THE ELECTRON MICROSCOPY OF THE CHOROID PLEXUS*

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Plates 125 to 128

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The role of the choroid plexus in elaborating the cerebrospinal fluid has been discussed in the reviews of Wislocki (22), Agduhr (1), and Flexner (6 and 7). It is apparent that a secretory process is involved. The first two papers make it clear that morphological specialization of the ependyma of this region has been recognized for many years. The cells are cuboidal and unusually large, with a prominent Golgi apparatus. "Secretory blebs" have been described on their surface. These features are presumably associated with their secretory function.

There has been no direct effort to extend existing morphological knowledge by electron microscopy, although both Dempsey and Wislocki (3) and van Breemen and Clemente (21) have had occasion to examine the choroid plexus by electron microscopy in relation to silver deposition in studies of the hematoencephalic barrier. Although these authors necessarily made some comments concerning the histology of this organ, it will become apparent that the descriptions are incomplete in important respects.

This study is part of a larger program of one of the present authors (D.C.P.) directed towards a comparative study of different tissues notable for their water transport. The tubular portions of the kidney are fairly well known already, particularly through the work of Sjöstrand and Rhodin (19), Rhodin (17), and Pease (14 and 15). Electron microscopy in this instance has disclosed a major specialization of the basal surface whereby the plasma membrane is infolded in a manner to increase vastly its surface area. The epithelium of the ciliary body of the eye, and of the submaxillary gland, show analogous specializations (Pease, (16)). It will become apparent below that there is a comparable folding of the basal cell surface in the choroid plexus epithelium, so that in at least one respect there is morphological similarity associated with a specific function in a variety of epithelial tissues.

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Material and Methods

Our main work with the choroid plexus has been done with adult rats. The specific descriptions and published micrographs are all limited to this species. However, samples from the frog, rabbit, and cat have also been examined without observing important differences.

Sodium pentabarbital anesthesia was used, administered intraperitoneally. Exposing the choroid plexus in the smaller animals while they remained alive presented some difficulty. In some cases a lateral ventricle was opened leaving the midline vasculature intact. Then ice cold 2 per cent osmic acid, buffered at pH 7.4–7.7 in the manner of Palade's method (9), was dripped on the choroid plexus for 15 to 20 minutes. The tissue was then removed and immersed in cold osmic acid, usually for another 15 minutes, although sometimes longer. Equally satisfactory results were obtained with plexus of the 4th ventricle exposed just after the animals' death. In exposing the 4th ventricle there seemed to be no practical way of maintaining an intact venous drainage from the brain, so the posterior calvaria was ruthlessly sacrificed and the cerebellum removed while the animal died from hemorrhage. These procedures could be completed within 2 minutes and the osmic acid drip started.

As a control measure designed to determine whether the barbital anesthesia was producing a morphological change, choroid plexus was removed without benefit of general anesthesia. In this case the top of the head and the back of the neck were infiltrated with procaine hydrochloride, and the spinal cord was transected through the atlanto-occipital membrane for immobilization. This specimen was not different from those specimens obtained in the more usual manner.

RESULTS

Three distinct elements must be recognized in the organization of the choroid plexus. These are the epithelium of ependymal derivation, vascular channels, and, between these two, interposed tissue of leptomeningeal origin. It will be convenient to consider observations of these structures in this order.

Epithelial Surface.—There are two striking features of the epithelial cells which are easily visible even with the relatively low electron microscopic magnification of Fig. 1. The first of these, at the apical ends of the cells, resembles a brush border with vast numbers of processes extending into the ventricular lumen. The second is an elaborate system of folds of the basal cell membranes in the vicinity of cell junctions. The latter is discussed in the next section.

The surface processes have been observed as clear globules by light microscopists (Agduhr (1) and Wislocki (22)) and are briefly noted in electron micrographs by van Breemen and Clemente (21) and Dempsey and Wislocki (3). A glance at Figs. 1 and 2 will indicate that they must be very watery in life, for they show almost no electron density.

It is apparent that the surface pedicels are simple extensions of the apical cytoplasm. They have no specific size or shape, and hence probably should not be considered as constituting a "brush border" in the true sense. The processes are so crowded together that they seemingly are often forced to assume forms with long stalks before room is afforded for the characteristic terminal expansions. Their shape suggests that the term "polypoid border" would be appropriate for this type of surface.

