

# Blood-brain barrier ultrastructural alterations in human congenital hydrocephalus and Arnold-Chiari malformation

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## Abstract

*Cortical biopsies of 13 patients with clinical diagnosis of congenital hydrocephalus, Arnold-Chiari malformation and hydrocephalus, and postmeningitis hydrocephalus were examined by transmission electron microscopy to study the damage of endothelial cells, basement membrane, astrocytic end-feet layer, and perivascular space. Capillaries from the parietal and frontal cortex showed increased vesicular and vacuolar transport, intact endothelial junctions, thin and immature basement membrane, swollen perivascular astrocytic end-feet layer, and enlarged perivascular space. In areas of severe oedema, open endothelial junctions, swollen basement membrane, absent perivascular astrocytic end-feet layer, enlarged perivascular space, and disrupted perivascular neuropil were observed. The electron microscopic findings demonstrated breakdown of the blood-brain barrier in all cases examined.*

**Key words:** blood-brain barrier, congenital hydrocephalus, Arnold-Chiari malformation, electron microscopy.

## Introduction

Earlier electron microscopic tracer studies have located the mammalian blood-brain barrier to protein at the level of the tight junctions between endothelial cells of cerebral blood vessels [22]. Most electron microscopic studies on brain oedema suggest that the breakdown of the blood-brain barrier is mainly due to enhanced vacuolar and vesicular transport rather than to opening of endothelial junctions [3,4,20,24,30], supporting the concept of Klatzo [13], who postulates that it is unlikely that the opening of tight junctions may play a significant role in conditions associated with brain oedema.

Few electron microscopic studies have been reported thus far on the damage of the immature

blood-brain barrier in experimental and human congenital hydrocephalus. Sada et al. [23] found well formed tight junctions between endothelial cells, partial defect of capillary basal membrane, protrusion of endothelial cells between pericytic cytoplasm, and swelling of astrocytic end-feet in congenitally hydrocephalic HTX rat brain. McAllister and Chovan [15] proposed brain barrier dysfunction in hydrocephalus as a primary mechanism for neurological impairment in neonatal hydrocephalus. Seyfert and Faulstich [26] and Seyfert et al. [27] reported altered blood-CSF barrier permeability in patients with normotensive hydrocephalus expressed by albumin and immunoglobulin variations in the patients studied. Sendrowski et al. [25], using S-100 protein as a mar-

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ker of blood-brain barrier disruption, described functional disturbances of blood-brain barrier in children with internal hydrocephalus. Del Bigio [6] elegantly reviewed the damage and prevention in childhood hydrocephalus, and expressed the need for further research on the blood-brain barrier in hydrocephalus. Brettschneider et al. [1] found isolated blood-cerebrospinal fluid barrier dysfunction in normal pressure hydrocephalus characterized by abnormal elevation of the albumin CSF/serum concentration ratio.

The above-mentioned studies prompted us to carry out a detailed electron microscopic study of the blood-brain barrier in human congenital hydrocephalus and Arnold-Chiari malformation to examine the cortical capillaries of different brain cortical

regions, using cortical biopsies taken during neurosurgical treatment. The aim of the present paper is to demonstrate that in addition to interstitial or hydrocephalic oedema there is also a superimposed vasogenic oedema. To the best of our knowledge such a study has not been performed until now.

## Material and Methods

Samples of cerebral cortex of 13 patients with clinical diagnosis of congenital hydrocephalus, Arnold-Chiari malformation, and postmeningitis hydrocephalus were examined in the present study (Table I). Cortical biopsies were performed according to basic principles of the Helsinki Declaration, and

