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Special Report

The Blood-Brain Barrier: An Important Concept in Neuroimaging

Michael R. Sage and Alan J. Wilson

Many of the modern techniques for brain imaging such as enhanced computed tomography (CT), enhanced magnetic resonance (MR) imaging, single-photon emission CT, and positron emission tomography rely on the presence or absence of a normal blood-brain barrier (BBB). Other techniques such as cerebral angiography, may have a direct effect on the BBB. We shall review the anatomy and physiology of the BBB to provide essential background information for neuroradiologists.

Anatomy of the BBB

The concept of a barrier between the blood and the extracellular fluid of the brain was established at about the turn of the century by German pharmacologists. In 1909, Goldmann (1) injected trypan blue intravenously and observed that it distributed rapidly into the extracellular fluid throughout the body, with the exception of the brain and spinal cord, which remained unstained; the choroid plexuses were stained. In 1913, Goldmann (2) injected trypan blue into the subarachnoid space and cerebral ventricles of dogs and rabbits and observed that the brain and spinal cord were heavily stained: the dye was unable to pass out of the brain into the bloodstream and thence into other tissues. This experiment demonstrated that the lack of staining in his first experiment was not attributable to a lack of affinity of the dye for brain tissue. These two experiments confirmed the existence of a BBB and located it at the level of the cerebral blood vessels, although their true significance was not widely appreciated at the time. One of the factors limiting greater understanding of the BBB was the limited resolution of the light microscope, which approximates the thickness of a cerebral endothelial cell.

With the development of the electron microscope it became possible to define the ultrastructural features of blood vessels throughout the body. Capillaries, the primary sites for exchange between the blood and surrounding tissues, were observed to fall into three categories (3). In *continuous* capillaries, the wall is composed of a single layer of from one to several endothelial cells, connected at the interendothelial boundaries by tight junctions. There are no discontinuities in the wall and the capillary is surrounded by a continuous basal lamina, which may split to enclose a pericyte. Continuous capillaries are located in smooth, cardiac, and skeletal muscle, in skin, in the lung, and in the brain. Figure 1 is an electron micrograph of a typical continuous capillary from the brain. *Fenestrated* capillaries have the same basic structure as continuous capillaries, the major difference being the presence of a number of circular fenestrations, or "pores," 30 to 100 nm in diameter, in the endothelial cells. These fenestrations are closed by diaphragms that are thinner than a plasma membrane and are of unknown composition. Fenestrated capillaries are located in areas where substantial exchange takes place between blood and tissues, such as endocrine glands, renal glomeruli, intestinal villi and the choroid plexuses. Fenestrated capillaries are also located in a number of small, specialized areas of the brain, known as the circumventricular organs (CVOs). These areas include the median eminence, area postrema, the subfornical organ, the pineal gland, and the organum vasculosum of the lamina terminalis. These areas are not typical of the rest of the brain, are highly vascularized, and lack a BBB. They are thought to be involved in neurohumoral regulation of the circulation. *Sinusoidal* capillaries are larger and more irregular than the continuous and fenestrated capillaries. The endothelial cells display large intercellular gaps and they are surrounded by a discontinuous basal lamina. They are found in the liver, spleen, and bone marrow, areas where processing of blood components takes place.

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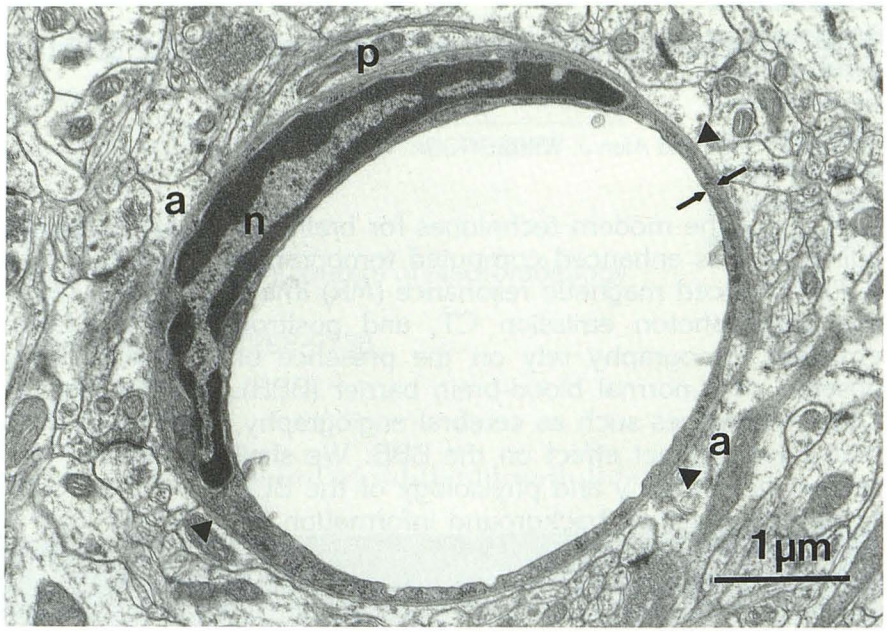

Fig. 1. An electron micrograph of a capillary in the rat cerebral cortex. A single endothelial cell surrounds the capillary lumen. The nucleus (*n*) occupies a thickened region of the cell, but elsewhere the cell is extremely attenuated (*arrows*). Surrounding the cell is a continuous basal lamina (*arrowheads*) which splits to enclose a portion of a pericyte (*p*). Abutting on the basal lamina are astrocytic processes (*a*), which form a complete sheath. No interendothelial tight junctions are visible in this vessel.

Development of the enzyme horseradish peroxidase (HRP) as a vascular tracer permitted new insights into vascular permeability. HRP is a glycoprotein with a molecular weight of 40 000, which can be demonstrated at both the light and the electron microscope level by cytochemical means. HRP injected intravenously into mice passed freely out of the capillaries in cardiac and skeletal muscle (4). The major route was between endothelial cells via discontinuities in interendothelial tight junctions, although there also appeared to be some transport via pinocytotic vesicles, which are numerous in the cytoplasm of noncerebral endothelia. It was concluded that the discontinuities in the tight junctions represented the morphologic equivalent of the small pores (4 to 5-nm radius) postulated on physiologic grounds to account for the passage of small, hydrophilic molecules, such as ions, glucose, and amino acids, across permeable endothelia (4). It was thought that passage across endothelia via pinocytotic vesicles might correspond to the much less frequent large pores (25-nm radius) postulated to account for the permeability of larger molecules up to about the size of albumin.

In 1967, Reese and Karnovsky (5) injected mice intravenously with HRP and studied its distribution in cerebral cortical blood vessels by electron microscopy in an experiment analogous to Goldmann's first experiment. They found that HRP was able to enter the interendothelial spaces only up to, but not beyond, the first luminal interendothelial tight junction in cerebral capillaries. Unlike muscle capillaries, these tight junctions appeared to be continuous, and pinocytotic vesicles were uncommon and not involved in HRP transport. Similar results were obtained with the intravenous injection of smaller protein tracers such as microperoxidase, with a molecular weight of 1900 and a diameter of 2 nm (6), and the even smaller ion, lanthanum (7), the smallest available electron-dense tracer, with an ionic radius of 0.115 nm. In another ultrastructural study, analogous to Goldmann's second experiment, Brightman and Reese (7) injected HRP and lanthanum into the cerebral ventricles of mice. Both tracers passed freely through the ependyma, into the interstitial space and from there passed between the gap junctions joining the almost complete sheath of astrocytic processes surrounding cerebral vessels, through the basal lamina and up to, but not beyond, the first abluminal tight junction. The astrocyte "foot processes" are a unique feature of cerebral vessels and were formerly thought to play a major structural role in the BBB. These experiments showed that this is not the case and established firmly that the interendothelial tight junctions represent a structural barrier to passive diffusion from blood to brain, and vice versa. Although astrocytes play no structural role in maintaining the BBB, evidence is accumulating that they play another, equally important role in the BBB (see below).

Freeze-fracture studies have confirmed the very tight nature of the tight junctions between endothelial cells of cerebral capillaries, as indi-

cated by the earlier tracer studies. In cerebral capillaries, tight junctions consist of 8 to 12 parallel junctional strands running in the longitudinal axis of the vessel, with numerous lateral anastomotic strands; no discontinuities are present in the strands (8, 9). This pattern extends into the postcapillary venules, although in a less complex fashion (9). In cerebral arteries, tight junctions consist of simple networks of junctional strands, with occasional discontinuities, whereas collecting veins, of which there are few, have tight junctional strands which are free-ending and widely discontinuous (9). A recent immunocytochemical study has demonstrated the presence of the protein ZO-1 in the interendothelial junctions of human and rat brain vessels (10); ZO-1 is the first protein to be identified as a specific component of mammalian epithelial tight junctions. In contrast to cerebral capillaries, freeze-fracture and ultrathin serial section studies have shown that noncerebral continuous capillaries have tight junctions composed of complex networks of junctional strands, but with occasional discontinuities (11, 12). These discontinuities almost certainly correspond to the small pores of permeable capillaries mentioned above.

