Review paper

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Increased vesicular and vacuolar transendothelial transport in traumatic human brain oedema. A review

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Abstract

The endothelial vacuolar and vesicular transports in traumatic human brain oedema have been reviewed and compared with experimental brain oedema in order to establish their role in both oedema formation and oedema resolution. Normal or "non-activated" and "activated" capillaries are found. The activated capillaries showed predominantly an enhanced abluminally orientated vesicular transport by means of small, medium and large uncoated and clathrin coated vesicles, as well as the presence of endothelial tubular structures. Activation of the endothelial nuclear zone is featured by the increased amount of micropinocytotic vesicles. Vesicles internalizing to the hypertrophic Golgi complex, lysosomes and multivesicular bodies are observed. The protein vacuolar transport is predominant in most cortical capillaries. A wide spectrum of endothelial cell mechanisms is observed increasing the vesicular and vacuolar transport, such as deep invaginations of the luminal surface, large coated vesicles, tubular structures, and transient and incomplete transendothelial channels formed either by chained plasmalemmal vesicles or elongated protein-containing vacuoles. Uncoated vesicles are seen surrounding lysosomes. Vesicular transport might be discriminated between abluminally orientated or transendothelial transport (oedema formation) and intraendothelial transport (oedema resolution) directed towards cell lysosomes to be degraded by lysosomal enzymes. The transendothelial passage via large vacuoles is mainly caused by macromolecular protein transport.

Key words: *brain trauma, brain oedema, vesicular transport, vacuolar transport, endothelial mechanisms.*

Introduction

Increased endothelial cell vesiculation has been earlier reported in anoxic-ischaemic lesions [30,31,36], experimental cerebral infarction [3], ionizing radiation [20], brain-stem lesion of thiamine-deficient rats [44], following intraventricular perfusion with serotonin, norepinephrine and cyclic AMP [63], ultraviolet irradiation [49], experimental seizures [46], hypertension [28,47,59], brain trauma [1,6,12-16,18,21,31,47,48], congenital hydrocephalus [10,17], and brain tumours [23,50,53-55].

The hypothesis of "vesicular transport" stands as a generally accepted process, explaining in particular macromolecular transport in capillaries [2]. The most convincing evidence to date for a vesicular transport of protein across

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reactive cerebral capillaries was earlier provided by Beggs and Waggener [1], who found that upon reproducible contusion of the spinal cord in cats, horseradish peroxidase escaped from the vessels by means of pleomorphic vesicles, tubules and large vacuoles.

The orientation of pinocytotic vesicles correlating with the stage of the oedematous process was first made by Wagner *et al*. [61], and reproduced by Cervós-Navarro *et al*. [19]. Sazaki *et al*. [49] reported a typical orientation of the micropinocytotic vesicles depending upon the stage of the oedematous process: in the early stages, they are found preferably at the luminal side of endothelial cells, and later on they predominate at the abluminal endothelial cell membrane. Ferszt *et al*. [29] reported diffuse macro- and micropinocytotic activity in focal brain oedema due to ultraviolet irradiation as tissue water content reaches maximum values.

Casley-Smith and Carter [4] found that large vacuoles in inflamed endothelium do indeed transport macromolecules across the cells. In addition, large endothelial vacuoles and transendothelial vacuolar transport have been described in a large variety of pathological entities, such as brain tumours [50,53-55], anoxic-ischaemic lesions [6,8-10,13,17,21,27,30,33,34, 40,41,45], injections of Gram-negative endotoxin [24], lead encephalopathy [39], experimental allergic encephalomyelitis [35], and human brain oedema [6,8]. These studies show that the formation of endothelial vacuoles could be interpreted as opening of the endothelial 'pore system' induced by different pathological conditions [6]. Lossinsky *et al*. [42,43] studied the vesicular and canalicular transport structures in endothelial cells after crude leptomeningeal damage in mice, and in cold lesion injury in cats. Castejón [6-9] described an increased vesicular and vacuolar transendothelial transport, and formation of transendothelial channels in endothelial cells and pericytes in human traumatic and complicated brain injuries. Shibata *et al*. [50] reported increased endothelial pinocytotic vesicle formation in human brain tumours. Claudio *et al*. [25] found an increased endothelial cell vesicular transport in rat experimental autoimmune encephalomyelitis. According to these authors, immunogold staining of endogenous albumin demonstrated the presence of albumin in cytoplasmic vesicles and in channel-like tubular structures adjacent to endothelial cell junctions. These results indicated that there is a role for vesicles in transendothelial cell transport and oedema formation in animals with experimental autoimmune encephalomyelitis. Takano *et al*. [53] observed an increased vesicular transport in human glioma capillaries. Kato *et al*. [38] demonstrated increased numbers of vesicles and vacuoles in endothelial brain capillaries in cerebral oedema from fulminant hepatic failure.