**Table I.** Human congenital hydrocephalus and Arnold-Chiari malformation

Sample identification	Age and sex	Clinical data	Diagnosis	Cortical Biopsy
HLCS	1 month, F	Increased cephalic circumference, disjunction of sutures, tense fontanelles	Uncompensated communicant congenital hydrocephalus	Right parietal cortex
EEAT	1 month, F	Increased cephalic circumference, poor feeding, tense fontanelles	Uncompensated congenital hydrocephalus	Right temporo-parietal cortex
LMBC	1 month, F	Increased cephalic circumference. Hypertensive fontanelles, vomiting, disjunction of sutures.	Congenital communicant hydrocephalus	Right frontal cortex
DEMY	6 month, M	Increased cephalic circumference. Disjunction of sutures. Tense fontanelles. Left peridural abscess.	Congenital hydrocephalus	Right temporo-parietal cortex
FJRA	10 days, M	Bulging of fontanelles and increased cephalic circumference after surgical correction of lumbar meningomyelocele.	Arnold-Chiari malformation. Communicant hydrocephalus	Frontal cortex
CMV	2 months, F	Increased cranial volume, hypertensive fontanelles, deviation of gaze to the right, external rotation of both legs and increased tendinous reflexes after treatment of lumbar meningomyelocele.	Arnold-Chiari malformation. Hydrocephalus. Parieto-occipital intraparenchymatous abscess	Right parietal cortex
NU	12 days, F	Increased cephalic circumference after treatment of lumbar meningomyelocele.	Congenital hydrocephalus. Meningomyelocele	Right parietal cortex
IATF	3 months, M	Febrile syndrome. Meningitis Obstructive hydrocephalus after two weeks	Congenital hydrocephalus. Postmeningitis hydrocephalus	Right frontal cortex
NSM	8 months, F	Increased cranial volume since three months of age, tense fontanelles, poor feeding	Congenital communicant hydrocephalus	Right parietal cortex
GAPG	3 months, M	Meningeal syndrome. Tonic-clonic convulsions, increased cephalic circumference, vomiting increased limb tone.	Congenital postmeningitis hydrocephalus	Right frontal cortex
RGG	4 months, F	Increased cephalic circumference, disjunction of sutures. Hypertensive fontanelles	Congenital hydrocephalus	Right frontal cortex
JLAR	3 months, M	Increased cephalic circumference observed 1 month after birth, disjunction of sutures, tense fontanelles	Congenital communicant hydrocephalus	Right frontal cortex
HR	2 years, F	Increased cranial volume since 4 months of age, poor feeding, disjunction of sutures	Congenital communicant hydrocephalus	Right frontal cortex

approved by the Ethical Committee of the Biological Research Institute. Parent consent for surgical biopsy was obtained for each patient studied. Table I contains the clinical data and lists the cortical regions from which the biopsies were taken. Two to five mm thick cortical biopsies were immediately fixed in the surgical room in 4% glutaraldehyde-0.1 M phosphate or cacodylate buffer, pH 7.4 at 4°C. Later they were divided into 1 mm fragments and immersed in a fresh, similar solution for periods varying from 2 to 72 h, followed by secondary fixation in 1% osmium tetroxide-0.1 M phosphate buffer, pH 7.4 for 1 h. They were then rinsed for 5 to 10 min in a buffer similar to that used in the fixative solution, dehydrated in increasing concentrations of ethanol and embedded in Araldite or Epon. For proper orientation of the electron microscope study, thick sections of approximately 0.1 to 1 µm were stained with toluidine blue and examined with a Zeiss photomicroscope. Ultrathin sections obtained with Porter-Blum and LKB ultramicrotomes were stained with uranyl acetate and lead citrate and observed in a JEOL 100B electron microscope. Observations

