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Invited Review

Blood-Brain Barrier (BBB). Review from Morphological Aspect

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1. Introduction

Compared with other organs of the body, the central nervous system (CNS) shows a unique behaviour in the exchange of metabolites: many metabolites are not freely transferred from, and to, the blood. This phenomenon has led to the concept of the «blood-brain barrier» (BBB). This concept is useful not only when dealing with questions about metabolic exchange in general, but also more specifically for understanding the mechanism of the production of and the changes in the cerebrospinal fluid (CSF), the permeability of the CNS for toxins, viruses and antibodies and, finally, the capability of various drugs to reach the CNS. This review will be focused on the different aspects of morphological characteristics oof the brain vessels related to the BBB function. Further barrier systems in the nervous system, such as the blood CSF (Milhorat et al., 1973), the CSF-blood barrier (Ramsay, 1965) and the blood-nerve barrier (Olsson et al., 1984) will not be discussed here.

Historically, presence of a barrier between the brain and blood vessels has been shown by Ehrlich (1985) and Goldmann (1913): the CNS is not stained by intravenously injected aniline dyes or trypan blue except for the choroid plexus, area postrema, tuber cinereum, median eminence, pituitary gland and pineal body. These regions in the CNS are regarded now to have no BBB function. In addition, the retina possesses a barrier (the blood-retina) which is structurally identical to that of the CNS (Casley-Smith, 1959; Shakib et al., 1966). The retina is embryologically an extension of the brain, including its barrier system.

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2. Structural profile of the BBB

a. Attempts to define a morphological basis for the BBB

In spite of the fact that the concept of the BBB itself has started as a physiological phenomenon, morphologists were repeatedly called upon to identify the localization of this barrier system. Morphologists were repeatedly tempted to find the structural principles underlying this barrier system. The study of the anatomical basis of the BBB should include all the vascular segments where the exchange of metabolites between CNS and blood takes place. This exchange occurs mainly in the capillaries. However, the importance of arterioles for the metabolic exchange could also be demonstrated through a number of findings in normal and pathological brains as well as in animal experiments (Cervós-Navarro et al., 1978). Under normal conditions arterioles are surrounded by a capillary-free space. This is demonstrated in picture from brain tissue obtained after injecting the vessels with India ink. The cylinder of brain tissue that is supplied through the surrounding capillaries does not cover the periarteriolar space. Therefore, the brain tissue around the arteriole must be supplied by this vessel. Since the periarteriolar space contains the same density of nerve fibers and glial cells as any other area in the cerebral cortex, it is logical to assume that in these areas metabolic exchange occurs through the arteriolar wall.

The basement membrane was once thought to have a main structural correlation with the BBB. The morphological grounds were that silver nitrate granules coming from the vessel remained attached to the basement membrane after their intravenous application (Dempsey et al., 1955). However, this behaviour is not specific for the basement membrane of the CNS, but for all basement membranes which function as a filter for large molecules.

Some authors thought that the function of the BBB was localized in the perivascular endfeet of astrocytes which cover all the capillary surface and the outer surface

of the brain. These astrocytic processes are connected with other astrocyte processes by gap junctions and/or punctate adhesions without zonula occludens. The gap junctions existing between the astrocyte processes do not even stop the larger molecules. If the large molecule tracers have once passed through the endothelium and reached the perivascular space, they permeate the entire extracellular space of the neuropil.

Sometimes less attention is paid to the intima of cerebral vessels other than capillaries. The endothelium is, however, the only continuous layer which is common to the different segments of the microvasculature engaged in the exchange of substances of the microvasculature engaged in the exchange of substances between blood and CNS. Consequently, structural characteristics of the endothelium of cerebral vessels are expected to be the expression of the BBB. Isolated brain microvessel endothelial cells provide a useful model for studying properties of the BBB (Goldstein et al., 1975; Bowman et al., 1983; Dorovini-Zis et al., 1987). The cultured cells resemble their *in vivo* counterparts in that they are bound together by tight junctions, that they restrict the movement of horse radish peroxidase (HRP) across the cell layer, and that they are characterized by a paucity of cytoplasmic vesicles.