It seems probable that the pedicels are capable of dynamic change. At times the terminal enlargements of a few blebs were seen to be enormously distended to dimensions of even a few microns. Such enlargement suggests that water was taken up as the plasma membrane was damaged during fixation. Occasionally, too, the apical ends of epithelial cells were greatly swollen, and watery and without pedicels. This is thought to represent more extreme fixation damage, although the other areas and organelles of the cells often were quite adequately preserved. In some instances the whole specimen was affected in this way, in other instances, only isolated cells. Thus this system of pedicels would seem to be labile and sensitive to osmotic forces. This perhaps explains why it seems to have been elusive in past histological literature.

It is reasonable to regard the polypoid border as a device to increase surface area. It is possible also that the tips of the processes may pinch off and become a part of the secretion. This is a suggestion of van Breemen and Clemente (21). If correct, the blebs should be large enough to observe with a light microscope in aspirated fluid. This sort of evidence should be sought and would then demonstrate an apocrine secretion.

Basal Folding of Epithelial Cells.—If one examines the basal portion of the epithelial cells in Fig. 1, it is apparent that there is no special modification of much of the surface. Yet in the vicinity of cell junctions, elaborate folding occurs. The extent of this is better appreciated at greater magnification in Figs. 4, 5, and 6.

In Fig. 4 the plane of section is parallel to the base of the cells, and the margins of two nuclei can be seen. The folded "double membranes" thus represent, in part, interdigitated walls of adjacent cells. The extent of this system shows to advantage in a wide field in Fig. 3 where zones of elaborate interdigitation are indicated by brackets.

The basal surface is also thrown into deep folds in these regions. This is very clearly shown in Fig. 5. A relatively dense basement membrane is apparent underlying the epithelial cells, and also a flattened sheet of pial cytoplasm.

At a high magnification in Figs. 5 and 6, it is apparent that the gap between the "double membranes" is not absolutely constant; yet the gap is sufficiently uniform to define its usual width as approximately 110–170 A. Particularly favorable views of the infolded membranes seen in the horizontal plane have been found in this material. When the section is in the plane of the membrane, the latter appears as a smudge of substantial density. This can be seen at a number of loci in Fig. 6. An effort has been made without success to resolve and observe any structural pattern that might be present. Even with great resolution the membranes appear continuous and without visible organization.

The folded basal and intercellular membranes certainly result in a major

increase in potential surface area. It seems likely this may be related to water transport for reasons considered in the discussion.

Experiments with a Functional Blocking Agent.—Of course it has occurred to us that the apical and basal surface specializations of the ependymal cells might be labile structures whose form would change with function. Diamox,[1] a carbonic anhydrase-inhibitor, is now known to be an excellent blocking agent for cerebrospinal fluid production (Tschirgi, Frost, and Taylor (20)). Rats were given large doses (150 to 500 mg./kg.) as a pretreatment. The system of basal infolding seemed unaffected. The polypoid border was still there, although possibly reduced in size. Other parts of the plexus seemed unaffected. Thus undoubtedly cerebrospinal fluid production can be greatly reduced without any important morphological change.

Organelles of the Ependymal Cells.—It seems worth while to comment that the mitochondria of the ependymal cells are randomly located, as a glance at Fig. 1 will show. Of course, this is at variance with what one often finds in highly polarized secretory cells.

There is little specialization or orientation of the Golgi apparatus. It consists of small clusters of packed double membranes and vesicles in the perinuclear region. It is most often seen laterally or apically, but sometimes decidedly basally. It is not figured in this article.

The system of cytoplasmic vesicles which can be observed in Figs. 1 to 6 represents the endoplasmic reticulum (*cf.* Palade (10)). The tiny granules accumulate on the surface of these cavities in typical fashion. This system is slightly polarized, and the vesicles are both more numerous and often larger at the apical ends of the cells. The tiny granules sprinkled rather sparsely throughout the cytoplasm are no doubt mostly the RNA granules of Palade and Siekovitz (12). Their tendency to accumulate on the surface of the endoplasmic reticulum is well established and obvious in this material.