Zumkeller and Dietz [65] described an increased protein vesicular transport in rats after treatment with nimodipine. Cicciarello *et al*. [26] found enhanced vesicular transport of horseradish peroxidase in an experimental model of whole brain after irradiation. Plateel *et al*. [45] examined the increased permeability of albumin after hypoxia, attributed to non-specific vesicular transport in a cell culture model of brain barrier.

Hofman *et al*. [37] showed an increased vascular permeability for plasma proteins *in vivo* induced by vascular endothelial growth factor-A (VEGF) in blood-retinal barrier endothelium predominantly caused by a mechanism involving active trans-endothelial transport via pinocytotic vesicles, and not by formation of endothelial fenestrations or vesiculo-vacuolar organelles.

Castejón *et al*. [15] reported an increased vesicular and vacuolar transendothelial transport in two patients with post-traumatic seizures. Lossinsky *et al*. [42] examined the vesicular and canalicular transport structures in the injured mammalian blood-brain barrier. Lossinsky and Shivers [43] reviewed in detail the structural pathways for macromolecular and endothelial cell transport during inflammatory conditions and brain injuries. Cipolla *et al*. [27] found increased apical and basolateral pinocytosis in cerebral endothelium during ischaemia/reperfusion, and elevated intravascular pressure. Castejón [10,17] described an increased vesicular and vacuolar transport in cortical capillaries from parietal and frontal cortex of patients with congenital hydrocephalus and Arnold-Chiari malformation.

Vesicular and vacuolar transport is a highly dynamic process currently studied in experimental animal works with electron dense tracers. The study of endothelial vesicular and vacuolar transport in human cortical biopsies is obviously limited for many reasons, such as: a) ethical principles that limit the use of electron dense probes *in vivo*, b) samples randomly obtained, c) ultrathin sections giving isolated images of endothelial cell substructures, d) great variability of the nature of traumatic agents and impact energy, and the patient's age and state of health.

In the present review we describe the morphological evidence of increased vesicular and vacuolar transports induced by human traumatic brain injuries. Emphasis will be placed on the role played by vesicular and vac-

uolar transports in oedema formation and oedema resolution. The variety of endothelial cell mechanisms observed in severe vasogenic brain oedema also are analysed.

Vesicular and vacuolar transendothelial transport as morphological signs of increased cerebrovascular permeability

Resting cortical capillaries

In traumatic moderate brain oedema, some gray matter cortical capillaries exhibit a normal appearance with an inactive, resting endothelial cytoplasmic peripheral zone, characterized by straight, almost smooth, luminal plasma membrane, without pseudopodic expansions and scarce micro- and macropinocytotic activity. In these normal capillaries, the basement membrane appears as a compact structure formed by filaments embedded in a homogeneous matrix. Only some free and membrane-bound clathrin-coated vesicles are encountered. The endothelial junctions are structurally intact, and the astrocytary glial end-feet appear intimately applied to the basement membrane [6,8,11]. These "non-leaky" capillaries are not activated by the mechanical injury exerted by the intensity of brain trauma.

Activated cortical capillaries

Other capillaries are activated by the traumatic injury and show an endothelial cell luminal surface activity, exhibiting abundant luminal microvilli, presence of coated vesicles, remarkable orientation of numerous abluminal uncoated pinocytotic vesicles toward the capillary basement membrane (Fig. 1), and a lesser amount of caveolae intracellularis or 'pits' connected to the luminal endothelial surface, suggesting abluminally orientated vesicular transport [6,8]. The non-leaky segments of capillaries contain "immobile" endothelial vesicles [58], which are activated in the leaky segments by the impact energy.