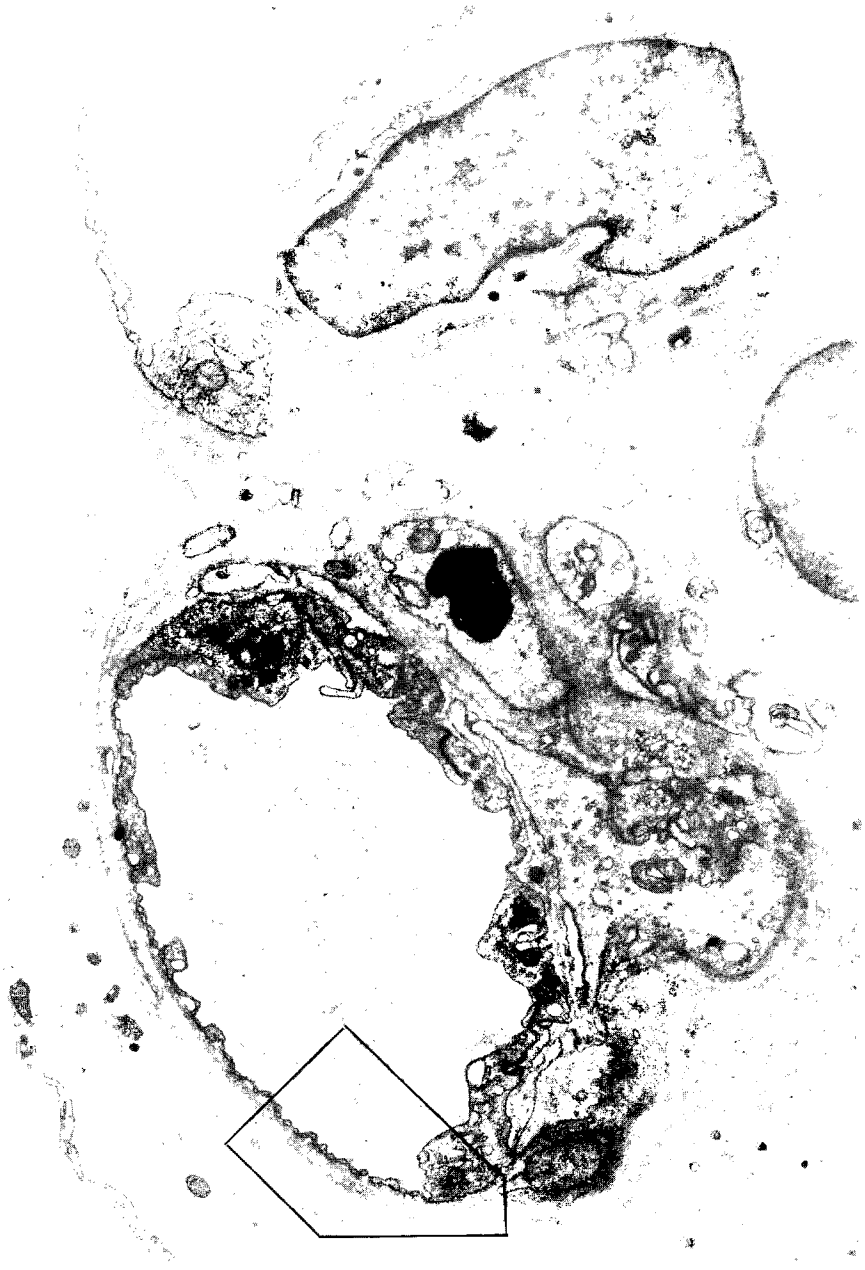

Fig. 1. a: Small venule in pituitary gland. Numerous fenestrations are observed in the endothelium, × 4,000. **b:** Inset. Higher magnification. Diaphragm in these fenestration separate the lumen from the perivascular space. × 25,000

b. Endothelium of the vessels in the CNS

The vascular endothelium of the brain shows various structures different from those of other organs (Manjo et al., 1965). In some organs such as kidney, intestine, endocrine glands, salivary and lacrimal glands, ciliary body and choiroidea of the eye, the endothelial cells become greatly attenuated and small pores or fenestrations about 50 nm in diameter are present (Fig. 1). In sections perpendicular to the cell surface, the fenestrations are seen to be closed by a 5 nm-thick diaphragm which separates the lumen from the perivascular space. These organs with fenestrated vessels show a high water- and metabolic exchange. Except for certain limited areas of the brain such as the area postrema, the choroid plexus, the pineal body, pituitary gland, the intercolumnar tubercle and certain nuclei within the hypothalamus, these fenestrations are not seen within the normal mammalian brain (Lee et al., 1971) (Fig. 2). These areas, supplied with fenestrated capillaries, corresponded to areas, where the BBB was absent.

In many other organs such as the striated muscle, the vessels are not fenestrated, but numerous vesicles can be seen within the endothelial cytoplasm (Fig. 3). About 1/3

