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SPECIAL ARTICLE

# MECHANISMS OF DISEASE: THE BLOOD-BRAIN BARRIER

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**OBJECTIVE:** The blood-brain barrier (BBB) is often perceived as a passive membrane. However, evidence has demonstrated that the BBB plays an active role in normal homeostasis and in certain disease processes.

**METHODS:** Approximately 300 peer-reviewed publications that discussed normal or abnormal BBB function were reviewed.

**RESULTS:** The role of the BBB and how it contributes to disorders of the central nervous system vary, depending on the specific disease process.

**CONCLUSION:** In health and disease and extending to old age, endothelial cells, neurons, and glia constitute a neurovascular unit that regulates the BBB. Advances toward penetrating the BBB must account for both normal and abnormal functions of the neurovascular unit.

**KEY WORDS:** Barrier, Blood, Brain, Central nervous system delivery

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The blood-brain barrier (BBB) consists of a layer of endothelial cells that line the blood vasculature throughout the brain (*Fig. 1*). This layer is held together by tight junctions produced in response to signals from astrocytes. These tight junctions prevent small molecules from diffusing through the gaps between the cells (i.e., the paracellular route) (*Fig. 2*). Because the diffusion of molecules across the cells via other means (e.g., pinocytosis) is minimal, these tight junctions and their inherent impermeability to water-soluble molecules or molecules larger than $M_r$ 200 to 400 create the BBB.

The BBB permeability of most molecules can be predicted on the basis of their octanol/water partition coefficients (68, 83, 84). For example, diphenhydramine (Benadryl), which has a high coefficient, easily accesses the brain, whereas water-soluble loratadine (Claritin) does not cross the BBB and has little effect on the central nervous system (CNS) (30). However, permeability is not absolute and diffusion across the BBB reflects the octanol/water partition coefficient, although it may be low. Therefore, virtually all albumin (30 mg/100 ml) in cerebrospinal fluid (CSF) is from the ultrafiltration of serum (3 g/100 ml), as is the small amount of immunoglobulin M (approximately $M_r$ 1,000,000) that is normally present in the CNS.

Substances with low partition coefficients that easily penetrate the CNS are generally ushered across the BBB via active or facilitated transport (*Fig. 2*). Transport is often asymmetric, depending on ion channels, specific transporters, energy-dependent pumps, and a limited amount of receptor-mediated endocytosis. Glucose, amino acids, and small intermediate metabolites, for example, are carried into the brain via facilitated transport mediated by specific proteins, whereas larger molecules, such as insulin, transferrin, and other plasma proteins, are carried across the endothelial layer via receptor-mediated or adsorptive endocytosis (55).

Some small solutes with high octanol/water partition coefficients are observed to poorly penetrate the BBB. Recent studies demonstrated that these molecules are actively transported back into the blood by efflux systems (55). These systems can be particularly troublesome for drug delivery across the BBB. For example, P-glycoprotein (P-gp), which is a member of the adenosine triphosphate-binding cassette family of exporters and is found in brain capillaries, has been demonstrated to be a potent energy-dependent transporter. P-gp contributes greatly to the efflux of xenobiotics from the brain and has increasingly been recognized as having a protective role and conferring drug resistance by impeding the delivery of therapeutic agents (70) (*Fig. 2*). The organic anion transporters and glutathione-dependent multidrug resistance-associated proteins (MRP) also contribute to the efflux of organic anions from the brain and CSF, and many (if not most) drugs with CNS permeability that is lower than predicted are substrates for these efflux proteins (*Fig. 2*).

Recent research suggested that blocking of these transporters, some of which can be inhibited by probenecid, for example (9), could improve delivery across the BBB. *MDR1*-deficient mice, for example, are phenotypically normal unless exposed to drugs that are normally pumped out of the endothelial cells by P-gp (70). Furthermore, it was observed that a colony of dogs that exhibited unexpected neurotoxicity with the administration of ivermectin, which is an excellent P-gp substrate, were deficient in the transporter (44). Attention has