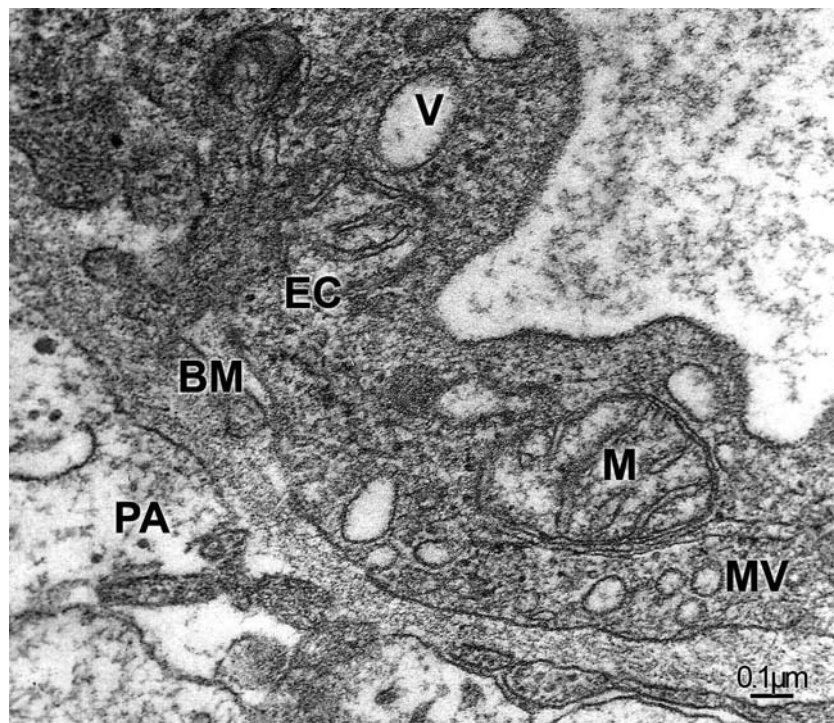
were made using intermediate magnifications ranging from 12,000 to 75,000 X.

## Results

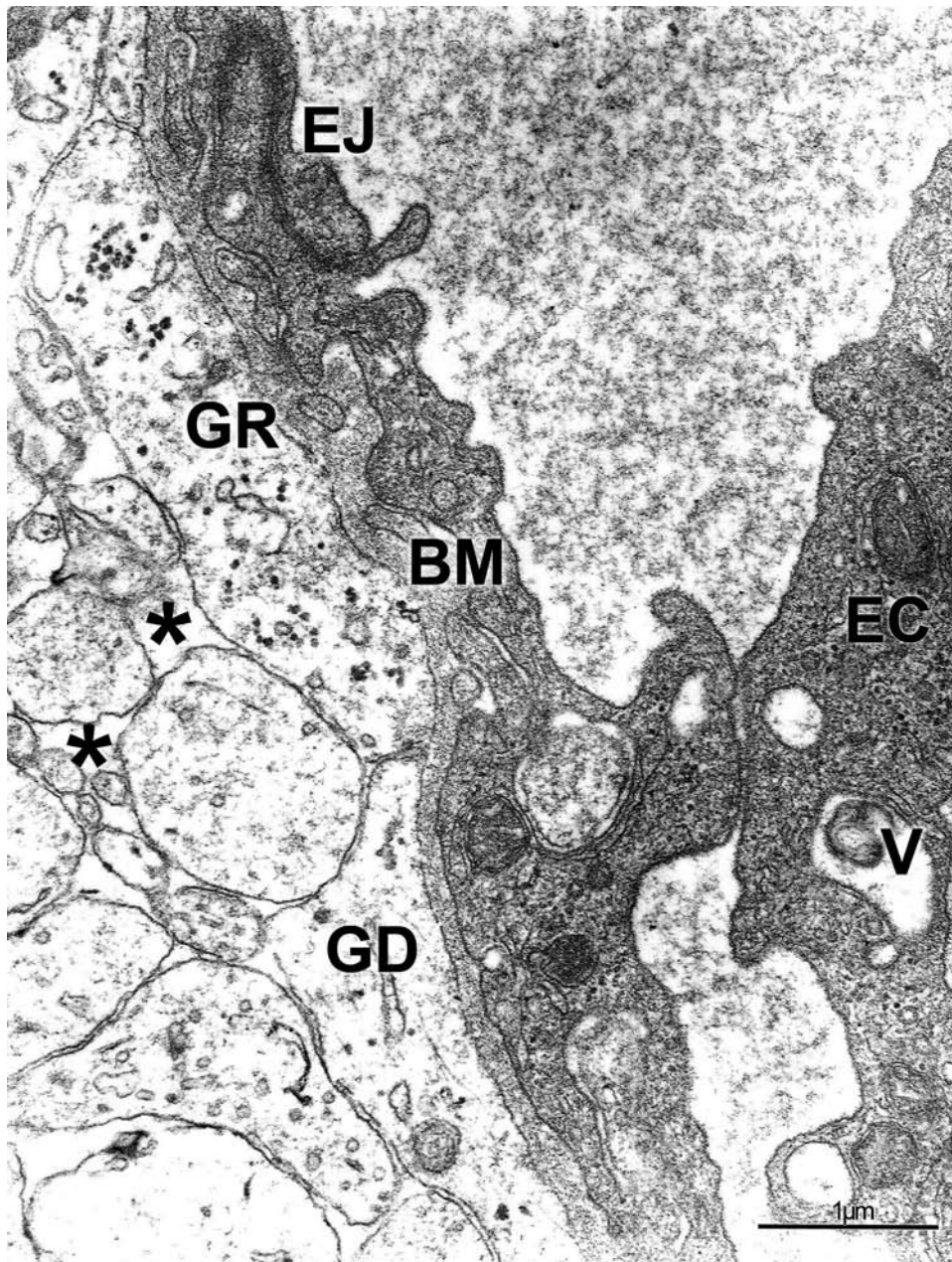
In most examined cases of congenital hydrocephalus (cases 1-4 and 7-13) the cortical capillaries of different brain regions exhibited increased amount of vacuoles and micropinocytotic vesicles at the level of endothelial cell peripheral cytoplasm, apparently intact endothelial junctions, an immature and thin basement membrane, swollen glycogen-rich and glycogen-depleted astrocytic perivascular end-feet, and a neighbouring oedematous neuropil (Figs. 1 and 2).