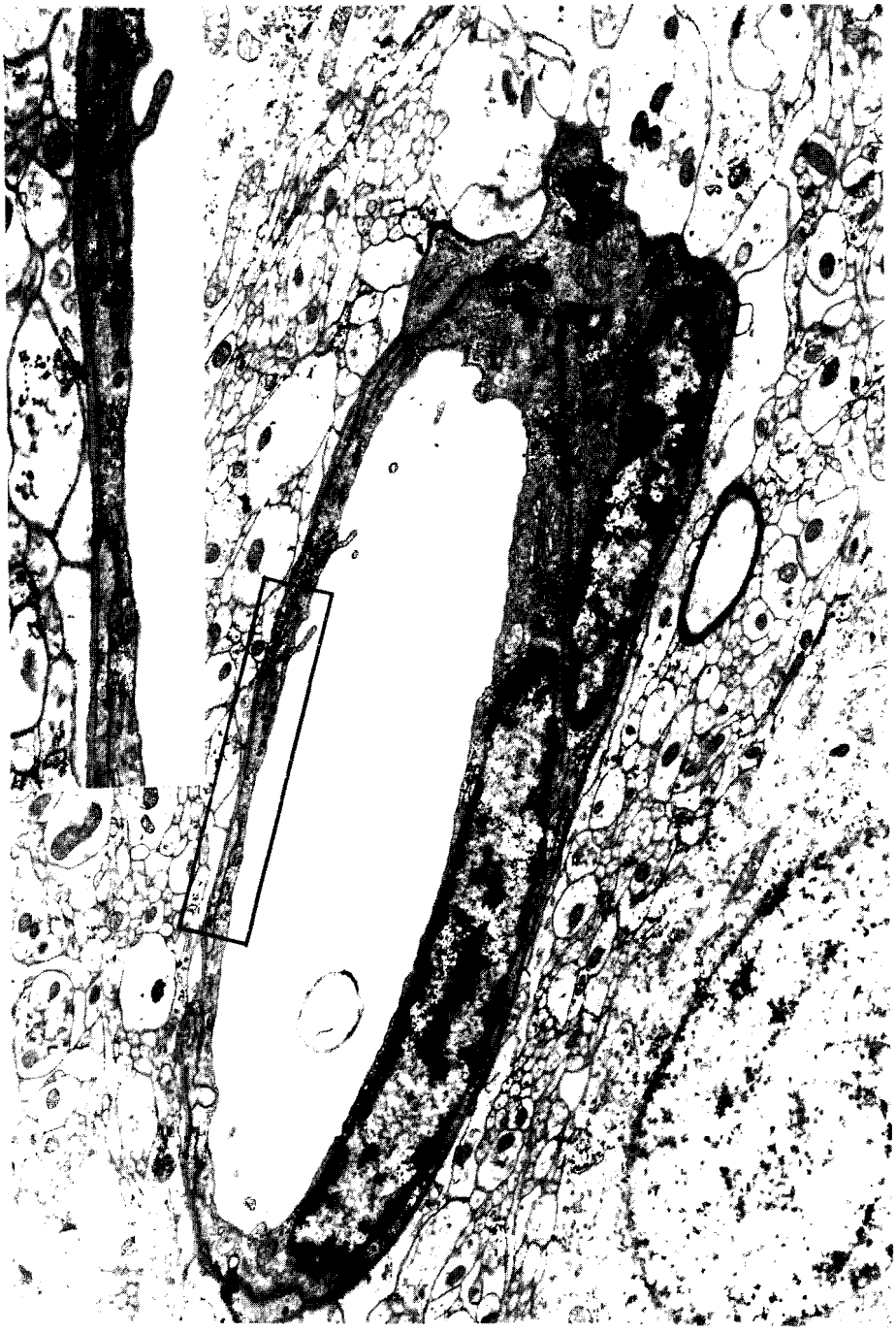

Fig. 2. Normal capillary in the cortex. No fenestration nor pinocytosis are found in the endothelium. × 6,150

of the endothelium in the vessels of striated muscle is occupied by these pinocytotic vesicles. Their diameter is about 70 nm, and they possess a transport function through the endothelium. Contrary to striated muscle, pinocytotic vesicles in the brain vessels are very few (Cervós-Navarro, 1963), which represents one of the morphological features of the BBB. In addition to the two basic processes (diffusion and facilitated process) that can drive plasma molecules across the capillary wall, vesicular transport represents a third, energy-requiring transport mechanism. In most tissues, it was found that mitochondria occupied about 4% of the capillary endothelial cell cytoplasm. In contrast, it occupied 10-11% of the endothelium in the rat (Oldendorf et al., 1981). Furthermore the number of mitochondria is greater in white matter capillaries than in gray matter. It can be assumed that the greater mitochondrial content of the cerebral capillaries is the consequence of a greater requirement for the regulation of a more selective permeability.

Endothelial cells of cerebral vessels also differ from those of other organs with respect to their attachment to each other (tight junctions of zonula occludens). A part of the opposing outer layers of adjacent cytoplasmic membranes fuse together over short segments and form a continuous belt of a pentalaminar structure around the vessel, although these junctions look like isolated structures in TEM (Brightman et al., 1969). In other organs, adjacent endothelial cells may come quite close to each other, but usually the outer layers are not fused. Even if areas of fusion may be seen occasionally, they do not constitute a continuous belt (Karnovski et al., 1967). These structures, the tight junctions, are considered to be another anatomical substrate of the BBB.

The cerebral endothelial cells also seem to differ from those in various other organs by the apparent absence of the tubular bodies or Weibel-Palade bodies. In many other endothelia, especially in large vessels including the capillaries of certain tissues, membrane-bound, elongated rod-like bodies filled with closely packed

