Electron-Microscope Study of the Effect of Increased Intracranial Pressure on the Arachnoid Villus*

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The prevailing concepts of the structure and function of the arachnoid villi are based on reports by Cushing,7 and by Weed;19–23 the familiar diagram of the latter is found in many textbooks. Weed reviewed the literature of the time, described the anatomy and embryology and demonstrated that material injected into the subarachnoid space found its way to the arachnoid villi. Since, the villus has been characterized as a blind diverticulum of arachnoid, separated from the venous channel into which it projects, by a layer of endothelial cells across which the cerebrospinal fluid may pass in returning to the blood stream. For excellent reviews of this subject, the reader is referred to the report of Turner18 or the Ciba symposium on the cerebrospinal fluid.26

Welch and Friedman24 took issue with the classical views of Weed and suggested that the arachnoid villus is "a labyrinth of coapted tubes which connect, from place to place, with each other and which open to the subarachnoid and subdural spaces on one hand and to the venous channels of the dura on the other." They proposed that the villus thereby provides direct flow from the cerebrospinal fluid to blood when the pressure in the subarachnoid space exceeds that in the venous systems and that the "valves" close to prevent flow in the opposite direction when the pressure gradient is reversed. Their studies are based on light microscopy of paraffin-embedded tissue and although they certainly demonstrate the expandable capabilities of the arachnoid villi with increased pressure, it is possible, as suggested by Turner,18 that the apparent defects in the endothelium represent artifacts.

Most of the available physiological data concerning flow of cerebrospinal fluid do not bear directly on the problem of the function of the arachnoid villus because, although tracer studies have verified that substances pass from cerebrospinal fluid to blood, the site of egress is not established.6–8,19–21 Welch and Pollay,25 however, demonstrated that in an excised system, polystyrene spheres of 1.8 μ, yeast (3–6 μ) and erythrocytes up to 7.5 μ would pass through the villus. They interpreted these data as confirming the presence of the 4–12 μ "valves" which they postulated from anatomic studies.

The increased resolving power of the electron microscope can be utilized to gain additional information about physiologic processes by allowing visualization of sub-microscopic changes in structure which result from altered physiologic variables. Applying this method, the present study was undertaken to compare the arachnoid villus under conditions of normal and increased intracranial pressure.

Materials and Methods

Six mongrel dogs were used for this study. The animals were anesthetized with intravenous Nembutal after which a craniotomy was performed bilaterally over the convexity to expose the sagittal sinus from the coronal suture to the internal. The sinus was opened at both ends and perfused without elevation of pressure with 2 per cent ice-cold osmium tetroxide buffered with colidine.4 In the 2 animals designated for studies on increased pressure a cisternal needle was utilized to maintain the intracranial pressure at 500 mm. of saline for 20 min. prior to perfusion of the sagittal sinus. In 1 animal 0.5 cc. of ferritin19 was injected as a tracer substance into the cisterna magna 1 hour before perfusion.

Once perfused, the sagittal sinus was removed, cut into 1 mm. tubular segments and fixed 1 hour in the same fixative. The tissue was dehydrated in ascending concentrations of alcohol and embedded in Epon.11 Two to 5 μ sections of the cross
section of sagittal sinus were made with either a Porter-Blum ultramicrotome and glass knives or an American Optical Rotary microtome with steel knife. These sections were observed with phase microscopy and the blocks were sectioned serially until an arachnoid villus was encountered. The block was then trimmed to include only the villus or a portion thereof. Thin sections were cut with glass or diamond knives on a Porter-Blum or Huxley ultramicrotome. The sections were stained by a modification of the Millonig lead stain\textsuperscript{12} and observed with an RCA EMU3C microscope. Pictures were taken at magnifications of 900 to 12,000 times and enlarged photographically.

Observations

The normal submicroscopic anatomy of the canine arachnoid villus has been reported previously.\textsuperscript{1} The villus is a porous structure composed of interlacing, elongated processes of cells containing the usual cytoplasmic organelles, i.e., mitochondria, endoplasmic reticulum, Golgi apparatus and vesicles. These cells are similar in appearance to the normal arachnoid cells described by Pease and Schultz.\textsuperscript{15} The over-all appearance suggests a lattice-work of interdigitating cellular processes which could be expandible in nature (Fig. 1). Between the cellular processes are various-sized spaces which are presumed to be in direct connection with the subarachnoid pathways as indicated by the free entrance of ferritin tracer (Fig. 1).

The meshwork of arachnoid-like cells is covered by a continuous layer of endothelium and separated from it by a continuous layer of basement membrane. The endothelium is in direct continuity with the endothelial lining of the sagittal sinus. Between the superficial arachnoid cells and the endothelium, bundles of collagen are present commonly (Fig. 6).

Under conditions of normal intracranial pressure the endothelium is characterized by numerous nuclei of cells and intervening intracellular junctions with well developed areas of attachment (Fig. 2). The endothelial cell has an irregular external surface because of the presence of frequent pseudopodia and clefts. Occasionally these clefts penetrate deeply into the cell and rarely the inner and outer cellular membranes are approximated or penetrated for short distances. The resultant single-membrane diaphragms or pores are never greater than 0.1 \( \mu \) across and represent less than 0.1 per cent of the endothelial surface. A continuous basement membrane is always present beneath such defects. The cytoplasm of the endothelial cell contains numerous vesicles ranging from 600 \( \AA \) to 1500 \( \AA \) in diameter—some of which appear to be forming by pinching off from small clefts in the cellular membrane (Fig. 3).

