Fluid secretion in arachnoid cysts as a clue to cerebrospinal fluid absorption at the arachnoid granulation

K. GWAN GO, M.D., HENDRIK-JAN HOUTHOFF, M.D., JOHANNES HARTSUIKER, B.Sc., ENGBERT H. BLAAUW, B.Sc., AND PIET HAVINGA, B.Sc.

Departments of Neurosurgery, Pathology, and Medical Electron Microscopy, University of Groningen, Groningen, The Netherlands

The morphological similarity of the lining of arachnoid cysts to subdural neurothelium and the mesothelium of arachnoid granulations suggested that the latter tissues might be the origin of arachnoid cysts. Transport Na⁺-K⁺-adenosine triphosphatase was shown by enzyme ultracytochemistry to be an indication of secretory activity in the lining of arachnoid cysts and in the endothelial lining of arachnoid granulations. This secretory activity suggests the existence of a biochemical mechanism for cerebrospinal fluid absorption at these granulations separate from the mechanisms already demonstrated.

KEY WORDS • cerebrospinal fluid absorption • arachnoid granulation • arachnoid cyst • Na⁺-K⁺-ATPase

In previous papers, we have suggested that the lining of arachnoid cysts shows an ultrastructural similarity to subdural neurothelium and the neurothelial (or arachnoid mesothelial) constituents of arachnoid villi and granulations. The localization of transport Na⁺-K⁺-adenosine triphosphatase (ATPase) in plasma membranes bordering the arachnoid cyst cavity, as demonstrated by enzyme ultracytochemistry, indicates the capacity for fluid transport toward the cyst lumen. It was surmised that arachnoid cysts derive from subdural neurothelium, which has differentiated toward arachnoid villus mesothelium.7,8

Since the investigations of Weed,26 the role of the arachnoid villi and granulations in the absorption of cerebrospinal fluid (CSF) has been well established. However, it has not yet been resolved whether the absorptive mechanism constitutes a closed or an open system, or both. On the basis of morphological observations describing the subarachnoid space as it extends into the core and the subendothelial spaces of the arachnoid villus, a closed system has been envisaged as being separated from the venous or sinus lumen by a continuous layer of endothelial cells.1,21,22 Under the influence of a pressure head, invaginations from the subendothelial spaces (presenting as giant vacuoles) form in the endothelial cytoplasm and eventually evacuate into the sinus lumen.10,33,24

Evidence of an open system was provided by the observation of endothelium-lined tubules in pacchionian granulations, which may provide a direct communication between the subendothelial CSF spaces and the sinus or venous lumen.3,12,17 Although the closed system has been described in the small arachnoid villi which occur in both smaller and larger animals, the open system has only been reported in the larger pacchionian arachnoid granulations of sheep and man. Apart from these biomechanical pathways of CSF absorption, a biochemical mechanism constituting fluid secretion by the neurothelial lining of the arachnoid villus or granulation should be considered, since, like the neurothelial lining of arachnoid cysts, the arachnoid granulation appears to possess the enzymatic apparatus for fluid secretion. The present study has been undertaken to elucidate this issue.

Clinical Material and Methods

Source of Specimens

Tissue was obtained for study from four patients. A specimen of arachnoid cyst was obtained from Case 1 and arachnoid granulation tissue was excised during tumor surgery in Cases 2, 3, and 4. A brief summary of the cases follows.

Case 1. This 38-year-old man had suffered chronic headaches for 10 years, with occasional intense exacerbations of his complaints. Neurological examination disclosed no abnormalities. Computerized tomography (CT) demonstrated a left-sided hypodense lesion of
Cerebrospinal fluid absorption

typical rectangular shape, which had replaced the entire left temporal lobe and part of the left frontal lobe. At surgery, there was obvious bulging of the left temporal squama. Opening the tense dura, which formed the roof of the cyst, gave access to the cyst cavity. A biopsy for scanning electron microscopy (SEM), transmission electron microscopy (TEM), and enzyme ultracytochemistry was taken from the cyst lining, which was firmly adherent to the dura. The cyst was filled with clear colorless fluid resembling CSF, and was entirely separated from surrounding CSF spaces by the translucent membranous lining. The bottom of the cyst was formed by the exposed insula, with the branches of the middle cerebral artery coursing on its surface. Communication with the surrounding subarachnoid spaces and basal cisterns was established, whereupon CSF flowed into the emptied cyst cavity through these perforations. The patient’s postoperative course was uneventful.