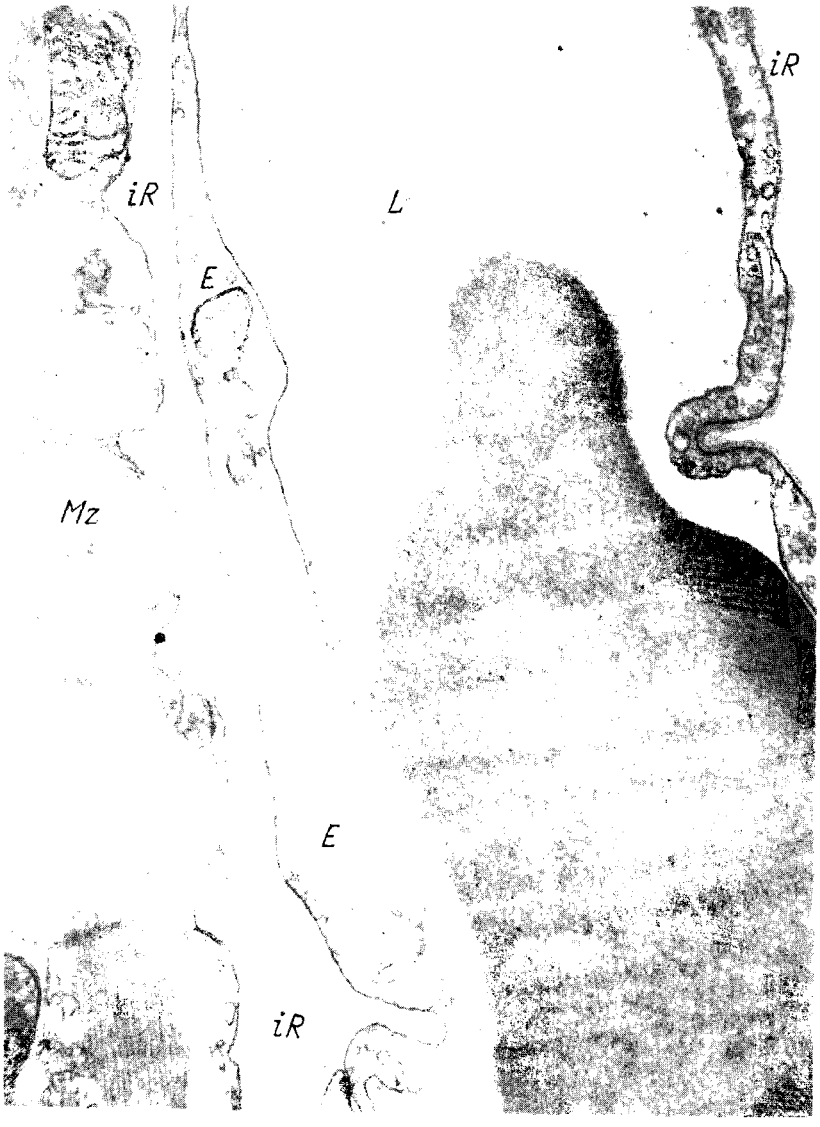

Fig. 3. Capillary of striated muscle. The endothelium has a lot of pinocytosis. L: Lumen, Er: Erythrocyte, E: Endothelial cell, iR: Interstitial space, Mz: Muscle cell. × 6.000

microtubular structures are present (Weibel et al., 1964). Although they are reported to be found sporadically in cerebral arterial endothelial, they are extremely rare in the capillaries of the normal brain (Stehbens, 1972). Increase of these bodies has been reported in certain kinds of brain tumors and in the aged brain. Von Willebrand protein (factor VIII), which is thought to be a specific glycoprotein in the cerebral endothelium, has been detected in the Weibel-Palade body (Wagner et al., 1982). It is not clear whether they have any effect on the BBB. However, such an influence cannot be ruled out.

Recently, the importance of the ionic environment around the vascular endothelium has been emphasized by several experimental models.

Net negative charge of the luminal surface of the cerebral endothelium influences the BBB and transport functions (Danon et al., 1980; Simionescu et al., 1982; Nag 1984; Vehaskari et al., 1984; Hart et al., 1987). Glycocalyx, especially its sialyl residues account for this net negative charge (Nag, 1986). The charge distribution is varied, some sequences are more heavily charged than others. As an indicator of this negative membrane charge, cationic ferritin has been extensively used because of its large, electron-dense polycation molecule and existence of a control, the native ferritin. Extracellular Ca++ also plays an important role in maintaining structural integrity of cerebral tight junctions (Nagy et al., 1983, 1985). In isolated cerebral microvessels or cultured cerebral endothelium, Ca++ dependent disassembly and reassembly of tight junctions was reported (Nagy et al., 1985).

c. Enzymatic barrier

It has been well documented that the BBB passage of the amine neurotransmitters, in contrast to their precursors, is insignificant under physiological conditions (Oldendorf, 1971). Further histofluorescence investigations have proven that cerebral but not peripheral capillary endothelia possess an enzymatic barrier for l-DOPA and dopamine by demonstrating accumulation of l-DOPA in capillaries after DOPA-decarboxylase and monoamine oxidase inhibition (Björklund et al., 1969, Hardebo et al., 1976). Cerebral microvessels themselves contain considerable activity of MAO, COMT and AAD that are involved in the monoamine degradation (Mrsulja et al., 1980; Hardebo et al., 1980). Therefore, all the accumulated histochemical and biochemical data indicate that the main capillary barrier for monoamines resides in the endothelium. A good possibility also exists that the cerebral capillary enzymes could participate in the inactivation of other neurotransmitters or neurotransmitter-like substances, especially since gamma-aminobutyric acid (GABA) does not pass the BBB (Van Gelder 1968). This idea is supported by the presence of capillary GABA-transaminase activity, as indicated by the histochemical and biochemical approaches (Barret et al., 1971; Mrsuja et al., 1980).

Some proteolytic enzymes are shown to be able to reversibly increase the permeability of the BBB to different tracers such as trypan blue. Collagenase was found to be the most potent. The injection of the enzyme was followed by the increase in the permeability due to the enzymatic degradation of collagen. In addition, Robert et al. (1977) demonstrated that the recovery of normal BBB permeability is a process dependent upon the protein synthesis. This finding supports the hypothesis according to which the basement membrane plays a role in the BBB function. Therefore, under normal conditions, the capillary basement membrane, especially its collagen, may be part of the BBB regulatory system against molecules that meet the BBB coming from the capillary blood.