Chained pinocytotic vesicles fused with each other and with tubular invaginations to form shuttle vesicles or incomplete transient transendothelial channels [7,42,43]. In addition, large spheroid or elongated vacuoles, containing hematogenous oedema fluid appear free in the endothelial cytoplasm (Figs. 2 and 3). These vacuoles discharge their content directly into the basement membrane or by means of plasmalemmal vesicles. The basement membrane appears swollen, and abundant proteinaceous oedema fluid is seen separating the basement membrane from the perivascular glycogen-rich and glycogen depleted astroglial end-feet [11,14]. The latter are remarkably swollen and their limiting membranes appear fragmented.

Some actin-like filaments are attached to the vacuole limiting membrane, suggesting that the cytoskeleton is involved in the transendothelial movement of these vacuoles [8]. In normal capillaries the small vesicular and vacuolar transport is a random process powered by Brownian motion [4], but in the case of an enhanced cerebrovascular permeability, the participation of cytoskeletal structures is required to speed up the vacuolar and vesicular transport. A detailed study of the longitudinal sections of the capillary wall show that, in addition to the endothelial peripheral zone, the nuclear and organelle endothelial zones (Fig. 4) also

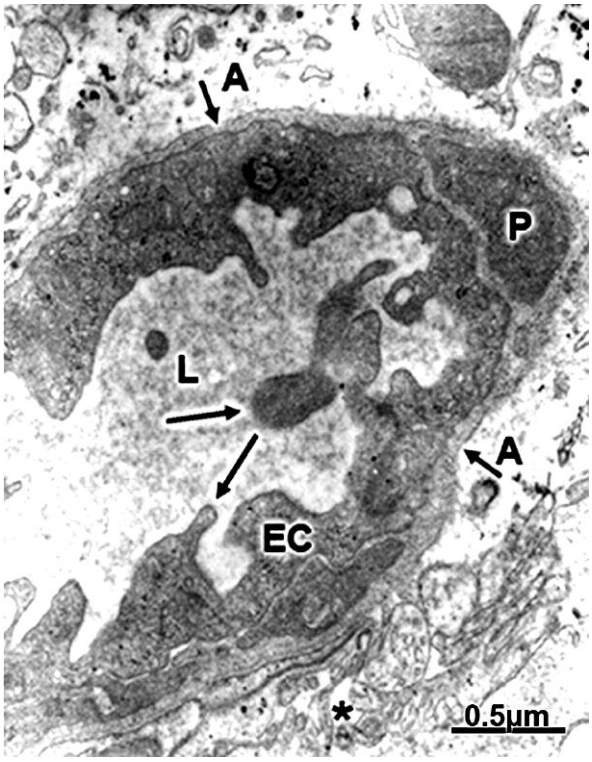

Fig. 1. Brain trauma. Subdural haematoma. Left parietal cortex. Cross section of a traumatically activated cortical capillary showing prominent protrusion of pseudopodic endothelial cell (EC) expansions (long arrows) engulfing the proteinaceous oedema fluid from the capillary lumen (L). The apparently normal basement membrane (short arrows), the enclosed pericital cytoplasm (P), and the astrocytic perivascular end-feet (A) also are seen. The asterisk labels the enlarged perivascular space.

