin of cerebrospinal fluid from sites other than the choroid plexus was produced by Pollay and Curl (1967). They perfused the isolated

aqueduct of Sylvius and the plexectomised IVth ventricle of the rabbit and measured the fluid and solute exchange occurring between the brain tissue and the perfusate. They showed by direct measurement and by inulin-dilution method (Pappenheimer et al., 1961) that there was a net fluid secretion of 0.33 ul cm^2 min^{-1}. This makes approximately 30% of the total CSF production. They considered this fluid to be produced by the ventricular ependyma. Sonnenberg, Solomon and Frazier (1967) perfused the central canal of the cat spinal cord and from the results of their experiments concluded that about 40% of the cerebrospinal fluid is secreted by the ependymal lining of the ventricular cavity.

The perfusion in both these experiments was done according to the inulin-dilution technique of Pappenheimer et al. (1961). The validity of the results was questionable as in both experiments drastic experimental procedures were performed, i.e. Pollay and Curl (1967) removed both the cerebral hemispheres while Sonnenberg et al. (1967) transected the spinal cord, procedures which might well have affected the results obtained.

Sweet et al. (1950) used radioactive isotopes to study the formation, flow and absorption of cerebrospinal fluid in man. They found that water, electrolytes and protein enter the cerebrospinal fluid both in the ventricles and in the subarachnoid space. They arrived at their results by measuring the rate of entry of sodium

isotope (Na^{24}) and heavy water (D_2O) into the ventricular, cisternal and lumbar cerebrospinal fluid in two human subjects.

Subject I.

Type of CSF	$T_{0.5}Na^{24}$ mins	$T_{0.5}D_2O$ mins
ventricular	83	8.1
cisternal	370	1.5
lumbar	600	18.6

Subject II.

ventricular	82	11
cisternal	370	1.4
lumbar	550	25.5

($T_{0.5}$ is the length of time taken for the isotope concentration in the CSF to reach half the blood plasma concentration).

The entry of heavy water into the cisternal CSF was 7 times more rapid than entry into the ventricular CSF. This would indicate that the appearance of D_2O in cisternal CSF was due to its diffusion directly from the blood into CSF and not due to flow of CSF from the ventricles. On the other hand, Na^{24} must have been actively produced inside the ventricles because it accumulated 4 times more rapidly in the ventricular CSF than in the cisternal CSF.

Indirect evidence for origin of cerebrospinal fluid from extrachoroidal sites includes the observations by Kappers (1958) that a fluid is formed in the neural tube of human fetuses long before the development of the choroid

plexus and that some lower vertebrates lacking a choroid plexus had cerebrospinal fluid in their ventricular cavities. Kappers made no suggestion as to the origin of such fluid, but the ependymal lining of the ventricles seemed to be the logical source. Although the structural evidence of choroid plexus secretion is not seen until the 24th week of gestation in human fetuses (Kappers, 1958), it is interesting to note that congenital hydrocephalus can occur in human fetuses considerably before this time (Milhorat, 1972).

So fluid is present in the lumen of the neural tube before the time of appearance of the choroid plexus though the dynamics of its production has not yet been established. It is this fluid, presumably of ependymal origin, which creates the pressure necessary for brain enlargement especially during neural tube occlusion.

Because of the important implications of neural tube occlusion for the subsequent development of CSF pathways, recent work on this subject will be reviewed.

Occlusion of the neural tube is a well documented transient event which occurs during early embryogenesis in chick (Desmond and Jacobson, 1977; Desmond and Schoenwolf, 1985), in man (Desmond, 1982), in mouse (Kaufman, 1983) and in rat (Freeman, 1972). Neural tube occlusion involves apposition, in the midline, of the lateral walls of the spinal cord.

It was postulated that complete occlusion of the lumen of the neural tube plays an important role in

inducing rapid enlargement of the brain (Desmond and Jacobson, 1977). They showed that rapid enlargement of the brain occurs only when the neural tube was occluded and became a closed compartment filled with CSF. They found that reducing the CSF pressure by intubating the brain with thin glass tubes, would lead to a marked decrease in brain enlargement, so ventricular CSF pressure is required to overcome the resistance to brain expansion offered by the surrounding mesenchyme and surface ectoderm. Similar observations were made by Coulombre and Coulombre (1958). Schoenwolf and Desmond (1984) found a close temporal relationship between neural tube occlusion and rapid brain enlargement and suggested that the former is important for initiating the latter.

It is interesting to note that Coulombre (1956), studying the development of chick eye, found that when the intraocular pressure was reduced by draining the vitreous humor by thin glass tubes, the growth of the eye was arrested. He concluded that intraocular pressure is important for the normal development of the eye and is needed to counteract the resistance to expansion offered by the walls of the eye.

Desmond and Schoenwolf (1985) established, on a sound morphological basis, that occlusion was a real event and not simply an artifact produced by histological processing. They studied neural tube occlusion in chick embryos by semithin sections and by injecting dye into the neural

tube. They reported that the process of occlusion occurred in three phases:

1. Preocclusion:

   Stage 8 (26-29 hours of incubation).

   The cranial neuropore is widely open, and no occlusion has taken place.

2. Incipient occlusion:

   Stages 9 and 10 (29-38 hours of incubation)

   By this stage the cranial neuropore is closed together with the roof of the hind brain, while the caudal neuropore is still open.

   Although histological examination showed that the lumen of the neural tube was occluded, dye injected under pressure flowed freely across the occluded zone. Incipient occlusion occurred at the mid-spinal cord level.

3. Definitive occlusion:

   Stages 11-14 (40-53 hours of incubation)

   Histological examination of transverse sections of neural tube showed that occlusion of the neurocoele had occurred at mid-spinal cord level. Unlike the situation in incipient occlusion, dye injected under pressure failed to traverse the occluded zone.

Neural tube occlusion is short lived. In chick embryos, reopening of the occluded segment starts at stage 15 and is complete by stage 17 (Schoenwolf and Desmond, 1986), while in man occlusion lasted about 2 days (Desmond, 1982).

Ultrastructural studies of occlusion in the mouse (Kaufman, 1983) showed that even in areas of apparently total occlusion, a narrow slit is still demonstrable between the opposing apical surfaces of neuroepithelial cells, and that no desmosomal junctions of any kind could be demonstrated. Factors initiating and maintaining neural tube occlusion have not yet been determined (Desmond and Schoenwolf, 1986).

Kaufman (1983) found in mouse embryos that occlusion of neural tube lumen was not a uniform occurence, i.e. animals of the same developmental age might show a completely different picture of the extent of occlusion or even its occurence at all. Occlusion usually occurs between early on 9th day to late on 10th day, at a time when the cephalic and caudal extremities of the neural tube were still open. The neural lumen becomes patent again along its whole length just before the closure of the caudal neuropore. So in the mouse, after occlusion has taken place, there is a short interval during which the lumen of the neural tube is not a closed system.

(c) Absorption

Absorption was regarded by some authors either as the sole function or as one of several functions of the choroid plexus. Askanazy (1914) found that patients suffering from intraventricular haemorrhage had haemosiderin deposits within their choroidal epithelial cells and he consequently suggested that the choroid plexus might be involved in

absorption as well as secretion.

More evidence for absorptive function of the choroid plexus was added by Tennyson and Pappas (1961), Pappenheimer, et al. (1961), Smith et al. (1964) and others. Tennyson and Pappas (1961) injected thorotrast intraventricularly in adult rabbits and demonstrated its pinocytotic uptake by the choroid plexus epithelium. Pappenheimer, et al. (1961) examined the IVth ventricular choroid plexus in goats after intraventricular injection of diodrast and phenolsulphonphthalein. They stated that both substances were actively transported from cerebrospinal fluid to the blood. Smith et al. (1964) using isolated chick choroid plexus reported transchoroidal transport of fluorescent-labelled albumin from the incubation medium.

(d) Endocrine

An endocrine function was attributed to the choroid plexus by Hollander and Spiegel (1930), who found that on intravenous administration of bovine choroid plexus extract there was a fall in the blood pressure of cats but not rabbits. This fall in blood pressure might as well be due to a mild anaphylactic reaction caused by the administration of foreign protein. Dixon and Halliburton (1914) found that intravenous administration of sheep, ox and human choroid plexus extracts caused increased formation of cerebrospinal fluid in dogs; however, the authors did not directly attribute endocrine function to the choroid plexus.

e) Pump-like action

Bering (1955) suggested that the choroid plexus acts as a mechanical pump for the CSF. He noted that pulsations of the choroid plexus, by creating a pressure gradient, forced CSF out of the ventricles and postulated that this pump-like action is important in embryonic development of the subarachnoid space. This pump-like action of the choroid plexus might explain the results of Weed (1917) who concluded that the extraventricular spread of cerebrospinal fluid was due to the secretory activity of the choroid plexus. Weed arrived at this conclusion because he found that the extraventricular spread of cerebrospinal fluid coincided with the appearance of the choroid plexus of the IVth ventricle. It might be argued, according to Bering (1955), that at the time when extraventricular spread of cerebrospinal fluid took place the choroid plexus was not functioning and it was the pump-like action of the pulsations of the choroidal blood vessels which forced the cerebrospinal fluid out of the ventricle.

(f) Blood-CSF barrier

Another important function of the choroid plexus is that it is one site of the blood-CSF barrier.

Goldmann (1913) made a detailed study of the vital staining of tissues of several small mammals after systemic injection of the acid dye, Trypan blue. The dye was distributed widely in most regions and organs. However, the brain and spinal cord remained strikingly white, as did the peripheral nervous system. The choroid plexus, on the

other hand, stood out as blue lines against the surrounding white brain.

Goldmann (1913) also observed that, while the placenta accumulated a large amount of Trypan blue, there was a lack of uptake of the dye by the fetus after its injection into the maternal systemic circulation. This suggested to him an analogy between the function of the choroid plexus and that of the placenta - both might have a protective function for the tissues beyond them, i.e. the central nervous system in one case and the fetus in the other. Indeed Goldmann called the choroid plexus "placenta cerebralis".

In most areas of vertebrate brain, the cells of the cerebral endothelium are joined by pentalaminar tight junctions (zonulae occludentes) which prevent the intercellular movement of proteins and other colloidal tracers (Brightman and Reese, 1969; Milhorat et al., 1973). Brightman and Reese used horseradish peroxidase (M.W. 401000, diameter 50-60 A) as tracer, while Milhorat et al (1973) used cytochrome C (M.W. 1000, diameter 25-30 A). After intravascular injection these tracers remained strictly within the lumina of the cerebral capillaries. However, in certain areas of the brain, including the choroid plexus, area postrema, median eminence, pituitary gland and others the tracers passed easily out of the capillaries. In these special areas, the cells of the cerebral endothelium were not joined by tight junctions.

The choroid plexus, as shown by Reese and Brightman (1968) and Brightman et al. (1970) was covered by choroid ependymal epithelium with tight junctions between its adjoining cells. This specialized epithelium was capable of stopping the intercellular movements of colloidal tracers such as ferritin, horseradish peroxidase and cytochrome C.

Milhorat et al. (1973) carried out experiments employing cytochrome C as an electron-dense marker. Following intravascular injection, in the rat, the marker stayed inside the cerebral capillaries but passed rapidly out of choroidal capillaries. Two minutes after the injection the marker was found in the following sites in the choroid plexus: within the capillary lumen, in the extracellular space between epithelial cells (but not beyond the apical tight junctions), within the epithelial cells in pinocytotic vesicles. An hour later the marker was progressively cleared from the tissue interspaces and taken up by intracytoplasmic pinocytotic vesicles, dense bodies and multivesicular bodies. No reaction product of the marker was found in relation to the apical plasma membrane. As cytochrome C did not appear in the ventricular cerebrospinal fluid, its uptake (and possibly that of other proteins) by the choroid epithelial cells does not constitute a mechanism of transcellular transport but might represent the initial step in the degradation of proteins and possibly other substances. This mechanism might be regarded as playing an important role in the

blood-CSF barrier which guards against the entry of certain substances into the cerebrospinal fluid and subsequently into the nervous system.

iii) Epiplexus and supra-ependymal cells:

Intraventricular macrophages are classified according to their relationship to the surface of the choroid plexus or the ependyma. Cells lying close to the ventricular surface of the choroid plexus, named epiplexus cells by Kappers (1953) were noticed by Chamberlain (1974), Peters et al. (1970) and Peters (1974). Similar cells resting on the ependymal epithelium of the ventricles (supraependymal cells) and some lying free in the ventricular cavity were also noticed by Clementi and Marini (1971), Allen and Low (1973), Ling (1976) and others. The ultrastructure of the epiplexus cells was shown to be similar to that of monocytes and macrophages (Ling, 1976; Chamberlain, 1974; Peters, 1974). The phagocytic properties of these epiplexus cells was investigated by Carpenter et al. (1970), who demonstrated the uptake of electron dense particles introduced into the cavity of the cerebral ventricles.

Sturrock (1979) described a developmental study of the intraventricular macrophages of the mouse lateral ventricle. He found that at 11 days postcoitum only epiplexus cells were present, followed a day later by supraependymal and free intraventricular macrophages. He thought that the clearance of products of epithelial cell

degeneration of the developing choroid plexus was the possible explanation for the early appearance of epiplexus cells. When they first appeared, the intraventricular macrophages were mostly spherical with smooth surfaces and were considered by Sturrock to be the most primitive form. By 14 days postcoitum, large, irregular and multivacuolated macrophages appeared, followed by the mature flattened type at 17 days postcoitum. This flattened type was predominant in the postnatal and adult mice.

Quantitatively, Sturrock (1979) found that the number of epiplexus cells (and supraependymal cells) increased to reach a peak at 17 days postcoitum, fell rapidly until 4 days after birth, increased again at 8 days followed by another fall up to 15 days postnatum. The decrease in the number of epiplexus cells (and supraependymal cells) after 17 days postcoitum, might be due to their migration into the brain to form microglia which showed a rapid increase in number at the same time (Sturrock, 1974b).

In aged mice (25-31 months) most of the intraventricular macrophages were of the flattened type, were found mainly on the surface of the choroid plexus and some were distended to a varying degree with lipid droplets (Sturrock, 1983).

The origin of epiplexus cells is controversial. Tennyson and Pappas (1964) assumed their origin to be from macrophages in the connective tissue of the choroid plexus. Carpenter et al. (1970) claimed a haematogenous origin for them because of their resemblance to intravascular

monocytes. Ling (1979) supported the haematogenous origin of epiplexus cells. He found that 5 days after the intravenous injection of colloidal carbon into postnatal rats, carbon-labelled epiplexus cells could be detected. He came to the conclusion that intravascular monocytes, after ingesting carbon particles, left the subependymal blood vessels, traversed the ependymal lining and entered the lumen of the cerebral ventricles. In a transmission electron microscopic study in the monkey (Ling, 1981) macrophages were found apparently lying within the choroid epithelial cells. In light of this finding, Ling (1981) suggested that macrophages entered the ventricular lumen by following an intracellular pathway through the choroid epithelium. He later substantiated this theory by SEM study of the monkey lateral ventricle where he observed ruptured apical membrane of choroid epithelial cells within which one or two cellular elements could be seen which resembled epiplexus cells (Ling, 1983).

Another type of supra-ependymal cell thought to be neuronal in nature was found in the lateral, IVth and (mainly) the third ventricle of many species (Clementi and Marini, 1971; Coats, 1973; Scott et al., 1977; and others). It was suggested by many that these neurone-like cells might serve to assess changes in the composition of cerebrospinal fluid and might function either as receptors or integrators of neuroendocrine function (Clementi & Marinia, 1971; Scott et al., 1977).

B. THE DEVELOPMENT AND HISTOGENESIS OF THE CHOROID PLEXUS

i) General features:

The development of the choroid plexus has been studied with the light microscope by Weed (1917), Maxwell and Pease (1956), Kappers (1958), Shuangshoti and Netsky (1966), Dohrmann and Ducy (1970); with the transmission electron microscope by Tennyson and Pappas (1964 & 1968), Dohrmann and Herdson (1969), Davis, Lloyd and Milhorat (1973); with transmission and scanning electron microscope by Cancilla et al (1966); with scanning electron microscope by Clementi and Marini (1971), Chamberlain (1973); with light microscope, transmission and scanning electron microscope by Sturrock (1979a).

Kappers (1958) gave a comprehensive account of the development of the telencephalic choroid plexus in man, which he described as occurring in three phases. In the first phase (6th-8th prenatal weeks) the plexus was covered by a pseudostratified epithelium in which glycogen was just beginning to appear and the cells of the mesenchymal stroma were rapidly differentiating into angioblasts and haemocytoblasts. He postulated haematopoiesis to be the main function of the choroid plexus at this stage of development. Tennyson and Pappas (1968), who examined the fine structure of the developing choroid plexus of lateral and IVth ventricles in the rabbit disagreed with this, and maintained that most of the primitive blood cells thought by Kappers to be lying free in the mesenchymal stroma were

actually within capillaries whose walls were too thin to be seen with the light microscope.

In the second phase (8th-15th prenatal week) Kappers (1958) found that the pseudostratified choroidal epithelium became columnar to cuboidal with increasing glycogen content. The choroid plexus became very large and almost filled the lumen of the ventricle but as yet had no real folds. This glycogen-laden choroidal epithelium had earlier been reported by Weed (1917) to secrete a significant amount of protein into the cerebrospinal fluid. Flexner (1938) described a presecretory stage in the choroid plexus of the fetal pig which would be equivalent to the second phase of Kappers (1958): he found that cerebrospinal fluid from these fetuses had a higher protein content than that of adult animals and its ion concentration was typical of extracellular fluid. This was confirmed in humans: premature infants had a much higher protein concentration in cerebrospinal fluid than full term infants; [Otila (1949) and Arnold and Zetterstrom (1958)].

In the third phase (15th-40th prenatal week), Kappers (1958) described the choroidal epithelial cells as low cuboidal with diminishing glycogen content. The stroma changed from a mesenchymatous into a fibrous form and as a result the size of the plexus decreased considerably. With the decrease in the amount of intracellular glycogen the position of the nuclei changed from apical or central to basal. Shuangshoti and Netsky (1966) noticed the same phenomenon when they were studying the development of the

choroid plexus in man. Their account of the choroid plexus development was generally similar to that of Kappers (1958). These authors also noticed the formation of what they called "epithelial tubules" in the stroma of the choroid plexus. According to them the number of "tubules" increased at the same rate as the number of choroid plexus folds indicating that the intervillous clefts were the precursors of the tubules. The tubules were formed by the tips of these intervillous clefts being pinched off to lie free in the stroma of the choroid plexus. They claimed to have proved by serial sectioning that the "tubules" were not sectional artifacts, attributable to the extreme convolution of the epithelium. However, these "tubules" have not been recognised by other investigators.

ii) Fine structure of developing choroid plexus epithelium

a) Rabbit

The fine structure of the developing choroid plexus of the lateral and IVth ventricles in rabbits was studied by Tennyson and Pappas (1964). Like Kappers, they also divided the development into three stages:

1. The pseudostratified epithelial stage (14th-16th prenatal day) with irregular microvilli, occasional cilia and "multivesicular bulbous protrusions" forming the apical surface of the epithelial cells. They thought that the "multivesicular bulbous protrusions" formed microvilli by coalescence of their vesicles. Mitochondria, endoplasmic reticulum and Golgi apparatus were present in the cytoplasm

but mitochondria were sparse and had few cristae.

Just beneath the surface, the lateral membranes showed an increased density characteristic of terminal bars. Near the base of the cells the lateral cell membranes were either simple or very slightly infolded. The base of the cell was plane and rested on a basement membrane.

2. The granular columnar epithelial stage (16th-18th prenatal day).

Here the apical microvilli and the infoldings in the basilateral membranes were well developed and there was an increase in the cytoplasmic cellular organelles.

3. Glycogen-laden columnar epithelial stage (17th-20th prenatal day).

The cells were distended with glycogen, the apical microvilli became shorter, the basilateral infoldings less convoluted and both the nucleus and cellular organelles were displaced towards the apex of the cells. This stage persisted till birth.

Since the myelencephalic choroid plexus was derived from a thin choroid epithelial lamina, rather than from a multilayered neural tube, it did not pass through the first stage of development described for the telencephalic choroid plexus. Apart from this difference, the development of both choroid plexuses was found to be similar.

b) Mouse

Sturrock (1979a) studied the development of the choroid plexus of the lateral ventricle of the mouse with the light, transmission and scanning electron microscopes. He found that the telencephalic choroid plexus first appeared at 11 days postcoitum, as a bilateral ridge, of pseudostratified epithelium, in the roof of the interventricular foramen. The plexus increased in size rapidly and developed many folds. Dark spherical masses, which were interpreted as pyknotic nuclei, were observed at the sites of foldings. Sturrock (1979a) suggested that choroidal cell degeneration at the sites of foldings might be advantageous, by allowing thinning of the epithelium at these sites, it enables folding of the plexus to occur more rapidly.

Although the part nearest the root of the plexus remained pseudostratified, most of the epithelium had changed into simple columnar by day 13 postcoitum. The increase in size of the plexus and the transformation of the epithelium from pseudostratified into simple columnar, was accompanied by differentiation. As early as 11 days postcoitum, Sturrock (1979a) noticed microvilli on the apical surface of choroid epithelial cells. They were more obvious in the TEM than SEM, and increased in number and size as development progressed. According to Sturrock (1979a) glycogen first appeared in the choroid epithelial cells at 13 days postcoitum and was found as aggregates on the basal aspect of cells as well as granules dispersed

throughout the cytoplasm.

From 14 days postcoitum, Sturrock (1979), noticed the presence of both "dark" and "light" epithelial cells. "Dark" cells had a much darker cytoplasm, formed about 11% of the total number of epithelial cells, with cytoplasmic organelles generally similar to those of light cells but had finer and narrower microvilli. He found it difficult on morphological grounds alone to interpret such a difference in density, in terms of a difference in function. "Light" and "dark" choroid epithelial cells were also identified in some mammalian species by Wislocki and Ladman (1958) who considered them to be modulations of the same basic cell; and by Van Deurs et al (1978) in the rat, who found no difference in the uptake of intraventricularly administered horseradish peroxidase between the two cell types.

"Dark" cells have also been described in tissues other than the choroid plexus, e.g. extra-adrenal and adrenal chromaffin tissue in the rabbit (Coupland & Weakley, 1968; 1970), developing thyroid of mouse (Romeyt & Gauguin, 1973), ultimobranchial body of sheep (Jordan et al., 1973), developing sheep parathyroid gland (Jordan et al., 1975) and others. Romeyt and Gauguin (1973) considered "dark" cells of the developing thyroid as fixation artifacts because they found them to be more numerous in poorly fixed material. "Dark" cells were, however, considered as precursors of thyroid C-cells

(Jordan et al., 1973), of parathyroid chief cells (Jordan et al., 1975) and of pancreatic B-cells (Wessels & Evans, 1968). It should be mentioned that the majority of "dark" cells were identified in glutaraldehyde-fixed material, and indeed Jordan et al. (1975) considered their identification to be glutaraldehyde dependent as they were absent in osmium-fixed specimens.

c) Rat

Chamberlain (1973) examined both the telencephalic and the myelencephalic choroid plexuses of the rat with the scanning electron microscope and concluded that early in fetal life (days 13 & 14) the surface projections of the choroid epithelial cells were well developed indicating an early onset of function of the choroid plexus.

Cancilla, Zimmerman and Becker (1966) on the other hand, found in fetal rats 1-3 days prior to birth that there was minimal development of the microvillous surface and almost no infolding of the lateral plasma membranes of the choroid epithelial cells; and in the new-born rat the microvillous apical surface was slightly better developed while the plasma membrane infoldings were still minimal. However, in the fetal stages they found that cellular organelles were similar to those of more mature animals.

d) Human

Kiszely (1951) observed in humans that mitochondria were sparse in the choroid ependymal cells during the greater part of the fetal life but became numerous in those infants several days old. He concluded that the function

of the fetal choroid plexus was initially absorptive in nature and that it became secretory later. According to Kappers (1958), however, the human choroid plexus became functional only after birth.

The time-relationship of the development of the myelencephalic to that of the telencephalic choroid plexus was found to be different in different species. In rabbits the telencephalic choroid plexus developed before the myelencephalic choroid plexus (Minot and Taylor, 1905; Cohen and Davis, 1938; Strong and Cohen, 1951; and Strong, 1956). In man and pig the myelencephalic plexus developed first (Weed, 1917 and Kappers, 1958). In mouse both developed at almost the same time (Rugh, 1968), the same applies to the chick (Longridge, 1966) and to rat and guinea pig (Cohen and Davis, 1938).

iii) Development of blood vessels of choroid plexus

Although many studies have been done on the development of the epithelium of the choroid plexus in different species, less attention has been paid to its developing capillaries, although Tennyson and Pappas (1964) studied them in detail in the rabbit. With the light microscope they found thin-walled narrow capillaries, containing immature blood cells, following the base of the epithelium of the primitive choroid folds. Wider capillaries were found in the central region of the connective tissue core. As the choroid plexus enlarged, the capillary lumen became wider and irregular. With the

transmission electron microscope the capillary wall was found to be very attenuated in many areas. Pores or fenestrae covered by a diaphragm were also noticed. These fenestrae were considered by earlier investigators as perforations (Maxwell and Pease, 1956), or interruptions (Wislocki and Ladman, 1958) because the available techniques at the time did not show the diaphragm spanning them. This diaphragm was formed by the apposed outer leaflets of the endothelial unit membrane (Luft, 1966). Except for the choroid plexus and certain limited areas of the brain such as the pineal body, the pituitary gland, the area postrema, these fenestrations were not seen at other sites within the normal mammalian brain (Lee, 1971) in which the endothelial cells of the blood vessels have a voluminous cytoplasm (Donahue, 1962).

The widely accepted view that fenestrations were actually formed from vesicles was challenged by a fascinating study on the pancreatic capillaries of the prenatal rat carried out by Daniel and Henderson (1979) who found that fenestrations preceded vesicles during the development of capillaries.

Tennyson and Pappas (1964) observed that the cytoplasm of the endothelial cells of the choroidal capillaries was more abundant in the perinuclear area where most of the cellular organelles were present. Vesicles were found all over the cytoplasm except in the most attenuated areas. Endothelial cells had desmosomal thickening at their junctions, with cytoplasmic sheaths

overlapping each other. The basement membrane of the choroidal capillaries in the embryonic stages (rabbit) was apparently absent or incomplete and did not reach maturity until a few weeks after birth (Tennyson and Pappas, 1961).

Capillaries were closely applied to the basal surface of the choroid plexus epithelium, while those on the outer surface of the adjacent neural tube were more randomly oriented, and separated from the immediate vicinity of the external limiting membrane except in the region where they penetrated it (Tennyson and Pappas, 1964). Here they ran perpendicular to the external limiting membrane rather than parallel to it, as was the case of the choroidal capillaries. That choroid plexus formation may be dependent on the inductive influence of capillaries on the future choroidal epithelium has been shown experimentally by Birge (1962). After ablation of the presumptive metencephalic alar plate of the chick embryo, choroid plexus formation occurred in the defect area only when vascularised leptomeningeal tissue was in direct association with the regenerated choroidal epithelium.

iv) Morphometric studies of choroid plexus

The choroid plexus is the site of blood-CSF barrier and the major site of CSF production. It plays an essential role in controlling the fluid environment of the brain by maintaining its composition relatively constant compared to that of blood plasma (Davson, 1967; Bradbury, 1979).

The choroid plexus consists of a typical secretory epithelium made of a single layer of cuboidal cells (Tennyson and Pappas, 1968) which are connected by apical tight junctions and have complex basilateral interdigitations and numerous apical microvilli (Brightman and Reese, 1969).

During development, qualitative changes have been described in the shape of epithelial cells, the complexity of apical and basilateral membranes and in the number of mitochondria (Cancilla et al., 1966; Sturrock, 1979).

These changes have been quantified in the developing rat, from 16 days of gestation to 30 days after birth, by Cawkwell, Jones and Keep (1985), Keep, Jones and Cawkwell (1986), Keep, Cawkwell and Jones (1986). They showed that the weight of the IVth ventricle choroid plexus increased rapidly from 0.15 mg at 19 days postcoitum to 1.09gm at 10 days after birth (Keep, Jones and Cawkwell, 1986). This result was similar to that obtained for the rat lateral ventricle choroid plexus (Johanson, Reed and Woodbury, 1976). This increase in weight was attributed by Keep et al (1986) to growth of the epithelial component whose volume fraction increased steadily from 54% at 16 days postcoitum to 88% at 30 days after birth, as calculated by the point-counting method. They also found that there was a 10-fold increase in the number of epithelial cells between 19 days postcoitum and 10 days after birth with no further increase up to 30 days. They did not, however, measure the number of epithelial cells between 16 and 19

days postcoitum. They suggested that, in view of the very few mitoses found in the body of the plexus and the greater number in its root, the increase in the number of epithelial cells was brought about by addition of new cells at the junction with neighbouring ependyma.

Keep, Cawkwell and Jones (1986) calculated the height of choroid epithelial cells of the rat IVth ventricle using the ratio of the basal membrane surface area to the epithelial volume by the intersection counting method. They found that cell height decreased, from 19.1um to 11.6um, between 16 days postcoitum and birth respectively, but thereafter it remained almost the same. This might correspond to the change in choroidal cell shape from columnar to cuboidal which occurs during development in the mouse (Sturrock, 1979).

Cawkwell, Jones and Keep (1985) measured the epithelial cell apical surface density (i.e. the surface area of the apical membranes per unit volume of cell), in developing rat, by using the intersection counting method and found a 3-fold increase (from 0.9 to 2.8 um^2/um^3) between 16 days postcoitum and 10 days after birth. The increase before birth is accounted for by 33% reduction in cell volume due to reduced height, while after birth the increase is due to a corresponding increase in microvillous number and height. Cawkwell, Jones and Keep (1985) also measured the volume fraction of mitochondria by the point counting method and found that although no change occurred

before birth, a 2-fold increase in value was noticed between birth and 30 days. They attributed this change to an increase in the volume of individual mitochondria rather than to an increase in number.

Although much emphasis has been put on the basilateral membrane foldings and interdigitations (Tennyson and Pappas, 1968) regarding active transport, Keep, Cawkwell and Jones (1968) found that there was no significant change in the surface density of basilateral membranes between 16 days postcoitum and 30 days after birth. They concluded that infolding makes a fairly small contribution to the total surface area of the basilateral membranes.

From these results (i.e. significant postnatal increase in volume fraction of mitochondria, and apical microvilli) Cawkwell, Jones and Keep (1985) suggested that the choroid plexus either increases a pre-existing secretory function or that it starts secretion only after birth.

It should be mentioned that the previous reports have concentrated on changes in the epithelial component of the developing choroid plexus, but no similar studies, to my knowledge, have been done to quantify changes in the blood vessels during development.

v) Mitosis in choroidal epithelium

Because the choroid plexus grows rapidly and because the choroidal epithelium is functionally the most important constituent of the plexus, several authors have studied

mitosis in the choroidal epithelium. No mitoses have been observed throughout development in the epithelium of the human choroid plexus while many have been seen in the ependyma (Kappers, 1958; Zand, 1930). Boyd (1957) was also unable to find mitoses in the epithelium of the choroid plexus of human embryos and added that this absence of the signs of normal cell division presented a general biological problem. Tennyson and Pappas (1964) and Sturrock (1979) also mentioned the presence of mitoses near the root of the developing telencephalic choroid plexus of rabbit and mouse respectively but they did not quantify their results. The word "root" here refers to that part of the choroid plexus nearest to, and continuous with, the ependyma at the point of invagination. All these attempts were on sections of normal material, without the use of drugs producing mitotic arrest. Kappers (1958) thought that the rapid surface growth of the lateral ventricle choroid plexus was provided for by the transformation of the pseudostratified epithelium into single-layer columnar epithelium. However, Knudson (1964) injected Colcemid subcutaneously in mice at different stages of pregnancy and removed the embryos four hours later. Colcemid arrests cell division at metaphase and so causes all cells in metaphase to accumulate whenever mitoses normally occur. He found that mitoses were always present in the choroid plexus at various stages of development. In the epithelium, mitoses were present only near the root of the

plexus; while in the connective tissue mitoses occurred everywhere. In the Colcemid-treated mouse embryos the number of mitoses in the choroid plexus as a whole was increased up to 20 times as compared to that of untreated embryos. He did not indicate the frequency of mitosis in the epithelium but mentioned that the mitosis-free peripheral zone was about 50% of the surface area of the plexus.

In adult mammals Volzhina (1957, 1958) showed that the choroid plexus of the third ventricle underwent compensatory hypertrophy and hyperplasia after removal of the plexuses in the lateral and IVth ventricles. In addition, regeneration occurred locally if a stump of the plexus remained. Mitotic activity was noted in both sites in these experiments.

Messier and Leblond (1960) found that tritiated thymidine-labelled epithelial cells in the choroid plexus of adult mouse and rat accounted for less than 1% of total epithelial cells. This could be regarded as the critical observation which showed the paucity of mitoses in adult choroidal epithelium. When mitotic figures were present they were always located in the luminal surface of the pseudostratified epithelium at the root of the choroid plexus as well as in the ependyma. This localisation may be compared with that occurring in the neuroepithelium of developing neural tube, as first described by Sauer (1935). He found that epithelial cells of the neural tube are attached to each other at the apical surface by terminal

bars. When a cell of the neural tube is about to divide, it becomes shorter and rounded causing the nucleus and cytoplasmic mass to move towards the lumen. After division is complete the daughter cells are found to be attached to each other and to surrounding cells by apical terminal bars. They then elongate to assume the previous columnar shape with the nucleus passing away from the free surface. It would be interesting to know if this phenomenon of interkinetic nuclear migration also occurs in the telencephalic choroid plexus.

vi) Ocurrence of glycogen in choroidal epithelium

The presence of large quantities of glycogen in the epithelium of developing choroid plexus has been well documented, by Weed (1917), Wislocki (1932), Kappers (1958), Tennyson and Pappas (1964, 1968), Brocklehurst (1969), Sturrock (1979) and others. Its accumulation in the basal part of the cell might be due to its role in the formation of glycosaminoglycans of the basement membrane of the epithelial cells as suggested by Kappers (1958), or simply due to its uptake from neighbouring capillaries as suggested by Sturrock (1979). Kappers (1958) suggested a nutritive role for glycogen, as an important anaerobic source of energy. Goldmann (1913) named the choroid plexus "placenta cerebralis" because of its function as a barrier between cerebrospinal fluid and the blood and its supposed nutritive function for the brain. Dempsey and Wislocki (1944) discussing the significance of glycogen in the

trophoblast of human placenta, suggested that it was deposited in regions showing poor vascularisation, thus providing an anaerobic source of energy. A similar argument was used by Brocklehurst (1969) in his discussion of the presence of glycogen in the ependymal cells surrounding the developing foramina in the roof of the IVth ventricle. He thought that the glycogen indicated "a provision for anaerobic metabolism required for the demands of rapidly developing cells in the absence of a fully developed local blood supply".

vii) Blood vessel casts

Injection replication of the vasculature of the choroid plexus of the IVth ventricle

Injection replication is a technique in which an alveolar or a tubular system or a cavity is filled with a fluid medium after the solidification of which, the covering soft tissue is removed. This will convert tubes into rods and the deep concavities into readily examined convex surfaces.

It is an old technique which has been used by anatomists since the sixteenth century when Leonardo de Vinci (1452-1519) made a wax replica of the cerebral ventricles. Others used different types of injection media in an attempt to illustrate the various systems of the body. William Cowper (1666-1709) and others used fusible metals (lead, tin and bismuth) to produce casts of the cavities of the lungs. Marcello Malpighi (1628-1694) was the first to use mercury to demonstrate the structure of

the lungs; and Anthony Nuck (1650-1692) used the same metal to inject the lymphatics. Johann Wepfer (1620-1695) described the course and distribution of the carotid arteries by injecting the vessels of the brain by saffron water.

Since that time a lot of improvements, many of which are described by Tompsett (1970), were made in the replication media. A notable improvement was the use of celloidin (cellulose nitrate) as an injection medium by Schiefferdecker in 1882. Its disadvantage was that it has to be kept in water as it explodes if it becomes dry. This problem was solved by substituting celluloid (cellulose acetate) for celloidin in 1899 by Storeh. The principal drawbacks of such injection media were the shrinkage on solidification after the evaporation of the solvent and the long time taken for solidification to take place.

Narat, Loef and Narat (1936) introduced the use of a vinyl resin whose casts were superior to those of celluloid as it required shorter time to solidify and it was perfectly soluble in acetone. This resin was used on a large scale to produce casts of ducts and blood vessels. Gelatin was used mainly to produce casts of large blood vessels and cavities for museum specimens (Tompsett, 1970). Latex, a synthetic rubber fluid, produces moderately strong and elastic casts but takes a long time to set, up to a month, depending on the volume of the cast. It is used to

inject blood vessels and lymphatics. Murakami (1971) produced latex corrosion casts of blood vessels of the rat which he observed under the scanning electron microscope. He found that latex casts, when dried, readily droop, shrink and adhere and concluded that such flexible casts were inadequate for scanning electron microscopy. Nowell and Lohse (1974) also noted that latex casts which were critical point dried showed evidence of shrinkage and distortion.

Microfil, a silicone rubber fluid of low viscosity, produces soft and weak casts, which not unusually disintegrate during washing, especially those of very small blood vessels. Nowell and Lohse (1974) found that microfil casts could not be rendered conductive for scanning electron microscopy and it was not possible to subject the casts to slow scan scanning electron microscope examination.

The introduction of unsaturated polyester resins as injection media in 1948 revolutionised the anatomical casting practice. They produced strong coloured casts, without shrinkage, of tubes and cavities which can last indefinitely without deterioration. They can successfully replicate the very small and delicate blood vessels of embryonic material (Dollinger and Armstrong, 1974) as well as the blood vessels of adult animals (Nowell et al., 1972; Murakami, 1971, 1972; Murakami, Miyoshi and Fujita, 1971; and others). If they are injected immediately after the death of the animal they cause severe muscular spasm which

might spoil the injection so it was recommended by Tompsett (1970) to store the animal at 4°C overnight before the injection is made. However, Murakami (1971) started the injection of the resin when the peristalsis of the small intestine disappeared and Dollinger and Armstrong (1974) perfused freshly isolated living chick embryos with 2% KNO_3 to paralyse the heart prior to resin injection.

The maceration of the soft tissue is done by immersing the injected organ or animal in a concentrated acid solution or in a strong alkali. The acid usually used is concentrated hydrochloric acid; nitric acid and sulphuric acid are not used as they attack the injected medium (Tompsett, 1970). Sodium hydroxide or potassium hydroxide in concentrations ranging from 10% to 34% and for periods of between one and several days have also been used for macerating soft tissues.

The improvements in the injection media and the techniques for macerating surrounding soft tissues have greatly improved the results. The greatest improvement, however, is the combination of the injection replication techniques with the use of the scanning electron microscope for the study of microstructures. The advantage of this lies in the three-dimensional nature of the results, the high resolution of the scanning electron microscope and its considerable depth of focus.

Microscopical examination of cleared whole mounts also gives three-dimensional results but its value is

limited if the anatomy of the structure under examination is not simple. This method has been employed by Strong (1956) to study the early development of the ependyma and vascular pattern of the fourth ventricular choroid plexus in rabbit embryos. He injected India ink into the circulation, fixed the animals in formalin, dehydrated them and then cleared them in benzylbenzoate. He described the choroid plexus of the fourth ventricle as consisting of two limbs, superior and inferior. The superior limb developed first and consisted of a medial, a lateral and glomar component. The capillary plexus of the choroid plexus was fed by branches of the pontobulbar artery caudally and the superior cerebellar artery rostrally. He pointed out that the vascular bed of the plexus consisted of a marginal tortuous capillary and a basal capillary with smaller capillaries connecting the two.

Although valuable information can be obtained from serial tissue sections many of the important features of the circulation such as capillary density and the three-dimensional nature of the distribution of the blood vessels in the tissue cannot be fully understood. The comprehension of three-dimensional relationship from two-dimensional sections is difficult. Construction of serial section models, Price (1972), image analysing computers, Pedl$\ddot{e}$r (1968) and complicated graphical reconstruction techniques, Bang and Bang (1957), Dunn (1972), Yamada and Yoshida (1972), might be necessary to find the third dimension of serial tissue sections.

Murakami (1971) pioneered the scanning electron microscopic study of vascular casts employing a polyester resin, methyl methacrylate. He studied the fine distribution of the blood vessels in the rat's renal glomeruli, gastric mucosa, intestinal villi and hepatic lobules. Murakami and his co-researchers used the same method to demonstrate the existence of a double efferent artery in the rat's renal glomeruli (Murakami, Miyoshi and Fujita (1971)), and to study the vasculature of the pancreas (Fujita and Murakami (1973)). Gannon, Campbell and Randall (1973) used scanning electron microscopy of corrosion casts to study the vascular connections in the trout gill. Nowell and Lohse (1974) simultaneously injected the respiratory system of the dog with cementex and the pulmonary circulation with vultex and microfil and then examined the replicas of these intimate but separate systems under the scanning electron microscope. They found it difficult to fill the whole pulmonary circulation and attributed this to the viscosity of the replicating media or the fact that the vascular injection was done after the airways have been filled with resin.

Replicas of the circulatory system were all done in adult animals and the only study, so far, done in embryonic material was conducted by Dollinger and Armstrong (1974). They produced vascular casts of the circulatory system of chick embryos 2-4 days incubation. They found it necessary to use a medium of low viscosity to replicate the thin and

very delicate blood vessels of young embryos. Batson's corrosion compound introduced by Batson (1955), was the medium of their choice, as it successfully replicated even the very small embryonic blood vessels without undue distension and fulfilled all the criteria suggested by Nowell and Lohse (1974) for a medium to be suitable for scanning electron microscopy. These criteria included that the medium should fill completely the system or structure to be examined, polymerize and dry without shrinkage or distortion, withstand corrosion, be rendered conductive and withstand electron beam bombardment. They found methyl methacrylate used by Murakami (1971) too viscous to replicate the delicate embryonic blood vessels.

C. COMPARATIVE ANATOMY AND DEVELOPMENT OF FORAMINA IN THE ROOF PLATE OF THE FOURTH VENTRICLE

In adult mammals, the foramina of Magendie and Luschka provided the route for escape of ventricular CSF into the cerebello-medullary cistern, and thence to the arachnoid villi, and return to the venous blood.

Although this pathway for bulk flow of CSF is well established for adult mammals, there has been controversy about:

i) whether or not foramina exist at all in the IVth ventricular roof in submammalian vertebrates;

ii) the time of appearance of the foramina in the mammalian embryos;

iii) the relationship, if any, between the establishment of foramina and the development of the subarachnoid space.

Although the first of these topics has not formed part of the laboratory work of this thesis, recent work on it by others will be reviewed, because it has a direct bearing on the other two topics.

i) Do intraventricular and subarachnoid CSFs communicate in submammalian vertebrates?

Brocklehurst (1978) reviewed the comparative morphology of vertebrate CSF systems and confirmed earlier findings of the presence of CSF both within the ventricles and in the subarachnoid space of birds, reptiles and amphibians, but did not show the existence of macroscopic foramina for escape of ventricular CSF. The problem was

re-examined recently by Jones and her colleagues who found, in amphibians, that the posterior tela was complete in three urodele species but variably deficient in anuran species, with the exception of Xenopus.

Jones (1978) looked in more detail at Rana pipiens, using whole membrane mounts and light microscopy of resin embedded tissue. She found that the posterior tela formed an entire membrane only at its rostral end close to the choroid plexus: "here the pia formed a complete layer of loosely overlapping cells and the ependymal cells form an underlying incomplete layer with one to four gaps". Further caudally there were 7-10 gaps in the ependyma, of sizes ranging from 10-200um, and small deficiencies also were seen in the pial layer, so that there were some total fenestrations. Fine processes of pial cells were described as enclosing the end of a group of ependymal cells on both their ventricular and subarachnoid surfaces, so that the pial cells were interpolated into the ependyma. When fluorescein labelled dextran (MW = 150,000) was infused into the fourth ventricle of anaesthetised frogs, it was found two hours later in the ventricle, subarachnoid space, and passing through gaps in the ependyma.

These findings were confirmed and extended in four adult amphibian species by Jones (1979), using SEM, which showed deficiences in the posterior tela of 5-100 um. She fully appreciated that deficiencies of this size are unusual in epithelial sheets but compared them with

fenestrations in the endothelium of liver sinusoids (3 um in diameter) and in the diaphragmatic mesothelium (4-10 um).

She extended her study to birds and found, using TEM, that the membranous roof of the IVth ventricle was a continuous layer of flattened ependyma, without fenestrations (Jones and Dolman, 1979).

ii) Development of foramina in IVth ventricular roof in mammalian embryos

Weed (1917) put forward the concept that the roof of the IVth ventricle of mammalian fetuses is permeable to allow the escape of CSF from the ventricular cavity into the subarachnoid space. He replaced ventricular CSF of pig fetuses with a solution of 1% potassium ferrocyanide and iron ammonium citrate. Following fixation in acid medium, a precipitate of prussian blue granules was obtained which is histologically identifiable. He found that in pig fetuses over 16mm CR length there was a clear extraventricular spread through the roof of the IVth ventricle, particularly through the posterior region which he called "area membranacea inferior". He described this region of the roof as a complete membrane but suggested that it loses its epithelial appearance and becomes more mesenchymal in nature. He concluded that the extraventricular spread of prussian blue precipitate coincided with development of the choroid plexus and consequently with CSF production. However, Cohen and Davis (1937, 1938) found, in prenatal rat, rabbit and guinea pig,

that no foramina were present in the roof of the IVth ventricle at a time when the choroid plexus was well developed.

According to Weed (1917), when production of CSF by the choroid plexus started, it created a rising intraventricular pressure which upon reaching a certain level, caused rupture of the roof of the IVth ventricle thus forming foramina connecting the ventricular and subarachnoid CSF compartments.

Brocklehurst (1968) studied the development of CSF pathways in man, and concluded that the appearance of foramina in the roof of the IVth ventricle was an active developmental process, and not the result of passive rupture of the roof by the secretory pressure of CSF as suggested by Weed (1917).

Brocklehurst (1968) demonstrated a midline opening in the roof of the IVth ventricle in human embryos of 26-30mm (45-52 days). This would provide the route for escape of CSF at the theoretically appropriate time. Lateral foramina did not appear until very much later (21-26 weeks) in embryos of 195-220mm. This finding of a midline foramen as early as the 7th or 8th week, was contrary to the views of other authorities such as Hamilton, Boyd and Mossman (1952) who considered that the foramen of Magendie did not appear until the end of the third month or even until the fourth. Bartelmez and Dekaban (1962) demonstrated what they called "the locus apertura medialis ventriculi IV"

which is composed of a very thin, but continuous epithelial layer, and they discussed the possibility that it constituted a physiological foramen.

A possible weakness of all these earlier studies on mammalian embryos is that they were based on wax embedded material and on the use of rather crude tracers.

The problem has been extensively investigated in recent years, particularly by Jones and her colleagues, with some interesting, if controversial, results.

In several species (mouse, rat, pig and sheep) it was shown that interependymal pores occurred transiently in the membranous roof of the IVth ventricle, during the interval between the formation of the choroid plexus and the opening of the foramina of Luschka (Jones et al, 1987). By SEM the pores were 5-40 um in diameter (rat), up to 100 um (pig) and up to 300 um (sheep). She emphasized that adult rats and pigs do not have a midline foramen of Magendie and that the ependymal pores allow for bulk flow of CSF from the IVth ventricle to the subarachnoid space during the period between the appearance of the choroid plexus and of the foramina of Luschka.

"In the mouse and rat the area of the membranous roof decreases as birth approaches owing to the growth of the choroid plexus and the surrounding neural structures, such that by the time the lateral foramina open the number of pores is greatly reduced".

It is evident that there are considerable variations in the escape route for CSF from the IVth ventricle to the

subarachnoid space, both between species and at different developmental stages. Some of the available information is summarized in table 27.

D. DEVELOPMENT OF THE SUBARACHNOID SPACE:

Although the adult subarachnoid space has been adequately examined (Allen and Low, 1975; Cloyd and Low, 1974; Malloy and Low, 1976, 1974) less attention has been paid to its development. Detailed developmental studies of the mammalian meninges and subarachnoid space were undertaken by Weed (1917), Sensenig (1951) and more recently by McLone and Bondareff (1975).

Weed (1917) used pig and human embryos for his study. He replaced the embryonic cerebrospinal fluid with a solution of 1% potassium ferrocyanide and iron ammonium citrate and then fixed the embryos in acid medium so as to obtain a prussian blue precipitate. He described two areas of ependymal differentiation in the roof of the IVth ventricle which he called "area membranacea superior and inferior". The former gradually regressed and disappeared while the latter developed and gradually occupied most of the inferior medullary velum. These differentiated areas provided the route of escape of cerebrospinal fluid from the ventricles into the periaxial tissue. He found that the time of escape of the replacement fluid coincided with the development of the choroid plexus of the IVth ventricle. Cohen and Davis (1937) using a similar method established a similar correlation in chick embryo. They showed, however, that the corresponding areas in rabbit, rat and guinea pig were impermeable to the replacement solution at stages when the choroid plexus was already well developed. Keegan (1917) recorded similar observations in

rabbit embryos. He also showed that when replacing cerebrospinal fluid with iron ammonium citrate alone extraventricular spread occurred even before the development of the choroid plexus. From these findings it appears that the specialised areas in the roof of the IVth ventricle of various animals showed a specific difference in permeability. Also the finding of protein coagulum and prussian blue granules in contact with the inner surface of the membranous areas might well represent a dialysis phenomenon of this semi-permeable membrane.

Weed (1917) stated that the development of the subarachnoid space was a process of breaking down the perimedullary syncytium and dilatation of existing mesenchymal spaces. The enlargement of mesenchymal spaces was associated with the presence, in the spaces, of an increased amount of protein derived from the flow of the protein-rich embryonic CSF from the ventricles. Weed considered this protein-rich embryonic CSF as the most important causative agent in initiating and possibly also in completing the transformation of the extracellular mesenchymal spaces, filled with ground substance, into fluid-filled subarachnoid space. According to Weed (1917), the cerebrospinal fluid passed first around the brain stem, then downward around the spinal cord and upward around the basal part of the brain and lastly around the superior part of the mid-brain and the cerebral hemisphere. He considered that the development of the subarachnoid space

followed the same sequence. If this statement were true then the spinal subarachnoid spaces ought to be considered as developing physiologically from above downwards and not from below upwards as Reford found (cited - Cushing, 1914).

According to Sensenig (1951) in 15-16mm human embryos the mesenchymal tissue lying between the pia and dura became less regularly dispersed and cavities developed within it, at first in the areas ventral to the spinal cord and later lateral and dorsal to the cord, the subarachnoid space was thus beginning to form. By the stage of 30mm the majority of the cells had disappeared and a large cell-free space separated the pia from the dura, apart from some arachnoid trabeculae coursing through the space. Sensenig, however, did not mention whether the cerebrospinal fluid played any role in the development of the spinal subarachnoid space.

These subarachnoid spaces were bounded by an outer layer of mesenchymal tissue which would ultimately form the skull, dura mater and arachnoid mater and by an inner layer forming the pia mater. This would mean that all the meninges develop from mesenchyme. The mesenchymal origin of the meninges was suggested by many earlier workers. Thus Kollmann (1861) reported that the cranial meninges developed from a single mass of embryonal tissue, which he compared to Wharton's Jelly, situated between the cerebral surface and the superficial ectodermal layer. His (1865), Salvi (1898), Sensenig (1951) and others were all in favour of the mesodermal origin of the three meningeal layers.

The pia and arachnoid, known as the leptomeninges, were first studied histologically by Key and Retzius (1876) who interpreted them as mesothelial cells because of their resemblance to the cells lining the body cavities. However, studies with the transmission electron microscope clearly indicated that these cells were representative of the connective tissue (Pease and Schultz, 1958; Morse and Low, 1972a).

Harvey and Burr (1924, 1926) performed transplants of neural tube tissue in amphibian embryos. They made two series of neural tube transplants; one including neural crest cells and the other devoid of such cells. They found that no pia-arachnoid layer developed in neural tube transplants which were devoid of neural crest cells. On the basis of their results they concluded that the pia-arachnoid was derived from neural crest cells while the dura was derived from mesoderm. Flexner (1929) repeated the same experiments but his results failed to support a neural crest origin for the leptomeninges. Brocklehurst (1968) on the other hand, considered that the pia mater was of neuroectodermal origin whereas the dura and arachnoid were of mesodermal origin. He arrived at this conclusion by observing that in human embryos stained with trichrome the pial layer stained like the adjacent neural tube and differently from the arachnoid and dural condensations.

A recent detailed study of the development of the subarachnoid space and meninges in mouse was reported by

McLone and Bondareff (1975). Their study was limited to the area over the developing cerebral cortex. They showed that the development took place in four stages:

The first stage (10th - 13th prenatal day) after the closure of the neural tube was the interposition of mesenchyme, between the developing neuroepithelium and the overlying layer of ectoderm.

In the second stage (14th-16th prenatal day) the limits of the future subarachnoid space were defined. This stage was heralded by the flow of cerebrospinal fluid, which they described as watery and similar to cerebrospinal fluid of mature animals, through the large extracellular spaces, replacing the mesenchymal ground substance. They believed, as did Weed, that the flow of cerebrospinal fluid was an important causative agent in initiating the transformation of the extracellular mesenchymal spaces filled with ground substance into fluid-filled subarachnoid space. However, they did not explain how they differentiated, in semithin sections, between a fluid-filled space and a space occupied by a well-hydrated mesenchymal jelly. The cells of the pia-arachnoid became aligned parallel to the surface of the brain. A compact cellular layer 3-4 cells thick was formed halfway between the surface of the brain and the future epidermis. This layer would form the outer limit of the future subarachnoid space and would later give rise to the skull, dura and arachnoid mater. By the end of this stage the subarachnoid space and the fetal meninges were identifiable.

In the third stage (17th prenatal day - birth) the meninges became more mature and the subarachnoid blood vessels became ensheathed by pia-arachnoidal cells.

In the fourth stage (up to 21st postnatal day) macrophages appeared in the subarachnoid space, smooth muscle cells were added to the walls of the larger blood vessels and there was a general increase in the amount of collagen and elastic fibres.

Subarachnoid (leptomeningeal) macrophages

Normally a small number of macrophages are present in the subarachnoid space (Cloyd and Low, 1974; Malloy and Low, 1976).

Sturrock (1988) described an ultrastructural study of the development of macrophages in the leptomeninges of the mouse and rabbit spinal cord. He found that a small number of leptomeningeal macrophages could be identified at 11 and 12 days postcoitum in the mouse and rabbit respectively. At this stage they contained few cytoplasmic organelles, had narrow convoluted processes, but became well differentiated by 14 days in the mouse and 16 days postcoitum in the rabbit. McLone and Bondareff (1975) while studying the development of the cerebral subarachnoid space in the mouse, noticed subarachnoid macrophages only after birth. It might be that macrophages appear at different sites of subarachnoid space at different times. Sturrock (1988) also found that leptomeningeal cells are phagocytic but they are structurally different from free

macrophages. He could not provide any evidence to suggest that macrophages arose from leptomeningeal cells. He suggested that macrophages could have arisen from circulating monocytes, given the presence of blood vessels in the mesenchyme surrounding the spinal cord at all ages examined. Another source might be intrinsic mitosis due to the presence of mitotic leptomeningeal macrophages during fetal life (Sturrock, 1981).

The role of leptomeningeal cells in the production of macrophages was previously studied by Essick (1920) who found that following injection of inert material into the subarachnoid space, leptomeningeal cells rounded up and acted as phagocytes. Woollard (1924) also found that dye injected into the CSF was taken up by leptomeningeal cells, and concluded that macrophages arose both from leptomeningeal cells and from cells in the stroma and perivascular spaces of the meninges.

E. THE ARACHNOID VILLI

It is well established in man that most of the CSF returns to the blood stream by way of the arachnoid villi and granulations. This, however, obviously cannot hold in the case of those adult animals in which these structures are lacking and in the embryonic stages before they develop. Jayatilaka (1964) found arachnoid villi in man, monkey, sheep, dog, cat, rabbit, guinea pig but not in rat.

Weed (1917) found in pig embryos that a considerable extent of the wall of the transverse sinus, lying in mesenchymal tissue which was breaking down to form arachnoidal spaces, served as a site for the passage of fluid from the subarachnoid space into the lumen of the venous sinus. In the adult, however, the process was not so diffuse but was confined to the arachnoid villi. Weed made no comment on the time and mode of development of the arachnoid villi. According to Jayatilaka (1964) the development of arachnoid villi in sheep embryos started at 90 days as proliferations of arachnoidal cells around cerebral veins. These proliferating arachnoidal cell masses were heaped on one or both sides of the cerebral veins at their confluence with the venous sinus. The core of these arachnoidal cell masses was continuous with the subarachnoid space. In the 140 day embryo the arachnoid villi were well developed.

The mode of passage of cerebrospinal fluid through the arachnoid villi is a controversial subject. There are three main conflicting theories:

1) The open theory: this maintains that the arachnoid villi contain endothelial-lined "tubules" which open to the subarachnoid space at one end and to the venous channel at the other. This theory was supported by Welch and Friedman (1960) who showed that the arachnoid villi acted as valves allowing the cerebrospinal fluid to pass in one direction only, from the subarachnoid space into the venous channel. Welch and Polley (1961) found in the monkey that injected particles in the subarachnoid space entered tubules in the core of the arachnoid villi which opened into the venous channels. Jayatilaka (1965) demonstrated the presence of two types of "tubules" in the arachnoid granulations of sheep. One type was shown to be blood vessels which filled with India ink when this marker was injected into the carotid artery; the other type of "tubule" did not fill under these circumstances but did so when the India ink was injected into the subarachnoid space. These latter tubules were found to open into the lumen of the venous sinus.

Gomez, Potts and Deonarine (1974) and Gomez and Potts (1974) examined the arachnoid villi of sheep with the scanning electron microscope and demonstrated the openings of the tubules through the endothelial lining of the venous sinus. However, foldings and invaginations of the surface of the arachnoid villi might give an appearance which could be mistaken for the openings of the tubules.

2) The closed theory holds that an intact, non-fenestrated endothelial lining separates the subendothelial space of

the arachnoid villi from the lumen of the sinus and that no tubules or other structures were found that could be interpreted as representing open channels of communication between the subarachnoid space and sinus lumen. This theory was supported by Shabo and Maxwell (1968) who studied the arachnoid villi in the monkey with the transmission electron microscope, and by Alkene and Lovings (1972) who examined the arachnoid villi of the dog. All agreed that the endothelial covering of the arachnoid villi is intact.

3) Vacuolar Transport Theory: Tripathi and Tripathi (1974) did not find any tubules in the arachnoid villi of the monkey. They stated that the passage of cerebrospinal fluid into the venous system was provided by giant vacuoles which formed temporary transcellular channels connecting the subarachnoid space and the lumen of the venous sinus. They did not, however, control the pressure differential between subarachnoid CSF and superior sagittal sinus. This giant vacuolar transport has also been suggested as the mode of escape of the aqueous humor from the anterior chamber of the eye (Tripathi, 1971).

Levine, Polvishock and Becker (1983) reported a TEM and SEM study of the arachnoid villi of the monkey (Macaca fascicularis). They perfused the arachnoid villi with fixatives at various rigorously controlled subarachnoid and superior sagittal sinus pressures. The arachnoid villi were fixed when the CSF pressure was respectively 5mm Hg (normal functional state), 20mm Hg and 50mm Hg greater than

the superior sagittal sinus pressure; and also when it was 15mm Hg lower than the superior sagittal sinus pressure. This enabled them to detect ultrastructural changes occurring in the endothelium of the arachnoid villi throughout this wide range of pressure differentials.

They found that at 5mmHg pressure differential, the endothelial surface of the arachnoid villi showed both concave and convex cellular contours and also the occasional pore (1-2 um in diameter) on SEM. With TEM they noticed the presence of intracellular cytoplasmic vacuoles of 2-10 um in diameter. Rarely, these vacuoles were found to open both into the subarachnoid space and the venous sinus; this created a conduit between the two which measured 1-2 um in diameter. This would support the results of the in vitro studies of Welch and Polley (1961) who found that particles of up to 2 um in diameter are transported unimpeded through the arachnoid villi of the monkey.

At higher CSF pressures, Levine et al. (1982) found that the number and size of intracellular vacuoles increased, and the pores on the endothelial surface of the villi are now frequently encountered. On the other hand, when the superior sagittal sinus pressure exceeded that of the CSF, it was found that the endothelial surface of the arachnoid villi was smooth and showed no pores; and that there was complete absence of the intracellular cytoplasmic vacuoles.

Levine, Polvishock and Becker (1982) concluded, in the light of their findings, that the intracellular vacuoles are the major route of CSF absorption.

The disagreement between the different theories might be due to a basic difference in the structure of arachnoid villi in different species or due to the difference in techniques used. Levine, Polvishock and Becker (1982) emphasized that these differences in interpretation were due to the fact that the functional morphology of arachnoid villi changes with the change of pressure differentials between CSF and superior sagittal sinus. To overcome this, they suggested that fixation of arachnoid villi should take place while controlling CSF and superior sagittal sinus pressures.

MATERIALS AND METHODS

The CBA mouse was used in this study as it is inexpensive and readily available in the Department. The embryonic age was estimated from timed-matings, which were done between 10.00am and 12 noon in order to allow as accurate an estimate of post-coital (p.c) age as possible. The breeding stock was kept in a light-reversal room (lights on 19.00h to 10.00h, lights off 10.00h to 19.00h). Evidence of successful mating was a vaginal plug, a coagulum of fluid from the vesicular and coagulating glands of the male, that occluded the vaginal orifice. When a vaginal plug was found, then that day was considered as day "0". The Crown-Rump (C-R) length of all embryos from two litters at each stage was measured by calipers.

1. SCANNING ELECTRON MICROSCOPY OF THE CHOROID PLEXUS

Mouse embryos aged 11,13,14,15,16,17,18 and 19 days (p.c), newborn mice and adult mice aged 6 weeks were used. Five animals were used at each stage. The mother was killed by cervical dislocation and the embryos were removed from the uterus and immediately decapitated. The heads of 11 day p.c embryos were immediately immersed in 5% glutaraldehyde in Millonig's buffer solution (see appendix). The heads of 13 to 19 day old embryos were skinned, bisected in the coronal plane and the caudal halves were immersed in 5% glutaraldehyde in Millonig's buffer solution. New born mice were killed by decapitation and then treated similarly. The heads of the embryonic

(13-19 day) and newborn mice were bisected to make smaller blocks which allowed more rapid penetration of the fixative into the tissues, with better results. Bisection of the head did not cause any difficulties during the exposure of the choroid plexus.

Adult mice were killed by an overdose of anaesthetic ether. Perfusion, through the left cardiac ventricle, was then carried out by a gravity feed system at a pressure of 150cm of water. The right atrium was opened to provide an outflow route. The circulation was first flushed with mammalian Ringer Solution for three minutes, then perfusion fixation with 5% glutaraldehyde in Millonig's buffer solution was started. After fixation was deemed to be complete the brains were removed from the skulls and immersed in the same fixative overnight. All the specimens were left in the fixative for 24 hours. They were then washed in buffer solution D for 4 hours. The brains of 15, 16, 17, 18 and 19 day embryos and new born mice were removed from the skulls.

Exposure of the choroid plexus of the IVth ventricle

Throughout the procedure, which was done under the dissecting binocular microscope, the specimens were immersed in buffer solution D to prevent drying.

A. 11 day old embryo:

It was somewhat difficult to expose the choroid plexus of the IVth ventricle because of the small size of the head. The thin roof of the IVth ventricle was

difficult to handle and was often damaged during dissection. Another difficulty was that the choroid plexus could not be seen under the dissecting binocular microscope as it had not yet invaginated into the cavity of the IVth ventricle and was not vascular enough to give the distinctive pinkish colour seen in the more advanced stages.

The whole extent of the thin roof of the IVth ventricle could be clearly seen; the thicker floor was then dissected away using the sharp point of a needle, leaving the roof behind. In this way the whole extent of the roof could be viewed from inside (as if one were standing in a room and looking up at the ceiling). The roof was then carefully reflected rostrally. Handling the midline portion of the roof was avoided so as not to damage the incipient choroid plexus.

B. 13 and 14 day old embryos:

As the cerebellum was not yet well developed, most of the transparent roof of the IVth ventricle could still be identified. The floor was then gently separated from the roof and detached using the sharp point of a needle. The pinkish colour of the now vascular choroid plexus could be easily seen attached to the roof of the ventricle.

C. 15 to 19 day embryos, new born and adult mice:

Here the inferior medullary velum was covered to a varying extent by the developing cerebellum. It was made more readily visible by gently pushing the brain stem ventrally. The floor of the IVth ventricle was then

carefully dissected away using the sharp point of a needle. The pinkish colour of the choroid plexus, hanging from the roof of the ventricle, could now be clearly seen (this was not of course the case in the adult mouse, in which perfusion-fixation had flushed out all the blood from the vascular system of the choroid plexus).

After exposure of the choroid plexus, all the specimens were placed in 30% ethanol for 24 hours to remove any debris from the surface of the choroid plexus. The specimens were then dehydrated in a graded series of ethanols (50 - 100%). Three changes of absolute ethanol were used. They were then placed in a 50:50 mixture of absolute ethanol and amyl acetate and then in two changes of pure amyl acetate. After replacement of absolute ethanol with amyl acetate, a polar solvent miscible with CO_2, the specimens were critical-point dried with CO_2 in a Polaron critical-point dryer. They were then mounted on stubs on a conductive carbon cement layer (Leitz). Orientation of the specimens on the stubs was done under the dissecting binocular microscope. Difficulty was encountered only in the 11 day embryo because of the small size of the specimen and the fact that the incipient choroid plexus could not be visualized; the thin roof of the IVth ventricle was often found to have been damaged. The specimens were then coated with gold (500 A) in a Polaron Sputter Coater and viewed in a Cambridge S_2 Stereoscan Electron Microscope. Some of the specimens were

examined in a Jeol 300 Scanning Electron Microscope. All the specimens were screened and suitable areas were selected for photography.

2. VASCULAR CASTS OF THE CHOROID PLEXUS

The injecting medium used was Batson's Corrosion Compound (Batson, 1955), marketed in Britain as Trylon resin. It was possible within wide limits to control the viscosity and the working time of the resin. The viscosity could be controlled by varying the amount of thinner added. The working time or "pot-life" of the resin is the time during which the resin remains injectable after its preparation. This could be prolonged by either reducing the amount of catalyst added or by placing the resin container in an ice-cold bath. Polymerization of the resin usually took place between 22°C and 23°C by an endothermic reaction.

Preparation and injection of the resin was always done in a down draught fume cabinet because of the toxic nature of the chemicals involved. Gloves and goggles were also always worn. Adults and newborn mice, 18, 17, 15, 14 and 13 day embryos were used.

A. Adult mouse:

The animals were killed by an overdose of anaesthetic ether. The superior vena cava was exposed and opened, as far away from the right atrium as possible, to provide an outflow route. It was found that when the right atrium itself was opened to provide an outflow route much of the resin would somehow pass from the left ventricle and out through the right atrium. The descending aorta was clamped just above the diaphragm. The circulation was then flushed with Ringer Solution through the left cardiac ventricle

using a 23G butterfly needle which was kept in place by a small clamp. When the perfusate was clear the resin was drawn into a 10ml disposable syringe and slowly injected through the butterfly needle. There was a severe muscular reaction to the injected resin. However, this did not interfere with the injection and did not seem to affect the final result. When the injection was complete (i.e. deep blue discolouration of the tongue, snout and upper limbs) the animals were quickly transferred to a hot water bath at 25 - 30^{o}C for 10 minutes and then into tap water. This high temperature accelerated polymerization of the resin and by doing so reduced the chances of its leaving the blood vessels. It was found to be better to use the slowly polymerizing resin with subsequent application of external heat, as less catalyst was used and fewer batches of resin were prepared. Moreover the slowly polymerizing resin would allow ample time for injection.

B. Newborn mice:

The procedure was done under the binocular dissecting microscope. The same procedure as for the adult was applied for the injection of newborn mouse, with some modifications. A smaller butterfly needle (30G) directly connected to a 5ml disposable syringe was used and the resin was injected without prior flushing of the circulation with Ringer solution. The descending aorta was not clamped. It was technically easier to use the inferior vena cava as an outflow route, but when this was done most

of the injected resin was found to pass into the distal part of the body rather than towards the head. This might be due to the decreased resistance to the flow of resin as a result of opening the inferior vena cava. The blue colour of the resin could be clearly seen filling the superficial venous sinuses of the skull, and blood vessels of liver, lung and intestine, as well as flowing out from the opening in the superior vena cava. After the injection was deemed to be complete the animals were placed in a hot water bath (25 - 30^{o}C) for 10 minutes and then transferred into tap water.

C. 17 and 18 day embryos:

The mother was killed by an overdose of anaesthetic ether and the embryos removed. The tips of the 30G needles were filed to make them shorter and more blunt so that they could fit into the relatively smaller left ventricular cavity of the 17 and 18 day embryos. Apart from this modification, the procedure was the same as that used for newborn mice.

D. 15 day embryos:

The mother was killed by an overdose of ether and the embryos removed with their amniotic sacs intact. The small size of the embryos (12mm C-R length) made it technically difficult to inject the resin through the heart and the vitelline circulation was chosen as an alternative route. For this purpose drawn out micropipettes were used. These were mounted on 23G butterfly needles using araldite resin, which was given 24 hours to set and effectively seal the

gap between the micropipette and the needle.

The resin was drawn into a 5ml disposable syringe which was then connected to the butterfly needle, which was filled with resin until it started to dribble from the broken end of the micropipette. Sometimes it was necessary to break the micropipette a little farther from the tip to increase its calibre, but at the same time making sure that it was not too big to be inserted into the vitelline vein. The micropipette was then introduced into the lumen of the vein. An assistant was usually needed to steady the micropipette after its insertion into the vitelline vein. The amniotic sac was then ruptured and the umbilical cord was severed to provide an outflow route. The resin was then injected very slowly. The liver, which was very large at this stage, became filled with resin which could then be seen to fill the whole circulation. When the injection was stopped the umbilical vessels were simultaneously crimped by a needle to stop the outflow of resin. The amniotic sac was then removed and any excess resin on the surface of the embryo was gently wiped by a wet piece of cotton. The embryo was placed into a hot water bath at 25-30^{o}C for 10 minutes and then into tap water. Floating in water helped to prevent the collapse of the cast.

24 hours after the injection, the brains of adult mice were removed from the skull and the new born mice were decapitated. 18, 17 and 15 day embryos were transected just below the neck level to make bigger specimens for easy

handling. All the specimens were then immersed in 10% potassium hydroxide solution for 24 hours. Potassium hydroxide macerated all the soft tissues without affecting the vascular cast. The specimens were then given several rinses in distilled water to remove the macerated soft tissue, helped by gentle agitation of the container. Complete clearance of macerated soft tissue was checked by the binocular dissecting microscope and more rinses in distilled water were done when necessary.

E. 13 and 14 day embryos:

The same procedure as for the injection of 15 day embryos was followed. Unfortunately, all attempts at injection were unsuccessful.

Exposure of the vascular cast of the choroid plexus:

The vascular cast of the choroid plexus of the IVth and lateral ventricles was exposed in the adult, in the newborn and in the 18 day embryo. Only the vascular cast of the choroid plexus of the IVth ventricle was exposed in the 17 and 15 day embryos.

The procedure was done under the dissecting binocular microscope using fine, sharp scissors and small dissecting forceps. The specimen was fixed to cardboard by small pins and submerged under water throughout as the vascular cast could not support its own weight. The technique to expose the vascular cast of the choroid plexus was similar at all stages; it was technically more difficult in the embryonic stages and especially in the 15 day embryo because of their small size.

Exposure of the vascular casts of the choroid plexus of the IVth ventricle:

Exposure of the vascular cast of the choroid plexus of the IVth ventricle was done by the same procedure as for exposing the choroid plexus for the scanning electron microscopy; the only difference was that here we were dealing with casts of blood vessels rather than fixed brain tissue.

The cast of the vascular plexus beneath the floor of the IVth ventricle was carefully removed, thus exposing the cast of the choroid plexus. This appeared as a solid mass and was distinctly different from that of the subependymal capillaries, which formed a loose interwoven mesh. This characteristic appearance was made use of in the final orientation of the specimen on the stub.

Exposure of the vascular cast of the choroid plexus of the lateral ventricle:

The cast of the cerebral capillaries was cut in the parasagittal plane on the superior surface. The edges were pushed apart and the cut was deepened until the cavity of the lateral ventricle was entered. The vascular cast of the choroid plexus was seen projecting into the cavity of the ventricle and was easily recognised by its characteristic pattern. The casts were then given several rinses in distilled water to remove any remaining macerated soft tissue. They were then dehydrated in graded series of ethanol (50 - 100%), placed in a 50:50 mixture of absolute

ethanol and amyl acetate solution and finally in pure amyl acetate. They were then critical-point dried with CO_2 in a Polaron critical point dryer. The specimens were mounted on stubs using a quick drying silver paint. Orientation of the cast was done under the binocular microscope. The specimens were coated with gold (500 A) in a Polaron Sputter Coater and screened in a Jeol 300 Scanning Electron Microscope and suitable specimens selected for photography.