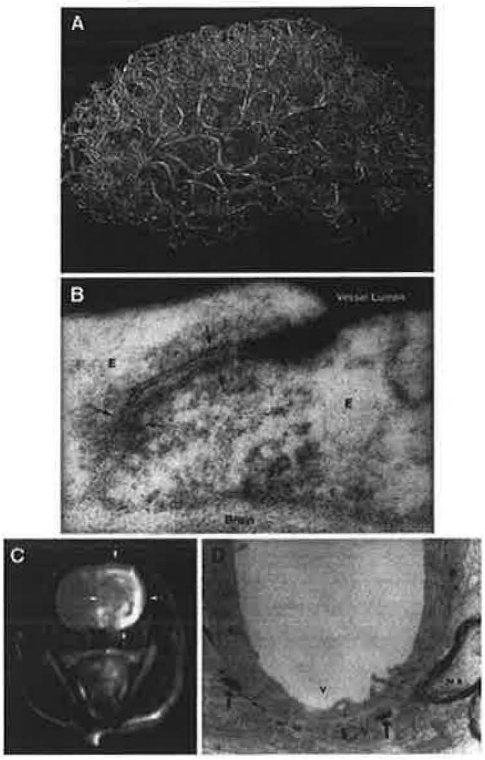


**FIGURE 1.** *Blood vessels in the human brain* (A *and* B) *and imaging of the BBB* (C *and* D). *There are approximately 400 miles of capillaries in the brain, with a surface area of approximately 12 $m^2$, which is 1000 times larger than the surface area of the choroid plexus, the origin of the blood-CSF barrier.* A, *photograph of blood vessels. A plastic emulsion was injected into the brain vessels, and brain parenchymal tissue was dissolved* (from, *Zlokovic BV, Apuzzo MLJ: Strategies to circumvent vascular barriers of the central nervous system.* ***Neurosurgery*** *43:877–878, 1998 [92]).* B, *electron micrograph demonstrating electron-dense lanthanum (dark areas in the vessel lumen) that is unable to penetrate beyond a tight junction* (arrows) *between endothelial cells* (E) *(courtesy of Dr. Milton Brightman, National Institutes of Health).* C *and* D, *imaging with superparamagnetic iron oxide particles partially coated with dextran (Feridex; Berlex Laboratories, Inc., Wayne, NJ), which are the size of adeno-associated virus virions and can be observed at the light and electron microscopic levels and with MRI.* C, *MRI scan obtained after reversible BBB opening and intravascular infusion of superparamagnetic iron oxide particles. Distribution of the superparamagnetic iron oxide particles throughout the right cerebral hemisphere* (arrows) *is indicated by increased MRI signal.* D, *electron micrograph demonstrating that all of the particles are trapped in the basement membrane* (arrows) *and are not exposed to brain parenchyma. Thereby MRI can suggest global CNS delivery across the BBB when the contrast agent (iron particles) is actually trapped at the basement membrane (46)* (D, *original magnification, ×19,000).* V, *vessel lumen;* MA, *myelinated axon.*

also been focused on other efflux transporters, particularly nucleoside transporters (21).

Inhibition of P-gp may also have consequences independent of drug delivery. Some glial tumors, for example, demonstrate increased levels of P-gp, which has generated interest in the development of inhibitors of this transporter as chemotherapeutic agents (70). Unfortunately, effective doses of P-gp inhibitors, such as cyclosporin and verapamil, are often toxic. More detailed information on the BBB can be found elsewhere (5, 7, 26, 40, 48, 49, 55).

## CHOROID PLEXUS AND OTHER CIRCUMVENTRICULAR ORGANS

In addition to the endothelium of the BBB, tight junctions are found in the epithelium of the choroid plexus (28) and in the arachnoid membrane that surrounds the surface of the brain and the spinal cord. These tight junctions limit the movement of solutes into and out of the CSF. Microvilli give the small choroid plexus a large surface area (48, 55), ensuring that the brisk blood flow through the plexus can replace the total CSF volume every 3 to 4 hours. Net sodium transport across the plexus from the blood is a cardinal feature of CSF production, with concomitant chloride and bicarbonate transport. Potassium and calcium ions, urea, and some drugs (such as penicillin) are pumped out of the CSF (48, 55) and micronutrients such as ascorbic acid are specifically pumped into the CSF at the plexus. After solutes enter the CSF, they can theoretically proceed to the brain unimpeded; however, the small extracellular space in the CNS ensures that diffusion into the brain is limited.