Case 2. This 44-year-old man underwent a craniotomy for a small meningioma of the right frontal pole. During the operation, arachnoid granulations were excised subdurally, as they were located beside a bridging vein running into the superior sagittal sinus.

Case 3. This 50-year-old woman was operated on for a malignant glioma of the left frontal lobe. A pacchionian granulation was excised, as it protruded through the dura into a lacuna lateralis of the superior sagittal sinus.

Case 4. This 55-year-old man underwent a craniotomy for a malignant oligodendroglioma of the left frontal lobe. An arachnoid granulation was subdurally excised beside a bridging vein.

Specimen Preparation

The specimens of arachnoid cyst wall were attached to pieces of cork by small entomology pins applied at the corners to prevent shrinking. For SEM and TEM, they were immersed in 2% paraformaldehyde 0.1% glutaraldehyde solution in 0.1 M sodium-cacodylate buffer with a pH of 7.38. After fixation they were washed for 2 hours at 0°C to 4°C in a 0.01-M Tris-maleate buffer containing 10% v/v dimethyl sulfoxide (DMSO) and 8% w/v sucrose at pH 7.38. For the demonstration of transport Na+-K+-ATPase (by means of the K+-nitrophenylphosphatase (K-NPase) reaction) and of alkaline phosphatase, the enzyme cytochemical method of Ernst5 was used, as modified by Mayahara, et al.16 A further modification11 consisted of the replacement of lead by cerium as a capturing agent to improve cytochemical localization.25 In final concentration the incubation medium consisted of: 60 mM Tris-maleate buffer with a pH of 8.95 (adjusted with 45 mM KOH and 120 mM NaOH); 25% v/v DMSO; 10 mM Mg-p-nitrophenylphosphate; 2.5 mM levamisole; and 1 mM cerium chloride. For the selective demonstration of Na+-K+-ATPase and of alkaline phosphatase, various constituents were added to or omitted from the above-mentioned medium (Table 1).

For enzyme ultracytochemistry, the sheets of cyst wall attached to cork and the excised arachnoid granulations were immersed for 1 hour at 4°C in 2% paraformaldehyde 0.1% glutaraldehyde solution in 0.1 M sodium-cacodylate buffer with a pH of 7.38. After fixation they were washed for 2 hours at 0°C to 4°C in a 0.01-M Tris-maleate buffer containing 10% v/v dimethyl sulfoxide (DMSO) and 8% w/v sucrose at pH 7.38. For the demonstration of transport Na+-K+-ATPase (by means of the K+-nitrophenylphosphatase (K-NPase) reaction) and of alkaline phosphatase, the enzyme cytochemical method of Ernst5 was used, as modified by Mayahara, et al.16 A further modification11 consisted of the replacement of lead by cerium as a capturing agent to improve cytochemical localization.25

The specimens were preincubated in the medium at 0°C for 12 to 16 hours and subsequently incubated in the same medium at 37°C for 12 to 15 minutes. The specimens were washed in Tris-maleate buffer at pH 7.2, postfixed in the same buffer containing 2% glutaraldehyde for 1 hour, and washed three times in distilled water for a few minutes. After postfixation in 1% osmium tetroxide, they were dehydrated and embedded in epoxy resin. Ultrathin and 1-μm thick sections were cut on an LKB ultramicrotome. The unstained ultrathin sections were viewed with an electron microscope. The 1-μm thick sections from the arachnoid granulations were stained with toluidine blue and basic fuchsin for light microscopy.

---

* JEOL scanning electron microscope, Model JSM-U3, manufactured by Japan Electron Optics Laboratory, Tokyo, Japan.

† Electron microscope, Model EM 300, manufactured by Philips Electronic Instruments, Mount Vernon, New York.

---

### TABLE 1

Final concentrations of constituents used in the incubation media

<table>
<thead>
<tr>
<th>Feature</th>
<th>K⁺ (mM)</th>
<th>Na⁺ (mM)</th>
<th>Mg²⁺ (mM)</th>
<th>Levamisole (mM)</th>
<th>Ouabain (mM)</th>
<th>pNPP (mM)</th>
<th>DMSO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-NPase</td>
<td>45</td>
<td>120</td>
<td>10</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>
| alkaline
phosphatase   | 120     | 10       | 2.5       | 10              | 10          | 25        |          |
| Inhibition of K-NPase | 120 | 10       | 2.5       | 10              | 10          | 25        |          |

*pNPP = p-nitrophenylphosphate; DMSO = dimethyl sulfoxide; K-NPase = K⁺-nitrophenylphosphatase (Na⁺⁻K⁺-adenosine triphosphate).
FIG. 1. Scanning electron micrograph of the arachnoid cyst from Case 1 showing scattered stubby microvilli of 0.3 to 1 μm and fenestrations on the luminal surface of the cyst dome. Bar = 1 μm; × 11,000.