Another anatomic feature of cerebral endothelial cells that has been thought to be responsible for the restricted permeability of the BBB is the low frequency of cytoplasmic vesicles in comparison with noncerebral capillary endothelial cells. This idea is based upon the supposition that endothelial cytoplasmic vesicles are involved in transendothelial transport of blood-borne solutes. Morphometric studies have confirmed that cerebral capillaries contain sevenfold fewer cytoplasmic vesicles than muscle capillaries (13), and three- to sevenfold fewer than fenestrated capillaries in several CVOs (13, 14). Three-dimensional reconstruction studies have shown, however, that most of the few vesicles in cerebral endothelia are connected to golgi or endoplasmic reticulum-like structures in the cytoplasm and are thus probably involved in cellular metabolic processes (15). Very few vesicles lie free in the cytoplasm, as would be expected if they were involved in transendothelial transport (15). Many of the vesicles appear to be artifacts of fixation with aldehydes (16). The number of vesicles in endothelia of some non-CVO regions of the brain is only slightly lower than the number of vesicles in endothelia of some CVOs, although the permeability of the CVOs is several hundred times greater than the permeability of the non-CVO brain regions (17). Thus, there is no correlation between capillary permeability and frequency of endothelial vesicles, suggesting that few, if any, cytoplasmic vesicles in these endothelial cells are involved in transendothelial transport under normal conditions (17).

No fenestrae or transendothelial channels have been found in cerebral (non-CVO) endothelia, although fenestrae are numerous in CVO endothelia (13, 15, 17). The walls of cerebral (non-CVO) capillaries are 40% thinner than those of muscle and area postrema capillaries (13). This is consistent with the requirement for a short diffusion pathway to satisfy the high metabolic requirements of the brain. Cerebral (non-CVO) capillaries contain up to six times more mitochondria per capillary profile than do CVO capillaries, suggesting a high energy requirement for BBB capillaries to transport materials into the brain (17). The area of pericytes associated with cerebral (non-CVO) capillaries is not significantly different from the area associated with CVO and muscle capillaries, and it has been proposed that cerebral pericytes do not play a major role in maintenance of the BBB under normal conditions (13). Cerebral pericytes appear to be unique, however, with regard to noncerebral pericytes, in their expression of a specific surface glycoprotein (18), which may relate to some specialized function of cerebral pericytes. The phagocytic properties of these cells may be activated as a backup defense mechanism when the BBB is disrupted (19).

In recent years, a number of mostly cytochemical or immunocytochemical studies have been done to define better the molecular anatomy of the BBB. These studies have demonstrated unique properties of cerebral endothelial cells that are quite likely to be involved in their special barrier function. These studies have been recently reviewed by Vorbrodt (20) and Dermietzel and Krause (21); only a brief description will be given here.

It is important to characterize the proteins involved in the maintenance and function of the BBB; to this end, investigators have raised antibodies against various components of cerebral microvessels. One antibody recognized a protein that appeared to be a component of the interendothelial tight junction complex in brain capillaries (22); another recognized an antigen present in the cytoplasm and on the luminal cell surface coat of cerebral endothelia (23). Several others recognized proteins or glycoproteins located only

on the luminal plasma membranes of cerebral endothelia (24–27); one recognized an antigen on luminal, abluminal, and lateral surfaces of endothelia (28). All these antibodies were tested against noncerebral vessels and CVO vessels and showed no staining, indicating that they recognize molecules specific to BBB capillaries that are probably involved in some aspect of the BBB. Interestingly, despite the lack of staining of noncerebral capillaries, several of the antibodies recognized nonvascular structures involved in transport processes, such as choroid plexus epithelia, bile canaliculi, and apical and basolateral membranes of proximal tubules of the kidney (22–24). This suggests that the proteins recognized by them in the BBB similarly may be involved in transport across the BBB. One of the antibodies, anti-endothelial barrier antigen (EBA), recognized a triplet of proteins located on the luminal plasma membrane of cerebral barrier endothelia only (25). Subsequent studies have demonstrated that, in experimental allergic encephalomyelitis, vessels showing BBB disruption, as evidenced by perivascular accumulations of inflammatory cells, no longer stain with anti-EBA (29). In animals that had recovered from one attack, most cerebral endothelial cells again stained with anti-EBA, showing that recovery of function was associated with re-expression of EBA (29). In another study, stab wounds to the brain caused BBB breakdown in directly injured and adjacent microvessels; this breakdown was associated with loss of anti-EBA staining, which was maintained for approximately the period during which the BBB remained compromised (30). Thus EBA and the other BBB-specific molecules identified by these antibodies may prove to be very useful markers in the identification of BBB damage and the molecular mechanisms that are involved.

Lectin studies have been used extensively to characterize the composition of saccharide and oligosaccharide residues projecting from the surface of endothelial cells as components of plasma membrane glycoconjugates (ie, glycoproteins, glycolipids, and proteoglycans). Just as in the antibody studies, the ultimate aim of the lectin studies is to identify those endothelial glycoproteins unique to particular vascular beds which may have specific functional roles. Receptors for a variety of lectins have been defined in cerebral microvessels in several species, and for many lectins there seem to be differences in the distribution of their receptors between luminal and abluminal endothelial plasma membranes (31, 32). Differences in the distribution of lectin receptors in the endothelia of arterioles, venules, and capillaries have been attributed to different functions carried out by those different components of the microvasculature (32). Distinct differences between the lectin-binding patterns of cerebral and noncerebral endothelia have been described (31, 33), and a 135-kDa glycoprotein that binds to the lectin wheat germ agglutinin is specific to the luminal plasma membranes of cerebral microvessels (34). Both cerebral cryoinjury and acute hypertension cause BBB breakdown, and in cerebral microvessels showing increased permeability as a result of these insults, marked changes in the distribution of lectin-binding sites were observed on luminal and abluminal plasma membranes (35, 36). It was suggested that the changes were associated with changes in the function of endothelial plasma membranes (36).

The plasma membranes of all endothelia carry a net negative charge attributable to anionic groups of plasma membrane constituents, including carboxyl groups of sialic acid and hyaluronic acid and sulfate groups of glycosaminoglycans such as heparan sulfate (37). Cerebral endothelia carry a negative surface charge that is easily demonstrated with cationic probes such as cationic ferritin or cationic colloidal gold (Fig 2). Vorbrodt (38) has demonstrated that the negative charge on the luminal plasma membrane of cerebral endothelia is mainly contributed by terminal sialic residues of acidic glycoproteins, whereas on the abluminal plasma membrane it is contributed by a mixture of proteoglycans and glycopeptides containing hydrophobic amino acids, sialic acid residues, and sulfate groups of heparan sulfate-bearing glycosaminoglycans. This configuration is different from that found in other continuous, noncerebral, and fenestrated capillaries and probably reflects its special role in BBB function (38). The exact nature of the special role of the negative surface charge of cerebral endothelia is unknown but its importance in the maintenance of normal BBB function has been demonstrated by several experiments in which animals receive intracarotid injection of polycations such as protamine sulfate and polylysine. This procedure leads to a reversible BBB breakdown that has been attributed to neutralization of the anionic surface charge by the polycationic molecules (39–42). Other insults leading to BBB breakdown, including cerebral cryoinjury and acute hypertension, have also been associated with a loss of

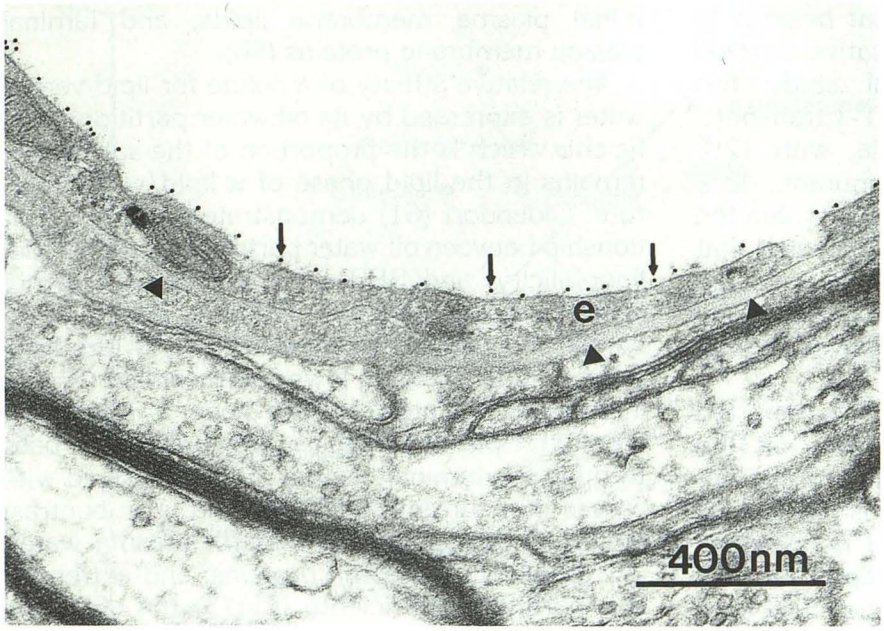

Fig. 2. The presence of negative surface charge on the luminal plasma membrane of a cerebral endothelial cell (*e*) is demonstrated in this electron micrograph by the binding of 10-nm-diameter cationic colloidal gold particles (*arrows*). *Arrowheads* indicate the basal lamina lying beneath the endothelial cell.

negative charge on the luminal surface of affected vessels (43, 44).