3. SCANNING ELECTRON MICROSCOPY OF THE INTERIOR OF BLOOD VESSELS OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE.

5 adult and 5 newborn mice were used. They were killed by an overdose of anaesthetic ether. The thoracic cavity was opened, descending aorta clamped just above the diaphragm and the right atrium was opened to provide an outflow route. The circulation was then flushed with Ringer Solution through the left cardiac ventricle. When the perfusate was clear, perfusion fixation with 3% buffered glutaraldehyde in Millonig's buffer solution was started. When the fixation was complete as judged by the rigidity of the perfused tissues, the brains were removed from the skulls and immersed in the same fixative for 24 hours. The brains were then given a quick wash in buffer solution D (see Appendix), dehydrated in a graded series of ethanol (50 - 100%), cleared in amyl acetate and then embedded in wax. 10 um thick horizontal sections were cut on a rotary microtome.

Every tenth unstained section was examined under the light microscope until a level was reached at which the cut surface of the choroid plexus of the IVth ventricle could be identified. The blocks were then dewaxed in many changes of xylene, critical-point dried with CO_2 in a Polaron Critical Point Dryer, mounted on stubs using a quick drying silver paint, coated with gold (500 A) in a Polaron Sputter Coater and then examined in a Jeol 300 Scanning Electron Microscope. Only the blood vessels which

were opened longitudinally were suitable for examination. The interior of cerebral capillaries was also examined to act as controls.

4. SEMITHIN LIGHT MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE

11, 13, 14, 15, 16, 17, 18 and 19 day embryos, and newborn and adult mice were used. Five animals were used at each stage.

The mother was killed by cervical dislocation and the embryos were removed and immediately decapitated. The heads of 11 day embryos were immersed in 5% glutaraldehyde in Millonig's buffer solution for 24 hours. Heads of 13 to 19 day embryos were bisected in the coronal plane and the posterior halves trimmed and then immersed in 5% buffered glutaraldehyde solution for 24 hours. New born mice were killed by decapitation, adult mice by an overdose of anaesthetic ether, and then treated similarly. The specimens were given several rinses in buffer solution "D". The brains of 15 to 19 day embryos and new born mice were removed from the skulls and given further rinses in Millonig's buffer solution D. All the specimens were then postfixed in osmium tetroxide in Millonig's buffer for 2 hours.

After a few rinses in buffer solution D, the specimens were dehydrated in a graded series of ethanols, cleared in propylene oxide and embedded in Spurr's resin. Polymerisation of the resin occurred overnight in a 60^{o}C oven. 1 um thick serial sections were cut in the coronal plane in all specimens except the 11 day embryo which was cut in the sagittal plane. Sections were stained with Azur

blue. Only the midline portion of the choroid plexus was examined. Suitable sections at each stage were photographed in a Leitz Orthomat photomicroscope.

Under the binocular microscope the cut surface of the choroid plexus of the IVth ventricle could be clearly identified on the face of the block. This was trimmed to a suitable size and thin sections, at 700 nm were cut with a Zeiss-Jung Ultracut microtome equipped with a glass knife. The sections were mounted, unsupported, on 200 mesh copper grids and stained with 0.5% uranyl acetate in 40% ethanol, and 0.75% aqueous lead citrate. Selected areas were examined in a Jeol transmission electron microscope, and images were recorded on Ilford film.

5. MORPHOMETRIC ANALYSIS OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE

Mouse embryos at daily intervals from 13 to 19 days and new born mice were used. Three animals were used at each stage. The mother was killed by an overdose of anaesthetic ether and the embryos removed. 13 to 19 day embryos were immediately decapitated and their heads were bisected in the coronal plane and the caudal halves immersed in 5% glutaraldehyde in Millonig's buffer solution for 24 hours. New born mice were killed by decapitation and then treated similarly. Immersion was chosen as a method of fixation because perfusion might cause distension of the blood vessels. Moreover, it would have been very difficult to perfuse the smaller embryos successfully. After 24 hours in the fixative the heads were washed in buffer solution D for 3 hours. The brains of 15 to 19 day embryos and new born mice were removed from the skulls. All the specimens were then dehydrated in a graded series of ethanols (50%, 70%, 90% and then absolute); cleared in propylene oxide and embedded in Spurr's resin. The specimens were then placed in a $60^{o}C$ oven and left to polymerise overnight. Serial sections were cut, at 1 um, and those which cut through the midline portion of the plexus were mounted, four sections to a slide, and stained with Azur blue. This provided from 25 - 50 serial sections from each of the 3 animals at each stage. From these, 5 sections from each animal were selected at random and

photographed. Each stage was therefore represented by a total of 15 photographs. The sections were photographed in a Leitz-Orthomat at an initial magnification of 400x. The prints were enlarged two and a half times making a final magnification of 1000x. Because of the initial high magnification (400x) a montage of two or more micrographs was necessary to show the whole area of the choroid plexus to be examined.

The lateral limits of the choroid plexus were defined by -

a) its invagination into the cavity of the IVth ventricle.
b) the difference in appearance between the choroid plexus and ependymal cells as seen under the microscope using an oil immersion lens, i.e. the well developed brush border of choroid plexus cells compared to that of ependymal cells.

The latter criterion was found to be useful for that part of the choroid plexus which was not invaginated into the cavity of the ventricle. A tangential line, or lines, were drawn to mark the limits of the choroid plexus. All the blood vessels lying within those limits were marked and double checked by simultaneously examining the corresponding section under the microscope using oil immersion lens.

In each micrograph the relative cross-sectional areas of the choroid plexus, of the epithelium and of the blood vessels were measured using the Reichert-Jung-AMO_2 MOP machine, with a magnitude of error of around 2%. The

relative cross-sectional area of the connective tissue was calculated by subtracting the relative cross-sectional area of blood vessels and epithelium from the relative cross-sectional area of the choroid plexus. The percentage of surface area of each component (epithelium, blood vessels and connective tissue) from the relative cross-sectional area of the choroid plexus was then calculated. The results would represent area per unit area (AA); and according to Weibel (1974):

$$AA = VV$$

where VV is volume per unit volume or volume density. There was a possible source of error when deriving volume and surface area estimates from a two dimensional image. This was minimised by cutting the sections at 1 um (Philip and Buchanan, 1971).

6. DEVELOPMENT OF THE SUBARACHNOID SPACE

11, 13, 14, 15 and 17 day embryos and newborn mice were used. Three animals were studied at each stage.

The mother was killed by cervical dislocation and the embryos were removed. They were immediately decapitated and the heads immersed in 5% glutaraldehyde in Millonig's buffer solution for 24 hours. Newborn mice were killed by decapitation and then treated similarly. After staying in fixative for 24 hours, the specimens were washed in buffer solution D for 4 hours. The heads of 15, 16 and 17 days embryos and newborn mice were decalcified in a phosphate buffered ethylene diamine tetrahydroacetate (EDTA) solution for 5 days. The solution was changed daily. After being given quick rinses in buffer solution D the specimens were dehydrated in a graded series of ethanols (50 - 100%), cleared in propylene oxide and embedded in Spurr's resin.

Polymerisation of the resin took place overnight in a $60^{o}C$ oven. 1.5 um sections were cut in the coronal plane and stained with Azur blue. The developing subarachnoid space around the hind brain was studied in detail, with more general reference to the areas on the lateral aspect of the cerebellum and the cervical spinal cord.

The subarachnoid space was also studied by the scanning electron microscope in the new born mouse. Two animals were used. These were given a lethal dose of ether and then perfusion-fixed through the left ventricle with 3% buffered glutaraldehyde solution. The heads were then

skinned and immersed in the same fixative overnight. After a few rinses in buffer solution D, the heads were decalcified in a phosphate buffered EDTA solution for 5 days, the solution being changed daily. The specimens were then dehydrated in a graded series of ethanols (50 - 100%), cleared in amyl acetate and embedded in wax. 10 um thick sections were cut in the coronal plane on a rotary microtome. Every tenth unstained section was examined under the light microscope until a level was reached where the cut surface of the cerebellum and the hind brain could be identified. The blocks were then dewaxed in a few changes of xylene, critical point dried with CO_2 in a Polaron Critical Point Dryer, mounted on stubs using a quick drying silver paint and coated with gold (500A) in a Polaron Sputter Coater. The subarachnoid space on the ventral aspect of the hindbrain and lateral aspect of the cerebellum was examined in a Jeol 300 Scanning Electron Microscope.

7. DEVELOPMENT OF THE ARACHNOID VILLI

Adult mice, newborn mice and newborn guinea pigs were used. Five new born mice were killed by decapitation. The head was divided in the coronal plane at the level of the coronal suture and immediately immersed in 5% glutaraldehyde in Millonig's buffer solution. Five adult mice were killed by an overdose of anaesthetic ether, decapitated and the heads immersed in 5% buffered glutaraldehyde solution. After 24 hours in the fixative all the specimens (adult and newborn mice) were given a quick rinse in buffer solution D and then decalcified in phosphate buffered EDTA solution for 7 days, the solution being changed daily. The blocks were then reduced in size to include the posterior half of the superior sagittal sinus, the confluence of sinuses and the first part of the transverse sinuses. The specimens were then dehydrated in a graded series of ethanols (50 - 100%), cleared in propylene oxide and embedded in Spurr's resin. The resin was allowed to polymerise overnight in an oven at a temperature of 60^{o}C. 2 um serial sections of the specimen were cut in the coronal plane and stained with haematoxylin and eosin. Two more adult mice were used. After fixation the calvaria was carefully removed without damaging the dura mater or the venous sinuses. This procedure was done with the head immersed in buffer solution D to prevent drying. A block of tissue containing the confluence of sinuses and the neighbouring parts of the superior sagittal

and trasverse sinuses, together with the adjoining dura mater and brain tissue, was removed. The block was embedded in Spurr's resin and 2 um serial sections were cut in the coronal plane, stained with Azur blue and examined in the light microscope for the presence of arachnoid villi. As no arachnoid villi were found in the adult or newborn mice, the prenatal stages were not examined.

Arachnoid villi in the Guinea Pig

Three new born guinea pigs were used. The animal was given a lethal dose of anaesthetic ether, decapitated, scalped and the head immersed in Bouin's solution for 24 hours. The head was then decalcified in phosphate buffered EDTA solution for 7 days. The EDTA solution was changed daily. The specimen was dehydrated in a graded series of ethanols (30 - 100%), cleared in amyl acetate and embedded in wax. 5 um serial sections of the whole head were cut in the coronal plane using a rotary microtome. The sections were stained with haematoxylin and eosin and examined in a light microscope for the presence of arachnoid villi.

8. DEVELOPMENT OF FORAMINA OF THE IVTH VENTRICLE

13, 14, 15, 16, 17, 18 and 19 day embryos and newborn mice were used. Three animals were examined at each stage. The mother was killed by an overdose of anaesthetic ether and the embryos were obtained by Caesarian section. They were immediately decapitated, heads bisected in the coronal plane and the caudal half placed in 5% glutaraldehyde in Millonig's buffer solution. Newborn mice were killed by decapitation and then treated similarly. After 24 hours in the fixative all the specimens were washed in two changes of buffer solution D. They were then decalcified in a phosphate buffered EDTA solution for 7 days. The solution was changed daily. The brain was not removed from the skull because it was found that this procedure causes disruption of the wall of the lateral recess and the thin roof of the IVth ventricle.

The specimens were then dehydrated in a graded series of ethanols (50% - 100%). From absolute ethanol the specimens were placed in propylene oxide, followed by 50:50 solution of Spurr's resin and propylene oxide and finally embedded in Spurr's resin. The resin was allowed to polymerise overnight in a 60^{o}C oven. 1 um sections were cut in the coronal plane. Every fourth section was mounted on a slide; this would help to estimate the dimensions of the foramina, that is if a foramen spans four slides then its diameter would be approximately 16 um. The sections were stained with Azur blue. The thin roof of the IVth

ventricle caudal to the choroid plexus and the lateral recesses were carefully examined for the presence of foramina.

APPENDIX TO MATERIALS AND METHODS

		No. of animals used
EXP I	SEM of choroid plexus	50
EXP II	SEM of interior of blood vessels	10
EXP III	Semithin and TEM of choroid plexus	45
EXP IV	Morphometry of choroid plexus	24
EXP V	Development of subarachnoid space	18
EXP VI	Development of foramina in the roof of IVth ventricle	24
EXP VII	Arachnoid villi	15

MILLONIG'S BUFFER SOLUTION (BUFFER SOLUTION D)

2.26% Na H_2PO_4, $2H_2O$ (Sodium Dihydrogen Orthophosphate)

2.52% NaOH (Sodium Hydroxide)

Made up in distilled H_2O

Solution made up of:

In 1 Litre: 830mls of 2.26% NaH_2PO_4

+ 170 mls of 2.52% NaOH.

Adjust pH to 7.2 - 7.4 (if necessary)

GLUTARALDEHYDE FIXATIVE

5% Glutaraldehyde in Millonig's Buffer.

In 1 litre: 800 mls of Buffer

+ 200 mls of Glutaraldehyde (Stock 25%)

+ 30 mls of 5.4% Glucose

pH 7.2 - 7.4

OSMIUM TETROXIDE (OsO_4)

1% of OsO_4 in Millonigs Buffer

1 gm OsO_4 in 90 mls Millonig's buffer + 10 mls of 5.4% Glucose.

Dissolve OsO_4 at room temperature and leave overnight.

Thereafter, store in fridge at $0^{o}C$

MAMMALIAN RINGER SOLUTION

NaCl	0.9g
KCl	0.04g
$CaCl_2$	0.025g
$MgCl_2$	0.0005g
Na HCO_3	0.05g
NaH_2	0.005g
Glucose	0.1g

Anhydrous salt per 100mls of distilled water

Rinse solution

500mls Ringer solution

50mls (2%) xylocaine chloride

AZUR BLUE STAIN

60% of 1% Azur II Blue *40% of 1.9% sodium tetraborate solution.

(Made up in distilled H_2O)

Filter before use

URANYL ACETATE

Saturated solution of uranyl acetate in 40% ethanol

LEAD CITRATE

1.33gms of lead nitrate + 1.76gms sodium citrate shaken with 30mls distilled H_2O for 30 minutes. Add 8mls NaOH and make up with distilled H_2O to 50mls

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H & E (Haematoxylin and Eosin)

1% Eosin

Harris' Haematoxylin

SPURR'S RESIN

Vinyl cyclohexene dioxide (ERL-VCD 4206)

Diaglycidyl ether of polypropylene glycol (DER 736)

Nonenyl succinic anhydride (NSA)

Dimethylamenoethanol (D.M.A.E. or S.I.)

VCD	10gms
DER	4gms
NSA	26gms
SI	0.3gms

EDTA

Ethylenediaminetetra-Acetic Acid (disodium salt)

Normally 10%

TRYLON CLEAR LOW-VISCOSITY RESIN (CL201P)

"BATSON'S" CORROSION COMPOUND

Formula

Resin	25gms
Catalyst	2.5gms
Activator	0.15gms
Thinner	10-15mls

+ Dye

Injection temperature = 24-26°C

TIMETABLE FOR SEMI-THIN SECTIONS

Spurr Embedded

Fixation	Glutaraldehyde	48hrs
	Buffer	2-3hrs total
	2 changes	
Dehydration	50% Ethanol	1-2hrs
	70% Ethanol	1-2hrs
	90% Ethanol	1-2hrs
	100% Ethanol	1-2hrs
	4 changes	4-8hrs total
Clearing	Propylene oxide	
	2 changes	45 mins each
Soaking	Propylene oxide and Spurr	
	1 = 1	12hrs
	1 = 3	12hrs
	Spurr	
	2 changes	7 days
Embedding & Curing	Spurr	8 hrs, 60^{o}C

Time in each solution depends on the size of the tissue block.

Before changing the Spurr solution, the bottles were placed in a 60^{o}C oven for one hour. This will cause the resin to be less viscous and thus more manageable and will enhance the penetration of the resin into the tissue.

TIMETABLE FOR PARAFFIN WAX SECTIONS

Fixation	Bouin's Solution	24hrs
Decalcification	E.D.T.A., Daily changes	7 days
Dehydration	30% Ethanol	2hrs
	50% Ethanol	4 hrs
	70% Ethanol	4 hrs
	90% Ethanol	4 hrs
	100% Ethanol	4 hrs
	(2 changes)	
Clearing	Amyl acetate	4 hrs
Soaking	Paraffin wax, 3 changes	24 hrs total
Embedding	Paraffin wax	

The time in each solution depends on the size of the block of tissue.

TIMETABLE FOR SCANNING ELECTRON MICROSCOPY SPECIMEN

Dehydration	30% Ethanol	Up to 24 hrs
	50% Ethanol	4 hrs
	70% Ethanol	4 hrs
	90% Ethanol	4 hrs
	100% Ethanol	4 hrs
	2 changes	
	100% Ethanol and amyl acetate	2 hrs
	1 = 1	
	Amyl acetate	4 hrs
	2 changes	

The time in each solution depends on the size of the block of tissue.

STAINING TECHNIQUES

HAEMATOXYLIN & EOSIN

Slides should always be left overnight in the 36^{o} oven to prevent sections floating off.

1) Place slides in Xylene to dewax - 10 mins

2) Hydrate through Alcohols. Absolute Alcohol.

3) 2nd change Absolute Alcohol

4) 90% Alcohol

5) 70% Alcohol

6) Wash thoroughly in running water

7) Haematoxylin - 4 mins

8) Wash in water

9) Differentiate in Acid Alcohol

10) Wash in water

11) Blue in Scott's. Microscopic check, if haematoxylin is too heavy, further differentiation in acid alcohol is required.

12) Wash in water

13) Eosin - 3 mins

14) Wash in water

15) Dehydrate through alcohols differentiating Eosin in 70% Alcohol

16) 90% Alcohol

17) Absolute Alcohol

18) Absolute Alcohol

19) Carbo-xylene till clear, i.e. there should be no milky patches on the slide - 3 mins

20) Xylene - 5 mins. Microscopic check. If further differentiation of Eosin is required, hydrate to 70% Alcohol

21) Mount in DPX

RESULTS

SCANNING ELECTRON MICROSCOPY OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE

The following account describes the appearance of the ventricular surface of the developing choroid plexus, as seen by SEM. At each stage the IVth ventricle was opened at its caudal end, and the caudal end of its roof turned rostrally to expose the ventricular surface of the plexus.

1. 11 day old embryo

a) Choroid plexus epithelium

It was difficult to expose the choroid plexus of the IVth ventricle at this stage, because of the small size of associated structures and partial damage to the roof of the IVth ventricle often occurred.

Fig. 1 shows the internal surface of the IVth ventricle, the roof of which has been opened by a transverse cut. The observer is looking rostrally into the rostral part of the ventricle, and towards the cavity of the mid brain. The thick floor of the ventricle shows alar and basal laminae on each side of the midline sulcus, and demarcated by the sulcus limitans. The thin ependymal roof of the ventricle has been reflected rostrally to expose its internal surface, and that of the incipient choroid plexus which was confined to a limited area in and around the mid-line portion of the roof. The anlage of the plexus was identifiable only by the fact that the cells covering its

surface were already clearly different from the ordinary ependymal cells. The apices of the choroid plexus cells were dome shaped and bore numerous, short microvilli (Figs. 2 & 3). Some cells also had a single cilium while others showed tufts of cilia (4-8 cilia per tuft). The microvilli and cilia did not however completely cover the apical surface of the cells and small areas of relatively smooth apical plasma membrane were seen between them. Many free, spherical cells were seen lying on the surface of both ependyma and choroid plexus. Their nature is uncertain, but they may represent epiplexus and supraependymal cells (see later). The choroidal epithelium had not yet been invaginated into the IVth ventricle by the tela choroidea, and no choroid plexus folds were visible at this earliest stage.

b) Ependyma

In contrast to the choroid epithelial cells the ependymal cells had flat and smooth apical surfaces, which were irregularly polygonal in shape (Fig. 4). Short microvilli were very sparsely distributed over the whole surface, but were more abundant and longer along the margins of the cells. Most cells had a single centrally-placed cilium, some had more than one.

2. <u>13 day old embryo</u>

Fig. 5 shows the paramedian part of the choroid plexus projecting from the roof into the cavity of the IVth ventricle. It had grown considerably in size and complexity and now formed numerous folds. The process of

differentiation of choroidal epithelium had extended laterally into the lateral recesses of the IVth ventricle (Fig. 6). In contrast to the central part of the plexus, the lateral part had not yet become folded, but its covering cells were identifiably differentiated as choroidal epithelium: they formed a tall columnar epithelium seen exposed in the cut surface at X in Fig. 6 with dome-shaped apices covered by microvilli (Fig. 6).

The convex apical surfaces of the choroid epithelial cells were now completely and densely covered by numerous microvilli, of varying shape: some were cylindrical, some broad and some clavate, while a few were enlarged to form blebs. Cytoplasmic processes apparently joining adjacent cells could be seen. Cilia, either single or in groups, were seen projecting beyond the general level of the microvilli (Fig. 7). Scattered over the surface of both the choroidal epithelium and the ependyma were numerous spherical cells, about 8-9 um, with a generally smooth surface, covered by ruffles and blebs. They are seen at low magnification in Figs. 5 & 6 and at high magnification in Fig. 8. They are interpreted as macrophagus (epiplexus and supraependymal cells).

3. 14 day old embryo

At 14 days the surface appearance of the choroid plexus epithelium was very similar to that at the 13 day stage (Fig. 9).

4. 15 day old embryo

Fig. 10 shows a general low power view of most of the choroid plexus of the IVth ventricle. The plexus as a whole was much larger than at previous stages. Its larger midline part was elaborately folded, with deep fissures between the folds. Traced laterally, the folding was progressively reduced, to be almost absent at the lateral end of the plexus. . The apical surfaces of its epithelial cells were convex and were now completely covered by a large number of microvilli of different sizes and shapes (Fig. 11). A few cilia were also present. Adjacent cells were connected by numerous cytoplasmic processes running across the deep fissures between the apical ends of the cells.

A very curious feature observed at this stage was what appeared to be ruptured blebs, lying on the apical surface of the cells. They were shaped like a thistle funnel about 1-2 um in diameter with a circular opening of 0.2 - 0.4 um diameter. They appeared to be connected by a narrow stalk to the surface of the choroid epithelial cells. At high power the external surface of these blebs was similar to that of the microvilli and the cytoplasmic processes connecting the adjacent cells. This suggests that these blebs were derived from the epithelial cells (Fig. 12).

5. 17 day old embryo

a) Choroid plexus epithelium

Fig. 13 shows a general low power view of the entire extent of the choroid plexus of the IVth ventricle. The characteristic folding of the central portion of the plexus had now extended to the lateral part, which reached into the lateral recess of the ventricle.

The convex apical surfaces of the choroid epithelial cells were completely covered by a dense mat of microvilli of various sizes and shapes. Groups of cilia were seen projecting beyond the general level of the microvilli (Fig. 14).

b) Ependyma

The apical surfaces of some of the ependymal cells were gently convex, others were flattened (Fig. 15). They were smooth apart from a centrally placed cilium and short microvilli which were sparsely distributed over the whole surface but more abundantly along the margins of the cells.

Comparison of the appearance at 11 days (Fig. 4) and 17 days (Fig. 15) shows that there has been little change in the structure of the apical surface of the ependymal cells. By contrast, there has been considerable change in the surface appearance of the choroidal epithelium (cf Figs. 3 & 14).

6. 18 day old embryo

The midline part of the choroid plexus of the IVth ventricle is shown in Fig. 16. Its surface was highly convoluted, and clearly demarcated from the smooth surfaced

ependymal roof.

As at all stages from 13 days onwards, the apical surfaces of the choroid epithelial cells were convex and completely covered by microvilli of different sizes and shapes. A few cilia could also be seen. Long cytoplasmic cellular processes were seen connecting adjacent cells to each other (Fig. 17).

7. 19 day old embryo

As at 18 days the apical surfaces of the choroid epithelial cells were convex and completely covered by dense, numerous microvilli of different sizes and shapes (Fig. 18). A few cilia could also be seen. Cytoplasmic cellular processes were seen connecting adjacent cells to each other.

8. New born mouse

The structure of the choroidal epithelium was found to be very similar to that of the late prenatal stages (Fig. 19). By contrast the surface appearance of the ependyma showed some differences from that of the prenatal stages. The boundaries of the cells were much less well defined. A large number of cilia was seen projecting from the apical surfaces of some ependymal cells, sometimes as many as 40 cilia per cell (Fig. 20), although some ependymal cells possessed only a single cilium. Short microvilli were also seen, their density varying greatly from one cell to the other.

9. Adult mouse

a) Choroid plexus epithelium

The apical surfaces of the choroid epithelial cells were completely and densely covered by numerous microvilli of different sizes and shapes (Fig. 21). Some were cylindrical, some broad and some club-shaped. A few cilia could also be seen. Apical blebs, larger in size than most microvilli were also seen. They seemed to have ruptured and started to flatten out.

b) Ependyma

Tufts of cilia (up to 40 cilia per tuft) were seen arising from the apical surfaces of most of the ependymal cells (Fig. 22). They were much longer than those of the newborn (Fig. 20). Some of the ependymal cells were devoid of cilia. Short, sparse microvilli were seen scattered on the surface of the ependymal cells.

What is interpreted as a supraependymal cell was seen lying on the surface of a non-ciliated ependymal cell. It had a few short processes. Similar cells were seen quite frequently.

Broad, flat irregular and branching processes were also seen lying on the surface of ependymal cells. The nature of these processes is a matter for speculation.

Another type of supraependymal cell was infrequently seen (Fig. 23). The cell lay on a non-ciliated ependymal cell, was oblong in shape and measured about 9 um along its widest diameter and 5 um across. It had long processes which branched and re-branched and ran for a considerable

distance on the surface of the ependyma. These branches contained varicosities or swellings along their course. This appearance suggested that it was neuronal in nature rather than phagocytic.

Summary

From the time of its first inception at the 11th day p.c., the epithelial cells of the choroid plexus could be recognised by their bulging apices and abundant microvilli and were clearly different from ependymal cells whose apices were flat with very few microvilli. At the 13th day p.c., the apical surfaces of the epithelial cells of the choroid plexus were already completely covered by numerous and dense microvilli. Between the 13th day p.c., and birth, the choroid plexus increased greatly in size and complexity but the surface specialization of its cells was very similar. If these surface specializations were important for the secretory function, then on the basis of this structural evidence it might be said that the choroid plexus started secretion at the 13th day p.c.

TRANSMISSION ELECTRON MICROSCOPY (TEM) OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE

1. 11 day old embryo - Fig. 24

The choroidal epithelial cells were columnar with convex apices, as already seen by scanning electron microscopy (SEM). Microvilli were short and slender and appeared surprisingly sparse as compared with what was seen by SEM until one recalls that the TEM profile is of a single very thin section of tissue. Also seen at the apical surface were occasional pedunculated structures, circular in sectional profile, and packed with vesicles.

The lateral plasma membranes were straight and parallel with no enlargement of the intercellular space and no lateral infoldings. They were joined at their apical ends by junctional complexes. The epithelial cell nuclei were ovoid, with two or more nucleoli, and were mostly situated towards the apical end of the cells.

The cytoplasm contained uniformly scattered mitochondria and sparse profiles of rough endoplasmic reticulum. The most conspicuous feature of the cytoplasm was a uniformly dense sprinkling of glycogen granules, not aggregated into clumps. The basal plasma membrane was gently folded and rested on a well defined basal lamina.

2. 13 day old embryo

Some significant developmental changes had occurred by this stage (Figs. 25, 26). The apical surfaces of the choroid epithelial cells were now covered by

abundant bulbous microvilli, an appearance correlating well with the scanning electron microscopic findings. The basilateral plasma membranes showed more complex interdigitations and infoldings, contrasting with the simple, straight basilateral plasma membranes at the 11th day p.c. The apical ends of the lateral plasma membranes were joined by junctional complexes. The nuclei were either lobated or irregular in shape and had one or more nucleoli.

Profiles of rough-surfaced endoplasmic reticulum were more numerous throughout the cytoplasm. No particular pattern of distribution was noted. Mitochondria were also seen scattered throughout the cytoplasm. Golgi complexes were more frequently seen than at 11th day p.c.; they consisted of tubules and vesicles and occupied a supranuclear position. Glycogen granules were still scattered uniformly throughout the cytoplasm, but now also formed conspicuous clumps and aggregates situated mainly at the basal parts of the cells (Fig. 26). Dark, spherical bodies, most probably lysosomes, were seen in the cytoplasm of some cells. Some cells had conspicuously paler cytoplasm (Fig. 26), with more widely dispersed organelles. Some of them showed basally situated lipid inclusions. Similar cells were again noticed at 16th day p.c.

The choroid epithelial cells rested on a well defined basal lamina, which did not take part in the basilateral plasmalemmal infoldings. Blood capillaries were found immediately beneath the bases of the choroid epithelial

cells with very sparse connective tissue elements intervening (Fig. 25). The basement membrane of the vascular endothelium was poorly developed or absent in contrast to its well developed epithelial counterpart. The capillary endothelium varied quite considerably in thickness when traced over a short distance, and showed frequent flap-like extensions on its adluminal surface.

3. 14 day old embryo

Fig. 27 shows features which were very similar to those of the 13 day embryos. These included lobated or irregular nuclei with one or more nucleoli; a well developed microvillous apical border; basilateral plasmalemmal infoldings; a slightly undulating basal surface resting on a well defined basal lamina which did not take part in the basilateral plasmalemmal infoldings. Ribosomes, mitochondria and profiles of rough endoplasmic reticulum were scattered throughout the cytoplasm. Prominent supranuclear Golgi complexes composed of tubules and vesicles were also seen.

Glycogen granules were scattered throughout the cytoplasm as well as forming clumps at the basal parts of some cells. Pinocytotic vesicles were seen arising from the basal and lateral plasma membranes as well as lying free in the cytoplasm. Part of a blood capillary is shown just beneath the choroidal epithelium. The endothelium continues to show marked local variations in thickness, and the basement membrane was ill-defined or completely absent,

compared to the well developed epithelial basal lamina. An incidental finding was a non-myelinated axon insinuated between the capillary wall and the bases of choroidal cells.

Fig. 28 shows an epiplexus cell resting on the microvillous border (including a single cilium) of a choroid epithelial cell. Its cytoplasm contained a large number of vacuoles, of varying size; numerous pinocytotic vesicles, dense bodies, mitochondria and rough endoplasmic reticulum. The cell also had many cytoplasmic processes. This cell is most probably phagocytic in nature.

4. 15 day old embryo

The choroidal epithelium showed little change from that described at 14th day p.c. (Fig. 29), with the cytoplasmic organelles generally similar in quantity and disposition.

A laminated body, like onion skin with an empty core, was rarely seen in the cytoplasm of some epithelial cells (Fig. 30). Also seen at the apical borders of the choroidal epithelium were occasional pedunculated structures, circular in sectional profile and packed with vesicles (Figs. 31 and 32). These structures might corrrespond to the apical blebs seen in the scanning electron microscope.

A choroidal blood capillary was present just beneath the epithelial cells with very sparse connective tissue elements intervening (Fig. 29). The endothelial cells were generally thinned out and occasionally fenestrated.

Compared with the well developed epithelial basal lamina that of the vascular endothelium was still poorly developed.

5. 16 day old embryo

Fig. 33 shows that the choroid epithelial cells had an appearance generally similar to that of the previous stages. They had a well developed microvillous apical border, lateral plasma membranes which were infolded near the base and joined at their apices by junctional complexes. The cells had lobated nuclei with up to four nucleoli. Mitochondria, glycogen granules and profiles of rough endoplasmic reticulum were randomly distributed throughout the cytoplasm whereas the Golgi complexes occupied a supranuclear position. Some choroid epithelial cells had conspicuously paler cytoplasm with more widely dispersed organelles. Some of them had lipid inclusions situated mainly in the basal parts of the cells. Similar cells were noticed at the 13th day p.c. (Fig. 26). Again these lipid inclusions were not seen in the other epithelial cells.

Fig. 33 also shows a blood capillary lying just beneath the epithelium with very sparse connective tissue elements intervening. The endothelial cells were now more uniformly thinned and showed extensively overlapping and interdigitating junctions with their neighbours. Flange-like cytoplasmic extensions were present on both adluminal and abluminal surfaces.

A higher power view of the capillary endothelium

earlier stages. The endothelial basement membrane was also better developed, but still less well developed than that of the adjacent choroidal epithelium.

A curious incidental finding was of part of the cytoplasm of a nucleated red blood cell apparently in the process of diapedesis through the endothelial wall (Figs. 35 and 36). Both these figures also clearly show glycogen clumps scattered in the cytoplasm of the basal part of choroid epithelial cells.

6. 17 day old embryo

Again, the structure of the choroid plexus cells was generally similar to that described at the previous stages (Fig. 37). The cells possessed lobated nuclei with one or more nucleoli. Mitochondria and rough endoplasmic reticulum were randomly distributed throughout the cytoplasm and well developed Golgi complexes consisting of tubules and vesicles occupied a supranuclear position. Glycogen was seen both as basal aggregates and widely distributed granules. The lateral plasma membranes of adjacent cells were joined apically by junctional complexes from where they ran straight and parallel to each other except near the base where they entered into interdigitations and infoldings.

The basal border of the cell was slightly undulating and rested on a well defined basal lamina.

Fig. 37 also shows another curious finding: two red blood cells lying free in the extracellular space between the base of the epithelial cells and the blood capillary.

This may represent completion of the process of diapedesis illustrated in Figs. 35 and 36.

7. 18 day old embryo

The choroidal epithelium showed little change from that described at 17th day p.c. (Fig. 38). A blood capillary was seen just beneath the epithelial cells. Its endothelial wall was uniformly thinned and occasionally fenestrated.

8. 19 day old embryo

Fig. 39 shows a blood capillary just beneath the epithelial cells with a long process of a connective tissue cell intervening. The endothelial wall was attenuated in places and occasionally fenestrated. The fenestrae were more frequent than in the previous stages.

Glycogen aggregates were seen in the basal parts of epithelial cells as noticed in previous stages (Figs. 26 and 29).

9. New born mouse - Fig. 40

The choroid epithelial cells had a well developed apical microvillous border, the microvilli being of different sizes and shapes. The lateral plasma membranes of adjacent cells were joined apically by junctional complexes and showed basilateral infoldings. The basal surfaces of the cells were slightly undulating and rested on a well developed basal lamina. Epithelial cells had irregular and lobated nuclei with up to three nucleoli. Mitochondria and glycogen granules were distributed

throughout the cytoplasm.

Some cells had conspicuously paler cytoplasm, otherwise showing little difference from other cells.

Fig. 40 also shows a blood capillary lying immediately beneath the epithelium. The endothelial wall was uniformly thin over considerable distances and was frequently fenestrated.

Choroidal capillaries

Randomly selected samples of choroidal capillaries at each stage from 13th day pc to new born and adult were examined in more detail, to determine if there were any changes in the structure of the lining endothelium.

1. 13 day old embryo

Fig. 41 shows a medium power view of a part of the wall. The endothelium was mostly quite thick, but there were scattered areas of thinning and an occasional fenestra. The junctions between adjacent endothelial cells were widely overlapping with flange-like flaps extending into the lumen. These junctions showed a substantial space between the overlapping partners, except at the site of discrete desmosomal junctions.

Pinocytotic vesicles were very sparse indeed and there was little, if any, sign of vesicular invaginations on either surface of the cells. The basement membrane was discontinuous and poorly developed.

2. 14 day old embryo

Again the endothelial cells were generally thick, with marked variations over a short distance, and thickest

in the perinuclear area where the cellular organelles were concentrated. Endothelial junctions were similar to those seen at 13th day p.c. No fenestrae were identified. Pinocytotic vesicles were much more frequently seen than at 13th day p.c. (Fig. 42).

3. 16 day old embryo

At 16th day p.c, the endothelium was generally thinner and more uniform in thickness. Some internally projecting endothelial flaps were still present, but much less frequently. Fenestrae were more frequent, and the basement membrane was better organized and becoming more continuous (Fig. 43).

4. 17 day old embryo

There was no change in the general structure of the endothelium, but fenestrae were more frequently identified within an endothelium which was more uniformly thinned (Fig. 44).

5. Adult mouse

The endothelium was characterized by a much more uniform and general thinning. Overlapping intercellular junctions were very infrequent. There were occasional fenestrations. The basement membrane remained discontinuous and poorly organized (Fig. 45).

Thickness of the endothelium

By inspection, the impression was that the endothelial lining was becoming more uniformly thinner between subsequent stages of development (Fig. 46). This

was most noticeable when capillary thickness at 13th day p.c. was compared with that in the adult. To substantiate this, five areas of greatest thickness were measured at each stage and the mean thickness calculated (Table 1). Results were then analysed by one-way analysis of variance (ANOVA) (Table 2) using the statgraphics programme on an IBM computer. This proved to be significant and therefore intergroup comparisons were performed by the least-significant difference (LSD) intervals both at 95% and 99% confidence levels. This showed a statistically signficant decrease in the maximum thickness of the endothelium between 14th and 16th day p.c. and a gradual fall thereafter (Tables 3 & 4).

Summary

1. Elaborations of plasma membrane of choroidal epithelium

At the 11th day p.c. the apical surface of the choroid epithelial cells bore relatively few microvilli and their lateral surfaces were straight and simple. From the 13th day p.c. onwards the apical surfaces of the epithelium bore numerous microvilli and the basilateral cell membranes were characterised by interdigitations. The possible significance of these two features for secretory activity of the epithelium will be returned to in the Discussion.

2. Intracellular organelles

In all stages examined, mitochondria and rough endoplasmic reticulum were distributed throughout the cytoplasm, while Golgi complexes usually occupied a supranuclear position. Although no quantitative studies

were made, the general impression was that there were no dramatic changes in the quantity and distribution of mitochondria, rough endoplasmic reticulum or Golgi complexes from 13th day p.c. to birth. Scattered glycogen granules were present in the choroid epithelial cells from the 11th day p.c. onwards, but it was only by the 13th day p.c. that they formed basally-situated aggregates.

Lipid inclusions were noticed during development; they were present only in those choroid epithelial cells with a conspicuously paler cytoplasm. The significance of this association is not clear.

3. Capillary endothelium

There was gradual thinning of the endothelial lining of choroidal capillaries during development. This was most noticeable when endothelial wall thickness at the 13th day p.c. was compared with that in the adult. Scattered fenestrae were already apparent in the endothelial capillary wall by the 13th day p.c., and they increased in frequency during prenatal development. The basement membrane remained discontinuous and poorly organised through development.

Very sparse connective tissue elements intervened between the blood capillaries and the overlying choroidal epithelium, whose relationship became more intimate as development progressed.

These structural features - a thin, fenestrated endothelium with a poorly developed basement membrane,

pinocytosis and close contact between capillaries and overlying choroidal epithelium would all be expected to facilitate the transfer of substances between the blood stream and choroid epithelial cells.

THE INTERIOR OF THE BLOOD VESSELS OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE (SEM STUDY).

The blood vessels of the choroid plexus of the IVth ventricle of both adult and new born mice were examined. The cerebral capillaries were also examined as controls. Five specimens from each stage were examined. The glutaraldehyde perfusion - fixed whole brains were embedded in wax and cut in the horizontal plane in series at 10 um on a rotary microtome. Every 5th section was examined under the microscope, until a level was reached where a cut surface of the choroid plexus of the IVth ventricle was obtained. Fig. 47 showed the cut-surface of the choroid plexus in the lateral recess of the IVth ventricle. At this stage the block was de-waxed and processed for SEM examination. Blood vessels cut in a favourable plane, i.e. opened up longitudinally, and especially those lying just beneath the choroid plexus epithelium were examined (Fig. 47 (arrow) & Fig. 48). Structures which were interpreted as holes or discontinuities were observed in the endothelial wall of the choroid plexus capillaries of both adult (Fig. 49), and new born mice (Fig. 50). They occurred in groups, were circular in outline, and measured about 0.1 um in diameter. They were completely or partially encircled by what appeared to be endothelial ridges. These holes or discontinuities in the endothelial lining of the capillaries of the choroid plexus were never seen in the cerebral capillaries. From their size and their grouping, they conformed to the fenestrae which are

familiar from TEM studies, but which have not, it is thought, been previously demonstrated in the choroid plexus by SEM.

VASCULAR CASTS OF THE CHOROID PLEXUS

The aim of this exercise was to study the nature and distribution of the developing choroid plexus capillaries and to see how the results compare with those obtained by light microscopy of semi-thin sections.

Casts were made by injecting a medium into the vascular system, and, after it had solidified, removing the surrounding soft tissue.

The injection of the medium was technically difficult due to the small size of mouse embryos (Table 5). This was reflected in the large number of attempts (Table 6). The injections were done in the order shown in the Table, i.e. new born mice were injected first, 18 day old embryos next, and so on.

Although the 15 day old embryos were of much smaller size (CR length ~ 12.2mm) than the new born mice (CR length 24mm), the success rate of injection was much higher in the former. This was presumably due to the level of experience and technical skill achieved by the time these injections were done.

Some of the successfully injected specimens were found to be unsuitable for scanning electron microscopy due either to damage of the choroid plexus cast during dissection or the presence of soft tissue debris obscuring the choroid plexus cast.

Exposure of the vascular casts of the choroid plexus was always done under water as the cast could not support its own weight. For proper exposure of the vascular cast

of the choroid plexus, blood vessels in the roof of the ventricle were either cut away and removed, or pushed to one side, so that the relationship of blood vessels to each other during life was not maintained, i.e. good exposure of the vascular cast of the choroid plexus was obtained at the expense of anatomical relationships. It was more difficult to expose the lateral part of the vascular cast of the choroid plexus of the IVth ventricle and in most cases it was damaged. Consequently, the examination was confined to that part of the choroid plexus cast which lay in the vicinity of the mid-line.

Even under the lower power of the dissecting binocular microscope, the vascular casts of the choroid plexus of the IVth ventricle of the embryonic stages and new born mice appeared different from those of the subependymal capillaries. The former appeared as a solid mass whereas the latter formed a mesh. This difference in appearance was useful in the final orientation of the "cast". The word "cast" here refers to the vascular replica of the whole brain, which was mounted on the grid in such a way that the vascular cast of the choroid plexus could be easily examined. The prepared specimens were screened by the scanning electron microscope and appropriate areas were selected for photography.

1. 15 day old embryo

This was the youngest age at which successful injections were obtained, despite 20 attempts in both 14 and 13 days embryos.

The vascular cast of the choroid plexus of the IVth ventricle showed the capillaries as broad, leaf-like structures with very irregular outlines (Figs. 51 & 52). Due to their irregular shape accurate measurements could not be made, but they were, on average, about 3-4 times the diameter of subependymal capillaries. The vasculature of the choroid plexus of the IVth ventricle at this stage was, therefore, composed of sinusoids, about 30-40 um in diameter, rather than of capillaries.

Part of the cast of a large blood vessel could be seen (Figs. 51 & 52), the main feeding vessel of the choroid plexus.

Subependymal cerebral capillaries appeared as thin, hair-like, interlacing and branching tubes about 10 um in diameter (Fig. 51).

2. 17 day old embryo

The vascular cast of the midline portion of the choroid plexus could be seen, the lateral part had been damaged during its exposure (Fig. 53). A long feeder vessel could be identified on either side of the mid-line portion of the plexus (Fig. 53) and branches arising from these feeder vessels to supply the choroid plexus could be clearly seen (Figs. 54 & 55).

The difference in size and shape between the vascular

cast of the choroid plexus and that of subependymal capillaries was very evident (Figs. 53 & 54). The vessels of the choroid plexus formed a highly tortuous dense mass of sinusoidal vessels of very irregular outline, while the casts of subependymal capillaries appeared as a fine mesh of rather straight, uniformly narrow capillaries.

Fig. 56 showed clearly the broad and irregular shape of the sinusoidal capillaries of the choroid plexus of the IVth ventricle.

3. 18 day old embryo

The difference between the vascular cast of the choroid plexus of the IVth ventricle and that of the subependymal cerebral capillaries was again obvious (Fig. 57). The subependymal cerebral capillaries appeared as thin, hair-like, interlacing and branching rods of 7 um in diameter. The choroid plexus sinusoids, on the other hand, were broad and very irregular in shape (Figs. 57 & 58). Because of this irregularity in shape satisfactory measurements of their size could not be made, but on average they were some 4-5 times the diameter of the subependymal capillaries.

Globular masses were present on the surface of the cast of some choroid plexus capillaries; they measured up to 5 um in diameter (Figs. 57, 58 & 59). These might represent soft tissue debris not adequately removed during the preparation of the cast. They might also represent resin which had passed through small ruptures in the vessel

wall.

Surprisingly, the vascular cast of the choroid plexus of the lateral ventricle was very different from that of the IVth ventricle (Fig. 60). Although the capillaries of the lateral ventricle choroid plexus (measuring 10 um in diameter) were still wider than the subependymal cerebral capillaries, they were very much narrower than those of the IVth ventricle choroid plexus and were not sinusoidal in nature. The criteria used in this comparison were both the size and shape of the capillaries. The choroid plexus of the lateral ventricle showed a characteristic vascular pattern, i.e. large vessels running in the long axis of the plexus and opening into a marginal vessel, all of them being linked by a large number of capillaries which also interconnected with each other forming a dense capillary meshwork. This was in contrast to the vascular arrangement of the choroid plexus of the IVth ventricle where branches from the feeder vessels seemed to expand rapidly into broad irregular sheets.

There were some breaks in the continuity of the capillary network (Fig. 60) which might be due either to imcomplete filling of the capillaries or to mechanical damage during preparation.

4. New born mice

The vascular cast of the choroid plexus of the IVth ventricle showed that its sinusoidal capillaries were similar to those described for earlier stages. They were wide, tortuous and very irregular in shape (Figs. 61, 62 &

63). One of the sinusoidal capillaries, which was almost discoid in shape (Fig. 61 - white arrow), measured about 100 um at its widest diameter. In the mid-line portion of the choroid plexus the capillaries were so crowded together that the feeder vessels and their branches lying behind them could not be seen (Figs. 61 & 62). More laterally, however, a feeder vessel could clearly be seen giving branches supplying the plexus (Fig. 61 - big arrow). Some of these branches entered into the sinusoidal capillaries of the plexus after a very short course (Fig. 61 - small arrows).

Some of the sinusoidal capillaries were bent on themselves to form U-shaped patterns (Figs. 62 & 63). This appearance was not very different from that of the earlier stages.

Subependymal capillaries were thin, hair-like, interlacing and branching rods measuring around 8 um in diameter and were obviously different from the sinusoidal capillaries of the choroid plexus of the IVth ventricle (Figs. 61, 62 63).

The vascular cast of the choroid plexus of the lateral ventricle continued to be very different from that of the IVth ventricle. Its vessels were of capillary dimensions and form, and were not sinusoidal. The vascular cast of the lateral ventricle choroid plexus again showed a characteristic pattern, i.e. large vessels running along the long axis of the plexus, joining up with a large

marginal vessel, all of these were connected together by a dense network of capillaries (Fig. 64). The surface of the cast of the larger vessels was studded with a large number of globular masses about 5 um in dimater. They were not very different from those seen on the cast of the choroid plexus capillaries of the IVth ventricle of 18 day old embryos. These globular masses were not found on the cast of choroid plexus capillaries or that of cerebral capillaries (Figs. 64 & 65).

5. Adult mouse

The vascular cast of the choroid plexus of the IVth ventricle showed that the vessels were now 6-10 um in diameter, much smaller than those of the new born and embryonic stages. Although satisfactory measurements could not be made on the sinusoidal vessels of the choroid plexus of the IVth ventricle in the new born and embryonic stages due to their very irregular shape, it was still obvious that the adult choroid plexus capillaries were of much smaller diameter. However, they were still wider than the subependymal cerebral capillaries.

The capillaries of the choroid plexus of the IVth ventricle branched and anastomosed with each other to form a dense network, but no characteristic pattern was observed (Figs. 66 & 67).

The branches supplying the capillary network of the choroid plexus could be seen arising from a main feeder vessel (Fig. 67 - arrows). These branches after running for a short distance broke up into capillaries.

The vascular cast of the choroid plexus of the lateral ventricle showed a very rich capillary network stretching between large vessels running along the long axis of the plexus and marginal vessels, thus maintaining the same characteristic vascular pattern seen in the new born and the embryonic stages. The choroid plexus capillaries were still wider than the subependymal cerebral capillaries (Fig. 68).

LIGHT MICROSCOPY OF SEMI-THIN SECTIONS OF THE DEVELOPING CHOROID PLEXUS OF IVTH VENTRICLE

Now that the constituent elements of the choroid plexus - the choroidal epithelium and the associated blood vessels - have been examined in detail by SEM and TEM, we can proceed to the next stage of the study, a qualitative description of the changing relationship between the choroidal epithelium, blood vessels and associated connective tissue as seen by light microscopy of semithin sections. This material was also used for several quantitative assessments.

The plexus was studied in serial 1 um sections of the heads of embryos of 11, 13, 14, 15, 16, 17, 18 and 19 days post coitum, and of new-born mice. All were cut in the coronal plane, except the 11 day embryos which were cut in the sagittal plane. To maintain comparability only the central part of the choroid plexus was studied at each stage, as different parts of the choroid plexus developed at different times; at each stage development of the central part of the plexus was more advanced than that of the lateral parts.

In describing the general histological features of the choroid plexus, particular attention was paid to the choroidal epithelium and especially to features highlighted by Sturrock (1979) in his study of the development of the choroid plexus of the lateral ventricle in the same species. The following characteristics will be described:

1. The general characters of the epithelium.
2. Nuclear shape and position.
3. Height of epithelium.
4. Cytoplasmic characteristics.
5. Presence or absence of "dark" cells.
6. Evidence of pyknotic nuclei.
7. Incidence of mitosis.

A. Qualitative Findings

1. 11 day embryo:

Fig. 69 is a parasagittal section of the head of the embryo. It shows the thin ependymal roof of the IVth ventricle. The site of the future choroid plexus was recognizable even at low magnification, because of the greater vascularity of the tela choroidea. Wide, thin-walled capillaries lay in close association with the presumptive choroid plexus epithelium, which consisted of a single layer of low cuboidal cells (Fig. 70). There was no definite invagination of the choroid plexus epithelium into the cavity of the IVth ventricle by the tela choroidea, in agreement with the scanning electron microscopic findings at the same stage.

2. 13 day embryo: (Fig. 71)

The choroid plexus had grown considerably in size and complexity. It was invaginated into the cavity of the IVth ventricle and had formed many folds, in conformity with the scanning electron microscopic appearance (cf. Fig. 5).

The choroidal epithelium was formed by a single layer

of low columnar cells, with centrally or apically placed nuclei. Most of the nuclei were oblong in shape and occupied most of the cytoplasm. In some places the epithelium appeared to be pseudostratified, but this appearance was most probably due to the plane of section passing tangentially through a fold. The stroma of the choroid plexus contained abundant connective tissue cells and fibres, and wide, thin-walled blood capillaries were seen lying beneath the epithelium, but separated from it by some connective tissue elements.

3. 14 day embryo: (Fig. 72)

The folds of the choroid plexus were more pronounced and better defined. The choroidal epithelium consisted of a single layer of low columnar cells, whose nuclei were located centrally or apically, and contained one or more nucleoli. Some of the nuclei were now circular in sectional profile but some were still oblong.

"Light" and "dark" cells:

In view of Sturrock's (1979) finding of the presence of "light" and "dark" cells in the developing choroid plexus of the lateral ventricle in mice, particular attention was paid to the staining characteristics of the choroidal epithelium of the IVth ventricle. The whole extent of the epithelium was first examined cursorily and then a sample of about 200 cells was examined more critically at each stage.

At 14 days post coitum, the nuclei of some choroidal

epithelial cells were more darkly stained than others (see Fig. 72, arrows) but there was not comparably darker staining of the cytoplasm. These cells were not therefore regarded as comparable to the "dark" cells described by Sturrock (1979), nor were "dark" cells seen at any of the subsequent stages. This problem will be returned to in the Discussion.

Glycogen

Vacuoles containing metachromatically stained material were seen in the basal part of some cells. These represented the site of large aggregates of glycogen identifiable by the transmission electron microscope (see Fig. 26).

Pyknosis

Evidence of pyknotic degeneration of epithelial cells was not seen at this, or subsequent, stages although this was very carefully looked for, especially at the sites of folding of the epithelium.

Blood vessels

Compared to those of the 13 day embryos, the choroid blood capillaries were generally wider, although they were very irregular in diameter. They were more closely applied to the base of the choroid epithelial cells with sparse connective tissue elements intervening, so that in places the epithelial cells seemed to rest directly on the capillary endothelium.

4. 15 day embryo: (Fig. 73)

There was further increase in the size of the choroid plexus and its folds became more complex. The choroidal epithelium was still formed by a single layer of low columnar cells with centrally or apically placed nuclei. The general structural features were little changed from those at 14 days.

The arrow on Fig. 73 identifies an epiplexus cell lying on the apical surface of the choroid plexus. Such cells have already been identified by the transmission electron microscope (see Fig. 28), and by the scanning electron microscope (see Fig. 5).

5. 16 day embryo: (Fig. 74)

Apart from a continuing increase in total size, the choroid plexus was similar to that at 15th day p.c. Although most of the epithelium was formed by low columnar cells with rounded nuclei, some cells, especially those nearer the root of the plexus, were tall columnar with oblong nuclei which were more darkly stained. Basal vacuoles containing metachromatically staining material was seen in some cells. They represent glycogen aggregates. Similar features were also seen in the subsequent stages. Note the presence of two epiplexus cells (arrow).

6. 17 day embryo: (Fig. 75)

The general features of the choroid plexus were little changed from those at 16 days.

Compared to the pale "potato-like" cell nuclei at the apex of the plexus, those at the root of the plexus, i.e.

adjacent to the ependyma, were mainly oblong, more darkly stained, and resemble the adjacent undifferentiated ependymal cells. The choroid blood capillaries were wide, irregularly shaped in sectional profile, and closely applied to the epithelium.

7. 18 day embryo: (Fig. 76)

The structural features of the choroid plexus were similar to those at 17th day p.c.

8. 19 day embryo: (Fig. 77)

The structural features of the choroid plexus were little changed from those at 18th day p.c. A single layer of low columnar cells formed the epithelium. The epithelial cells at the root of the plexus were smaller, more basophilic and less differentiated than those at the apex of the plexus. A few cells were seen with lighter stained cytoplasm (arrow). These probably corresponded to those already described with TEM (Figs. 26 and 29).

9. New-born mouse: (Fig. 78)

The structure of the choroid plexus was unchanged compared to that at the 19th day p.c. Note cells with lighter stained cytoplasm (arrow).

B. Quantitative findings

1. Incidence of mitoses

In view of previous conflicting reports on the incidence of mitoses in the choroid plexus epithelium, this was quantified at all stages by counting the number of

dividing cells in a total of 500 - 900 choroid epithelial cells. Similar counts were made of mitoses in the epenedyma of the roof of the IVth ventricle on either side of the choroid plexus. The results are given in Tables 7 and 8.

Three points emerge from this:-

i) low incidence of mitoses in the choroidal epithelium

ii) much higher incidence of mitoses in the ependyma

iii) in the choroidal epithelium mitoses were found only in the root of the plexus, i.e. adjacent to undifferentiated ependyma.

The implication of these findings for the mode of growth of the choroid plexus will be returned to in the Discussion.

2. Height of choroid epithelial cells

Because the nuclei appeared to occupy a smaller proportion of the total cell volume at 14th day p.c. than at 13th day p.c., it was decided to measure the height of the epithelium at these and subsequent stages. Measurements were made on photographic montages at a constant magnification of x 1000. Measurements were confined to cells with clearly defined apical and basal surfaces, and areas of pseudostratification were avoided. The height of 20 cells, selected at random, was measured at each stage. The mean cell height and the standard deviation (SD) was then calculated (Table 9). The results were analysed by one-way analysis of variance (ANOVA) (Table 10), using the statgraphics programme on an IBM computer. This proved significant, consequently intergroup

comparisons were then performed by the least-significant difference (LSD) intervals both at 95% and 99% confidence levels.

Although the choroid epithelial cells were still low columnar in shape, their mean height decreased significantly (by more than a third) between 13th and 14th day p.c. The decrease in the mean height of epithelial cells between the 14th and 15th day p.c., and between subsequent stages was not statistically significant (Tables 11 & 12).

3. Epiplexus cells:

Epiplexus cells were enumerated in 13, 14, 16, 18 day embryos and new-born mice. A total of 20 1um coronal sections, from three animals at each stage (only 2 newborns), showing the midline part of the plexus were examined and the total number of epiplexus cells was noted. The outline of the invaginated choroidal epithelium was then drawn using the camera lucida at a constant magnification of 200x. The length of the epithelium was then measured from these drawings by the MOP-AM_{02} machine, and the total length of the epithelium at each stage was then calculated. The number of epiplexus cells per unit length was then calculated (Table 13). There was a three fold increase in the number of epiplexus cells between 13th and 14th day p.c. It is worth mentioning that large numbers of macrophages first appeared in the subarachnoid space at the 14th day p.c. (see Development of the

Subarachnoid Space). There might be a relationship between the two events.

The number of epiplexus cells decreased steadily from the 14th day p.c. to the new born stage. Note that the number was 10 times greater in the 14th day p.c. than that in the newborn.

MORPHOMETRY OF THE CHOROID PLEXUS OF THE IVTH VENTRICLE

The choroid plexus is made up of three components, i.e. epithelium, blood vessels and connective tissue. In order to determine if any changes occurred in the relative contribution of each of these components during development, morphometric analysis was carried out.

Measurements were done with the Reichert-Jung-AMO_2 MOP machine on montages at a magnification of 1000x. At each stage the relative cross-sectional areas of the choroid plexus and its components were measured (Tables 14, 15, 16 & 17). From these measurements the volume density of each component (volume per unit volume) was calculated (Tables 18, 19 & 20).

The results were analysed by one-way analysis of variance (ANOVA) using the Statgraphics programme on an IBM computer. Where these proved significant, intergroup comparisons were then performed by the Least Significant Difference (LSD) intervals both at 95% and 99% confidence levels.

i) Volume density of connective tissue

The means table (Table 21) shows that the volume density of connective tissue increases from 13th to 15th day p.c. where it reaches its highest level and then decreases progressively to newborn.

Analysis of variance (Table 22) shows that it is significant.

$$(F = 5.14;\ df = 7 \times 16;\ p < 0.01)$$

Intergroup comparisons (Tables 23 & 24 and Graphs 1 & 2)

show that:

a) Value at 15th day pc is significantly higher than those at all other stages except 16th day pc ($p < 0.01$).

b) Value at 16th day pc is higher than that at 13th day pc and newborn ($p < 0.05$).

c) The results are made difficult to interpret by what appears to be an anomalously high value for volume density of connective tissue at 15th day pc: the figure is significantly higher than that at any other stage. The reason for this is not clear; the only other significant changes are an increase between 13th day pc and 16th day pc, and a decrease between 16th day pc and newborn.

ii) Volume density of epithelium

Table of means (Table 25) shows that the volume density of epithelium decreased between 13th and 15th day pc from where it increases gradually to newborn.

Analysis of variance was performed (Table 26) and was found to be significant.

($F = 5.26$; $df = 7 \times 16$; $p < 0.01$)

Intergroup comparisons (Tables 27 & 28 and Graphs 3 & 4) reveal that:

a) At 15th day pc the volume density of epithelium is significantly lower than those at all other stages ($p < 0.01$).

b) The value at 13th day pc is higher than at 19th day pc ($p < 0.05$).

c) Again the figure at 15th day pc is anomalous. The only other significant finding is that the value at 13th day pc is higher than at any of the other stages. In interpreting this, it should be pointed out that, at 13th day pc, much of the more laterally placed choroidal epithelium has not yet been invaginated by the underlying connective tissue and blood vessels. Because of the standardised method of measurements adopted, no connective tissue or blood vessels was "credited" to these lateral parts of the plexus: the epithelium component was therefore (artificially) exaggerated.

iii) Volume density of blood vessels

The table of means (Table 29) shows that the volume density of blood vessels increased between 13th and 15th day pc, and thereafter fluctuates.

Analysis of variance was performed (Table 30) and found to be significant:

$$(F = 4.75;\ df = 7 \times 16;\ p < 0.01)$$

Intergroup comparisons (Tables 31 & 32 and Graphs 5 & 6) reveal that:

a) Apart from 18th day pc, the volume density of blood vessels at 15th day pc is significantly higher than that at all other stages ($p < 0.05$).

b) Value at 13th day pc is significantly lower than that at 18th day pc ($p < 0.01$).

c) Changes in the volume density of blood vessels do not show any particular pattern.

Conclusion

The volume densities of the different components of the choroid plexus (epithelium, blood vessels, connective tissue) did not show any progressive significant changes between 13th day pc and newborn.

DEVELOPMENT OF FORAMINA OF THE IVTH VENTRICLE

The brains of pre-natal (13, 14, 15, 16, 17, 18 and 19 day pc) and new born mice were examined. Three specimens from each stage were used. 1 um thick plastic serial sections were cut in the coronal plane. Every fourth section was mounted on a slide.

A. Lateral Foramina

Until the 16th day p.c. the lateral recesses of the IVth ventricle were covered by a single layer of cuboidal ependymal cells. On the 17th day p.c. however, part of the wall of the lateral recess started to become thinner in an area caudal to the point of invagination of the ependymal lining of the lateral recess to form the choroid plexus (Fig. 79). This thinning area was always covered with pia-arachnoidal cells and their processes. No foramen was seen.

By the 18th day p.c. the ependymal epithelium became even more attenuated (Fig. 80), but still no foramen could be seen.

By the 19th day p.c. the attenuated area in the ependymal lining of the lateral recess became extremely thin, but still no definite foramen could be seen (Fig. 81). It was always covered with pia-arachnoidal cells and their processes.

In the new-born animals the attenuated area in the wall of the lateral recesses of the IVth ventricle had disappeared and was replaced on each side by a foramen

(Figs. 82, 83, 84 & 85). The foramen was bridged by pia-arachnoidal cells and their processes and was situated caudal to the point of invagination of the choroid plexus into the lateral recess. The foramina correspond to the attenuated areas seen in the prenatal stages. In one animal tufts of choroid plexus were seen protruding through the foramen (Fig. 84) but still covered by pia-arachnoidal cells and their processes.

B. Midline Foramina

In view of the finding of interependymal pores in the caudal part of the roof of the IVth ventricle of rat and pig (Jones, 1980), and of mouse, rat, pig and sheep (Jones, 1987), this area was carefully examined in the 13, 14, 15 and 16 day embryos and newborn mice for the presence of holes.

Coronal, semithin serial sections of the hind brain showing the caudal part of the roof of the IVth ventricle were meticulously examined with the light microscope and double-checked under oil immersion, for the presence of discontinuities in the ependymal roof. The posterior tela was examined over its whole extent, starting rostrally at the caudal end of the choroid plexus. From one embryo at each stage, three representative sections, at intervals of approximately 20 um, were photographed at a magnification of 1000x and a montage constructed.

1. 13 day embryo

Montages from one of the embryos are shown in Figs. 86, 87 & 88. Each shows the roof of the IVth ventricle

from its attachment to the hindbrain, up to the midline. It was collapsed ventrally into the cavity of the ventricle and was formed by a single layer of extremely thinned ependymal cells in which no discontinuities were seen. The overlying pial layer, on the other hand, consisted of a discontinous layer of flattened cells, indicated by arrows in Figs. 87 & 88.

2. 14 day embryo

The three photographic montages prepared from one of the embryos are shown as Figs. 89, 90 & 91. Each shows one half of the ependymal roof which was convex ventrally, and consisted of a single layer of highly attenuated ependymal cells in which, again, no discontinuities were found. By contrast, the overlying pia consisted of widely scattered, very flattened cells, which in none of the sections formed a complete layer, and which were always clearly distinguishable from the ependymal layer.

There was no evidence of interpolation of the pial cells into the ependyma.

Pial cells were identified by arrows in Figs. 90 & 91.

3. 15 day embryo

The three montages prepared from one of the embryos are shown in Figs. 92, 93 & 94. The roof of the ventricle was formed by a single layer of ependymal cells which could be traced, without any break in continuity, from the hind brain to the caudal end of the midline part of the choroid

plexus. In places, the cytoplasm of the ependymal cells was extremely attenuated, so that it appeared only as a thin membrane, but no discontinuities, resolvable at a magnification of 1000x, were found. By contrast, the pia consisted of a single discontinuous layer of flattened cells, in places very close to, but always separate from, the ependymal layer (arrows).

The three montages extended over a cephalo-caudal extent of about 100 um and were representative of appearances throughout the serial sections.

Some macrophages were present in the subarachnoid space, some between pia and ependyma, and some close to the ventricular surface of the ependyma.

4. 16 day embryo

Montages from one of the embryos are shown in Figs. 95, 96 & 97. The sections illustrated in Figs. 95 & 97 were separated by about 65 um. The ependymal roof was still clearly seen as a continuous layer of highly attenuated cells. Closely applied to it was an even thinner but discontinuous pial layer. It should be emphasized that it is very difficult to differentiate the nuclei of pial and ependymal cells on morphological grounds; it is only by tracing the two types of cells as separate layers that they can be distinguished.

An interesting feature was the presence of numerous macrophages between the pia and the ependyma, serving to emphasize the separate character of pia and ependyma (Fig. 96).

What looked like a precipitated proteinous material was seen inside the cavity of the IVth ventricle adjacent to the thin ependymal roof (Figs. 98 & 99 colour print). No such material was present in the subarachnoid space. The appearances suggested that the thin ependymal roof of the IVth ventricle was preventing the spread of this material from the ventricular cavity into the subarachnoid space.

5. Newborn mouse

Three montages prepared from three representative sections from one newborn mouse are shown in Figs. 100, 101 & 102.

The ependymal layer was again a continuous one, although in some areas it was extremely attenuated, with extensive stretches in which nuclei were not included in the plane of section. A clump of ependymal cells which were not flattened, but showed characteristics of choroid plexus cells, was seen (Figs. 101 & 102). It is noteworthy for two reasons: the first, that it represented an unusual development of choroid plexus cells away from the midline, and the second, that the clump bulged dorsally towards the subarachnoid space, lifting up the pial layer, which elsewhere ran closely parallel to the ependyma.

As in all the earlier stages there were substantial gaps in the pial layer, but none were seen in the ependymal layer. Nor was there any evidence of intercalation of pial cells into the ependymal layer.

DEVELOPMENT OF THE SUBARACHNOID SPACE

Mouse embryos aged 11, 13, 14, 15, 17 days and new born mice were used. The head was embedded in plastic and sectioned at 1 um, the 11 day embryo in the sagittal plane, and all the others in the coronal plane. The study was concentrated on the area around the hind brain, and the cerebellum. In addition the subarachnoid space at the anterior aspect of the hind-brain and lateral aspect of the cerebellum in the new born mouse was studied by scanning electron microscopy.

1. 11 day embryo - Figs. 69 & 70.

At the 11th day p.c. a loose, cellular vascular mesenchyme was interposed between the neuroepithelium and the epidermis, represented by a simple squamous epithelium. The mesenchymal cells were large - with large spindle shaped nuclei and long slender cytoplasmic processes. They were arranged in laminae parallel to the squamous layer of ectoderm and to the surface of the neuroepithelium. Abundant blood vessels were seen on the surface of the neuroepithelium. No subarachnoid space was seen at this stage.

2. 13 day old embryo

Fig. 103 shows a great increase in the thickness of the mesenchyme over the roof plate of the IVth ventricle. This is due to the plane of sectioning. The mesenchymal cells became widely spaced as compared to those at 11th day p.c. They were spindle-shaped with fusiform nuclei and

and long slender cytoplasmic processes (Fig. 104). In the outer part of the mesenchyme, a compact layer of condensed mesenchyme, 4-7 cells thick, formed the anlage of the future skull and dura mater and marked the outer limit of the future subarachnoid space. In the loose mesenchyme the cells had lost their laminar arrangement and formed a wide-meshed reticulum, the interstices of which were filled by palely stained intercellular matrix. No definite subarachnoid spaces were present.

The mesenchyme immediately adjacent to the neuroepithelium contained abundant small capillaries, anticipating the future appearance of the pial plexus.

3. 14 day old embryo

Fig. 105 illustrates a coronal section of the hind brain, in continuity with a longitudinal section of the cervical spinal cord.

Many structural changes had occurred between 13th and 14th day p.c. Chondrification of the skull ventrolateral to the hind brain was established. Constituent parts of the subarachnoid space were now well established, as intercommunicating loculi bounded by mesenchymal condensations, one or two cells in thickness, which could be traced into continuity with the pia mater covering the hind brain, and with the future arachnoid in contact with the skull and the dura. These continuities are shown at X, X^1 in Fig. 105, and at higher power in Fig. 106.

Fig. 107 shows the subarachnoid space on the ventral surface of the hind brain, and Fig. 108 another loculus of

the space dorso-lateral to the hind brain. In both these situations strands of mesenchymal cells form a very loose network, linking pia and arachnoid across the space. A large space, largely free of mesenchymal remnants, was present in relation to the cranial end of the cervical spinal cord (Fig. 109). It was not possible to decide whether any of these spaces were filled with fluid or with a well hydrated jelly.

It is important to emphasize that, on the dorsal aspect of the hind brain adjacent to the thin roof of the IVth ventricle, no definite spaces were yet present, although the mesenchyme existed as a very loose reticulum (Fig. 110).

A conspicuous new feature, not seen at earlier stages, was the presence of a large number of large round cells with vacuolated cytoplasm lying in the subarachnoid space (Fig. 111). Some had large, rounded or ovoid nuclei with one or more nucleoli; others had bilobed nuclei. These large mononuclear cells are interpreted as macrophages.

4. 15 day old embryo

Fig. 112 shows a coronal section through the hind brain and cerebellum. The subarachnoid space has extended medially into the mesenchyme separating these two structures.

Fig. 113 is a high power montage from Fig. 112 and shows further detail of the subarachnoid space. On the

dorsolateral aspect of the hind brain the subarachnoid space was traversed by strands of mesenchymal cells and their processes, linking the pia and arachnoid across the space. Abundant small capillaries, on the surface of the hind brain and cerebellum form the pial plexus. Blood vessels were also seen running through the space.

5. 17 day old embryo

By this stage further extension of the subarachnoid space had occurred, so that it virtually surrounded the hind brain. In the most recently formed areas of the subarachnoid space, dorsal to the hind brain, remnants of the mesenchymal reticulum still persisted (Fig. 114).

6. New-born mouse

Fig. 115 shows a coronal section through the hind brain and cerebellum. Endochondral ossification of the skull is advanced. A definite subarachnoid space was present all round the hind brain. It was almost completely free of mesenchymal reticulum apart from a few cellular strands on the dorsal and ventral aspect of the hind brain. Large nerves and vessels were seen coursing through the space.

The interval between the cerebellum and the skull was still packed with loose vascular mesenchyme (Fig. 116). Development of the subarachnoid space had not yet begun here. An earlier stage in the development of the subarachnoid space in the area between the cerebellum and the skull is illustrated dramatically in the low and higher power scanning electron micrographs (Figs. 117 & 118).

These show flattened sail-like bodies of the mesenchymal cells, interlinked by slender cytoplasmic extensions into a 3-dimensional reticulum. Sectional profiles of blood vessels are particularly numerous in the future pial layer, but some are also seen traversing the mesenchymal network.

Fig. 119 shows, en face, a scanning electron microscopic view of the cerebral aspect of the subarachnoid space on the ventral aspect of the hind brain. The cerebral surface is covered by what, at first sight, appears to be a continuous flattened epithelial layer. Close inspection shows its true nature: a sheet of flattened pial cells, of mesenchymal origin, between which are small discontinuities. From this surface layer a slender branching network of pia-arachnoid trabeculae is seen. Lying on the surface of the pia is a large thin-walled blood vessel, a small branch, or tributary, of which penetrates the pia to enter the brain substance.

ARACHNOID VILLI

a) In the mouse

Since in some other species, the arachnoid villi were frequent in the confluence of sinuses (Jayatilaka, 1964), this particular area was carefully examined for the presence of arachnoid villi.

Five adult and five newborn mice were used. A block of tissue containing the confluence of sinuses was examined in 2um serial, coronal sections. The entire decalcified heads (snout removed) of two further adult mice were examined by 2 um coronal sections for the presence of arachnoid villi in association with the superior sagittal sinus, the confluence of sinuses, the transverse sinuses and the cavernous sinuses.

No arachnoid villi were found in any of the adult (Figs. 120 & 121) and newborn mice (Figs. 122 & 123).

The dura mater surrounding the superior sagittal sinus (i.e. the supporting dura) appeared thin, and loosely arranged, with its collagen fibres randomly orientated This was in contrast to the periosteal dura mater which appeared thick and compact, with its collagen fibres running parallel to each other. The functional significance of this very loosely organized dural connective tissue in the presumed return of cerebrospinal fluid to the superior sagittal sinus, in the complete absence of any specialized arachnoid villi, will be returned to in the Discussion.

b) New born Guinea pig

Three newborn guinea pigs were used. 5 um wax sections of the confluence of sinuses, cut in the coronal plane were examined. Arachnoid villi were consistently found. They were 3-5 in number, and consisted of a mass of arachnoidal cells which invaginated through the dura mater into the lumen of the venous sinus (Fig. 124). The arachnoid villi were covered by a layer of endothelial cells lining the venous sinus. The supporting dura mater surrounding the venous sinus was thick and compact, quite unlike that in the mouse.