Some brain capillaries located in severe areas of interstitial brain oedema showed a notably swollen basement membrane and a glio-basal dissociation process characterized by detached swollen glycogen-depleted astrocytic end-feet floating in the enlarged perivascular space (Fig. 3).

In Arnold-Chiari malformation, hydrocephalus and lumbar meningomyelocele (cases 5 and 6), we have found in severe oedematous regions vacuola-



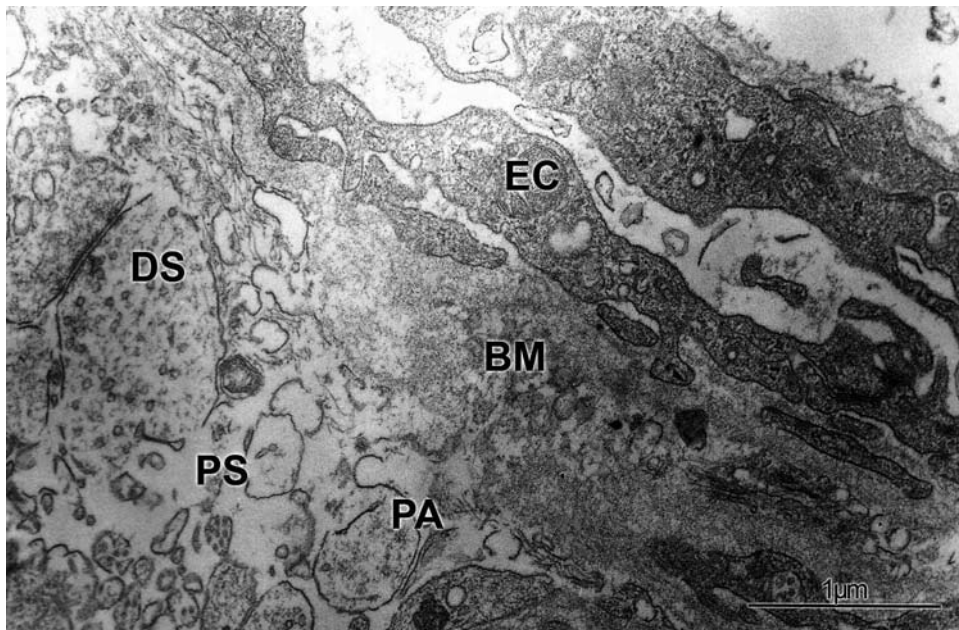
**Fig. 1.** Case No. 1. Congenital hydrocephalus. Right parietal cortex. Cortical capillary showing the peripheral cytoplasm of endothelial cell (EC) exhibiting vacuoles (V), micropinocytotic vesicles (MV), a thin basement membrane (BM), and notably swollen perivascular astrocytic end-feet (PA)