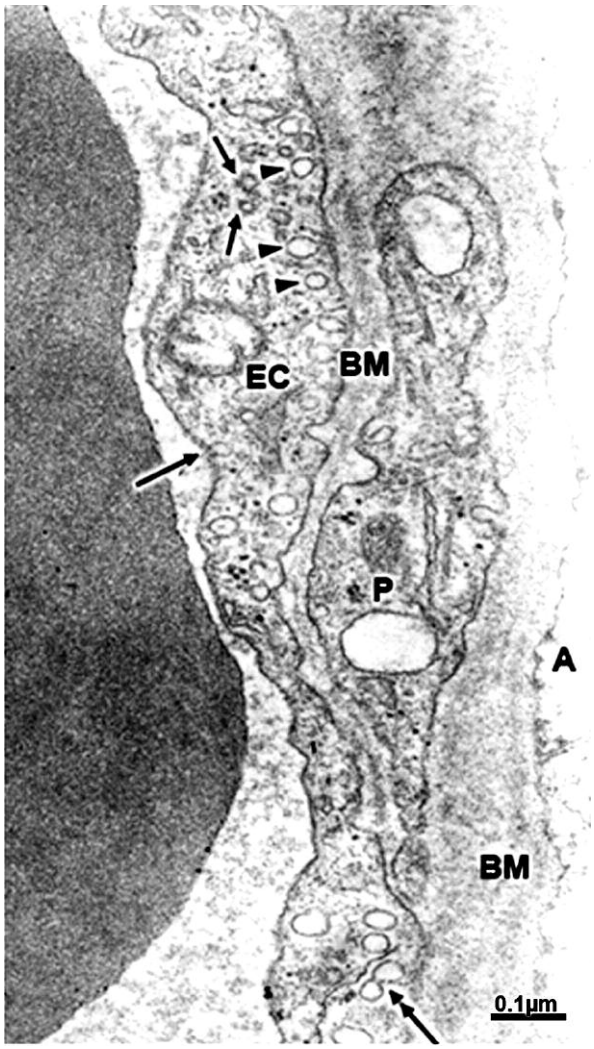

Fig. 2. Brain trauma. Right epidural haematoma. Right temporal cortex. Endothelial peripheral cytoplasm (EC) showing predominant vesicular transport (arrowheads) polarized toward the swollen basement membrane (BM). A caveolae intracellularis (long arrow) and fused micropinocytotic vesicles (double head arrow) are seen. The swollen pericyte (P) and basement membrane (BM) are noted. The limiting membrane of astrocytic (A) perivascular end-foot appears irregularly applied to the basement membrane outer surface.

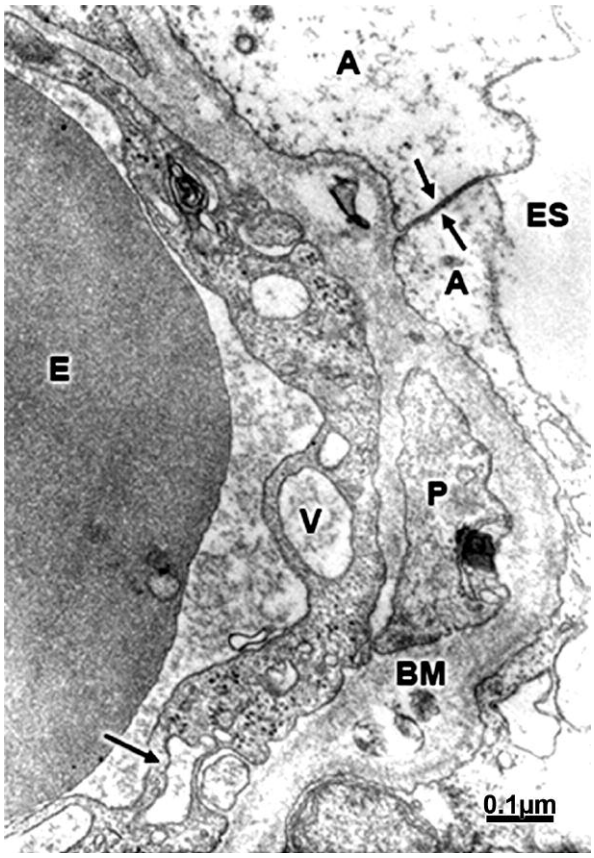

Fig. 3. Brain trauma. Subdural haematoma. Left parietal cortex. Endothelial cell peripheral zone showing large round (V) and elongated (long arrow) vacuoles. An erithrocyte (E), the swollen and vacuolated basement membrane, the oedematous astrocytic perivascular end-feet (A), and the distended extracellular space (ES) also are distinguished. The short arrows indicate the astrocytic end-feet gap junction.

exhibit surface activity, with shallow and deep invaginations of luminal plasma membrane to form protein-transporting vacuoles [6,8]. Deep invaginations of endothelia cell luminal membranes were also reported by Lossinski *et al.* [42] in experimental models of brain injury. The endothelial cell nuclear zone in normal capillaries shows a little or no micropinocytotic activity [6,51,52].

Vacuoles and vesicles actually participating in the transendothelial transport toward the basement membrane could be distinguished from those internalizing to some cell organelles, such as multivesicular bodies, Golgi apparatus and lysosomes (Figs. 5 and 6). The Golgi complex appears hypertrophic with dilated endoplasmic sacs and Golgi vacuoles. Deep invaginations of the luminal surface are formed in the vicinity of the Golgi formation phase, and numerous clathrin-coated and uncoated vesicles are observed in this area [8].