A number of enzymes have been demonstrated in cerebral endothelia. Some of these are common to all endothelia, whereas some are found exclusively in cerebral endothelia and may therefore play a role in the BBB. In endothelia, alkaline phosphatase is found only in cerebral barrier vessels and skeletal muscle vessels; its distribution in the muscle vessels differs from that in cerebral endothelia, where it is located predominantly on the luminal plasma membrane (45). The gradual appearance of alkaline phosphatase activity during development coincides with the increasing tightness of the BBB (45). Its exact role is uncertain but it is thought to be involved in the transport of phosphate esters and ions across the BBB (20). Opening of the BBB by cryoinjury has been shown to be associated with major redistribution of the enzyme from the luminal to the abluminal plasma membrane (44). Another enzyme that is a specific marker for cerebral endothelia is γ-glutamyl transpeptidase, which is also located on the luminal plasma membrane (46). This enzyme is believed to be involved in the transport of amino acids from blood to brain (47). Nucleoside diphosphatase is not found in nonbarrier CVO endothelia but is located on the abluminal plasma membrane of barrier endothelia (48); the role of the enzyme is not known. The enzyme Na^+,K^+-adenosine triphosphatase (ATPase) is found in no other endothelia except cerebral barrier endothelia, where it is located predominantly on the abluminal plasma membrane. There it acts as an ion pump, removing potassium from the brain to the blood against the concentration gradient and removing sodium from the cytoplasm into the brain interstitium, also against the concentration gradient (49). This action maintains the constant low levels of potassium in the brain extracellular fluid that are required for optimal neural function. The enzymes aromatic amino acid decarboxylase and monoamine oxidase are located in the cytoplasm of cerebral endothelia but in no other endothelia (50). Here they form an enzymatic barrier to circulating neurotransmitter monoamines and their precursors. Neurotransmitter precursors such as levodopa and 5-hydroxytryptophan pass readily into cerebral endothelial cells by a facilitated transport process. Here they are rapidly decarboxylated by aromatic amino acid decarboxylase (to dopamine and 5-hydroxytryptamine, respectively) and are then degraded by monoamine oxidase.

Much recent attention has focused on the identification and localization of glucose transporter proteins. A number of studies have identified a glucose transporter of the GLUT-1 isoform, identical to that found in human erythrocytes, in cerebral barrier endothelia (51, 52). The transporter is not present in CVO vessels and so far has been observed only in other endothelia in microvessels of the testis and placenta (53, 54). It is a 55-kDa glycoprotein that spans the plasma membrane and appears to differ from the glucose

transporter of neurons and glia that belongs to the GLUT-3 isoform (55). A quantitative electron microscopic immunocytochemical study has demonstrated the presence of GLUT-1 transporters in cerebral capillary endothelia, with 12% located on the luminal plasma membrane, 48% on the abluminal membrane, and 40% within the endothelial cytoplasm (56). It was suggested that the cytoplasmic component represents a reserve that can be mobilized to the plasma membrane for rapid up-regulation of cerebral glucose transport (56).

An immunocytochemical study has reported the presence of a transferrin receptor on the luminal plasma membrane of rat and human cerebral capillaries. The receptor was not detectable in capillaries from a variety of noncerebral tissues (57). It is likely that the receptor permits the transport of transferrin, and hence iron, into the brain. Immunocytochemical studies have also identified a 170-kDa surface membrane glycoprotein, the P-glycoprotein, in human cerebral barrier capillaries and in capillaries in some other locations where a blood-tissue barrier exists (eg, testis), but not in any other endothelia (58, 59). This glycoprotein is responsible for the multidrug-resistant properties of certain tumors and seems to function as an energy-dependent efflux pump whose role in the BBB is to protect the brain from the entry of harmful nonpolar solutes, such as certain xenobiotics (eg, doxorubicin, actinomycin D, and vincristine) (58).

Physiology of the BBB

The anatomic features of the BBB described above, particularly the presence of the very tight interendothelial junctions and the lack of transendothelial vesicular transport, mean that the cerebral endothelium has the permeability properties of a continuous plasma membrane, with the virtual elimination of passive diffusion across the endothelium. Thus, unlike noncerebral vessels, blood-borne solutes passing into the brain must first cross the endothelial luminal plasma membrane, negotiate the endothelial cytoplasm, and then cross the abluminal plasma membrane. Whether or not a solute is able to do so is dependent on its relative affinity for the four major molecular groups present at the blood-luminal plasma membrane interface, and on its resistance to cytoplasmic enzymes. The four groups are plasma water, plasma proteins, luminal plasma membrane lipids, and luminal plasma membrane proteins (60).

The relative affinity of a solute for lipid versus water is expressed by its oil:water partition coefficient, which is the proportion of the solute that remains in the lipid phase of a lipid/water mixture. Oldendorf (61) demonstrated a direct relationship between oil:water partition coefficient (ie, lipophilicity) and BBB permeability with highly lipophilic solutes such as caffeine, ethanol, and heroin showing high permeability and less lipophilic solutes showing lower permeability (Fig 3). If lipophilic solutes do not have a significant affinity for plasma proteins, they are able to pass through the luminal plasma membrane lipid with ease. The current iodinated and MR contrast media all have high affinities for plasma water, low affinities for plasma proteins, and extremely low partition coefficients (Fig 3) (64, 65) and therefore do not penetrate the intact BBB. On the other hand, radiopharmaceuticals used in positron and single-photon emission CT to demonstrate regional cerebral blood flow are highly lipophilic because it is desirable that they cross the intact BBB completely during the first pass through the cerebral vasculature (66, 67). Positron emission tomography agents used to investigate the integrity of the BBB, such as ^{68}Ga-EDTA, must not cross the intact BBB and therefore have low partition coefficients and high affinities for plasma water.

As soon as lipophilic solutes cross the luminal plasma membrane they enter the endothelial cytoplasm, where they are exposed to a number of enzymes that have been shown to be involved in the metabolism of lipophilic xenobiotics (68). In addition, the plasma membrane P glycoprotein, mentioned above, may function to remove certain lipophilic xenobiotics from endothelial cells back into the circulation (58, 59). A high degree of lipophilicity, therefore, does not automatically ensure passage of a solute across the BBB.

Many lipophilic solutes, such as steroid and thyroid hormones, free fatty acids, and some drugs, have a high affinity for plasma proteins and for practical purposes do not circulate in the unbound state. It was formerly thought that such protein-bound solutes would be unavailable for movement across the BBB; however, recent studies have shown that they are available and do cross the BBB, although the plasma proteins to which they are normally bound do not cross it (69, 70). It has been proposed that the solutes become available by a process of enhanced dis-

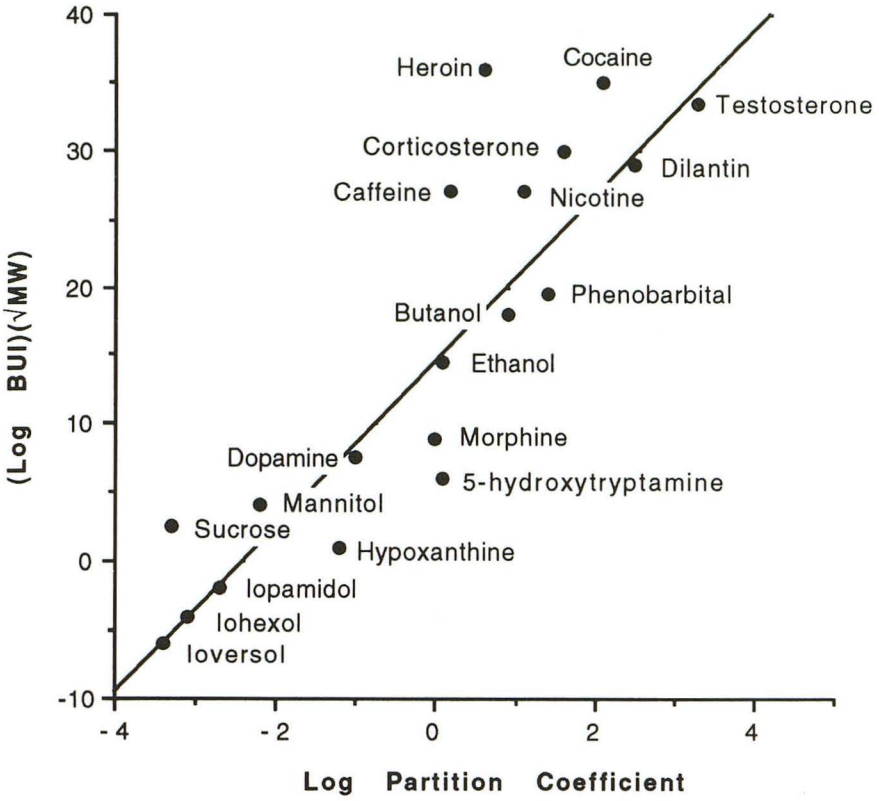

Fig. 3. The correlation between the octanol:water partition coefficients of a number of substances and their passage across the BBB, as measured by their brain uptake index (BUI) and in relation to their molecular weight (MW) (modified from Cornford et al [62]). The permeability of the three nonionic contrast media (iopamidol, iohexol, and ioversol) has been interpolated by fitting their octanol:water partition coefficients (63) to the regression line derived by Cornford et al.

sociation of the ligand from plasma proteins as they pass through the cerebral microcirculation. This dissociation may be caused by transient interactions with components of the endothelial surface causing conformational changes in the plasma proteins, which in turn release the ligand (69, 70). Having been dissociated, the solutes would rapidly cross the BBB by virtue of their lipophilicity. The endothelial surface components involved in the conformational changes may be the oligosaccharide residues associated with plasma membrane glycoconjugates that comprise the glycocalyx and which were discussed above. The specialized composition of the luminal plasma membrane of cerebral endothelia in relation to noncerebral endothelia, as discussed above, is likely to account for reported tissue-specific differences in dissociation of certain plasma protein-bound solutes in various vascular beds (69).