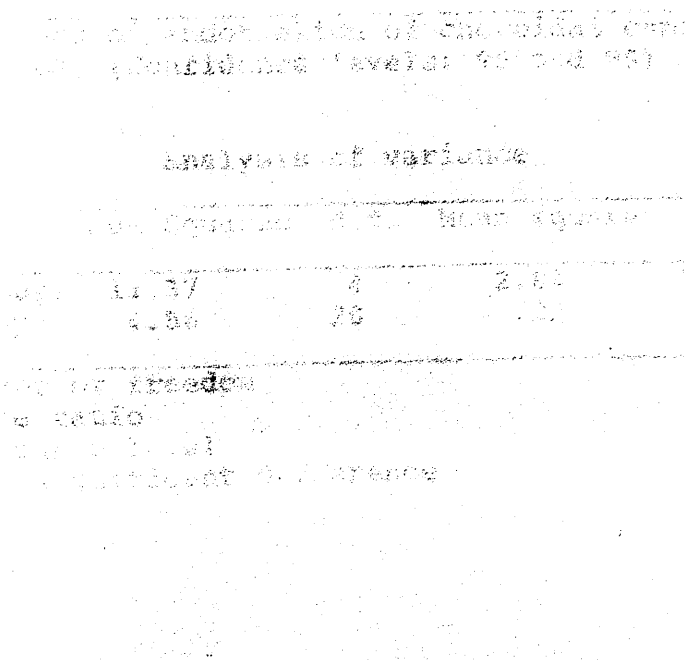


Table 1

Shows maximum thickness of the endothelial lining of choroid plexus capillaries at different ages.

Age	Thickness of endothelial lining (um)	Mean thickness (ums)
13th day pc	2.6, 2.5, 2.1, 2.0, 1.4	2.1 ± 0.47
14th day pc	3.0, 2.6, 2.6, 1.4, 1.2	2.1 ± 0.80
16th day pc	1.3, 0.9, 0.9, 0.8, 0.7	0.9 ± 0.22
17th day pc	1.3, 0.9, 0.5, 0.5, 0.4	0.7 ± 0.37
Adult	1.1, 0.7, 0.7, 0.5, 0.4	0.6 ± 0.26

Table 2

Statistical analyses of changes in thickness of endothelium of the choroid plexus capillaries at different ages.

One-Way Analysis of Variance

Data: Thickness of endothelium of choroidal capillaries
Range test: LSD (Confidence levels: 95 and 99)

Analysis of variance

Source	Sum Squares	d.f.	Mean square	F	P
Between groups	11.37	4	2.84	12.46	.0001
Within groups	4.56	20	.22		

d.f. = degree of freedom
F = variance ratio
P = significance level
LSD = least significant difference

Table 3

Multiple range analysis for thickness of endothelium.

Method: 95 Percent Confidence Intervals Age	Mean	Homogeneous Groups
13th day pc	2.16	*
14th day pc	2.12	*
16th day pc	.92	*
17th day pc	.72	*
Adult	.68	*

Table 4

Multiple range analysis for thickness of endothelium.

Method: 99 Percent Confidence Intervals Age	Mean	Homogeneous Groups
13th day pc	2.16	*
14th day pc	2.12	*
16th day pc	.92	**
17th day pc	.72	**
Adult	.68	*

Asterisks at different ages which share the same vertical column indicate no significant change, while those lying in different vertical columns indicate a statistically significant change.

Table 5

Crown-Rump (C-R) length of mouse embryos at different ages.

Prenatal age in days	11	13	14	15	16	17	18	19	New born
Average C-R length in mm.	5.5	9.2	10.4	12.3	14.9	17.5	19.6	22.8	24.8

Two litters were used at each stage

Tables 6

VASCULAR CASTS OF THE CHOROID PLEXUS

Age	No. of attempted injections	No. of successful injections	No. suitable for SEM	No. not suitable for SEM
Newborn	23	7	5	2
18th day pc	20	8	5	3
17th day pc	22	10	8	2
15th day pc	20	11	5	6
14th day pc	20	-	-	-
13th day pc	20	-	-	-
Adult	10	8	6	2

Table 7

Shows the mitotic index in the choroidal epithelium at different ages.

Age	No. of epithelial cells examined	Total No. of mitoses	No. of mitoses per 100 cells
13th day pc	500	2	0.4
14th day pc	600	2	0.33
16th day pc	728	1	0.14
17th day pc	927	3	0.32
18th day pc	950	1	0.11
19th day pc	900	3	0.33

Table 8

Shows the mitotic index in the ependymal roof of the IVth ventricle at different ages.

Age	No. of ependymal cells examined	Total No. of mitoses	No. of mitoses per 100 cells
13th day pc	520	24	4.4
14th day pc	535	28	5.2
16th day pc	500	12	2.4
17th day pc	520	6	1.1
18th day pc	521	2	0.38
19th day pc	545	3	0.55

Table 9

Shows height of choroid epithelial cells at different ages.

Age	Measured height of epithelial cells in ums	Mean height and standard deviation (SD)
13th day pc	20,22,17,15,18,21,15,20,18,18 20,23,19,18,20,18,15,20,16,18	18.5 ± 2.2
14th day pc	13,12,15,12,14,11, 8,15,10,10 10,15,10,10,16,10,13,13,11,12	12.0 ± 2.2
15th day pc	14,11,16,11, 8,10,13, 8,12, 9 10,15, 8,10,12,10, 9,11,14,13	11.2 ± 2.3
16th day pc	14,12,10,10, 7,11, 7, 8, 9,12 9,12,10,10, 8,13,11,10, 9,10	10.1 ± 1.8
17th day pc	17, 9,11, 7,10, 9, 7, 9, 8,10 7,10, 8,11, 9,10,10, 7,13, 7	9.4 ± 2.4
18th day pc	13,12,12, 8, 8,11, 7,10, 7, 9 11, 8, 8,12, 8, 8,14,10,11,10	9.8 ± 2.0
19th day pc	12, 7,13,10, 8, 8,10, 7, 6, 7 6,10,11, 7, 8,10,11, 6, 9, 8	8.7 ± 2.0

Table 10

Statistical analyses of changes in height of epithelium of the choroid plexus of the IVth ventricle at different ages.

One-Way Analysis of Variance

Data: Height of choroid epithelium.
Range test: LSD (Confidence levels: 95 and 99)

Analysis of variance

Source	Sum Squares	d.f.	Mean square	F	P
Between groups	1334.14	6	222.35	46.23	.0001
Within groups	639.65	133	4.80		

d.f. = degree of freedom
F = variance ratio
P = significance level
LSD = least significant difference

Table 11

Multiple range analysis for height of epithelium.

Method: 95 Percent Confidence Intervals Age	Mean	Homogeneous Groups
13th day pc	18.55	*
14th day pc	12.00	*
15th day pc	11.20	**
16th day pc	10.10	***
17th day pc	9.45	**
18th day pc	9.85	**
19th day pc	8.70	*

Table 12

Multiple range analysis for thickness of endothelium.

Method: 99 Percent Confidence Intervals Age	Mean	Homogeneous Groups
13th day pc	18.55	*
14th day pc	12.00	*
15th day pc	11.20	**
16th day pc	10.10	**
17th day pc	9.45	**
18th day pc	9.85	**
19th day pc	8.70	*

Asterisks at different ages which share the same vertical column indicate no significant change, while those lying in different vertical columns indicate a statistically significant change.

Table 13

Shows the number of epiplexus cells at different ages.

Age	Total length of epithelium in ums.	Total number of epiplexus cells	No. of epiplexus cells per unit length (No. per 100ums)
13th day pc	1082.3	7	0.6
14th day pc	1376.6	31	2
16th day pc	2565.7	21	0.8
18th day pc	6067.1	19	0.3
New-born	6364	19	0.2

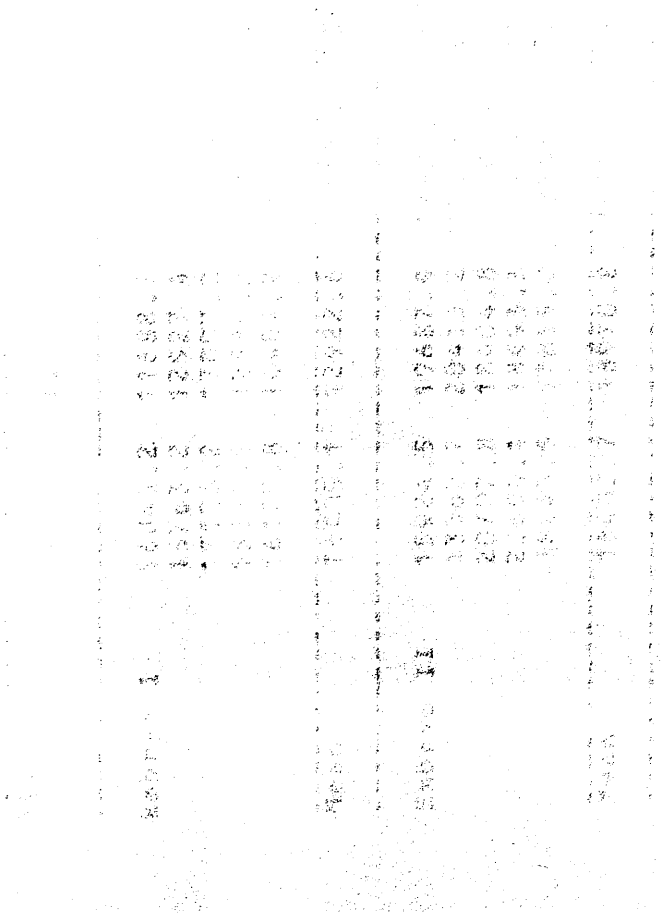


Table 14

Relative cross-sectional area of the choroid plexus of the IVth ventricle at different ages.

Age	DAYS PC							
	13	14	15	16	17	18	19	NB
Embryo I	16049.2	11688.3	16928.9	32868.8	45617.3	43297.6	39712.8	49983.6
	15763.2	12927.4	17535.1	37863.8	42815.0	48198.4	39394.5	53974.1
	15123.2	13007.2	17988.4	35278.1	41662.8	48404.0	44317.9	54871.8
	15512.1	12586.2	17904.6	36459.8	43291.1	49092.0	44489.1	57398.6
	16127.8	14404.2	18071.6	37264.3	44029.6	38174.4	45587.4	55200.4
Mean	16075.1	12922.6	17685.7	35946.9	43483.1	45433.2	42700.3	54285.6
Embryo II	15924.5	19687.9	17804.6	23041.9	48008.3	48504.4	51139.7	60318.9
	13362.7	20413.2	19081.6	22164.9	53880.0	57091.4	51330.2	60586.4
	20797.8	18404.8	19604.8	20977.8	54866.5	49822.6	52525.3	59264.8
	23690.1	18493.3	20860.4	21250.0	57429.5	57726.6	51381.2	58744.2
	18243.5	17855.2	18845.2	21635.7	52713.9	55954.2	52998.9	62385.9
Mean	18403.7	18970.8	19239.3	21814.3	53379.6	53819.8	51875.3	60260.3
Embryo III	11757.6	20781.7	17853.7	18252.6	40027.6	60281.7	39414.2	52514.9
	11444.0	22006.6	17412.6	22502.6	39529.2	58731.6	41682.6	52900.9
	11010.7	18764.4	18503.8	24002.3	40058.0	62780.3	42396.3	51420.4
	13984.3	21198.7	21870.4	25215.3	42887.0	59741.0	42506.4	50349.7
	12539.2	20161.1	22774.6	18020.6	43209.8	60666.8	44898.2	53959.7
Mean	12147.1	20582.5	19683.3	21598.6	41142.2	60436.6	42179.5	52229.1

Table 15

Relative cross-sectional area of the connective tissue of the choroid plexus of the IVth ventricle at different ages.

Age	DAYS PC							
	13	14	15	16	17	18	19	NB
Embryo I	4039.3	3213.8	5241.4	10453.8	11449.9	10403.3	8269.1	11470.2
	4091.8	3586.9	5523.7	12840.8	10746.6	10216.7	7963.2	10200.0
	3786.1	2983.6	6140.0	10462.1	10457.2	11557.8	8762.7	10350.9
	4088.2	3508.4	5860.1	11153.1	10822.8	16721.6	9484.2	14207.1
	4050.1	4265.6	6152.7	10962.9	11095.5	11649.7	9857.4	14167.4
Mean	4011.1	3511.6	5783.5	11174.0	10913.8	12109.2	8867.3	12078.8
Embryo II	4328.7	5456.8	5760.0	8561.0	12701.5	12227.9	10683.8	16431.6
	2409.5	5068.4	6652.7	7498.7	11347.8	15668.5	11889.6	14005.6
	3095.7	5503.8	7336.2	5542.1	11479.1	12107.2	12062.9	12992.8
	5411.5	5224.3	6764.3	6107.2	15266.7	19966.1	14314.6	15163.0
	5226.7	5178.4	5678.5	5183.1	13262.5	14532.2	13768.4	16930.3
Mean	4074.4	5286.3	6628.3	6578.4	12811.5	14900.3	12543.2	15104.6
Embryo III	3228.0	4788.2	6232.0	5123.1	9326.9	16296.6	11805.5	12126.7
	3100.3	6617.0	7144.5	5468.1	14176.6	14337.0	12681.7	13707.9
	2257.6	2022.7	6935.2	6917.9	12785.2	16690.3	12542.7	11959.5
	3244.1	4452.2	6774.1	6219.8	10508.1	12854.0	12935.9	11576.1
	2769.6	5026.7	7001.8	4514.6	12231.8	13252.7	12585.7	13174.2
Mean	2919.9	4581.3	6817.5	5248.7	11805.5	14685.8	12510.3	12508.4

Table 16

Relative cross-sectional area of the blood vessels of the choroid plexus of the IVth ventricle at different ages.

Age	DAYS PC							
	13	14	15	16	17	18	19	NB
Embryo I	1417.7	1042.9	2443.9	4438.1	5200.4	6360.2	4322.4	5884.3
	1667.1	958.2	2364.2	3321.4	4581.2	5831.1	4554.3	6044.7
	811.4	1415.9	2544.4	4054.7	5041.2	5216.3	5608.4	7351.7
	1084.3	1290.0	3017.3	4821.9	4762.0	7018.0	5494.5	7409.3
	996.7	1129.4	3496.9	4905.0	5195.5	4473.4	5165.8	7409.8
Mean	1195.4	1167.2	2773.3	4307.8	4956.0	5778.8	5028.6	6819.9
Embryo II	1042.2	2339.0	3018.0	1740.1	4166.6	6840.6	6145.7	9224.1
	684.1	3260.4	3596.9	1999.6	4687.5	9312.6	6556.6	6482.1
	1401.6	3212.7	3568.2	1799.7	6203.8	7914.4	5990.7	7506.7
	1933.5	3296.0	3292.0	1822.0	6410.4	8611.7	5819.7	6368.5
	1296.7	2268.1	2768.4	2012.7	5288.5	9799.5	6525.3	7046.4
Mean	1271.6	2875.2	3248.7	1874.8	5351.3	8495.7	6207.3	7325.5
Embryo III	1398.6	1892.1	2546.0	1680.0	5398.5	9125.1	4549.9	5988.5
	1027.5	1815.3	2405.9	2571.7	4018.6	6970.9	5199.7	6497.6
	1803.4	1687.3	3068.2	2118.8	4626.5	7237.6	5548.3	6549.9
	1675.8	2345.6	3792.2	2361.3	5723.9	7531.0	6049.6	5253.6
	1591.0	2116.5	4529.6	1312.1	5801.4	6445.5	7057.9	7102.3
Mean	1499.2	1971.3	3268.3	2008.8	5113.7	7715.7	5780.4	6278.3

Table 17

Relative cross-sectional area of epithelium of the choroid plexus of the IVth ventricle at different ages.

Age	DAYS PC							
	13	14	15	16	17	18	19	NB
Embryo I	10637.2	7431.6	9243.6	17978.9	28967.0	26534.1	27121.3	32629.1
	10004.3	8355.3	9647.2	21701.6	27487.2	30150.6	26877.0	37729.3
	10525.7	8607.7	9304.0	20761.3	26164.4	31629.9	29946.8	37169.2
	10339.6	7787.8	9027.2	20484.8	27706.3	25352.4	29510.4	35782.2
	10981.0	9009.2	8422.0	21396.4	27738.6	22050.9	30544.2	33623.2
Mean	10497.5	8238.3	9128.8	20464.0	27612.7	27143.3	28799.9	35386.6
Embryo II	10553.6	11892.1	9026.6	12740.8	31140.2	29435.4	34310.2	34663.2
	10269.1	12084.4	9432.0	12666.6	37844.7	32110.3	32884.0	40098.7
	16300.5	9688.3	8700.4	13636.0	37183.6	29801.0	34471.7	38765.1
	16345.1	9973.0	10804.1	13320.8	35752.4	29148.8	31246.9	37212.7
	11720.1	10408.7	10398.3	14439.9	34162.9	31622.5	32705.2	38409.2
Mean	13037.6	10809.3	9672.2	13360.2	35216.7	30423.6	33123.6	37829.4
Embryo III	7131.0	14108.4	9075.7	11449.5	25302.2	34860.0	23058.8	34399.7
	7316.2	13574.0	7862.2	14462.8	21335.0	37423.7	23801.2	32695.4
	6949.7	15054.4	8500.4	14965.6	22646.3	38852.4	24305.3	32911.0
	9064.4	14400.9	11304.1	16634.2	26655.0	39356.0	23520.9	33520.0
	8178.6	13017.9	11243.2	12193.9	25176.1	40908.6	25255.6	33683.2
Mean	7727.9	14031.1	9593.2	13941.2	24222.8	38280.1	23987.8	33441.8

Table 18

Calculated volume density of epithelium of choroid plexus of the IVth ventricle at different ages.

Age	DAYS PC							
	13	14	15	16	17	18	19	New born
Embryo I	66.3	58.3	49.4	57.3	63.5	67.4	56.3	62.4
	76.8	52.6	50.8	58.8	64.2	65.9	55.3	65.5
	78.4	60.4	45.9	54.7	62.8	61.9	58.5	66.6
	69.0	53.9	51.6	56.2	64.0	57.8	57.3	61.8
	64.2	59.2	45.1	57.4	63.0	63.7	57.1	64.0
Mean	70.9	56.9	48.5	56.9	63.5	63.3	56.9	64.1
Embryo II	60.6	64.6	46.6	55.3	56.5	56.2	61.7	57.5
	63.9	63.6	50.4	65.0	63.2	50.5	65.6	65.4
	63.1	62.5	51.7	66.7	62.2	59.8	60.8	61.6
	64.8	66.2	55.0	62.7	58.3	60.7	67.1	66.2
	65.2	61.9	54.6	57.2	54.0	56.5	64.1	63.3
Mean	63.5	63.8	51.6	61.4	58.8	56.7	63.9	62.8
Embryo III	66.1	67.9	50.6	64.3	70.2	61.3	68.3	69.9
	63.5	61.7	44.1	62.7	64.0	65.3	67.0	67.7
	69.6	80.2	44.3	62.4	62.2	51.6	68.2	62.3
	66.6	67.9	51.7	66.0	67.8	65.3	67.6	60.9
	68.1	64.6	55.1	67.6	64.8	57.8	66.3	65.3
Mean	66.8	68.5	49.1	64.6	65.8	60.3	67.5	65.2

Table 19

Calculated volume density of blood vessels of choroid plexus of the IVth ventricle at different ages.

Age	DAYS PC							New
	13	14	15	16	17	18	19	born
Embryo I	6.5	9.1	19.9	9.4	11.4	10.6	15.7	13.2
	5.1	8.2	14.2	7.3	10.7	12.6	14.2	11.4
	6.7	9.0	16.5	8.8	12.1	11.5	11.5	10.4
	8.2	11.1	17.3	9.2	11.0	15.1	13.1	12.3
	7.1	10.5	13.8	11.4	11.8	11.9	12.5	12.7
Mean	6.7	9.6	16.3	9.2	11.4	12.3	13.4	12.0
Embryo II	11.9	16.0	19.3	9.0	11.5	16.3	12.3	15.3
	9.0	7.6	16.8	8.6	13.5	14.9	11.4	12.7
	16.4	8.9	14.3	9.3	13.3	15.9	11.3	11.3
	12.0	7.8	13.4	8.6	13.4	17.5	12.0	10.7
	12.7	10.9	14.4	7.6	10.2	14.1	12.8	10.9
Mean	12.4	10.2	15.6	8.6	12.3	15.7	11.9	12.1
Embryo III	8.8	12.2	16.9	13.2	8.7	14.7	11.9	11.2
	10.6	12.7	18.8	8.8	8.7	12.6	11.3	13.4
	5.4	11.5	18.2	11.5	11.8	14.3	11.6	12.9
	7.0	11.9	15.7	13.5	11.3	10.8	12.6	13.4
	6.2	17.8	14.6	13.2	10.0	11.7	12.4	11.8
Mean	7.6	14.4	16.8	12.0	10.1	12.8	11.7	12.5

Table 20

Calculated volume densities of connective tissue of choroid of the IVth ventricle at different ages.

Age	DAYS PC							
	13	14	15	16	17	18	19	New born
Embryo I	27.2	29.0	30.7	33.9	25.1	22.0	28.0	24.4
	18.1	30.0	35.0	29.7	25.1	21.5	30.5	23.1
	14.9	27.7	37.6	31.8	25.1	26.6	30.0	23.0
	22.8	28.3	31.1	30.6	25.0	27.1	29.6	25.9
	28.7	24.8	41.1	29.4	25.2	24.4	30.4	23.3
Mean	22.3	28.0	35.0	31.1	25.1	24.3	29.7	23.9
Embryo II	27.5	27.8	34.1	37.1	32.0	27.5	26.6	27.2
	27.1	27.5	32.8	26.4	23.3	34.6	23.0	21.9
	20.5	29.7	34.2	24.0	24.5	24.3	27.9	27.1
	23.2	22.9	31.6	28.7	28.3	25.2	20.9	23.1
	22.1	27.9	31.0	33.0	35.8	26.0	23.1	25.8
Mean	24.1	27.2	32.7	29.8	28.8	27.5	24.3	25.0
Embryo III	25.1	23.0	32.5	24.3	21.1	24.0	20.8	22.9
	25.9	30.1	37.1	28.1	26.4	22.1	21.7	25.7
	25.0	10.8	37.5	28.8	27.0	34.1	20.2	24.8
	26.4	21.0	32.6	24.6	20.9	23.9	19.8	18.9
	25.7	24.9	30.3	25.1	25.2	30.5	21.3	18.9
Mean	25.6	22.0	34.0	26.2	24.1	26.9	20.8	22.2

Table 21

Table of means for volume density of connective tissue

Age	Mean	Stnd. Error	95 Percent LSD intervals	
13th day pc	24.00	.95	21.78	26.21
14th day pc	25.73	1.88	23.52	27.94
15th day pc	33.90	.66	31.68	36.11
16th day pc	29.03	1.46	26.82	31.24
17th day pc	26.00	1.42	23.78	28.21
18th day pc	26.23	.98	24.02	28.44
19th day pc	24.93	2.58	22.72	27.14
Newborn	23.70	.81	21.48	25.91

Table 22

STATISTICAL ANALYSES OF CHANGES IN THE VOLUME DENSITY OF CONNECTIVE TISSUE OF THE CHOROID PLEXUS OF THE FOURTH VENTRICLE AT DIFFERENT AGES.

One-Way Analysis of Variance

Data: volume density of connective tissue
Range test: LSD (Confidence levels: 95 and 99)

Analysis of variance

Source	Sum Squares	d.f.	Mean square	F	P
Between groups	235.01	7	33.57	5.142	.0032
Within groups	104.46	16	6.52		

d.f. = degree of freedom
p = significance level
F = variance ratio
LSD = least significant difference

Table 23

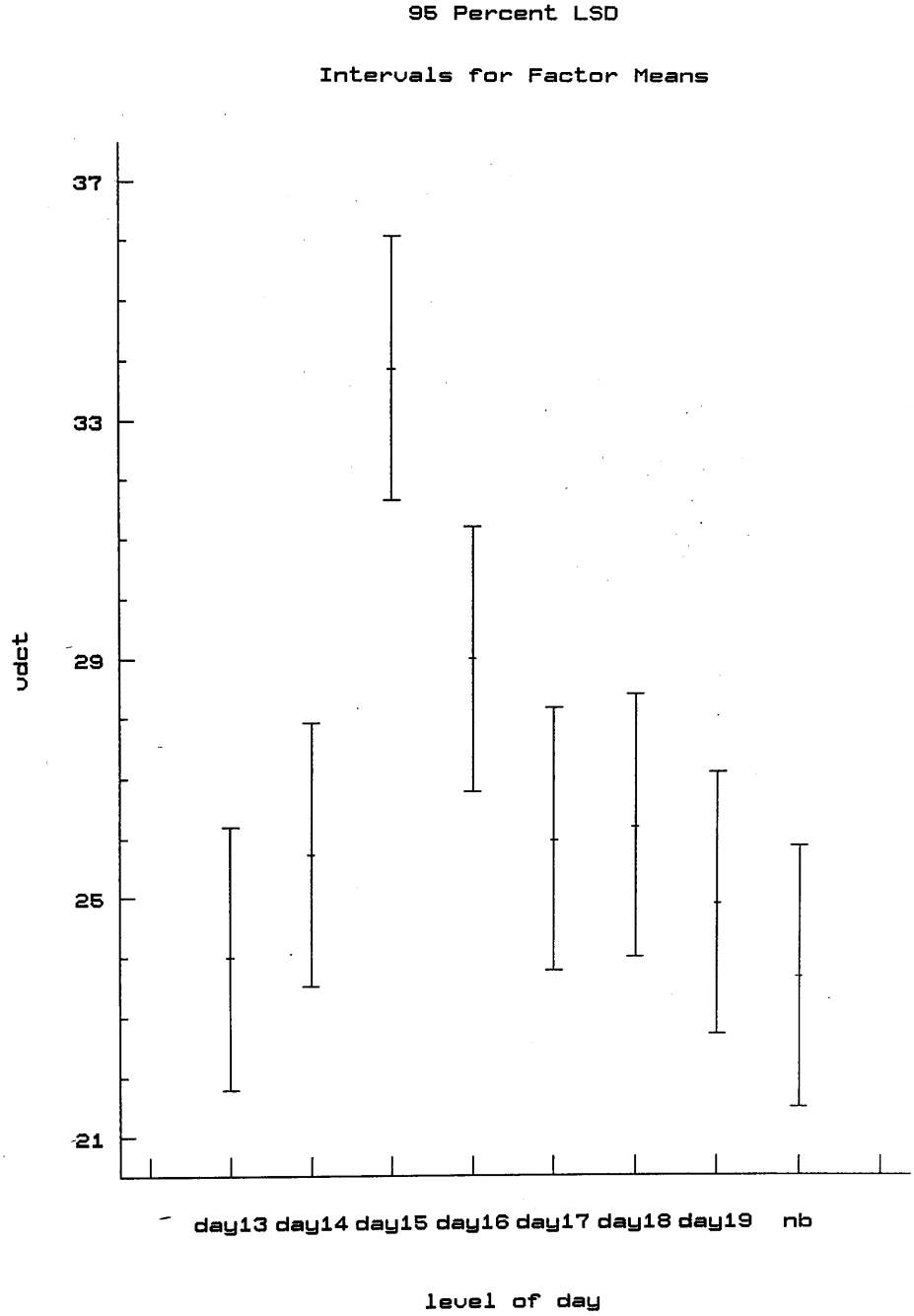
Multiple range analysis for volume density of connective tissue

Method: 95 Percent LSD Intervals Age	Mean	Homogeneous Groups
13th day pc	24.00	*
14th day pc	25.73	**
15th day pc	33.90	*
16th day pc	29.03	*
17th day pc	26.00	**
18th day pc	26.23	**
19th day pc	24.93	**
Newborn	23.70	*

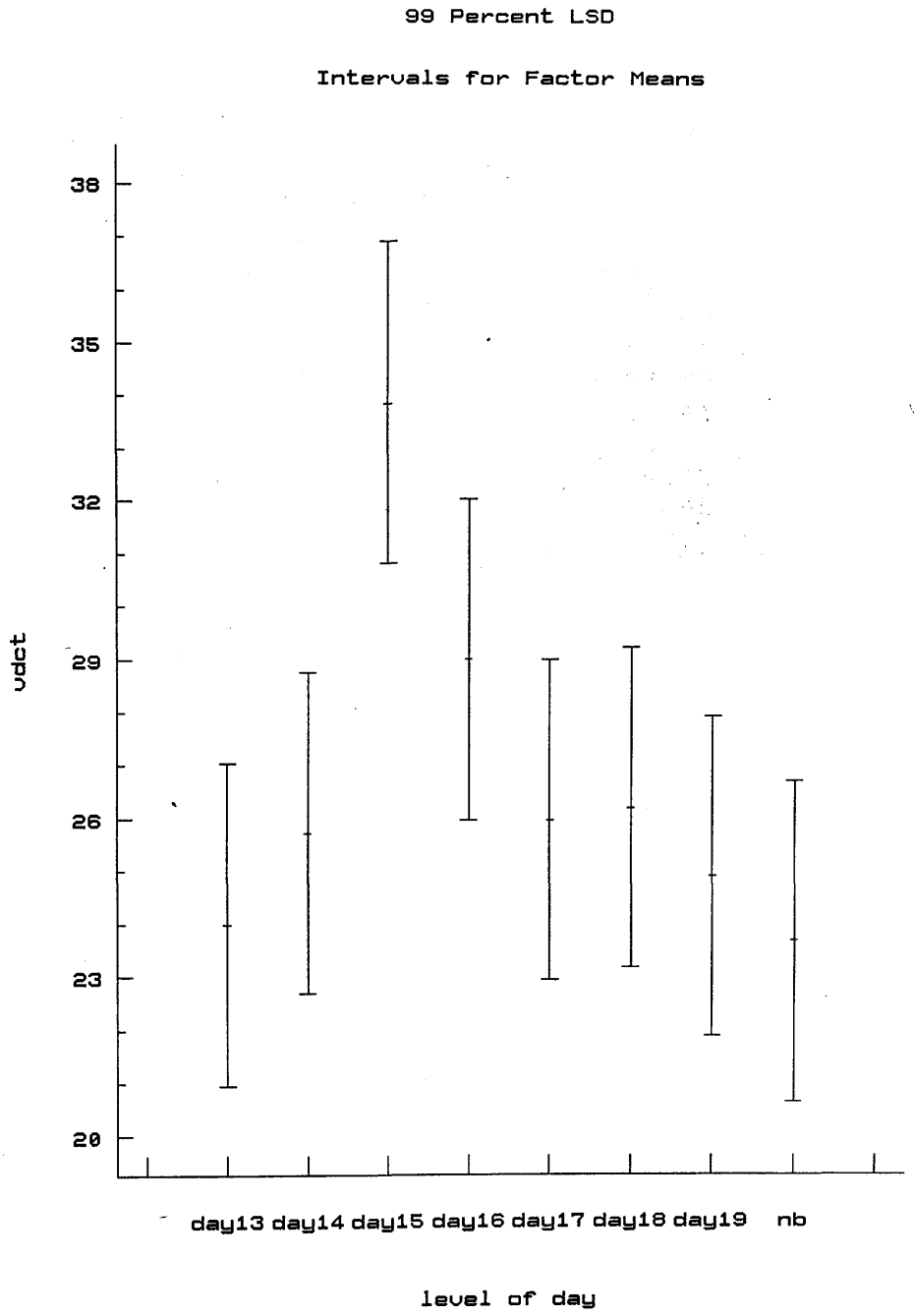
Table 24

Method: 99 Percent LSD Intervals Age	Mean	Homogeneous Groups
13th day pc	24.00	*
14th day pc	25.73	*
15th day pc	33.90	*
16th day pc	29.03	**
17th day pc	26.00	*
18th day pc	26.23	*
19th day pc	24.93	*
Newborn	23.70	*

Asterisks at different ages which share the same vertical column indicate no significant changes, while those lying in different vertical columns indicate a statistically significant change.



vdct = Volume density of connective tissue
nb = Newborn



vdct = Volume density of connective tissue
nb = Newborn

Table 25

Table of means for volume density of epithelium.

Age	Mean	Stnd. Error	95 Percent LSD intervals	
13th day pc	67.08	2.14	63.73	70.42
14th day pc	63.04	3.35	59.69	66.38
15th day pc	49.73	.94	46.38	53.07
16th day pc	60.96	2.23	57.62	64.31
17th day pc	62.70	2.05	59.35	66.04
18th day pc	60.10	1.90	56.75	63.44
19th day pc	62.76	3.11	59.42	66.11
Newborn	64.03	.69	60.68	67.37

Table 26

STATISTICAL ANALYSES OF CHANGES IN THE VOLUME DENSITY OF EPITHELIUM OF CHOROID PLEXUS OF THE FOURTH VENTRICLE AT DIFFERENT AGES.

One-Way Analysis of Variance

Data: volume density of epithelium
Range test: LSD (Confidence levels: 95 and 99)

Analysis of variance

Source	Sum Squares	d.f.	Mean square	F	P
Between groups	550.07	7	78.58	5.26	.0029
Within groups	238.88	16	14.93		

d.f. = degree of freedom
P = significance level
F = variance ratio
LSD = least significant difference

Table 27

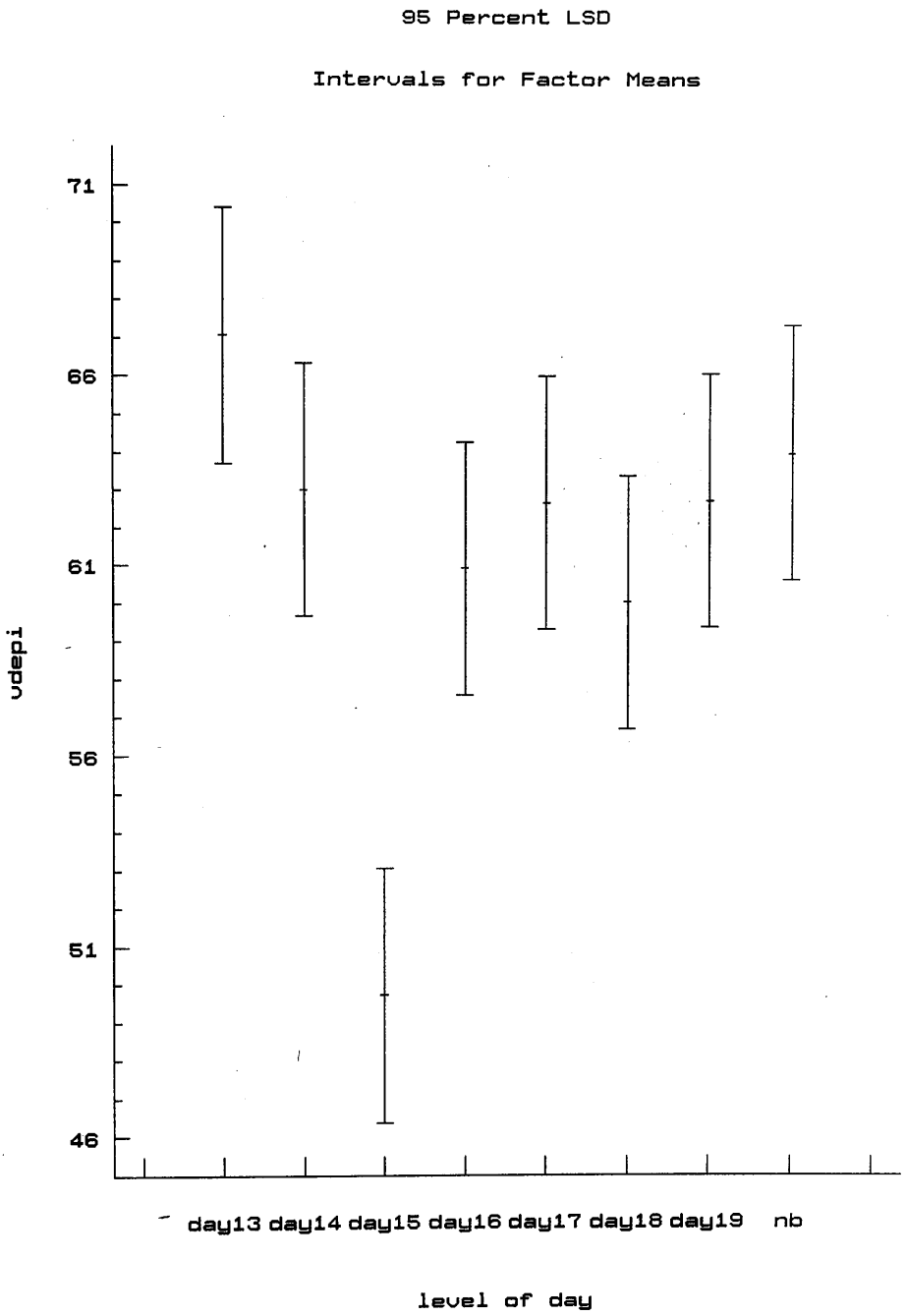
Multiple range analysis for volume density of epithelium

Method: 95 Percent LSD Intervals Age	Mean	Homogeneous Groups
13th day pc	67.08	*
14th day pc	63.04	**
15th day pc	49.73	*
16th day pc	60.96	**
17th day pc	62.70	**
18th day pc	60.10	*
19th day pc	62.76	**
Newborn	64.03	**

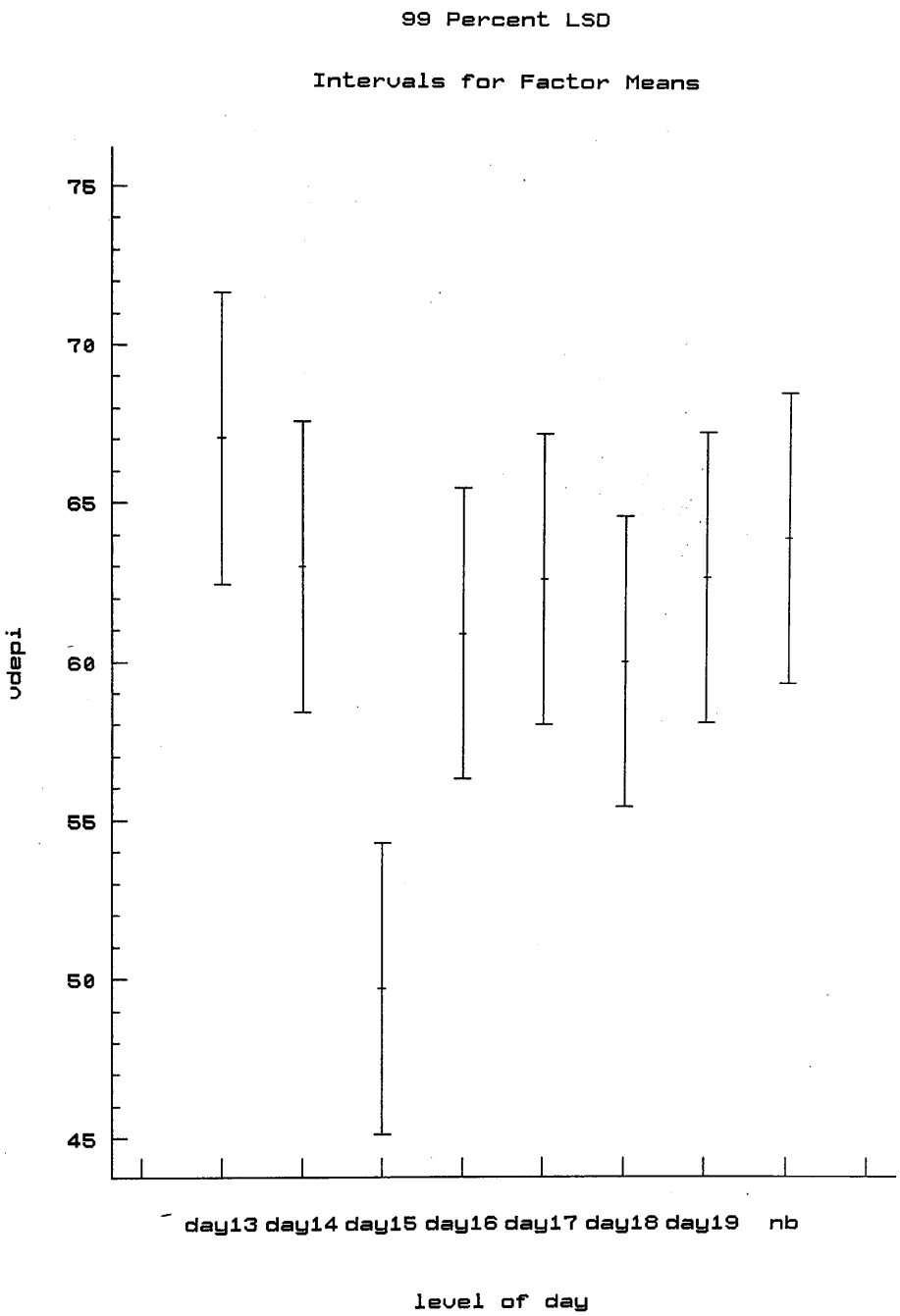
Table 28

Method: 99 Percent LSD Intervals Age	Mean	Homogeneous Groups
13th day pc	67.08	*
14th day pc	63.04	*
15th day pc	49.73	*
16th day pc	60.96	*
17th day pc	62.70	*
18th day pc	60.10	*
19th day pc	62.76	*
Newborn	64.03	*

Asterisks at different ages which share the same vertical column indicate no significant change, while those lying in different vertical columns indicate a statistically significant change.



vdepi = Volume density of epithelium
nb = Newborn



vdepi = Volume density of epithelium
nb = Newborn

Table 29

Table of means for volume density of blood vessels

Age	Mean	Stnd. Error	95 Percent LSD intervals	
13th day pc	8.90	1.76	7.35	10.44
14th day pc	11.38	1.52	9.83	12.92
15th day pc	16.23	.34	14.68	17.78
16th day pc	9.93	1.04	8.38	11.48
17th day pc	11.26	.63	9.71	12.81
18th day pc	13.60	1.05	12.05	15.14
19th day pc	12.33	.53	10.78	13.88
Newborn	12.20	.15	10.65	13.74

Table 30

STATISTICAL ANALYSES OF CHANGES IN THE VOLUME DENSITY OF BLOOD VESSELS OF CHOROID PLEXUS OF THE FOURTH VENTRICLE AT DIFFERENT AGES.

One-Way Analysis of Variance

Data: volume density of blood vessels
Range test: LSD (Confidence levels: 95 and 99)

Analysis of variance

Source	Sum Squares	d.f.	Mean square	F	P
Between groups	106.29	7	15.18	4.755	.0047
Within groups	51.10	16	3.19		

d.f. = degree of freedom
P = significance level
F = variance ratio
LSD = least significant difference

Table 31

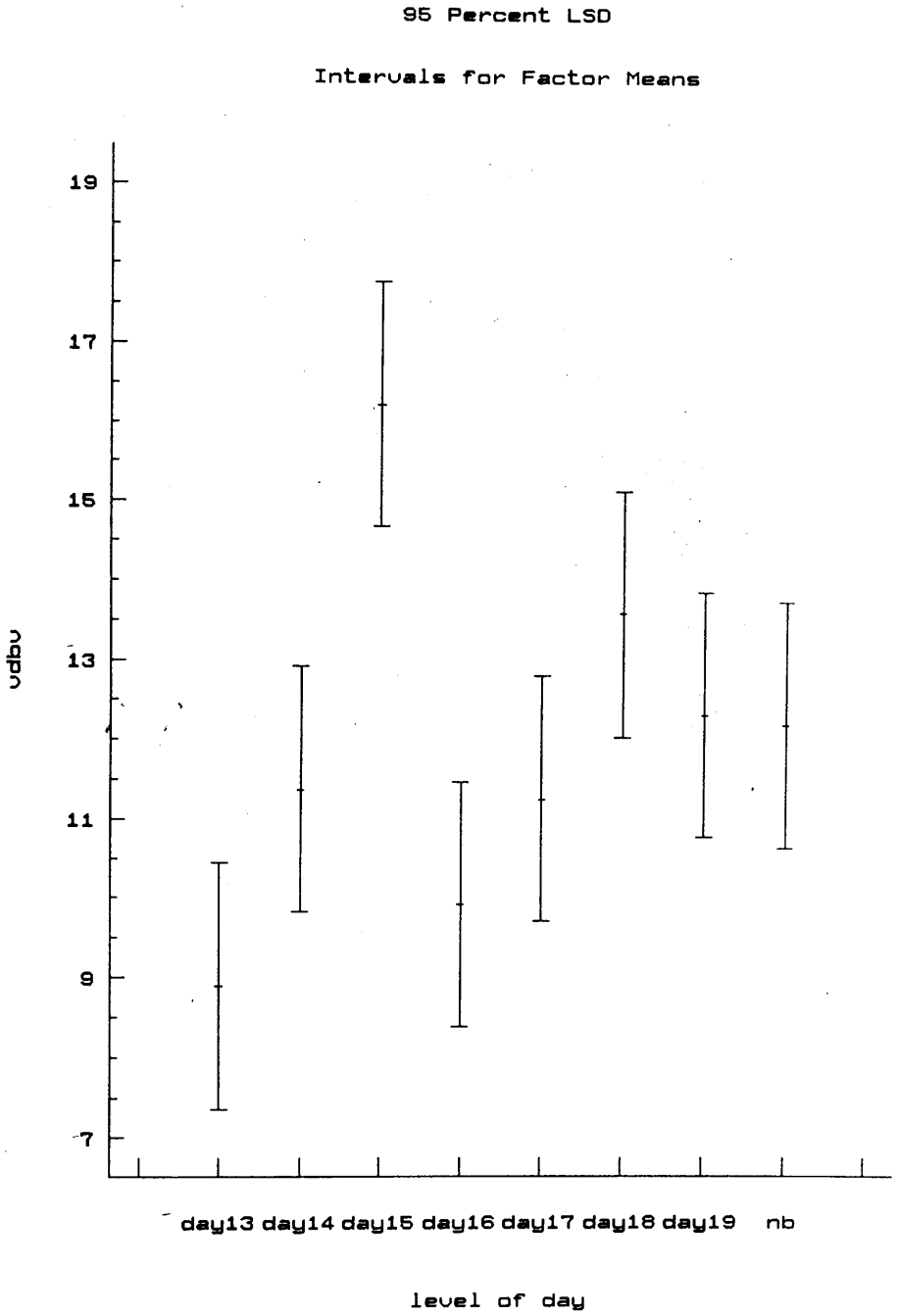
Multiple range analysis for volume density of blood vessels

Method: 95 Percent LSD Intervals Age	Mean	Homogeneous Groups
13th day pc	8.90	*
14th day pc	11.38	***
15th day pc	16.23	*
16th day pc	9.93	**
17th day pc	11.26	***
18th day pc	13.60	**
19th day pc	12.33	**
Newborn	12.20	**

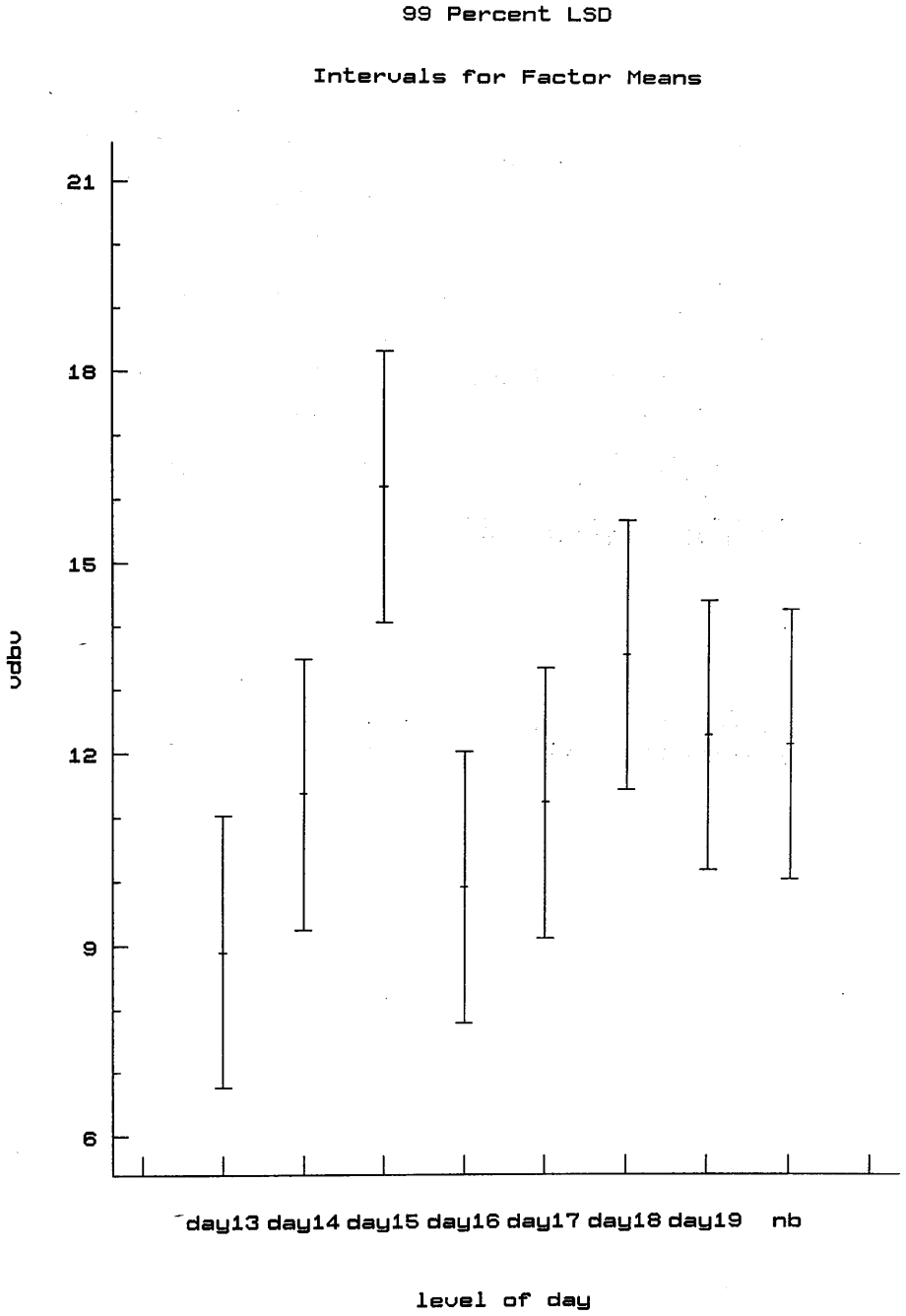
Table 32

Method: 99 Percent LSD Intervals Age	Mean	Homogeneous Groups
13th day pc	8.90	*
14th day pc	11.38	**
15th day pc	16.23	*
16th day pc	9.93	**
17th day pc	11.26	**
18th day pc	13.60	**
19th day pc	12.33	***
Newborn	12.20	***

Asterisks at different ages which share the same vertical column indicate no significant change, while those lying in different vertical columns indicate a statistically significant change.



vdbv = Volume density of blood vessels
nb = Newborn



vdbv = Volume density of blood vessels
nb = Newborn

Table 33

Caudal roof plate of the IVth ventricle

	Adult	Prenatal
AMPHIBIA		
urodeles	Complete (Blake 1900)	No information
anurans	Fenestrated (interependymal pores) Jones (1979)	No information
BIRDS	Complete Jones & Dolman (1979)	No information
MAMMALS		
mouse, rat	lat. foramina, no midline foramen Blake (1900)	No lat. foramina midline interependymal pores Jones (1987 et al)
pig	lat. foramina, no midline foramen Bradley (1906)	lat. foramina, midline interependymal pores Jones (1987 et al.)
sheep	Blake (1900)	
humans	lat. foramina midline foramen	> 22mm development of macroscopic and microscopic deficincies Blake (1900) Brocklehurst (1969)

DISCUSSION

I. Development of the choroid plexus

The findings of the present study on the development of the choroid plexus of the IVth ventricle of the mouse will first be summarised and then discussed in relation (i) to previous studies and (ii) to the problem of the time of onset of secretory activity of the plexus.

A. General features

The choroid plexus of the IVth ventricle made its first appearance on the 11th day p.c. and was first confined to an area in and around the midline part of the roof of the ventricle. Later, it extended laterally to reach the lateral recess on either side.

Even at this early stage of development the choroid plexus of the IVth ventricle could be recognised:

1. On SEM - by the fact that the choroid epithelial cells have bulging apices and some microvilli, contrasting with the neighbouring ependymal cells which have flat apices, without any microvilli.
2. On TEM - again by apical microvilli (less noticeable than on SEM) and abundant glycogen granules scattered all over the cytoplasm.
3. On light microscopy of semithin sections - here the choroid plexus was recognised mainly because the tela choroidea in relation to its epithelium is very vascular.

At this stage the choroid plexus is a simple structure

with no foldings, and invagination into the cavity of the ventricle has not yet begun.

B. Morphometric analysis

Morphometric analysis of the three components of the choroid plexus (epithelium, blood vessels and connective tissue) showed that there was no significant progressive change in their volume density during development.

C. Histogenesis

1. Of epithelium:

The choroid plexus of the IVth ventricle was formed by the invagination of a single layer of simple columnar epithelium as seen by TEM and by light microscopy of semithin sections.

The height of the epithelium decreased significantly between the 13th and 14th days p.c., and then gradually thereafter so that at birth the epithelial cells were low columnar to cuboidal.

At the 11th day p.c. the apical surface of the choroidal epithelium, as shown by SEM and to a lesser extent by TEM, bore few, short microvilli but by the 13th day p.c. it was completely and densely covered by longer microvilli and a few cilia. In addition multivesicular bodies (TEM) and blebs (SEM) were seen arising from the apical surface. These surface projections (microvilli, cilia and blebs) were basically similar between 13th day p.c. and birth.

TEM showed that at the 11th day p.c. the lateral plasma cell membranes of choroidal epithelium were straight

and simple and were joined together by apical junctional complexes, but by the 13th day p.c. they entered into complex interdigitations and infoldings which persisted until birth.

From the 11th day p.c. and until birth the choroidal epithelium rested on a well developed basement membrane.

Internal features: At the 11th day p.c. TEM examination revealed that the choroid epithelial cells possessed centrally-to-apically placed nuclei with one or more nucleoli. The cytoplasm contained uniformly scattered mitochondria, glycogen granules and sparse profiles of rough endoplasmic reticulum.

By the 13th day p.c. profiles of rough endoplasmic reticulum were more numerous, and a Golgi complex was more frequently seen and usually occupied a supranuclear position. Glycogen granules were now present as basal aggregates as well as being scattered throughout the cytoplasm. These glycogen clumps were also seen on light microscopy of semithin sections as basally situated metachromatic masses. No noticeable change occurred in the internal features of the choroid epithelial cells between the 13th day p.c. and birth.

"Light" and "Dark" Cells

No "dark" cells were seen in the choroidal epithelium of the IVth ventricle in both TEM and light microscopy of semithin sections. "Light" cells (those with paler-staining cytoplasm) were infrequently seen at 13th and 16th

days pc and in the newborn. These were structurally similar to other choroid epithelial cells apart from the fact that their cytoplasmic organelles were more widely dispersed and they contained cytoplasmic lipid inclusions.

Mitosis

Examination of semithin sections at different stages of development (13th day to 19th day pc) showed a low incidence of mitosis in the choroid epithelial cells ranging from 0.1% to 0.4%. This mitotic activity occurred only in the root of the choroid plexus. In the adjacent ventricular ependyma, on the other hand, the incidence of mitosis was about 10 times higher.

2. Of blood vessels:

a) SEM of vascular casts -

The vascular casts of the choroid plexus of the IVth ventricle of 15, 17 and 18 day embryos, newborn and adult mice were examined. In the prenatal stages and in the newborn the choroid plexus capillaries were found to be sinusoidal, with irregular outlines and measuring about 30 - 50 um in diameter. In the adult, however, the sinusoidal capillaries had become transformed into linear capillaries forming a dense anastomosing meshwork. The vascular cast of the choroid plexus of the lateral ventricle of 18 day embryos was different from that of the IVth ventricle. It was composed of a network of anastomosing linear capillaries running between larger marginal vessels. This arrangement was maintained throughout development and into adult stage.

b) TEM and SEM of Endothelium -

At 13th day p.c. TEM showed that the endothelial wall of choroidal capillaries was generally thick (average thickness 2.1 um) with scattered areas of thinning and occasional fenestrae. Neighbouring endothelial cells were joined together by desmosomal junctions. With development there was progressive, uniform and generalised thinning of the endothelial wall so that in the adult the average thickness was only 0.6 um; there was also an increase in the frequency of fenestrae.

The vascular basement membrane remained discontinuous and poorly organised throughout development.

SEM of the interior of choroidal capillaries of the newborn and adult mice showed that the fenestrae in the endothelial wall, which measured about 0.1 um in diameter, occurred in groups in areas which were apparently encircled by what looked like endothelial ridges.

c) Proximity to Epithelium -

TEM and light microscopy of semithin sections showed that throughout development choroidal capillaries were closely related to the base of epithelial cells with few connective tissue elements intervening. This relationship became more intimate as development progressed, so that in places the epithelial cells seemed to rest directly on the capillary endothelium.

D. Epiplexus and Supraependymal cells

Epiplexus and supraependymal cells, which were present throughout development, first appeared at the 11th day p.c. as shown by SEM. Their ultrastructure showed them to be spherical, 8-9 um in diameter with smooth surface covered by ruffles and blebs; their cytoplasm contained a large number of pinocytotic vesicles and vacuoles, dense bodies, mitochondria and rough endoplasmic reticulum. It was shown that the number of epiplexus cells increased significantly between 13th day and 14th day p.c, and decreased gradually thereafter (the relative number of epiplexus cells at 14th days p.c. was six times greater than that in the newborn).

Now that the findings of this study on the development of the choroid plexus of the IVth ventricle of mouse have been summarised, they will be discussed in relation to previous studies.

a) General Considerations

In this study, the choroid plexus of the IVth ventricle of the mouse has been shown to arise by an invagination of the single-layered ependymal roof plate. This is in contrast to the telencephalic choroid plexus of the same species which is formed by the invagination of the multi-layered medial wall of the cerebral hemisphere (Sturrock, 1979). Another difference between the choroid plexus of the IVth ventricle and lateral ventricle is that the former is formed by a process of invagination of the roof plate which started in the midline and then extended

laterally, whereas the latter is formed by one or two points of invagination of the ependymal wall. So one could speak of the choroid plexus of the lateral ventricle as having a pedicle, but this is not true of that of the IVth ventricle.

The myelencephalic choroid plexus of the mouse first appeared in the roof of the ventricle around the midline; the process of invagination then extended laterally and turned sharply forwards to pass into the lateral recess on each side. Therefore, the choroid plexus of the IVth ventricle might be considered as having a medial component which developed first, and two lateral components which developed secondarily, although the three components form a continuous structure. This was in contrast to the situation in the rabbit, in which the lateral parts of the choroid plexus of the IVth ventricle developed first (Strong, 1964).

In this study, as early as the 11th day p.c. the epithelial component of the choroid plexus of the IVth ventricle could be recognised by its bulging apices and relatively abundant microvilli. At the same stage the choroid plexus of the lateral ventricle of the mouse could be recognised as a bilateral ridge in the roof of the interventricular foramen (Sturrock, 1979), with a degree of differentiation similar to that of the IVth ventricle. Thus the choroid plexuses of both IVth and lateral ventricles of the mouse appeared at the same time (11th

day p.c.) and then increased rapidly in size and complexity.

b) Mode of growth of the choroid plexus of the IVth ventricle

At the 11th day p.c. the choroid plexus of the IVth ventricle was formed by a single layer of simple columnar epithelium which had not yet been invaginated into the cavity of the ventricle by the vascular tela choroidea. By the 13th day p.c. the choroid plexus was invaginated into the cavity of the ventricle. It had increased considerably in size and complexity, forming numerous folds except in its lateral parts. Growth of the choroid plexus of the IVth ventricle continued throughout prenatal development.

In this study only very few mitoses were found in the epithelium of the choroid plexus of the IVth ventricle and these were all present in the root of the plexus; but more were found in the neighbouring ependyma. The root of the choroid plexus refers to that part of the plexus which is nearest to, and continuous with, the ependyma at points of invagination. This result agreed with that of Knudson (1964) who studied mitosis in the developing mouse choroid plexus, and with those of Kappers (1958) and Sturrock (1979) who studied the development of the choroid plexus of the lateral ventricle in man and mouse respectively, and found very few mitoses in the choroidal epithelium.

This paucity of mitosis has raised the question as to the mode of growth of the choroid plexus. It has been suggested by Kappers (1958) that the transformation of the

pseudostratified epithelium of the choroid plexus of the lateral ventricle into a single-layered cuboidal epithelium, accounted for most of the growth of the plexus. This transformation seems unlikely to be the sole or even the most important factor in the great increase in size of the choroid plexus. Moreover, in the mouse, the choroid plexus of the lateral ventricle continued to grow even after all the epithelium had been transformed from pseudostratified into a single layer (Sturrock, 1979). This further growth must therefore be due to other mechanisms. The presence of mitosis mainly in the neighbouring ependyma may offer a possible explanation as to the mode of growth, and suggests that new epithelial cells were being added only in the root of the plexus and from the neighbouring ependyma, and not diffusely, since the free part of the choroid plexus was always devoid of any mitotic activity.

In this study it has been shown that, unlike the choroid plexus of the lateral ventricle, that of the IVth ventricle developed by the invagination of the single-layered ependymal roof plate, therefore excluding the transformation of epithelium from pseudostratified to a single-layer as a mode of growth. It has also been shown that mitoses were found only in the root of the plexus and in the neighbouring ependyma. From these findings it appears that growth of the choroid plexus of the IVth ventricle took place by the addition of new epithelial

cells in the root of the plexus, but mainly from the neighbouring ependyma, in which mitoses, in all stages examined, were 3 to 4 times more frequent than in the root of the choroid plexus. This would be in conformity with the findings in this study, that choroid epithelial cells nearer to the root of the plexus were undifferentiated and looked more like ependymal cells, compared to the more mature and differentiated epithelial cells nearer the free border of the plexus (free border of the choroid plexus is that point of the plexus farthest away from the point of invagination). These findings suggest that as a new generation of choroid epithelial cells was added to the root of the plexus, previous generations were pushed away from the root and towards the free border where they became more differentiated.

c) "Light" and "dark" cells

"Dark" cells were noticed in the choroid plexus of the lateral ventricle of some mammalian species (Wislocki and Ladman, 1958) and of the rat by Van Deurs et al. (1978). Sturrock (1979) also reported the presence of similar cells in the choroid plexus of the lateral ventricle of the mouse. According to him, dark cells first appeared by the 14th day p.c. and maintained the same ratio (about 11% of the total number of epithelial cells) throughout development, leading him to suggest that these cells are unlikely to be degenerating cells.

Sturrock (1979) also found that epiplexus macrophages were more commonly seen attached to "dark" cells than to

"light" cells, although the latter were more numerous. Could this be an indication that "dark" cells are dying cells and that the epiplexus macrophages are there to remove any products of degeneration?

The majority of studies which reported "dark" cells in epithelial tissue have involved glutaraldehyde fixation, postfixation in osmium and examination of plastic embedded material by electron microscopy or of toluidine blue stained sections by light microscopy. Jordan et al. (1973) found the detection of "dark" cells to be glutaraldehyde-dependent in sheep parathyroid gland, but Coupland and Weakley (1968), and Murakami et al. (1985) described "dark" cells in the developing chromaffin tissue in the rabbit and "dark" keratinocytes in mouse skin, respectively, despite the fact that in both studies the specimens had been fixed in formalin and embedded in paraffin.

In the present study, although all the specimens examined had been fixed in glutaraldehyde and postfixed in osmium, "dark" cells could not be identified in the epithelium of the choroid plexus of the IVth ventricle.

Cells with paler-staining cytoplasms, however, were seen both in TEM and light microscopy of semithin sections. Their presence was considered to be unimportant because (i) they were extremely uncommon; (ii) they were not present in all developmental stages (only in 13 day and 16 day pc and in the newborn), and (iii) they were in the minority whereas in all previous reports it was "dark" cells which

formed a minority.

Lipid inclusions were noticed in the choroid epithelium but only in those cells with paler staining cytoplasm; the significance of this association is not known.

d) Glycogen

Sturrock (1979) reported that glycogen first appeared in the choroid epithelial cells of the mouse lateral ventricle by the 13th day p.c. In the present study, glycogen granules were found to be uniformly and densely scattered throughout the cytoplasm of epithelial cells of the choroid plexus of the IVth ventricle as early as the 11th day p.c., and formed basal clumps and aggregates by the 13th day p.c. These glycogen granules and aggregates persisted until birth. Therefore, although the choroid plexuses of the IVth and lateral ventricles of the mouse both developed at the same time (11th day p.c.), glycogen was present in the choroid epithelium of the IVth ventricle at a much earlier stage. The significance of this difference in the time of appearance is not clear.

The reason for the formation of basally situated glycogen clumps from scattered granules is not known, although Kappers (1958) suggested that it might play a role in the formation of glycosaminoglycans of basement membrane of the choroidal epithelium.

The function of glycogen in the developing choroid plexus has not yet been determined. Kappers (1958) suggested a nutritive role for glycogen as an important

anaerobic source of energy. His suggestion, which was widely adopted, was based on the finding of poor vascularity of the human choroid plexus during early development (6th-8th prenatal week) and thus the need for glycogen to supply needed energy by anaerobic glycolysis. Dempsey and Wislocki (1944), discussing the significance of glycogen in human placenta, pointed out that it was deposited in regions showing poor vascularisation, thus providing an anaerobic source of energy. The question must be asked; "How was the glucose which is used as building blocks for glycogen delivered to these epithelial cells in the first place?"

The finding in this study of highly vascular choroid plexus, even in early prenatal development (when glycogen is abundant in the choroid epithelial cells), makes the nutritive role of glycogen less plausible. Scothorne (1955) found in a circumscribed area of one section from a 20mm sheep embryo that the ultimobranchial body contains much glycogen, the endodermal thymus contains a moderate amount and thyroid and parathyroid contain none. It is clear from this that in such a small area, this difference in glycogen content must be due to differences between the cells themselves and not to local variations in availability of nutrients.

e) Epiplexus cells

In the present study, epiplexus cells appeared in relation to the apical surface of the developing choroidal

epithelium since its first inception in the 11th day p.c., and were found to be morphologically similar to macrophages. The number of epiplexus cells in the mouse IVth ventricle increased significantly between the 13th and 14th day p.c. This coincided with the appearance of a large number of subarachnoid macrophages. The significance of the association between these two events is not clear. The function of the epiplexus cells is not known but they might be concerned with the removal of products of cell degeneration. The fact that they were found by Sturrock (1979) to be related to dark cells, which are probably degenerative cells might lend support to this possible function.

II. What light do these morphological studies throw on time of onset of function of the choroid plexus?

By the 13th day p.c., scanning and transmission electron microscopy showed that apical microvilli, lateral plasma membrane infoldings and interdigitations, mitochondria and other cellular organelles were all well developed and resembled those of the late fetal and adult stages. The formation of apical microvilli and infolded basilateral cell membranes is pertinent to the question of the onset of secretion of CSF by the choroid plexus since these surface elaborations are thought to be important for secretion in the adult. So the present study supports the view of Shuangshoti and Netsky (1966) that the fetal choroid plexus shows morphological differentiation commensurate with early onset of function. However, Cancilla et al (1966) and Dohrman (1970) found in rat and man respectively that even late in prenatal life the choroid plexus showed minimal surface development and questioned if the choroid plexus has any secretory function at this stage.

It has long been accepted that microvilli and basilateral membrane infoldings increase the surface area of the apical and lateral cell membranes respectively. Morphometric analysis of the developing choroid plexus of the IVth ventricle of the rat (Keep, Jones and Cawkwell, 1986) showed that microvilli had increased significantly the surface area of the apical cell membrane (3-fold

increase between 13th prenatal day and 10th postnatal day). However, they found that although the infoldings and interdigitations of the basilateral region of the epithelial cells seemed to increase in complexity with age, they made a quantitatively insignificant contribution to the total surface area of the basilateral cell membrane. In the past much emphasis has been placed on the basilateral membranes infoldings of choroid plexus cells (Maxwell and Pease, 1958; Tennyson and Pappas, 1968) and they were considered to be important for active transport, but in the light of this recent evidence they might not be as important as previously thought. These infoldings could, of course, be important in respects other than increasing the surface area.

An examination of the properties of some other tissues or organs which are involved in active transport might prove useful in understanding the changes during development of the choroid plexus with the onset of function. The ciliary body of the eye and the choroid plexus have many features in common. They both develop from nervous tissue. The electron microscopic structure of both the choroid plexus and ciliary body epithelium showed features considered by Pease (1956) to be important for water and ion transport. The aqueous humor and CSF are both secretions which have electrolyte composition different from that of a plasma dialysate.

Kinsey, Jackson and Terry (1942) biochemically analysed the aqueous humor of the postnatal rabbit and

found that at 28 days the level of ascorbic acid in the aqueous humor was many times that in the blood and was similar to that in adult rabbits. They concluded that the aqueous humor of rabbit became a secretion by the 28th postnatal day.

Holmberg (1959) carried out an ultrastructural study of the epithelium of the ciliary body of the rabbit's eye before and after secretion of the aqueous humor started (at 28 days after birth). Prior to secretory activity the non-pigmented epithelium of the ciliary body did not show many apical folds and there was minimal infolding and interdigitation of the lateral plasma membranes. He also found that the nucleus was apically located and there was much endoplasmic reticulum basally. After the onset of secretion by the ciliary body, as judged by the high level of ascorbic acid in the aqueous humor compared to plasma, the non-pigmented epithelium showed deep apical folds and complex and elaborate infoldings of the lateral plasma membranes. The nucleus moved towards the cell base and the rough endoplasmic reticulum was much reduced.

From the results of the study of Holmberg (1959) it is clear that the infoldings of the apical cell membrane, together with interdigitations and infoldings of the lateral plasma membranes were important structural correlates of the onset of secretory activity by the non-pigmented epithelium of the ciliary body of the eye.

In the present study, microvillous apical border and

lateral plasmalemmal infoldings and interdigitations were both well developed by the 13th day p.c. This stage might therefore be regarded, on structural grounds, as the stage at which the secretory activity of the choroid plexus of the IVth ventricle of the mouse started. On the basis of the structural similarity between the choroid plexus epithelium and that of the ciliary body of the eye, and that both epithelia produce secretions, it is probable that the mode of production of the two secretions is also the same. This evidence is, of course, only circumstantial. Proof would depend on correlated biochemical analysis of CSF to determine the developmental stage at which it becomes a secretion rather than a dialysate.

In addition to the changes which occurred in the epithelium, choroidal blood capillaries also showed some morphological changes which were thought to be important for the secretory function of the choroid plexus. These include progressive thinning of the capillary endothelium, and the appearance of fenestrae in the endothelial wall (by the 13th day p.c.) which increased in frequency during development. Both these morphological changes would facilitate the exchange of substances between the capillaries and epithelium, which is further augmented by the intimate relationship between the two.

Apical secretory blebs thought to represent an apocrine type of secretion by the choroid plexus, were observed by some authors (Wislocki and Ladman, 1958; Millen and Rogers, 1956; Hetzel, 1978; and others). Tennyson and

Pappas (1961) on the other hand took the view that these blebs were fixation artifacts, although they did mention that all other cellular organelles were well preserved. No such artifacts were reported in other secretory epithelia and they seemed to be peculiar to the choroid plexus epithelium. Such peculiarity would favour the supposition that these blebs were indeed genuine structures. In the present study both transmission and scanning electron microscopy of the choroid epithelial cells showed only a few apical secretory blebs. In the scanning electron microscope they were clearly shown as pot-like structures apparently discharging their contents into the ventricular cavity. In the transmission electron microscope they appeared as large multivesicular protrusions of the apical plasma membrane containing a large number (up to 50) of vesicles and connected to the cell surface by a narrow stalk. Tennyson and Pappas (1964) noticed the same appearance in fetal rabbit choroid plexus. They called them "multivesicular bulbous protrusions" and considered them to be the precursors of microvilli (forming microvilli by the coalescence of their vesicles).

In the present study, secretory blebs were also found in the form of distended microvilli containing few vesicles. Microvilli of the choroid plexus epithelium differ greatly in size and shape and might be considered as dynamic structures giving rise to secretory blebs which were then extruded into the cavity of the ventricle.

The paucity of secretory blebs found in the present study could be explained in one of two ways:

1. that they were randomly occurring artifacts, as previously suggested by Tennyson & Pappas (1961), or
2. that because the animals were killed (by an overdose of anaesthetic ether) before starting fixation, the dynamic secretory process of the choroid plexus might have stopped by the time the fixative was delivered to the tissue. When live anaesthetized animals were killed by perfusion of the fixative, large numbers of secretory blebs were usually observed (Collins and Morris, 1975). An argument in favour of an apocrine secretion by the choroid plexus was the tremendous increase in the number of secretory blebs when cerebrospinal fluid production was stimulated (by pilocarpine), and a marked reduction in their number when cerebrospinal fluid production was inhibited (by acetazolamide) (Santolaya and Echandia, 1968; Collins and Morris, 1975).