Occasionally cilia can be seen as a persistent embryological remnant; these usually occur as a small group near the apex of a cell. They frequently spring from a fairly deep pocket which indents the cell surface. There does not seem to be any specialization of the root area of these remnants such as described by Fawcett and Porter (4), although their internal structure is typical, with 9 pairs of radially arranged filaments plus one pair centrally located. These cilia are not figured in this article.

The Capillary Wall.—The small blood vessels of the choroid plexus consist of little more than a thin endothelium resting on a definite basement membrane. On the venous side this simple wall structure is found even in vessels of considerable size (up to six or more red cells in width). It is this expanded capillary or venule that is morphologically most intimately associated with the choroid plexus epithelium. The cytoplasm of the endothelial

[1] Diamox (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide), Lederle Laboratories, Pearl River, New York.

cells is extremely attenuated in many places except in the vicinity of nuclei. Sudden transitions to a thin sheet show in Fig. 8 (lower left) and 10.

The fine structure of the capillary wall closely resembles what Pease (13) described in peritubular capillaries of the kidney. In Fig. 10 a very thin, dense basement membrane (*b.m.*) underlies the endothelium. It is separated from the cytoplasmic sheet by a layer of low density material that may be termed a cement layer (*cem.*). The endothelial sheet shows characteristic perforations (arrows).

In the peritubular capillaries of the kidney cortex the endothelial pores were found in such numbers, with such a regular distribution, and of such a constant size that they were regarded as true features of this capillary type. In the choroid plexus only patches of endothelial sheet show a comparable pore structure. They are sufficiently rare so that one would be inclined to dismiss them as fixation artefacts, if it were not for their demonstration in the kidney. In view of this, however, we believe that the endothelium of the choroid plexus is probably sparsely and irregularly fenestrated. This may be regarded as a specialization facilitating the transfer of fluid.

Meningeal Elements.—The tissue between the blood vessels and the ependymal epithelium belongs fundamentally to the leptomeningeal system, and it would seem justified to speak of the cells of this region as pial. In spite of the probable ectodermal origin of this tissue, it has the characteristics of a very loose connective tissue. Findlay (5), however, early noted certain peculiarities, particularly the absence of a definitive adventitia around the blood vessels.

The tissue is characterized by large aqueous spaces, a fact which is not surprising, as these are fundamentally extensions of the subarachnoid space. Such a cavity is labeled in Fig. 9, and narrow spaces also can be seen in Figs. 8 and 10 between pial cells and other components. In the extracellular spaces occasional bundles of collagen may be found.

The relation of pial cells to ependymal epithelium and blood vessels is the most interesting feature of this tissue. These cells are greatly flattened and spread out under the ependyma as in Fig. 9, and also often flattened cytoplasmic extensions more or less invest blood vessels. Thus, pial cells tend to interpose sheets of cytoplasm between epithelium and endothelium. When blood vessels are close to ependyma, they usually share a single pial sheet as in Figs. 7, 8, and 10. It is by such shared pial cells, too, that capillaries are attached here and there to the ependymal layer.

The pial sheets are not continuous as is apparent in Figs. 8 and 10. No attempt has been made to determine accurately how nearly complete the pial barrier may be, but the authors' estimate from a perusal of many micrographs suggests it is about 85 per cent continuous. Thus, although this system might serve as a baffle in slowing diffusion, it would not interpose a definitive barrier.

The pial tissue probably should not be regarded as static, however. The reticuloendothelial system, which includes these cells, was activated in some rats by successive injections of trypan blue over a 10 day period. The choroid plexus at sacrifice was brilliantly stained. It seemed that there had been a substantial increase in pial cell number. Also, the cells tended to be globular rather than flattened, with much cytoplasm and unusual numbers of inclusions. It is likely that the inclusions consisted in large part of trypan blue, although this was not positively identified. No alterations were observed in either the epithelium or endothelium.

Unmyelinated nerve fibers also were to be seen in the meningeal tissue spaces. Ordinarily they were in obvious association with the arterioles and are presumed to have been vasomotor. No epithelial endings as described by Clark (2), or intracellular endings as described by Junet (8) were observed. The techniques of electron microscopy preclude examining large areas, however, so we hesitate to deny their existence.