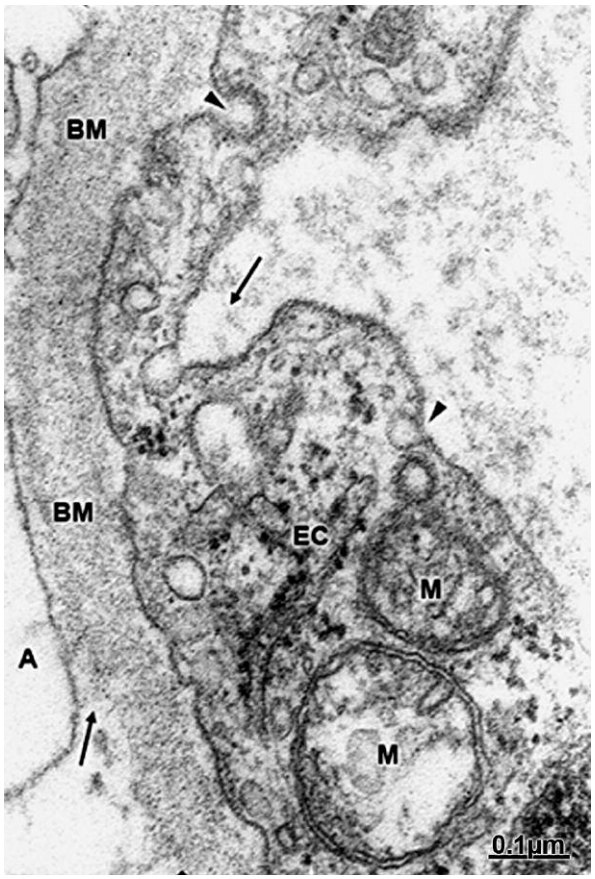

Fig. 4. Brain trauma. Rigt parieto-temporal subdural haematoma. Endothelial cell (EC) organelle and peripheral zones showing the formation of pinocytotic and clathrin-coated vesicles (arrowheads). A deep invagination of the endothelial luminal membrane (long arrow) ends in a micropinocytotic vesicle. Note the swollen mitochondria (M), the rough endoplasmic reticulum (ER), and the perivascular astrocytic end-foot (A) dissociated (arrow) from the thickened and rarefacted basement membrane (BM).

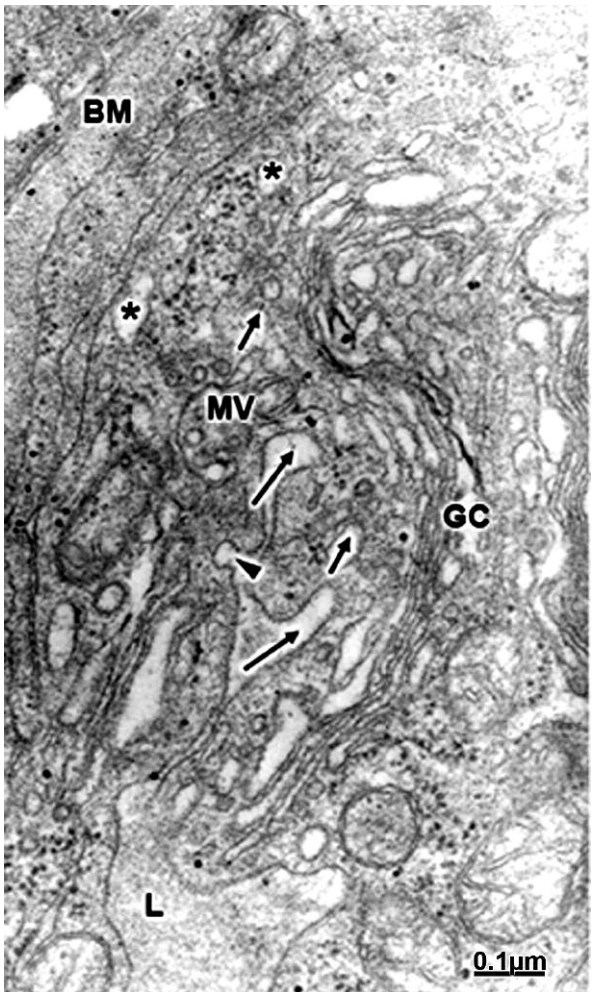

Fig. 5. Brain trauma. Contusion of frontal region. Right frontal cortex. Deep invaginations of the endothelial cell luminal membrane (long arrows) are formed at the endothelial peripheral cytoplasm. A caveolae intracellularis (arrowhead) is observed at the endothelial cell luminal membrane. Some coated and uncoated vesicles and vacuoles are internalized (small arrows) towards the hypertrophic Golgi complex (GC). Note that other endothelial vacuoles (asterisks) follow a transcapillary route toward the basement membrane (BM). Some vesicles appear surrounding a multivesicular body (MB). Note also the hydropic changes of Golgi endoplasmic sacs and vacuoles. The capillary lumen (L) also is noted.