In the light of the present study, it is not possible to resolve these conflicting views.

III. Evidence for presence of fluid in cerebral ventricles before differentiation of choroid plexus

Indirect evidence for the presence of fluid in the ventricular cavity before choroid plexus development was provided by the observations of Kappers (1958) that a fluid is formed in the neural tube of human fetuses long before the development of the choroid plexus, and that fluid is present in the ventricular cavity of some lower vertebrates in which the choroid plexus is completely lacking.

Longridge (1966) studied the functional development of the choroid plexus of chick embryos, and was able to obtain fluid from the ventricular cavity before the development of the choroid plexus. Coulombre and Coulombre (1958) and Desmond and Jacobson (1977), who investigated the relationship between neural tube occlusion and brain enlargement, drained fluid from the lumen of the neural tube of chick embryos at a time before the development of the choroid plexus.

There was no direct evidence for the presence of fluid in the cerebral ventricles of the mouse before the development of the choroid plexus, but it could be assumed, from evidence in other species, that that was the case.

Possible functional role of this (Pre-choroid plexus) "CSF"

Kaufman (1983) reported, in the mouse, that occlusion of the neural tube occurred on the 9th prenatal day after the closure of the cephalic neuropore, thus transforming the lumen of the neural tube into a closed system filled with "CSF". In chick embryos occlusion of the neural tube

was thought by Desmond and Jacobson (1977) and Schoenwolf and Desmond (1984) to be important for initiating rapid brain enlargement because of the close temporal relationship between the two. Brain enlargement seemed to be brought about by the creation of a "CSF" pressure in a closed system which is essential to counteract the resistance to brain expansion created by the surrounding mesenchymal elements and surface ectoderm. This "CSF" pressure is maintained by the continued production of fluid in the lumen of the closed system, i.e. the neural tube. Any breach in the integrity of this closed system would lead to leakage of fluid and reduced "CSF" pressure and consequently a decrease in brain enlargement as proved experimentally by Desmond and Jacobson (1977).

The present study has established beyond reasonable doubt that, in the mouse, after closure of the neuropores, the neural tube remained as an anatomically closed system until birth when the lateral foramina (of Luschka) appeared in the roof of the IVth ventricle to establish a communication between the ventricular cavity and the subarachnoid space.

The (pre-choroid plexus) "CSF" is presumably produced by the ependymal lining of the neural tube but the dynamics of its production are not known. Theoretically it is being produced against the pressure created by the elastic recoil of the neural tissue and the surrounding mesoderm and surface ectoderm. Thus the main role of this (pre-choroid

plexus) "CSF" seemed to be purely mechanical: to keep the ventricular cavity open and prevent its collapse.

IV. Vascular casts of the choroid plexus of the IVth ventricle

Vascular casts of the choroid plexus of the IVth ventricle have never, to my knowledge, been done before in the developing or newborn animals; although a few have been done in adult animals, these were mostly of the lateral ventricle choroid plexus. In the present study, vascular casts of the choroid plexus of the IVth ventricle of prenatal, newborn and adult mice were done.

Batson's corrosion compound as suggested by Dollinger and Armstrong (1974) was used with some modifications. These modifications were mainly to reduce the viscosity and prolong the working (pot) life of the resin. This was achieved by adding more thinner and less catalyst and by cooling the resin before the injection was made. Reducing the viscosity of the resin was found to be necessary for successfully replicating the delicate blood vessels of the developing choroid plexus. It must be mentioned here that Dollinger and Armstrong (1974) produced corrosion casts of the heart and great vessels of the embryonic chick. It was clear from the present study that a less viscous resin is needed to replicate the smaller blood vessels.

In the present study, the most striking feature of the results was the wide sinusoidal nature of the choroid plexus capillaries of the IVth ventricle of the prenatal mice, an appearance which was maintained until birth and was distinctly different from that of the subependymal

cerebral capillaries. This was in contrast to the situation in the lateral ventricle choroid plexus, of both prenatal and newborn mice, where although the capillaries were wider than the subependymal cerebral capillaries, they were much narrower than those of the choroid plexus of the IVth ventricle, and they were not sinusoidal in nature.

The sinusoidal nature of the capillaries of the IVth ventricle choroid plexus proved by the injection replication method, is consistent with their wide and very irregular appearance in light microscopy of semi-thin sections.

Tennyson and Pappas (1964) studying the fine structure of the developing telencephalic and myelencephalic choroid plexus in the rabbit noticed no difference in the size of the capillaries between the two. This might be due to species difference between mouse and rabbit; but it must be said that it is much easier to appreciate the difference in capillary size when studying vascular casts rather than light micrographs.

The maintenance of the sinusoidal nature of the capillaries of the choroid plexus of the IVth ventricle during the prenatal mouse development and in the newborn might explain the finding, in this study, that there was no significant change in the volume density of choroidal capillaries during development (see later).

The fact that wide sinusoidal capillaries were present in the choroid plexus of the IVth but not the lateral ventricle of the prenatal and newborn mice might

suggest a difference in the composition of cerebrospinal fluid produced in the two ventricles, but the exact functional significance of this difference in the nature of the choroidal capillaries is not known.

Ames et al. (1964) and Davson (1966), showed that the concentration of the chloride ion in the cerebrospinal fluid in the IVth ventricle was higher than that in the lateral ventricle of adult animals. The situation in the prenatal and newborn animals is not known. It may be relevant that the choroid plexus of the IVth and lateral ventricles of the mouse developed at approximately the same time (i.e. 11th day p.c.) (R ugh, 1968).

It was shown, in the present study, that the blood vessels of the choroid plexus of the IVth ventricle changed from sinusoidal to linear capillaries sometime after birth. Theoretically, this change might increase the surface area of capillary endothelium across which exchange of substances takes place. Morphometric studies are needed to prove this assumption.

In this study, the globular masses seen on the surface of the vascular cast of the choroid plexus could represent resin which has passed through fenestrae in the capillary wall. However, the absence of these globular masses from most of the specimens examined make this assumption less likely. It should be mentioned that no such globular masses were observed on the surface of the casts of hepatic sinusoids by Murakami (1974) although it

is well known that liver sinusoids contain holes or discontinuities in their endothelial walls. The presence of these globular masses on the casts of large choroidal veins might indicate that they were due to rupture of the vessel wall possibly as a consequence of increased pressure during injection. In another paper Murakami (1971) saw similar globular masses in vascular casts of the blood vessels of the small intestine but dismissed them as artifacts due to rupture of the vessel wall. In the present study, the globular masses were found to be inconstantly present, occurring mainly at the 18th day p.c., were very infrequent in the newborn and were absent in the 15th and 17th days p.c. and the adult mouse. It seems, therefore, that they too were most probably artifacts.

V. Morphometric Analysis

Morphometric analysis of the choroid plexus of the IVth ventricle was carried out in order to determine if any changes occurred in the relative contribution of epithelium, blood vessels and connective tissue during development.

The results showed that:-

1. The volume density of epithelium is higher at 13th day p.c. than at any of the other stages. In interpreting this, two points should be mentioned:

a) At the 13th day p.c. much of the laterally placed choroid plexus epithelium has not yet been invaginated by the underlying connective tissue and blood vessels. Due to the method of measurements adopted, no connective tissue or blood vessels have been measured in these lateral parts, thus making the volume density of the epithelium to appear deceptively high.

b) The height of epithelial cells fell sharply (by almost a third) between 13th and 14th days p.c., and then decreased gradually until birth. This fall in epithelial cell height might have partially accounted for the high volume density of epithelium at 13th day p.c.

In the developing rat the IVth ventricle choroid plexus epithelial cell height fell significantly between 16th prenatal day and birth (Keep, Jones and Cawkwell, 1986). This correlates well with the results of the present study. It is interesting to note that other tissues which, like the choroid plexus, form a barrier

between two surfaces and are involved in the transport of substances, also shared a reduction in the height of its epithelium during prenatal development. Such tissues include the placental barrier and the alveolo-capillary barrier.

The mode of thinning of airway epithelium and the formation of the capillary-air barrier is not agreed upon. Campiche et al (1963) and Boyden (1974) suggested that capillaries play an active role in the formation of the capillary-air barrier by penetrating the connective tissue layer which invests the terminal branches of the bronchopulmonary tree, to lie closer to the cuboidal respiratory epithelium. They also suggested that the capillaries, by exerting pressure on the overlying epithelium, might bring about thinning of the epithelium, and also insert themselves between epithelial cells. Prentice (1980) disagreed with the views of Campiche et al. (1963) and Boyden (1974). He found that, in the developing guinea pig lung, the alveolo-capillary barrier is brought about by the thinning epithelium of the expanding airways coming into close contact with newly formed capillaries; the capillaries do not play a direct role in the thinning of the epithelium. Prentice (1980) concluded that the airways, rather than the capillaries, are the dynamic component in the formation of the alveolo-capillary barrier.

In the porcine placenta, Friess et al. (1980) found

that there was a progressive decrease in the height of both the uterine epithelium and the trophoblastic layer. They did not mention, however, how this thinning of the epithelium is brought about. Friess et al. (1980) also noticed that fetal and maternal capillaries indent into the trophoblast and uterine epithelium respectively, but could not determine if this played any active role in the thinning of the epithelium. The thinning of the uterine epithelium and the trophoblastic layer in conjunction with the indentation of capillaries into the uterine and trophoblastic epithelium would lead to a marked reduction of the transplacental intervascular distance, and consequently facilitate the exchange of substances between the fetal and maternal circulation.

Although thinning of epithelium of the choroid plexus of the IVth ventricle has been shown in this study, and also in the rat by Keep, Jones and Cawkwell (1986), the exact mechanism is not known. Further studies are needed to determine the factors which bring about the thinning of the epithelium.

2. Blood vessels and connective tissue.

In the present study, it was shown that no significant progressive change occurred in the volume density of the connective tissue and blood vessels of the developing choroid plexus between the 13th day p.c. and birth. This would parallel the results of Keep, Jones and Cawkwell (1980) who found that the weight of the vascular core of the choroid plexus of the IVth ventricle of the rat

did not increase appreciably during development.

In the present study, although no significant progressive change in the volume density of choroidal blood vessels has taken place, it was noticed from light microscopic and TEM studies that capillaries were becoming more intimately related to the basal surface of the epithelium as development progressed, until epithelial cells came to rest directly on the endothelial wall of the capillaries. In some situations capillaries even appeared to be intraepithelial. This intimate relationship between epithelium and capillaries could be brought about either by the epithelium moving towards the blood vessels or vice versa. In either case the intervening connective tissue elements must either disappear or be displaced to allow for this closer contact between the epithelium and blood vessels. Displacement was considered to be the most probable event as the volume density of the connective tissue did not change significantly during development.

Prentice (1980) carried out a morphometric analysis of the developing guinea pig lung and found a significant increase in the endothelial surface area between the pseudoglanduler stage (39 days postcoitum, 56mm) and the canalicular stage (52 days postcoitum, 88mm). He attributed this increase in the endothelial surface area to the formation of a capillary bed from the sinusoid-like vessels of the pseudoglandular stage. This transformation of sinusoidal vessels into capillaries did not occur in the

mouse choroid plexus, as it has been proven in this study, that the blood vessels of the choroid plexus remained sinusoidal throughout prenatal development and at birth. This might possibly explain why there was no significant change in the volume density of choroidal blood vessels during mouse development.

The close contact between capillaries and epithelium seems to be important for more efficient function, possibly by making easier the exchange of substances between the two. In this respect an analogy could be drawn between the choroid plexus, the pig's placental barrier and the alveolo-capillary barrier. In all these organs early in development, there were always some connective tissue elements intervening between the capillary endothelium and the overlying epithelium. As development progressed these intervening connective tissue elements either disappeared or became very sparse, allowing closer contact between epithelium and capillaries. Friess et al (1980) found, in pig's placenta, that the relationship between the fetal capillaries and trophoblasts was so intimate that the endothelial basal lamina and its epithelial counterpart fused into one.

In conjunction with the closer contact between the capillaries and the choroidal epithelium, qualitative changes which have occurred in the capillary endothelium (i.e. attenuation and the appearance of fenestrae) would improve the exchange of substances between the blood stream and the choroidal epithelium.

VI. Foramina in roof of IVth ventricle

1. Midline foramen

The continuity between the ventricular and subarachnoid CSF was investigated in the amphibian, Rana pipiens, by the light microscope and fluorescent techniques (Jones, 1978), and by scanning electron microscope (Jones, 1979); and in some mammalian species (mouse, rat, pig and sheep) by the scanning and transmission electron microscope (Jones, 1980; Jones et al., 1987).

In the mammalian species examined, multiple, interependymal pores appeared in the caudal part of the roof of the IVth ventricle shortly after the development of the choroid plexus. They increased in number and size as development progressed. In the rat the pores measured up to 40 um in diameter. It was claimed that these interependymal pores provided a route for the bulk flow of CSF from the ventricular cavity into the subarachnoid space, before the development of the lateral foramina (of Luschka).

SEM examination of the ventricular aspect of the roof of the IVth ventricle necessitates dissecting open the cavity of the ventricle; preparation of the tissue also involves severe chemical and physical insults. When these are coupled with the very thin nature of the caudal part of the roof of the IVth ventricle, then the creation of artifactual discontinuities becomes a possibility.

In the present study, despite meticulous examination,

no midline interependymal pores were found in the caudal part of the roof of the IVth ventricle. There is clearly fundamental disagreement between these findings and those of Jones and her colleagues, and the disagreement probably hinges upon the validity of the techniques used in the two studies. The pores described by Jones et al. are so large and so numerous that, if they really exist, significant numbers of them must have been included in the region examined by serial semithin sections in the present study. In producing semithin sections the roof of the IVth ventricle was fixed in situ and was physically undisturbed in all subsequent processing. It seems highly unlikely, to say the least, that foramina should disappear following application of a technique which is usually accepted as faithfully preserving tissue relationships. It seems far more likely, again to say the least, that during the course of processing the very attenuated and delicate roof plate for SEM examination, artificial openings were produced by the rigours of the technique.

Further objections can be raised to the interpretation of the findings of the light microscope study of the roof of the IVth ventricle of the amphibian, Rana pipiens, (Jones, 1978) which is open to question for the following reasons:

a) It is dificult to differentiate between pial and ependymal cells on morphological grounds alone. The differentiating criterion she used was the shape of the nucleus, "a pial nucleus is oval while ependymal nuclei are

oval or indented". It is clear that if a pial nucleus is oval and an ependymal nucleus could also be oval, then this structural criterion for differentiation is not particularly useful.

b) A magnification of 500x might not be enough to show that the very thin ependymal layer is continuous, and that pial and ependymal membranes each formed a separate layer. In the present study, areas in the roof of the IVth ventricle which were initially interpreted as foramina at 500x magnification, were found, at 1000x magnification, to be spanned by an extremely attenuated ependymal layer.

c) In Fig. 7 and Fig. 8 (Jones, 1978) part of the ventricular roof was labelled as an interependymal pore, but on close inspection two distinct but extremely attenuated layers representing ependymal and pial membrane, can be identified.

From the foregoing points and from the results of the present study, it seems likely that the interependymal pores in the roof of the IVth ventricle of mouse found by Jones et al (1987) were artifacts.

2. Lateral foramina

The establishment of an anatomical continuity between the ventricular and subarachnoid compartments of cerebrospinal fluid was a late feature of development in the mouse. It was only at birth that such continuity in the form of lateral foramina (of Luschka) was established in spite of the fact that the choroid plexus of the IVth

ventricle was well developed at a much earlier stage. This would mean that there was no close relationship between the development of the choroid plexus and appearance of foramina in the roof of the IVth ventricle. In man, pig and chick on the other hand, the appearance of a mid-line foramen in the roof of the IVth ventricle coincided with the development of the fourth ventricular choroid plexus, a fact which has been taken to indicate that the development of foramina was dependent on the production of cerebrospinal fluid by the choroid plexus. In fact Weed (1917; 1938) put forward the widely accepted view that the roof of the IVth ventricle was actually burst by the secretory pressure of cerebrospinal fluid. This view was supported by Cohen and Davis (1937) who also found a close relationship between the development of the choroid plexus and the appearance of mid-line foramina in the roof of the IVth ventricle in chick embryos.

In the present study this was not found to be true in the case of the mouse. If we assumed the correctness of the views of Weed, then one would expect the foramina of the IVth ventricle in the mouse to appear at the 13th day p.c., or shortly after, at which time the choroid plexus of the IVth ventricle was well developed. Furthermore, these foramina might have been expected to appear in the mid-line part of the roof of the IVth ventricle in the region of the inferior medullary velum, where the roof was made of a single layer of very attenuated ependymal cells throughout development, rather than in the lateral recesses, which

were covered by a single layer of cuboidal ependymal cells up to the 16th day p.c. By the 17th day p.c. the walls of the lateral recesses started to thin out and this process continued until birth, when lateral foramina appeared. The appearance of the lateral foramina seemed therefore to be an active developmental process and not due to simple rupture of the lateral recesses caused by the secretory pressure of the cerebrospinal fluid. Although Brocklehurst (1968) found a close relationship between development of the choroid plexus of the IVth ventricle and the appearance of the mid-line foramen in human embryos, he also maintained that the development of the mid-line foramen was an active developmental process and not due to passive rupture of the roof by the secretory pressure of cerebrospinal fluid.

VII. Development of the subarachnoid space

Weed (1917) studied the development of the cerebrospinal fluid pathways by substituting, in living pig embryos, a solution of potassium ferrocyanide and iron ammonium citrate for cerebrospinal fluid. The embryos were kept alive for some time and then fixed in an acid medium thereby obtaining an insoluble prussian blue precipitate. He put forward the view, since largely unchallenged, that the embryonic cerebrospinal fluid was rich in protein and that it was the most important causative agent in initiating and probably also in completing the transformation of the periaxial mesenchymal spaces, filled with ground substance, into fluid-filled subarachnoid space; and that the presence in the periaxial mesenchymal spaces of large amounts of protein derived from the embryonic cerebrospinal fluid played a major role in gradually increasing the size of the mesenchymal mesh and in breaking down its syncytial strands, thus forming larger channels or spaces.

Weed (1917) also maintained that the specialised areas in the roof of the IVth ventricle acted as filters to the embryonic cerebrospinal fluid keeping proteins inside the cavity of the ventricle; and because he did not find actual anatomical communication between the ventricular and the subarachnoid cerebrospinal fluid compartments, he could not account for the presence of large amounts of protein in the periaxial mesenchymal spaces which he said was derived from the embryonic cerebrospinal fluid. Keegan (1917) also

confirmed the fact that the specialised areas in the roof of the IVth ventricle were semi-permeable membranes allowing the escape of only small molecular weight compounds. McLone and Bondareff (1975) also stated that the cerebrospinal fluid which passed out into the periaxial mesenchymal spaces was watery and very poor in protein. So the substance found by Weed in the periaxial mesenchymal spaces, which he interpreted as protein, was either not protein in nature, or protein which was not derived from the ventricular cerebrospinal fluid and which might have been produced locally. When Weed carried out his experiments it was the popular belief that all the cerebrospinal fluid was produced by the choroid plexus. It is now known that only about half of the total amount of cerebrospinal fluid is produced by the choroid plexus, the rest being produced by the ependyma and the blood vessels and lining of the subarachnoid space. One might add that it is difficult to interpret the results obtained by replacing physiological fluid by an artificial one, because any results obtained might as well be due to abnormal response of the embryo to the presence of the artificial fluid.

In the present study a precipitate was found in the cavity of the IVth ventricle up to the 16th day p.c. but none was found extraventricularly. This would lend support to the argument that cerebrospinal fluid passing out of the IVth ventricle into the periaxial tissue was very poor in

protein, and that the specialised area in the roof of the IVth ventricle acted as a semi-permeable membrane keeping the proteinous material inside the cavity of the ventricle.

In the present study, definite subarachnoid spaces were first noticed at the 14th day p.c., particularly over the ventral and lateral aspects of the hind brain; the cervical spinal subarachnoid space was also well developed at this stage. It must be emphasised that dorsal to the hind-brain and immediately adjacent to the thin roof of the IVth ventricle no definite space was noticed. These spaces were presumed to be fluid-filled, although it was extremely difficult to determine, from the examinations of semithin sections, whether they were filled with fluid or a well-hydrated mesenchymal jelly.

McLone and Bondareff (1975) mentioned that in the second stage of development of the subarachnoid space (in relation to cerebral hemispheres) in mouse (14th to 16th days p.c.), the mesenchymal spaces filled with ground substance were transformed into fluid-filled subarachnoid spaces. However, they did not show how they could differentiate between spaces filled with ground substance and those filled with fluid by examining semithin sections alone.

Weed (1917) found regional differences in the time of establishment of the subarachnoid space in pig and human embryos and attributed this to the fact that the CSF circulated through the different regions at different times. If this were true then one would expect the

subarachnoid space to develop first in the region nearest to the point of escape of CSF (i.e. the dorsal surface of the hindbrain).

In the present study, on the contrary, the subarachnoid space was found to develop first in regions farthest from, and only secondarily in regions nearest to, the point of egress of CSF. This argues against the idea that the flow of cerebrospinal fluid might be the initiating factor in the development of the subarachnoid space as suggested by Weed (1917) and acknowledged by McLone and Bondareff (1975).

In the present study, it was shown that direct anatomical communication between ventricular and subarachnoid space occurred only at birth, after the opening of the lateral foramina (of Luschka). Therefore, there was no bulk flow of CSF out of the ventricles and into the subarachnoid space during the prenatal period to initiate the development of subarachnoid space. This would indicate that bulk flow of CSF was not an important initiating factor in the development of the subarachnoid space. Other initiating factors or mechanisms might be in operation and CSF merely flowed into preformed subarachnoid spaces.

It is suggested here that the following mechanisms might be responsible for initiating the development of the subarachnoid space:-

a) Mesenchymal absorption due to cell death or atrophy.

Although no sign of cell death could be found on light microscopic examination of semithin sections, a small number of phagocytic cells was seen in the future subarachnoid space around the hindbrain by the 13th day p.c. These were specially found in areas where the mesenchymal cells were becoming separated and a space was about to be formed. By the 14th day p.c. the number of phagocytes had increased significantly. These phagocytes might originate from pre-existing leptomeningeal cells, as evidenced by the work of Essick (1920) who found that the lining cells of both pia and arachnoid, notably the latter, transform into free cells with the characteristics of macrophages, when the area was challenged by a foreign material. Sturrock (1988) studied the development of leptomeningeal macrophages in the mouse and found that some leptomeningeal cells showed phagocytic properties, although he did not find evidence that they transform into macrophages.

In the present study, the simultaneous appearance of a large number of phagocytic cells and the first appearance of subarachnoid space, might suggest that some of the leptomeningeal cells occupying the future subarachnoid space undergo atrophy and die while others transform into phagocytes and remove the dead mesenchymal cells thus creating a space. It is interesting to note that in the later prenatal stages and in the newborn mouse, the subarachnoid space seems to be almost devoid of phagocytic as well as leptomeningeal cells. This might be the result

of continued death and phagocytosis of mesenchymal cells. Further work is needed to verify this.

Sensenig (1951) mentioned that the development of the spinal subarachnoid space in human embryos was by the disappearance of mesenchymal cells, but he did not elaborate on that.

McLone and Bondareff (1975), who studied the development of the cerebral subarachnoid space in mice, found that although the subarachnoid space was well developed by the late 16th prenatal day, subarachnoid macrophages did not make their first appearance until after birth. This would suggest that macrophages have no role in the development of the cerebral subarachnoid space. McLone and Bondareff (1975) thought that the flow of CSF was the important initiating factor in the development of the subarachnoid space; and as the role of flow of CSF has been questioned in the present study, other factors need to be identified. It might be that different factors were involved in the development of different regions of subarachnoid space.

b) Cleavage or simple separation of the periaxial mesenchyme into two cellular layers which move apart thus creating a pouch or space, this cleavage being independent of the flow of CSF. Proof is needed to substantiate this hypothesis.

VIII. Arachnoid villi

Jayatilaka (1964) found arachnoid villi in adult man, sheep, monkey, dog, cat, rabbit, guinea pig but not in rat. In the present study, no arachnoid villi were found at any stage of development in the mouse, but they were found in the newborn guinea pig. They consisted of a mass of arachnoid cells which passed through the thick supporting dura mater and invaginated into the lumen of the venous sinus. They were always covered by endothelial cells which were continuous with those lining the venous sinus. No tubules could be identified within the arachnoid villi.

It is well established in man that the CSF returns to the blood stream principally by way of the arachnoid villi and granulations. This, however, obviously cannot be the case in the mouse and rat in which these structures are lacking.

Because of the absence of arachnoid villi in the mouse, uptake of CSF must involve other mechanisms. In the present study, the sagittal sinus was found to be lying within a thin supporting dura mater with very loosely arranged fibres. This arrangement would make it possible for cerebrospinal fluid to percolate through and reach the endothelial wall of the venous sinus where it passes into the blood stream.

Weed (1917) found the same loose arrangement of the supporting dura around the transverse sinus in pig fetuses, and suggested it to be the route for the escape of CSF back into the blood stream. He reached his conclusion by

injecting the double solution of potassium ferrocyanide and iron ammonium citrate into the ventricular cavity of living pig fetuses. The fetuses were then fixed in an acid medium so as to obtain the prussian blue precipitate. On histological examination of these fixed fetuses, he found the prussian blue granules in the ventricular cavity, in the subarachnoid space, in the interstices of the loosely arranged supporting dura mater around the transverse sinuses and also in the lumen of the sinus.

In the adult pig, however, this situation ceased to exist: the supporting dura mater around the venous sinuses became thick with compactly arranged fibres, and the route for the escape of CSF was limited to the, now developed, arachnoid villi.

It seems that the brains of large animals (e.g. pig), by virtue of their size, might need thick supporting dura mater for protection and stability. In these animals CSF cannot pass through this thick supporting dura mater to reach the venous sinuses, so there is the need for specialised structures in the form of arachnoid villi to act as the route for the uptake of cerebrospinal fluid from the subarachnoid space into the venous sinuses.

In the mouse and other small animals, on the other hand, the thin and loosely arranged supporting dura mater might be enough for the protection and stability for their small-size brains. In these animals cerebrospinal fluid could pass through this thin and loosely arranged

supporting dura mater to reach the venous sinuses without the need for arachnoid villi. Cerebrospinal fluid might also be absorbed into the subarachnoid blood vessels, but further work is needed to prove both these assumptions.

In the prenatal stage of many mammalian species including cat, rabbit and guinea pig, arachnoid villi were absent but they were present in the adult (Jayatilaka, 1964). It might be assumed here that the absorption process of cerebrospinal fluid in the prenatal stages might be entirely lacking due to the fact that the production of cerebrospinal fluid was being balanced by the growth of the nervous system and its ventricular and meningeal spaces.

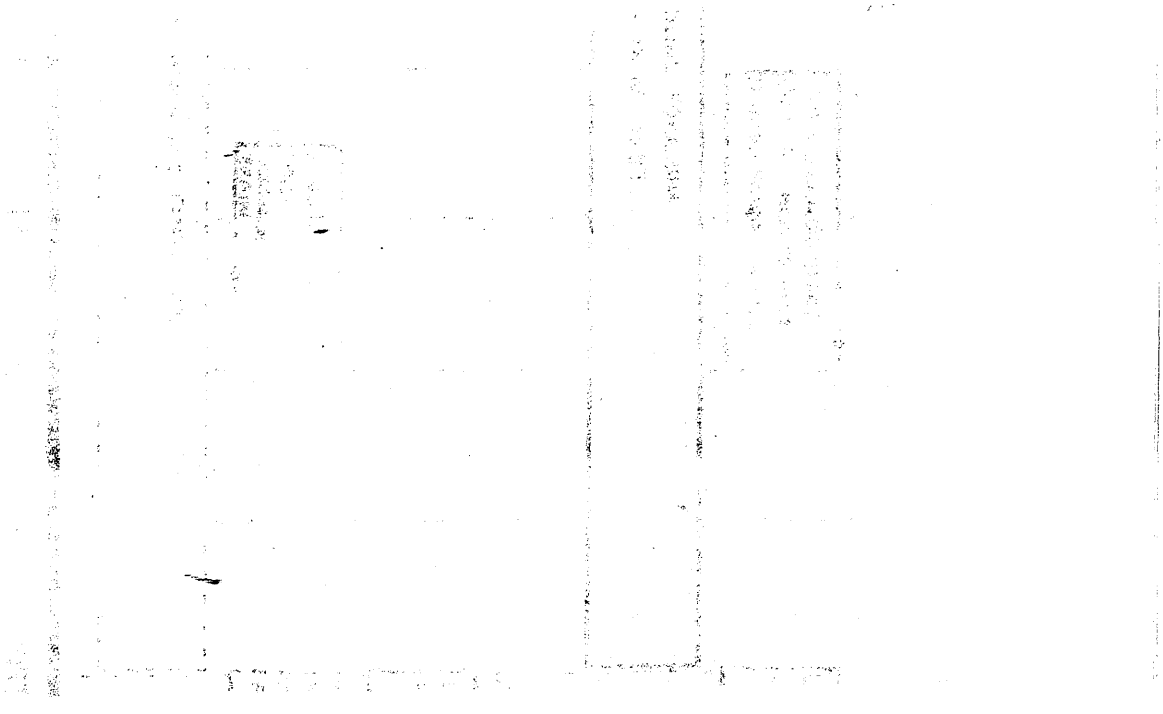
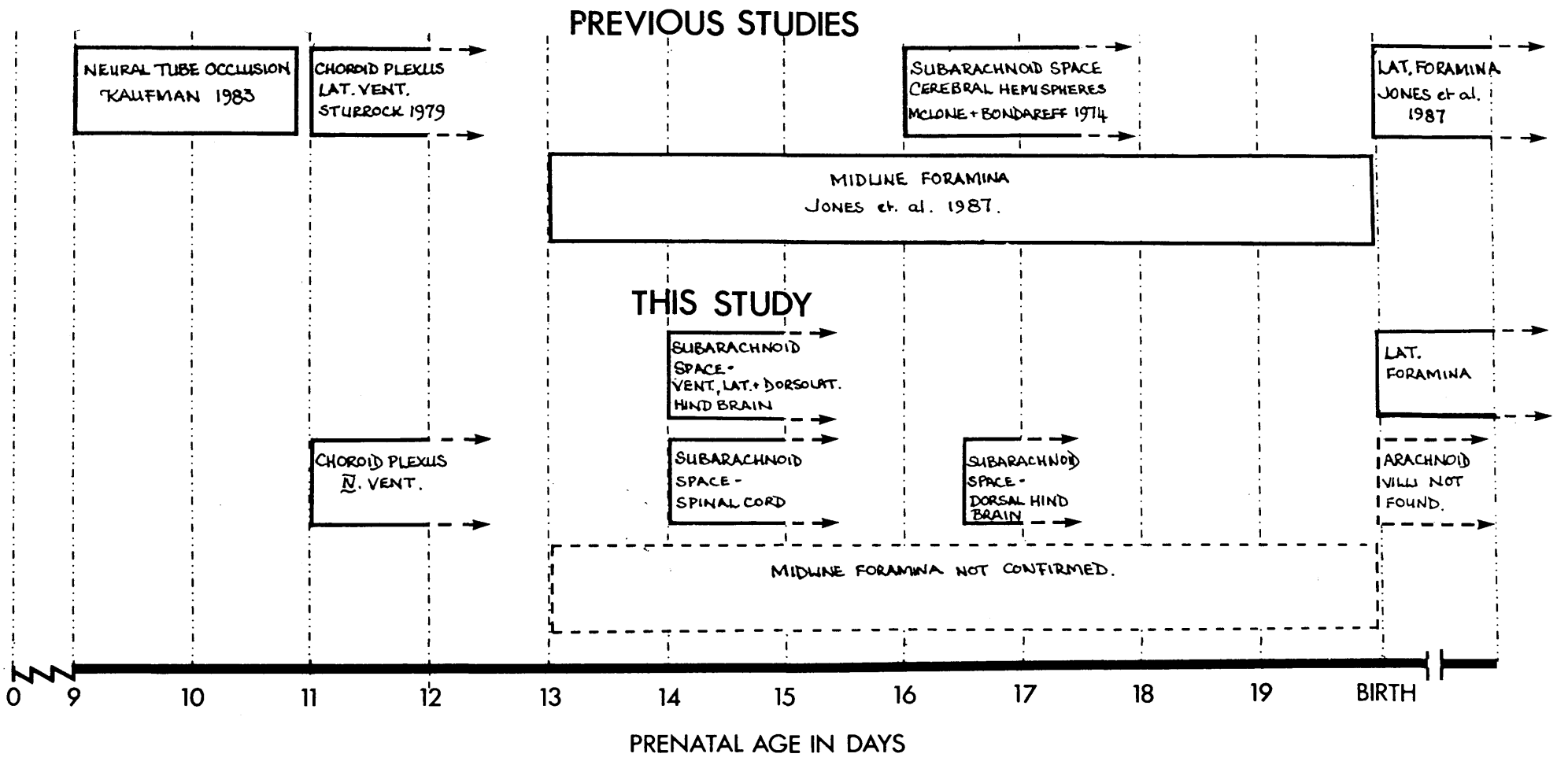


Fig. 125

SEQUENCE OF EVENTS IN THE DEVELOPMENT OF CSF PATHWAYS IN THE MOUSE



CONCLUSIONS

The purpose of this study was:

1. to detect changes during development
2. to relate such changes to the onset of function

From the semithin light microscopic and the scanning electron microscopic studies the impression was that there was a progressive increase in the total volume of the choroid plexus. The increase in the total volume of the plexus was particularly noticeable between 11th and 13th day pc. There was also a substantial increase in the number of microvilli between 11th and 13th day pc, as shown by S.E.M. and to a lesser extent, by T.E.M. T.E.M. studies also showed that at 13th day pc the lateral plasma membranes of epithelial cells started to show complex infoldings. These two features would increase the surface area available for exchange.

Morphometric analysis showed that there is no significant progressive change in the volume density of blood vessels, epithelium and connective tissue between 13th day pc and newborn. As for the blood vessels, this result correlates well with the fact that the choroid plexus capillaries maintained their sinusoidal nature up to birth, as shown by vascular cast studies.

Although the choroidal capillaries remained sinusoidal until birth, the endothelial lining was shown by T.E.M. to become progressively thinner, and to contain fenestrae from 13th day pc to newborn. This would

presumably facilitate the exchange of substances through the capillary wall. The height of choroidal epithelium decreased progressively from 13th day pc to newborn.

Although there was no significant progressive change in the volume density of connective tissue, it was noticed from light microscopic studies that fewer connective tissue elements were interposed between the choroidal capillaries and the epithelium, as development progressed. This would encourage exchange of materials between blood capillaries and epithelium.

Features associated with active secretion (i.e. lateral plasma membrane infoldings, abundant microvilli) appeared at 13th day pc and showed no dramatic change up to the newborn, and on this structural basis alone one can assume that the choroid plexus started active secretion at 13th day pc.

All the structural changes mentioned above would not, by themselves, prove that the choroid plexus started active secretion at a particular stage of development. To determine this, further investigation is required, e.g. quantitative study of mitochondria; biochemical studies of CSF to determine the stage at which it becomes a secretion rather than an ultrafiltrate of plasma; histochemical studies to detect enzymes involved in active transport.

From the information so far available, one can present a correlated morphological account of the development of structures involved in the production, circulation and removal of CSF in the mouse (see Fig. 125).

CSF is known to be present in the cavities of the central nervous system long before the development of the choroid plexus. This was based on the observation by Kappers (1958) of the presence of fluid in the neural tube of human embryos at a time when the choroid plexus is absent; and on the experiments of Longridge (1966) in which he obtained fluid from the ventricular cavity of chick embryos before the appearance of the choroid plexus.

Occlusion of the neural tube is a well documented phenomenon which occurs during early embryogenesis in chick (Desmond and Jacobson, 1977; Desmond and Schoenwolf, 1985); in man (Desmond, 1982) and in mouse (Kaufman, 1983). Kaufman (1983) found that, in mouse, occlusion of the neural tube occurs between early on the 9th day and late on the 10th day. This took place when the cephalic and caudal extremities of the neural tube were still open. The lumen of the neural tube became patent again along its whole length just before the closure of the caudal neuropore. The neural tube could thus be considered as a closed system from the 11th prenatal day and until the appearance of foramina in the roof of the IVth ventricle.

The choroid plexus of the IVth ventricle made its first appearance on the 11th day p.c., but it was by the 13th day p.c. that it assumed morphological characteristics commensurate with secretory function. According to Sturrock (1979) the choroid plexus of the lateral ventricle of the mouse also appeared on the 11th day p.c., and showed

morphological evidence of activity early in the prenatal period.

Midline interependymal pores were found by Jones, Cawkwell and Ellis (1987) in the caudal part of the roof of the IVth ventricle of the mouse at times from shortly after the development of the choroid plexus, up to the time when the medullary velum was completely covered over by the cerebellum. They considered these pores to be essential for the escape of CSF from the ventricular cavity into the subarachnoid space. According to them these pores disappeared completely by the time of birth, when their role was taken over by the lateral foramina (of Luschka).

In the present study, no interependymal pores could be found in the roof of the IVth ventricle, and the lateral foramina (of Luschka) appeared at birth. It is concluded therefore that after closure of the caudal neuropore, the ventricular system of the mouse remains an anatomically closed one until the appearance of the lateral foramina (of Luschka) at birth.

Desmond and Jacobson (1977) showed that rapid enlargement of the brain of chick embryos occurred only when the neural tube was occluded and had become a closed compartment filled with CSF. In the mouse, the fact that the neural tube remained as a closed system until birth, might be important for the prenatal development of the brain.

The absence of foramina from the roof of the IVth ventricle before birth, would mean that in the prenatal

period there was no extraventricular bulk flow of CSF. This would exclude bulk flow of CSF as the initiating factor in the development of the subarachnoid space. In the mouse, the subarachnoid space first appeared on the 14th day p.c. in relation to the lateral, ventral and dorsolateral aspects of the hind brain, and to the cervical spinal cord. The subarachnoid space did not appear in relation to the dorsal aspect of the hind brain until the 16th day p.c.

According to McLone and Bondareff (1975) the subarachnoid space in relation to the cerebral hemispheres appeared between the 16th and 17th days p.c. It is clear, therefore, that different parts of the subarachnoid space developed at different times. In the present study, it was concluded that the development of subarachnoid space is not dependent on bulk flow of CSF.

No arachnoid villi were present in the mouse, but it was thought that the thin and loosely arranged supporting dura would allow the CSF to percolate through and reach the sagittal venous sinus where it is absorbed into the blood stream.

SUGGESTIONS FOR FURTHER WORK

1. Biochemical analysis of CSF during mouse development to determine the stage at which it becomes a secretion, combined with histochemical study of the choroid plexus to determine the time of appearance of enzymes which are known to be important for active transport, in order to establish any relationship between these and the morphological findings.
2. Injection replication of the vasculature of the choroid plexus of the IVth ventricle of the postnatal mouse to determine the time at which the vascular bed changes from sinusoidal to linear capillaries.
3. Injection replication of the vasculature of the developing choroid plexus of the IVth and lateral ventricles of other mammalian species to see if the findings in the mouse could be reproduced.
4. Morphometric analysis of the choroid plexus of the IVth ventricle of postnatal mouse to determine any change in the volume density of blood vessels and connective tissue, particularly after the stage at which the blood vessels change from sinusoidal to linear capillaries.
5. Detailed ultrastructural study (TEM & SEM) of the development of the subarachnoid space in the mouse coupled with experimental work to determine the origin of the subarachnoid macrophages.
6. Reappraisal of the presence of mid-line foramin or foramina in the roof of the IVth ventricle in man and

other mammalian species.

7. Due to the absence of arachnoid villi in the mouse, a study is needed to determine the route of escape of CSF from the subarachnoid space and into the blood stream, in both prenatal and adult mice.

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A CORRELATIVE DEVELOPMENTAL STUDY OF STRUCTURES INVOLVED IN THE PRODUCTION, CIRCULATION AND REMOVAL OF CEREBROSPINAL FLUID

By

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A thesis presented for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow.

VOLUME II: FIGURES

Department of Anatomy,
University of Glasgow.

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Fig. 1

An SEM micrograph showing the hindbrain cut in transverse section and the cavity of the IVth ventricle (IVth). The roof of the ventricle has been reflected rostrally to show the surface of the incipient choroid plexus (P).

11 day mouse embryo. x 125

Fig. 2

An SEM micrograph (higher power from area outlined in Fig. 1) which shows the choroid plexus cells with their bulging apices. Invagination of the plexus into the ventricular cavity has not yet occurred.

11 day mouse embryo. x 1250

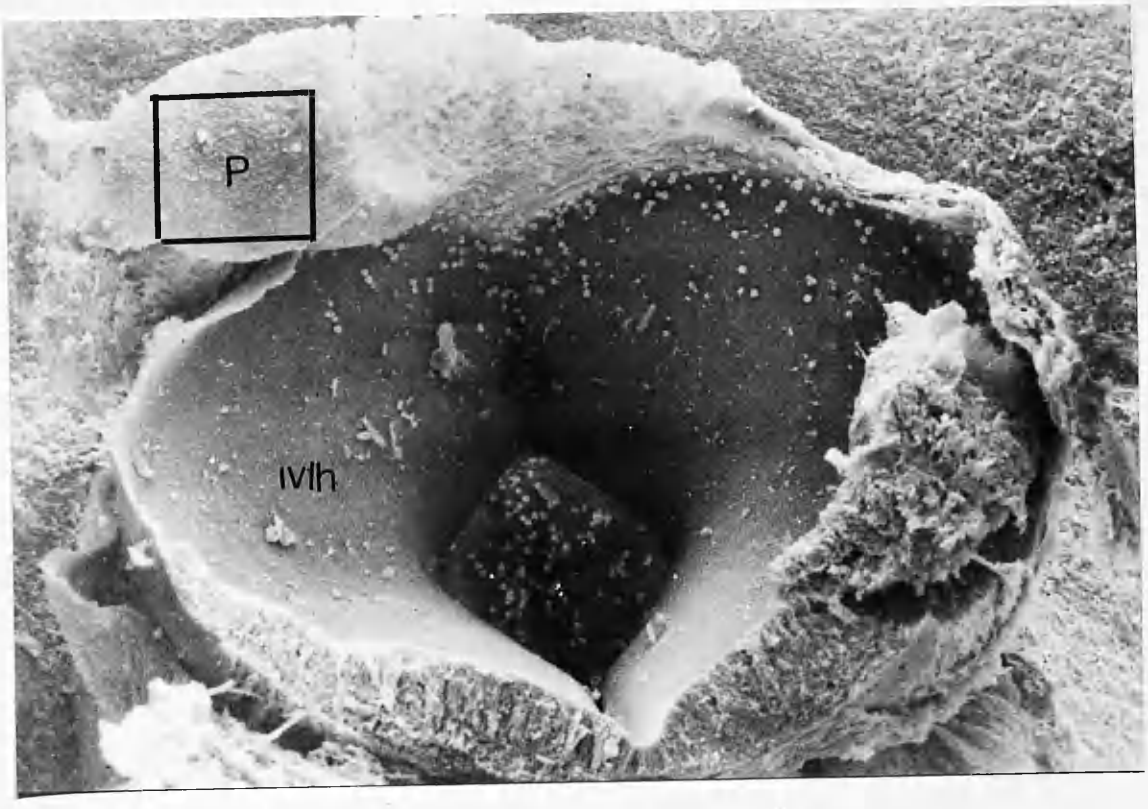

P
ivlh

Fig. 3

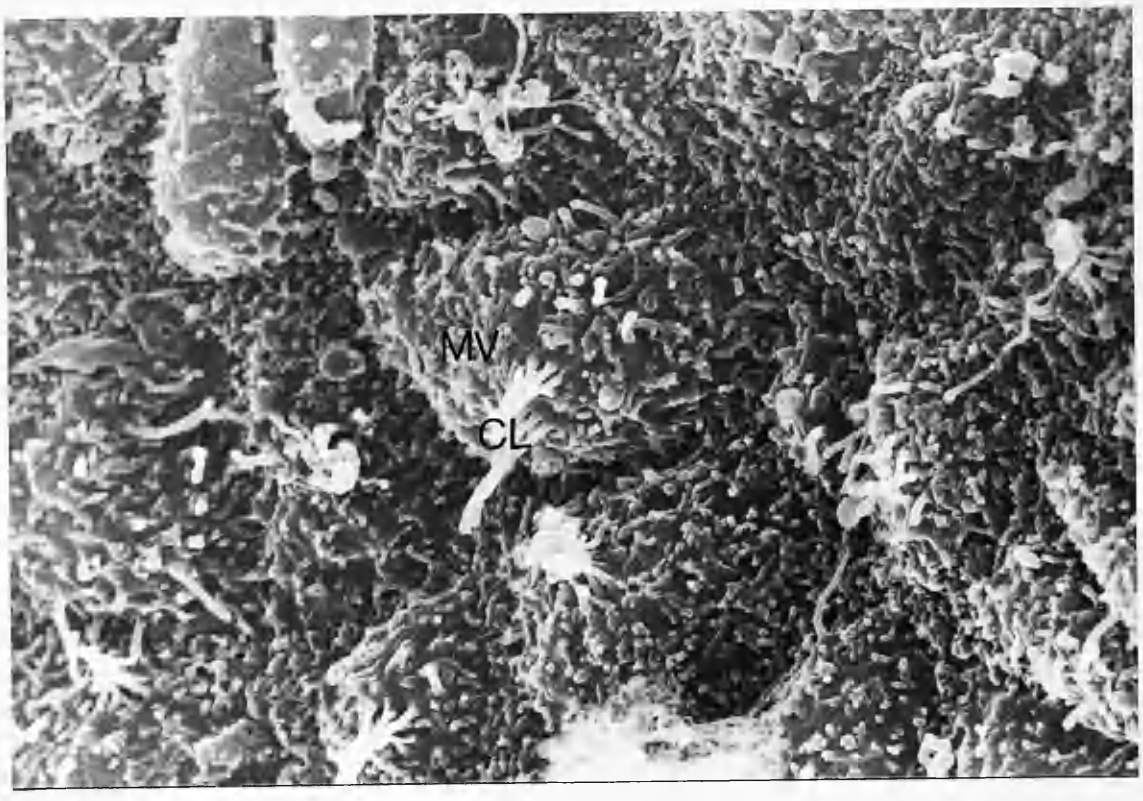
A high power SEM micrograph of the apical surface of the choroid plexus cells. They are dome-shaped with numerous microvilli (MV) and centrally placed cilia (CL).

11 day mouse embryo. x 6300

Fig. 4

High power SEM micrograph of the ependymal surface of the roof of the IVth ventricle. It shows ependymal cells as flat, irregular rectangles, with centrally placed single cilium (CL), and short sparse microvilli (MV), mostly abundant at the cellular margin.

11 day mouse embryo. x 6300


MV
CL

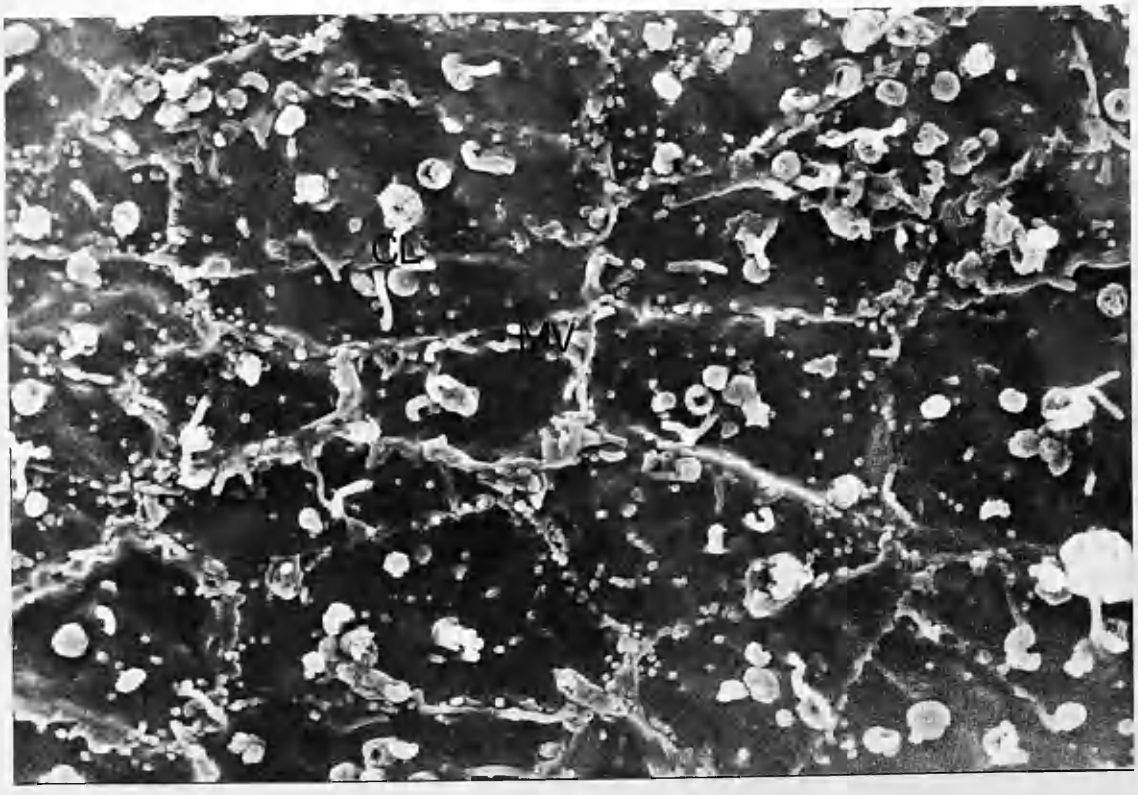

CL
MV

Fig. 5

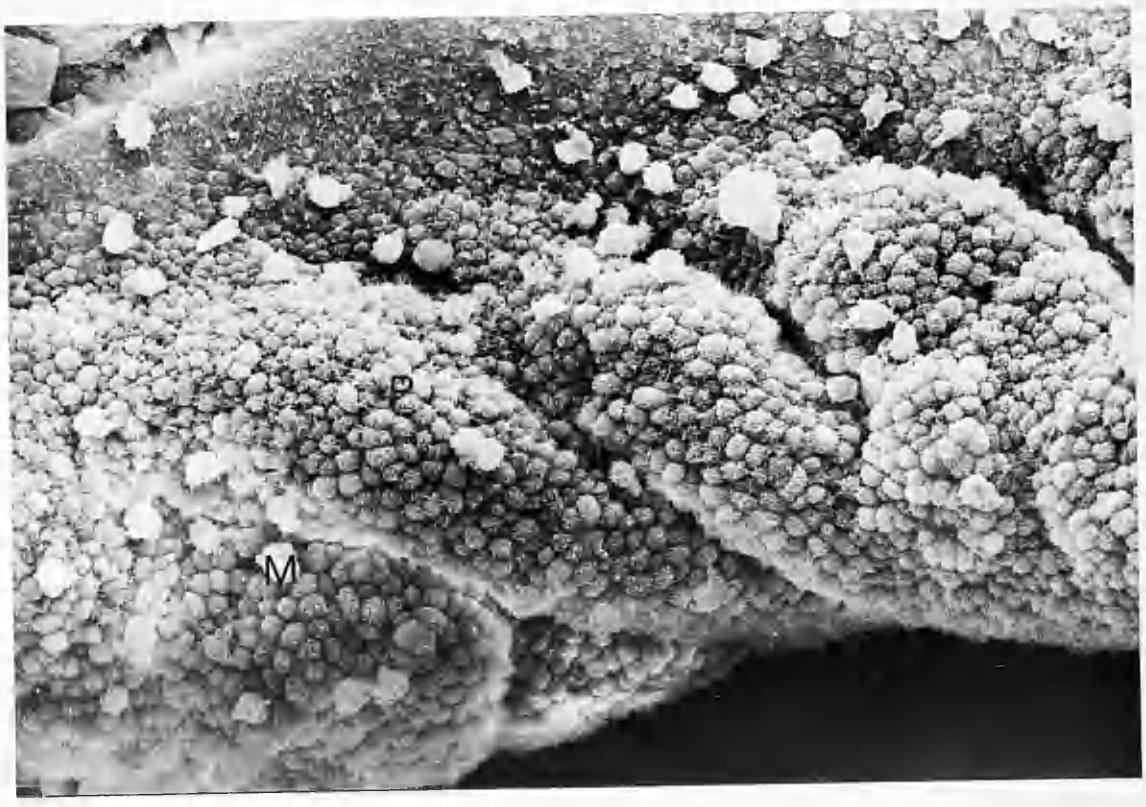
SEM micrograph of the parasagittal part of the choroid plexus (P) of the IVth ventricle. It has grown in size, become folded and invaginated into the ventricle.
M = macrophages

13 day mouse embryo. x 650

Fig. 6

This figure shows the lateral part of the choroid plexus (P). No folds were seen (compare with the parasagittal part, Fig. 5).
M = macrophages
X = cut surface of plexus

13 day mouse embryo. x 600


P
M

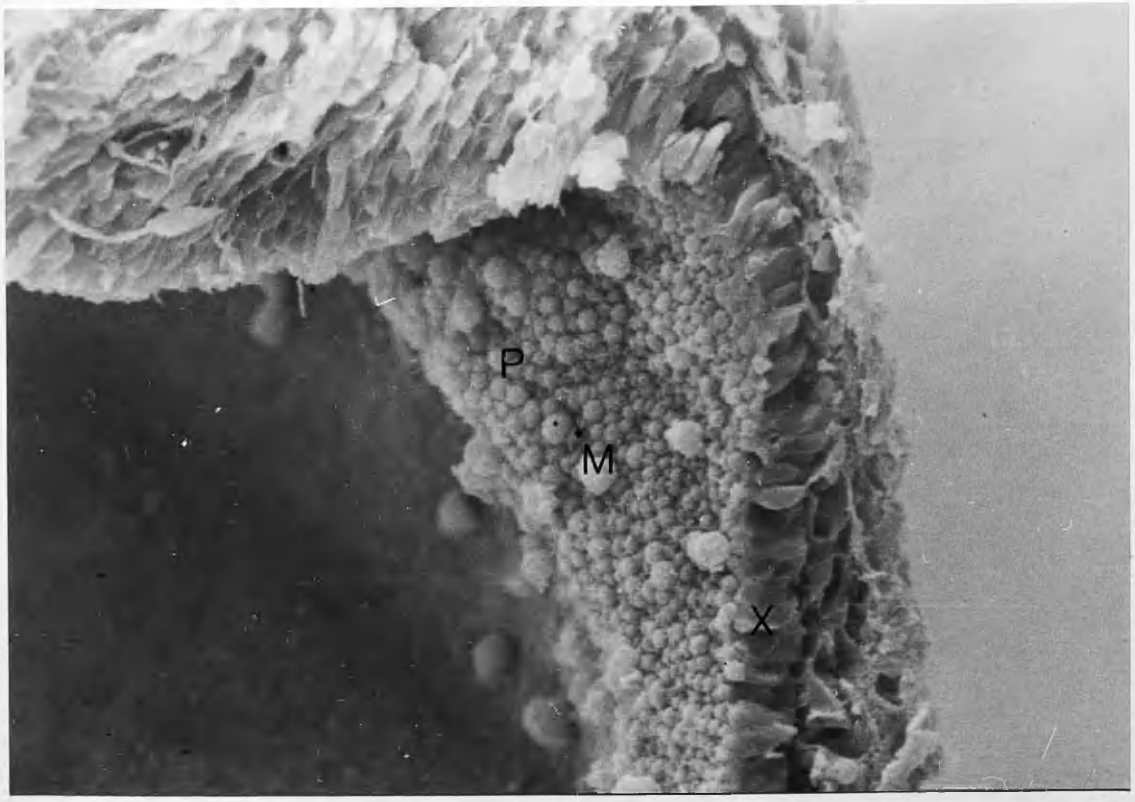

P
M
X

Fig. 7

A general view of the apical surfaces of the choroid plexus cells.
CL = cilia
MV = microvilli

13 day mouse embryo. x 6300

Fig. 8

A high power view showing a typical example of the cells (M) shown on the surface of the choroid plexus in Figs. 5 and 6. Its surface is generally smooth with several blebs and ruffles.

13 day mouse embryo. x 7500


CL
MV

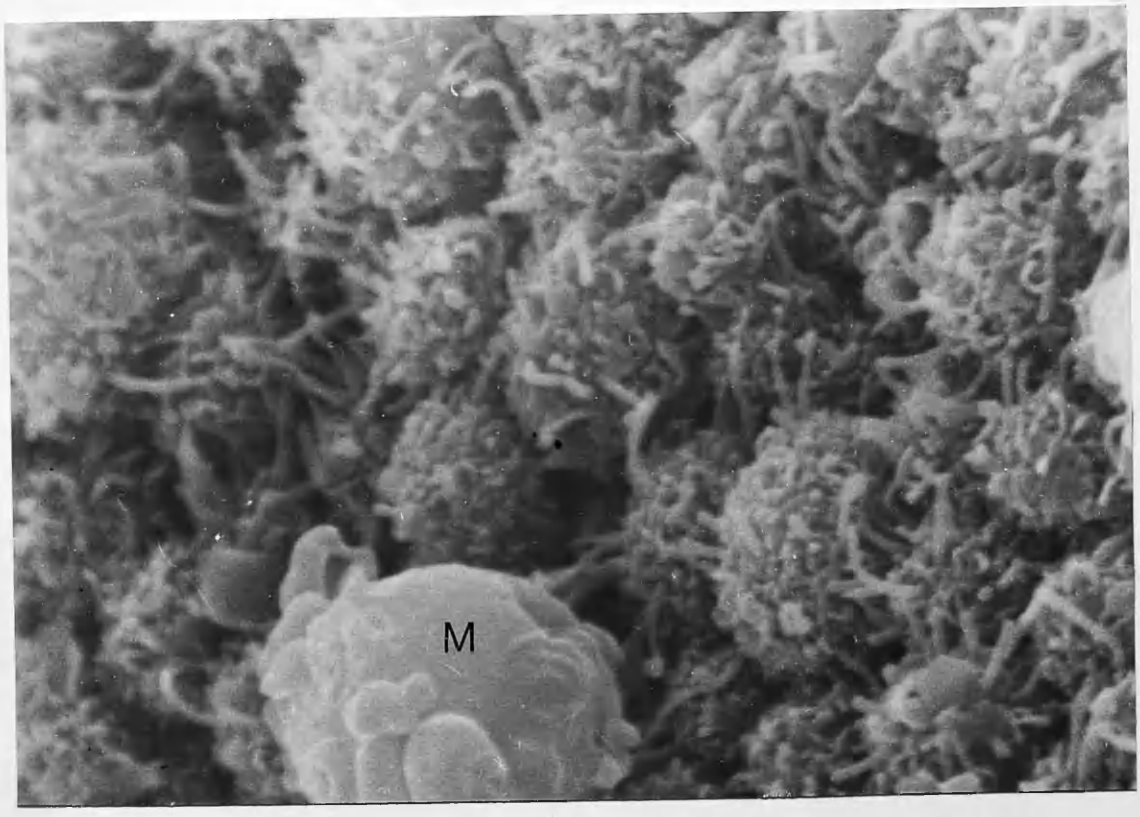

M

Fig. 9

SEM micrograph showing the convex apical surface of the choroid plexus cells completely covered by microvilli, and occasional cilia (CL).

14 day mouse embryo. x 5000

Fig. 10

Low power SEM micrograph of the choroid plexus (P) of the IVth ventricle. It shows the complex folding of the plexus.
IVth = IVth ventricle cavity

15 day mouse embryo. x 200


GE

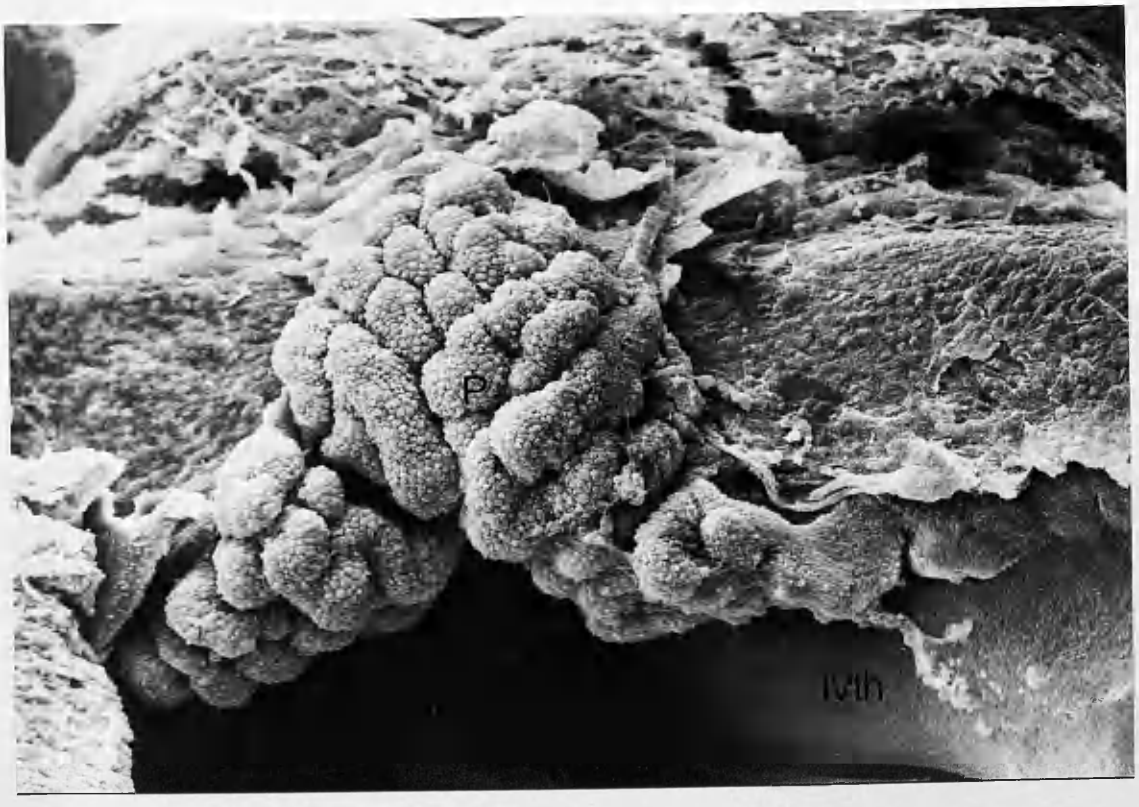

P
IVth

Fig. 11

A high power SEM micrograph of the convex apical surfaces of choroid plexus cells (P).
Numerous cytoplasmic processes (CP) are seen joining adjacent cells to each other.
What looks like ruptured blebs are also seen (arrows).
Inset - see Fig. 12

15 day mouse embryo. x 7500

Fig. 12

Higher power of the area outlined in Fig. 11.
CP = cytoplasmic process connecting adjacent cells together.
Arrow = Ruptured bleb - possibly connected to the cell surface with a stalk (S).

15 day mouse embryo. x 30,000


P
cp
P

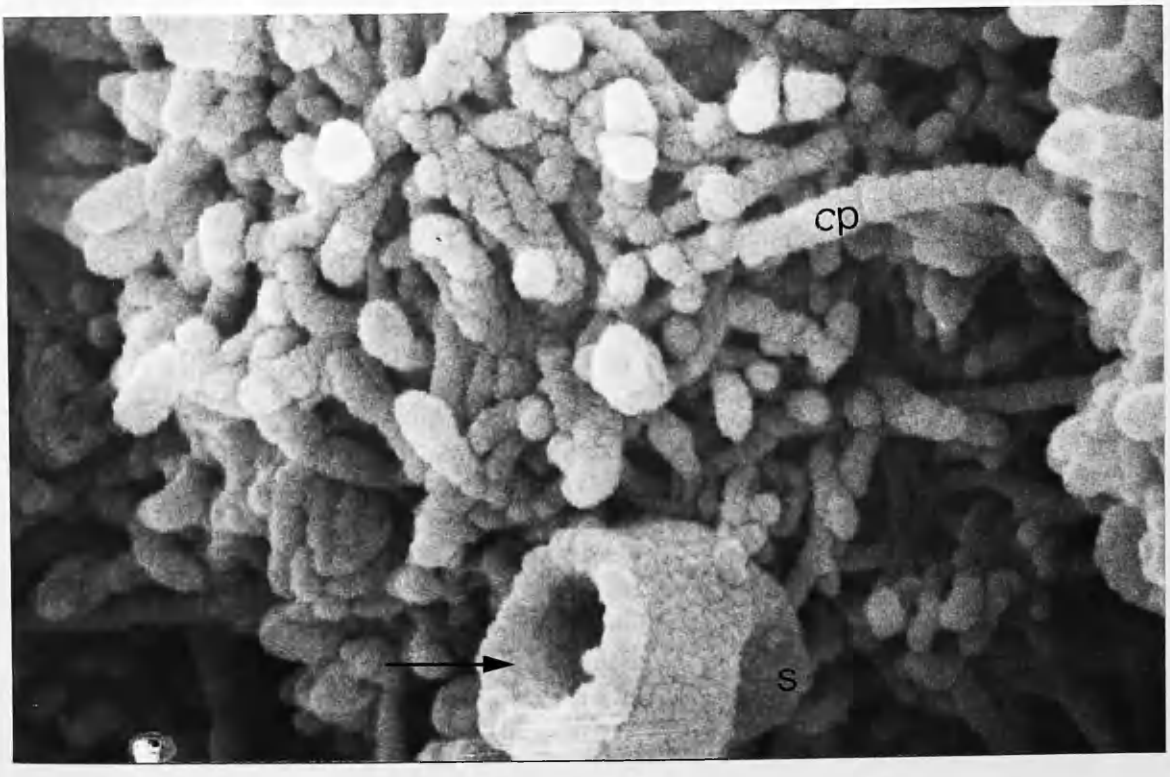

cp
s

Fig. 13

A general low power view showing the entire extent of the choroid plexus of the IVth ventricle.
P = choroid plexus
IVth = IVth ventricle

17 day mouse embryo. x 125

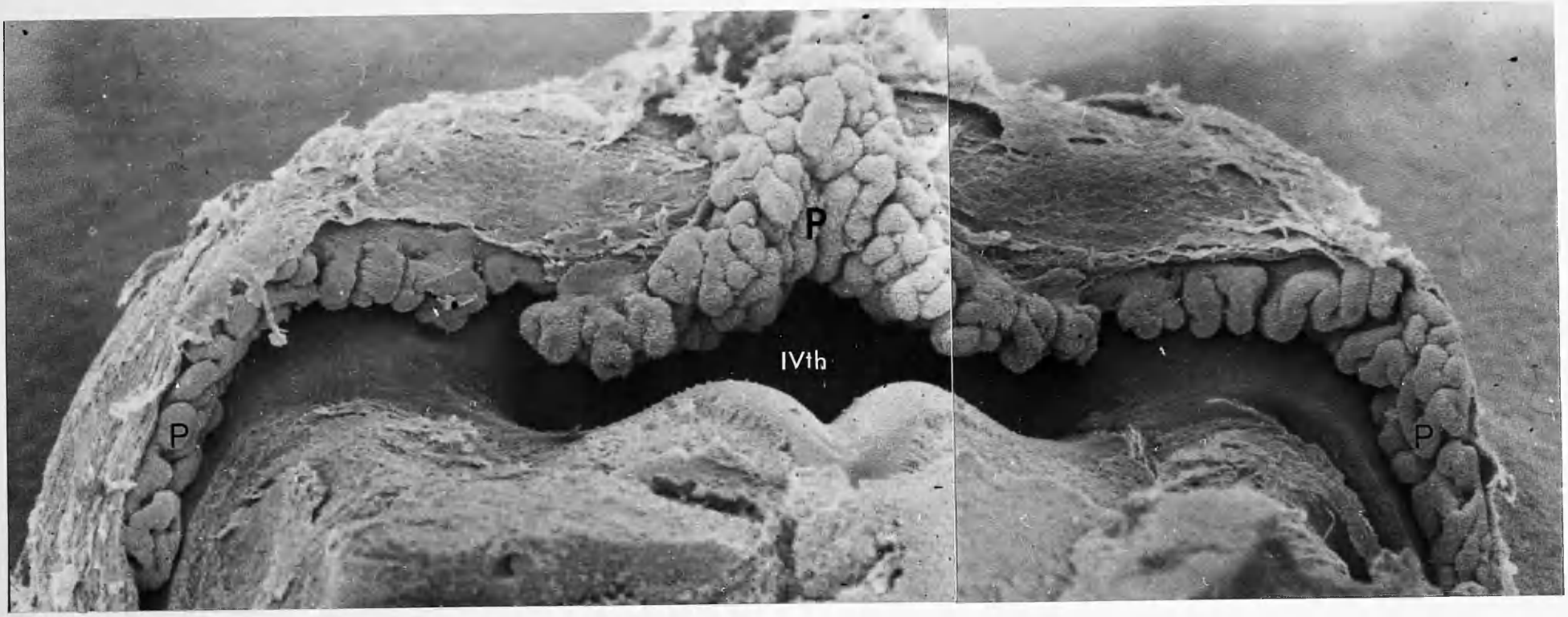

P
IVth
P
P

Fig. 14

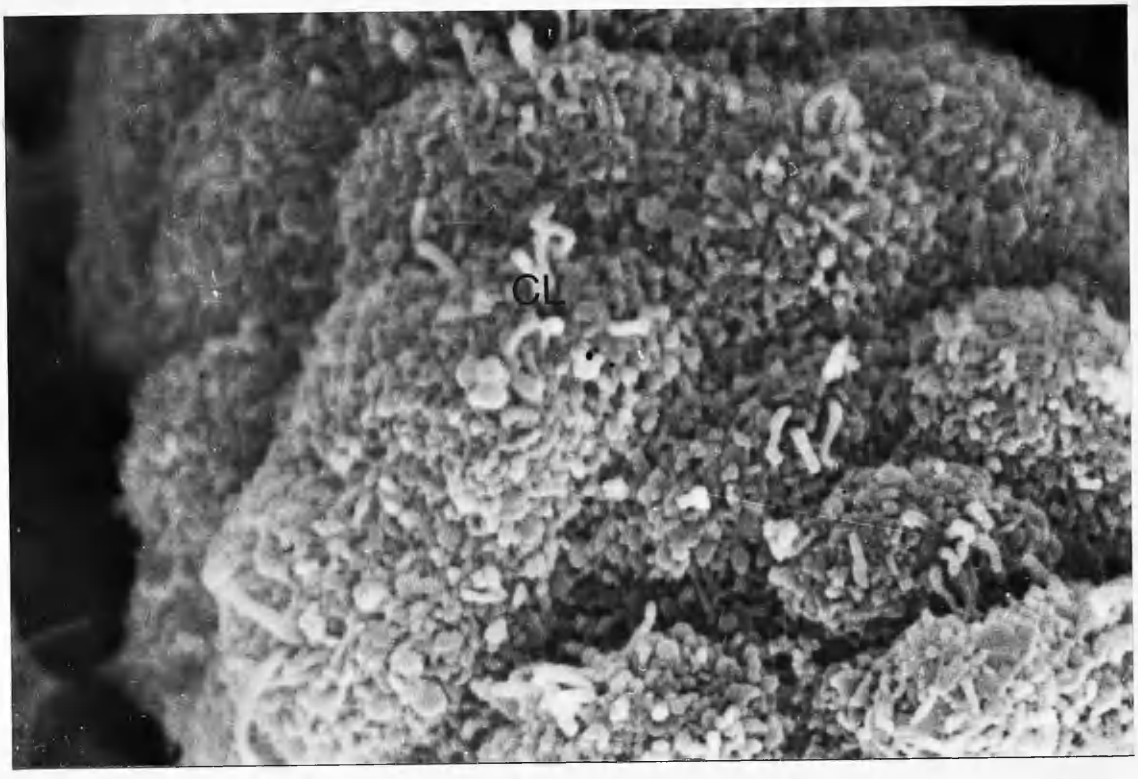
High power view (SEM) of the apical surfaces of choroid plexus cells. They are completely covered by numerous microvilli of different sizes and shapes; and cilia (CL).

17 day mouse embryo. x 10,000

Fig. 15

High power SEM micrograph of the ventricular surface of the ependymal roof of the IVth ventricle.
The ependymal cells are sparsely covered by short microvilli, more abundant at the margin (arrows).
CL = centrally placed cilium
The appearance is not dissimilar from that at 11 day stage (see Fig. 4).