Results

Arachnoid Cyst Wall

In studies of the arachnoid cyst wall, SEM showed scattered microvilli of 0.3 to 1 μm and fenestrations on the luminal surface of the roof of the cyst (Fig. 1). These findings were similar to previous studies.7 Transmission electron microscopy of the luminal lining depicted multiple layers or arachnoid mesothelial cells and tortuous intercellular clefts with desmosomal junctions. At some places vacuoles could be seen in the cytoplasm (Fig. 2). In the enzyme ultracytochemistry studies, the reaction product of Na⁺-K⁺-ATPase was mainly localized at the apical plasma membranes of the most superficial cells (Fig. 3 left). The reaction product of alkaline phosphatase was present both on the apical and basal plasma membrane of two or more of the superficial cell layers (Fig. 3 right).

Arachnoid Granulations

On light microscopy, the arachnoid granulations typically consisted of a core of arachnoid mesothelial cells and a lining of endothelial cells. The general structure, comprising a meshwork of mesothelial cells, collagen fibers, and wide intercellular clefts (presumably confluent with the subarachnoid CSF space through the pedicle of the granulation) is best illustrated on low-magnification photomicrographs (Fig. 4).

Ultrastructurally, the endothelium of the arachnoid granulation lacked a continuous basement membrane, and was morphologically identical to the underlying arachnoid mesothelium. In some granulations, arachnoid capillaries underlying the endothelial covering of the granulation could be observed. The core of the granulations consisted of layers of arachnoid mesothelial cells with interspersed bundles of collagen fibers. The mesothelial cells were of both the dark and light types and had elongated nuclei with marginated chromatin. The cytoplasm notably contained vacuoles; multivesicular bodies and lysosomal structures could also be observed. The cytoplasmic vacuoles occasionally seemed to open into the tortuous intercellular clefts, but it was difficult to discern whether they constituted true cytoplasmic vacuoles opening into the interstitial spaces or were merely the cross sections of invaginations of the tortuous intercellular clefts into the cytoplasm. Furthermore, many scattered desmosomal junctions could be seen, upon which tonofilaments in the cytoplasm abutted.

Enzyme ultracytochemistry depicted the location of Na⁺-K⁺-ATPase activity at the apical plasma membrane of the outermost layer of cells bordering the venous lumen (Fig. 5). Alkaline phosphatase activity was localized at the apical and basal plasma membranes of two or more of the superficial cell layers. Also some reaction product could be observed at the plasma mem-
Cerebrospinal fluid absorption

FIG. 3. Enzyme ultracytochemical studies of the arachnoid cyst in Case 1. **Left:** K+-nitrophenylphosphatase reaction. The reaction product is localized at the luminal (that is, apical) membrane of the most superficial neurothelial cells of the cyst dome. Bar = 1 μm; × 10,000. **Right:** Alkaline phosphatase reaction. The reaction product is situated at the plasma membranes of a number of the most superficial cell layers lining the cyst lumen. Bar = 1 μm; × 15,000.

branes of deeper-situated cells. The results were similar for all the specimens of arachnoid granulation tissue obtained from the three patients in whom this tissue was studied (Fig. 6).

**Discussion**

In accordance with a previous report, the arachnoid cyst wall in these studies showed the characteristic features of subdural neurothelium or arachnoid mesothelium. Moreover, the microvilli, which are tortuous intercellular clefts with their junctions, are features that are observed in fluid-secreting or fluid-absorbing tissues. Also, the ultracytochemical evidence of Na+-K+-ATPase in the plasma membranes of the lining cells suggests a capacity for fluid secretion. In the specimens of arachnoid cyst and arachnoid granulations that we studied, the polarity of distribution of Na+-K+-ATPase and alkaline phosphatase (the former apical and the latter basal), which is seen in other tissues, was not evident. The location of transport ATPase at the apical membrane, as has been observed in secretory epithelia, is similar to the situation in choroid plexus epithelium.