**Fig. 2.** Case No. 3. Congenital hydrocephalus. Right frontal cortex. Longitudinal section of a cortical capillary showing the dark peripheral endothelial cell cytoplasm (EC), a closed and undulated endothelial cell (EC) junction (EJ), vacuoles (V), a thin basement membrane (BM), intimately applied glycogen-rich and glycogen-depleted perivascular astrocytic end-feet (GR, GD). Note the enlarged perivascular space (asterisks) in the neighbouring neuropil

ted endothelial cells, tightly closed and open endothelial junctions, thin and discontinuous basement membrane, focal absence of perivascular astrocytic end-feet layer, enlarged perivascular space, and a fragmented neighbouring neuropil (Figs. 4 and 5).

In some congenital communicant hydrocephalus (cases 12 and 13) we found swollen and glycogen-depleted perivascular astrocytic end-feet joined by irregularly dilated gap junctions (Fig. 6). Other capillaries displayed swollen glycogen-rich and glycogen-deple-



**Fig. 3.** Case No. 4. Congenital hydrocephalus. Right temporal cortex. Longitudinal section of a cortical capillary showing dark endothelial cell (EC), a remarkably swollen basement membrane (BM), swollen perivascular astrocytic end-feet (PA) dissociated from the basement membrane, and the enlarged perivascular space (PS). A degenerate synaptic contact (DS) is also observed in the neighbouring and disrupted neuropil