Application of ultrastructural techniques has already resulted in better insight into the role of some capillary enzymes relating to the function of BBB. Buthyrylcholinesterase (BThE) activity, described originally by Koelle (1954) has been found within the endothelium of BBB regions (Joo et al. 1966), while BThE within the capillaries of the area postrema has also been reported (Karesu et al. 1977). The enzymatic end product was normally localized in the rough endoplasmic reticulum and perinuclear cisternae. In contrast to this, basement membrane of the BBB capillaries showed high enzymatic activity, while fenestrated capillaries were free of enzymatic activity. The presence of BThE in the rough endoplasmic reticulum indicates the site of its synthesis in the endothelial cell. Although BThE is suggested to be involved in the mechanism maintaining the permeability of cerebral vessels, its role in the function of the BBB remains unclear. More likely, the enzyme plays a protective role preventing the entrance of toxic choline esters into the brain (Kreutzberg et al., 1979). There is no doubt that further studies are necessary to elucidate the function of BThE in cerebral capillaries.

In contrast to nonspecific cholinesterase, the activity of which is observed in capillaries of all brain regions, acethylcholinesterase (AChE) capillary activity in rather restricted to brain regions with high activity of this enzyme in neuronal elements. Also AChE positive capillaries have been observed preferentially in cholinergic and cholinoceptive brain structures. Therefore, AChE activity in brain capillaries is more likely to be related to neurotransmission than to the BBB. The presence of AChE within the basement membrane and pinocytotic vesicles of the brain vessels speaks in favour of its non-endothelial origin.

Another important enzyme which takes an active part in the regulation of transport at the level of capillaries is Na+-K+ ATPase. Light microscopic examination showed that accumulation of the reaction product was always found within the wall of capillaries (Joo 1979; Mrsulja et al., 1980). Electron microscopic investigations revealed the presence of electron dense reaction products almost exclusively in the basement membrane of the capillary wall (Joo 1979). The electron-dense reaction product was confined to the basement membrane and, to a lesser degree, to the luminal

membrane (Joo, 1979; Mrsulja, 1984). Besides the vacuolar endothelium, astrocytic end feet appeared to be the prominent site of localization K+- dependent p-nitrophelphosphatase; the active part of Na+K-ATPase (Mrsulja, 1984). Since the essential contribution of Na+-K+-ATPase in active transport of ions is a well-known fact in animal tissue, the capillary localization of the enzyme is of utmost interested.

d. PET scan and NMR scan

Recent technological advances have made it possible to investigate BBB permeability in living human cases. Using Rb82 (Yen et al., 1981, 1982; Lammerstsma et al., 1984), Rb86 or Ga+ EDTA (Phelp et al., 1979; Hawkins et al., 1984) as a PET tracer, kinetic and/or quantitative analysis of BBB functions in human cases as well as in animal experiments are studied in both normal and pathological conditions such as brain tumors, hydrocephalus, cerebral infarction etc. Rb is an alkali metal cation with a smaller hydration sphere than potassium, but with similar chemical properties as previously measured (Kilpatrick et al., 1956). Glucose or ammonium transport across the BBB could also be measured when PET tracer was used against them. Potassium transport is regulated by ATPase localized in the endothelium, and thought to be increased when BBB is disturbed. GA + EDTA is labelled molecule that does not cross cell membranes to any significant degree, and is largely excluded from diffusing into the brain from the blood through an intact BBB. With alterations in BBB function, forward and backward diffusion of GA + EDTA occurred across the BBB. In addition, measurement of metabolic exchange across the BBB, such as glucose transport of ammonium transport, also became possible (Brooks et al., 1986). NMR enabled us to measure water distribution in living human individuals. Intrinsic NMR parameters, T1 and T2, are correlated with the tissue water content (Naruse et al., 1982; Kato et al., 1986). The main advantage of both new methods is that BBB function, transport of metabolic substances and brain water content can be measured repeatedly in living humans.

3. Pathology of the BBB

Experimental and clinical observations on the abnormal BBB function completed the concept of this barrier system, which derived from the studies on the normal cerebral barrier system. In various diseases, the breakdown of the BBB results in an accumulation of water in the brain; i.e. in brain edema formation. The pathophysiology of brain edema has been extensively investigated both experimentally and clinically because of its importance in many intracranial diseases. The experimental alterations of the BBB permeability are usually produced by drastic procedures, and are not strictly comparable with those of clinical cases. Nevertheless, the results of experimentation often elucidate the basic principles underlying the pathogenesis of related diseases. The following discussion will be focussed on the cerebral microvasculature changes in different experimental models of brain edema. The study of the exact structural changes during BBB breakdown has led to somewhat discrepant views: i.e. transendothelial transport by vesicles across tubular channels or by opening of tight junctions have been postulated.

a. Increased transendothelial transport

A considerable amount of experimental studies indicate that the enhancement of transendothelial vesicular transport occurs in brain edema (Fig. 4). This transport has been reported in both necrotic and non-necrotic models: the non-necrotic model includes radiation (Cervós-Navarro, 1967), non-necrotic trauma (Beggs et al., 1976), acute hypertension (Westergaard et al., 1977; Nag et al., 1977, 1979), seizures (Petito et al., 1977; Nitsch et al., 1986), portocaval anastomosis (Laursen et al., 1977), thiamine deficiency (Manz et al., 1972), mercury intoxication (Ware et al., 1974), and air embolism (Persson et al., 1978). In necrotic brain edema following cold injury and UV irradiation capillary endothelial cells also show an increased number of pinocytosis (Sasaki et al., 1977).

Our results, and those of other authors (Cervós-Navarro et al., 1976; Ferstz et al., 1978), indicate that it is practical to differentiate between macro- and micropinocytosis. Macropinocytosis is thought to be a sign of some kind of metabolic disturbance in the endothelial cell. The significance of the tracer experiments for proving transendothelial transport has been questioned, especially since tracer has been administered even post mortem.