The morphology of arachnoid villi and granulations has been the subject of many studies, which usually agree upon general structure, but may differ considerably as to minor structural detail. Andres and Rascol...
and Izard described the most differentiated structure of the arachnoid villus or granulation, consisting of a core of arachnoid mesothelial cells deriving from those of the subarachnoid space, surrounded by a layer of subdural neurothelium (which is the outer layer of the arachnoid bordering the dura), and most superficially by a lining of endothelial cells, continuous with those of the venous or sinus lining. Most other authors only distinguished two layers: an endothelial lining covering the bulk of the villus, and the mesothelial cells making up the villus. According to Jayatilaka, the lining endothelial cells cannot be distinguished from the underlying mesothelial cells ultrastructurally. The basal lamina underlying the endothelial layer, as described in the arachnoid villi of many animals, has been reported to be discontinuous in human material, or even absent.

**FIG. 5.** Enzyme ultracytochemical tests for K+-nitrophenylphosphatase (K-NPPase) of arachnoid granulation tissue. *Left:* Specimen from Case 2. The reaction product is situated at the apical membrane (facing the sinus lumen) of the outermost cell layer covering the arachnoid granulation. Note the large cytoplasmic vacuoles and desmosomal junctions (arrow). Bar = 1 μm; × 15,000. *Right:* Specimen from Case 4 showing localization of the reaction product of K-NPPase at the apical membrane of the most superficial layer of endothelial cells. Bar = 1 μm; × 20,000.

**FIG. 6.** Enzyme ultracytochemical tests for alkaline phosphatase of arachnoid granulations. *Left:* Specimen from Case 2 showing localization of the reaction product at the apical membrane and basal membranes of the upper layers of the endothelial covering of the arachnoid granulation. Bar = 1 μm; × 30,000. *Right:* Specimen from Case 3 showing localization of the reaction product at cell membranes lining the outermost intercellular spaces of endothelial covering of arachnoid granulation. Bar = 1 μm; × 7000.

646
Cerebrospinal fluid absorption

and replaced by an irregular subendothelial space filled with collagen fibers and basal lamina material. Although the endothelial covering of the villus has been observed to originate from sinus endothelium during embryonic development, its later morphological similarity to the underlying mesothelium or neurothelium and the different aspect of the underlying basal lamina seem to indicate a differentiation of this vascular endothelium toward mesothelium. We therefore tend to agree with Tripathi in considering it as mesothelium, or "neurothelium" as the outer mesothelial layer is termed by Andres and Rascol and Izard. Moreover, only the larger arachnoid granulations appeared to protrude into the sinus lumens, whereas the smaller ones abut onto a subdural space.

The ultrastructural activity of Na⁺-K⁺-ATPase in the outermost cell layer of arachnoid granulations would support the assumption that there is a capacity for fluid secretion, and opens the question whether there exists a biomechanical agency, separate from the open or closed pathways of CSF absorption, for the transport of CSF from the subendothelial CSF spaces of arachnoid villi to the venous lumen. In evaluating the various mechanisms of CSF absorption, not only the morphological but also the pathophysiological data have to be considered. It is well known that CSF absorption is dependent upon CSF pressure. This pressure-dependence has been shown to induce the formation of cytoplasmic vacuoles in the villus endothelium, although it is difficult to estimate from morphological observations how efficient a transport mechanism vacuole formation as a cell biological process can be. Moreover, as with other two-dimensional cross-sectional images, it is difficult to discern whether they represent true vacuoles belonging to the cytoplasm and occasionally opening into the interstitial space or invaginations of the tortuous intercellular clefts, possibly constituting transcellular channels. Pressure-dependence of CSF absorption would also be more compatible with an open system of tubules, as described in pacchionian granulations. On the other hand, the open system has only been reported in larger granulations of larger animals, not in the villi of smaller animals where other mechanisms have to be considered. Furthermore, corticosteroids have been shown to enhance CSF absorption, which would suggest a hormonal action upon enzyme activity and support the significance of the biochemical mechanism.

Acknowledgments

We are indebted to Mrs. A. H. Thijs-Ipema and Dr. W. L. Jongbloed for their assistance.

References

22. Shabo AL, Maxwell DS: The morphology of the arach-


Manuscript received November 5, 1985.
Accepted in final form April 30, 1986.
This work was supported by a grant from The Netherlands Foundation for Medical Research (FUNGO).
Address reprint requests to: K. Gwan Go, M.D., Kliniek voor Neuro-chirurgie, Academisch Ziekenhuis Groningen, Oostersingel 59, Postbus 30.001, Groningen 9700 RB, The Netherlands.