The CSF acts as a "sink," draining proteins and other metabolites from the interstitial fluid flowing from the ventricles over the surface of the brain; protein levels are generally approximately 5 mg/100 ml in the ventricles, compared with 30 to 40 mg/100 ml in the subarachnoid CSF. The brain is drained by the CSF as it egresses, mainly via arachnoid granulations but also, at least in animal models, via nerve roots, the carotid sheath, and olfactory tracts. The volume of CSF that leaves the CNS via these pseudolymphatic pathways may nearly equal the volume of CSF that leaves via arachnoid granulations (47). Although influx into the CNS via tight junctions is very restricted, this egressing fluid may contain components up to the size of intact white blood cells. These contrasting properties ensure that the brain is highly protected by a unidirectional barrier (48, 55).

The circumventricular organs of the hypothalamus, which lack a BBB, and the choroid plexus may also play key roles in metabolism. Both are densely packed with leptin receptors, which, after binding leptins produced by peripheral adipocytes, initiate a signal cascade that results in appetite suppression (90). Exactly how leptins access the hypothalamus is not well understood (43), but evidence suggests that decreased access across the hypothalamus BBB mediates obesity (93).

Because of these blood-CNS barriers and the lack of true lymphatic vessels, the CNS is often considered immunologically privileged. This privileged status is readily demonstrated by the fact that allogenic and even xenogenic grafts, which would be rapidly rejected if placed systemically, survive in the

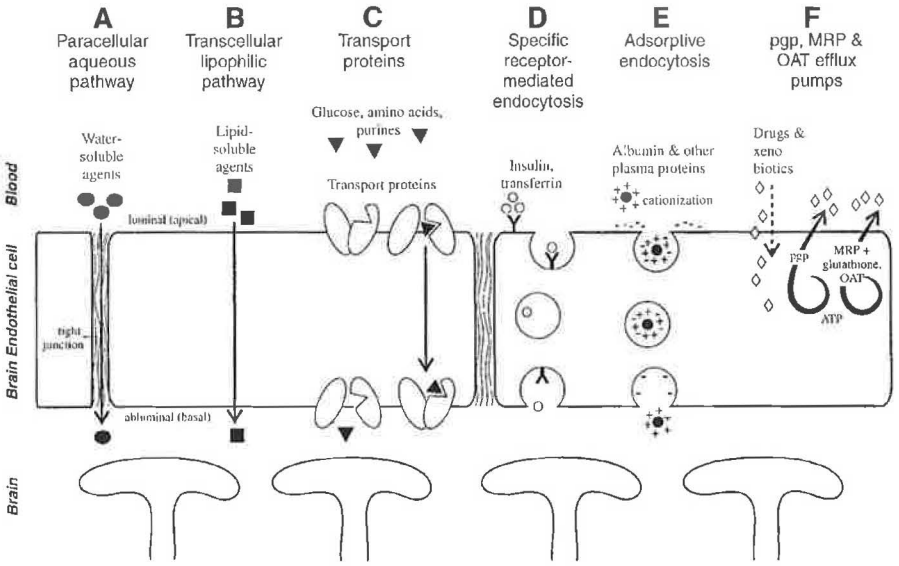


**FIGURE 2.** *Mechanisms and routes through the BBB (1, 22, 41, 58, 67, 81, 89). For normal homeostasis, carrier-mediated transport systems exist for hexoses (glucose, mannose, and galactose), monocarboxylic acids (acetic, lactic, and pyruvic acids), large neutral amino acids (tyrosine, phenylalanine, and isoleucine), acidic amino acids (glutamate and aspartate), basic amino acids (arginine and lysine), nucleic acid precursors (adenine, adenosine, and guanine), choline, and thyroid hormones (via transthyretin) (22). Concern regarding the consumption of sucrose and/or the artificial sweetener aspartame by children leading to increased delivery across the BBB via transporters, resulting in hyperactivity, was not documented with assessments of behavior and cognition, even after unusually high intake (89). The large neutral amino acid transporter, like the glucose transporter, is present on both the luminal and abluminal membranes of endothelial cells (67). At least 10 neutral amino acids compete for transport via the large neutral amino acid transporter, as indicated in a recent study of phenylketonuria, a disorder that causes extremely high levels of the neutral amino acid phenylalanine in the CNS, resulting in retardation unless dietary restriction is implemented (58). Transporters such as the monocarboxylic acid transporter for lactate and ketone bodies are particularly important during the neonatal period, with seizures, and during long-term fasts, when lactate and ketone bodies are important energy sources for the brain and levels of the transporter increase 8- to 25-fold (41). Whereas the glucose transporter and large neutral amino acid transporter are bidirectional (although they are most important for influx), other transporters are unidirectional efflux systems. For example, the inhibitory neurotransmitters glutamate and glycine are present in blood at concentrations 1000-fold greater than those in the CNS. Such levels in the CNS would be highly neurotoxic, as they are when the BBB is compromised after craniocerebral trauma or stroke (81). Therefore, the carrier for small neutral amino acids is primarily located on the abluminal membrane and allows only efflux. Similarly, brain extracellular potassium levels are maintained at approximately two-thirds the levels in blood, so as to not interfere with neural transmission. Potassium efflux is also accomplished with an energy-dependent process exchanging sodium for potassium at the abluminal membrane, with adenosine triphosphate (ATP) as the energy source ($Na^+/K^+$-adenosine triphosphatase). Encoded by the multidrug resistance gene (MDR1), P-gp is localized to the luminal membrane of endothelial cells and pumps amphipathic organic cations or neutral compounds out into the capillaries. Multidrug resistance-associated protein (MRP) and organic anion transporter (OAT) pump anions out of the CNS as efflux proteins* (modified from, *Abbott NJ, Romero IA: Transporting therapeutics across the blood-brain barrier.* ***Mol Med Today*** *2:106–113, 1996 [1]).*