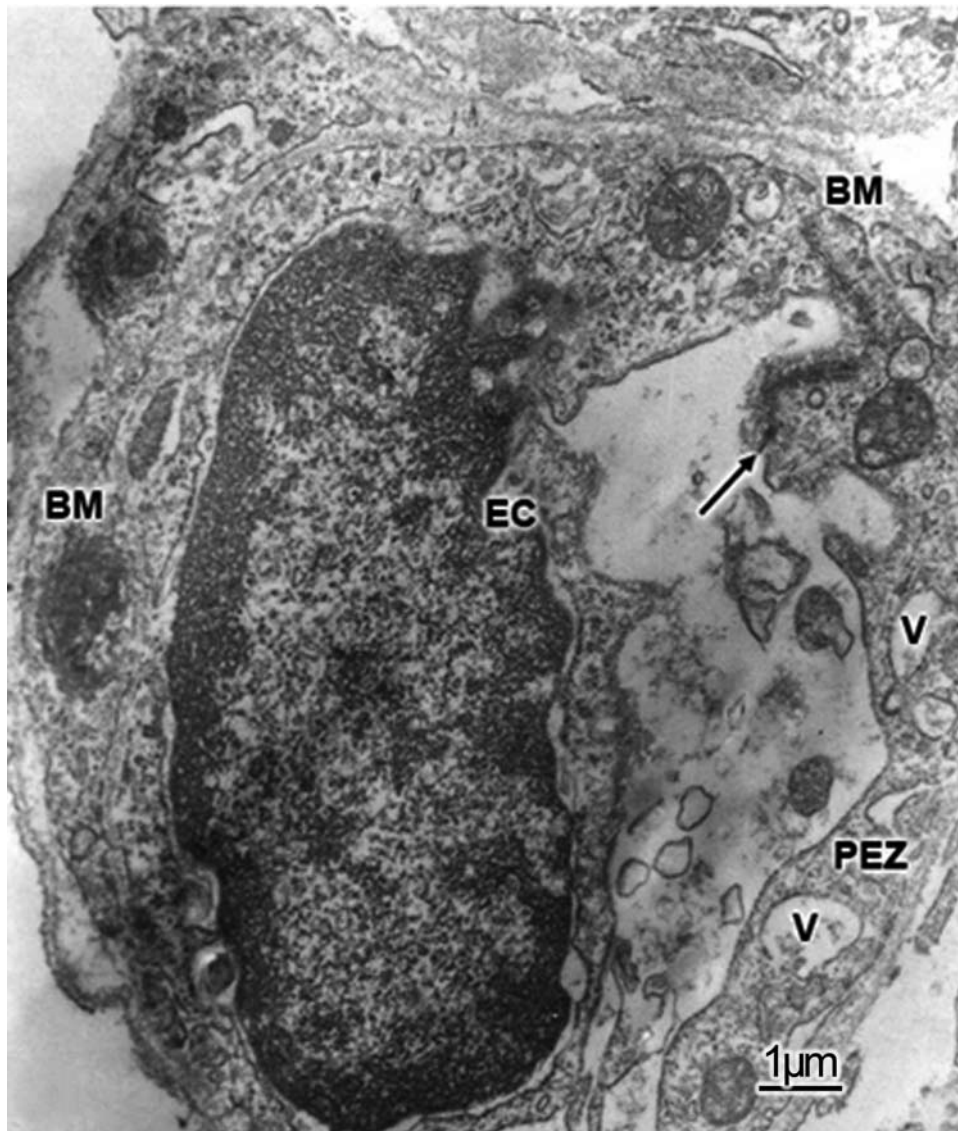
ted perivascular astrocytic end-feet which remained attached to the basement membrane (Fig. 7).

## Discussion

In the present paper we have shown brain capillaries of different cortical regions of infant patients with congenital hydrocephalus and Arnold-Chiari malformation exhibiting increased capillary permeability, characterized by augmented transendothelial vesicular and vacuolar transport, and with structurally closed endothelial junctions. In very severe oedematous areas of Arnold-Chiari malformation, the endothelial junctions appeared open, and the basement membrane was thickened. The perivascular space was considerably enlarged, and the neighbouring neuropil appeared disrupted. These morphological findings demonstrate that blood-brain barrier damage occurs in congenital hydrocephalus and Arnold-Chiari malformation, and that vasogenic oedema increases the pre-existing hydrocephalic or interstitial brain oedema. Similar findings have been earlier reported by the author in severe and complicated human traumatic brain oedema [3,4].

The significance of junctional dehiscence in the pathogenesis of human hydrocephalic severe brain oedema remains to be elucidated. Several possibilities should be considered: a) mechanical cleavage of endothelial tight junction by the pressure exerted by the interstitial oedema and the associated hypoxic-ischaemic process; b) serotonin or histamine release, which induces endothelial cell contraction and opening of endothelial junctions [30]; c) increased elevation of intracellular c AMP [31]; d) osmotic disruption due to solute and plasma protein movement into the compartments of the endothelial tight junction and the subsequent movement of water [2,17,21,29]; e) alteration of the architecture of actin cytoskeleton; f) posttranslational modification of junctional proteins; proteolytic degradation of junctional constituents; g) effect of proinflammatory cytokines [10].