One must also be cautious about equating increase in vesicle density with increased transcellular transport, because in many cells endocytotic vesicles are described that merge with lysosomes and undergo internal digestion rather than being engaged with exocytosis. In fact, the small amount of horseradish peroxidase found in endothelial cells after vacuolar perfusion of normal brain capillaries does not appear to be released onto the abluminal surface of the capillaries. Although numerous vesicles and tubules are observed in the entire endothelium, they are not always involved in the transendothelial transport of micromolecules.

Nevertheless, it seems to be a fairly useful hypothesis to presume that in principle the transendothelial transport of substances of higher molecular weight is possible. The tubular channel systems suggested by Simionescu et al., (1975) may form as a result of fusion of individual cytoplasmic vesicles. This channel has been observed in acute hypertension.

The unilateral localization of micropinocytotic vesicles indicates the direction of vesicular transport (Wagner et al., 1974). In our studies on UV irradiation edema, micropinocytotic vesicles are located preferably at the luminal side in the early stages, later predominating at the abluminal side. The reserve

vesicular transport of HRP from abluminal to luminal surface across the endothelium was also seen after cold lesion edema (Vorbolt et al., 1986). This represents one of the resolution routes for extravasated protein.

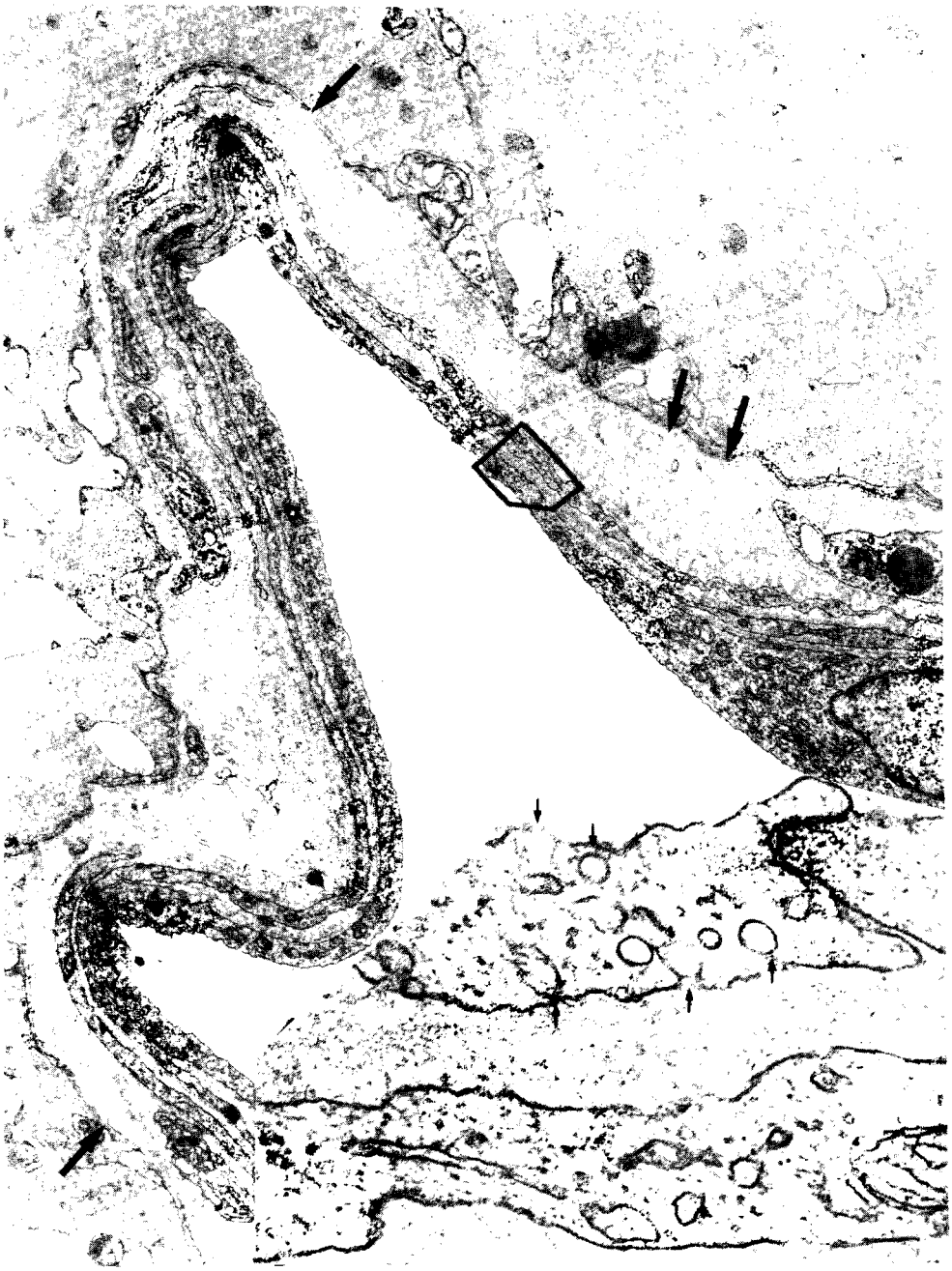

Fig. 4. a: Venule in the white mater of post-irradiation brain edema in the monkey. Cellular structure surrounding venules are distended by marked protein-rich edema. × 6,000. **b:** Inset. Higher magnification of endothelium. Marked increase of pinocytosis is observed. × 60,000