CNS. However, extracellular fluid and CSF can flow to superior cervical lymph nodes, via the olfactory nerves, to activate the systemic immune system (particularly the humoral arm) against antigens such as albumin (32). In addition, although transport across the BBB is tightly regulated, a slow influx of white blood cells into the CNS after bone marrow transplantation has been noted, and findings of 1 to 3 lymphocytes/$mm^3$ of CSF are considered normal. These findings indicate that the immunological privilege is only partial and may be subject to changes in disease states. The infiltration of white blood cells into the CNS after bone marrow transplantation, for example, can have a major effect in preventing neurological symptoms among children with globoid leukodystrophy and metachromatic leukodystrophy (35); lymphoma cells can preferentially target the CNS via the choroid plexus and cranial nerves (25).

## OUTWITTING THE BBB

The treatment of Parkinson's disease represents a model of successful treatment using the BBB (38). The introduction of L-dopa revolutionized therapy for Parkinson's disease, because L-dopa crosses the BBB via the neutral amino acid transporter (which is normally only half-saturated) and is then converted by dopa decarboxylase to biologically active dopamine. Treatment with L-dopa also demonstrates the "metabolic BBB." The dopa decarboxylase inhibitor carbidopa, which cannot enter endothelial cells, systemically blocks the metabolism of L-dopa. However, the precursor (dopa) can be metabolized to dopamine as it crosses the endothelium and can be trapped there (i.e., the metabolic BBB).

Unfortunately, analogous therapies using acetylcholine precursors, such as phosphatidylcholine or diaminoethanol, have not been effective for the treatment of movement disorders such as Huntington's chorea (48). However, direct infusion into CSF of the γ-aminobutyric acid agonist baclofen has been effective for the treatment of spinal spasticity (48). Intranasal administration is another promising avenue, allowing drugs to access the CNS via the olfactory nerves (7).

Sometimes the formulation and route of delivery can be modified to increase BBB permeability. Smith et al. (78) demonstrated that intra-arterial infusion of chlorambucil, which is normally tightly bound to proteins, in a protein-free infusate increased delivery to the CNS, because of the high lipophilicity of the drug. The opposite approach is also sometimes successful. Drugs bound to cationized albumin bind to the negatively charged cerebral endothelium and are transported

across the BBB by adsorption-mediated transcytosis. Other delivery vectors also demonstrate promise (54). Friden et al. (19) shuttled nerve growth factor across the rat BBB by conjugating it to the monoclonal antibody Ox-26, which targets the transferrin transporter. Avidin linked to an insulin fragment or an antibody to the BBB insulin receptor allowed Shi and Pardridge (54, 73) to shuttle biotin-coupled drugs or imaging agents (up to 4% of the injected dose) across the endothelium. Even an intact enzyme ($\beta$-galactosidase) has been delivered across the BBB, by being coupled to the human immunodeficiency virus (HIV) TAT protein (71).