The osmotic gradient would produce focal discontinuities in the junctional protein fibril while the osmotic disruption would induce interendothelial cleft dehiscence [18]. According to Fischer et al. [7-9] and Mark and Davis [14], hypoxia disrupts the continuity of the tight junction protein zonula occludens-1 (ZO-1). In contrast, Hamm et al. [11] considered that



**Fig. 4.** Case No. 6. Congenital hydrocephalus. Arnold-Chiari malformation. Right parietal cortex. Isolated capillary located in a disrupted neuropil. The endothelial cell (EC) peripheral cytoplasmic zone (PEZ) appears vacuolated (V), and the endothelial junction (arrow) is structurally closed. Note that the basement membrane (BM) focally lacks the attached astrocytic glial end-feet layer

opening of tight junctions is not accompanied by any change in the molecular composition of the endothelial junction. As mentioned above, Sada et al. [23] found well formed tight junctions between endothelial cells, partial defect of capillary basal membrane, protrusion of endothelial cells between pericytic cytoplasm, and swelling of astrocytic end-feet in congenitally hydrocephalic HTX rat brain. More recently, Nagy et al. [19] reported the opening of paracellular

avenues in human microvessel endothelial cell culture in ischaemic/hypoxic conditions.

We have shown the presence of swollen glycogen-rich and glycogen-depleted astrocytic end-feet applied to the basement membrane. We have described the presence of glycogen-rich and glycogen-depleted astrocytes in the injured cerebral cortex as astrocyte subtypes present in a heterogeneous astrocyte population within the oedematous cere-

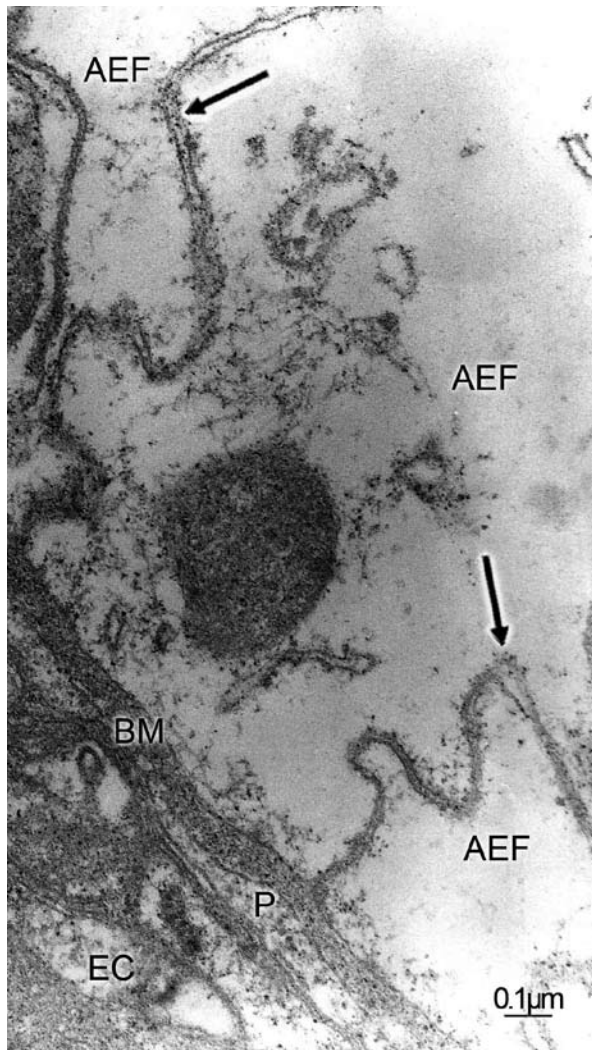




**Fig. 5.** Case No. 5. Congenital hydrocephalus. Arnold-Chiari malformation. Frontal cortex. Isolated capillary located in a disrupted neuropil in a severely oedematous area. The endothelial junction (long arrows) appears partially open. The endothelial cell (EC) shows vacuoles (V) containing proteinaceous material. A thin and discontinuous basement membrane (BM) is seen. Note the enlarged perivascular space (PS), and the neighbouring disrupted neuropil (asterisks)

bral cortex [5]. This finding suggests the possibility of glycogen degradation and anaerobic mobilization of glycogen stores in anoxic-ischaemic conditions. Smirnov and Akopian [28] earlier reported glycogen particles predominantly localized in the perivascular astrocytic processes after experimental craniocerebral injuries. The astrocyte cells would rapidly switch