DISCUSSION

In considering the functional role of the choroid plexus, we see that the capillaries may be regarded as specialized to present a minimal barrier to diffusion processes. Like the peritubular capillaries of the kidney, the endothelial sheet seems actually fenestrated. A very thin, dense basement membrane and an associated "cement" layer of little density constitute the only continuous barriers between blood and meningeal spaces. It is not surprising, then, that dye molecules get into these spaces (Rodriguez (18)), and that silver colloids deposit here (Dempsey and Wislocki (3), van Breemen and Clemente (21)).

Insofar as the choroid plexus does secretory work in manufacturing the cerebrospinal fluid, it must be the ependymal epithelium that is involved, since even colloids reach this layer. It is the ependyma, also, that is morphologically specialized in a manner reminiscent of the kidney tubules with a luminal surface resembling a brush border and a basal surface expanded by complex folding. Somewhat comparable infoldings of the basal surface have been observed by one of us (Pease (16)) in still other epithelia noted for their water transport. These include, besides kidney tubules, the epithelium of the ciliary body and the serous cells and secretory duct epithelium of the submaxillary gland. It may also be noted that this type of surface infolding has been related by Palade (11) to pinocytosis as well as to phagocytosis in macrophages.

Thus, the comparative study suggests that the morphological specializations of the ependyma are not without physiological implications. Yet the dependence of function on form may not always be direct as the experiments with diamox show, for the induced morphological change, if real at all, was altogether too slight to account for the major physiological effect.

SUMMARY

1. The choroid plexus of the rat has been studied in detail by electron microscopy. Samples from the frog, rabbit, and cat have also been examined without noting significant differences.

2. The surface of the ependymal epithelium is covered by pedicels of variable size. There is reason for thinking of these structures as labile. They may actually pinch off and contribute to the secretory product. In any case, the surface area is vastly increased by their presence. Polypoid border seems an apt term to apply to this type of surface.

3. There is also a great expansion of the basal surface of ependymal cells. In the vicinity of cell junctions this surface is deeply infolded, and continuous with elaborate interdigitations of the lateral intercellular surfaces. Analogous infolding of the basal cell surface is known to exist in other epithelia also noted for their water transport (kidney tubules, salivary gland, and ciliary body).

4. Pretreatment of rats with diamox, an agent known to block cerebrospinal fluid production, did not produce an important morphological change in the features of the ependyma, or any other part of the choroid plexus.

5. Capillaries of the choroid plexus have a very attenuated endothelium. This is seen to be fenestrated. It is thought this probably represents the condition in life, and is not simply a fixation artefact.

6. Pial cells tend to interpose sheets of cytoplasm between the capillaries and ependyma. The sheets are not continuous, however, and so would not constitute a serious diffusion barrier. These cells belong to the reticuloendothelial system, and undergo shape changes, and probably increase in number, when the system is stimulated by the repeated injection of trypan blue.