For decades, direct instillation into the brain and CSF produced only modest benefits; however, this approach is currently undergoing a resurgence of interest. Prophylactic intrathecal or intraventricular infusion of methotrexate and/or cytosine arabinoside has been demonstrated to markedly reduce the rates of death attributable to leukemic meningitis, which was virtually unknown in the 1950s but, with the advent of effective systemic chemotherapy, has become a major cause of death for children with lymphocytic leukemia. This approach is less effective for the treatment of overt CNS leukemia or lymphomas, because tumor cells fill the perivascular Virchow-Robin spaces, which limits the ability of drugs placed in the CSF to reach the cells (8). Another strategy that has demonstrated little success to date but is promising involves direct injection of fetal neuronal precursor cells or neurons directly into the brain for treatment of clinical movement disorders such as Parkinson's disease (18).

The major limitation with the instillation of drugs, proteins, viruses, and cells directly into the brain is the small extracellular space available. Secondary impediments to success include agent size, adsorptive properties, and efflux. For example, treatment of gliomas with surgical implantation of polifeprosan 20 with wafer carmustine (10), a polymer that slowly degrades and releases the lipophilic nitrosurea *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea, has demonstrated only limited success because of the slow diffusion of *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea and its high lipophilicity, which permits efflux back across the BBB. Similar diffusion problems were encountered when fibroblasts that released retroviruses carrying cytotoxic genes were placed in the brain; infected tumor cells were observed only within a few cell diameters of the injection site (64). However, direct injection into rodent brains could reverse symptoms of genetic neurodegenerative disease (77). The key problem with direct intracerebral injection models is that drugs with good therapeutic efficacy in 1-g rat brains usually do not demonstrate the same efficacy in human brains, which weigh more than 1000 g, unless the target volume is small (i.e., basal ganglia in Parkinson's disease).

One approach to solving delivery problems involves coupling the direct instillation of drugs or vectors with mechanical manipulation, to maximize the distribution of the agent. Arterial pulsations, for example, may accelerate drug movement in the brain and along perivascular spaces by increasing bulk flow. Chen et al. (13) promoted the use of this strategy, which is currently referred to as convection or clysis, to increase the volume of distribution. Testing in animals and among patients with brain tumors has demonstrated that chronic convection can increase brain sucrose levels up to 10,000-fold, compared with intravenous infusion (23). Convection may be best suited for treatment of focal rather than global CNS disease, because global delivery is not possible with current clysis methods (*Fig. 3, A* and *B*).

An alternative to direct instillation or the use of vectors is reversible opening of the BBB. Focal and global BBB openings have been attempted with a variety of strategies (49, 51). Chemicals such as dimethyl sulfoxide, bradykinin or its stable potent analog RMP-7, and the antineoplastic agent etoposide and mechanical stresses such as hypertension and hypercapnia have all been used, but none has been as consistent and safe as osmotic BBB disruption (BBBD). Osmotic BBBD is facilitated by hypertonic nonmetabolizable solutes that are injected intra-arterially and can open the BBB to components from small drugs to proteins and even the herpesvirus (180 nm in diameter). Intra-arterial infusion at a rate to yield a pressure just higher than the blood pressure for at least 20 seconds reversibly opens the BBB in the region of the infused arterial circulation for approximately 30 minutes. This increases delivery of drugs to the CNS up to 100-fold, compared with levels achieved with intravenous infusion; even greater relative increases are achieved for proteins and viruses (16, 34). An advantage of BBBD is that, after the BBB has closed, drugs in the systemic circulation can be neutralized (cleared) with chelators or modifying agents (60), because the resealed BBB once again creates two distinct compartments (*Fig. 3, C* and *D*).

## IMAGING OF THE BBB

Standard imaging of BBB integrity is performed with small, water-soluble, contrast agents with short plasma half-lives (usually $<1$ h). Iodinated contrast agents produce enhancement in the brain on computed tomographic (CT) scans, which indicates where there is a loss of BBB integrity (such as with malignant tumors, abscesses, or other lesions that cause vasogenic edema). The degree of enhancement on CT scans (measured in Hounsfield units) increases linearly with the amount of contrast agent entering the brain. For magnetic resonance imaging (MRI), chelated gadolinium is used as a water-soluble, paramagnetic, contrast agent. As with enhanced CT scanning, BBB breaches can be observed as enhancement on T1-weighted MRI scans, but with greater sensitivity than on CT scans. Signal intensity changes attributable to gadolinium enhancement on MRI scans are not linear, unlike CT scanning results.