Golgi vesicles are the structural vectors of the recycled membranes [51,52]. Presumably a considerable amount of Golgi complex-derived membrane is inserted into the endothelial luminal plasmalemma to provide new membranes for the increased vesicular and vacuolar transport. This sustained over-function might induce Golgi complex hypertrophy [8,16].

Some pinocytotic vesicles are found surrounding dense cored vesicles and small lysosomes, suggesting that some amount of plasma protein is transported by plasmalemmal vesicles towards the lysosomes to be degraded by lysosomal enzymes, as an endothelial cell mechanism of oedema resolution [8,58]. At the Golgi region, uncoated and coated pinocytotic vesicles and microtubules appear topographically related to lyso-

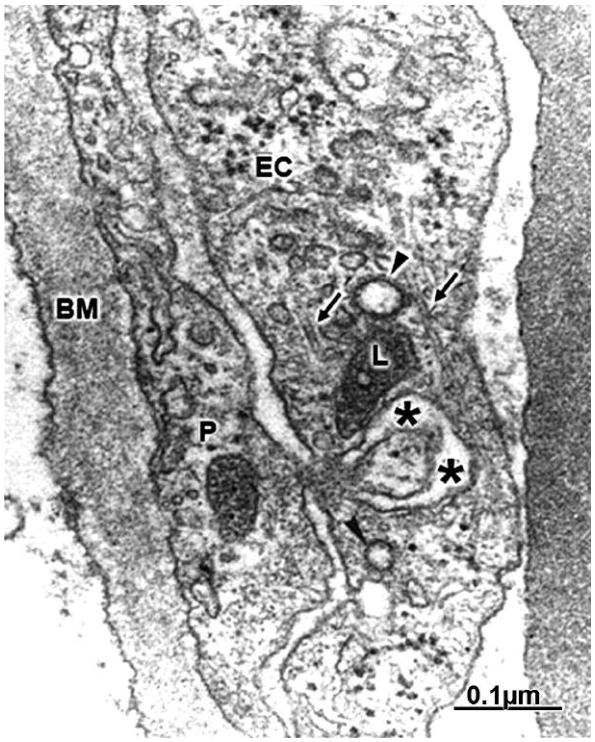

Fig. 6. Brain trauma. Epidural haematoma. Right temporal cortex. Endothelial peripheral cytoplasm (EC) showing a clathrin-coated vesicle (arrowhead) and microtubules (short arrows) in topographic relationship with a lysosome (L). A dilated curved basement membrane bifurcation (asterisks) appears in a sublemmal location. The pericyte cell (P) and the swollen basement membrane (BM) also are observed.

somes (Fig. 6). Coated pits and clathrin-coated vesicles are also seen.

These electron microscopic findings suggest that endothelial vesicular transport can be discriminated between abluminally orientated transendothelial transport toward the capillary basement membrane inducing oedema formation, and cell organelle orientated intraendothelial vesicular transport directed towards Golgi complex, lysosomes and multivesicular bodies for oedema resolution [8]. Van Deurs [58] has also shown evidence of transport of exogenous material to lysosomes by uncoated endothelial vesicles to be degraded by hydrolytic enzymes.

Numerous free and abluminal membrane-bound pinocytotic vesicles and vacuoles are also found discharging their content into the capillary basement membrane, suggesting the orientated increased pinocytotic and vacuolar transport toward the tissue front (Fig. 7). These capillaries display notably oedematous pericytes also with an increased vesicular and vacuolar transport [9,18], and swollen glycogen-rich and glycogen-depleted astrocytic perivascular end-feet [14], indicating the route followed by the hematogenous oedema fluid.