Permeability studies have demonstrated that some highly polar solutes such as glucose and some amino acids have a high affinity for plasma water and a low affinity for plasma membrane lipids (ie, low partition coefficients) and yet cross the BBB rapidly. This suggested the existence of luminal plasma membrane-bound transport systems with a high affinity for these solutes and ability to transport them across the BBB. A number of carrier-mediated transport systems have been demonstrated that facilitate the blood-to-brain uptake of hexoses, monocarboxylic acids, large, neutral amino acids, basic amino acids, purine bases, nucleosides, amines such as choline, and thyroid hormones (Fig 4) (reviewed by Pardridge [70] and Cornford [71]). These mechanisms are stereospecific, saturable, and energy independent, may be competitively inhibited, and are present on both luminal and abluminal plasma membranes.

The hexose transporter, the major substrate of which is D-glucose, is highly enriched in cerebral vessels and has been well studied (72, 73). It has been described in more detail above. Although D-glucose is the main substrate of the hexose transporter, it displays a lesser affinity for other hexoses such as D-galactose and D-mannose. Only 2-deoxy-D-glucose has a higher affinity for the transporter than does D-glucose (51) and this property is used in positron emission tomography scanning with ^{18}F-2-deoxy-D-glucose to image cerebral metabolism. The monocarboxylic acid transporter facilitates diffusion through the BBB of a number of short-chain monocarboxylic acids, such as acetic, propionic, butyric, and lactic acids, although their lipophilicity means that

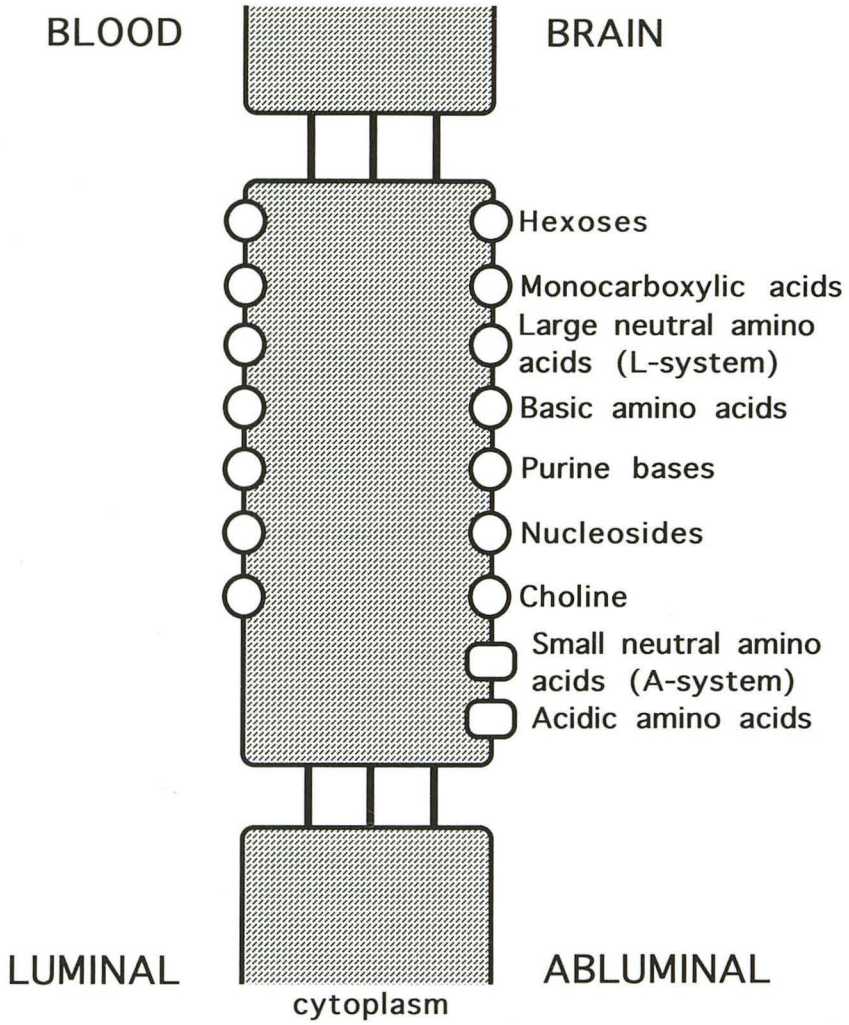

Fig. 4. A schematic representation of a portion of the wall of a cerebral blood vessel showing the currently known endothelial transport systems involved in transport between blood and brain. The transport systems indicated by *circles* are involved in facilitated diffusion and are located on both luminal and abluminal plasma membranes of endothelial cells. The transport systems indicated by *rectangles* are involved in active transport and are located only on the abluminal plasma membrane.

some diffusion will occur independent of the transporter (74).

Large, neutral amino acids and basic amino acids each have their own separate transport system (75). The L (leucine-preferring) system transports large, neutral amino acids such as phenylalanine, tryptophan, and tyrosine, all of which compete for the same transporter. The basic amino acid transporter is specific for amino acids such as lysine, arginine, and histidine (76). In addition to these two energy-independent amino acid transport systems, studies on isolated brain capillaries have demonstrated a third amino acid transport system, the A (alanine-preferring) system (77). This system is located only on the abluminal membrane of capillary endothelia and is energy dependent. It serves to remove small, neutral amino acids such as glycine and alanine, which are inhibitory neurotransmitters, from the brain extracellular fluid into the blood. A fourth amino acid transport system has been identified and is also energy dependent and located only on the abluminal plasma membrane of capillary endothelia (78). This system removes the acidic amino acids glutamate and aspartate, which are excitatory neurotransmitters, from the brain extracellular space to the blood.

Binding studies on isolated human brain capillaries have demonstrated the presence of high-affinity receptors for transferrin (79), insulin (80), and insulin-like growth factors 1 and 2 (81). As mentioned above, immunohistochemical studies also have shown the presence of the transferrin receptor on cerebral vessels (57). It has been postulated that the receptors are involved in the transport of their respective peptides from the blood into the brain. In vivo studies in animals have shown that insulin and transferrin cross the BBB via receptor-mediated transcytosis, which is thought to involve receptor-mediated endocytosis at the luminal endothelial surface, diffusion through the endothelial cytoplasm, and receptor-mediated exocytosis at the abluminal endothelial surface (82, 83). A similar mechanism is likely to

be involved in the movement of insulin-like growth factors 1 and 2 across the BBB (81). Movement of transferrin across the BBB is thought to be the way that the brain satisfies its requirement for iron, and also may result in the movement into the brain of other, potentially toxic, metals such as aluminum and zinc, which have an affinity for transferrin (83). There is evidence that other peptides and proteins (eg, IgG) are transported across the BBB by specific transport mechanisms (84).

Astrocytes and the BBB

Although astrocytic foot processes, forming an almost complete sheath around cerebral vessels, play no direct physical part in the operation of the BBB, nevertheless they appear to play a vital role in inducing and maintaining many of the specialized properties that characterize the BBB (reviewed by Abbott [85]).

Several in vivo studies have shown that when avascular embryonic brain was transplanted into extracerebral sites, the invading noncerebral vessels developed characteristic features of BBB vessels (24, 26, 86). Grafts of purified astrocytes onto the chorioallantoic membrane of chick embryos were vascularized by vessels impermeable to intravenous Evans blue, unlike vessels outside the graft (87). These studies suggested that the neuronal environment is responsible for the induction of BBB properties. In another in vivo study, however, neonatal rats were injected with a gliotoxin that caused the degeneration of all astrocytes and oligodendrocytes in the brain (88). The loss of perivascular astrocytic endfeet was not associated with loss of integrity of the BBB to intravascular HRP (88); however, the study did not investigate whether other specialized properties of cerebral endothelial cells not directly related to structural integrity to exogenous proteins were affected. In addition, membrane fragments of degenerated astrocytes remained in contact with endothelial cells; these fragments may have retained some inductive influence, as has been shown in some tissue culture experiments.

Tissue culture experiments in which cerebral endothelial cells were cocultured with astrocytes demonstrated that astrocytes induced the formation of complex tight junctions between adjacent endothelial cells (89, 90). Astrocytes also induced the expression in cultured cerebral endothelial cells of γ-glutamyl transpeptidase, an enzyme localized exclusively in cerebral, but no other, endothelia (91, 92). A soluble protein released from astrocytes stimulated glucose uptake in cerebral endothelial cells, probably by inducing the synthesis of more glucose transporters in the endothelial cells (93). The synthesis of the membrane-bound enzymes Na^+,K^+-ATPase and alkaline phosphatase and their normal localization on the abluminal and luminal plasma membranes, respectively, of cerebral endothelial cells, was induced by astrocytes (94). Similarly, astrocytes were responsible for inducing the normal polarized transport of small, neutral amino acids, via the A-system transport mechanism, from abluminal to luminal in cerebral endothelia (95). Plasma membrane fractions of C6 glioma cells and neurons induced much increased expression of γ-glutamyl transpeptidase and Na^+,K^+-ATPase in cultured cerebral endothelial cells (96).