17 day mouse embryo. x 5000


CL

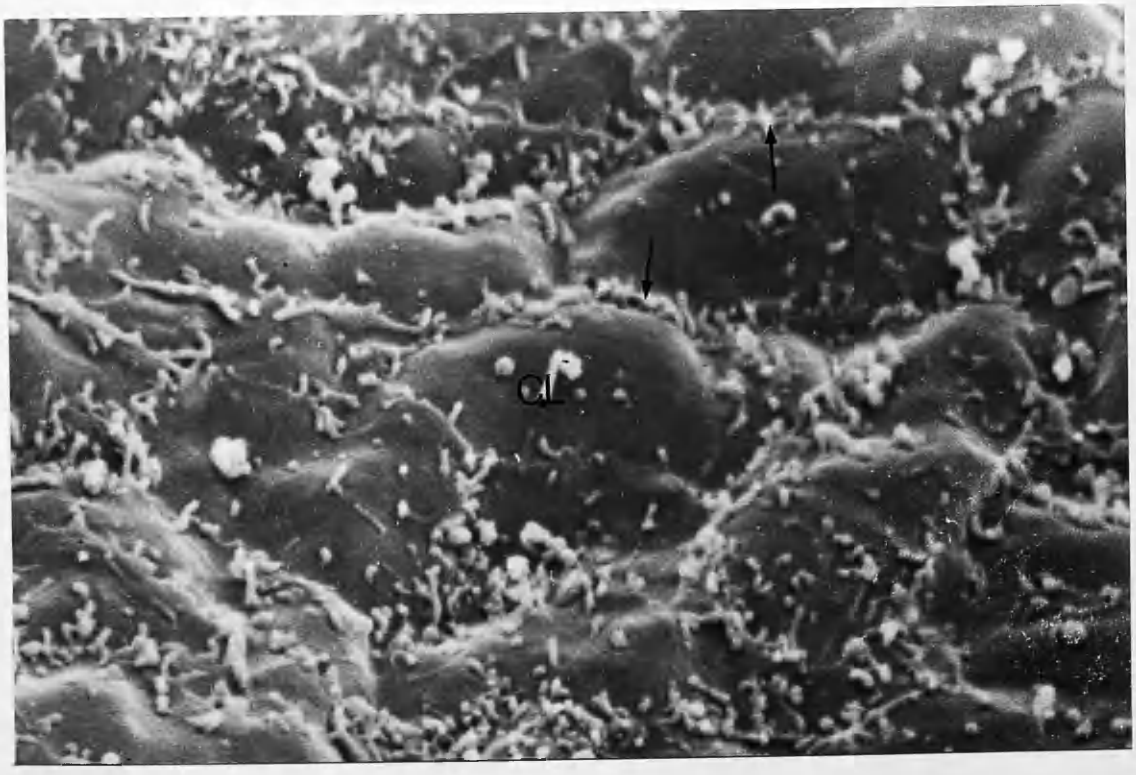

CL

Fig. 16

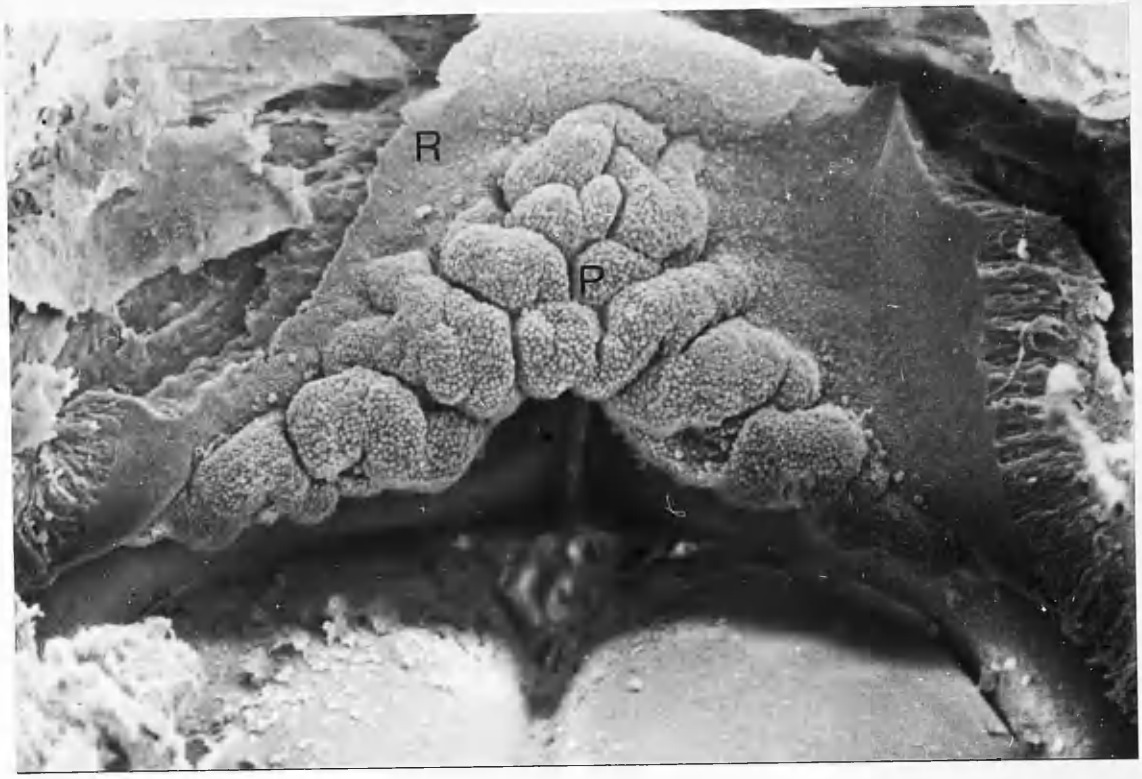
A low power view of the midline part of the choroid plexus (P) of the IVth ventricle. Its surface is very convoluted.
The smooth ependymal roof (R) is shown.

18 day mouse embryo. x 150

Fig. 17

High power view of apical surfaces of choroid plexus cells (P) covered with microvilli.
CP = Cytoplasmic processes between adjoining cells.

18 day mouse embryo. x 10,000


R
P

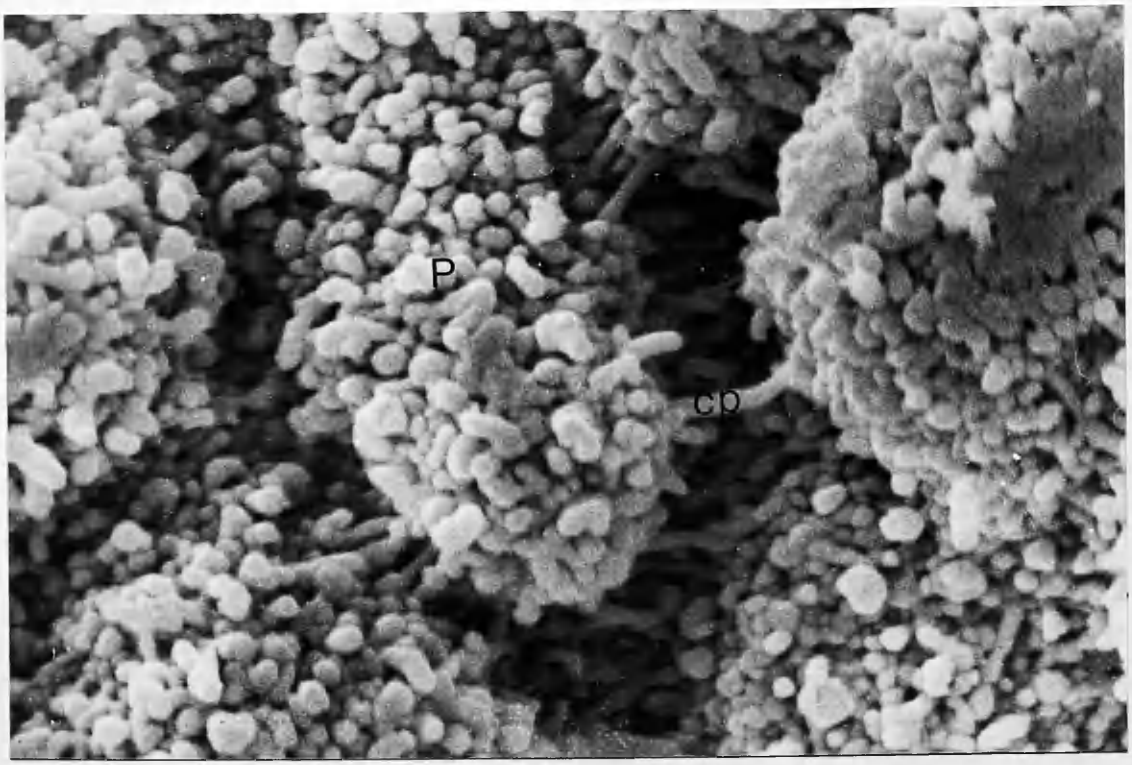

P
cp

Fig. 18

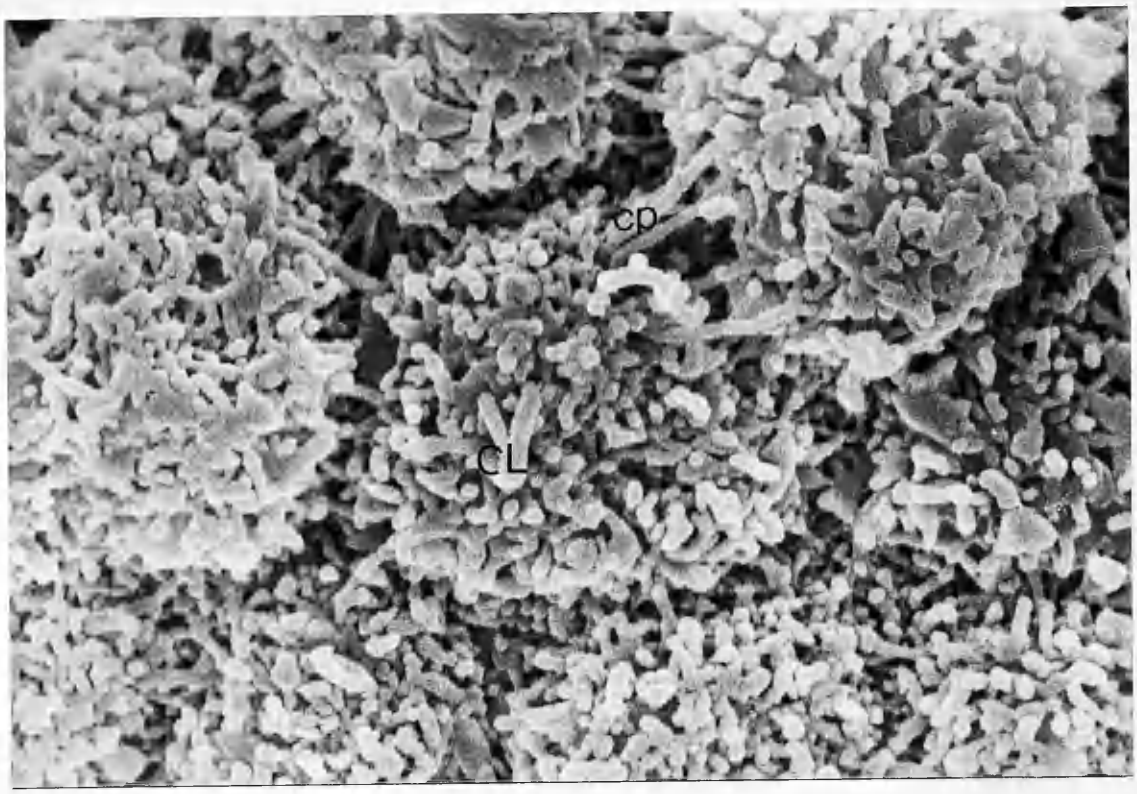
High power SEM micrograph of the surface of the choroid plexus showing similar features to those at 18 day stage (Fig. 17).
CL = cilia
CP = cytoplasmic processes

19 day mouse embryo. x 10,000

Fig. 19

Shows the numerous microvilli of different sizes and shapes covering the apical surfaces of choroid plexus cells.

Newborn mouse. x 15,000


cp
CL

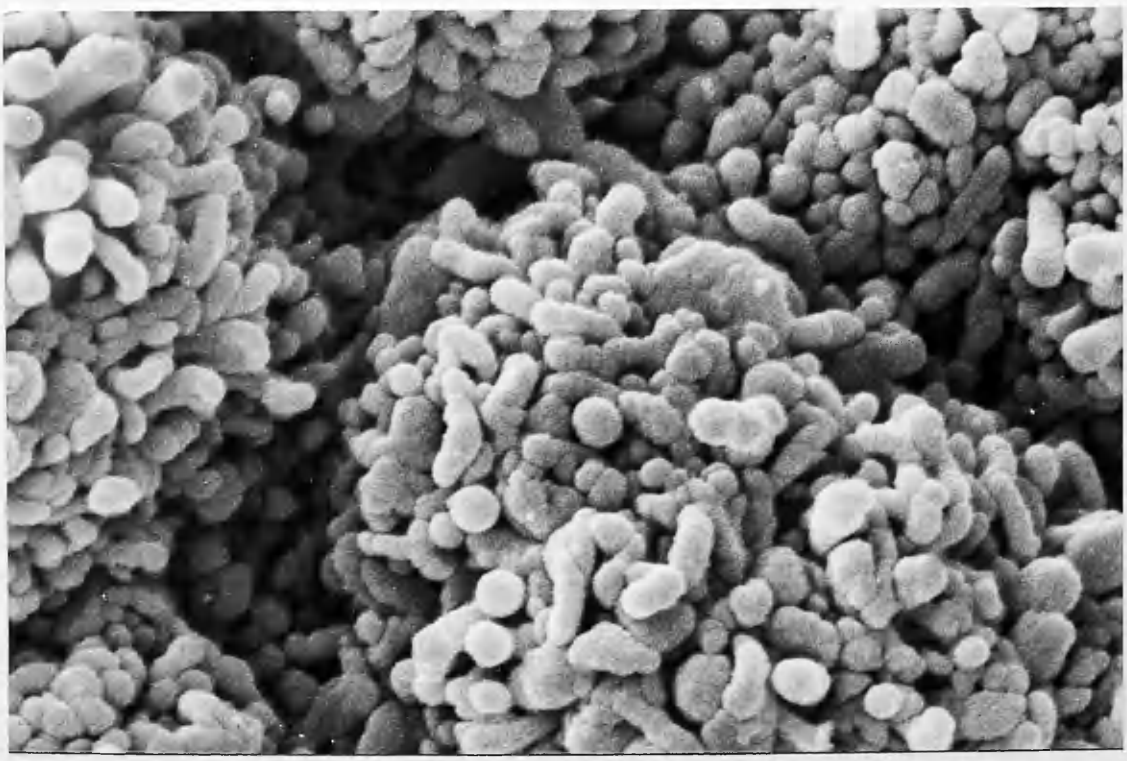


Fig. 20

High power view of the inner aspect of the ependymal roof of the IVth ventricle. The cell surfaces are incompletely covered by short microvilli (MV). Some cells have tufts of cilia (CL).
Cell borders are sometimes seen (arrows).

Newborn mouse. x 6000

Fig. 21

High power view showing the apical surface of choroid plexus cells densely covered by microvilli.
Secretory blebs (arrows) can also be seen.

Adult mouse. x 22,500


Cl

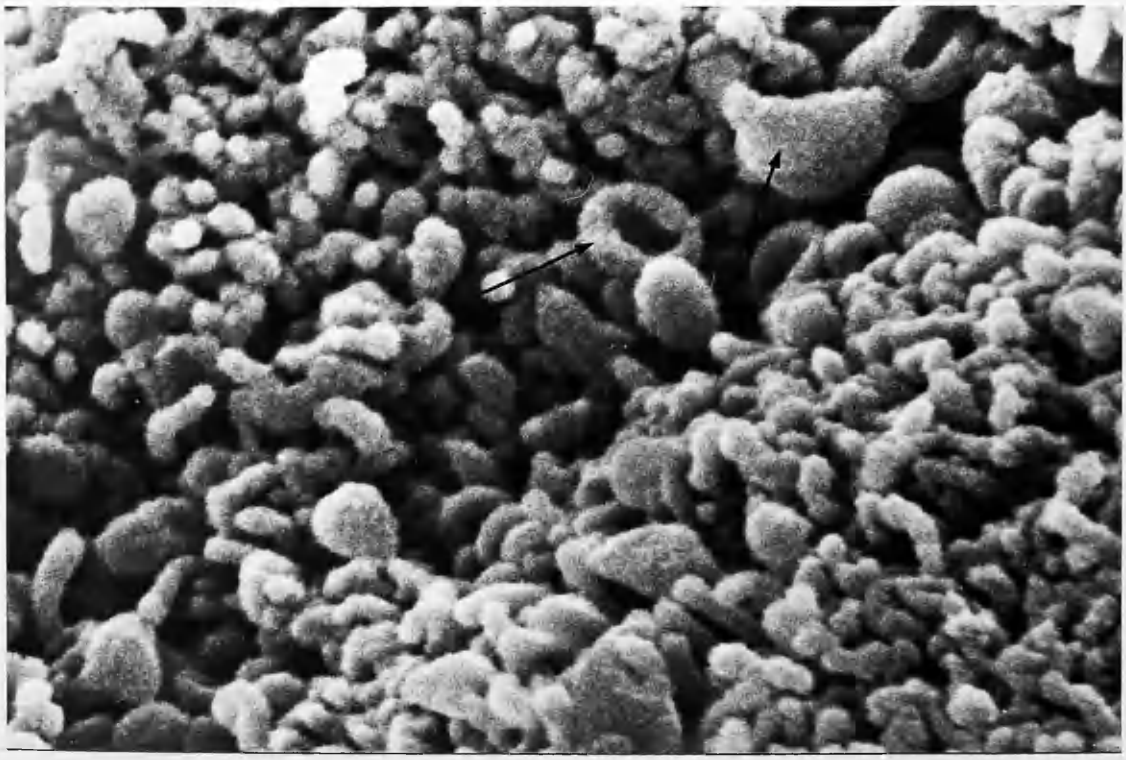


Fig. 22

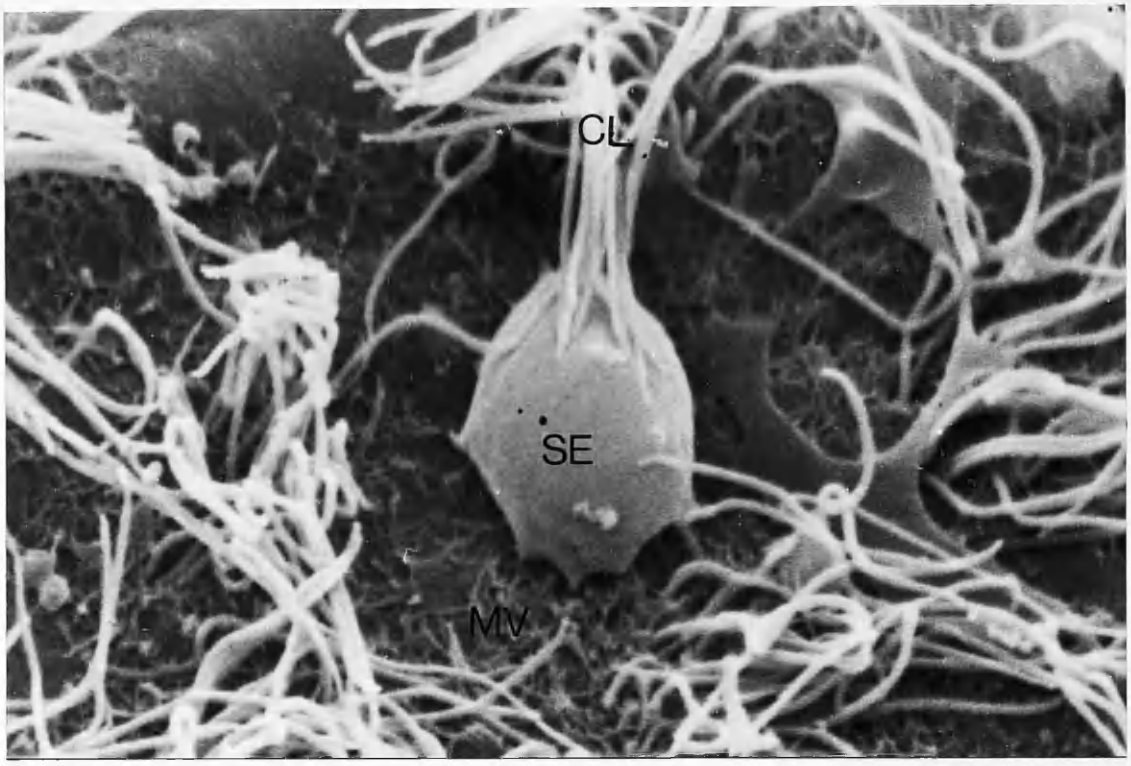
High power SEM micrograph of the ventricular surface of the ependymal roof of the IVth ventricle. Tufts of long cilia (CL) arise nearer the border of the cells while the rest of the surface is incompletely covered by short microvilli (MV).
SE = A smooth spherical supraependymal cell.

Adult mouse. x 6000

Fig. 23

Another supraependymal cell (SE) is shown. It has long processes (arrows) which branched and rebranched. Some of the branches contain varicosities (asterix).
CL = cilia

Adult mouse. x 6000


CL
SE
MV

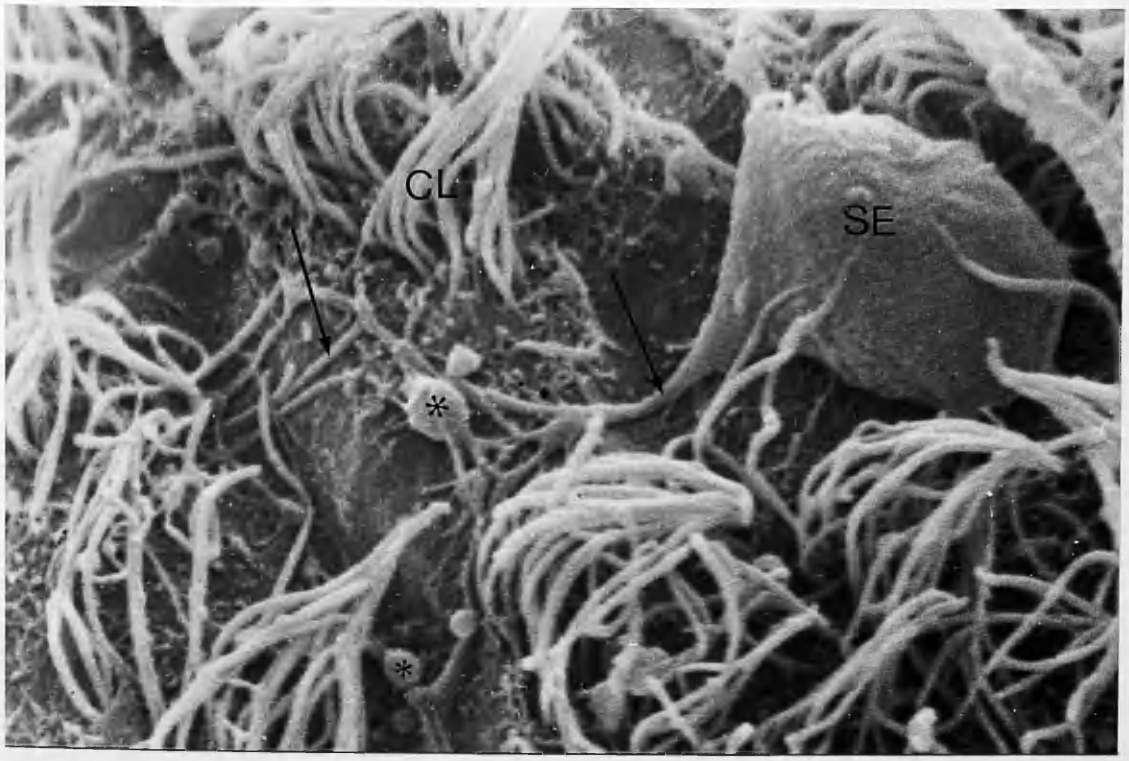

CL
SE
*
*

Fig. 24

TEM micrograph of the developing choroid plexus. The cells rest on a well defined basal lamina (arrows). The bulging apical surfaces have very few microvilli (MV).
M = mitochondria
N = nucleus
Arrowheads = glycogen granules
Thick arrow = pedunculated body packed with vesicles.

11 day mouse embryo. x 8400

2802715

Figs. 25 and 26

Choroid plexus cells now show numerous microvilli (MV), interdigitation of the basilateral membrane (I). Cells with paler cytoplasms were seen.

G = golgi apparatus
BV = blood vessel, closely applied to the epithelium
Arrows = basal lamina
GL = Glycogen clumps
L = lipid inclusions
LY = lysosomes

13 day mouse embryo. x 8400 x 8400

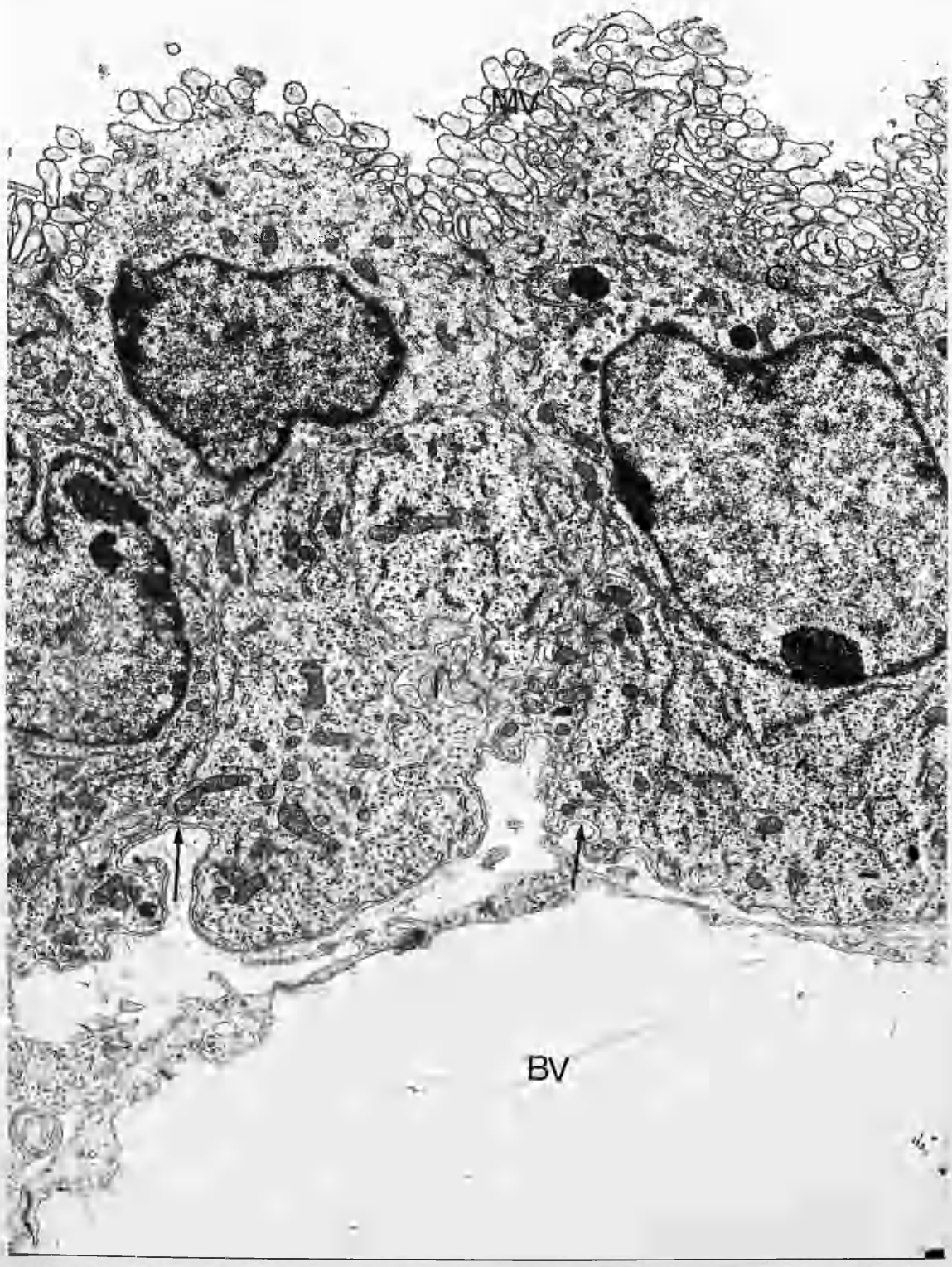

MV
BV

Fig. 27

TEM micrograph of the choroid plexus epithelium showing features similar to those of 13 day mouse stage (Fig. 25).
MV = microvilli
I = interdigitation of basilateral membrane
GL = glycogen clumps
G = Golgi apparatus
Small arrows = basal lamina
Large arrows = pinocytotic vesicles
A nerve fibre (NV) is seen in contact with a blood vessel (BV).

14 day mouse embryo. x 8400

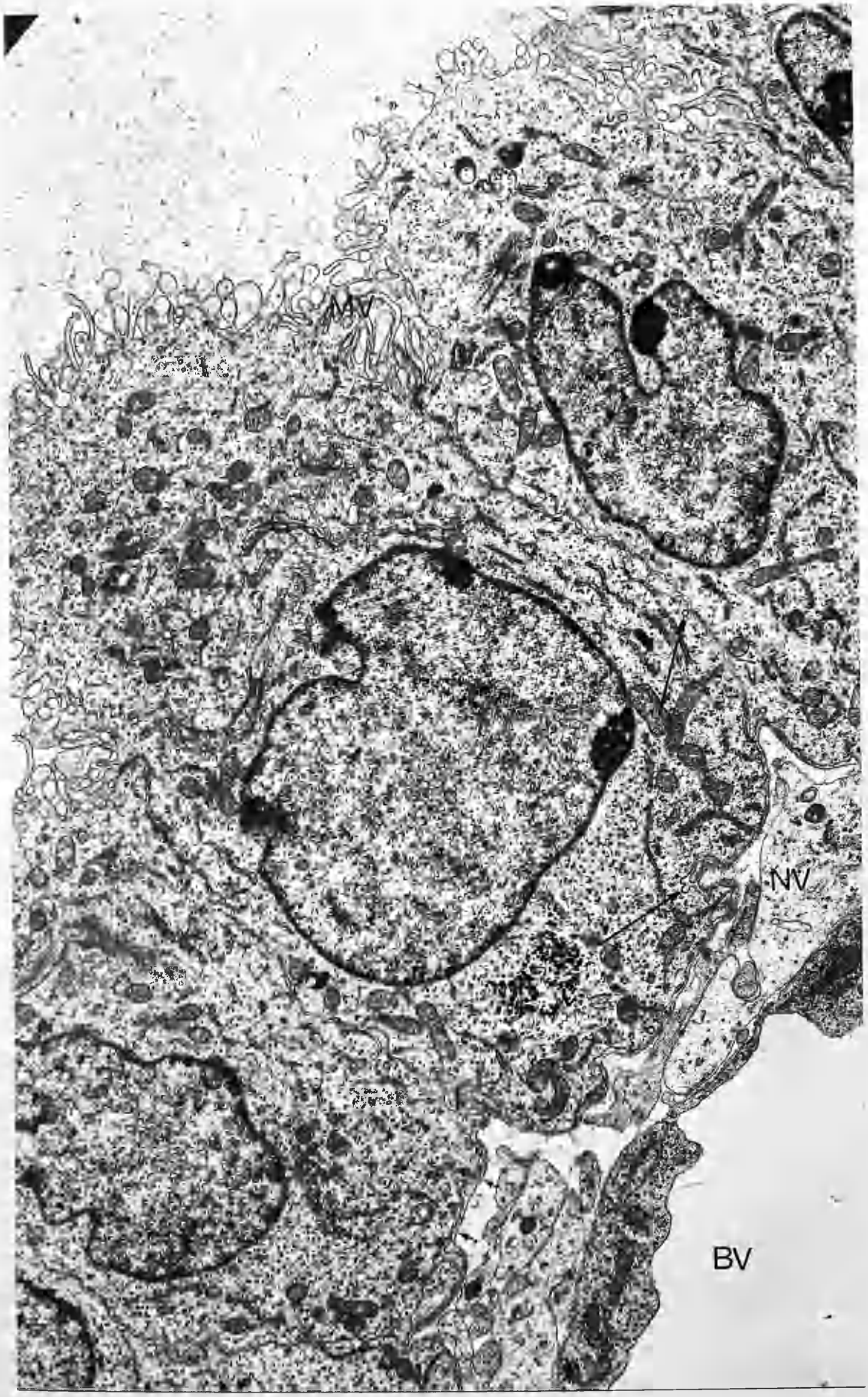

MV
NV
BV

Fig. 28

A phagocytic (epiplexus) cell is lying on the apical border of a choroid plexus cell.
MV = microvilli
CL = cilium

14 day mouse embryo. x 18000

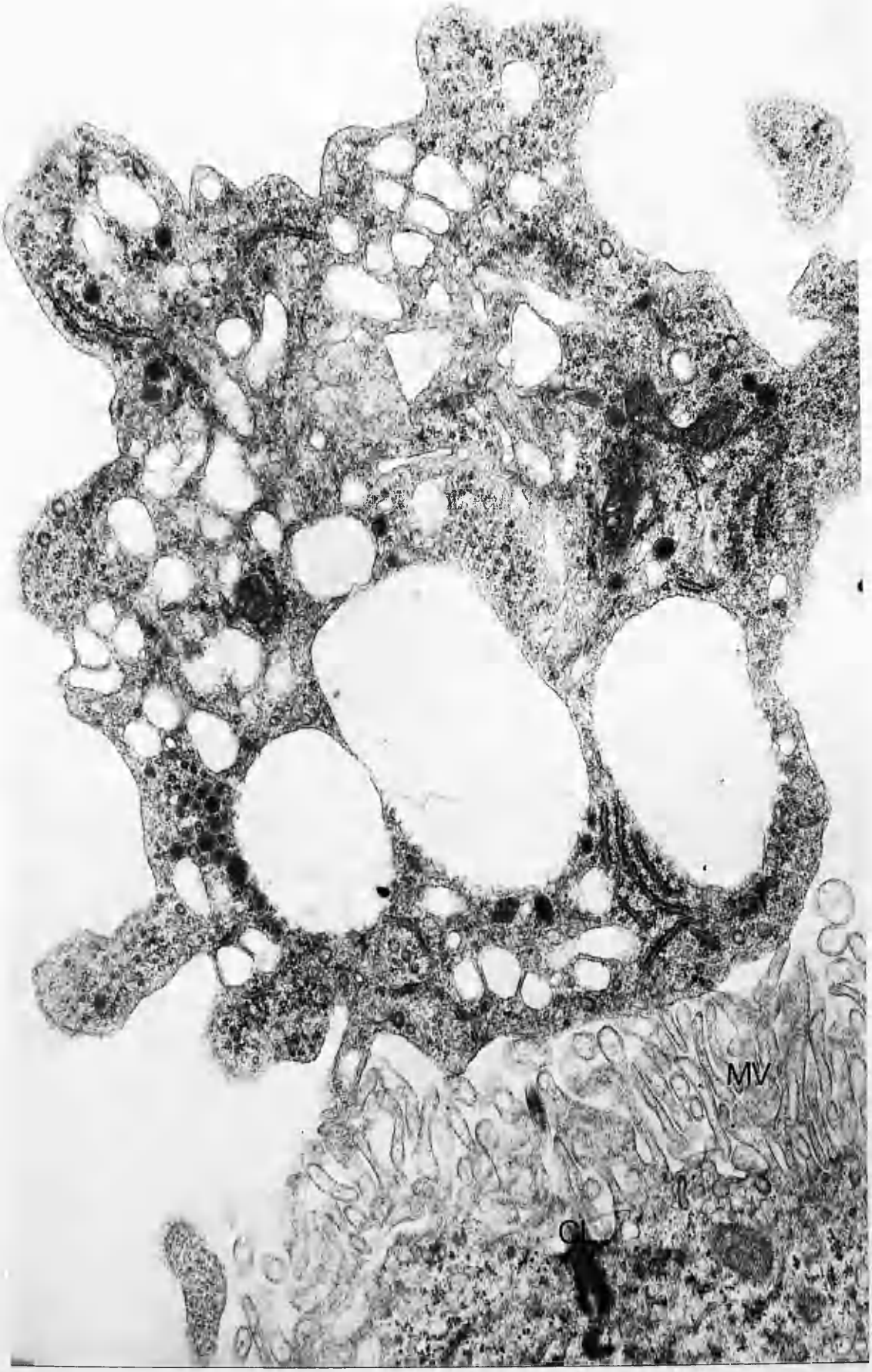

MV

Fig. 29

A blood vessel (BV) containing red blood cells (RBCs) lies just beneath the choroidal epithelium. This rests on well defined basal lamina (arrows), has interdigitated basilateral membrane (I), and numerous microvilli (MV) on the apical border.
GL = glycogen aggregate

15 day mouse embryo. x 8400

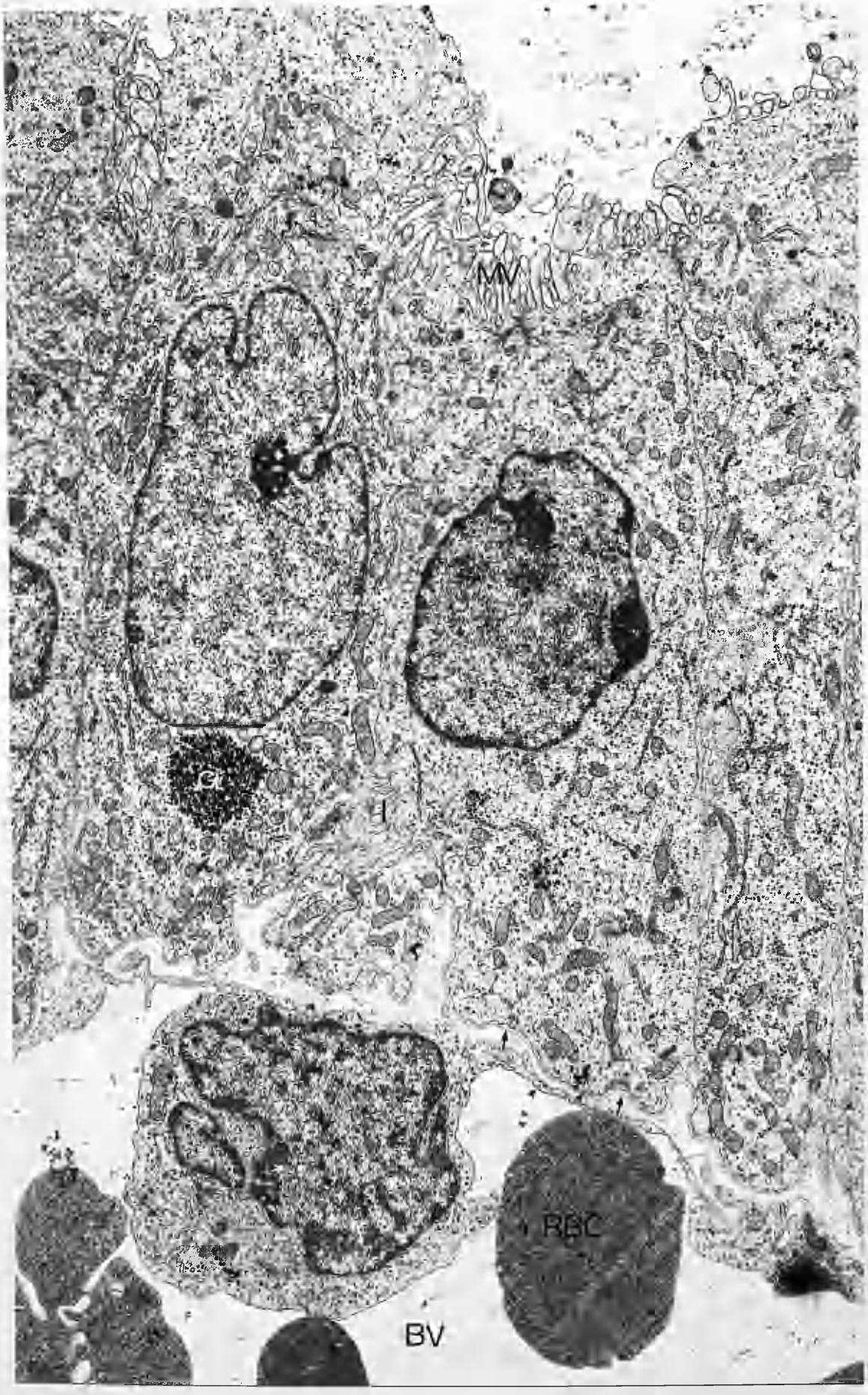
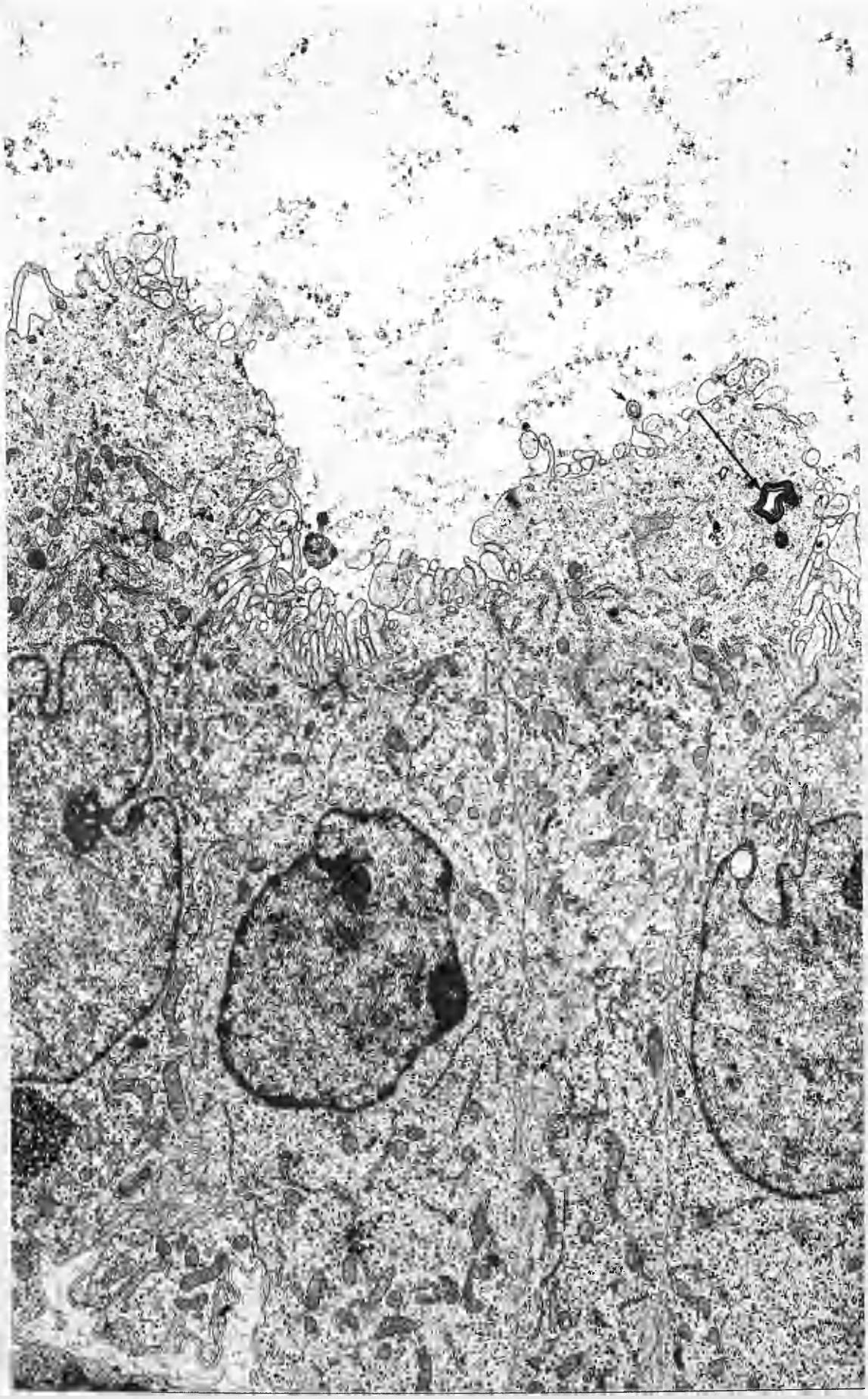

MV
RBC
BV

Fig. 30

A laminated body with vacuolated centre is seen (large arrow).
A cross section of a cilium is identified by a small arrow.

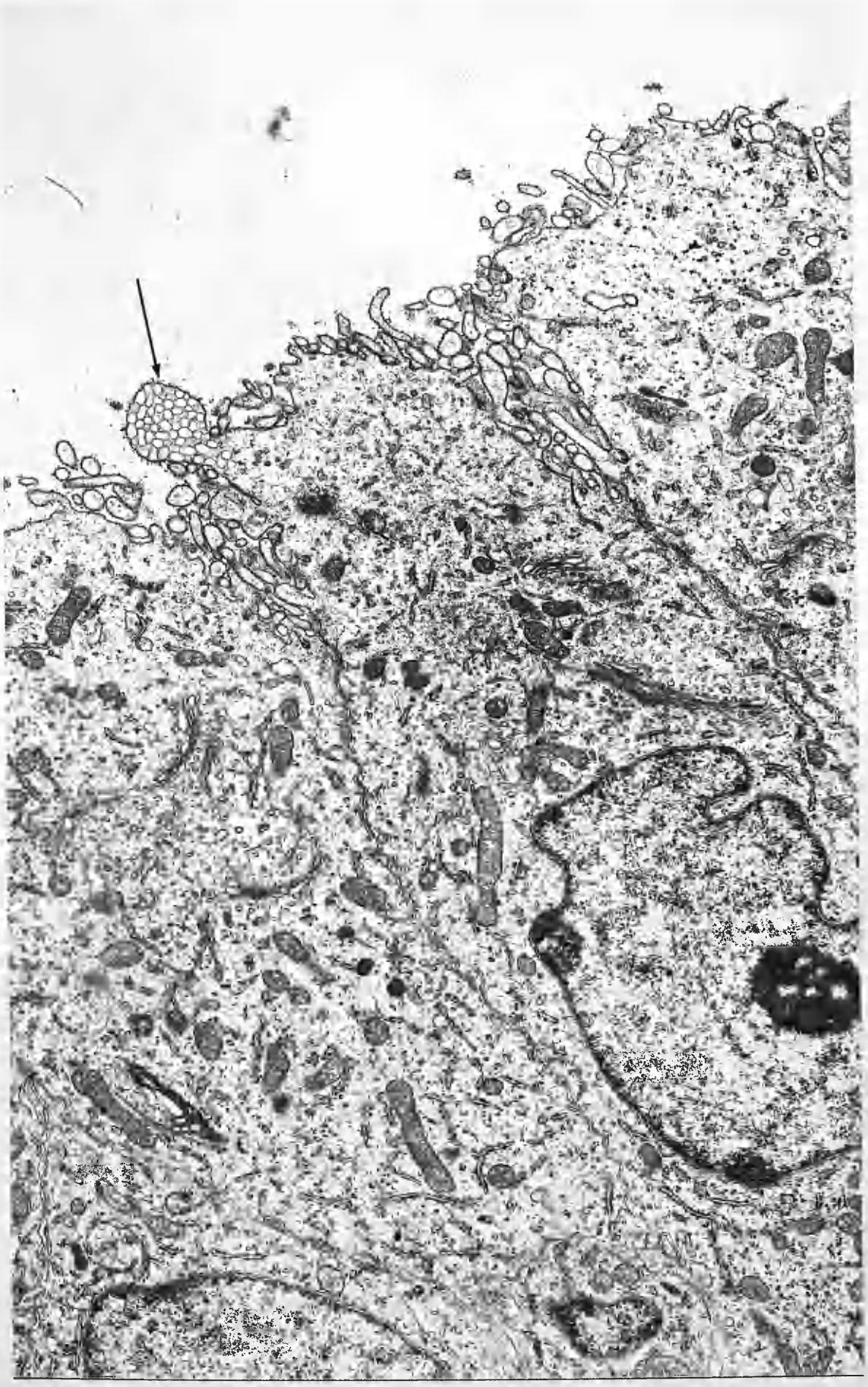
15 day mouse embryo. x 8400



Figs. 31 and 32

A pedunculated body containing numerous vesicles is seen on the apical border of the cell (arrow).

15 day mouse embryo. x 12,500 x 16,800



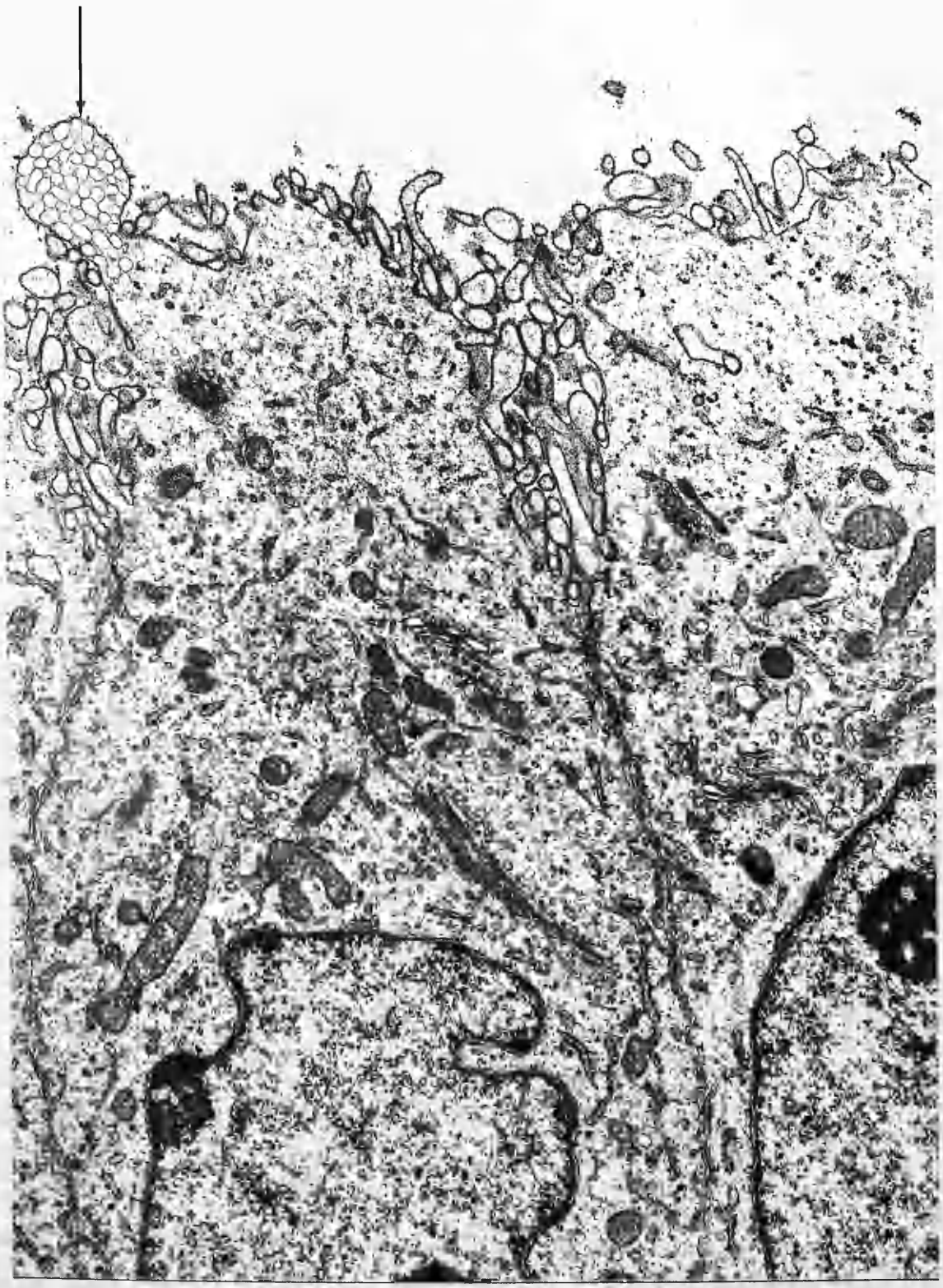


Fig. 33

Shows that the general structure of the choroid plexus cells is little changed compared to that of previous stages.
A cell with a paler cytoplasm containing lipid inclusions (L) is seen (also see Fig. 26).
MV = microvilli
I = interdigitations of basilateral cell membranes
BV = blood vessel - closely applied to the epithelium.
Arrows = well defined basal lamina

16 day mouse embryo. x 8400

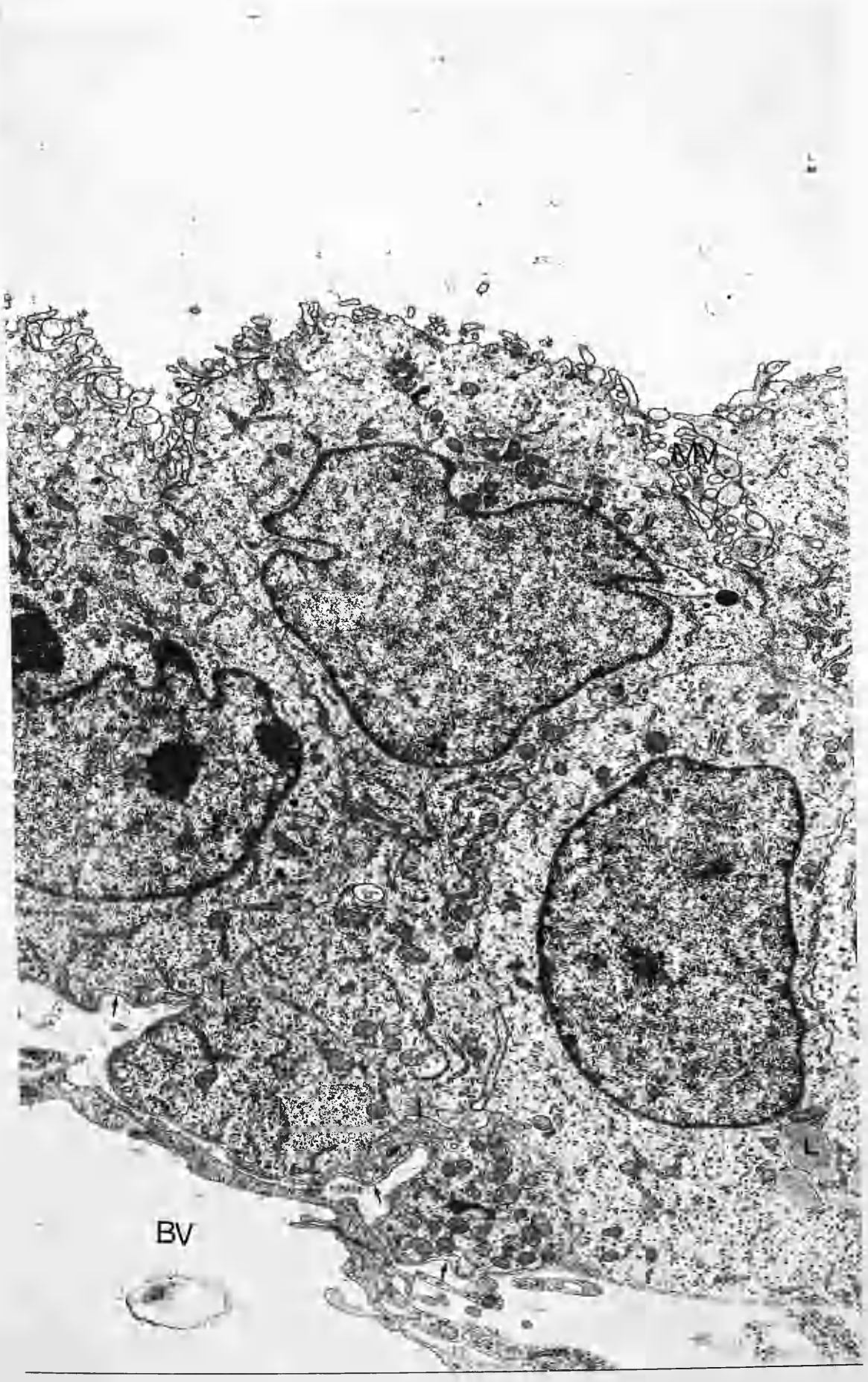
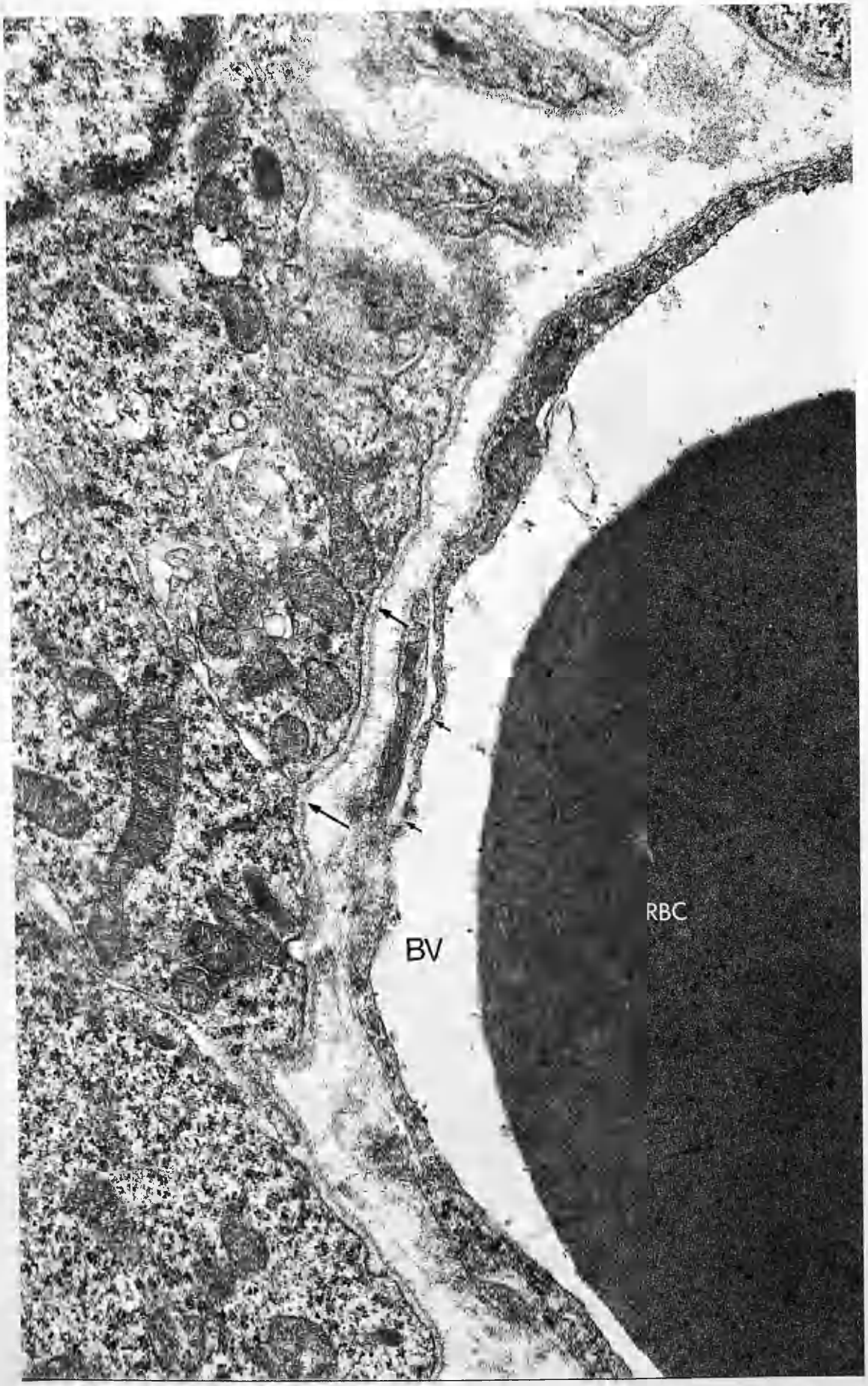

BM
L
BV

Fig. 34

High power TEM micrograph of endothelial wall of choroidal plexus capillary (BV), containing a red blood cell (RBC) lying just beneath the choroidal epithelium.
Small arrows = fenestrations in endothelium
Large arrows = epithelial basement membrane. This is well defined compared to its vascular counterpart.

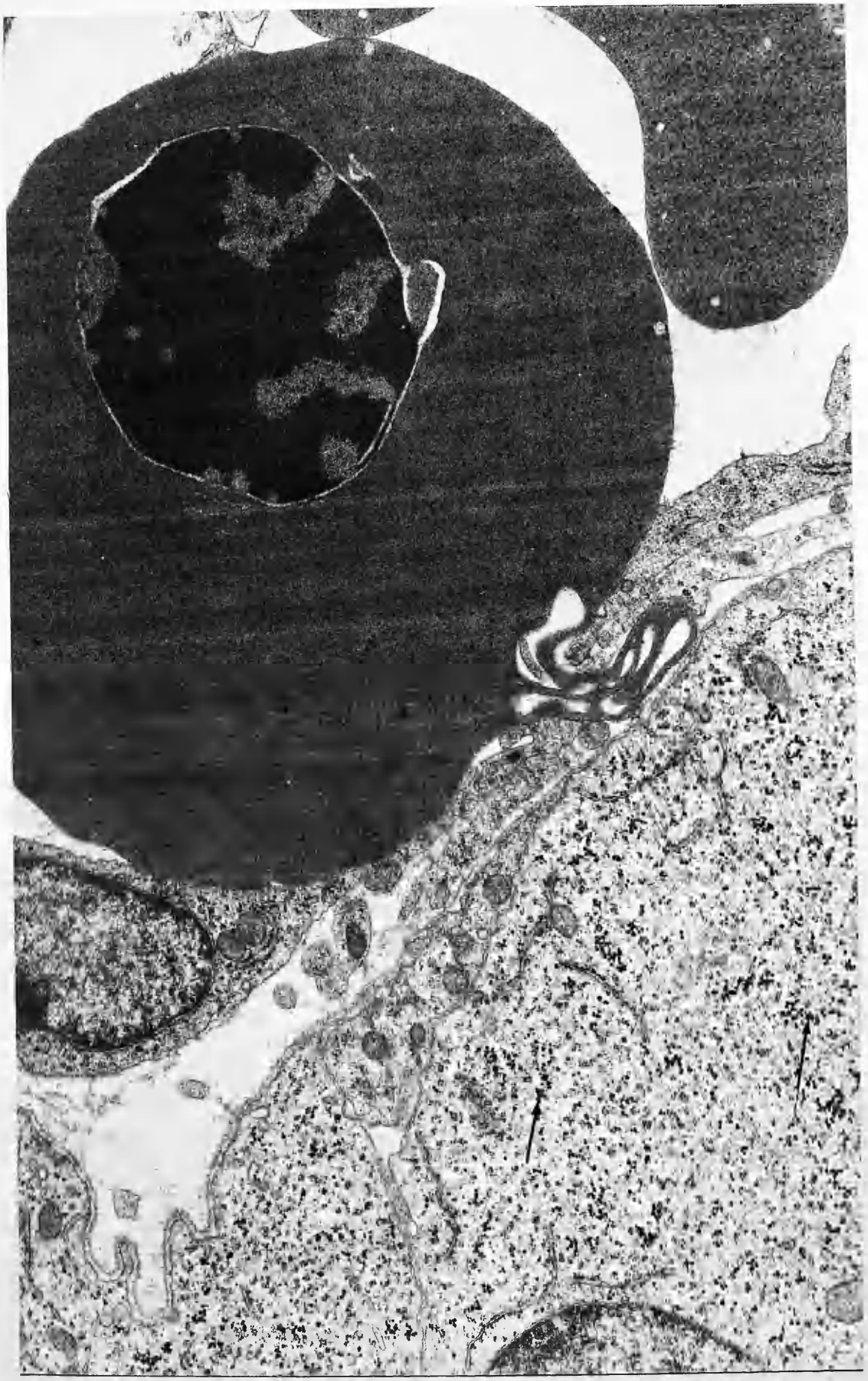
16 day mouse embryo. x 42,000


BV
RBC

Figs. 35 and 36

Showing a nucleated red blood cell in the process of extravasating through the endothelial wall. Glycogen granules forming small clumps are clearly shown in the basal part of choiroid plexus cells (arrows).

16 day mouse embryo. x 12,500 x 30,000



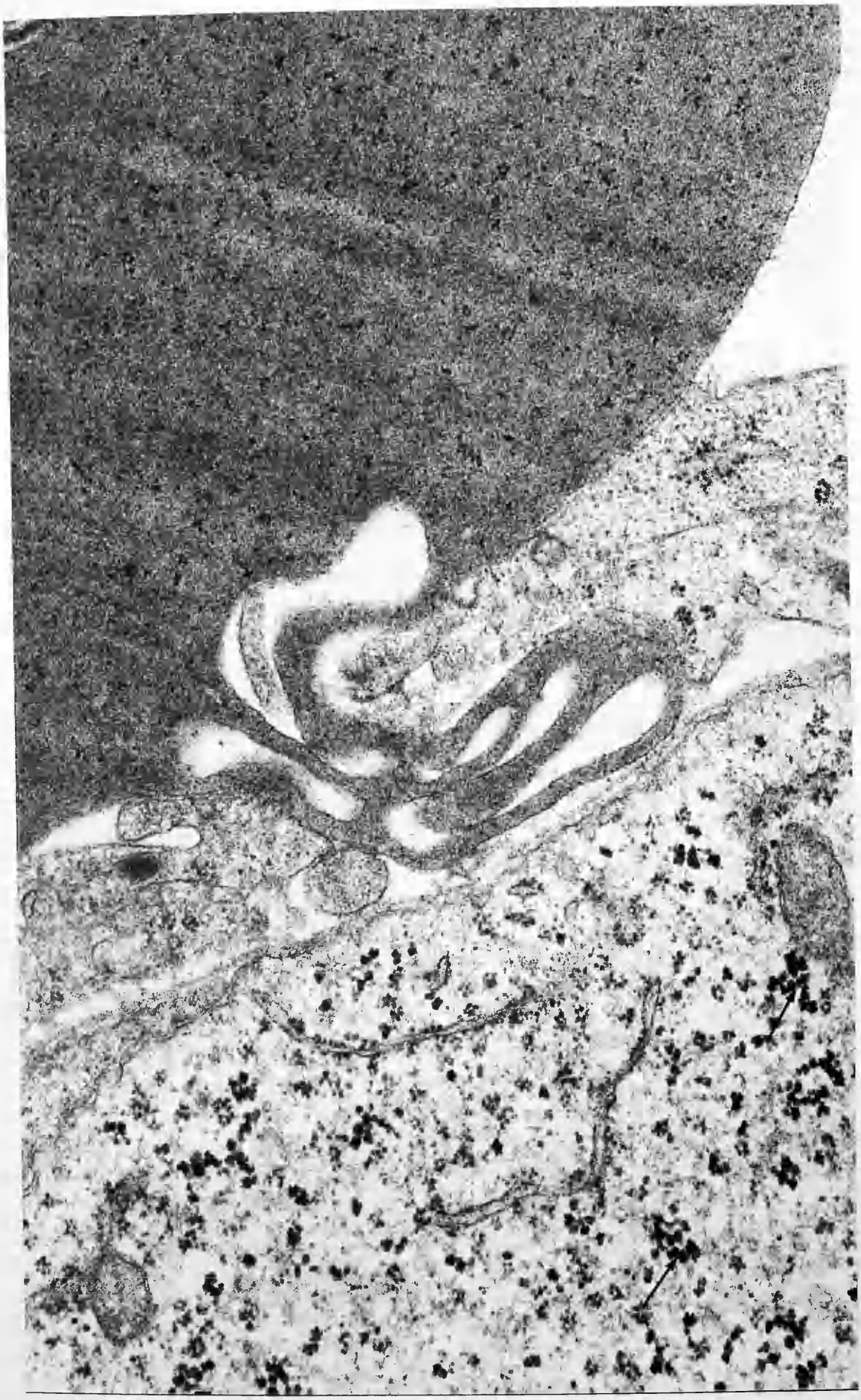


Fig. 37

The general appearance of the choroidal epithelium is unchanged.
N = nucleus
G = Golgi apparatus
M = mitochondria
RER = rough endoplasmic reticulum
GL = glycogen clumps
Arrows = basal lamina
BV = blood vessel
Two red blood cells (RBC) are lying free in the extracellular space.

17 day mouse embryo. x 16,800

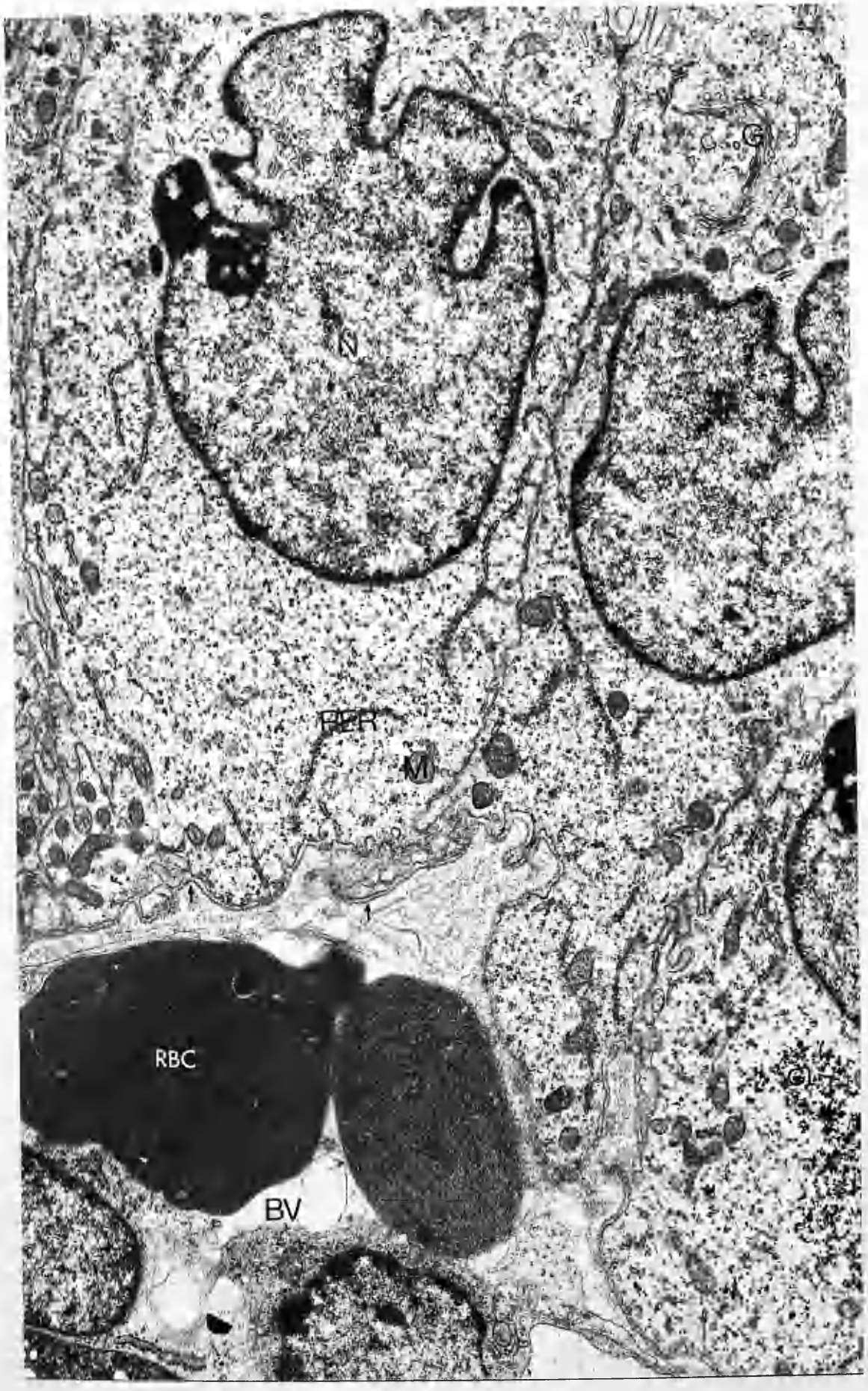

G
N
RER
M
RBC
BV

Fig. 38

A choroidal capillary (BV), with red blood cells (RBCs) in its lumen, is in close proximity to the choroidal epithelium. Its wall is thinned out and occasionally fenestrated (arrow).
MV = microvilli
M = microchondria
N = nucleus
I = basilateral interdigitations

18 day mouse embryo. x 8400

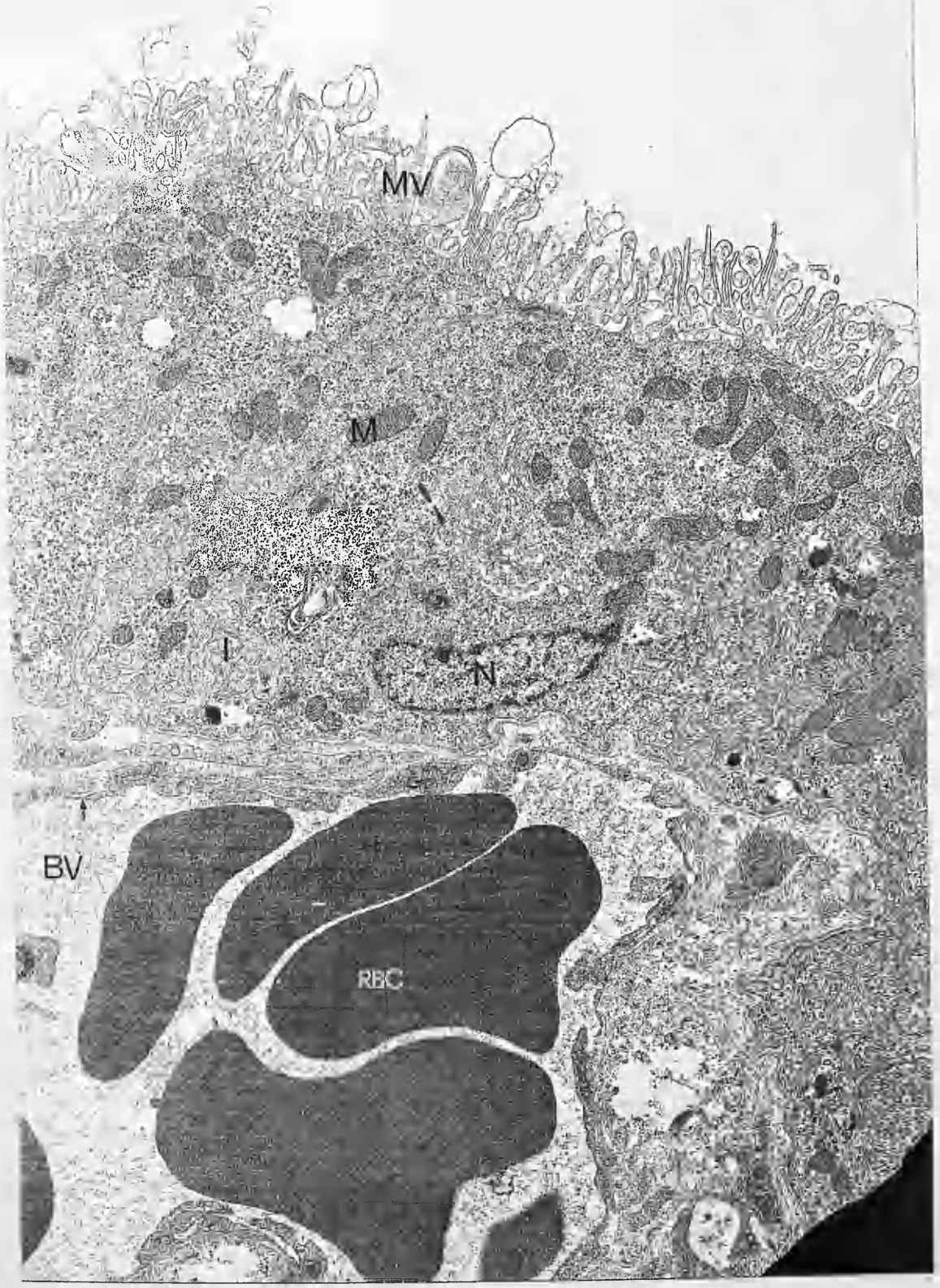

MV
M
I
N
BV
RBC

Fig. 39

TEM micrograph showing a choroidal capillary (BV), with multiple fenestrae in its wall (arrows).
The basal part of a choroid epithelial cell containing abundant glycogen granules (GL) is also shown.