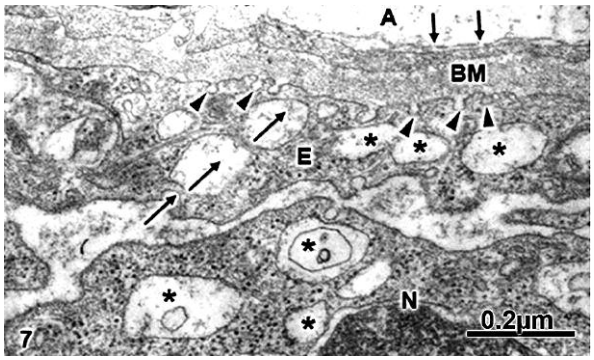

Fig. 7. Brain trauma. Fronto-parieto-occipital subdural haematoma. Left paritetal cortex. Activated endothelial cytoplasm of nuclear (N) and peripheral zones showing increased vacuolar and vesicular transports. The large arrows indicate the direction of vacuolar transcapillary passage. The dilated rough endoplasmic reticulum also is vacuolated (asterisks). There is a clear contraluminal orientation of micropinocytotic transport (arrowheads) since the micropinocitotic vesicles appear discharging their content to the swollen basement membrane (BM). Note the disrupted limiting membrane (short arrows) of the swollen perivascular astrocytic end-foot (A).

Some endothelial vacuoles are formed by a well-known mechanism of emission of microvilli, which initially projects towards the capillary lumen and afterwards refolds over the neighbouring endothelial luminal surface, or either by interdigitation of a pseudopodic expansion and a microvillus [5,6] (Fig. 8).

In relationship to the identification of endothelial vacuoles, much confusion has been caused in electron microscopy by ultrathin sections of simple indentations of endothelial cell plasma membrane of peripheral cytoplasmic zone, by phagocytosis, and by dilated endoplasmic reticulum [5]. In our studies we have clearly differentiated between these possibilities, and considered that unidirectional transendothelial passage of true protein-containing vacuoles is primarily concerned with oedema formation [6,8].

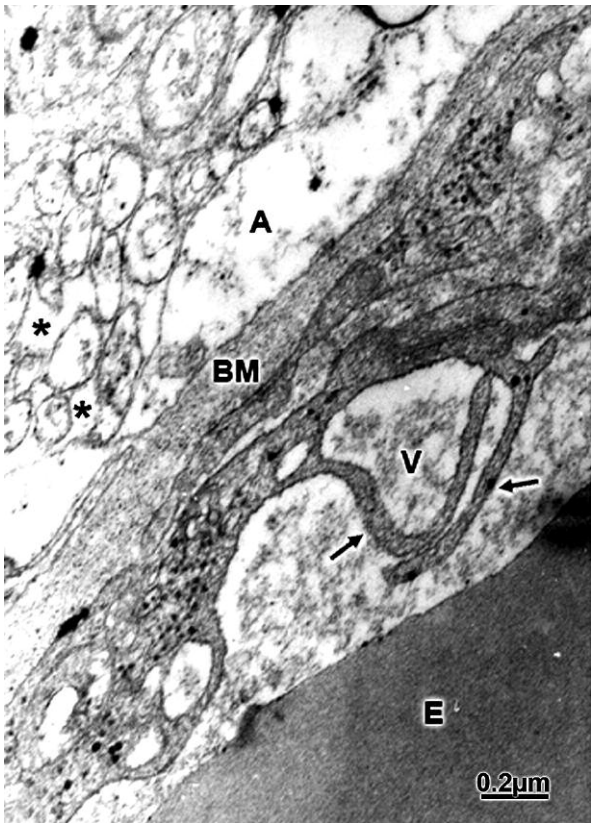

Fig. 8. Brain trauma. Parieto-temporal subdural haematoma. Left parietal cortex. Endothelial cell peripheral zone showing emission of two microvilli (arrows) entrapping haematogenous oedema fluid, and forming protein-containing vacuoles, as the most frequent mechanism of oedema formation. An erythrocyte (E), the basement membrane (BM), the swollen astrocytic end-foot (A), and the enlarged extracellular space (asterisks) also are seen.