It is possible that the BBB breakdown seen in some cerebral tumors is a consequence of an altered metabolism in affected astrocytes, leading to a change in the release of inductive factors and a loss of specialized features in associated capillary endothelia. It is also possible that the very different arrangement of tight junctional strands seen by freeze-fracture in cerebral arteries and collecting veins (9) is caused by the astrocytic sheath being separated from the endothelia by a layer of smooth muscle. In some of the tissue culture studies, a close physical approximation of astrocytes and endothelia was necessary for the inductive process to occur.

Although astrocytes seem to play a major role in maintaining the specialized properties of cerebral endothelia, the reverse also seems to be true. Cultured endothelia induced changes in associated cultured astrocytes (97). Thus there is apparently a complex relationship between astrocytes and endothelia.

Intravascular CM and the BBB

Current neuroradiologic practice uses the intraarterial injection of iodinated contrast media (CM), and intravenous injection of iodinated CM and MR contrast agents, to investigate cerebral pathology. There is an extensive body of literature which will be reviewed here, reporting the effects of these agents on the BBB, both intact and disrupted, in animal and clinical studies.

Most of the animal models have used rabbits because the pattern of major vessels supplying their brains is the same as in humans; dogs and rats have been used to a lesser extent. The earliest

studies used extravasation of Evans blue or trypan blue, both of which rapidly form a strong dye-albumin complex, as a gross indicator of BBB damage. Later studies measured the extravasation of radionuclides such as ^{99m}Tc, ^{197}Hg, and ^{125}I; these methods continue to be the most popular for investigating the effects of CM on the BBB. A few studies have used the vascular tracer HRP, which, after suitable histochemical treatment, can demonstrate sites of BBB breakdown at both the light and electron microscopic level. The HRP method is able to detect very subtle BBB damage, down to the level of a few damaged vessels in an entire brain. Most recently, contrast-enhanced CT and MR have been used to investigate CM-induced BBB damage. Contrast-enhanced CT does not seem to be as sensitive as the radionuclide and contrast-enhanced MR methods.

Intraarterial Iodinated CM and the BBB

Ionic CM.

Animal studies. Although a number of ionic monomeric CM have been developed, many are now obsolete. The most common agents in current use for cerebral angiography are methylglucamine (meglumine, sometimes combined with sodium) salts of iothalamate and diatrizoate. It has been shown in a number of animal studies that the intracarotid injection of these CM leads to significant BBB breakdown (98–102), as does intravertebral injection (103). Gonsette (101) and Velaj et al (102) reported that meglumine diatrizoate was more damaging to the BBB than meglumine iothalamate, although Jeppsson and Olin (98) found the reverse. Several of these studies observed that the sodium salts of the CM were more damaging than the meglumine salts (98, 101).

These studies have all used regimens with injection times and iodine doses much greater than those used clinically. A few studies have used injection regimens that resemble the clinical situation. These more clinically relevant studies, using HRP as a marker at the light and electron microscopic levels, have also demonstrated significant BBB breakdown in rabbits injected with iothalamate and diatrizoate (104–106) (Fig 5). Although diatrizoate and iothalamate are similar in structure, molecular weight, and osmolality, there were differences between them in terms of the areas of brain (106) and the types of vessels (ie, arterioles, venules, capillaries) most affected (105).

At the iodine concentrations at which the ionic CM are normally used, they have osmolalities of approximately 1500 mOsm/kg. It has been established by Rapoport (107) that the intracarotid injection of hyperosmotic inert solutions such as mannitol and arabinose causes a reversible BBB breakdown and that both the solution osmolality (ie, concentration) and the duration of the injection are critically interrelated factors. The threshold osmolality of inert solutions, above which significant BBB damage occurs in a rat model, is approximately equal to the osmolalities of the ionic monomeric CM at their usual concentrations. The hyperosmolality of the ionic monomeric CM has been demonstrated to be a factor in the BBB breakdown seen in experimental models (100). The mechanism involved in hyperosmotic opening of the BBB remains controversial but the weight of evidence suggests that interendothelial tight junctions separate as a consequence of shrinkage of the endothelial cells exposed to the hyperosmotic solution (108).

In several studies it was observed that the intracarotid injection of ionic CM caused significantly worse BBB damage than did mannitol solutions of approximately equal osmolality (102, 109). This suggests that some chemotoxic property of the CM is having a disruptive effect on the BBB in addition to hyperosmolality. The separate contributions of hyperosmolality and chemotoxicity were also demonstrated by Waldron et al (110) with repeated intracarotid injections of sodium diatrizoate, meglumine iothalamate, or equiosmotic saline in cats. It was reported that CM hyperosmolality caused increased pinocytosis in cerebral endothelial cells, whereas CM chemotoxicity caused opening of interendothelial tight junctions (110).

Acute hypertension, above a threshold level, is known to open the BBB reversibly (111). It was shown recently that subthreshold levels of acute hypertension caused BBB damage in rats receiving intracarotid sodium/meglumine diatrizoate, whereas normotensive rats injected with the same CM showed significantly less damage (112). This suggests that acute hypertension may be a risk factor for BBB damage in cerebral angiography with ionic CM.

The ionic dimer sodium/meglumine ioxaglate (Hexabrix, Guerbet) has an osmolality of 675 mOsm/kg at its normally used concentration (320 mg/ml), which is similar to the osmolalities

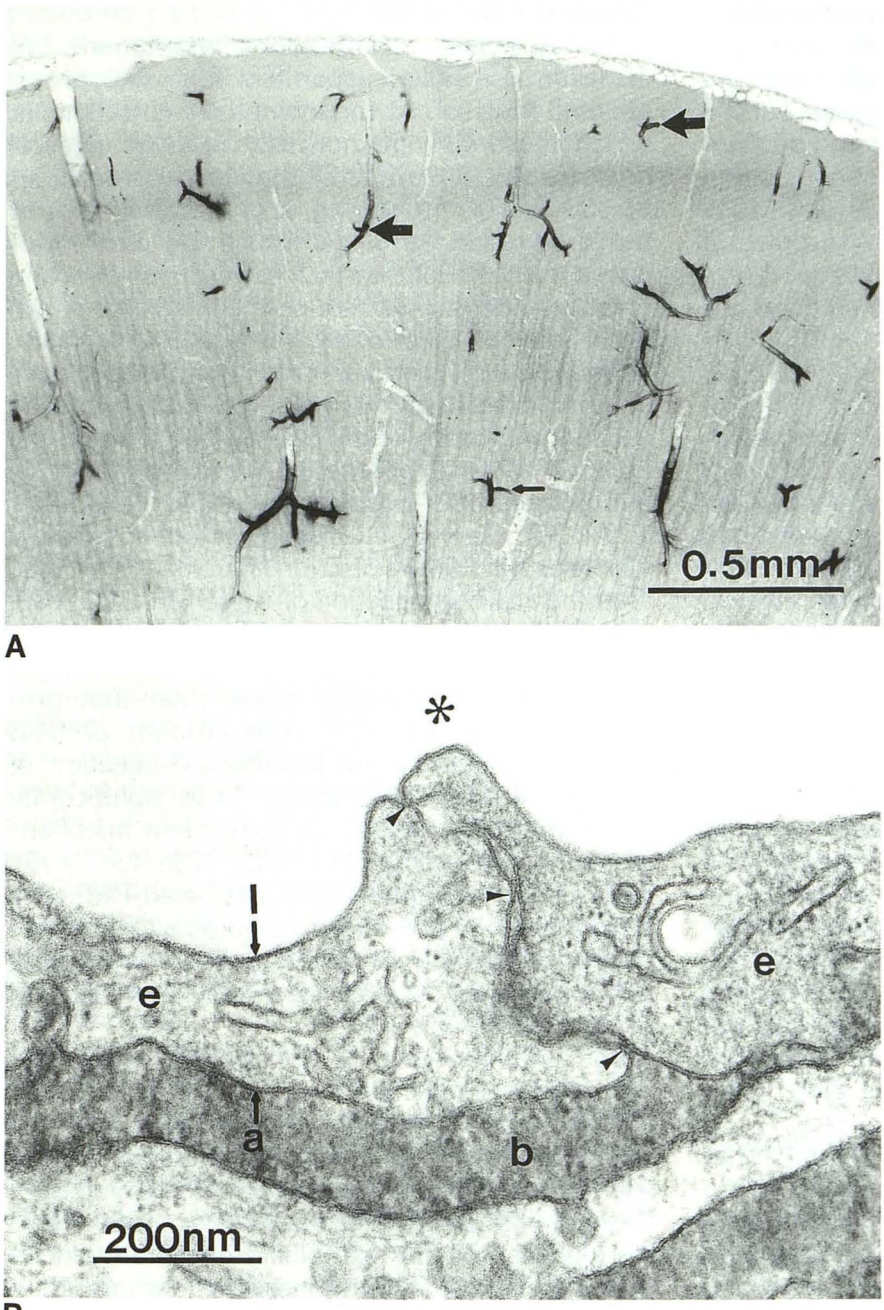

Fig. 5. *A*, A low-power light micrograph of a section of brain from a rat that has received an intracarotid injection of meglumine iothalamate. BBB breakdown has been demonstrated using HRP as an intravascular marker. A cytochemical technique produces a visible reaction product at sites of HRP extravasation. A number of blood vessels in this section show extravasated HRP in their perivascular spaces (*arrows*), indicating that they have undergone BBB breakdown. Many more vessels have not undergone breakdown but are less visible because they are not outlined by HRP. *B*, A high-power electron micrograph of a portion of a cerebral blood vessel from a rat that has received intracarotid meglumine iothalamate. Portions of two apposed endothelial cells (*e*), joined by interendothelial tight junctions (*arrowheads*), separate the vessel lumen (*asterisk*) from the surrounding brain. The luminal (*l*) and abluminal (*a*) endothelial plasma membranes can be seen. The vessel has undergone BBB breakdown, as evidenced by the presence of electron-dense amorphous reaction product of extravasated HRP within the basal lamina (*b*).