19 day mouse embryo. x 21,000

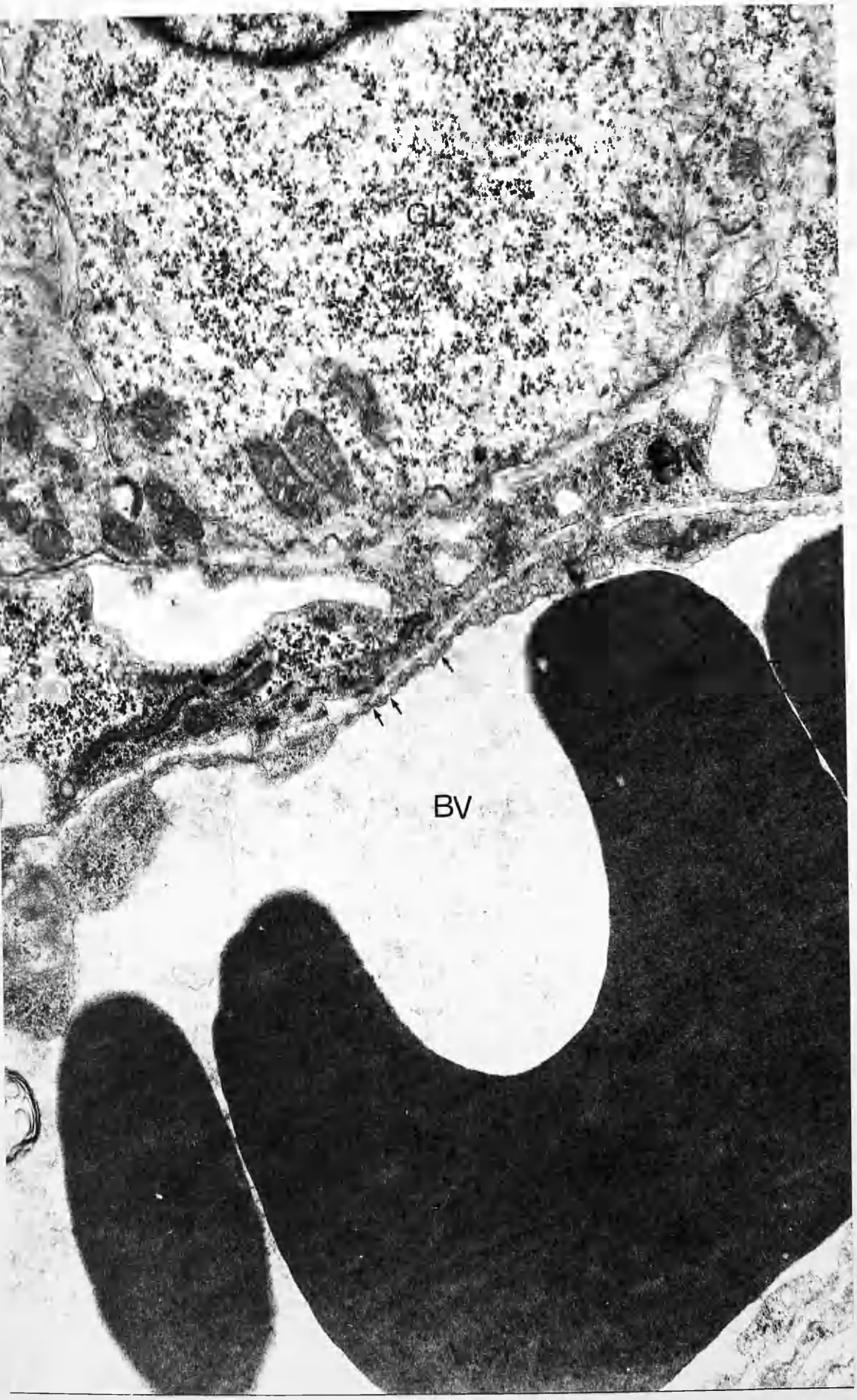

GL
BV

Fig. 40

A choroidal cell with lighter-stained cytoplasm is shown.
Also shown is a choroidal capillary (BV) with attenuated endothelial wall with occasional fenestrations (arrows).
MV = microvilli
N = nucleus

Newborn mouse. x 8400

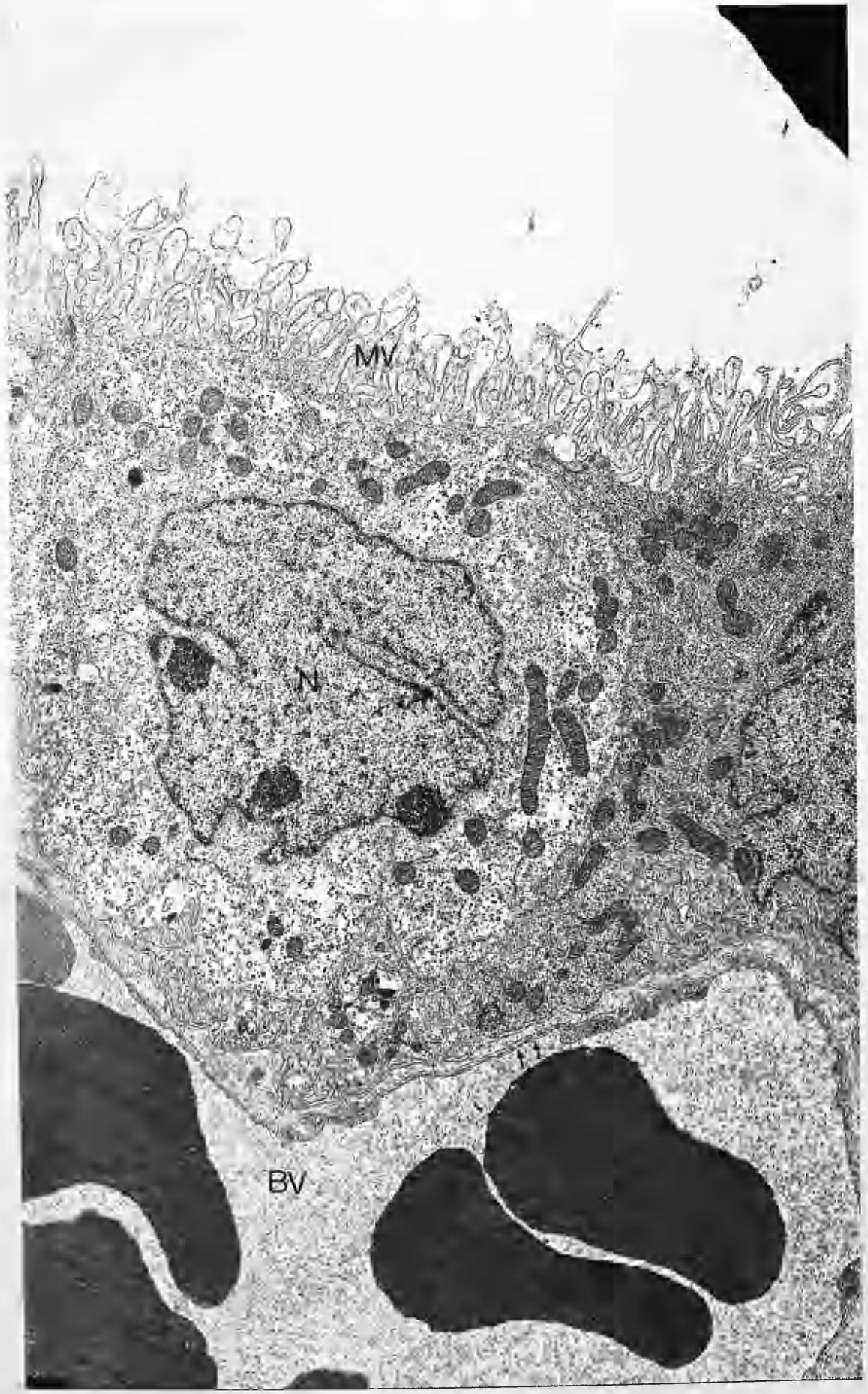

MV
N
BV

Fig. 41

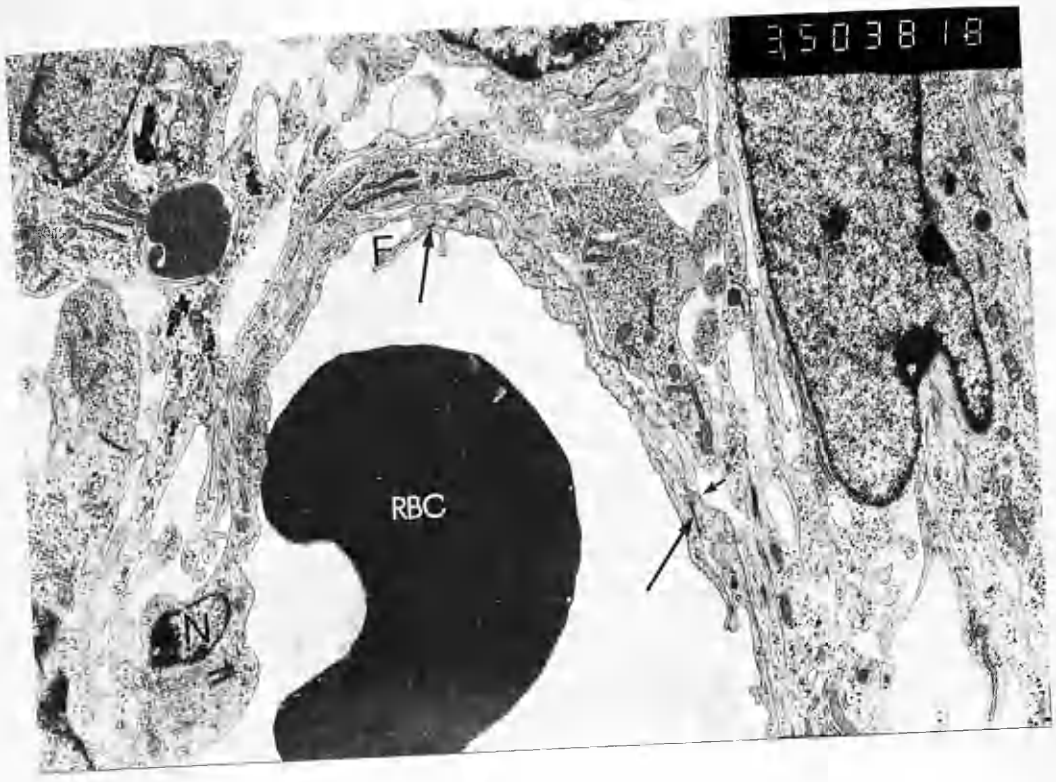
Shows choroidal capillary (BV) with a red blood cell (RBC) in its lumen. Cytoplasmic flaps (F) were seen at endothelial junctions.
N = nucleus
Small arrows = ill-defined and discontinuous vascular basement membrane
Large arrows = Desmosomal junctions between adjacent endothelial cells

13 day mouse embryo. x 5000

Fig. 42

A high power TEM micrograph of a choroid blood capillary (BV). There is a great variation in the thickness of the endothelium. Numerous pinocytotic vesicles are present (arrows).
N = nucleus

14 day mouse embryo. x 15,000


F
RBC
N

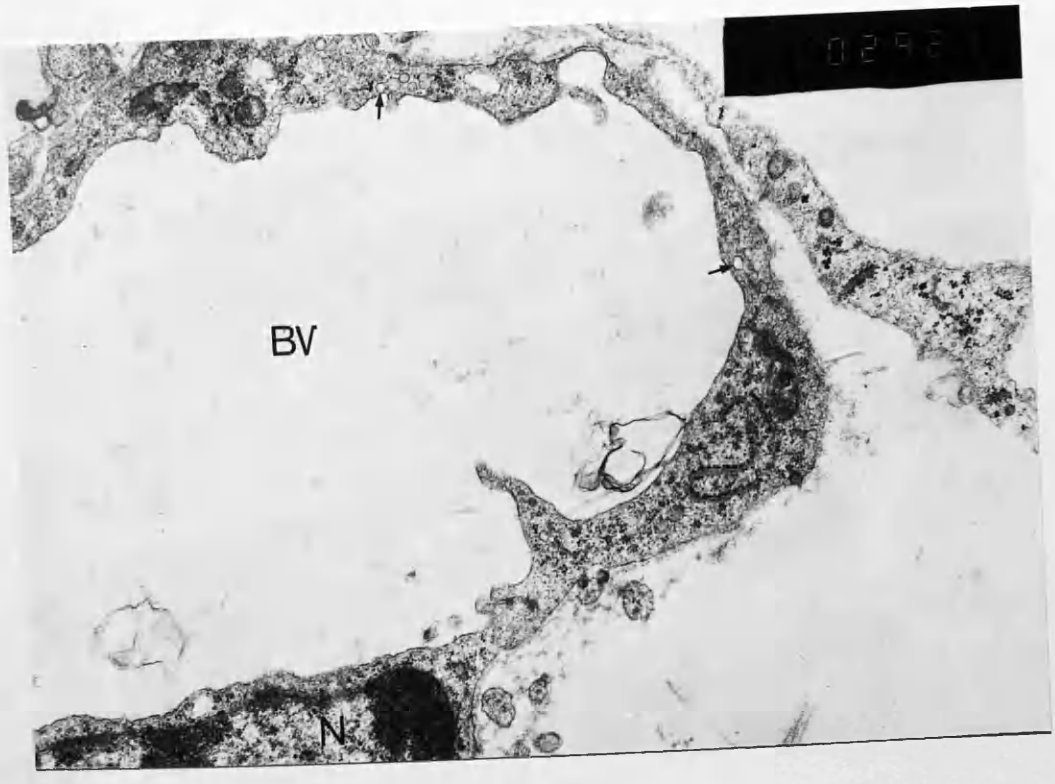

BV
N

Fig. 43

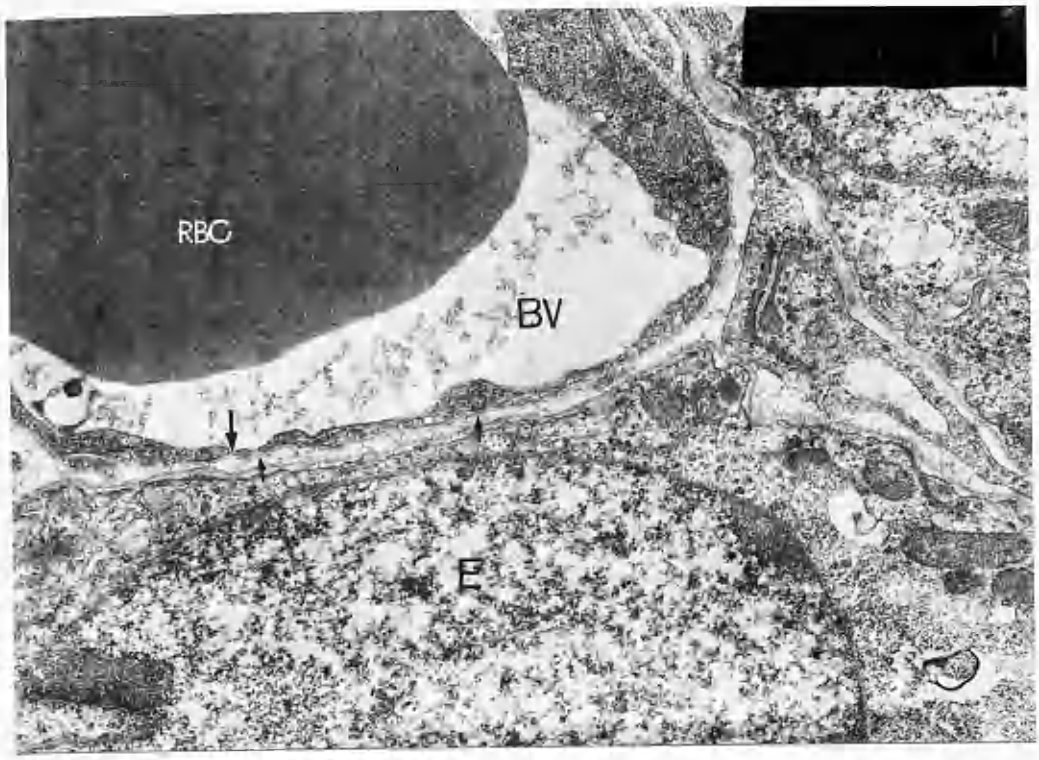
TEM micrograph of choroidal blood capillary (BV) containing a red blood cell (RBC) and lying just beneath the choroidal epithelial cell (E).
Large arrows = fenestrae
Small arrows = discontinuous vascular basement membrane

16 day mouse embryo. x 15,000

Fig. 44

High power TEM micrograph showing the attenuated wall of a choroidal blood vessel (BV) with occasional fenestrae (arrows).

17 day mouse embryo. x 15,000


RBC
BV
E

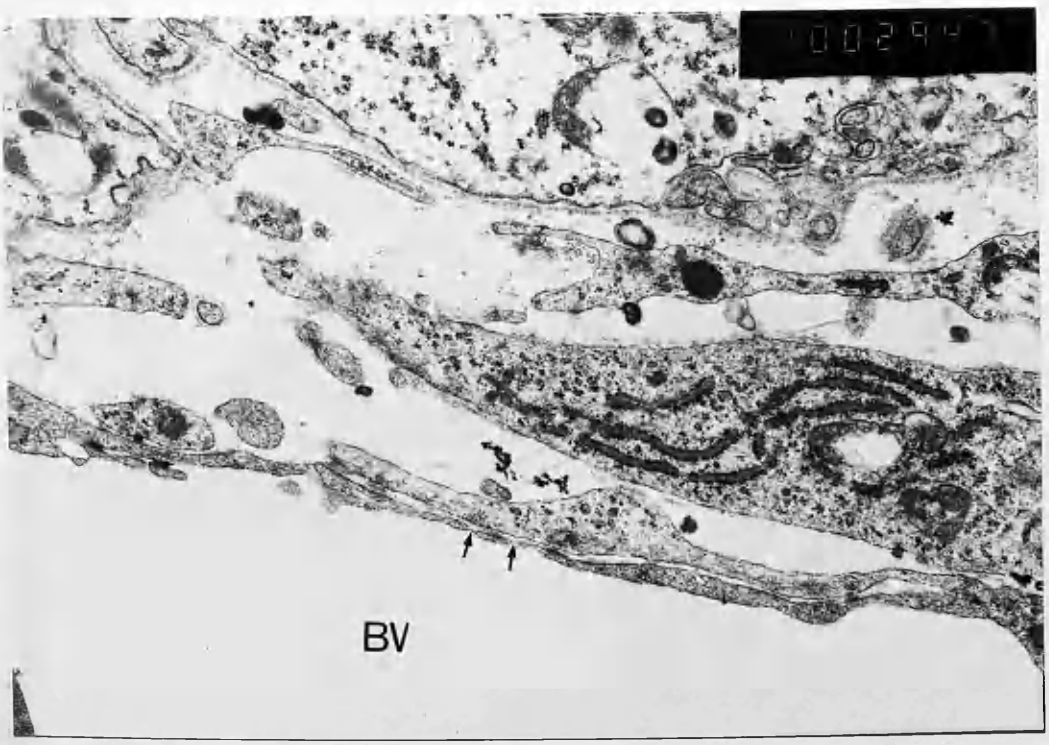

BV

Fig. 45

Shows the attenuated wall of a choroidal capillary (BV).

Adult mouse. x 6000

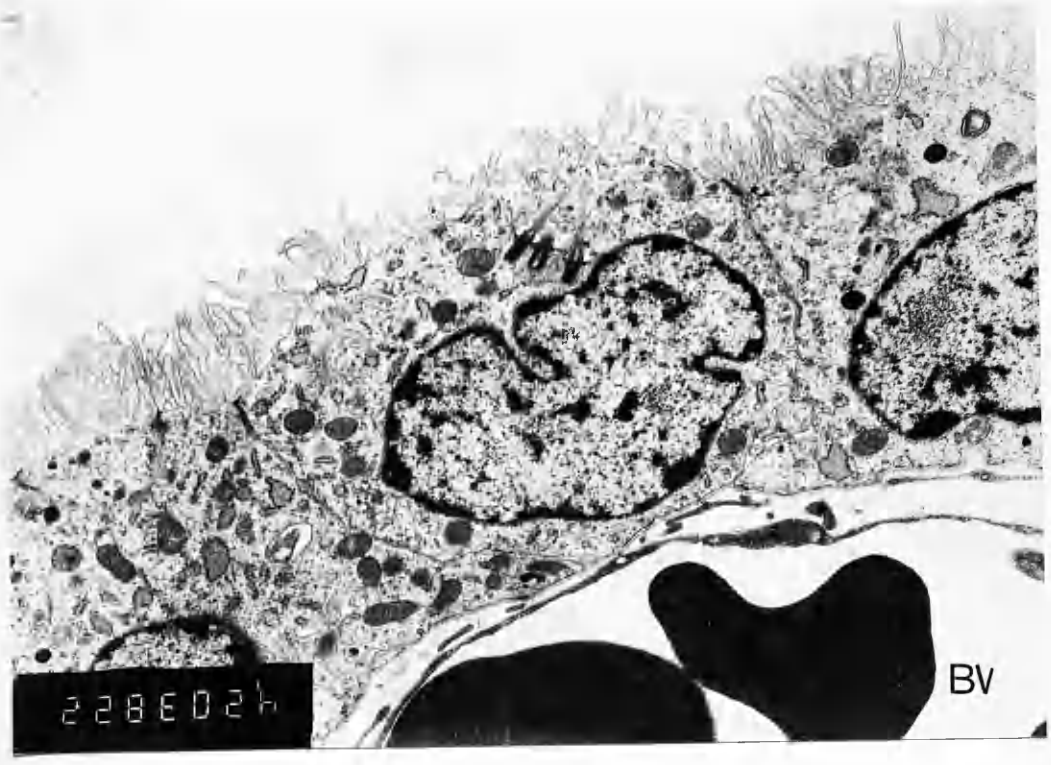

228E02h
BV

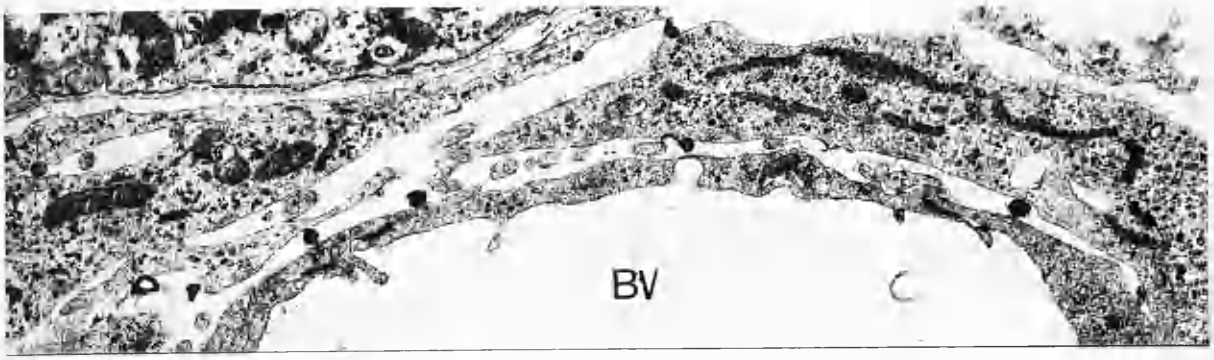
Fig. 46

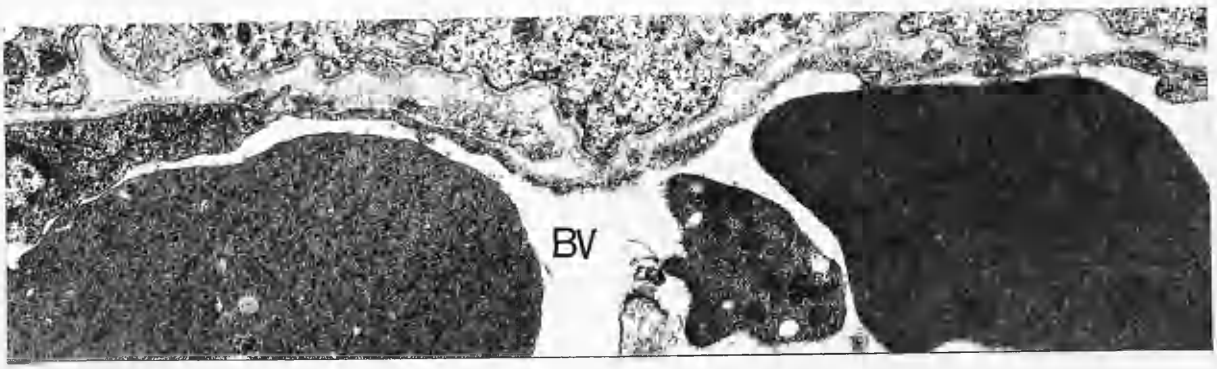
Shows choroidal capillaries at different stages of development (13th day pc, 14th day pc, 16th day pc, 17th day pc and adult mouse respectively from top to bottom). Note the difference in thickness of endothelium - it becomes progressively thinner as development progressed.

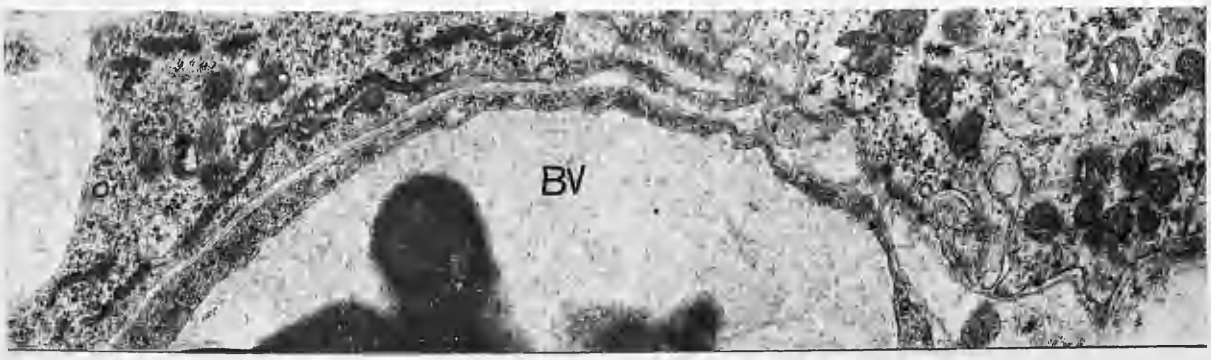
All micrographs at same magnification.

x 6000


BV


BV


BV


BV

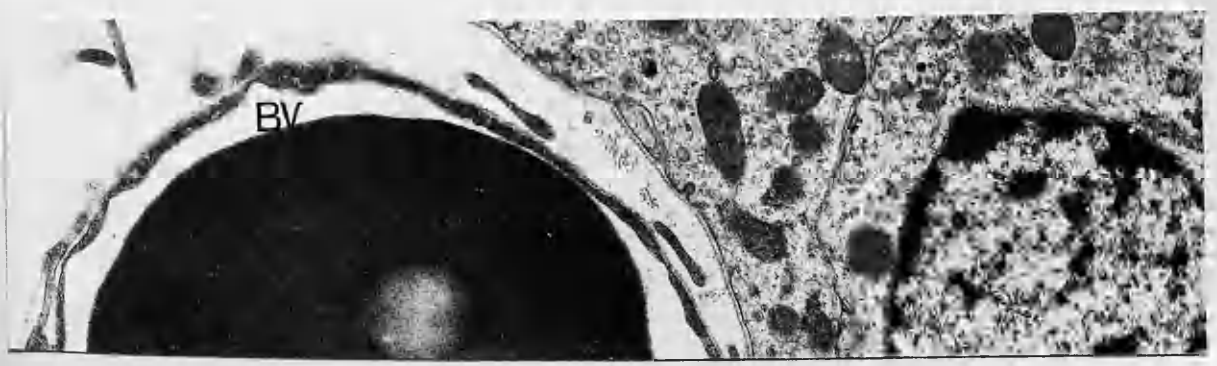

BV

Fig. 47

A low power SEM view showing the cut surface of the choroid plexus (P), in the lateral recess (LR) of the IVth ventricle.
E = ependymal lining of the lateral recess
SAS = subarachnoid space
Small arrow = a blood capillary lying just beneath the choroidal epithelium which has been cut tangentially.

Adult mouse. x 100

Fig. 48

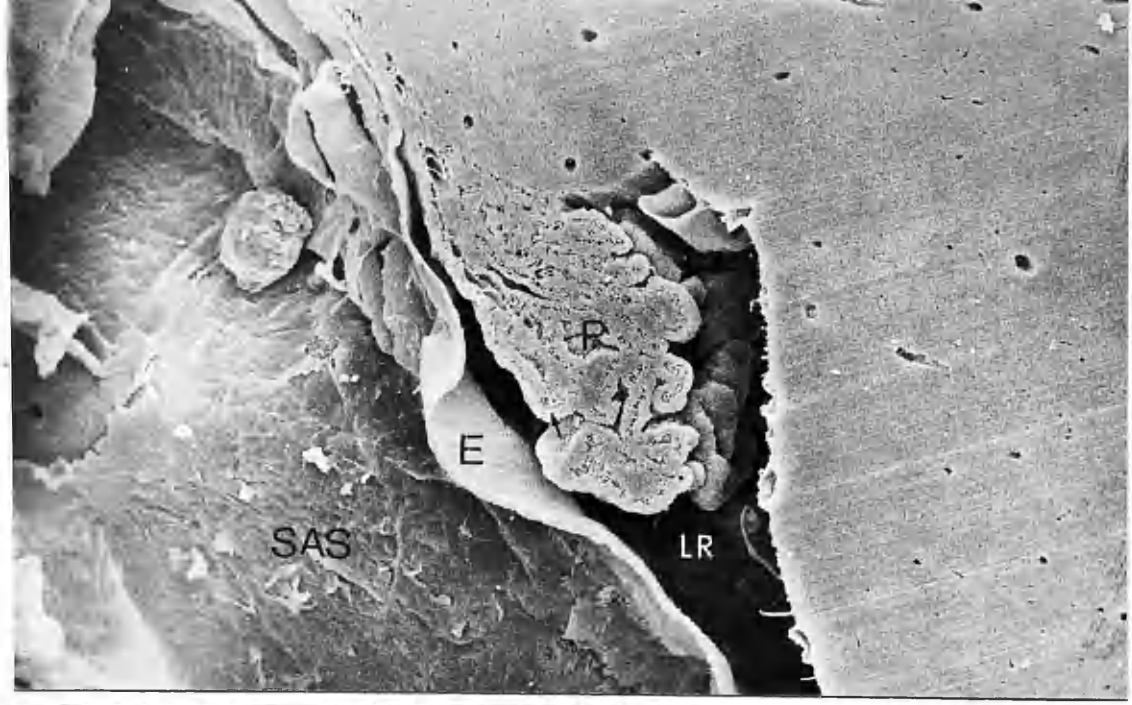
High power SEM micrograph of the interior of the blood vessel arrowed in Fig. 47.
Arrows = endothelial ridges.

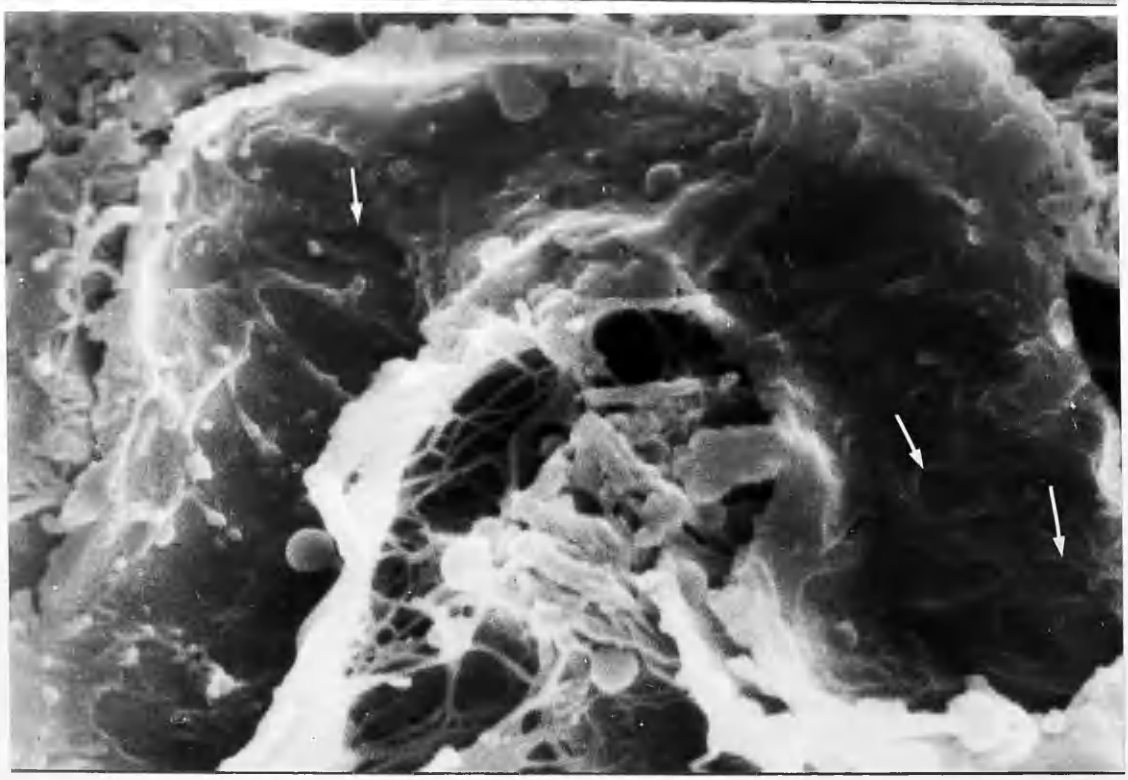
Adult mouse. x 4000

Fig. 49

High power SEM micrograph showing the fenestrae in the endothelial wall (arrows). They measure 0.05 - 0.1 um in diamter.

Adult mouse. x 30,000


R
E
SAS
LR



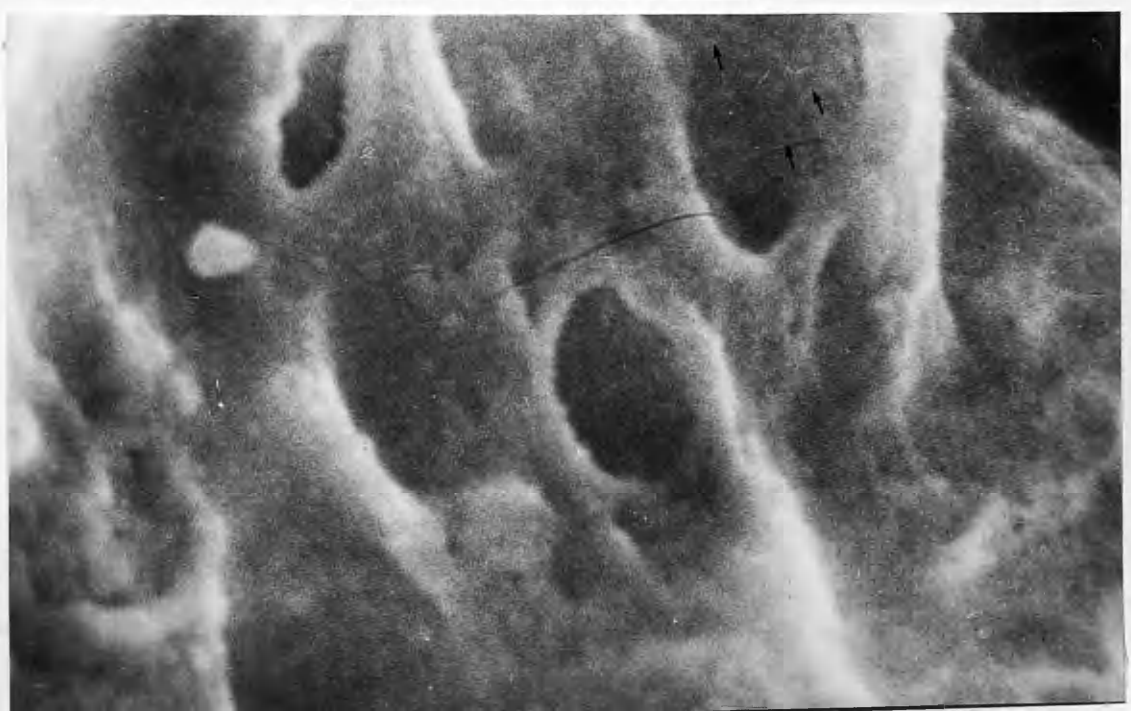


Fig. 50

SEM micrograph of the interior of a choroidal capillary showing fenestrae (arrows).
E = endothelial ridges.

Newborn mouse. **x 32,000**

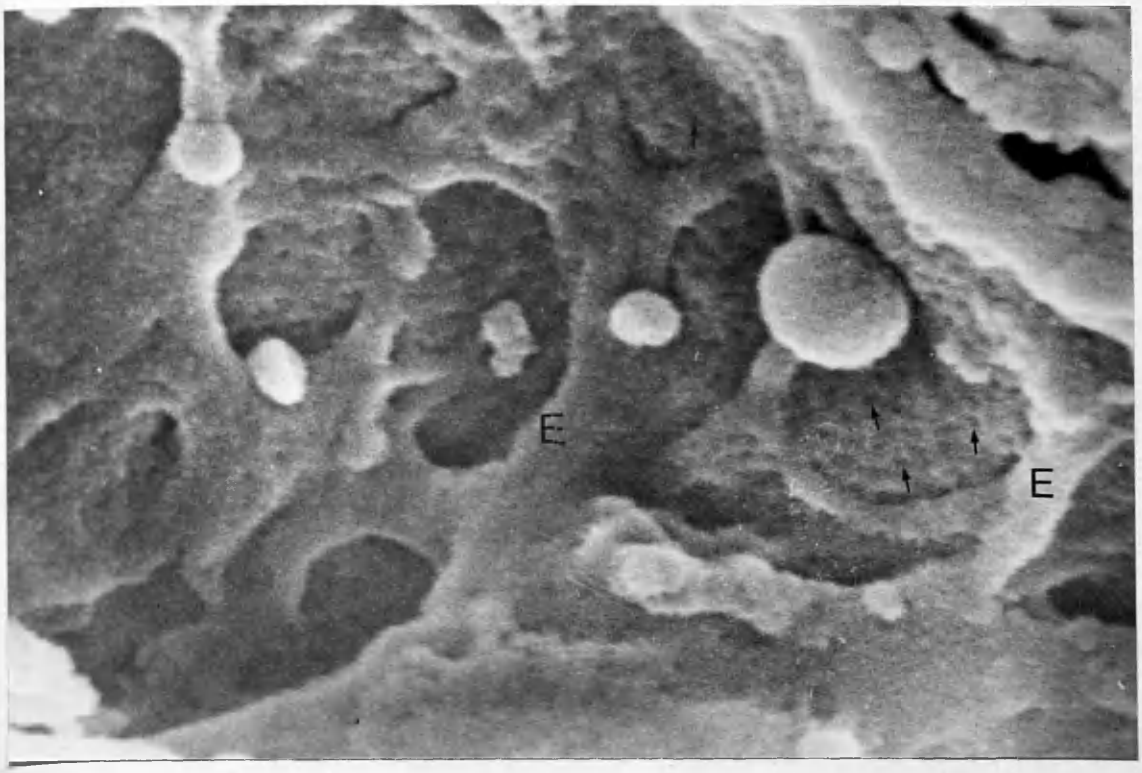

E
E

Fig. 51

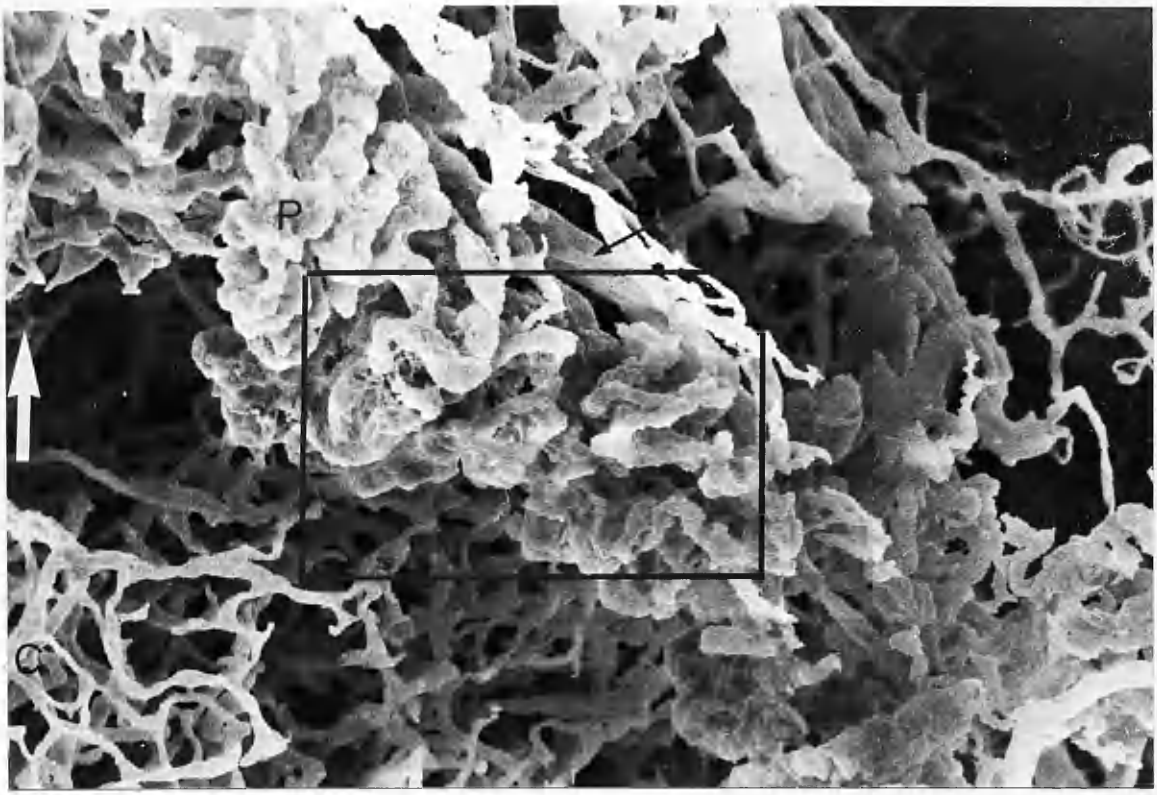
Vascular cast of the midline part of the choroid plexus of the IVth ventricle (P). The great difference in size between the choroid plexus and subependymal capillaries (C) is apparent.
White arrow = midline
Black arrow = feeding vessel
Inset - see Fig. 52.

15 day mouse embryo. x 250 Cast 2

Fig. 52

Higher power of area outlined in Fig. 51.

15 day mouse embryo. x 750


P
Q

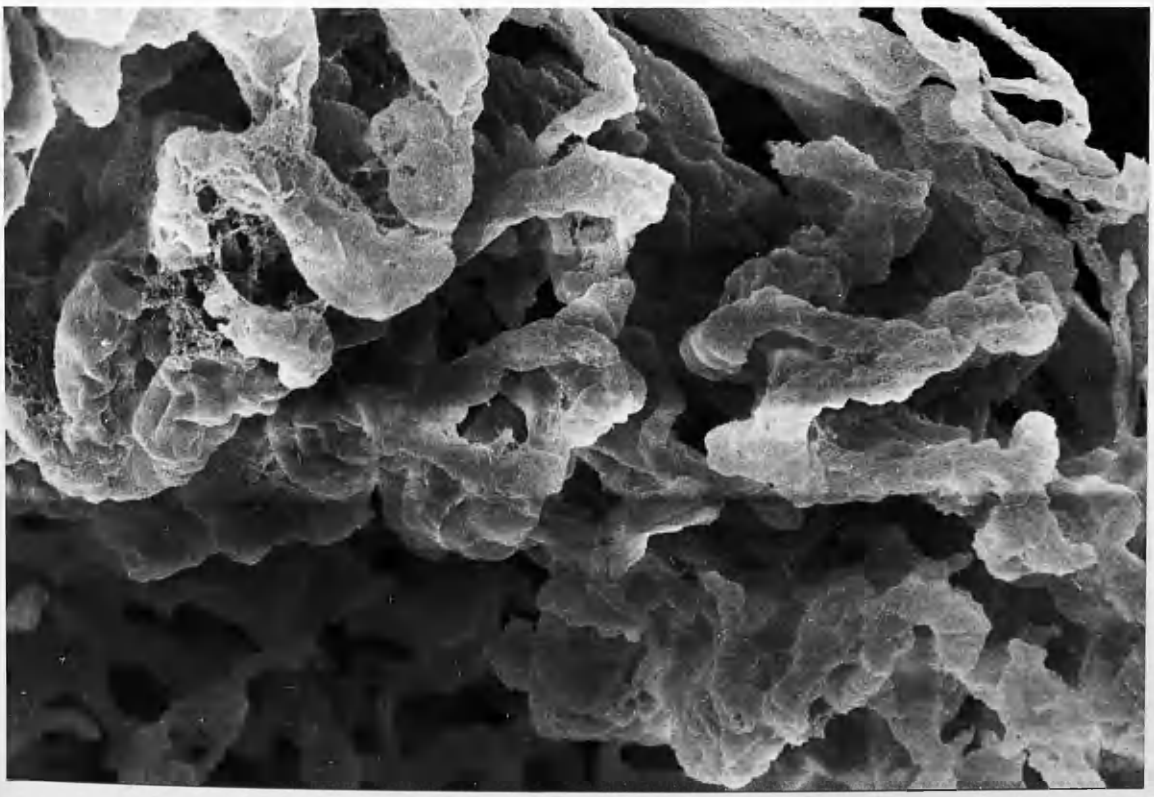


Fig. 53

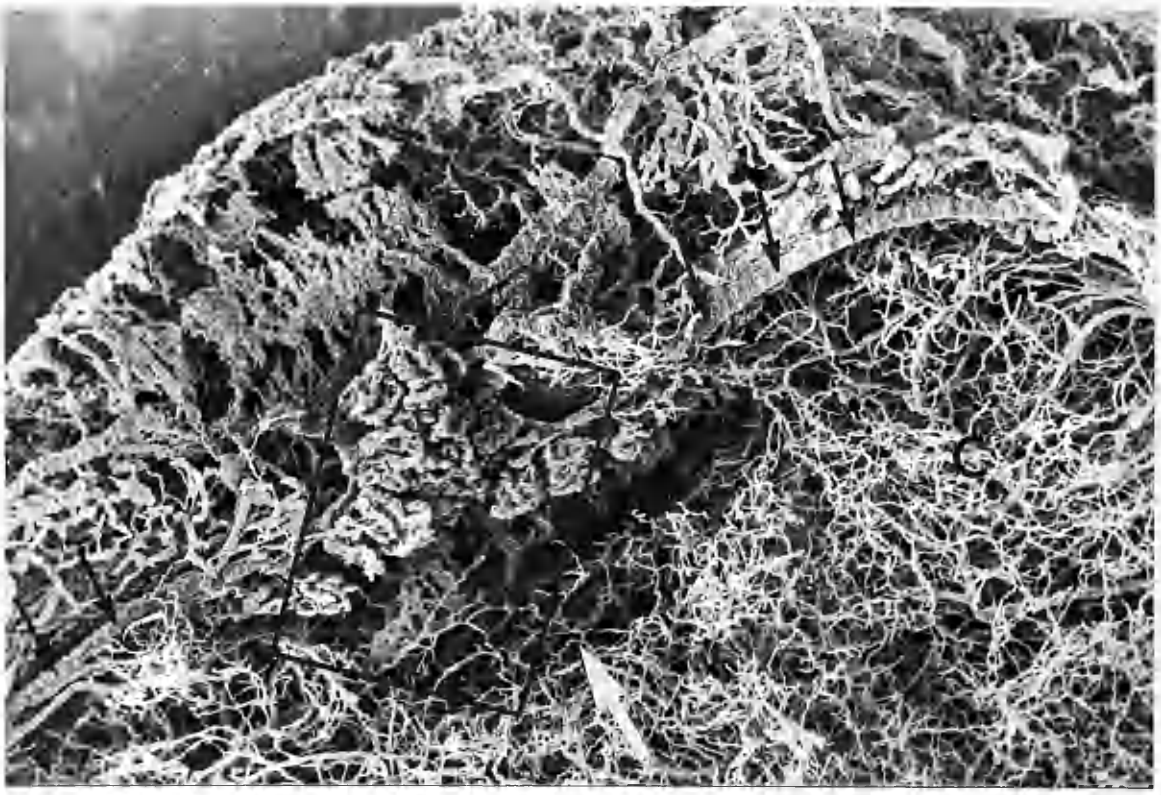
Low power SEM micrograph of the vascular cast of the choroid plexus of the IVth ventricle (P). The more laterally placed choroid plexus cast has been damaged revealing large feeding vessel (small arrows) on either side of the midline (large arrow).
C = subependymal capillaries.
Inset - see Fig. 54.

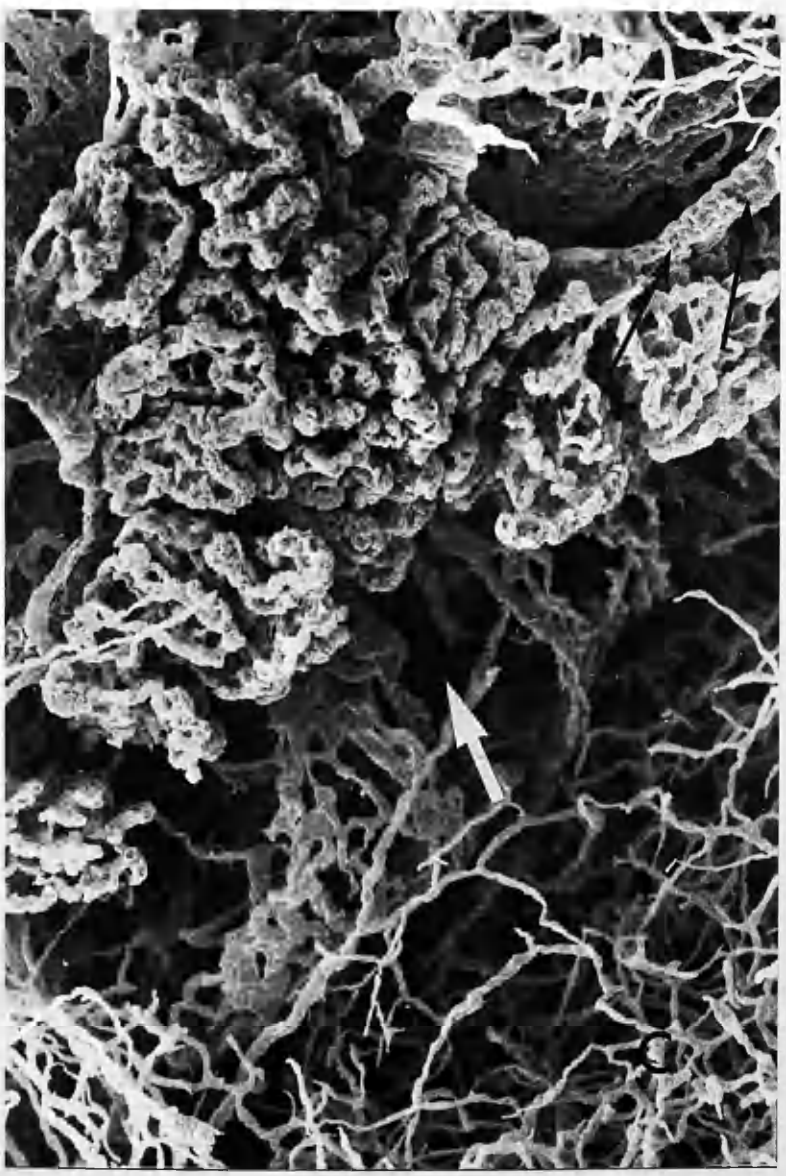
17 day mouse embryo. x 50 Cast 1

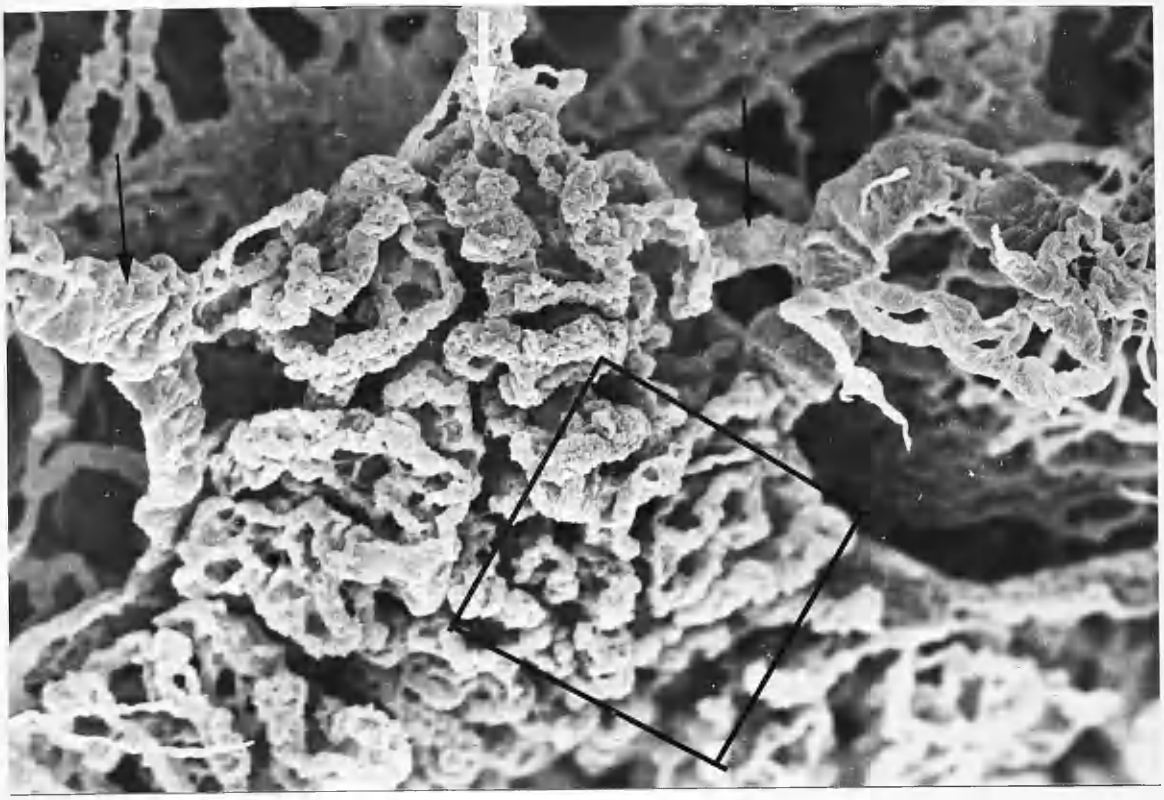
Fig. 54

Higher Power of the area outlined in Fig. 53. The choroid plexus capillaries are wide and irregular compared to the hair-like subependymal capillaries (C).
White arrow = midline
Black arrow = feeding vessels

17 day mouse embryo. x 150







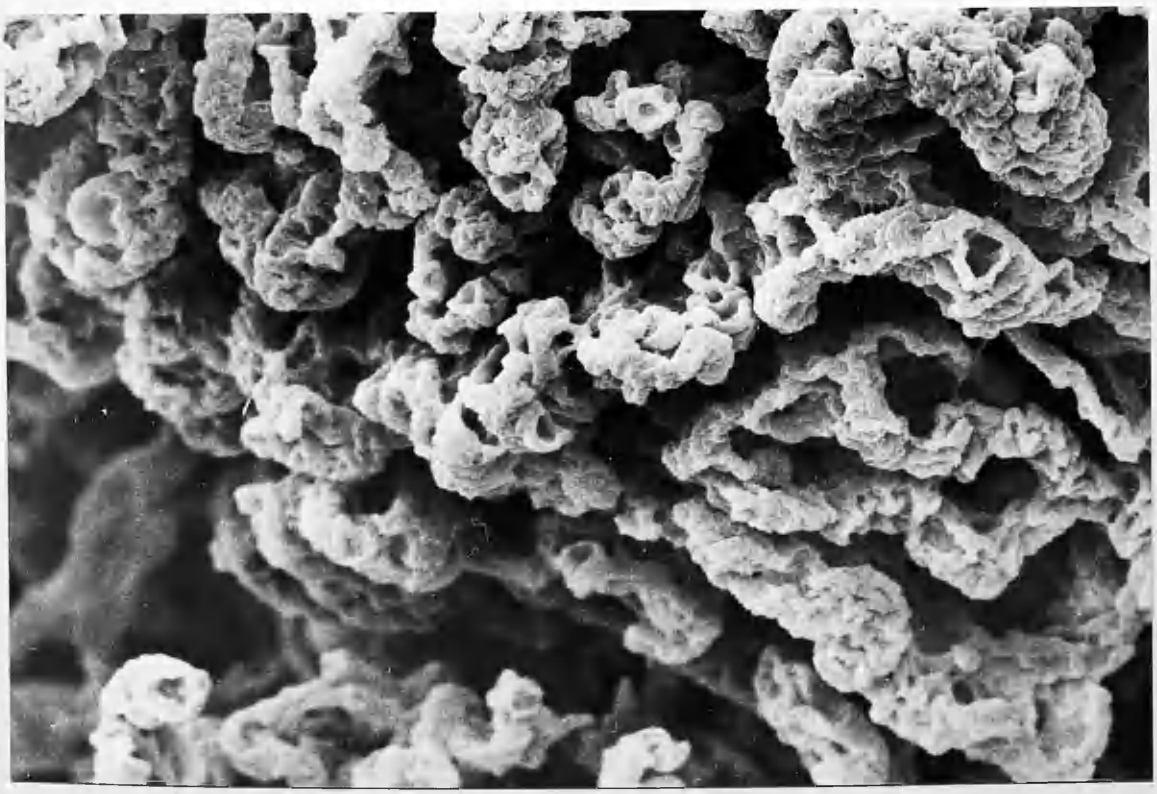


Fig. 55

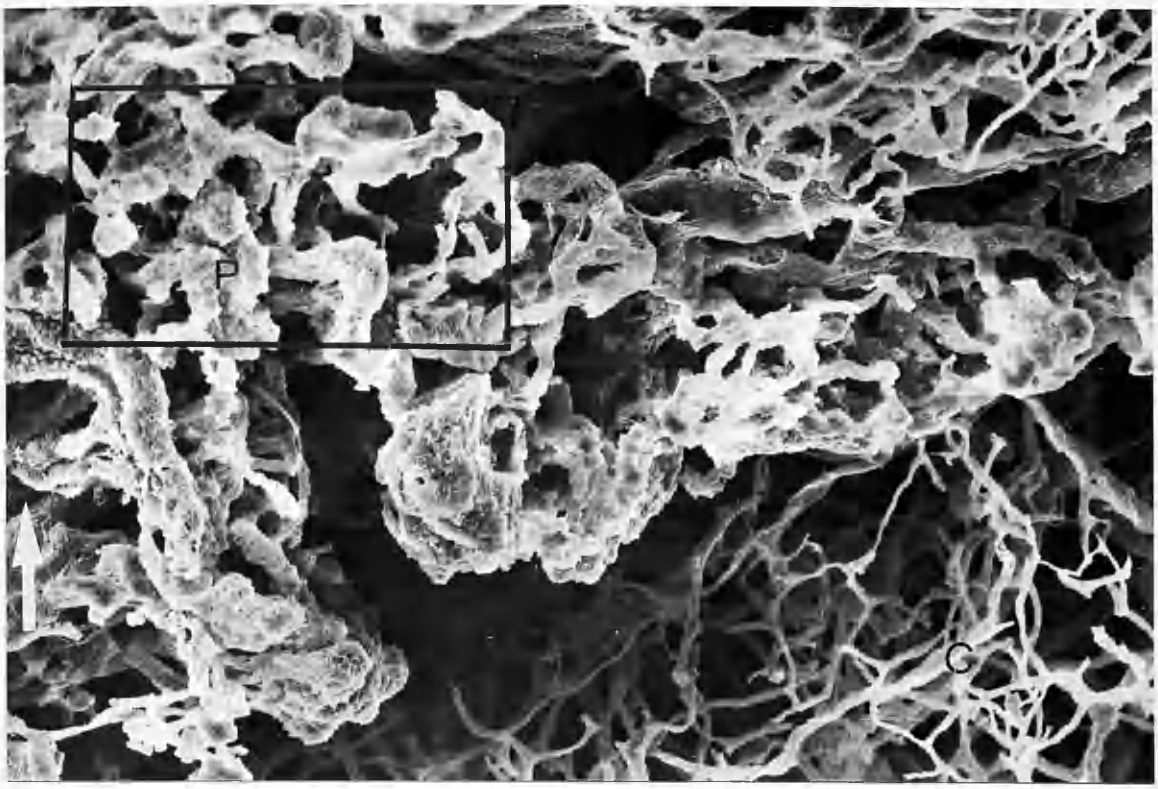
Higher power view from Fig. 54 to illustrate the wide and irregular nature of the choroid plexus capillaries.
White arrow = points to the midline
Black arrows = feeding vessels giving branches to the plexus

x 250

Fig. 56

Inset in Fig. 55.

x 500


P
G

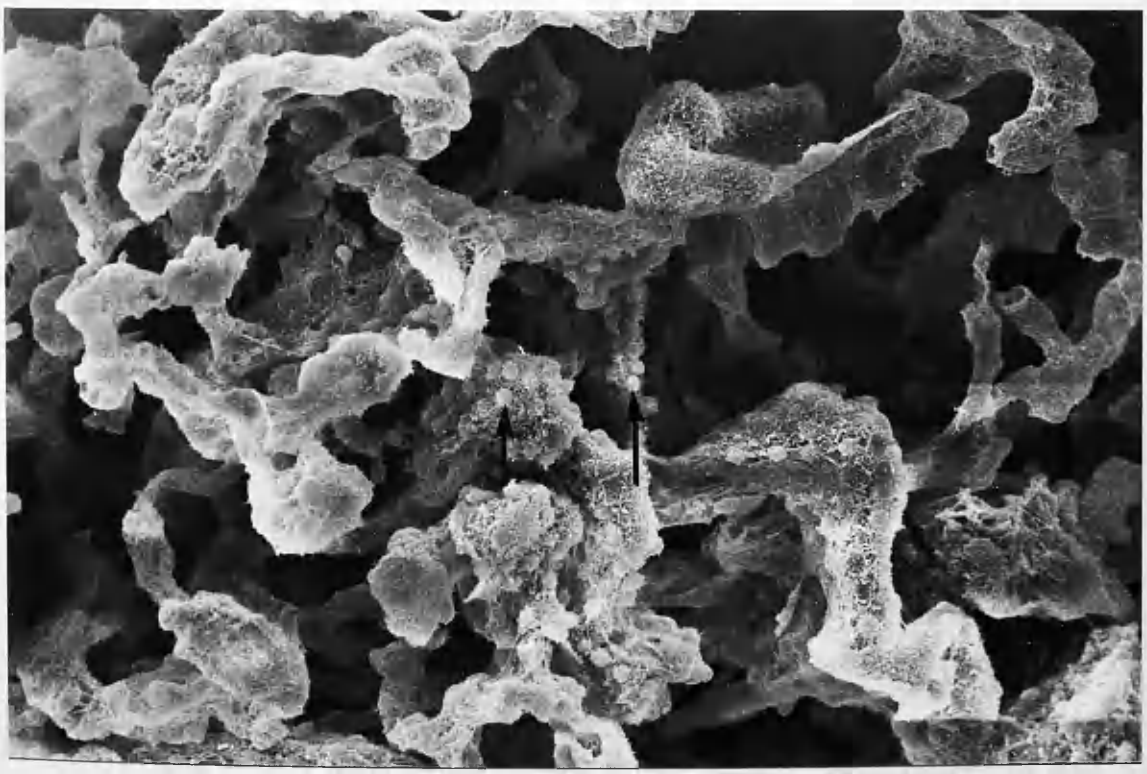


Fig. 57

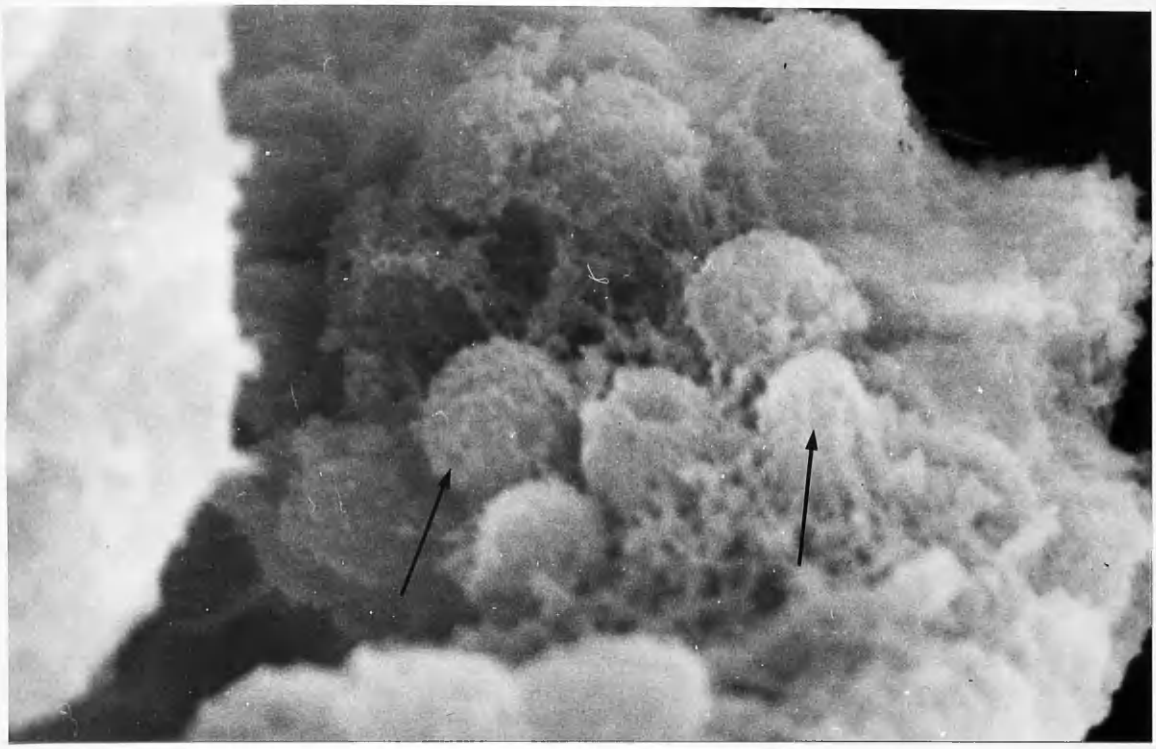
SEM micrograph of the vascular cast of the midline part of the choroid plexus of the IVth ventricle (P). It shows the great difference in diameter between the subependymal capillaries (C) and those of the choroid plexus.
White arrow = midline
Inset - see Fig. 58

18 day mouse embryo. x 225 Cast 3

Fig. 58

A high power view of the area oulined in Fig. 57. It shows the irregular shapes and different sizes of the choroid plexus capillaries. Globular masses were visible (arrows).

18 day mouse embryo. x 500 Cast 3



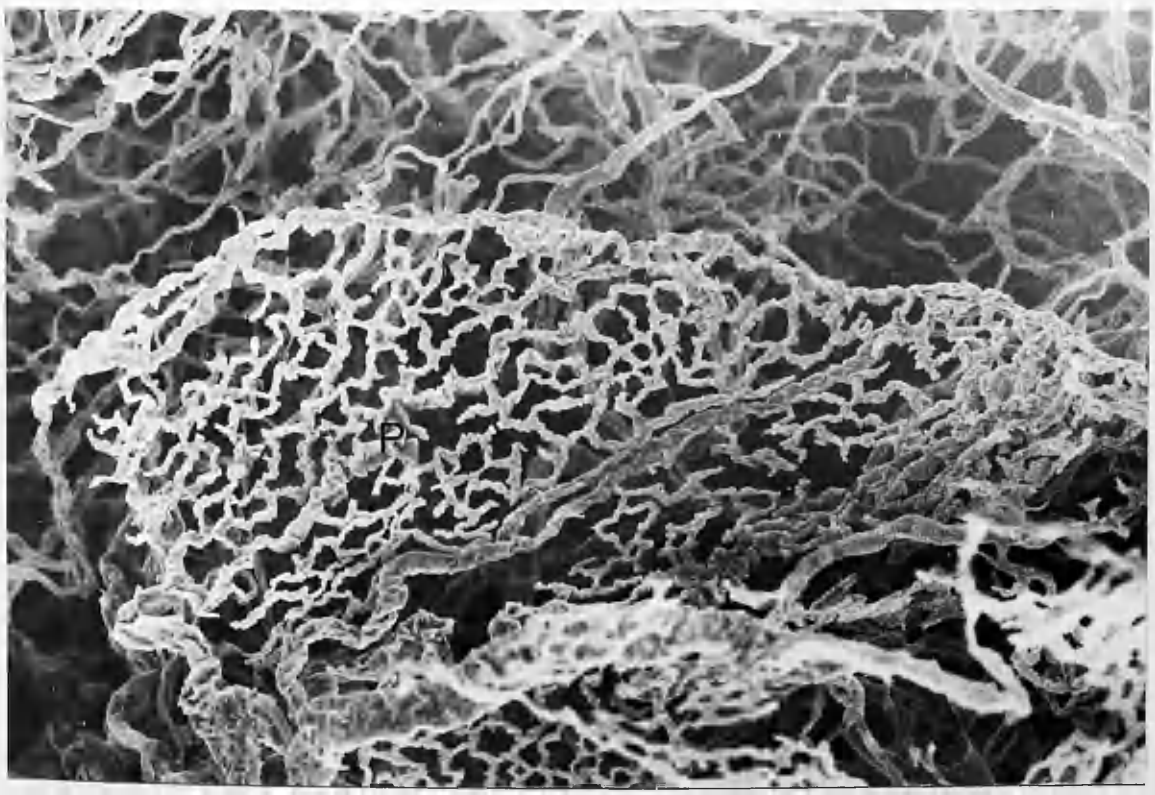

P

Fig. 59

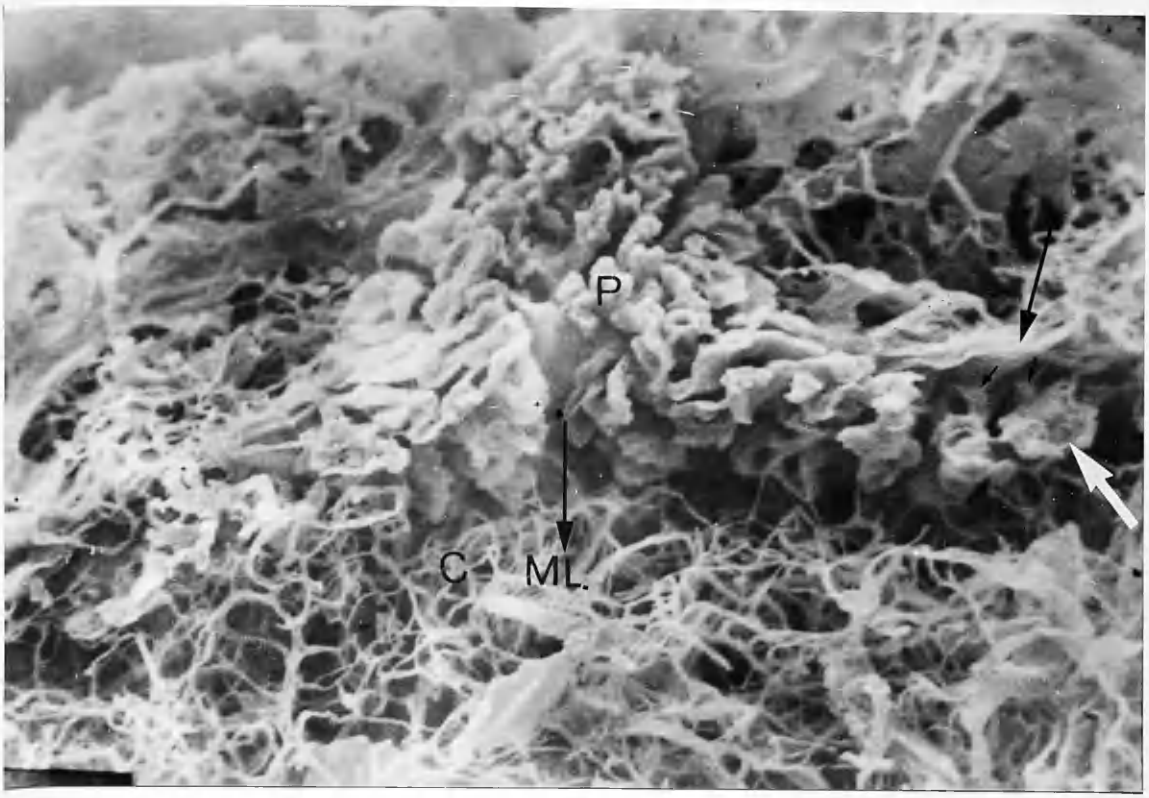
High power SEM micrograph of some of the globular masses (arrows) shown in Fig. 58.