Under conditions of increased intracranial pressure the endothelium is altered in that the nuclei of the cells are much farther apart and the endothelial cytoplasm is thinned. Well-developed areas of attachment persist, however, with no evidence of separation of cells (Figs. 4 and 5). The rare penetrations are unchanged in size or frequency. The cytoplasm remains vesicular although the vesicles generally are smaller. The outer cellular membrane has prominent pseudopodia in some areas (Fig. 6) but is smooth in others. A condensation of electron-dense material is present along the outer cellular membrane which is not present in the normal.

In contrast to the situation under normal conditions, the arachnoid cells from a villus under increased pressure show either a marked thinning and elongation of their cellular processes or marked vesiculation of their cytoplasm (Fig. 7). These two changes generally occur randomly throughout the villus except that thinning is more common immediately beneath the endothelium. The ferritin tracer in the normal reveals that the vesiculation is attributable either to deep channels extending into the cells from the exterior or to large vesicles pinching off from infoldings in the surface of the cell (Fig. 8). Further observations on the fate of the tracer substance will be the subject of a later report.

Discussion

Our observations demonstrate that during periods of increased intracranial pressure the canine arachnoid villus remains covered by a layer of endothelium which separates the cerebrospinal-fluid compartment from the
FIG. 1. Electronmicrograph from the body of an arachnoid villus from an animal injected with ferritin. Note the interlocking process of arachnoid cells (AC) and the ferritin tracer in the intercellular space (CSF). The cell processes contain mitochondria, endoplasmic reticulin, Golgi apparatus and vesicles with engulfed ferritin (V). Scale 1 μ.
Fig. 2. (above). Electronmicrograph of a segment of endothelium overlying a normal arachnoid villus showing portions of three cells. Note the well-formed intercellular junctions (J), the thickening of the cells at the closely-

SSL

N

AC

BM

CSF

2

AC

SSL

C

V

P

3
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Figs. 4, 5 and 6. Electronmicrographs of three segments of the continuous endothelium overlying a distended arachnoid villus from an animal with artificially elevated intracranial pressure. In one area (arrow) the endothelium is thinned to less than 0.1 μ; however, the intercellular junctions (J) are intact. Adjacent to the nucleus, the outer-cell membrane shows numerous pseudopodia, clefts, and vesicles. Along the entire luminal surface of the endothelium is a layer of electron-dense material (M) which extends amongst the pseudopodia. In the CSF space there is a bundle of collagen fibers (CF). The processes of the arachnoid cell (AC) are thinned and layered. Scale 1 μ.

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spaced nuclei (N), and the basement membrane (BM). Lumen of sagittal sinus is SSL. Cerebrospinal-fluid space is CSF. Small projections of arachnoid cells are AC. Scale 1 μ.

Fig. 3. (below). Higher magnification electronmicrograph of endothelium showing prominent vesicles (V), clefts (C) and pseudopodia (P). Some of the vesicles contain material of increased density. Scale 1 μ.
blood compartment. The occasional penetrating channels of less than 0.1 μ are always bridged by an intact basement membrane and are in no way comparable to the 4–12 μ openings postulated previously.2 These channels are presumed to represent a momentary phase in the dynamic activity of the endothelial cell. The prominent pseudopodia, clefts and vesicles in the endothelium are interpreted as indicative of the importance of the endothelium of the arachnoid villus in transporting substances from cerebrospinal fluid to blood.2,3,10,14

The electron-dense material along the luminal surface of the endothelium is noteworthy in that it could be related to activity of transport. Roth and Porter16 described increased density in clefts and vesicles in oocytes engaged in transporting protein. Some of the vesicles in the present study contained material of increased density and some did not. None of the radially arranged spines described by Roth and Porter16—external to the vesicle6—was seen. The possibility remains that the dense layer represents an artifact of fixation although an attempt was made to perfuse and fix all specimens in the same manner.

Under conditions of increased intracranial pressure, the endothelium of the arachnoid villus is beyond the limit of resolution of the light microscope. It seems possible that such a membrane could be disrupted in the course of in vitro experiments. If such damage were to occur, it would be difficult to apply the results to in vivo phenomena.

The thinning of the endothelium and expansion of the interdigitating network of arachnoid cellular processes were expected results of the distension of the villus demonstrated by Welch and Pollay25 under increased pressure. The marked vesiculation of arachnoid cells was unexpected. Although it is not possible to state whether this appearance is caused by actual large vesicles of tortuous channels within the cytoplasm either situation would suggest that arachnoid cells may participate in some active manner in cerebrospinal-fluid resorption. Arachnoid cells in general are known to have phagocytic properties9 so no interpretation has been made about their proclivity to remove ferritin from the cerebrospinal fluid. Further study is necessary to determine if any information about the handling of spinal-fluid proteins by the villus can be obtained by this method.

Unfortunately, no insight was gained into the route taken by intact red blood cells as they pass from cerebrospinal fluid to blood.5,17 It seems unlikely, however, that these cells would pass unaltered through an intact basement membrane and endothelium, inferring that some other site of egress may be important.

Summary

The ultrastructure of the canine arachnoid villus has been studied to determine the effects of increased intracranial pressure. It was found that the layer of endothelium normally separating the villus from the venous blood remains intact but is markedly thinned. The arachnoid cells of the villus develop a vesicular appearance which may be ascribable to vesicular formation or to the presence of deep tortuous channels in the cytoplasm. The possible significance of these findings to cerebrospinal-fluid resorption are discussed.

The authors wish to express their appreciation to the Department of Anatomy, University of Washington, for providing the electron microscope used for this study and to Dr. John Luft for his guidance and technical advice.
References