of the nonionic monomeric CM. There have been relatively few animal studies of the effects of this CM on the BBB, but most suggest that it is safer than the ionic monomers (101). Wilcox and Sage (113) were unable to find any disruptive effect of intracarotid ioxaglate on the BBB in a contrast-enhanced CT dog model but found slight Evans blue staining and a slight, but not statistically significant, increase in ^{99m}Tc extravasation in a rabbit model. Similarly, Aulie (114) reported slight trypan blue extravasation and a slight increase in ^{197}Hg extravasation in rabbits receiving intracarotid ioxaglate. A contrast-enhanced MR study in rabbits found that intracarotid ioxaglate caused significantly more BBB damage than did physiologic saline, but was not significantly different from meglumine diatrizoate (115). Ioxaglate caused slightly more BBB damage than did iohexol, which has a slightly higher osmolality, suggesting that the increased damage was caused by a chemotoxic effect of ioxaglate (115).

Clinical studies. BBB breakdown after cerebral angiography with ionic CM has been described in several case reports. Sage et al (116) reported a patient who demonstrated cortical CT enhance-

ment after a common carotid injection of sodium/meglumine diatrizoate. This patient developed seizures, as did two other patients also showing cerebral CT enhancement after common carotid angiography with meglumine diatrizoate or meglumine iothalamate (117). In an analogous situation, injection of sodium/meglumine diatrizoate into the ophthalmic artery caused breakdown of the blood-ocular (blood-aqueous and blood-retinal) barriers, which share many of the characteristics of the BBB (118).

Transient cortical blindness secondary to BBB disruption is a recognized complication of angiography with ionic CM and has been described in three cases of patients undergoing cerebral angiography. All patients showed CT enhancement of the occipital lobes and two patients later had seizures (119, 120). Coronary angiography with sodium/meglumine diatrizoate led to cortical blindness and CT enhancement in the occipital lobes in two patients (121).

Another recognized complication of cerebral angiography with ionic CM is transient global amnesia. Ten cases of this condition have been reported after cerebral angiography with ionic CM (122–124). Haley (125) reported three cases of global encephalopathy after cerebral angiography with meglumine iothalamate.

The only clinical report involving the adverse effects of the low osmolality ioxaglate involves a single patient who received a large quantity of ioxaglate during coronary angiography. This patient subsequently displayed diffuse CT enhancement of the cerebral cortex, suggesting widespread BBB breakdown (126).

The seizures, cortical blindness, and encephalopathy in the above cases are thought to be caused by a direct neurotoxic effect of the ionic CM on neurons to which the CM have gained access, either by causing BBB breakdown or, in some cases, passing across a BBB disrupted as a result of preexisting disease (127). The demonstration of CM within the brain via CT enhancement in many of these patients supports the idea of a direct neurotoxic effect as does the finding that patients undergoing hyperosmotic BBB breakdown for tumor chemotherapy are at increased risk of seizures when the breakdown is monitored with meglumine iothalamate-enhanced CT (128). Transient global amnesia has been attributed to ischemic complications of the catheterization technique (122, 127), but a direct neurotoxic effect of ionic CM also has been proposed as a mechanism (124).

Nonionic CM.

Animal studies. The nonionic monomeric CM in current use include iopamidol, iohexol, iopromide, and ioversol. At the iodine concentrations at which these CM are normally used in cerebral angiography (300 mg/ml), they have osmolalities of 600 to 700 mOsm/kg, less than half that of the ionic monomeric CM. These CM were introduced in the belief that their lower osmolality and chemotoxicity would reduce the incidence of adverse effects caused by the ionic CM. Many animal studies, using a variety of models, have demonstrated that the nonionic CM do indeed cause significantly less BBB damage than do the ionic CM after experimental intravertebral (103) and intracarotid angiography (102, 103, 115, 129–136). Several studies reported no grossly visible extravasation of Evans blue or trypan blue after intracarotid injection of these CM (102, 103, 130–132), whereas others reported slight staining (114, 129, 134, 137, 138); the level of staining was always substantially lower than that produced by the ionic CM. The amount of BBB damage caused by the intracarotid injection of nonionic CM has been shown to be significantly worse than that caused by equivalent injections of physiologic saline (115, 133, 135, 137, 138). Two studies, in rabbits and rats, used HRP as a vascular tracer to demonstrate a small but measurable population of cerebral vessels showing HRP extravasation after the intracarotid injection of iopamidol (139, 140).

The small amounts of dye and HRP extravasation and the significantly worse effects of nonionic CM in comparison with saline suggest that the nonionic CM are not completely inert with regard to the BBB. It was not possible to determine from these previous experiments whether these effects were caused by CM osmolality or chemotoxicity or some combination of these two factors. Several investigators observed that nonionic CM of similar osmolalities had considerably different effects on the BBB, suggesting that CM chemotoxicity must play some role in these effects (114, 129). A recent study estimated the relative contributions of osmolality and chemotoxicity to BBB damage caused by nonionic CM (141). Rabbits received intracarotid injections of iohexol, ioversol, or mannitol, all at approximately 700 mOsm/kg, and BBB damage was assessed by measuring ^{99m}Tc extravasation. Mannitol caused no detectable BBB breakdown whereas both iohexol and ioversol caused significantly more BBB breakdown than did mannitol. Thus osmolality

played no part in the BBB breakdown detected, and the chemotoxicity of the CM was solely responsible (141). This study suggested that CM osmolality is no longer a factor in BBB damage at levels below 700 mOsm/kg. The chemotoxicity of the nonionic CM might explain why the intracarotid injection of iopamidol at 20°C caused increased local cerebral glucose use compared with iopamidol at 37°C (142). Control experiments showed that the metabolic change was not caused by either solution temperature or osmolality; the increased viscosity of iopamidol at 20°C was proposed to be the cause. It seems likely that the increased viscosity prolonged the contact time of the CM with the cerebral endothelium and that this permitted CM chemotoxicity to have an effect on the BBB.

A recent study has demonstrated that, as with the ionic CM, acute hypertension may be a risk factor in cerebral angiography with nonionic CM (140).

The nonionic dimeric CM, of which iotrolan and iodixanol are the only examples currently available, have osmolalities approximately equal to blood at their normally used concentrations (300 mg/ml). Relatively few studies have been performed to test their effects on the BBB. Iodixanol was found to cause some slight extravasation of Evans blue and to produce significantly more extravasation of ^{99m}Tc than did an equivalent injection of saline (138). Iotrolan was also found to cause slight extravasation of Evans blue and caused significantly less ^{99m}Tc extravasation than did othalamate and iopromide, but was not significantly different from saline (137). Another study, using enhanced MR, showed that iotrolan caused significantly more BBB damage than did saline (115). The observations that iodixanol and iotrolan, both isoosmotic with plasma, cause significantly more BBB damage than does saline (115, 138) suggest that their effects are attributable to chemotoxicity. This was confirmed by Wilson et al (141), who showed that iodixanol and iotrolan caused significantly greater BBB breakdown than did mannitol solution of greater osmolality (700 mOsm/kg), which itself caused no detectable BBB damage.

Clinical studies. Few clinical reports of adverse effects of nonionic CM on the BBB are available. Cortical blindness has been reported in three patients undergoing angiography with nonionic CM (120, 121). Two patients underwent cerebral angiography and showed bilateral CT enhancement of the occipital lobes; one underwent coronary angiography without CT. Diffuse cortical CT enhancement also has been reported in one case after abdominal angiography with iohexol (143). During angiography the patient developed acute hypertension; it is possible that this potentiated the damaging effects of iohexol on the BBB. This is in accordance with animal studies showing acute hypertension to be a risk factor for BBB damage in cerebral angiography (112, 140). The patient developed generalized seizures after the procedure; these may reflect a direct neurotoxic effect of iohexol after crossing the disrupted BBB.