18 day mouse embryo. x 6500 Cast 3

Fig. 60

Shows the vascular replica of the choroid plexus of the lateral ventricle (P) showing regular pattern of arrangement, i.e. large central vessel joining a marginal vessel with a dense network of capillaries between them. (Compare with that of the choroid plexus of the IVth ventricle in Fig. 57).

18 day mouse embryo. x 225 Cast 5


P
C
ML.

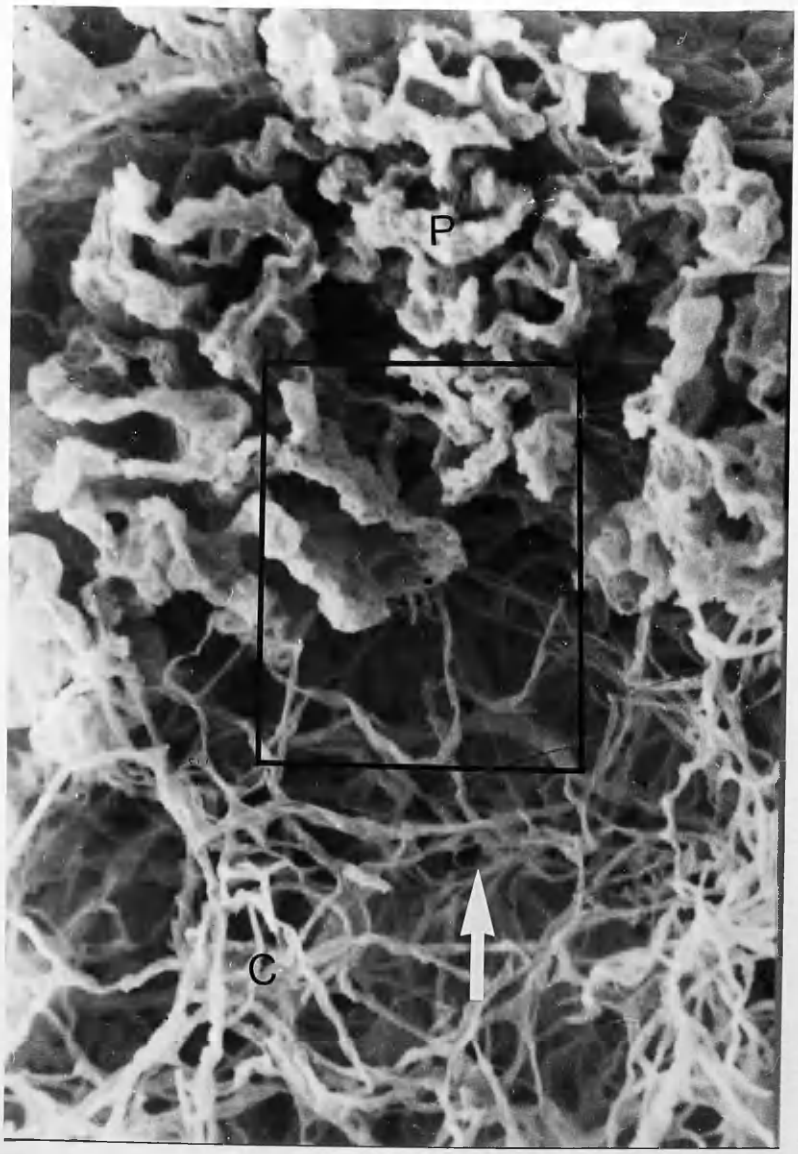

P
C

Fig. 61

Low power view of the vascular replica of the midline part of the choroid plexus of the IVth ventricle (P). It shows different sizes and shapes of the choroidal capillaries - some appear discoid (white arrow). A feeding vessel (large arrow) supplying branches (small arrows) to the plexus is seen.
C = subependymal capillaries.
ML = midline

Newborn mouse. x 100 Cast 1

Fig. 62

Shows the contrast in the size and shape between the choroid plexus capillaries (P) and subependymal capillaries (C).
White arrow = midline

Newborn mouse. x 180 Cast 3

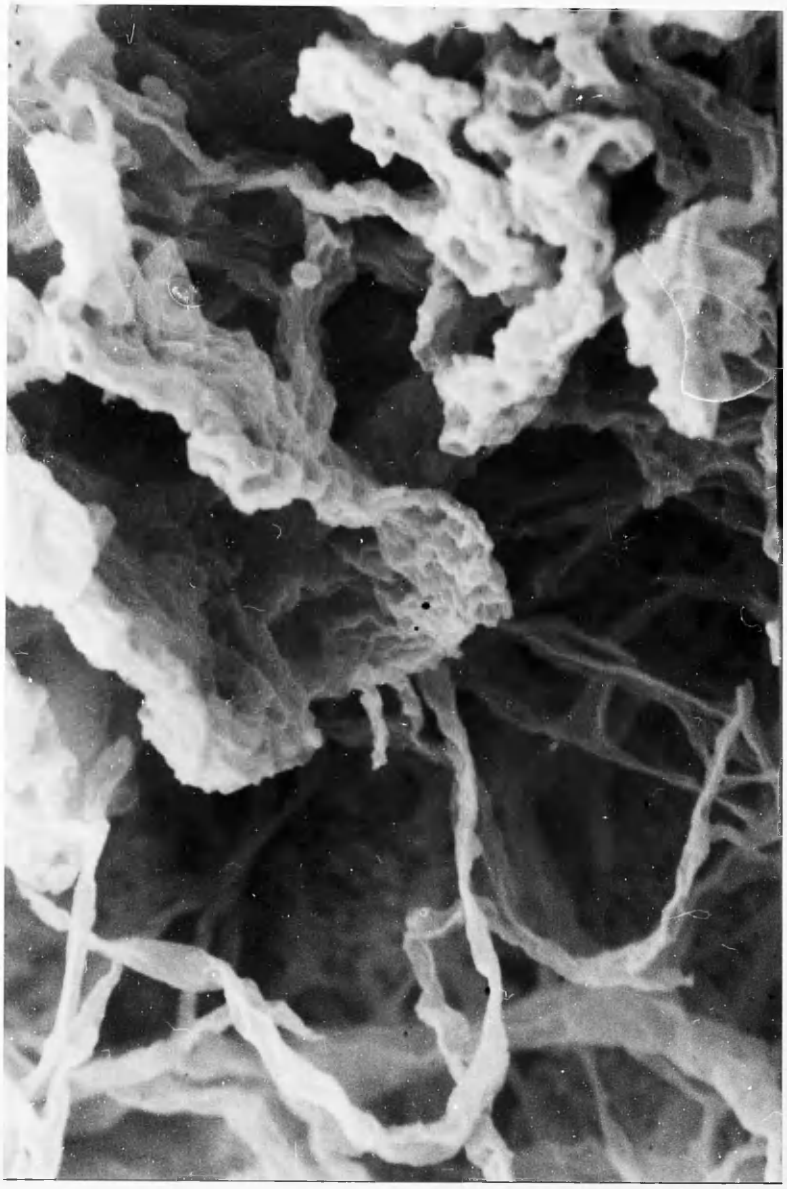
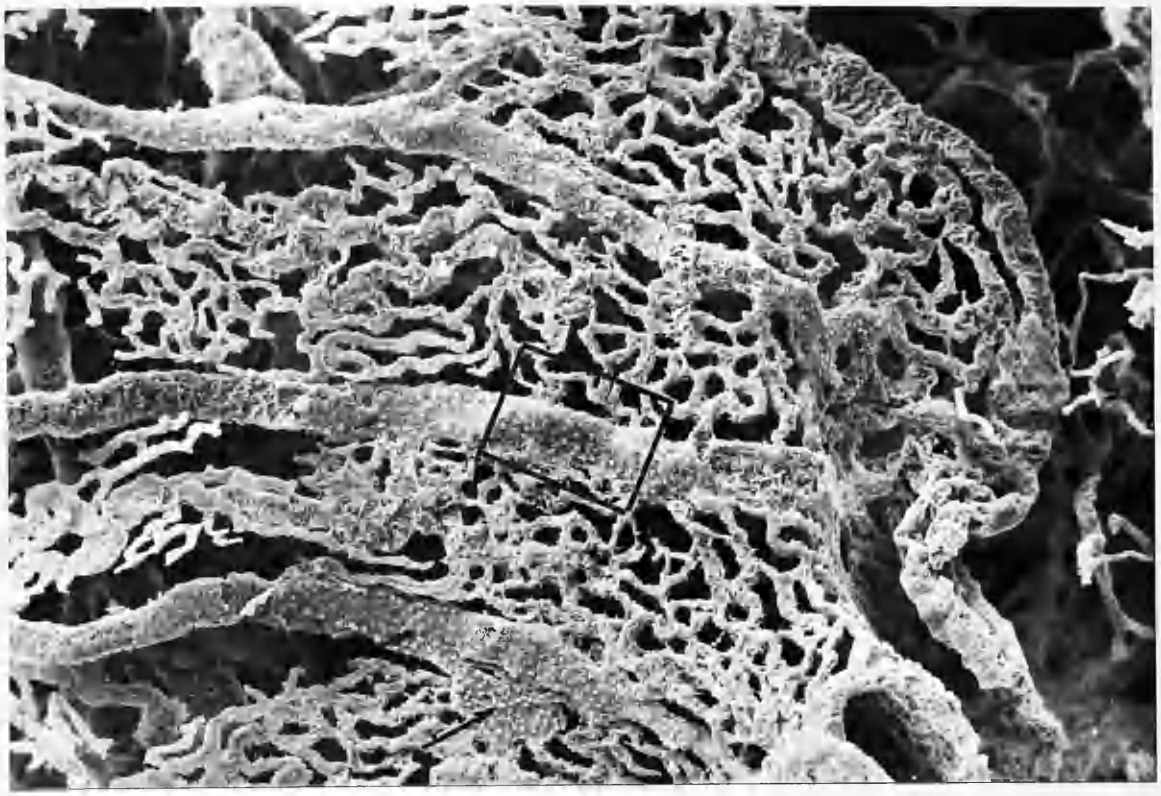


Fig. 63

Inset in Fig. 62.

x 450



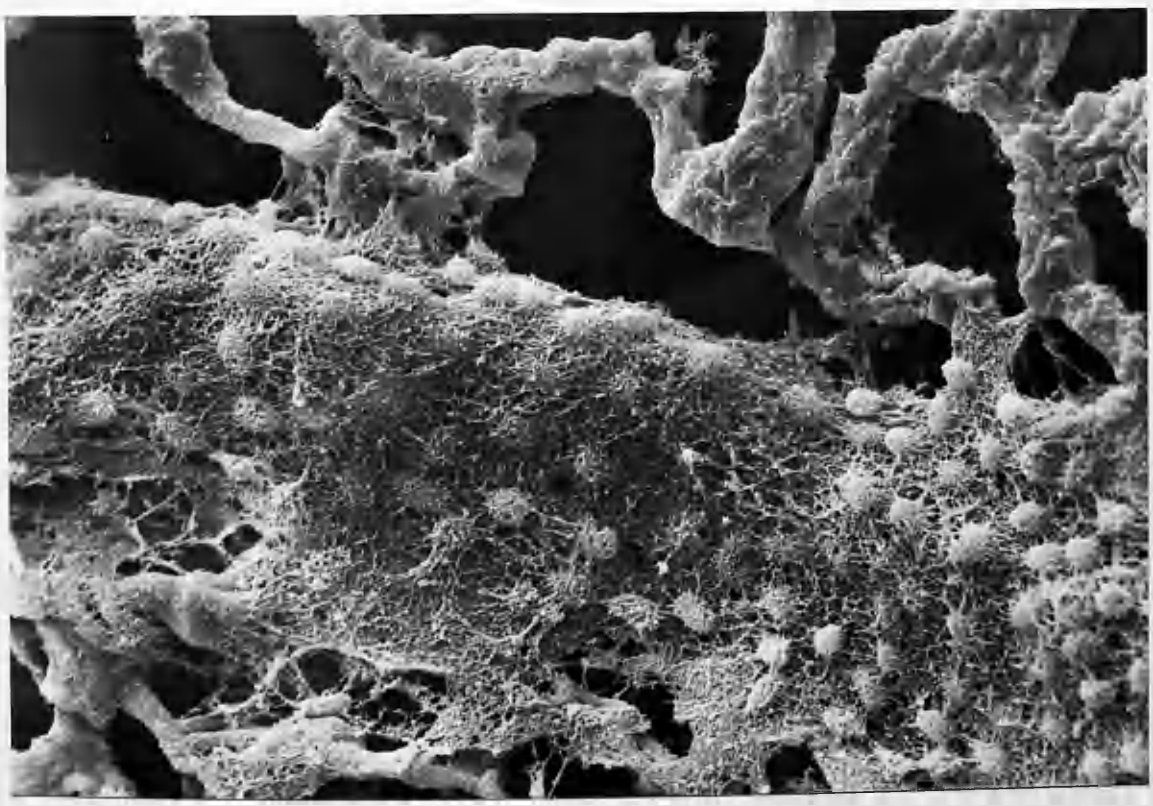


Fig. 64

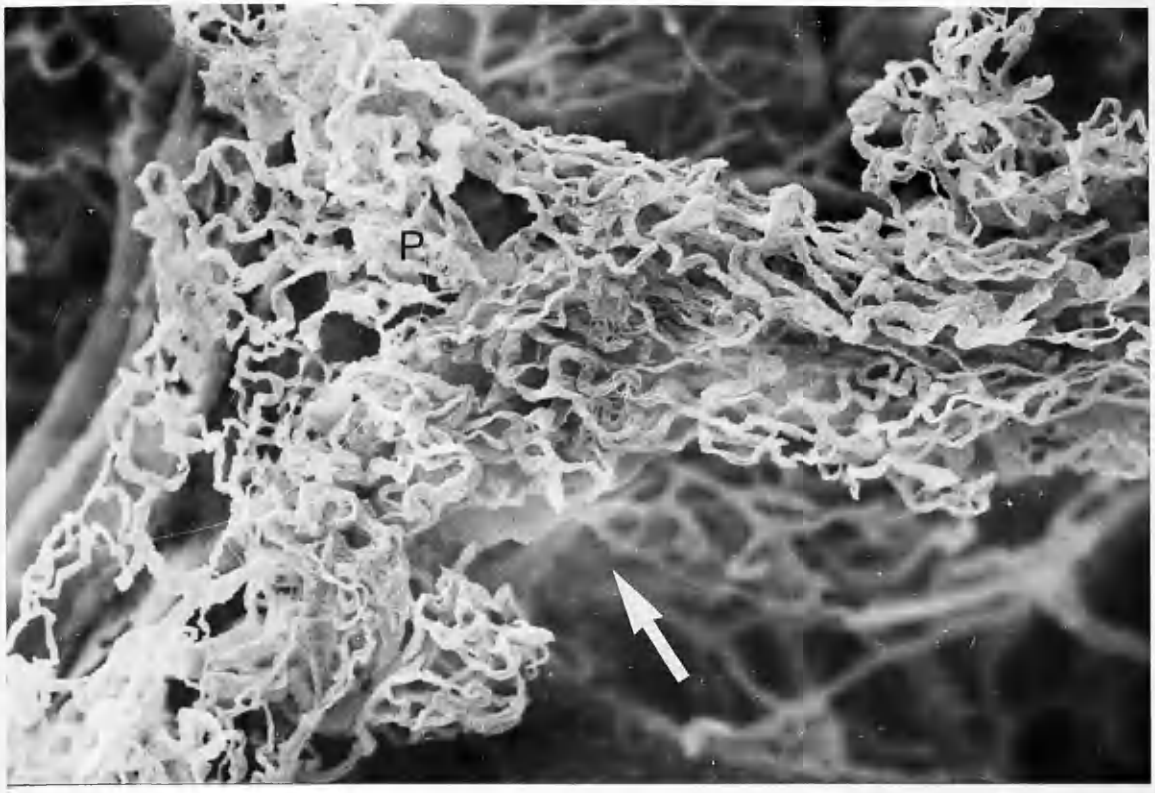
SEM micrograph of the vascular cast of the choroid plexus of the lateral ventricle. It shows large vessels running along the long axis of the plexus to join with a marginal vessel. All are connected with a meshwork of capillaries. Globular masses visible on large central vessels (arrows).
Inset - see Fig. 65.

Newborn mouse. x 150 Cast 2

Fig. 65

Inset - Fig. 64.

Newborn mouse. x 1200 Cast 2


P

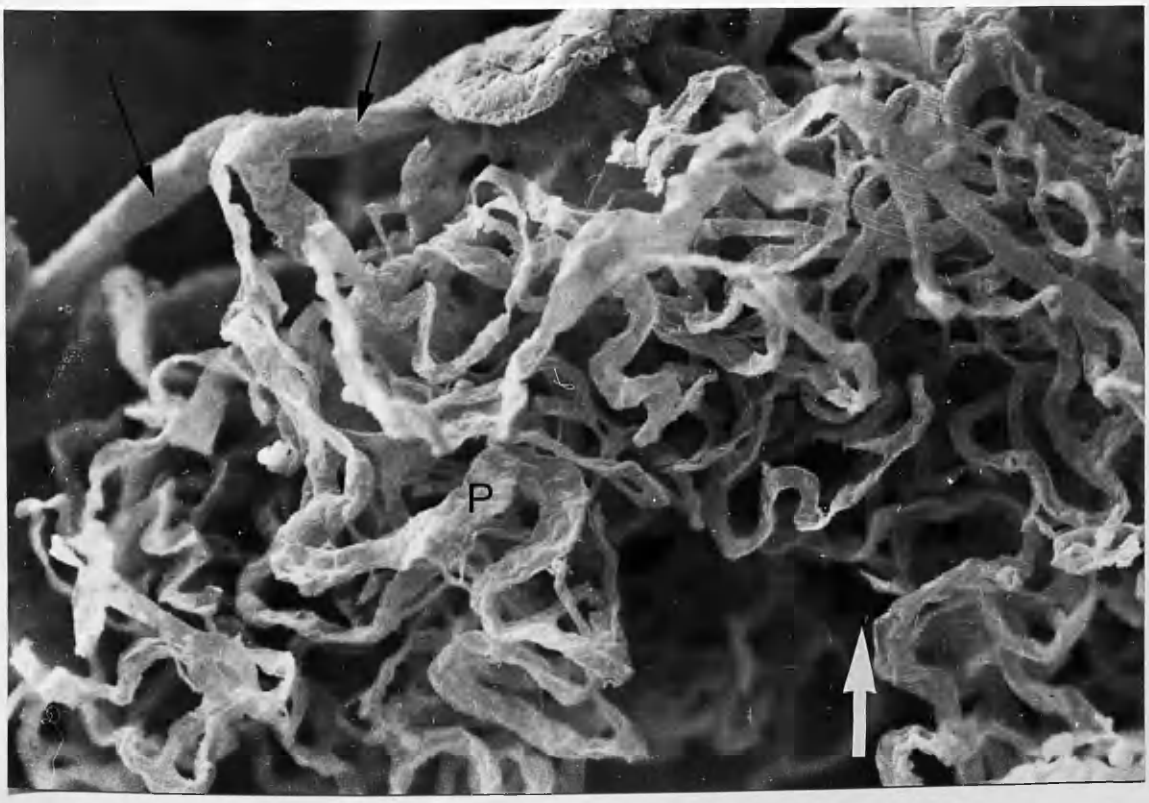

P

Fig. 66

Medium power view of the vascular cast of the midline part of the choroid plexus (P) of the IVth ventricle It is composed of meshwork of capillaries.
Arrow = midline

Adult mouse. x 225 Cast 1

Fig. 67

SEM micrograph of the vascular cast of the choroid plexus of the IVth ventricle showing a feeding vessel giving branches (black arrows) which divide and redivide forming a dense network of capillaries (P).
White arrow = midline.

Adult mouse. x 500 Cast 3

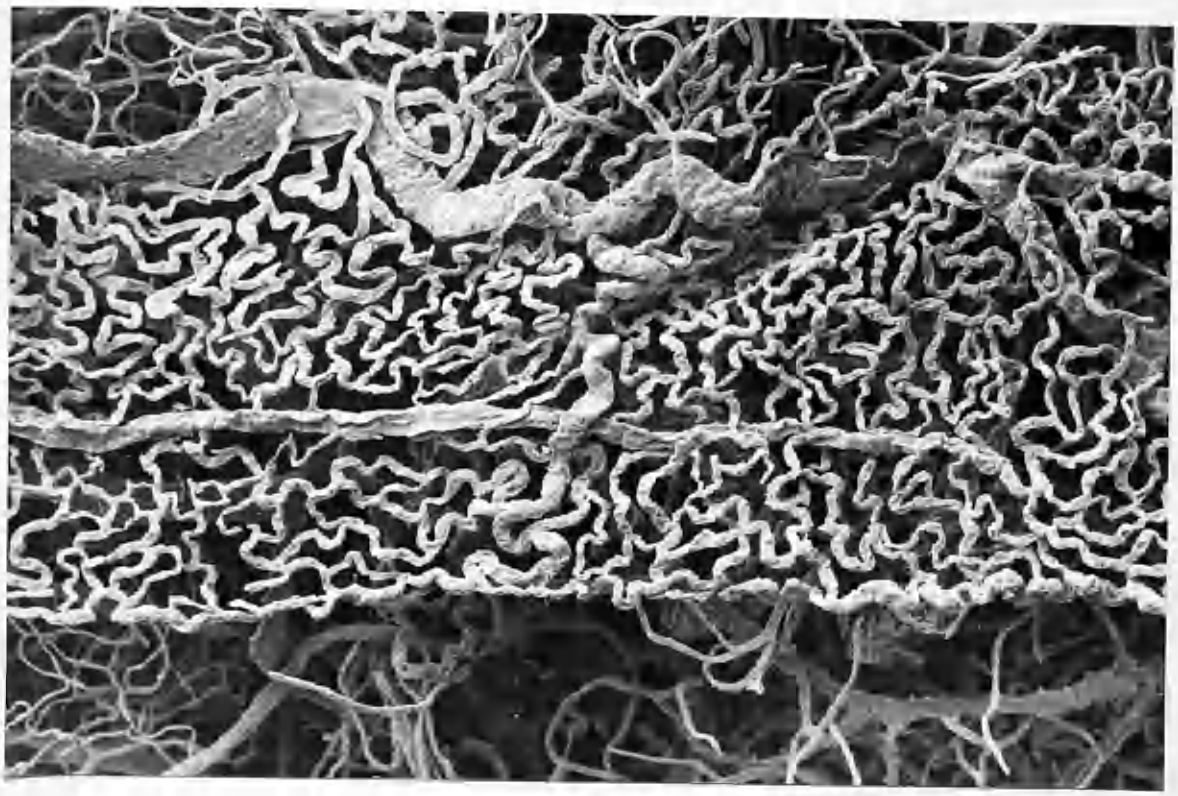
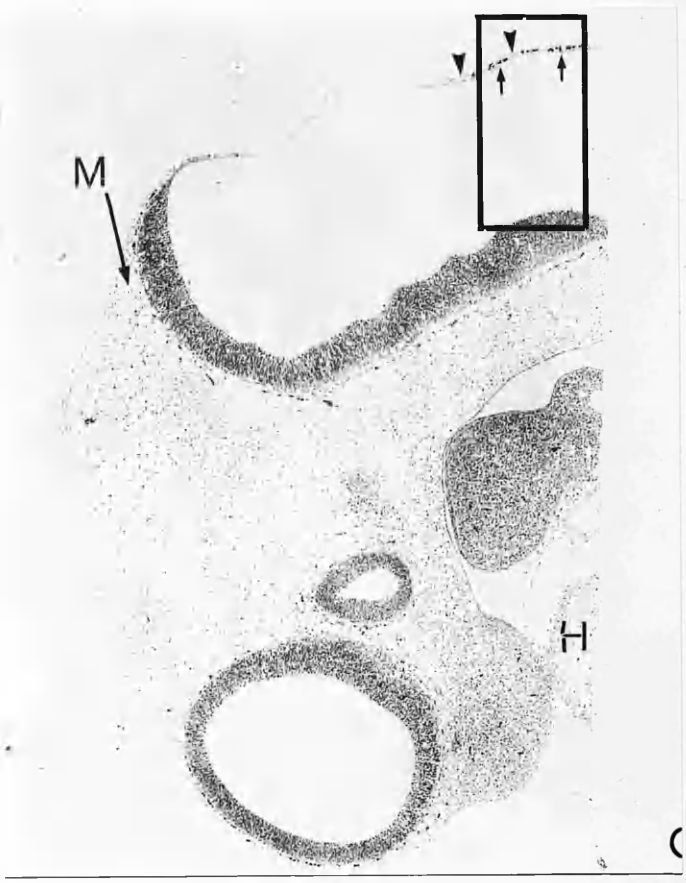


Fig. 68

Part of the vascular cast of the choroid plexus of the lateral ventricle. It shows large vessels running along the long axis of the plexus joined together by a rich network of capillaries. This pattern is different from that of the IVth ventricle (Fig. 66).

Adult mouse. x 300 Cast 4


M
H

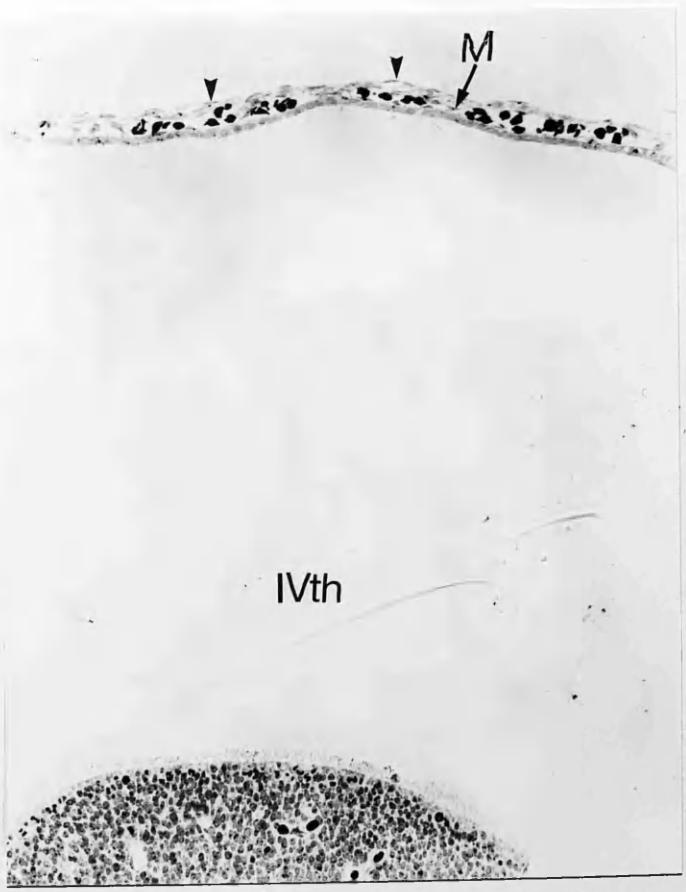

M
IVth

Fig. 69

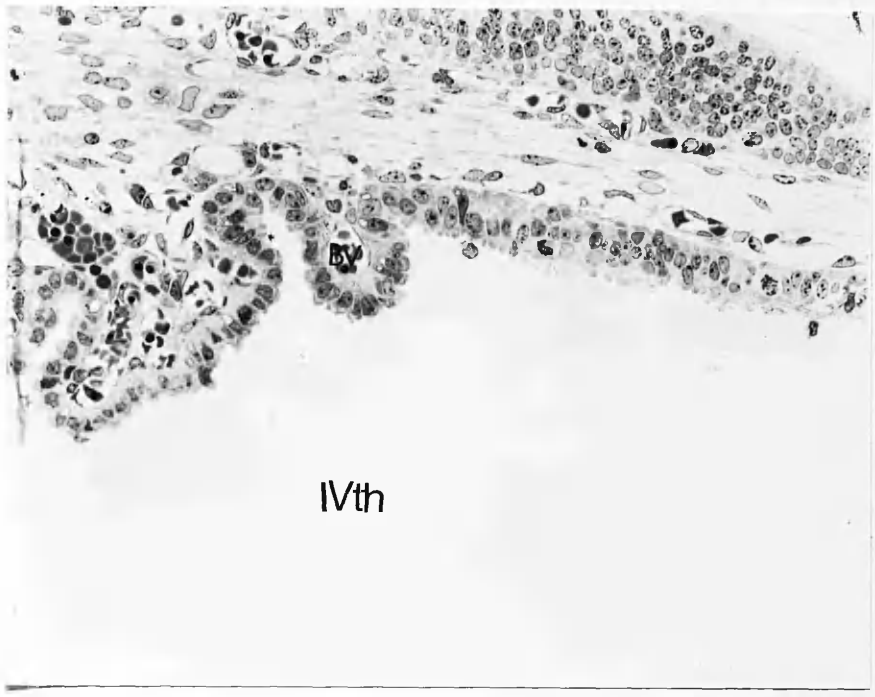
Low power view of a parasagittal section of the head.
It shows the incipient choroid plexus (arrows) of the IVth ventricle.
H = heart
Arrow heads = surface ectoderm
M = mesenchyme

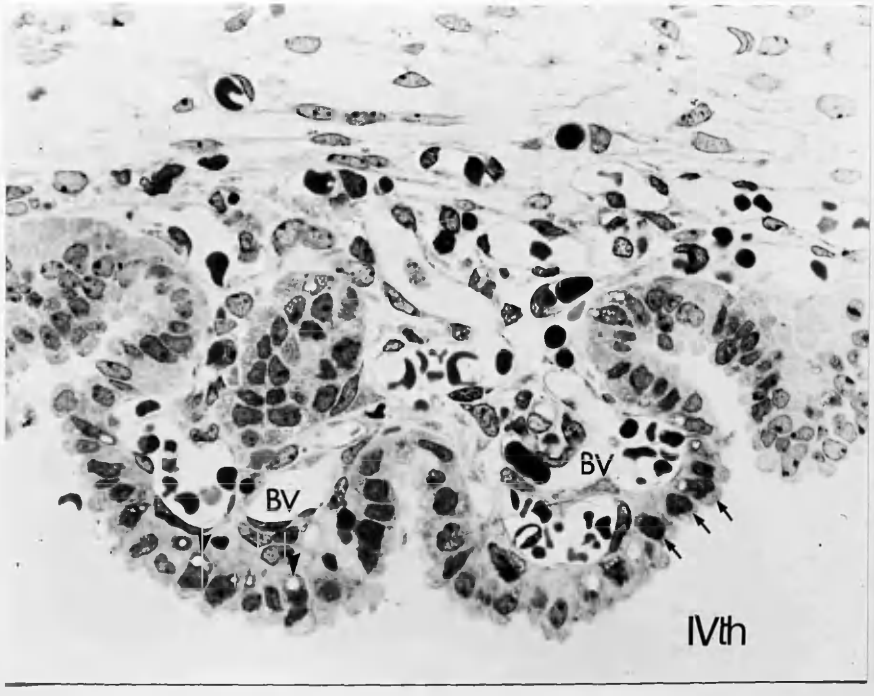
11 day mouse embryo. x 25 Azur II.

Fig. 70

(Inset in Fig. 69)
This shows the vascular tela choroidea overlying the single layer of cells forming the analage of the future choroid plexus.
IVth = IVth ventricle
Arrow heads = surface ectoderm
M = mesenchyme

11 day mouse embryo. x 100 Azur II


BV
IVth


BV
BV
IVth

Figs. 71 - 78

These figures represent coronal section of the midline part of the choroid plexus of the IVth ventricle at different stages of development. The general structure is bascially similar, i.e. epithelium formed by a single layer of low columnar cells, with a vascular connective tissue core.

Fig. 71

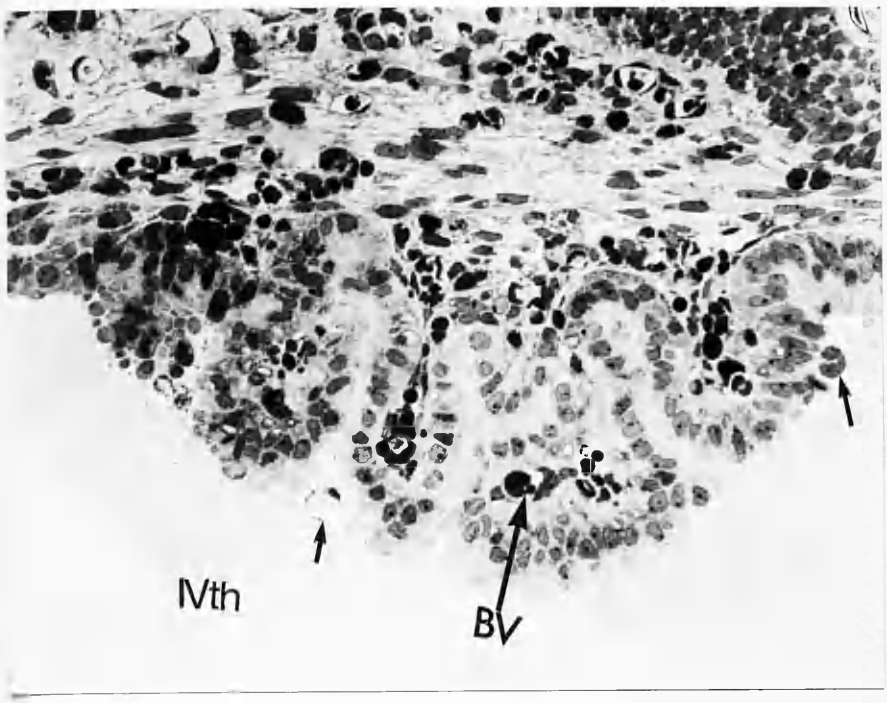
IVth = cavity of IVth ventricle
BV = blood vessels

13 day mouse embryo. x 500 Azur II

Fig. 72

BV = blood vessels closely applied to the epithelium.
arrows = epithelial cells with darker stained nuclei.
Arrowheads = metachromatic masses (representing glycogen)
IVth = Cavity of IVth ventricle.

14 day mouse embryo. x 500 Azur II


IVth
BV

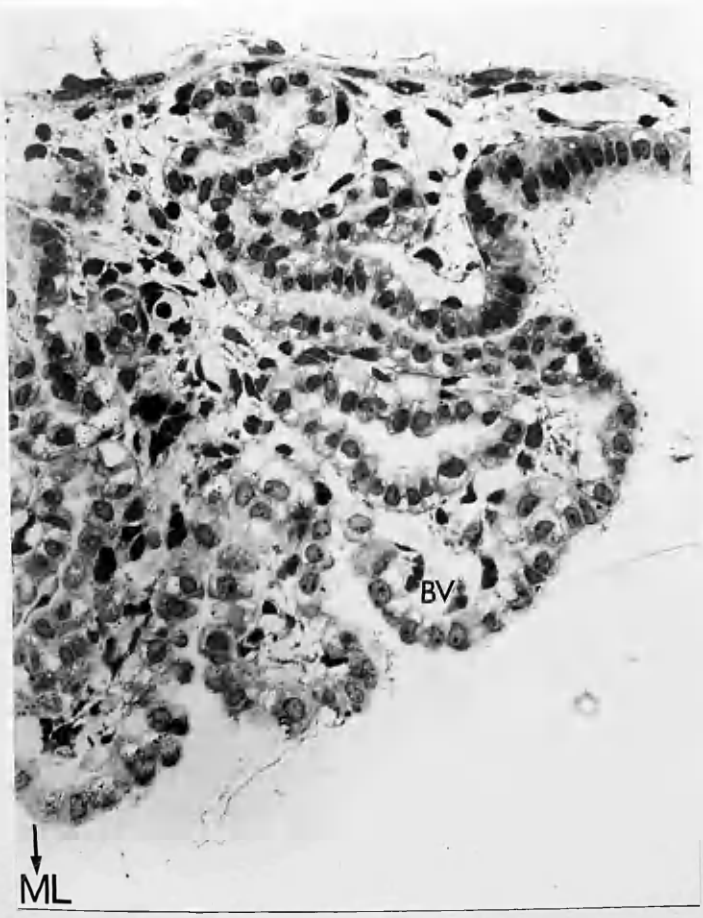

BV
ML

Fig. 73

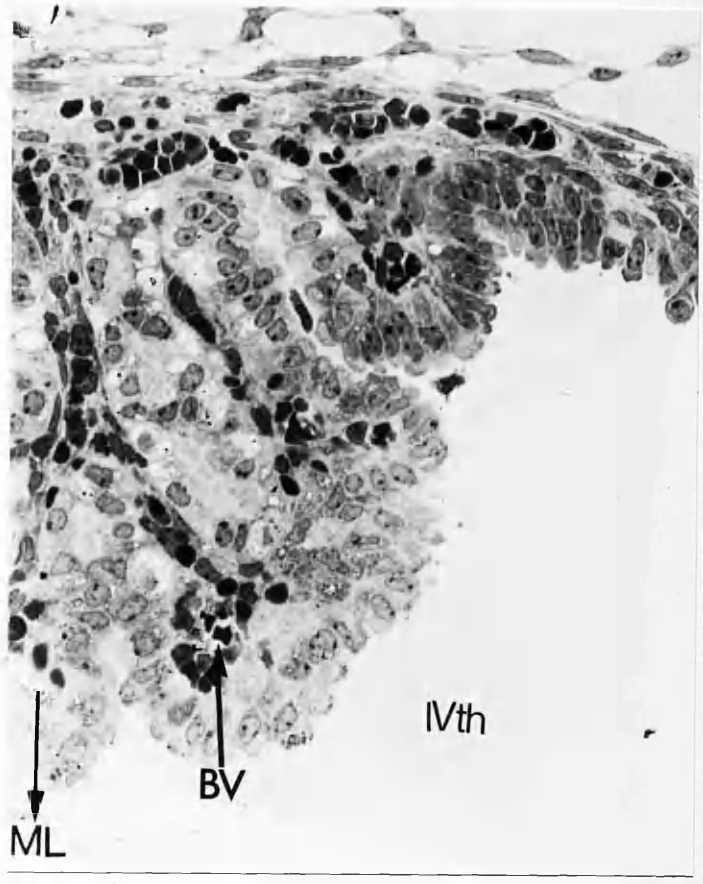
Arrows = Epiplexus cells
IVth = Cavity of IVth ventricle
BV = Blood vessels

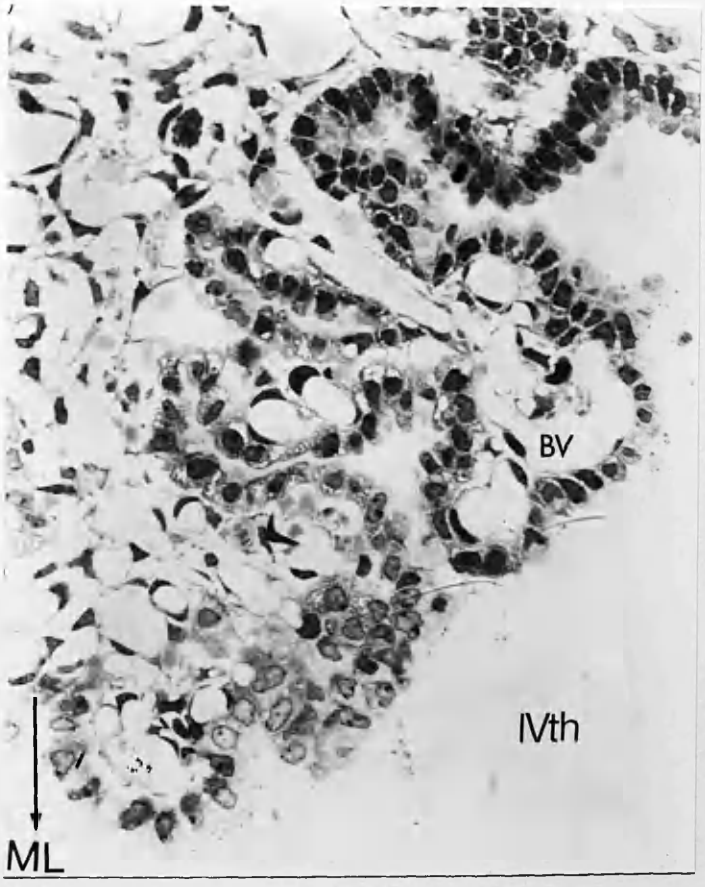
15 day mouse embryo. x 500 Azur II

Fig. 74

ML = midline
BV = blood vessels

16 day mouse embryo. x 500 Azur II


IVth
BV
ML

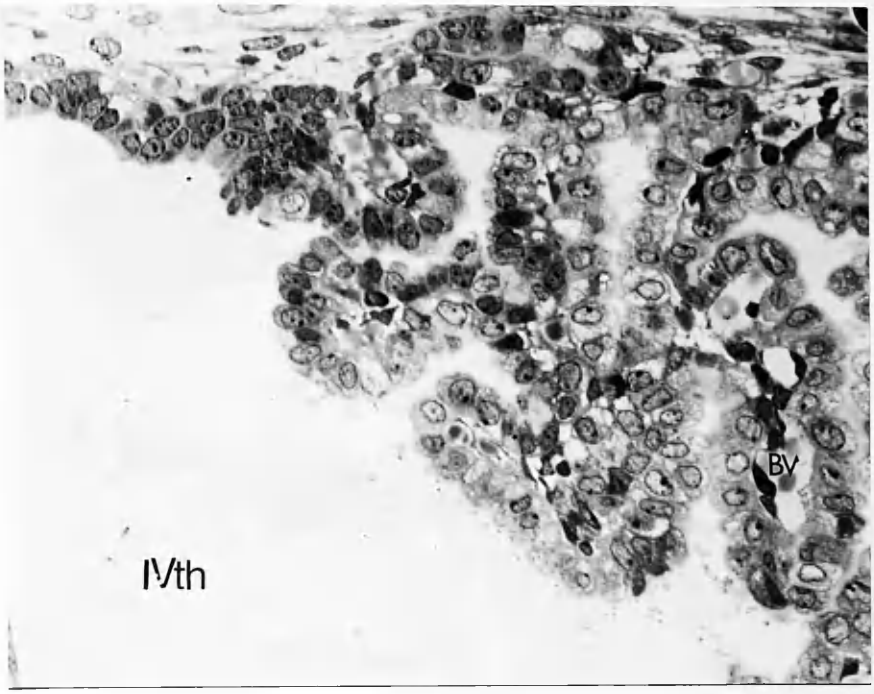

BV
IVth
ML

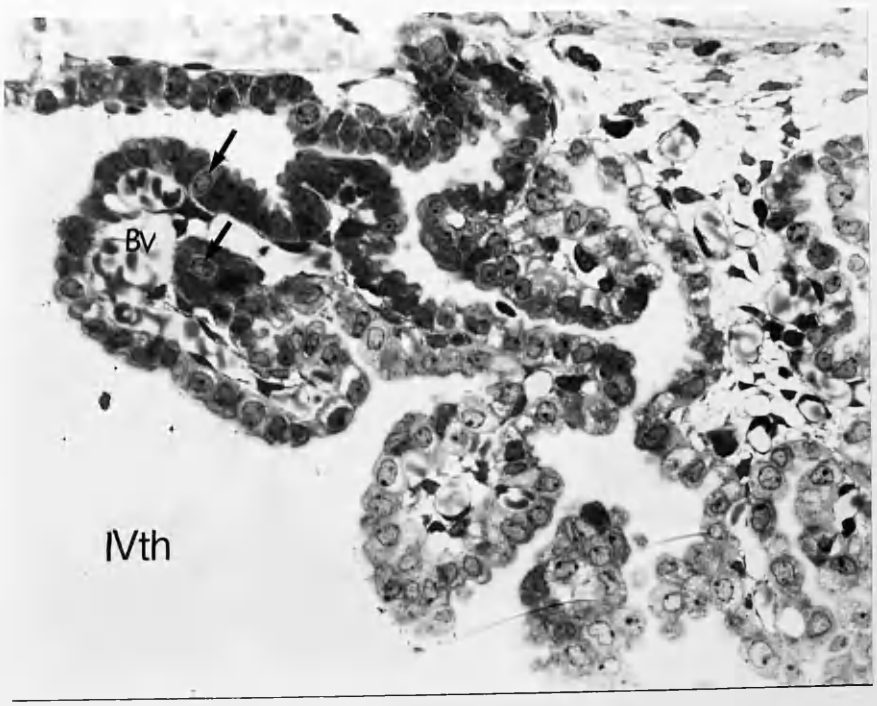
Figs. 75 & 76

BV = wide thin-walled blood vessels closely applied to the choroidal epithelium
IVth = Cavity of IVth ventricle
ML = Midline

17 day mouse embryo. x 500 Azur II

18 day mouse embryo. x 500 Azur II


BV
IVth

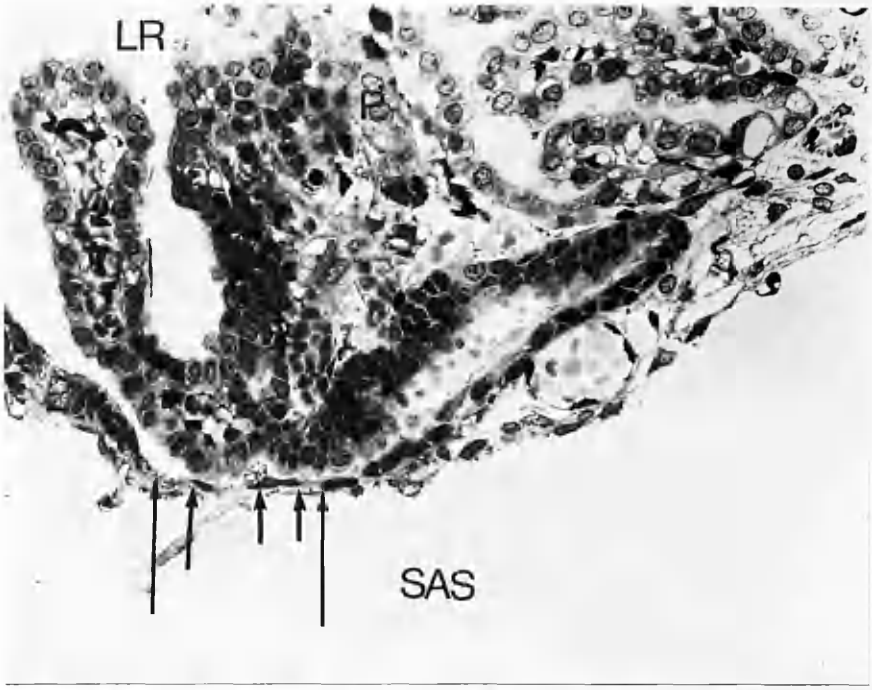

BV
IVth

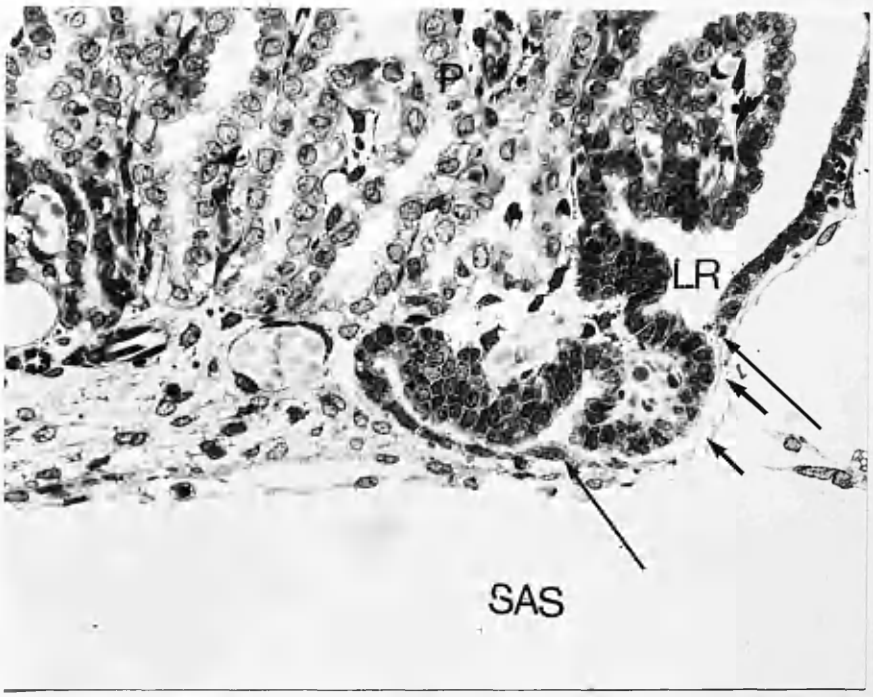
Figs. 77 & 78

Arrows = epithelial cells with lighter-stained cytoplasm
BV = blood vessels
IVth = cavity of IVth ventricle

19 day mouse embryo. x 500 Azur II

Newborn mouse. x 500 Azur II


LR
P
SAS


P
LR
SAS

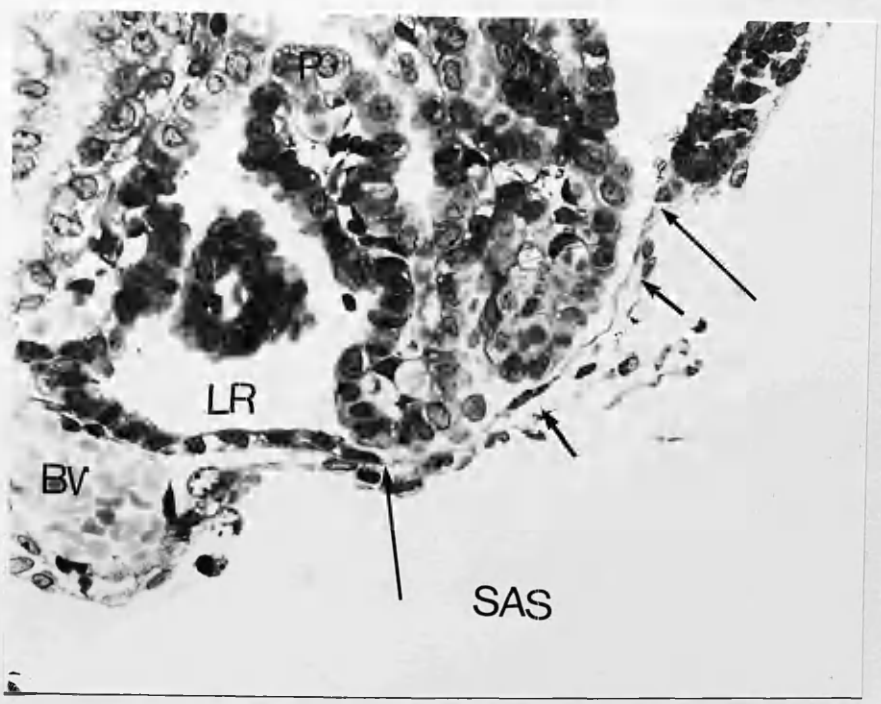

P
LR
BV
SAS

Fig. 79

A coronal section of the left lateral recess (LR) of the IVth ventricle containing folds of choroid plexus (P). It shows an extremely attenuated, though continuous, area of the ependymal lining (between large arrows). A thin, discontinuous pial layer (small arrows) overlies the attenuated ependyma.
SAS = subarachnoid space

17 day mouse embryo. x 320 Azur II

Fig. 80

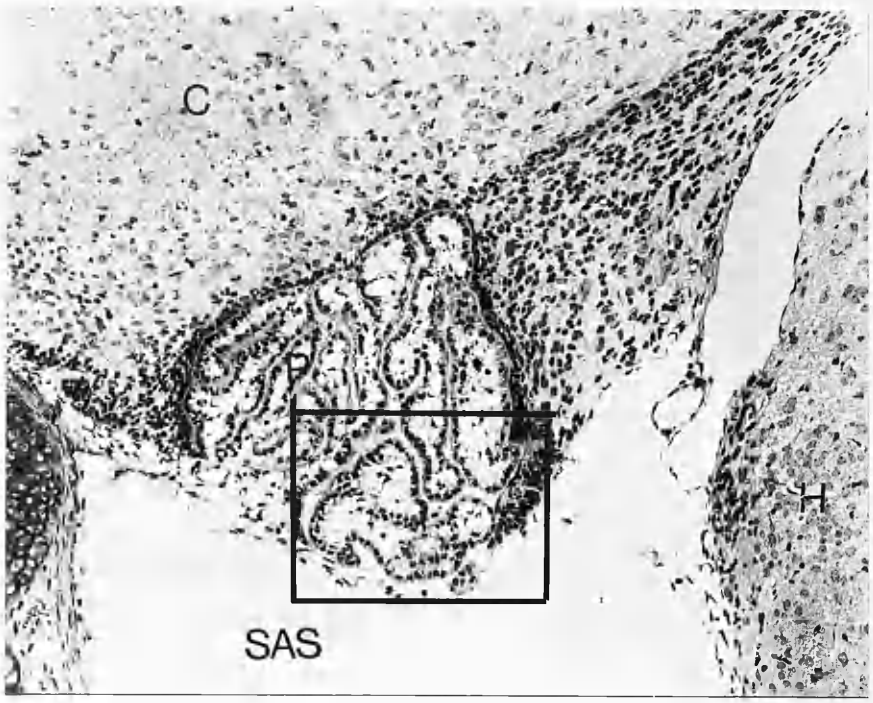
Shows the right lateral recess (LR) of the IVth ventricle with part of its wall thinned out (between large arrows).
Small arrows = pial layer overlying the ependyma
P = choroid plexus
SAS = subarachnoid space

18 day mouse embryo. x 400 Azur II

Fig. 81

A high power view of the left lateral recess of the IVth ventricle (LR). Part of the ependymal wall (between large arrows) is very attenuated but no break in continuity could be seen.
A pial layer (small arrows) runs parallel to the ependyma although in places it is separated from it by a blood vessel (BV).
P = choroid plexus
SAS = subarachnoid space

19 day mouse embryo. x 400 Azur II


C
P
H
SAS

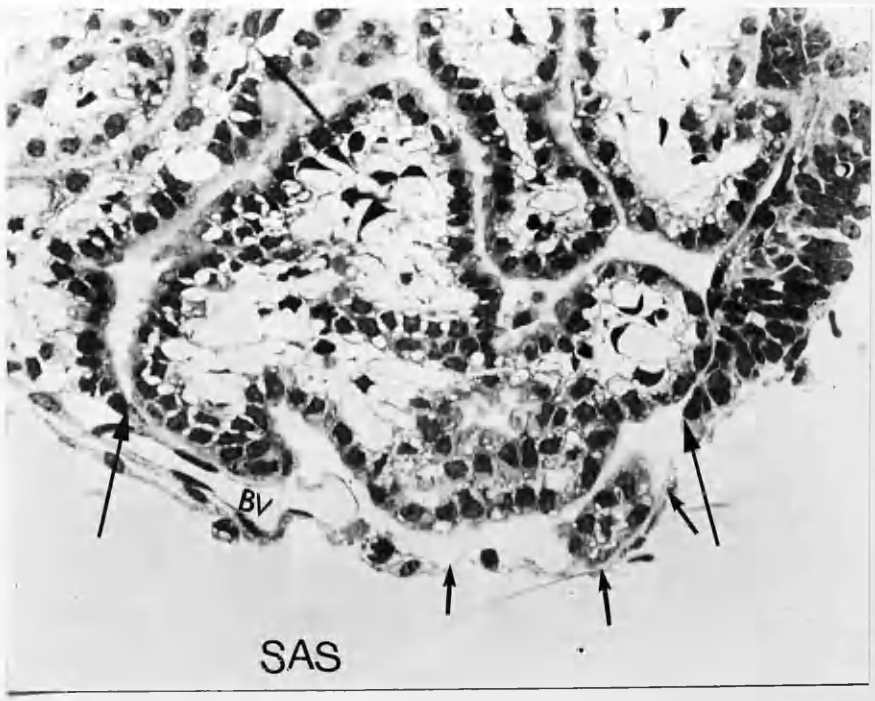

BV
SAS

Fig. 82

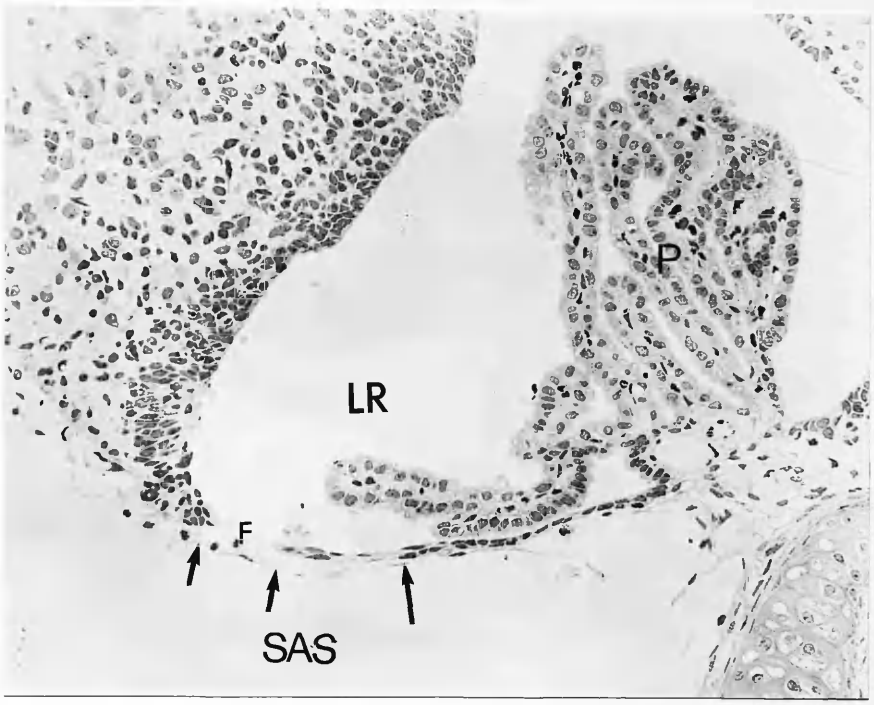
A medium power view of a coronal section showing part of cerebellum (C), hind brain (H) and the left lateral recess packed with choroid plexus folds (P).
SAS = subarachnoid space.
Inset - see Fig. 83

Newborn mouse. x 150 Azur II

Fig. 83

High power of area outlined in Fig. 82. A foramen appears in the wall of the lateral recess, the limits of which are shown by large arrows. The foramen is bridged by a pial layer (small arrows). A blood vessel (BV) is seen lying between the pial layer and the foramen.
SAS = subarachnoid space

Newborn mouse. x 500 Azur II


P
LR
F
SAS

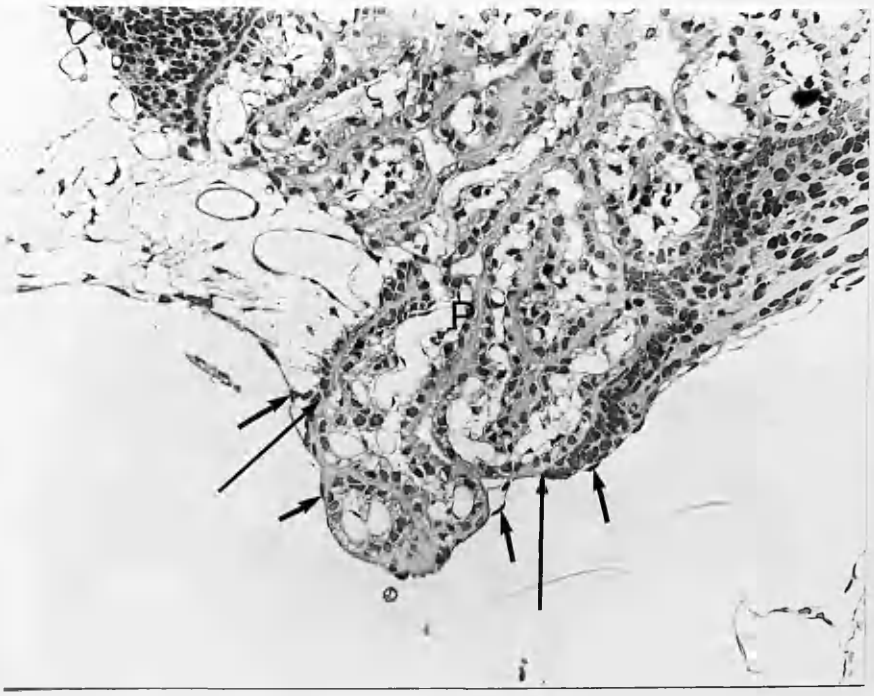

P

Fig. 84

Medium power view showing a coronal section of the right lateral recess (LR).

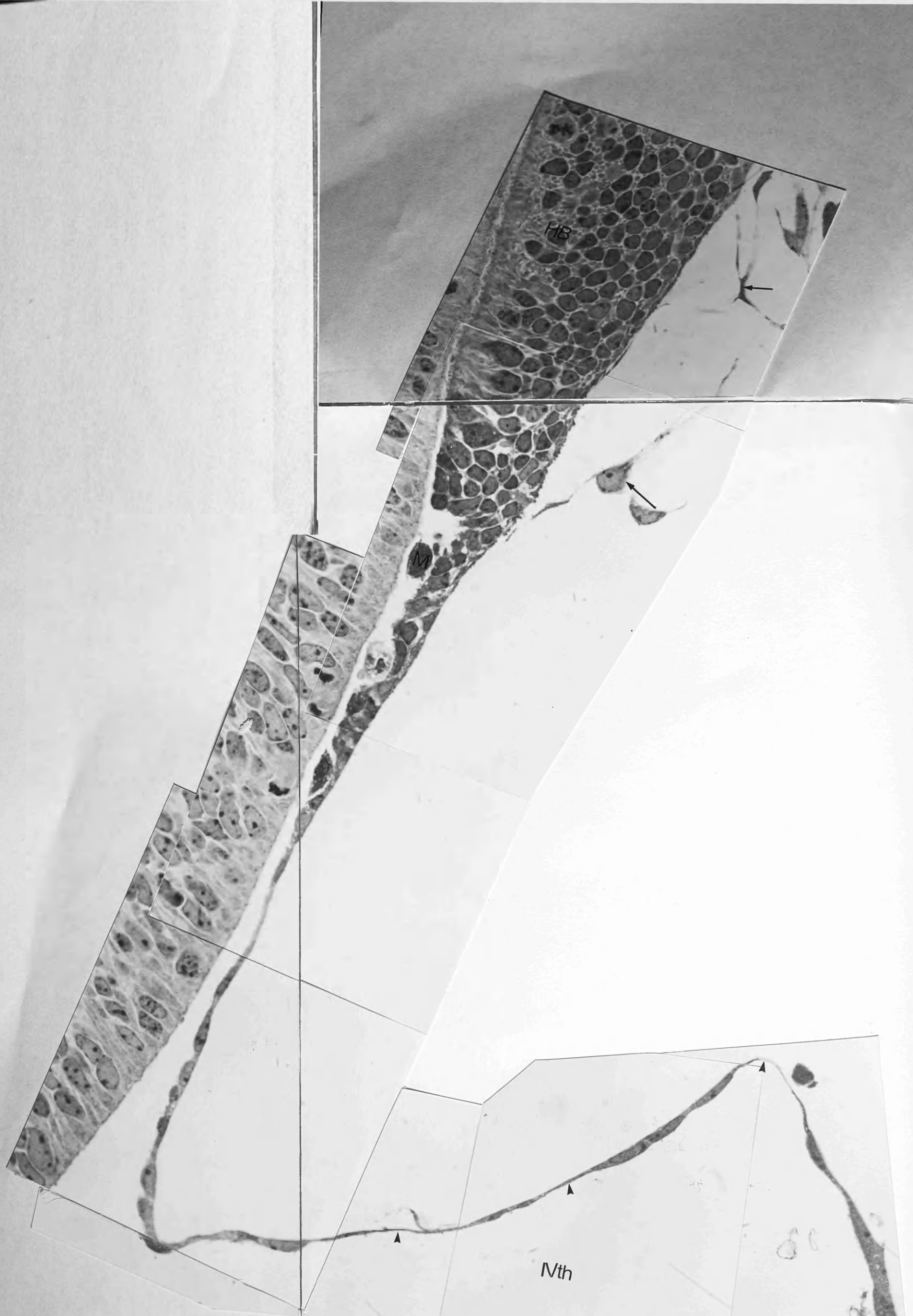
P = choroid plexus
F = foramen
SAS = subarachnoid space
Small arrows = pial layer

Newborn mouse. x 250 Azur II

Fig. 85

The left lateral recess containing folds of choroid plexus (P). A foramen in its ependymal wall is shown (between large arrows).
Folds of choroid plexus are seen prolapsing through the foramen, but they are still covered by an incomplete pial layer (small arrows).