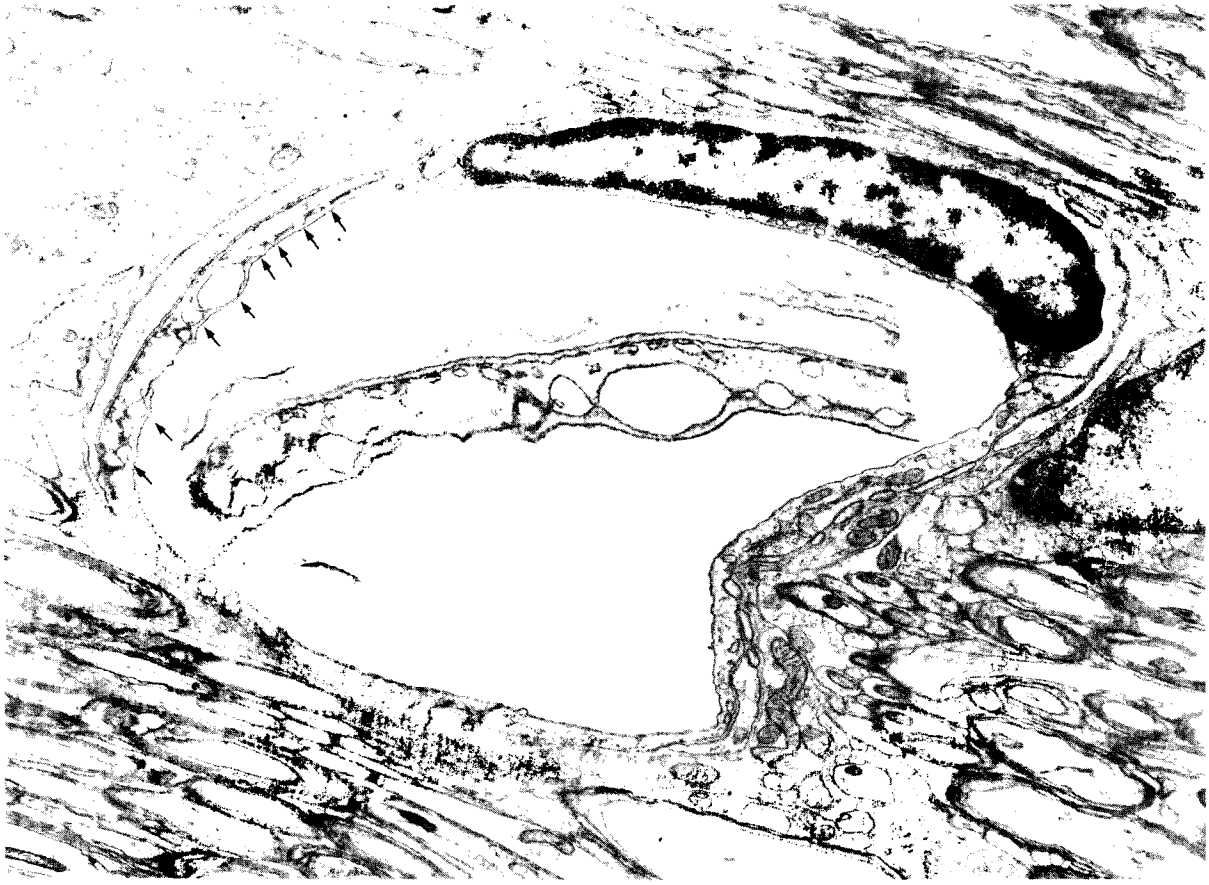

Fig. 5. a: Small venule of kaolin-induced hydrocephalus rat. Multiple vacuoles (interendothelial dehiscences and blisters; arrow) were seen, × 5,000. **b:** Inset. Higher magnification. Note that the multiple interendothelial blisters and dehiscences communicated with one other. × 12,500