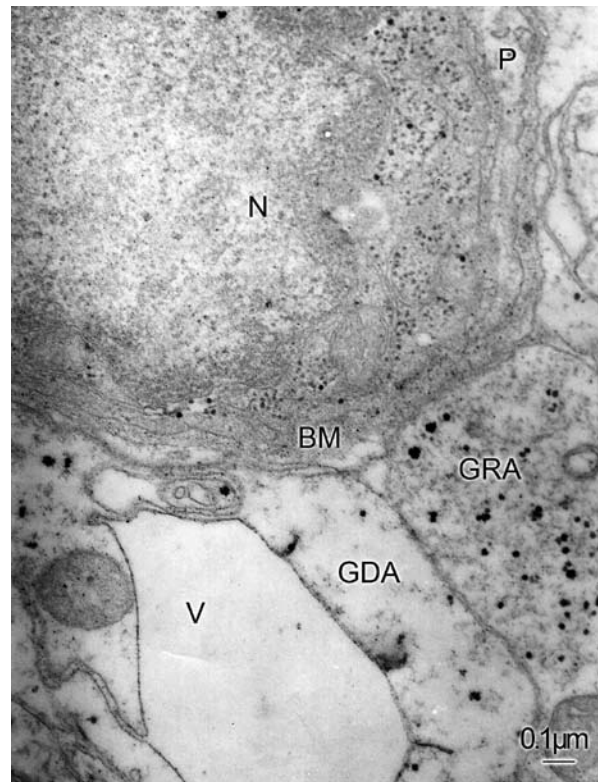
between production and consumption of glycogen depending on activities and needs of the neighbouring ischaemic neurons, and oligodendroglial cells. It is also important to consider that astroglial cells behave as a store of lactic acid (12) rather than of glucose. These authors postulated that damaged neurons and oligodendrocytes may well be using



**Fig. 6.** Case No. 12. Congenital communicant hydrocephalus. Right frontal cortex. Swollen astrocytic end-feet (AEF) joint by irregularly dilated gap junctions (arrows), which appear closely applied to the thin basement membrane (BM). The pericytic (P) and endothelial (EC) cytoplasm also are distinguished

endogenous lactate produced or released by neighbouring astrocytes as a valuable fuel material in the oxidative pathways of energy generation.

In relation with the disrupted perivascular neuropil, the extravasated haematogenous oedema fluid and the pressure of the non-circulating cerebrospinal fluid produced separation, indentation and rupture of nerve cell profiles. In this context, human congenital hydrocephalus differs from the hy-3 mouse neo-



**Fig. 7.** Case No. 13. Congenital communicant hydrocephalus. Right frontal cortex. The glycogen-rich and glycogen-depleted swollen perivascular astrocytic end-feet (GRA, GDA) are seen applied to the capillary basement membrane (BM). The glycogen-depleted swollen astrocytic end foot exhibits a huge vacuole (V). The endothelial cell nucleus (N), and the pericytic cytoplasm (P) are also seen

natal hydrocephalus, in which a normally operating blood-brain barrier is suggested by the presence of intact interendothelial tight junctions, no indication of increased pinocytotic activity and perivascular astrocytes of apparently normal submicroscopic morphology [16]. The increased perivascular space of the human cortical neuropil in congenital hydrocephalus also suggests the establishment of a transparenchymal route for cerebrospinal fluid absorption through the damaged cortical capillaries.

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