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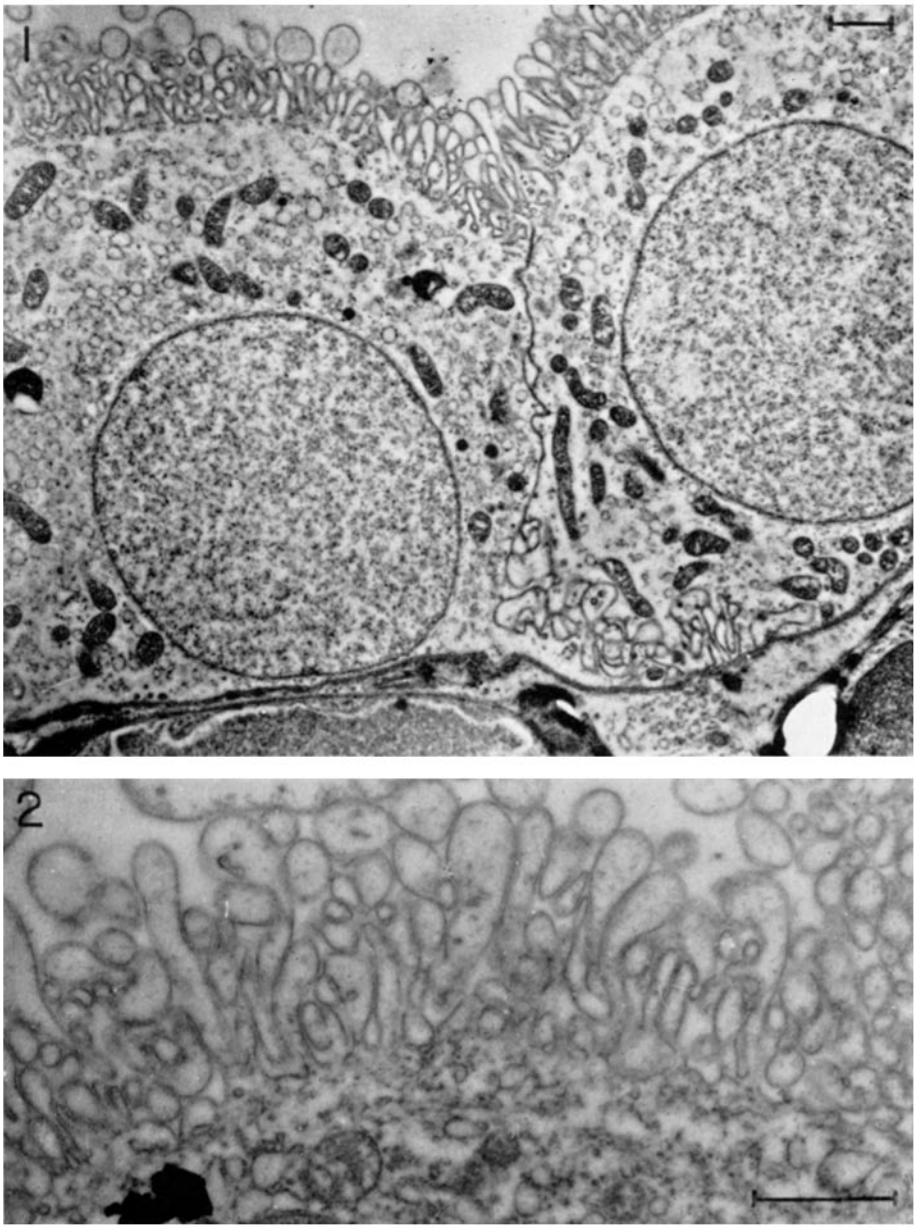
EXPLANATION OF PLATES

PLATE 125

FIG. 1. Transverse section of ependymal epithelium. The "polypoid border" is at the apical ends of the cells, with pedicels protruding into the ventricular lumen. The basal ends of the cells are unspecialized except near regions of cell contact laterally where the surface membranes can be seen to be extensively infolded. Edges of capillary loops can be seen at the bottom of the figure. Micron marks accompany all figures. ×8,100.

FIG. 2. The apical surface of an ependymal cell to show the polypoid border. Individual pedicels are simple extensions of the cytoplasm of variable size. They lack cytoplasmic organelles other than a few RNA granules. ×18,200.