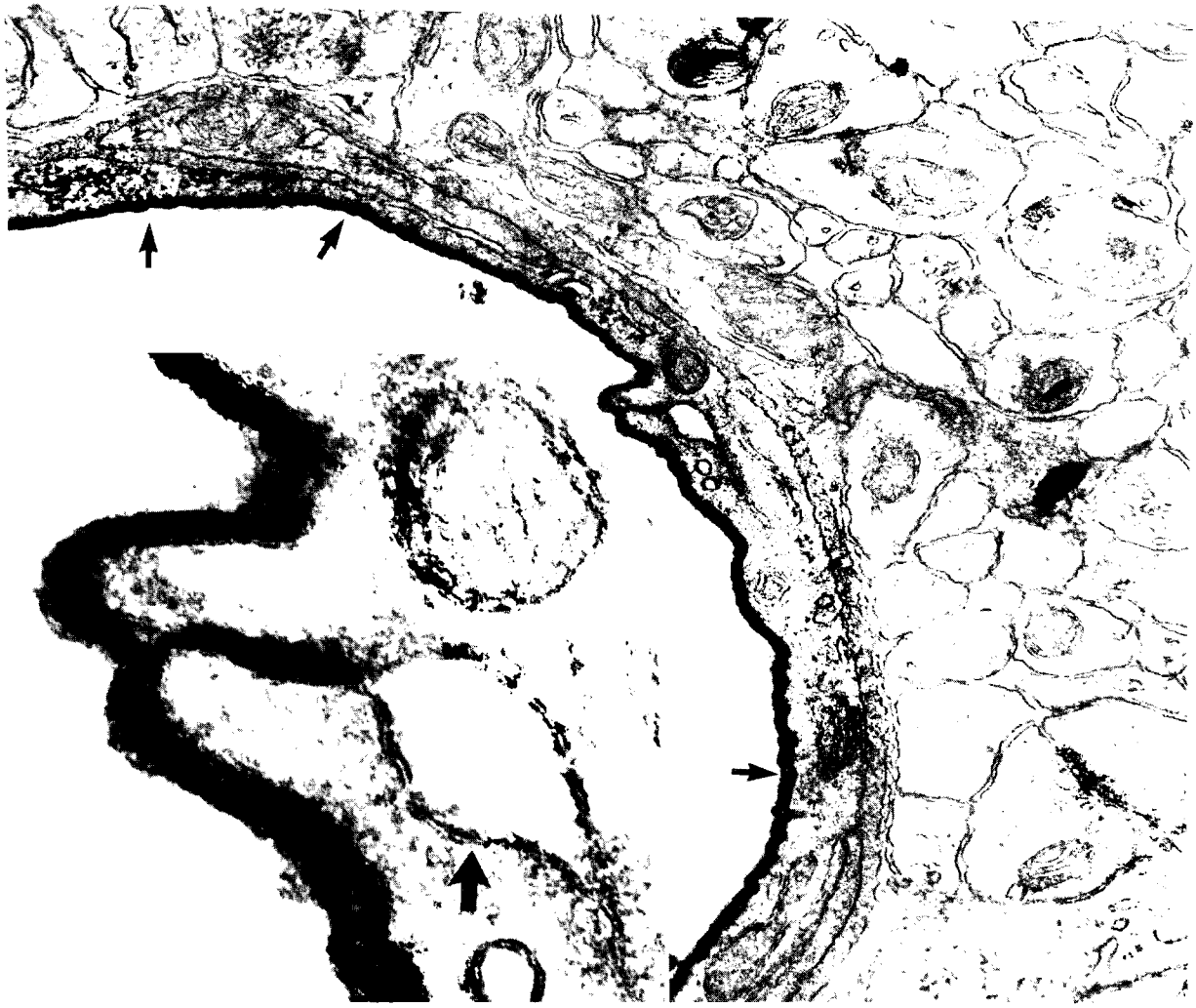

Fig. 6. a: Wall of capillary in experimental hydrocephalus rat. Dark HRP reactive product (arrows) were seen in the lumen. × 11,500. **b:** Inset. Higher magnification of the interendothelial cleft (arrows). Note that blisters were completely devoid of the marker substance. × 18,420

b. Paracellular shunt pathway

Under various experimental conditions tracers could be found lining the spaces between the endothelial cells during edema. The opening of the tight junctions as a possible route for hematogenous protein was proposed by several authors. The opening of the tight junction against protein tracers was reported in case of an acute arterial hypertension (Rapoport et al., 1976), hyperosmolar BBB injury (Hicks et al., 1976) and acute radiation injury (Olsson et al., 1975). In recent experiments dehiscence of tight junctions was induced in brain vessels by means of both perfusion with polycations, Ca2+ depletion or low pH solution (Nagy et al., 1981, 1985). These findings suggest that either the electric charge of the endothelial cell membrane or the active contractile mechanisms in the endothelial cells or both are relevant factors for the integrity of tight junctions.

Rapoport et al. (1972) suggested that the mechanism leading to the endothelial monolayer opening of tight junctions by hyperosmolar solutions is the shrinkage of the endothelial cells. Using cultured endothelium system, altered shrinkage of endothelium can be observed paralelly with opened tight junctions (Dorovini-Zis et al., 1987). However, the presence of reaction products between two neighboring tight junction does not necessarily imply their opening, since tracer-filled pinocytotic vesicles may eject their content into the interendothelial cleft. Therefore, sometimes it is difficult to be absolutely sure that part of the intercellular space has not been filled from the basal surface and that the tight junction had not remained intact. Nevertheless, a great number of the published illustrations are completely convincing, thus the opening of the tight junctions in cerebral vessels under certain conditions must be accepted as a proved fact.

Our observations on hypoosmolar brain edema (Artigas et al., 1983), early stage of incomplete cerebral ischemia (Nakagawa at al., 1985) and kaolin-induced experimental hydrocephalus (Nakagawa at al., 1985) suggest that the tight junction between two endothelial cells may constitute a part of a paracellular shunt pathway for the small solutes (Figs. 5 and 6), although the movement of large molecules is prevented. It has been reported that the net water content in incomplete cerebral ischemia increases during the first two hours in spite of a normal BBB against protein tracers of HRP (Kamijo et al., 1977; Sadoshima et al., 1983). In our observation of the rat MCA occlusion model La+++ was found in the interendothelial cleft of venules and arterioles 30 and 60 minutes after occlusion, although HRP molecules were blocked (Nakagawa et al., 1985). This different attitude of tight junction against small- and large molecule tracers is also observed in the experimental hydrocephalus (Nakagawa et al., 1985).

This interpretation is supported by findings of different authors working with freeze-fracture preparations indicating that there are systems of various widths. These findings should be taken into consideration in the attempt to clarify the mechanism of formation of cytotoxic edema without leakage of serum protein.

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