A new class of MRI contrast agents, namely, superparamagnetic iron oxide compounds (ultra-small-particle iron oxide), are now being used to assess BBB integrity (*Fig. 1, C* and *D*). One such agent, ferumoxtran-10 (Combidex; Advanced Magnetics, Cambridge, MA), has a long plasma half-life (1–2 d) and is taken up by phagocytic cells (microglia and reactive

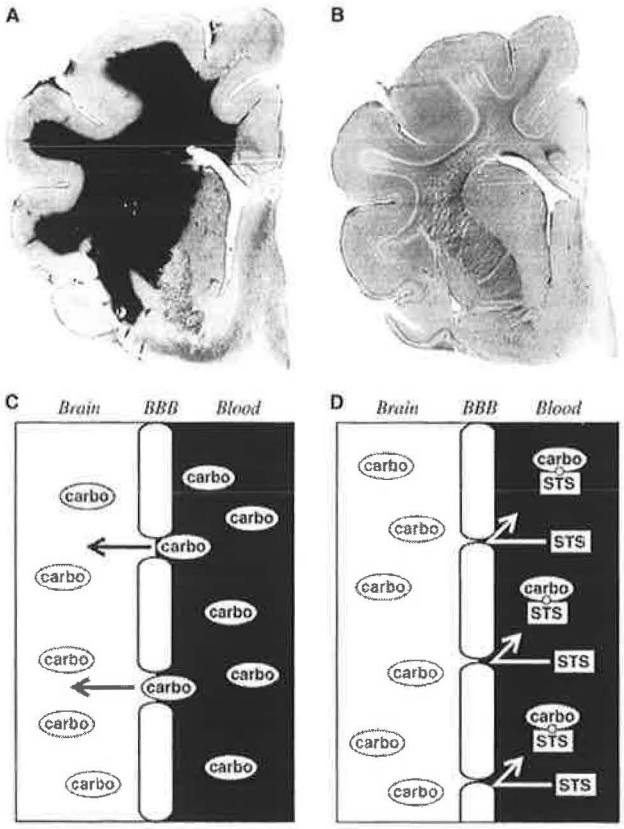


**FIGURE 3.** *Novel approaches for drug delivery. With a current focus on dose intensity and targeted delivery in CNS therapy, there is an increasing need to protect non-CNS tissues. The BBB permits novel approaches for dose-intensive and/or targeted delivery, with minimization of systemic toxicity (16). One method of targeted delivery that minimizes systemic toxicity is administration of therapeutic agents directly into the brain via convection.* A *and* B, *sections in which iron was infused into the cerebral hemisphere of a cat* (A *and* B, *original magnification,* ×5). A, *staining for iron, showing distribution* (black areas) *throughout the white matter* (A, *modified from, Muldoon LL, Nilaver G, Kroll RA, Pagel MA, Breakefield XO, Chiocca EA, Davidson BL, Weissleder R, Neuwelt EA: Comparison of intracerebral inoculation and osmotic blood-brain barrier disruption for delivery of adenovirus, herpesvirus and iron oxide particles to normal rat brain.* ***Am J Pathol*** *147:1840–1851, 1995 [45]).* B, *hematoxylin and eosin staining, showing no evidence of tissue damage. Myelin staining yielded similar results (data not shown).* C *and* D, *diagrams indicating that the BBB offers a unique two-compartment model to separate cytotoxic drugs aimed at killing CNS tumors from chemoprotectant agents designed to protect tissues such as the cochlea and bone marrow. For example, carboplatin* (carbo) *can be administered intra-arterially after opening of the BBB* (C) *and then, 4 to 8 hours after BBB closure, high doses of thiols such as sodium thiosulfate* (STS) *can be administered intravenously* (D). *Sodium thiosulfate does not cross the BBB and covalently binds non-CNS carboplatin, preventing most ototoxicity and even mitigating thrombocytopenia (16)* (C *and* D, modified from, *Neuwelt EA, Brummett RE, Doolittle ND, Muldoon LL, Kroll RA, Pagel MA, Dojan R, Church V, Remsen LG, Bubalo JS: First evidence of otoprotection against carboplatin-induced hearing loss with a two compartment system in patients with central nervous system malignancy using sodium thiosulfate.* ***J Pharmacol Exp Ther*** *286:77–84, 1998 [50]).*

astrocytes) and generally not by tumor cells. Therefore, despite their large size, relative to standard gadolinium contrast agents, these compounds facilitate imaging of brain tumors with slow leakage into the tumor and brain tissue around the tumor and uptake (trapping) by reactive cells in and around the tumor. With this approach, additional and/or larger lesions (*Fig. 4*) have been identified among some patients with brain tumors, in comparison with standard gadolinium-based scans (86). These agents may also facilitate imaging of inflammatory brain lesions, including multiple sclerosis (MS) and stroke. A National Institutes of Health-sponsored clinical trial of iron oxide MRI agents for observation of intracerebral tumors and other CNS lesions is ongoing.