Transient global amnesia was reported in two patients undergoing cerebral angiography with iopamidol (144). The exact pathophysiologic mechanism behind this condition is not clear, but in one case the existence of recent cerebral infarction suggested that CM neurotoxicity may have been involved (144). Another possible mechanism was raised with the observation that vertebral angiography with iohexol, leading to transient global amnesia, caused spasm of the intracranial portion of the vertebral artery and the basilar artery (145). It was suggested that ischemia, secondary to vertebrobasilar spasm, may have been the cause. Similar spasmogenic effects of intravertebral nonionic CM have been reported in two animal studies (146, 147); significant vasoconstriction of the internal carotid artery and larger middle cerebral vessels occurred in baboons given intracarotid injections of iohexol and ioxaglate (148).

Intravenous CM and the BBB

The use of high intravenous doses of iodinated CM in enhanced CT and digital subtraction angiography has led to a concern that plasma osmolality may be increased sufficiently to cause osmotic BBB breakdown. In rabbits, elevation of plasma osmolality to 370 mOsm/kg has been shown to cause focal BBB opening and elevation to 400 mOsm/kg caused widespread BBB opening, although it is not known how long these osmolality levels must be maintained for damage to occur (149). High circulating levels of sodium/meglumine diatrizoate (4 ml/kg), maintained for 48 hours with plasma osmolalities reaching 380 mOsm/kg, caused no BBB breakdown in a sheep model (150). This experiment was undertaken to simulate the conditions in a patient who underwent renal and abdominal angiography with sodium/meglumine diatrizoate and, as a conse-

quence of renal dysfunction, experienced prolonged high levels of plasma CM, equivalent to an intravenous injection of 4 ml/kg. This patient suffered cortical blindness and showed bilateral CT enhancement of the basal ganglia and occipital lobes, indicating BBB breakdown. The analogous sheep experiment suggested that the BBB breakdown was not caused by either the increased plasma osmolality levels or by a chemotoxic effect of the high plasma CM levels by themselves; the patient's additional complications of severe vascular disease and chronic hypertension may have lowered the threshold of the BBB to damage by these factors (150).

The intravenous injection of meglumine diatrizoate at 4 and 6 ml/kg, normal clinical doses for enhanced CT, caused BBB breakdown in a small number of dogs (151). When this study was later repeated, no BBB damage could be detected (152), and even higher intravenous doses of more concentrated CM (8 ml/kg of sodium iothalamate at 420 mg/ml) caused no detectable BBB damage in rabbits (153). Low doses (0.45 ml/kg) of intravenous CM caused no detectable HRP extravasation but did cause significant increases in the number of pinocytotic vesicles in rat cerebral endothelial cells, suggesting a subtle chemotoxic effect of the CM on these cells (154).

A clinical complication of contrast-enhanced CT studies of the brain is the development of seizures. Seizures have been reported in a number of patients with underlying focal brain lesions; patients with metastatic brain tumors appear to be at particular risk of developing seizures (154, 156). Fischer (157) has suggested that the already increased permeability of vessels in these lesions, coupled with a slowing of blood flow in the region of the lesion, may cause the lesion to be exposed to a prolonged concentration of CM; this in turn may lead to increased permeability of the blood vessels, permitting the CM to pass into the brain and exert direct neurotoxic effects. The use of nonionic CM, with their reduced osmolalities and lower chemotoxicities, might be expected to reduce the incidence of seizures in patients undergoing enhanced cranial CT, particularly in those with suspected cerebral neoplastic lesions.

The gadolinium compounds used as MR contrast media, gadopentetate dimeglumine, gadodiamide, and gadoteridol, have extremely low partition coefficients (65) and therefore do not cross the intact BBB. Currently there is no evidence that the intravenous injection of these CM has any effect on either the normal BBB or on the focally compromised BBB, either at normal doses (0.1 mmol/kg) (158, 159) or at increased doses (0.2 mmol/kg) (160). A recent study in dogs showed that intravenous injection of gadopentetate dimeglumine at clinical doses (0.1 and 0.2 mmol/kg) to image BBB breakdown caused by hyperosmotic mannitol was followed by seizures, some of which were intractable, in most of the dogs (161). It was suggested that the seizures were a chemotoxic effect of the CM on the brain as a consequence of the diffuse opening of the BBB brought about by the hyperosmotic insult (161). In neural tissue exposed to brain extracellular fluid concentrations of gadopentatate dimeglumine and gadoteridol that could be expected clinically in areas of BBB breakdown, it has been shown that the CM interfere significantly with tissue metabolism (162). Similar concentrations of gadopentetate dimeglumine and gadoteridol have been shown to inhibit the activity of the brain enzyme glutamate decarboxylase by about 15%; the enzyme acetylcholinesterase was unaffected (163).

Disease and the BBB

In certain diseases the permeability of the BBB is increased and this forms the basis of common neuroimaging techniques such as contrast-enhanced CT and MR. Such alterations in the BBB may be illustrated by intraaxial tumors, cerebrovascular disease, and infections.

Intraaxial Brain Tumors

In 1989 Greig (164) reviewed the morphology of blood vessels within brain tumors and their associated increase in permeability. Such tumors vary in their malignancy but tumor blood vessels have certain general characteristics in common (164). These include an increase in the number of capillaries except in avascular areas (165); an increase in the overall diameter of the lumen of tumor blood vessels compared with normal brain blood vessels (166–168); a greater number of endothelial cells indicating endothelial proliferation, which is a diagnostic criterion for glioblastoma multiforme (164); an increased number of cytoplasmic vesicles within capillary endothelial cells (166, 168–171); alterations in the basement membrane of capillaries consisting of reduplicating layers or folds and fragmentation (166, 168–171); development of a significant perivascular space that is not present in the normal brain

(166); and defects such as open tight junctions, gap junctions, and fenestrae (166). Another obvious feature is an apparent loss of the normal astrocytic investiture of the capillary endothelium of tumor blood vessels with the outer surface of the basement membrane becoming incompletely invested by tumor processes instead (166, 168–171).

Tumor cells have been demonstrated to release an angiogenic factor that stimulates the formation of new capillaries (172, 173) and these abnormalities of the blood vessels of central nervous system (CNS) tumors suggest a highly active but poorly controlled hyperplastic response to tumor-released angiogenesis factors (164). An angiogenic factor has been demonstrated in human gliomas (174) that is modified by the presence of macrophages (175) and promotes capillary growth. However, what determines the characteristics of endothelial cells of these new capillaries is unclear. It does appear that the tight junctions between cerebral capillary endothelial cells are normally under the inductive influence of the investing astrocytic endfeet, but it is not clear whether the tight junctions are sometimes lacking during the rapid initial formation of the tumor-induced capillary loops or break down as a consequence of poor structural support or vasoactive peptides (164). The formation of fenestrated endothelium, on the other hand, occurs during the angiogenic process (164).

The open interendothelial junctions, fenestrae, gap junctions, and increased pinocytic vesicles in the capillaries of CNS tumors (166, 167, 169, 171) have been reported to form the basis of the increased permeability of tumor vessels. Open interendothelial junctions in primary human brain tumors (166, 168, 169) may arise from a lack of structural support caused by the absence of the normal investing astrocytic endfeet, which are replaced by tumor cells or an enlarged perivascular space (166). It is also thought that when there are tight junctions between tumor capillary endothelial cells, they may be ultrastructurally simpler than those of normal brain capillaries (164) and therefore have increased permeability and fragility. The occurrence of a fenestrated endothelium in the vasculature of certain brain tumors has been reported (170, 171), and these pores apparently permit the direct flow of material between the capillary lumen and the perivascular space (164). On the other hand, the occurrence of endothelial gaps has been described in brain tumors, but it is not clear whether these represent incomplete capillary formation, tumor invasion, or postmortem changes (164, 167). Whereas endothelial vesicles are rare in normal cerebral capillaries, they occur in increased number in brain tumor vessels (166, 168, 169, 171). Most are found at the luminal and abluminal surfaces. However, it is not clear whether they play any role in increased capillary permeability (164). Greig et al (166) hypothesized that rather than playing a role in capillary permeability, the vesicles are involved with the expulsion of extravasated material, rather than with its initial transport into the brain.

Of recent interest has been the release of certain substances such as leukotrienes (176), bradykinin, the kallikrein-kinin system, glutamate (177), polyamine, and platelet-activating factor (178) from malignant gliomas, which may contribute to an increase in permeability of the BBB (178).

Although angiogenesis within CNS tumors leads to capillaries with features that increase the permeability of such vessels, both experimental (179) and clinical (180) studies have demonstrated that there is considerable variation in regional vascular permeability within the same tumor. This variation in the permeability of tumor vessels and to a lesser extent in regional blood flow between different types of brain tumors and within the same brain tumor is relevant to the delivery of chemotherapeutic drugs or the therapeutic manipulation of the BBB to increase the delivery of such agents.

A variety of protocols for hyperosmolar opening of the BBB have been developed (181) and enhanced delivery of chemotherapeutic agents has been documented (182) in such patients compared with chemotherapy without BBB disruption. Osmotic BBB disruption has proved to be generally safe and clinically effective (181). However, variations in permeability of tumor vessels and, to a lesser extent, regional blood flow may limit the effect.

Vasogenic edema caused by an increase in BBB permeability occurs with certain brain tumors and may contribute to the clinical symptoms and signs. The major strategy for reducing such edema is the use of corticosteroids (178). The effect of steroids in reducing cerebral edema is no doubt multifactorial (178), but a reduction in barrier permeability seems to play a part (178).