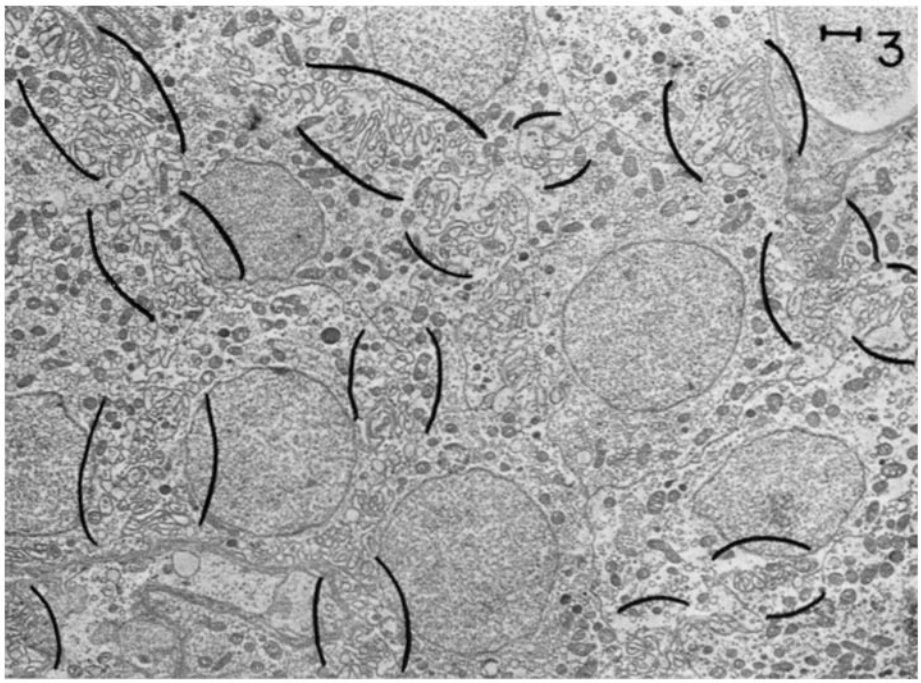


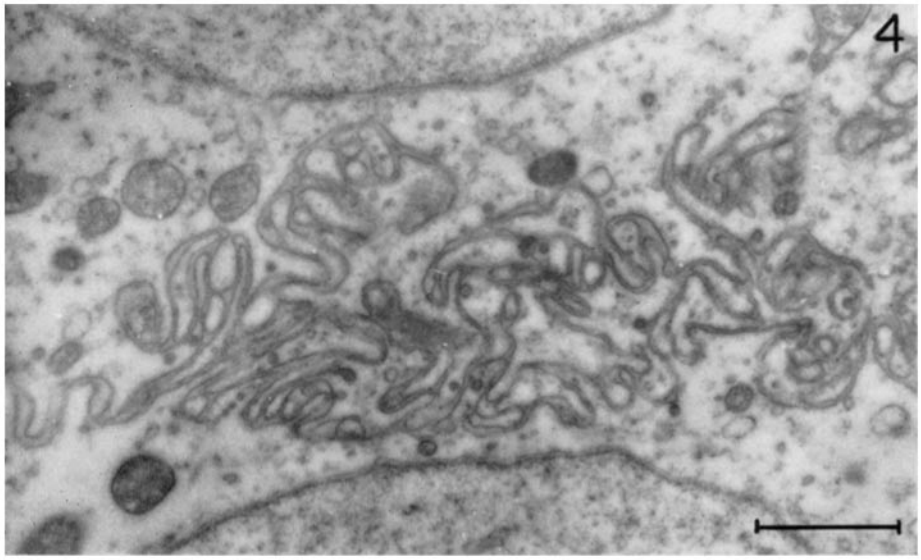
(Maxwell and Pease: Fine structure of choroid plexus)

PLATE 126

FIG. 3. A section of ependyma in the horizontal plane, through the basal portions of many cells. Extensively interdigitated intercellular membranes can be seen, particularly in bracketed areas. ×5,000.

FIG. 4. Plane of section as in Fig. 3. The elaborated folded and interdigitated intercellular membranes between the lateral margins of two nuclei show to advantage. ×18,600.