Newborn mouse. x 250 Azur II

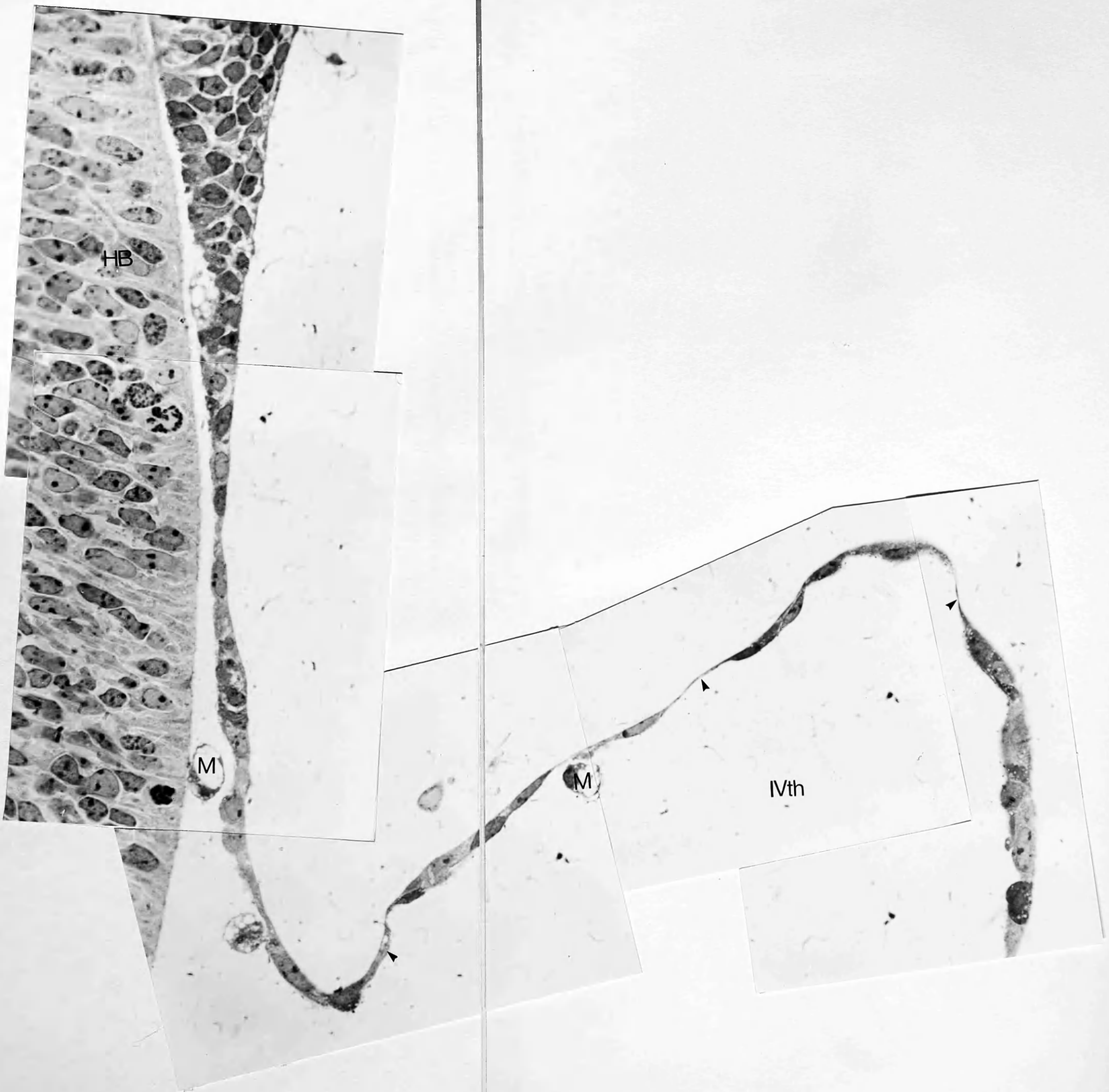

HB
M
IVth

Figs. 86, 87 & 88

Show the thin, but continuous roof (arrowheads) of IVth ventricle (IVth) from its attachment to hindbrain (HB) up to the midline.
Arrows = pial cells
M = macrophages

13 day embryo. x 1000 Azur blue


HB
IVth


HB
M
M
IVth

IVth

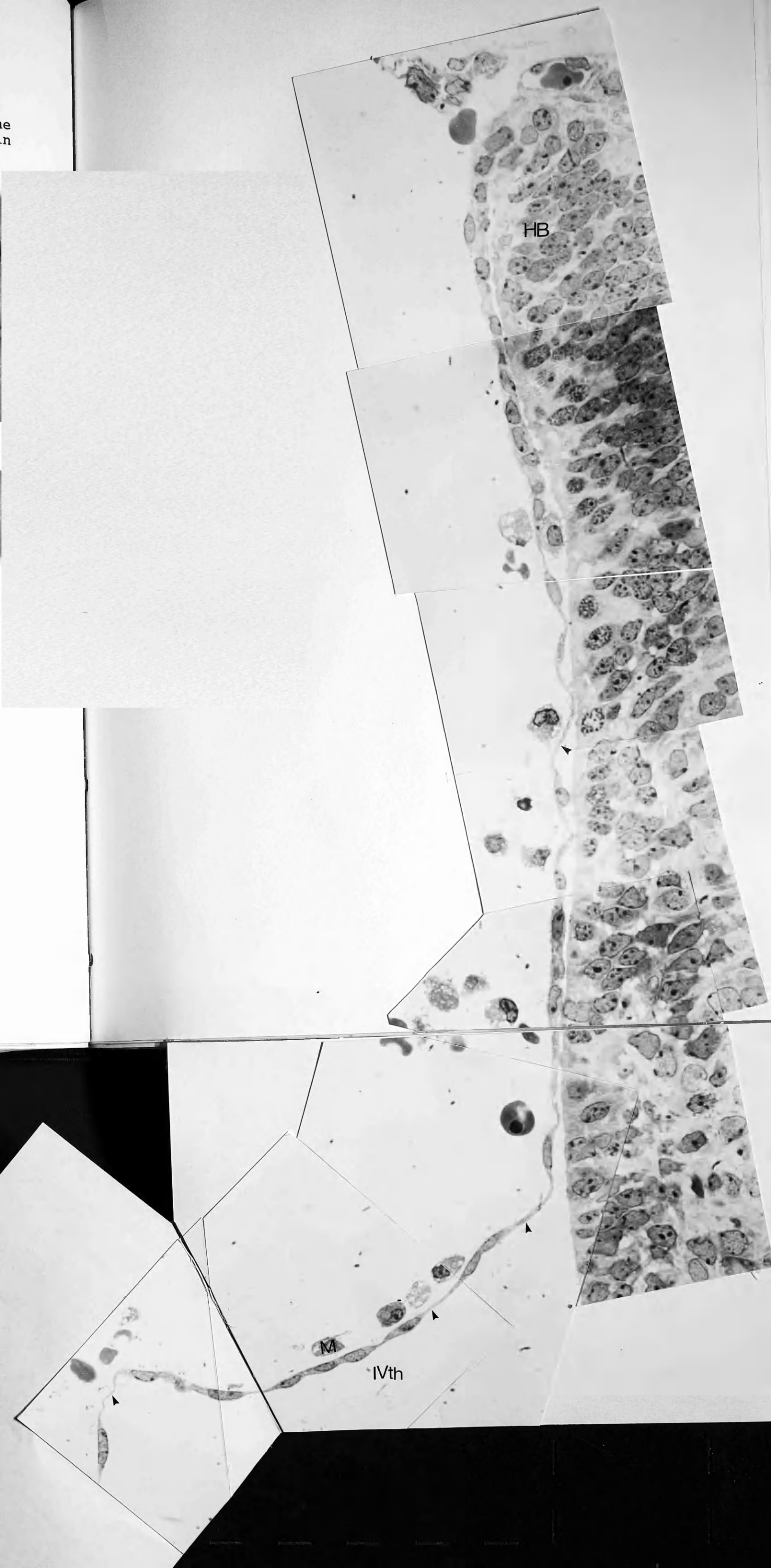
HB

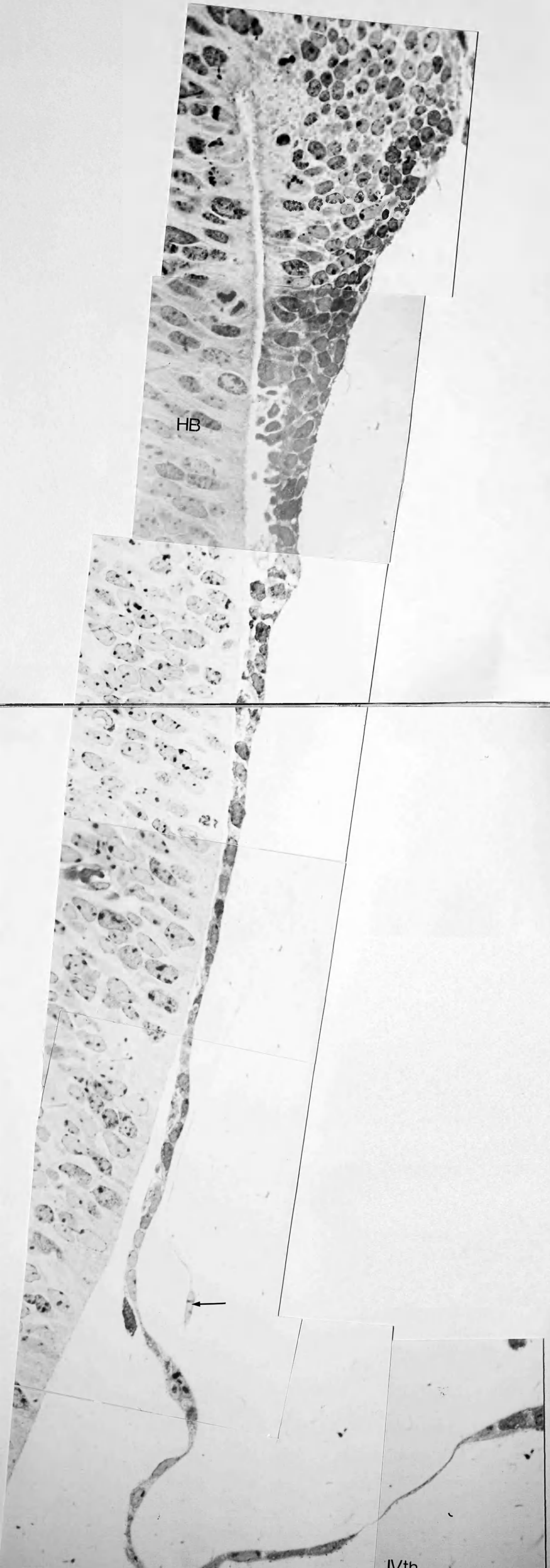
M

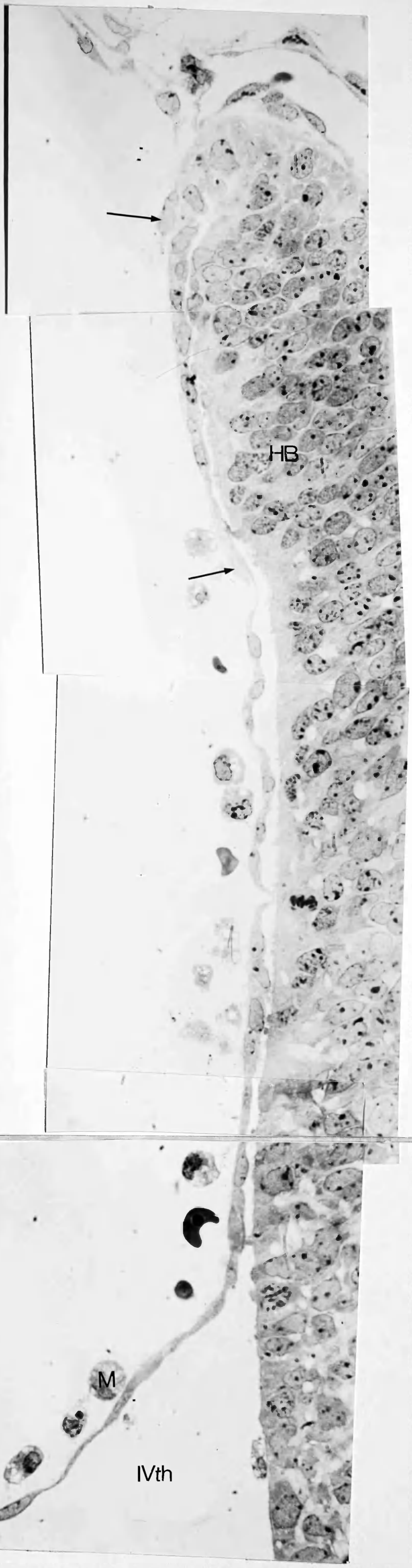
Figs. 89, 90 & 91

These montages show the ependymal roof of the IVth ventricle from its attachment to hindbrain (HB) up to the midline.
Arrows = pia arachnoid cell
M = macrophages

14 day mouse embryo. x 1000 Azur blue


HB
M
IVth


HB
IVth


HB
M
IVth

Figs. 92, 93 & 94

These montages show the thin but continuous roof of IVth ventricle (arrow heads), and the overlying discontinuous pial layer (arrows).
M = macrophages
SAS = subarachnoid space

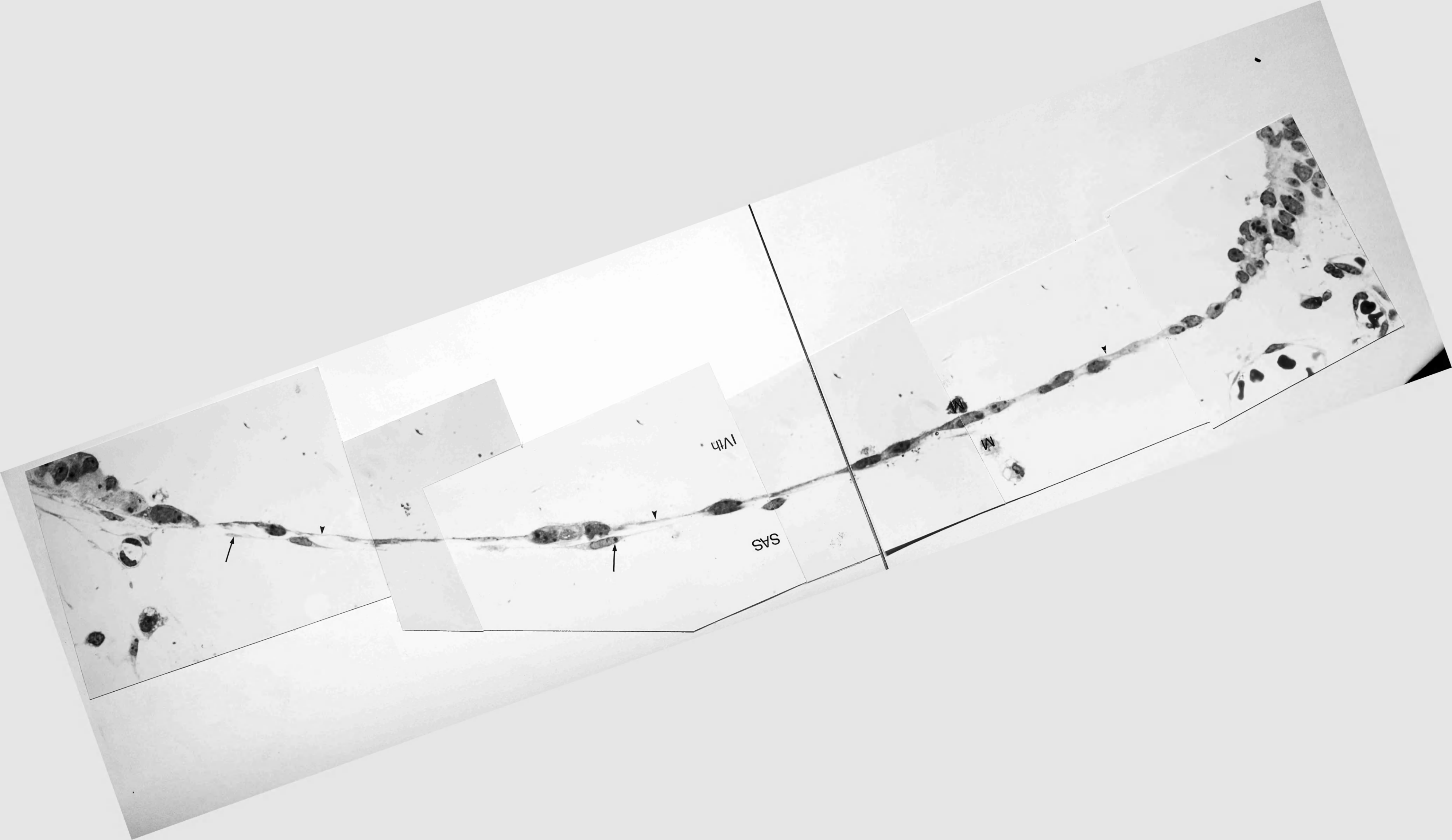
15 day mouse embryo. x 1000 Azur blue

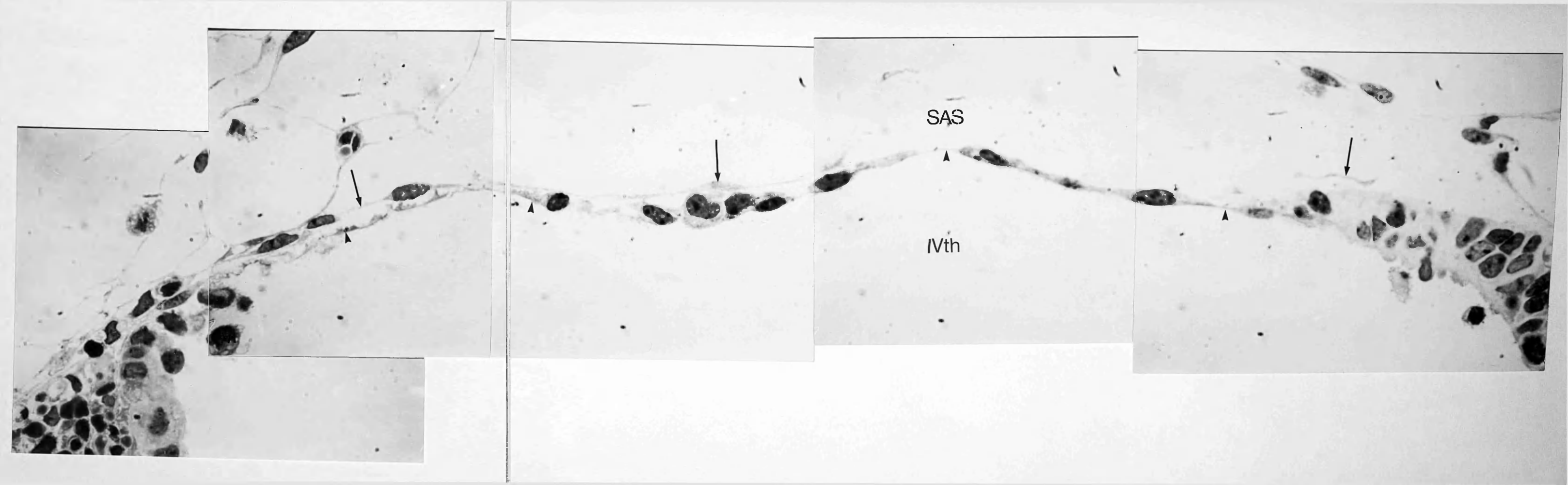
M

M

SAS

IVth


IVth
SAS
M
M


SAS
IVth

Figs. 95, 96 & 97

Three montages showing the roof of the IVth ventricle.
Arrowheads = indicate thin but continuous ependymal layer
Arrows = pial layer
Note macrophages (M) between ependymal and pial layer.

16 day mouse embryo. x 1000 Azur blue

Plate IV

IVth

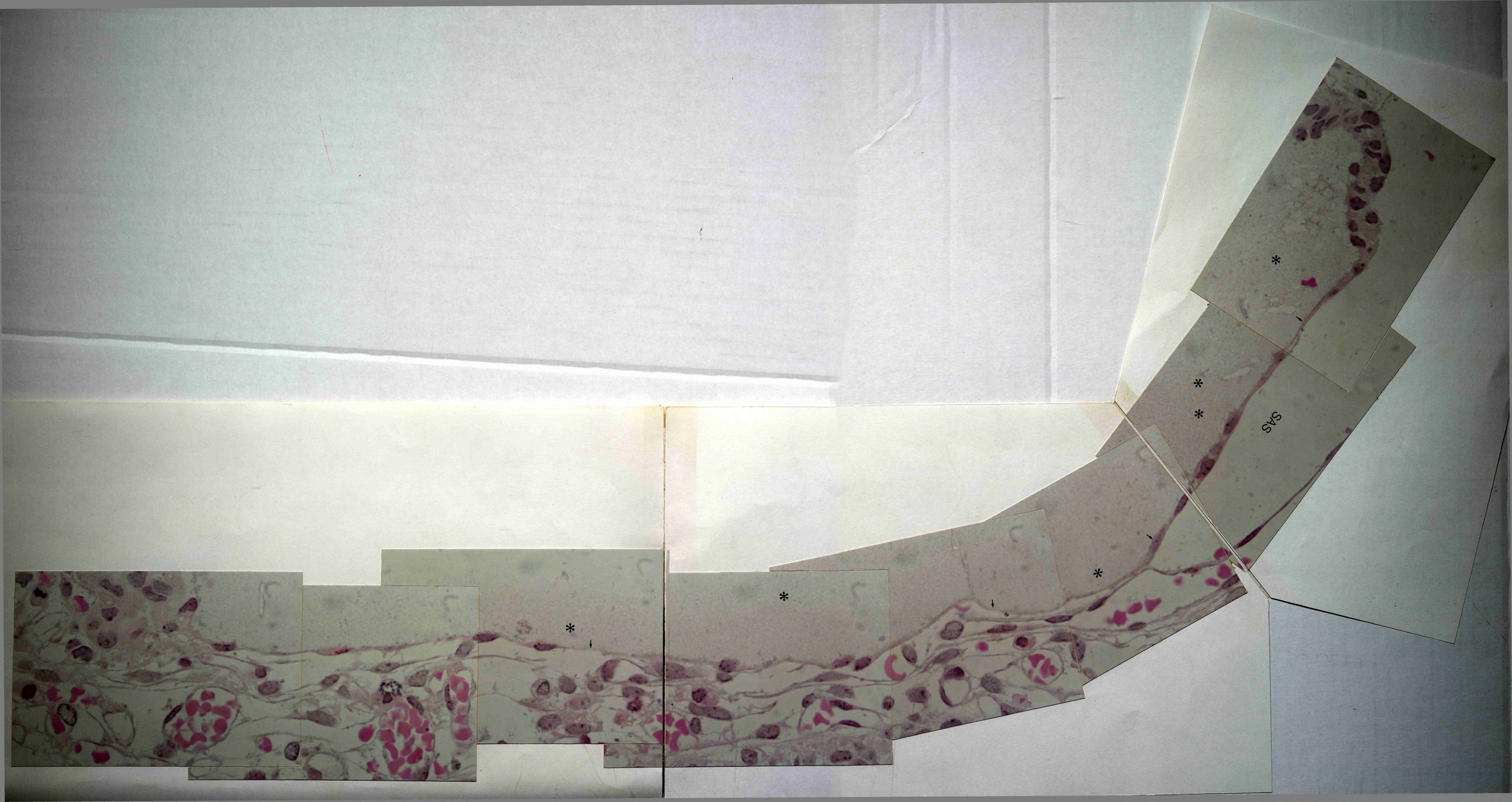
M

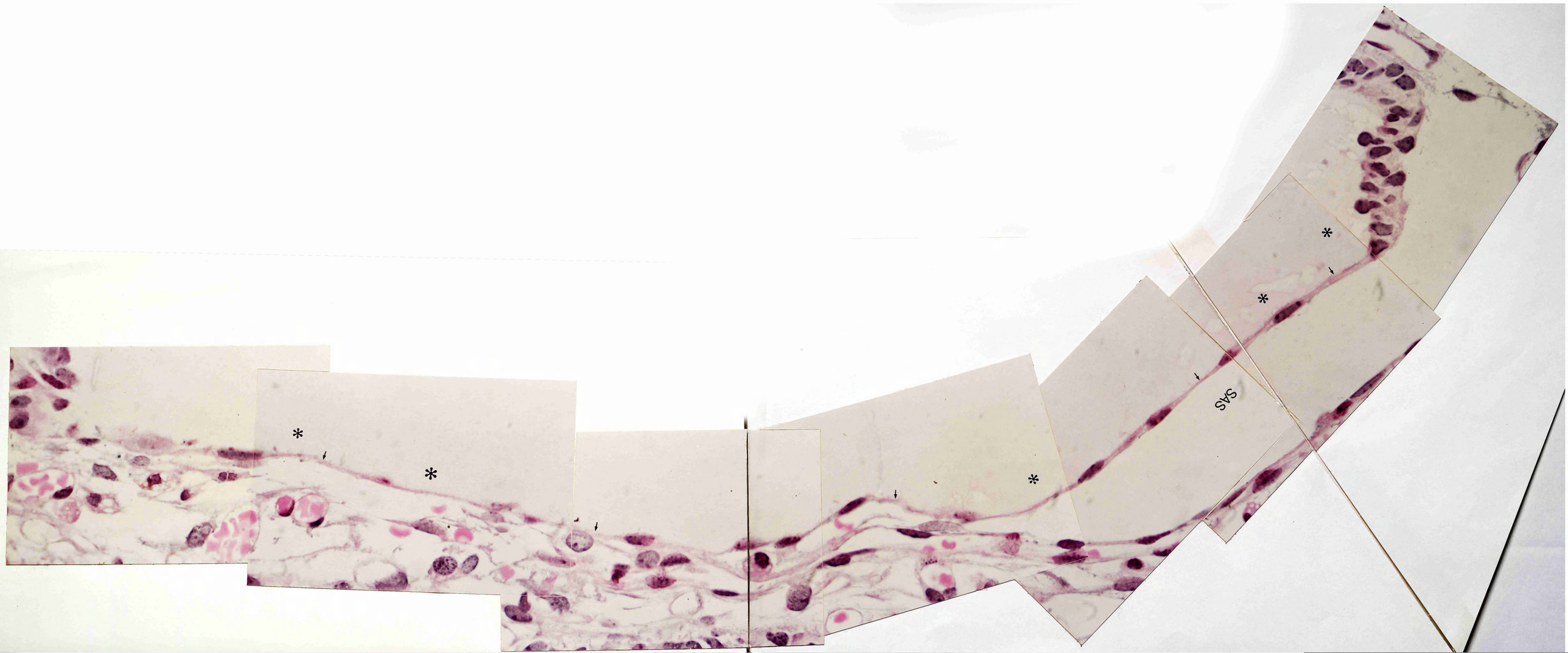

IVth

Figs. 98 & 99

These montages show a precipitated material (asterisks) in the cavity of IVth ventricle but not in subarachnoid space (SAS).
Arrows = roof of IVth ventricle

16 day mouse embryo. x 1000 H & E

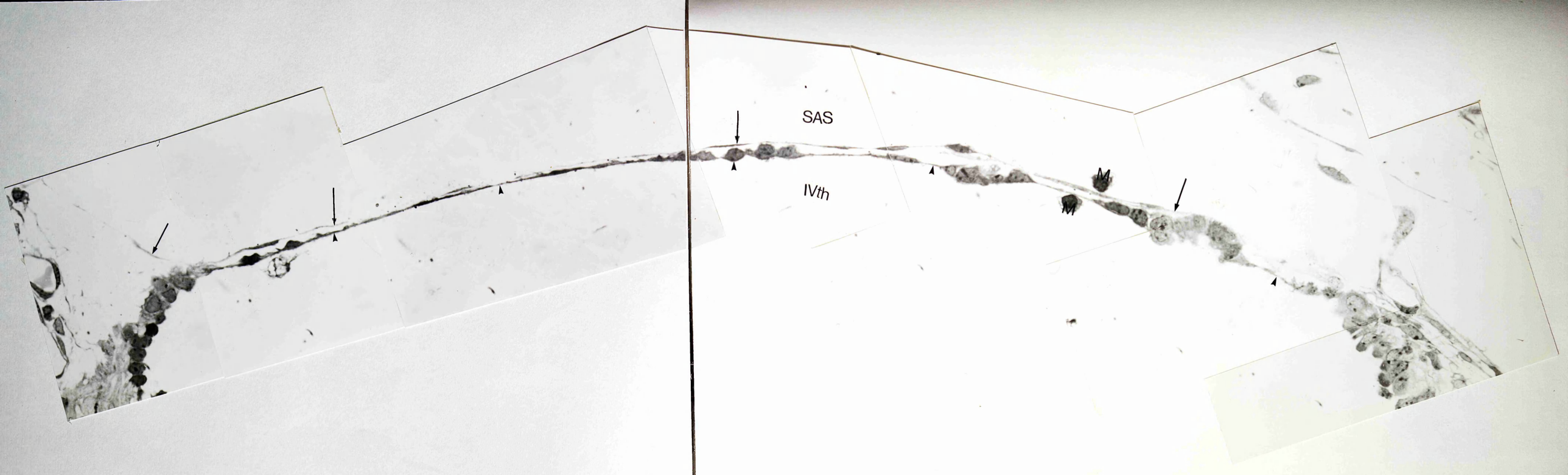

SAS


SAS

Figs. 100, 101 & 102

Three montages showing the roof of IVth ventricle.
Arrowheads = ependymal roof of IVth ventricle, extremely attenuated in places
Arrows = pial layer
M = macrophages
SAS = subarachnoid space

Newborn mouse. x 1000 Azur blue


SAS
IVth
M
M

SAS

IVth

SAS

IVth

Fig. 103

Coronal section of the hind brain, showing the cavity of the IVth ventricle with choroid plexus (CP) in its roof. Condensed mesenchyme (S) forms the future skull and duramater. The mesenchyme (M) is formed of widely separated spindle-shaped cells.
NO subarachnoid space is present.

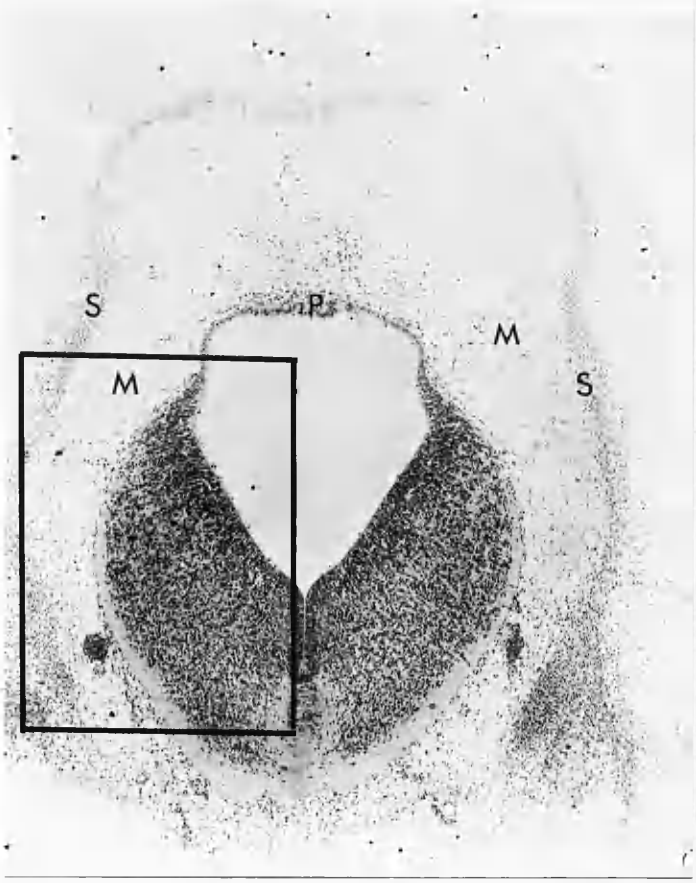
13 day mouse embryo. x50 Azur II

Fig. 104

(Inset in Fig. 103).

BV = blood vessel.

13 day mouse embryo. x 200


S
P
M
M
S

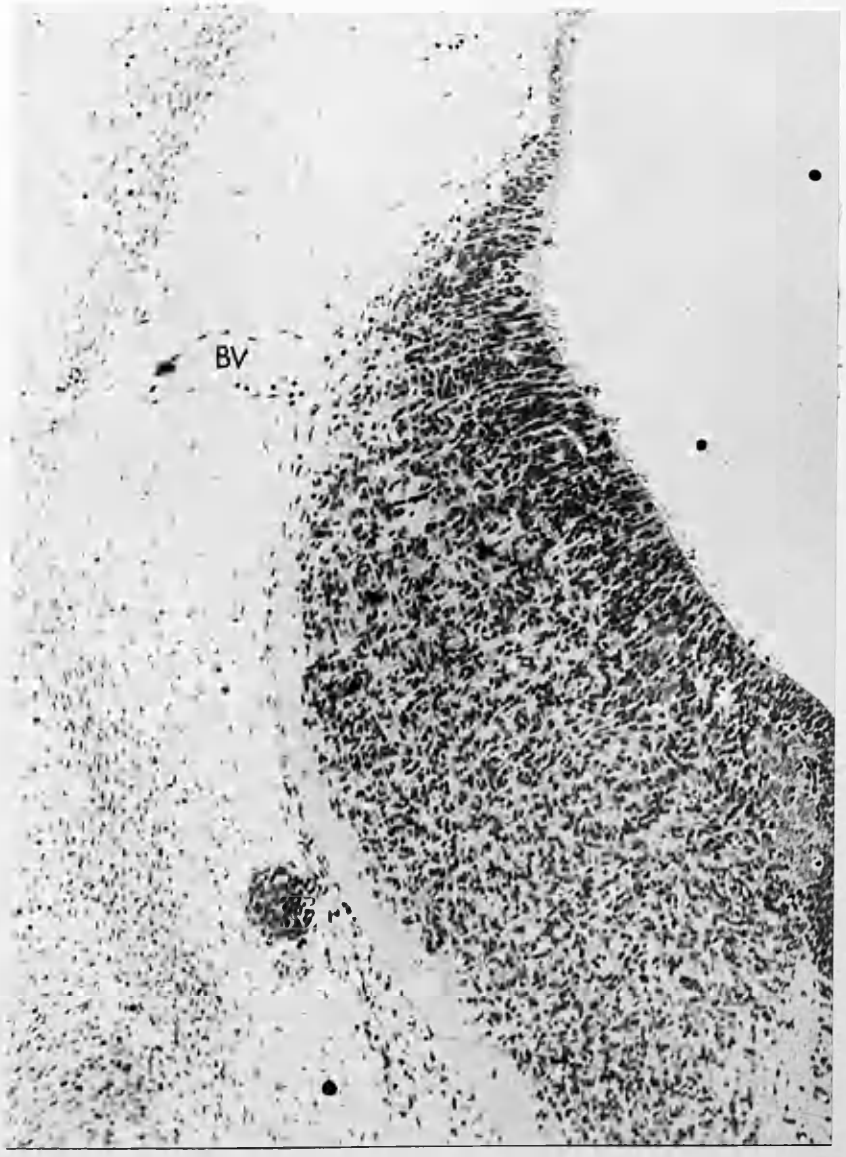

BV

Fig. 105

This is a coronal section of the hind brain (H) with a longitudinal section of cervical spinal cord (SC).
Subarachnoid spaces develop around the hind brain and cervical spinal cord.
Chondrofication of ventrolateral aspect of skull is well established (S).
Insets - see following figures.

14 day mouse embryo. x 32 Azur II

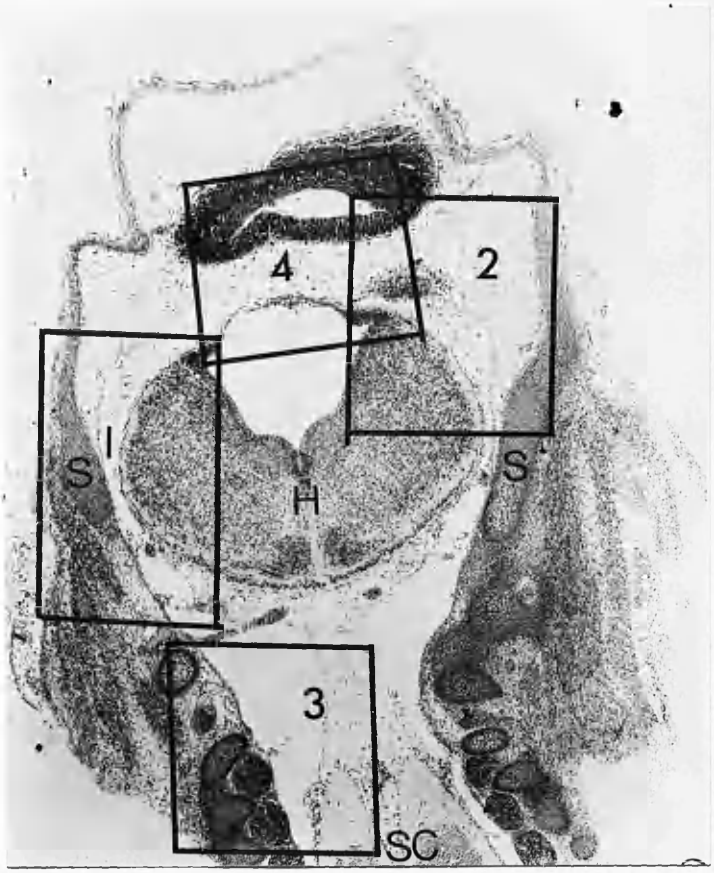

4
2
I
S
H
S
3
SC

Fig. 106

Higher power of Inset 1, Fig. 105, shows the area lateral to the hind brain, with definite subarachnoid spaces (SAS).
$X-X_2$ = mesenchymal condensations connecting pia and arachnoid
Many phagocytic cells are seen (arrows).
A = arachnoid
P = pia

14 day mouse embryo. x 100

Fig. 107

Medium power view showing subarachnoid spaces (SAS) on the ventral aspect of the hind brain (H).

14 day mouse embryo. x 100 Azur II


X1
A
X2
B
SAS

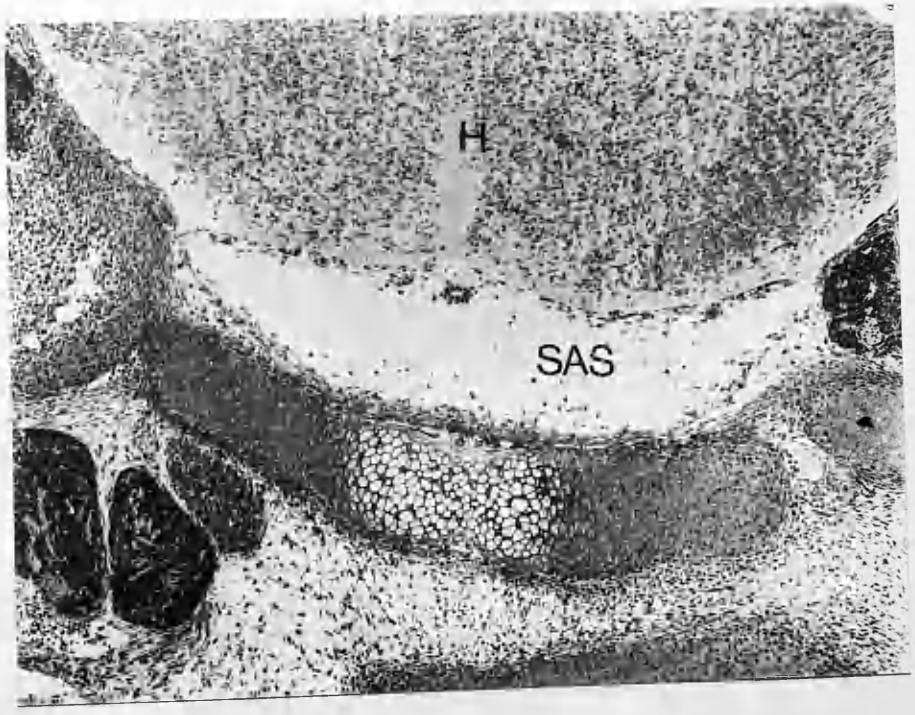

H
SAS

Fig. 108

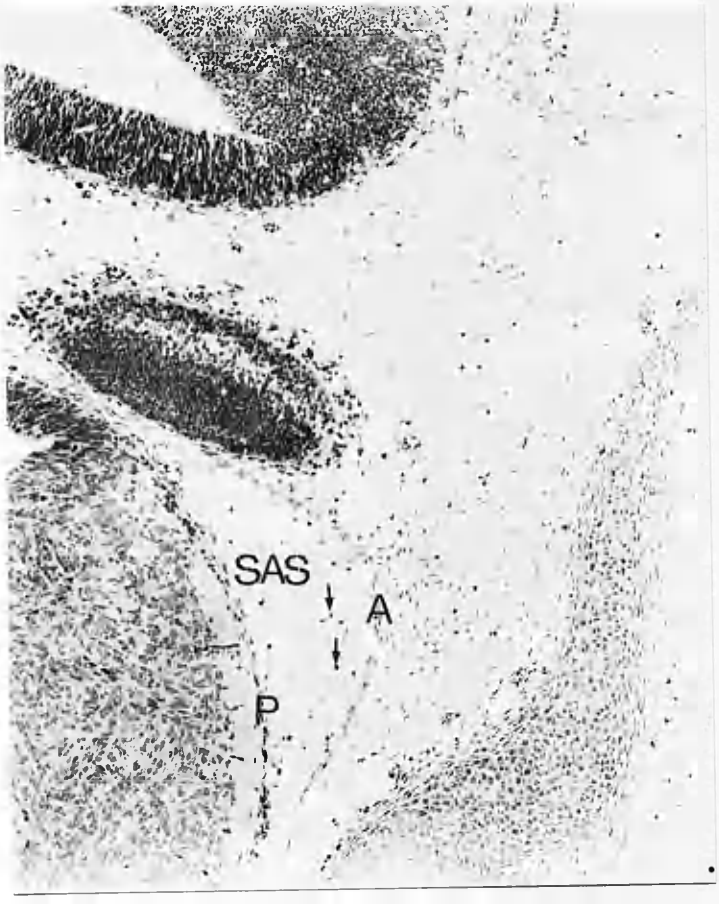
Higher power of Inset 2, Fig. 105. It shows development of subarachnoid spaces on the dorsolateral aspect of the hindbrain (SAS).
A = arachnoid
P = pial layer
Arrows = phagocytic cells

14 day mouse embryo. x 100

Fig. 109

Higher power of Inset 3, Fig. 105.
Subarachnoid spaces (SAS) are seen on the ventrolateral aspect of the hind brain, and on the lateral aspect of cervical spinal cord.
D = dorsal root ganglion.

14 day mouse embryo. x 100


SAS
A
P

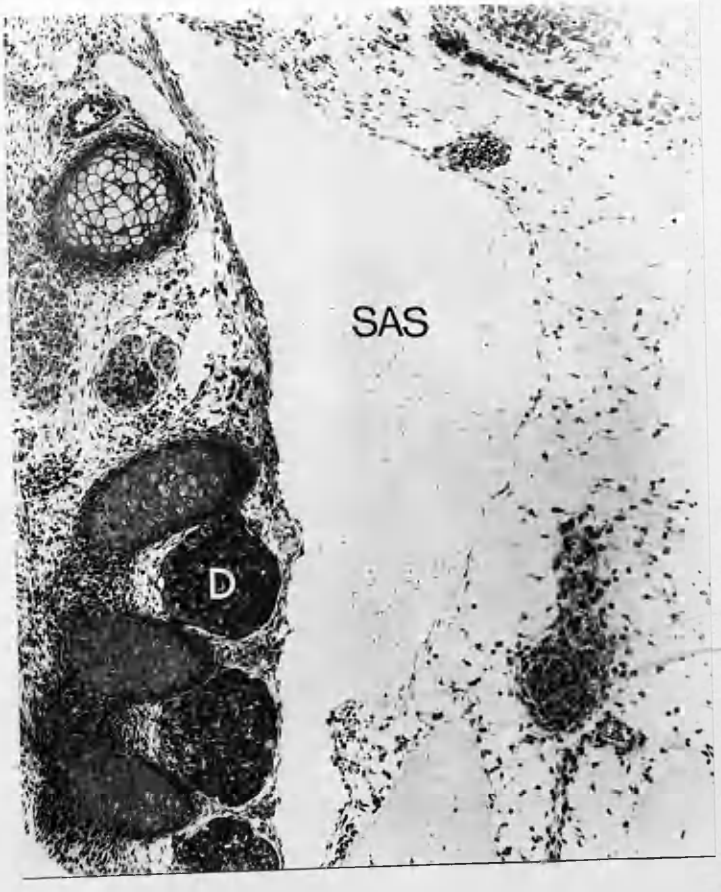

SAS
D

Fig. 110

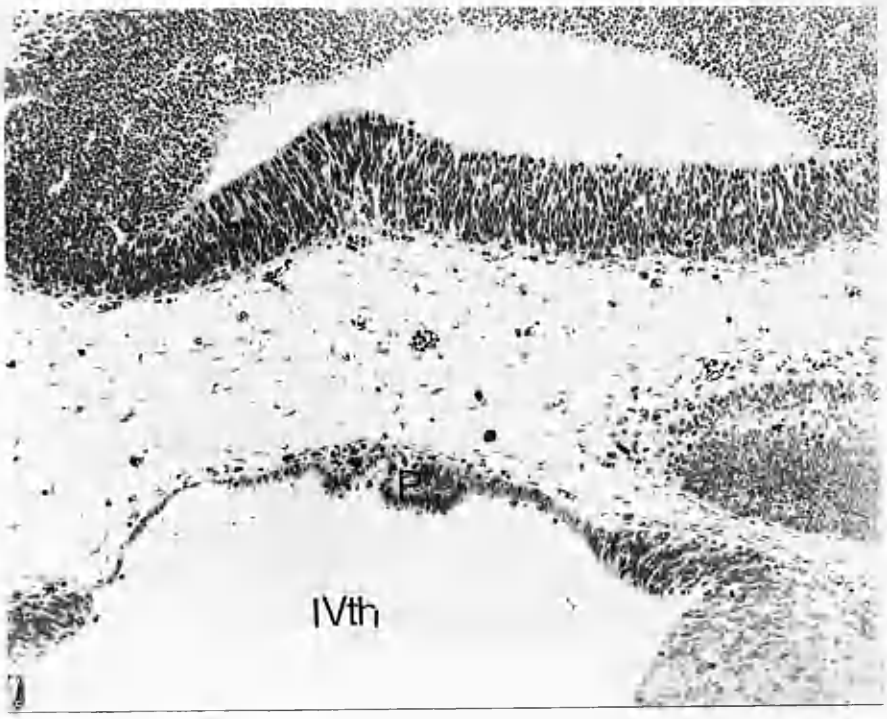
Medium power of Inset 4, Fig. 105.
This shows the area dorsal to the roof of the IVth ventricle.
No subarachnoid spaces develop here.
P = choroid plexus
IVth = cavity of IVth ventricle

14 day mouse embryo. x 125

Fig. 111

High power view showing some of the phagocytic cells (arrows) seen in the subarachnoid space.

14 day mouse embryo. x 1000 Azur II


IVth

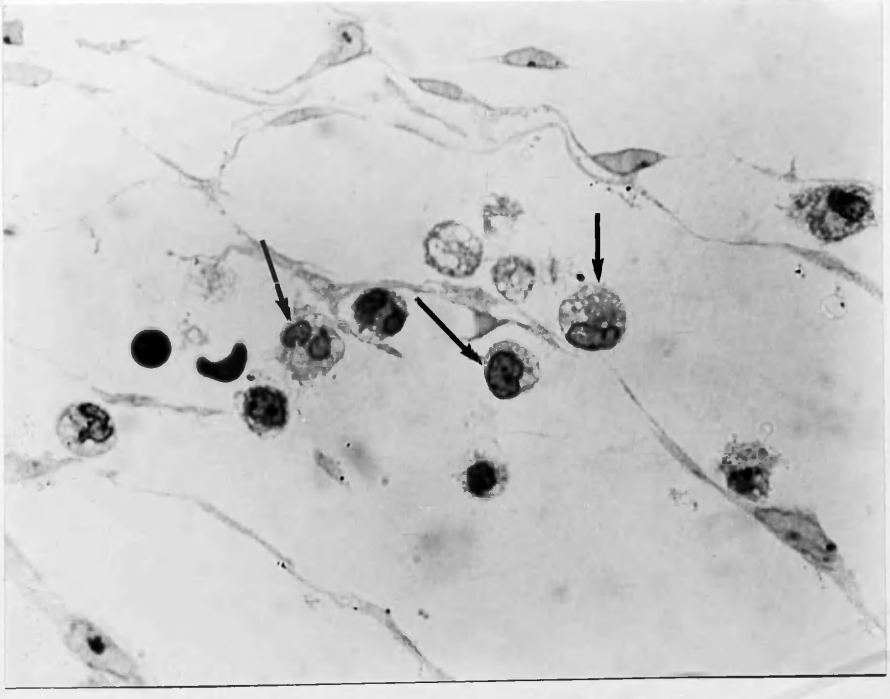


Fig. 112

Low power light micrograph showing subarachnoid spaces (SAS) on the dorsal and dorsolateral and ventrolateral aspect of the hind brain.

15 day mouse embryo. x 32 Azur II

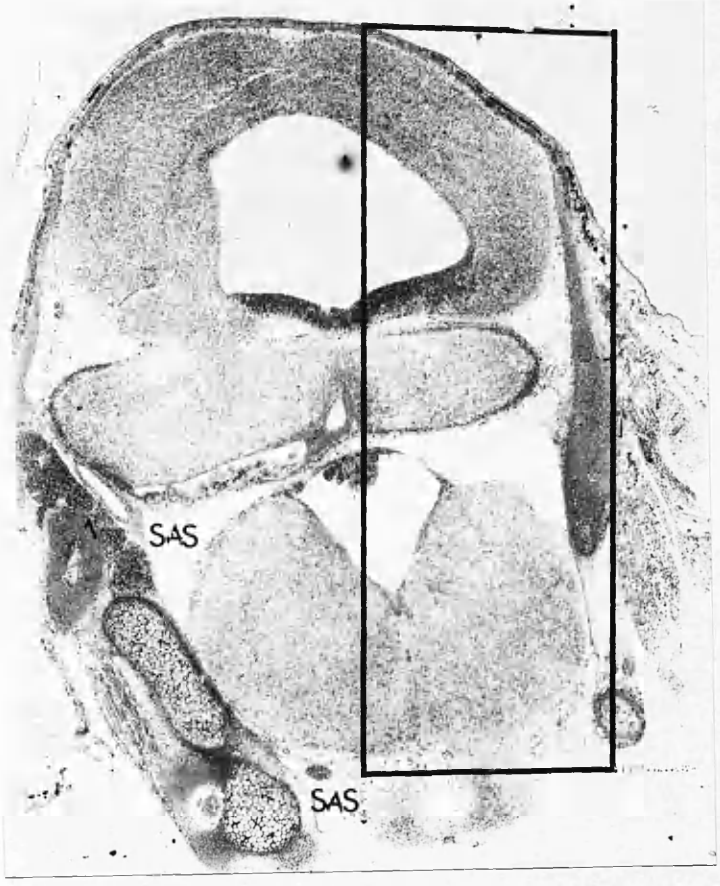

SAS
SAS

Fig. 113

Medium power photographic montage of the area outlined in Fig. 112.
SAS = subarachnoid space.

15 day mouse embryo. x 150

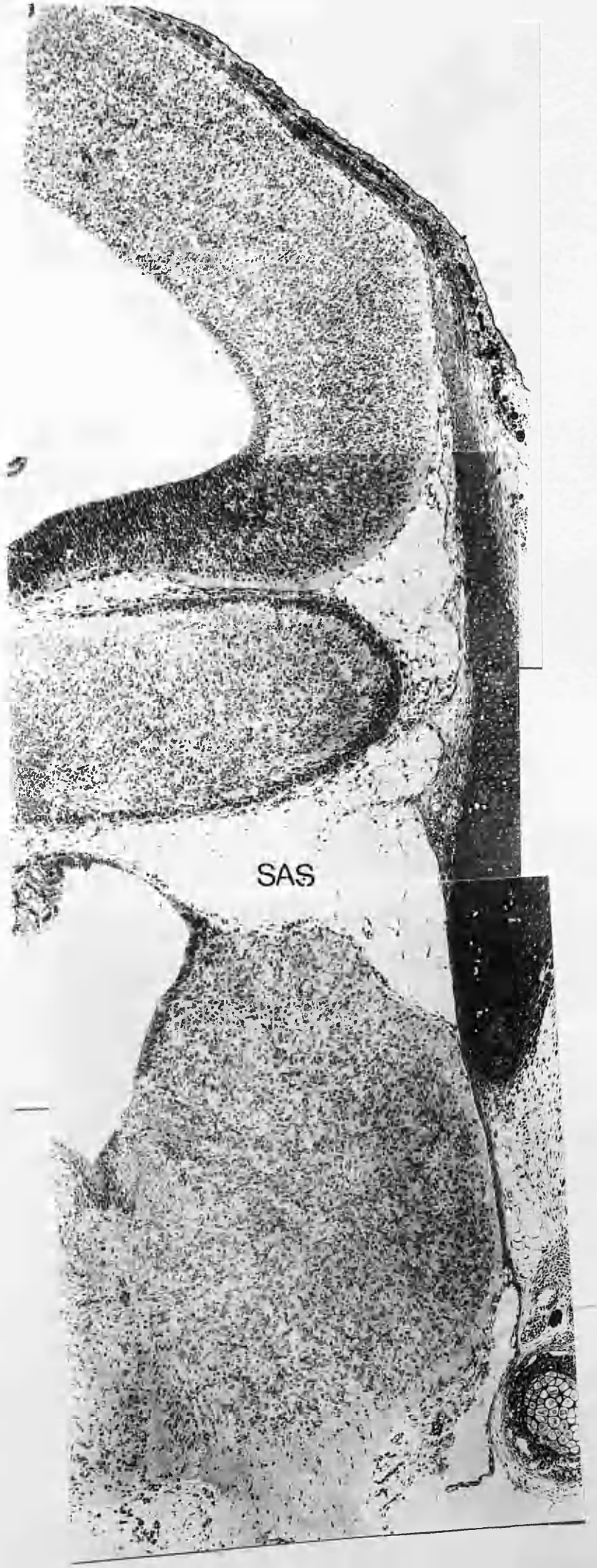

SAS

Fig. 114

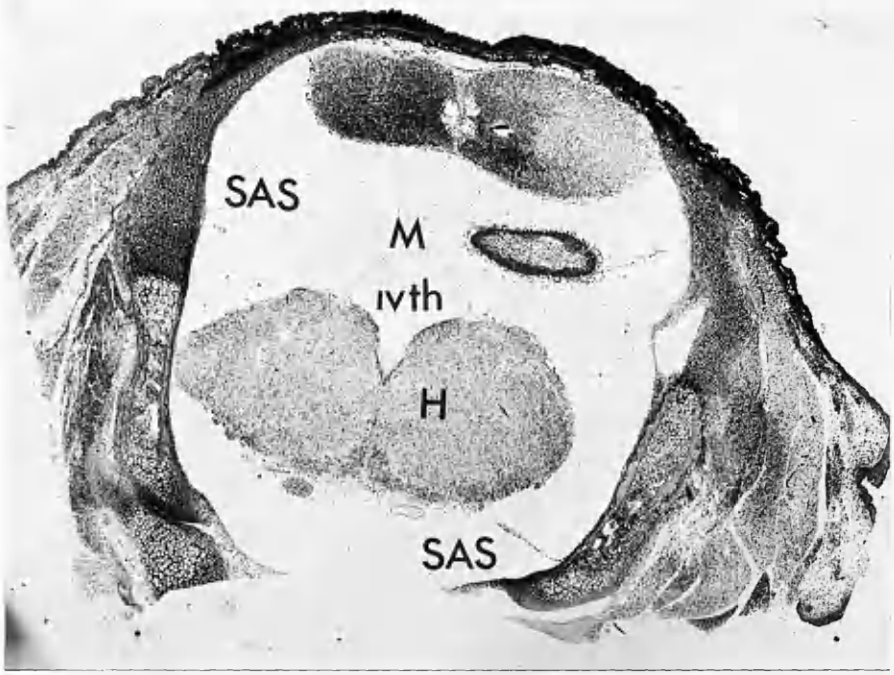
The subarachnoid spaces (SAS) now virtually surround the hind brain (H), with few strands of mesenchyme (M) dorsal to the roof of the IVth ventricle (IVth).

17 day mouse embryo. x 25 Azur II

Fig. 115

A low power view showing the subarachnoid space (SAS) surrounding the hind brain (H). Endochondral ossification is advanced (S).
C = cerebellum
IVth = caudal part of the IVth ventricle
arrow = nerve passing through SAS
Inset - see Fig. 116

Newborn mouse. x 30 Azur II


SAS
M
IVth
H
SAS

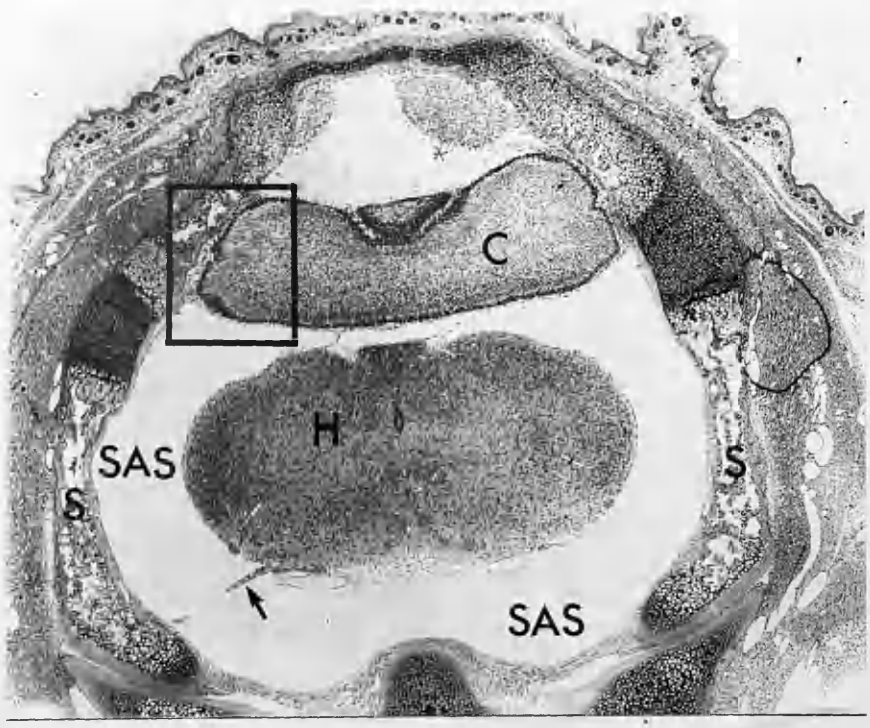

C
H
SAS
S
S
SAS

Fig. 116

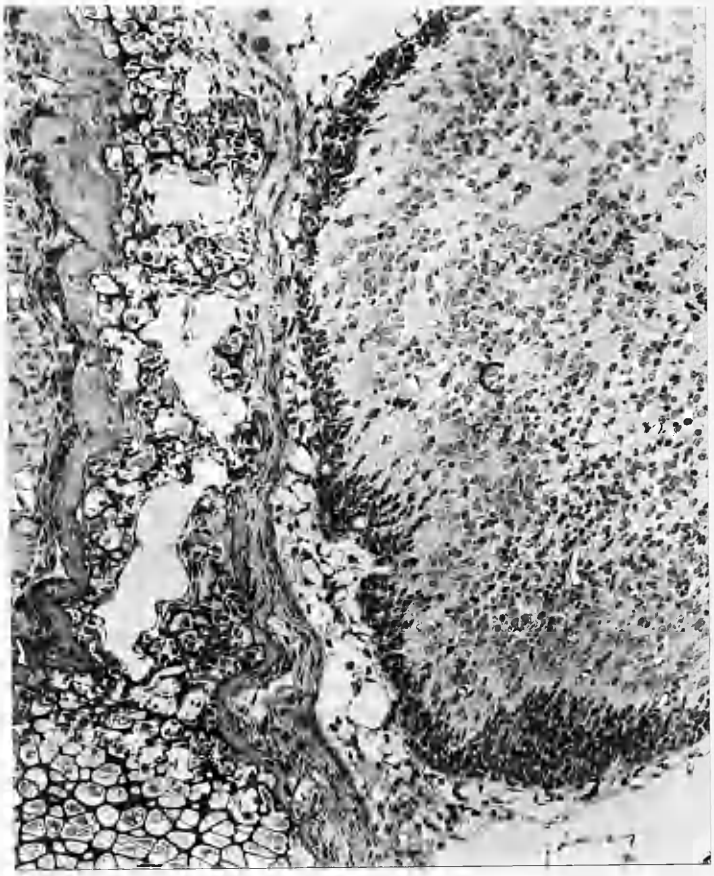
Higher power of area outlined in Fig. 115. It shows that lateral to the cerebellum no subarachnoid spaces exist.

Newborn mouse. x 175

Fig. 117

SEM micrograph of the interval between the cerebellum (C) and the skull (S). It is occupied by mesenchymal cells and their processes, and blood vessels (arrow).
No actual subarachnoid space exists (cf Fig. 116).

Newborn mouse. x 275


C

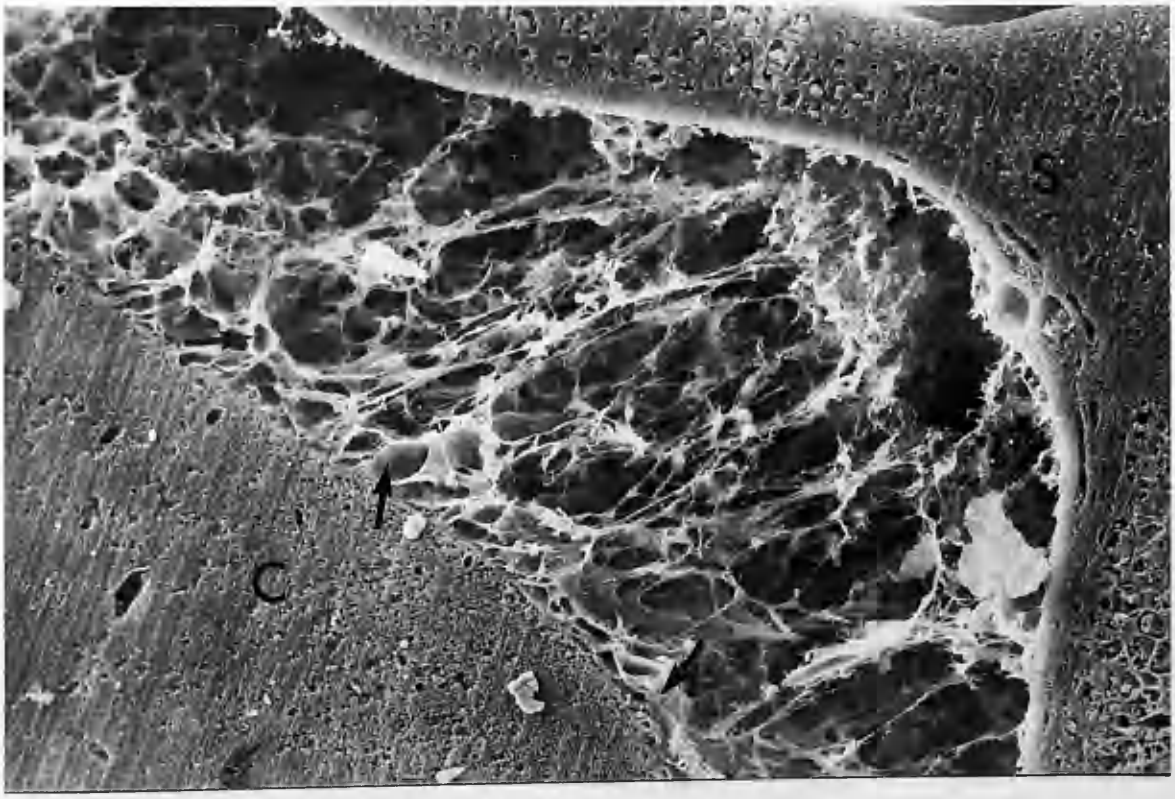

S
C

Fig. 118

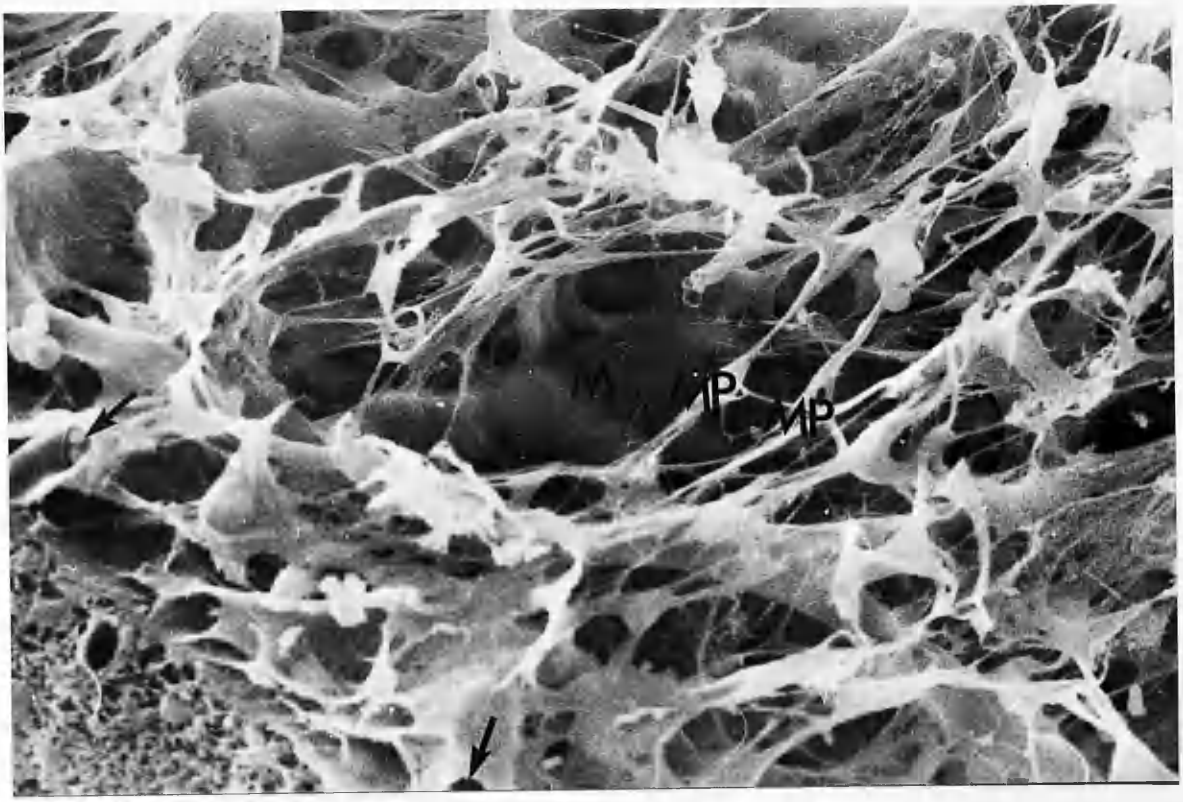
High magnification from Fig. 117.
M = mesenchymal cells
MP = mesenchymal cell processes
arrows = blood vessels.

Newborn mouse. x 1000

Fig. 119

An en face view of the subarachnoid space on the ventral aspect of the hind brain (H), on the surface of which a large, branching blood vessel (BV) is lying Mesenchymal cell processes course through the space (MP).
S = skull

Newborn mouse. x 450


MP
MP

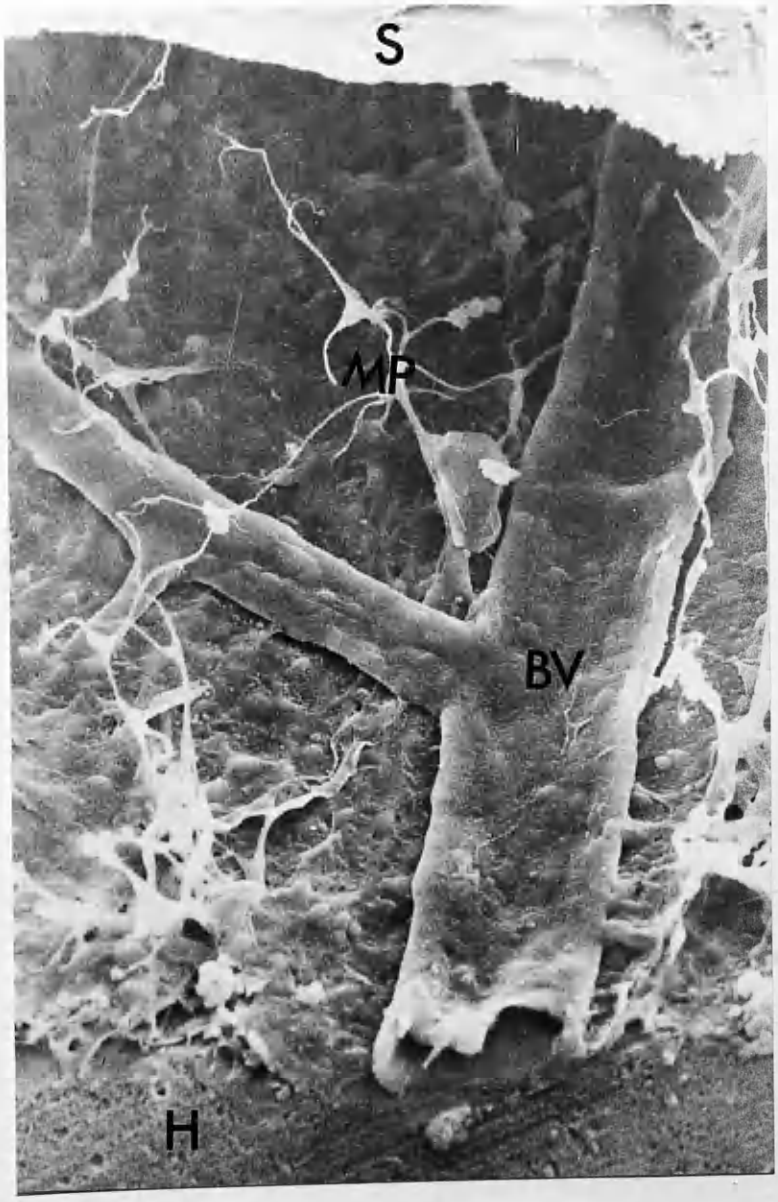

S
MP
BV
H

Fig. 120

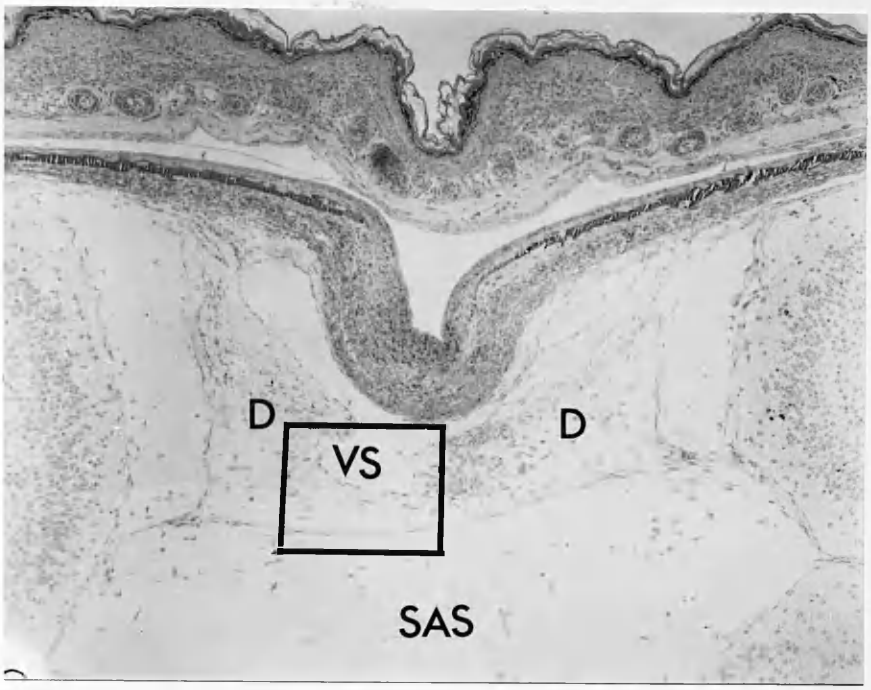
Low power view of a coronal section showing the confluence of sinuses (VS), surrounded by thin supporting dura (D).
SAS = subarachnoid space
Inset - see Fig. 121

Adult mouse. x 50

Fig. 121

High power of area outlined in Fig. 120. It shows the extremely thin and loosely arranged dura (D) surrounding the venous sinus (VS).
SAS = subaracnhoid space.

Adult mouse. x 500


D
VS
D
SAS

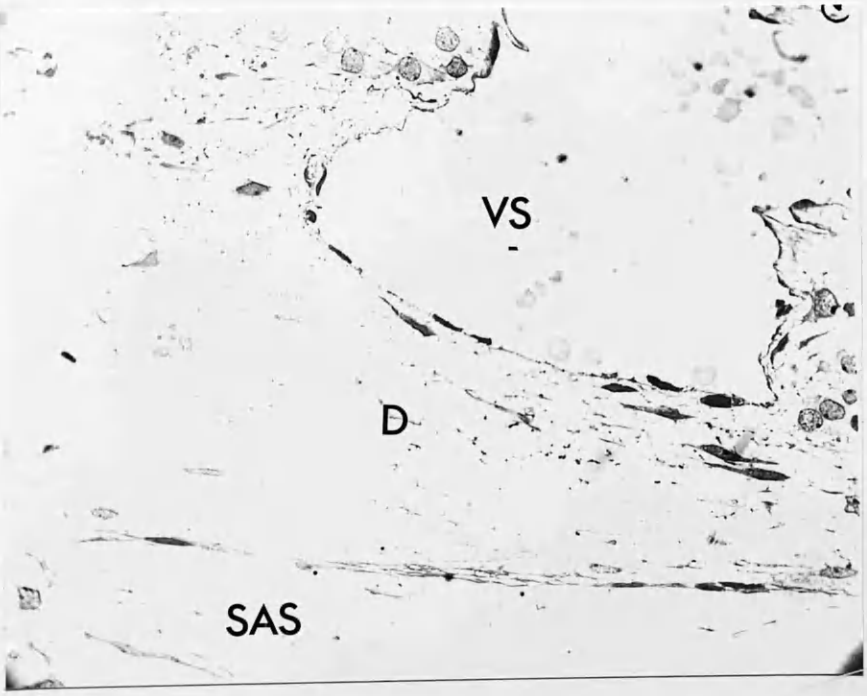

VS
D
SAS

Fig. 122

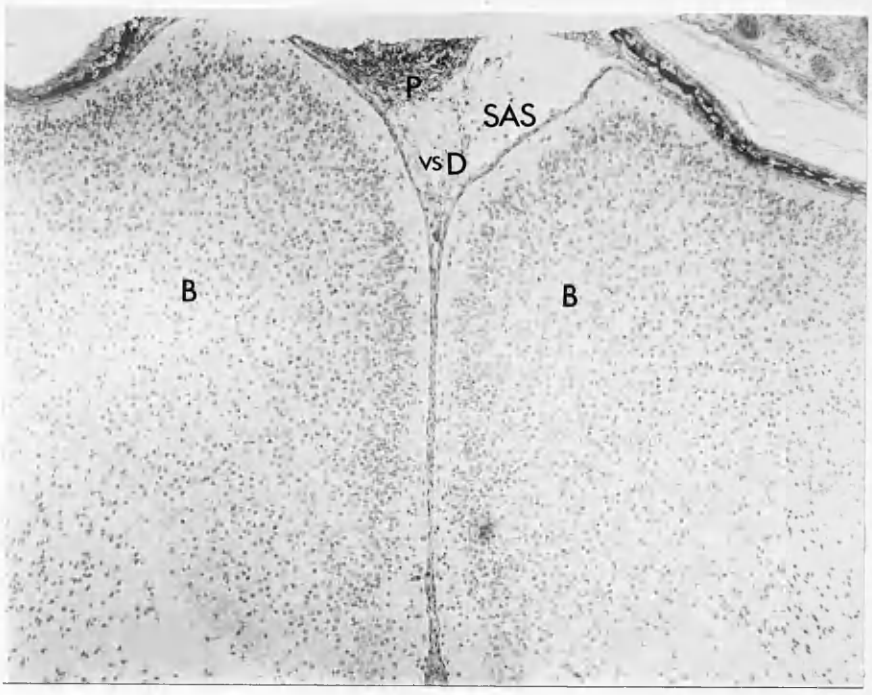
Shows the superior sagittal sinus (VS) surrounded by thin, loosely arranged dura (D).
No arachnoid villi are present.
P = periosteal dura which is thick and compact
SAS = subarachnoid space
B = brain tissue

Newborn mouse. x 175

Fig. 123

High power view showing the very loosely arranged and extremely thin dura mater (D) around the superior sagittal sinus (VS).

Newborn mouse. x 800


P
SAS
vsD
B
B

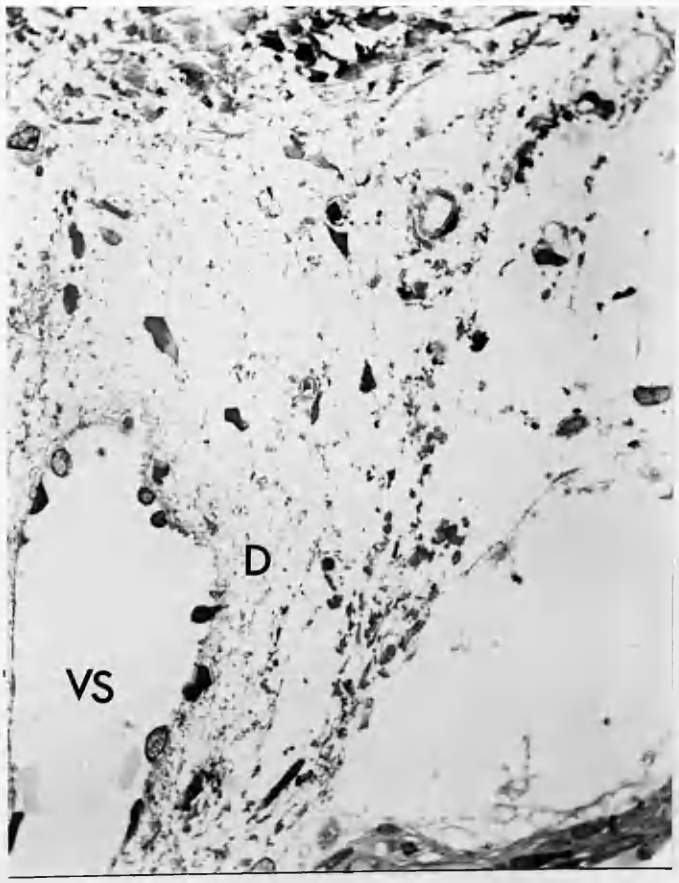

D
VS

Fig. 124

Shows an arachnoidal villus (arrow) passing through thick supporting dura (D) and into the venous sinus (VS). It is lined by endothelial cells (small arrows).

guinea pig. x 100