Cerebral Hypoxia, Ischemia, and Infarction

The effect of hypoxia alone on BBB integrity is controversial (183) but in the presence of stable blood pressure, the BBB is thought to remain intact except in severe hypoxia and hypocapnia of significant duration (183).

In the presence of ischemia, the cerebral capillary endothelium is relatively resistant to damage compared with neuronal cells (183–186). It has been suggested that energy deprivation associated with hypoxia and ischemia may actually inhibit barrier opening (178).

The literature relating to the effects of cerebrovascular disease on the BBB is confusing because of a failure sometimes to separate clearly reversible ischemia from ischemia that progresses to infarction. Cerebral ischemia may be complete or incomplete, focal, regional, or global whereas the causative vascular occlusion may be either permanent or temporary and of variable duration. At the same time, the experimental animals used to investigate ischemia have different cerebral circulations. For example, whereas rabbits have a circle of Willis similar to humans, many gerbils have an incomplete circle of Willis. Gummerlock (183) reviewed the extensive experimental data relating to the BBB and cerebral ischemia and concluded that the time course of BBB disruption in ischemia remains controversial. Although certain works have suggested an alteration in the integrity of the BBB immediately after or within a few minutes of cerebral ischemia (187–190), many others have indicated that barrier disruption caused by ischemia is a delayed phenomenon.

This confusion may be resolved by Kuroiwa's theory (191) of biphasic barrier opening. In a cat model, opening of the BBB was demonstrated after vascular occlusion for 1 hour, followed by reperfusion and associated marked reactive hyperemia of previously ischemic brain tissue. This initial opening is thought to be caused by the reperfusion, leading to an onrush of blood through vessels that are already dilated by acidosis and devoid of autoregulation (192). The role of reactive hyperemia in this first ischemic opening is supported by the fact that a reduction of the hyperemia by hypovolemia or by aminophylline prevents such opening and reduces vasogenic edema (192). Kuroiwa et al (191) demonstrated the barrier was again closed within 3 hours of recirculation. The second phase of BBB opening was seen after 5 hours of recirculation and remained for at least 72 hours. This second opening appeared to be related to severe ischemic injury. The mechanism for this delayed BBB disruption remains undefined (183), but the release of various substances from damaged neuronal cells such as kinins, fatty acids, free radicals, serotonin, and prostaglandins has the potential to induce barrier opening from the abluminal side (192).

Therefore, the biphasic opening of the barrier observed by Kuroiwa et al (191) may explain the variable observations of others with the first transient opening being related to reperfusion hyperemia and the second delayed opening reflecting ischemic damage to neuronal cells.

If ischemia progresses to infarction, the early changes consist of intracellular or cytotoxic edema, and no immediate change in the BBB permeability is observed in the first few hours (193, 194).

Although there is a significant variation in experimental and clinical observations, a review of such studies would suggest that a breakdown of the BBB is generally observed to begin between 4 hours and 3 days after the onset of infarction, which corresponds to the onset of vasogenic edema (183). Ito et al (145) concluded that the BBB disruption with infarction requires reperfusion, and the longer the duration of vascular occlusion, the shorter the reperfusion period before BBB changes are seen. This role of recirculation is supported by the observation of others that the barrier opening with ischemic infarction is first recognized at the periphery, which has access to collateral circulation with hyperemia caused by acidosis and loss of autoregulation (192, 196). Extravasated serum proteins passing through the altered BBB are seen initially at the periphery of such lesions and may then spread through the affected brain tissue, entering injured cells, particularly neurons, and migrating further, preferentially through the white matter (192).

A very recent report seems to encompass the biphasic opening caused by ischemia reported by Kuroiwa et al and the delayed opening caused by neuronal death and infarction (197).

Infectious Diseases

Meningitis. Most cases of meningitis are thought to develop as a result of hematogenous spread but the site at which bacteria leave the blood stream and enter the cerebrospinal fluid is not known (198). Experimental animal studies indicate that the BBB protects the nervous system from infection by microorganisms in the blood-

stream (199) and clinical evidence also attests to protection conferred by the BBB (198).

Immunocompromised patients and experimental animals lacking in immunoglobulins and complement components are at an increased risk of meningitis (200), indicating that immunoglobulins and complement and phagocytes also help protect the nervous system from hematogenous infection.

How microorganisms pass from the bloodstream to the subarachnoid space or meninges during a bacteremia or fungemia to produce meningitis is unclear. The anatomic site of entry could involve the choroid plexus epithelium or the vascular endothelium and may require passage between epithelial or endothelial cells or intracellular transport (198). Factors released by the microorganisms themselves may alter barrier function to allow invasion by blood-borne organisms (200).

After meningitis is established, there is an increase in the permeability of the BBB to serum proteins and also antimicrobiotic agents (198). This increase in BBB permeability is related to the inflammatory reaction and in the convalescent phase of meningitis as the inflammation resolves, the permeability of the BBB to antimicrobiotic agents declines (201). Experimental meningitis in the rat has confirmed that the increased permeability of the BBB in meningitis is associated with opening of tight junctions between endothelial cells and increased pinocytosis (202).

Brain abscess. Like meningitis, the development of a brain abscess may result from microorganisms introduced by trauma, spread from adjacent structures or by a hematogenous source.

It is convenient to classify the histologic evolution of an abscess into a cerebritis stage and late capsule stage (203). With organisms of low virulence, the cerebritis stage can be subclassified further into an early and a late phase (204), but this is not possible with more virulent organisms (205). Many of the observations of the effect of bacterial or fungal parenchymal infection have been made using enhanced CT (203). In the very early cerebritis stage, there is minimal BBB damage as evidenced by contrast enhancement but contrast enhancement does develop rapidly and is present before a collagen capsule is present (203). Therefore, the classical CT ring enhancement does not equate with an encapsulated abscess (204), but has been shown to be related to the acute inflammatory reaction. Therefore, in the cerebritis stage the effect on the BBB permeability is diffuse, so the CT contrast enhancement also may be diffuse.

In the capsule stage of an abscess, a collagen capsule develops around the necrotic center. The typical CT ring enhancement reflects the inflammatory infiltrate associated with the capsule, whereas the lack of central enhancement reflects the necrotic center.

Therefore, it is apparent that any change in BBB permeability during the development of brain abscess is closely related to the presence of an inflammatory infiltrate (203), and the changing patterns observed on CT of the BBB between the cerebritis and capsule stages reflects the changing pattern of the inflammatory infiltrate (203). The absence of contrast enhancement is thus important because it indicates a lack of a significant inflammatory response. This may reflect an early cerebritis stage but it is also seen in immunocompromised patients with a more ominous significance (203, 206).

The increased permeability of the BBB and the presence of infection may help the delivery of antibiotics to the site of a brain abscess, but this permeability has been shown to be variable (207) and the consistent delivery of antibiotic therapy across the BBB remains a problem.

Viral infections. Compared with the prevalence of other systemic viral diseases, viral infections of the CNS are infrequent, reflecting the apparent success of the BBB and other defenses against invasion by viruses (208).

Although virus infection may occur by both the neural and the olfactory routes, it is now recognized that the hematogenous route is the major pathway of CNS virus infection and that blood-borne viruses may indeed penetrate the BBB by various methods (208). Direct infection of the vascular endothelium may compromise the BBB (208). Leukocytes have been shown to cross an intact BBB; this is a potential source of spread of viruses into the brain tissue. Viruses may also traverse the BBB by viropexis (209).

The brain and spinal cord have evolved as relatively immunologically inert sites, presumably because an intact BBB shields the CNS not only from infection but also from antigenic stimulation (208). However, in the presence of a viral infection, there is an inflammatory response associated with an accumulation of mononuclear cells. This leads to compromise of the BBB, allowing the CNS to generate an immune response (208). Lymphocytes, immunoglobulins, and complement components pass into the extraneural

spaces to clear the viral infection. Immunosuppression obviously reduces the strength of any such immune response; therefore, it also alters the pattern of CNS viral infections, as dramatically illustrated by patients with acquired immunodeficiency syndrome (210). Because inflammation is the major factor in altering the integrity of the BBB, the limited inflammation exhibited during viral infections in the immunosuppressed patient may allow the BBB to remain relatively intact (208).

The infrequency and often sporadic nature of most human viral CNS infections provides limited opportunity for observing changes in the BBB. In herpes simplex encephalitis, cerebrospinal fluid albumin concentrations are variable, whether early or late in the course of the disease. This presumably reflects a varying degree of BBB disruption in different cases (208). Imaging of the BBB in herpes simplex encephalitis has shown this variability of BBB breakdown, but in general imaging of the BBB reveals abnormalities a few days after the onset of symptoms, although early findings can be subtle and apparent only in retrospect (208). The precise contribution of the BBB to the pathogenesis of human immunodeficiency virus–induced neurologic disease, acquired immune deficiency syndrome dementia complex, is uncertain. A lack of inflammatory response during acquired immunodeficiency syndrome would suggest that the BBB remains relatively intact, but some permeability must exist at least at low levels because human immunodeficiency virus–infected lymphocytes and macrophages apparently find their way from the blood into the CNS to initiate infection (210). An intact BBB has the potential to interfere with the delivery of antiviral drugs, but several nucleoside analogues such as acyclovir have achieved good cerebrospinal fluid levels and hence have proved effective in herpes simplex encephalitis.

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