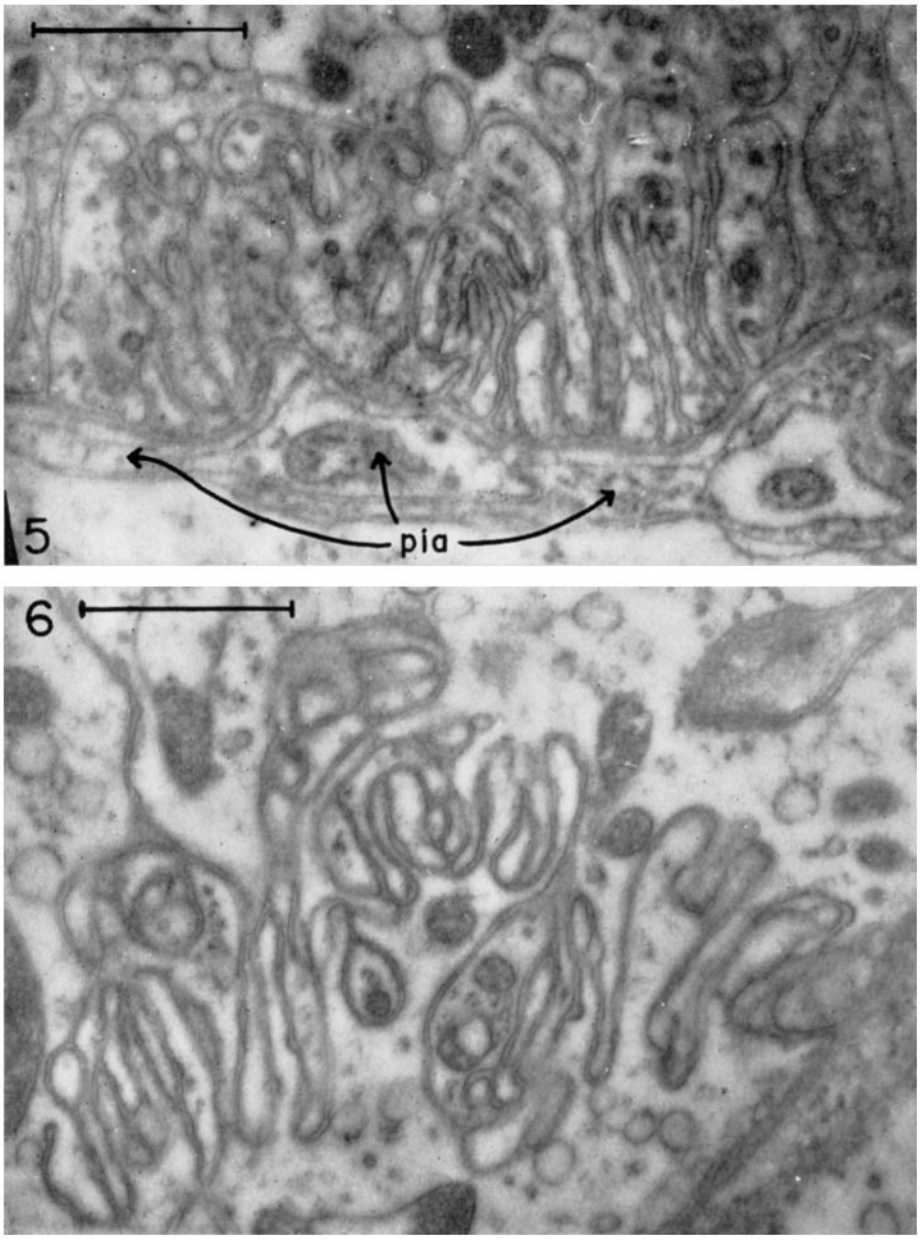
(Maxwell and Pease: Fine structure of choroid plexus)

PLATE 127

FIG. 5. The basal portion of an ependymal cell in the vicinity of an intercellular boundary. The basal surface is deeply infolded into the cytoplasm. This system of folds no doubt is continuous with the folds of the intercellular surface.

The cell rests on a basement membrane demarked by a thin but dense component. Flattened sheets of pial cytoplasm underlie this in the subarachnoid space. ×27,700.

FIG. 6. Folding of the basal and intercellular surfaces. In places the folds are viewed in the horizontal plane where they are seen as smudges of considerable density. The membranes appear structureless even under these conditions. ×27,700.



(Maxwell and Pease: Fine structure of choroid plexus)