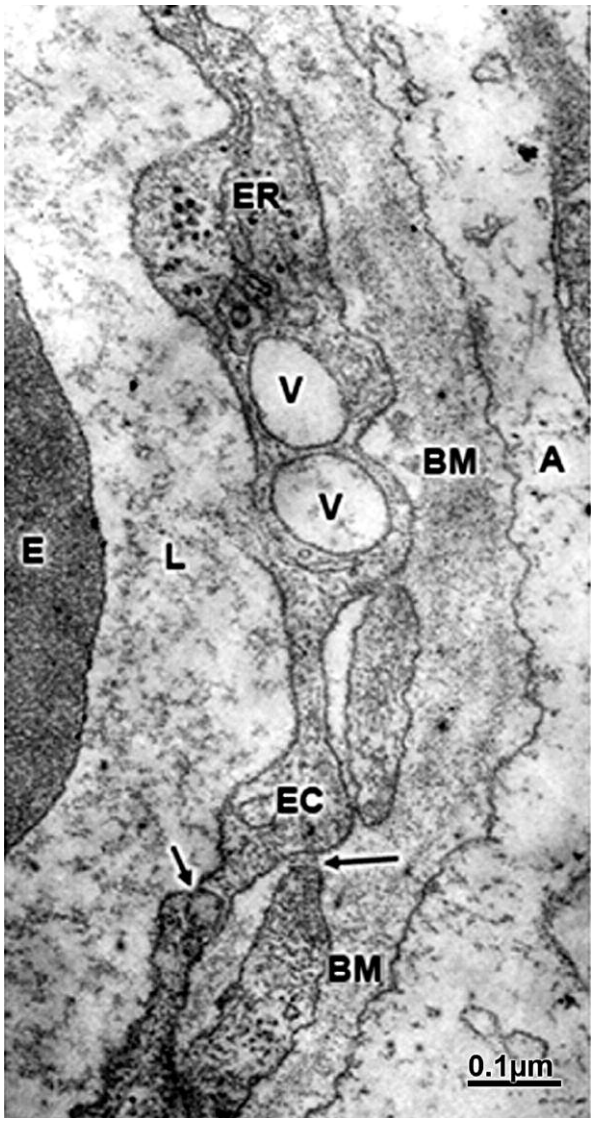

Fig. 9. Brain trauma. Subdural haematoma. The attenuated endothelial peripheral zone (EC) with a large micropinocytotic vesicle (small arrow) and vacuoles (V). An erythrocyte (E), the basement membrane (BM), the endoplasmic reticulum (ER), the swollen astrocytic end-foot (A), the vessel lumina (L).

In some capillaries, the cytoplasm of the endothelial peripheral zone becomes so attenuated that a large micropinocytotic vesicle and vacuoles can function almost as incomplete and transient transendothelial channels [6] (Fig. 9).

Earlier studies on experimental brain trauma have demonstrated that protein leakage occurs as early as three (3) minutes after brain injury [48]. In addition, plasma protein extravasation has been reported ten minutes after impact injury in spinal gray matter microvasculature [31]. In traumatic human brain injuries we have shown evidence that there is an accumulation of proteinaceous oedema fluid in the interspace between the basement membrane and the astrocytary perivascular end-feet twenty four (24) hours after a severe brain injury [8,11-13]. Extravasation of proteinaceous oedema fluid in moderately oedematous regions is mainly due to the enhanced vesicular and vacuolar transport because most tight endothelial junctions are intact and structurally closed [8,22].

Concluding remarks

The endothelial vacuolar and vesicular transports have been studied in traumatic brain injuries in order to establish their role in both oedema formation and oedema resolution. Normal or "non-activated" and "activated" capillaries are found. The activated capillaries show predominantly an enhanced abluminally orientated vesicular transport by means of small, medium and large uncoated and clathrin-coated vesicles, as well as the presence of endothelial tubular structures. Vesicles internalizing to the hypertrophic Golgi complex, lysosomes and multivesicular bodies are observed for oedema resolution. The vacuolar transport is predominant in most cortical capillaries. The basic endothelial cell mechanisms found in complicated human brain injuries are deep invaginations of luminal surface, formation of large coated vesicles, tubular structures, and transient and incomplete transendothelial channels formed either by chained plasmalemmal vesicles or elongated protein-containing vacuoles. Uncoated vesicles are seen surrounding lysosomes. The endothelial vesicular transport can be discriminated between abluminally orientated or transendothelial transport (oedema formation), and intraendothelial transport (oedema resolution) directed towards multivesicular bodies and lysosomes. The transendothelial passage via large vacuoles is mainly due to protein transport. Most endothelial junctions examined in moderate oedematous regions are structurally intact.

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