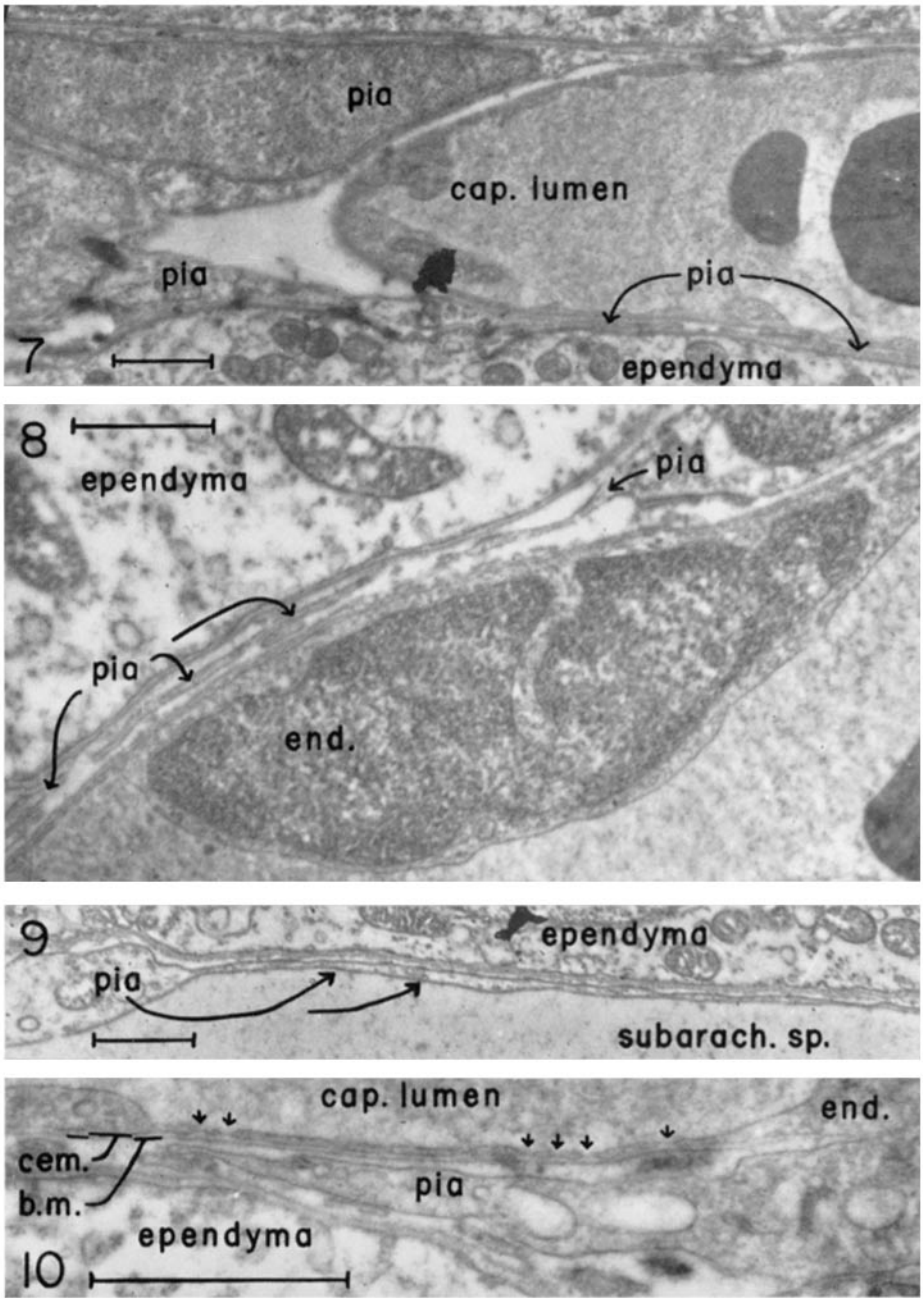
PLATE 128

FIG. 7. A capillary in the subarachnoid space with ependyma on both sides. Pial cells tend to interpose sheets of cytoplasm between these two. ×13,000.

FIG. 8. The edge of a capillary indicating clearly the incomplete pial sheet existing between it and the overlying ependyma. Note also the sudden transition from the endothelial perikaryon (*end.*) to an extremely attenuated endothelial sheet at the left. ×28,200.

FIG. 9. A portion of a pial cell showing an attenuated sheet of cytoplasm underlying the ependyma. ×13,000.

FIG. 10. A portion of a capillary wall with an attenuated endothelial sheet. The sheet is interrupted where small arrows indicate pores. Underlying the endothelium is a layer of low density that may be regarded as a cement substance (*cem.*), and a thin dense layer that probably represents the structural portion of the basement membrane (*b.m.*). Ependyma is nearby, with an incomplete interposed sheet of pial cytoplasm. ×33,700.



(Maxwell and Pease: Fine structure of choroid plexus)