## ROLE OF THE BBB IN DISEASE

### Infections

Much is known about bacterial infections of the CNS (62). Bacteria such as meningococci (61) colonize the nose, enter the vascular system, and bind, via pili, to the endothelium of both the brain and the choroid plexus. In particular, the PilC protein, which is found on the tip of the pilus, is up-regulated in bacteria isolated from CSF, compared with bacteria in the blood, and this protein may play a crucial role in adhesion.

In the case of pneumococci (65), bacteria bind to the endothelial cell surface via platelet-activating factor receptors. The ensuing CNS infection induces increased vesicular transport across cells and separation of tight junctions, leading to the release of inflammatory peptides such as interleukin-1, tumor necrosis factor, and metalloproteinases (56). To decrease the inflammatory damage, corticosteroids are sometimes administered, but they can restore the BBB integrity, thus decreasing antibiotic delivery and slowing bacterial clearance from the

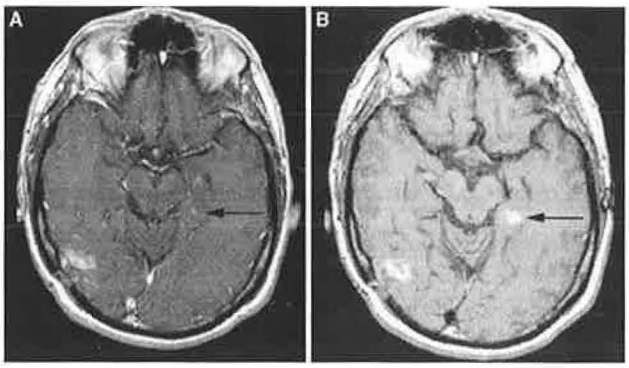


**FIGURE 4.** *MRI scans for a patient with a glioblastoma after an excellent response to temozolomide, a new oral chemotherapeutic drug that crosses the BBB very well. Residual disease was assessed on T1-weighted MRI scans, with two intravenously administered contrast agents.* A, *gadolinium-enhanced scan.* B, *ferumoxtran-10-enhanced scan. The residual lesion in the right hemisphere, which can be clearly observed with both gadolinium and ferumoxtran-10, should be noted. A left-side lesion that is not well detected with gadolinium but is easily observed with iron* (arrows) *should also be noted.*

CNS. However, corticosteroids can also decrease the rates of morbidity, such as hearing loss among young children with *Haemophilus influenzae* meningitis, and the benefits of adjunctive corticosteroid treatment thus remain controversial. Antioxidants such as *N*-acetylcysteine may be safer for decreasing CNS injury (4).

Some viruses (such as herpesvirus) enter the CNS via the olfactory nerves, and others (such as rabies virus) enter via spinal nerves (48). Some may enter via the circumventricular organs, but most probably cross the brain endothelium or the choroid plexus. In the case of HIV, brain endothelial cells lack the CD4 and galactosylceramide binding sites that are used by the virus to enter most cells. There is evidence that adsorptive endocytosis in response to cytokines can mediate uptake, although most HIV entry is via infected monocytes (57), which traverse abnormal tight junctions (14) and/or penetrate endothelial cells. After HIV has penetrated the BBB, it can proliferate in microglia and other non-neuronal parenchymal cells, to be released systemically to the venous blood via the arachnoid granulations and to the cervical lymph nodes via the olfactory tracts and nerves. Therefore, the CNS can function as both a reservoir and an HIV factory that releases the virus to the systemic circulation and the cervical lymph nodes, while being protected from systemically administered antiviral drugs by the BBB (82).

It is fortunate that infection may facilitate the influx of antibiotics, such as penicillin, across the BBB. For example, the CSF concentrations of penicillin G are usually approximately 0.4% of the steady-state serum levels in normal animals but CSF levels increase approximately 10-fold in animals with pneumococcal meningitis (48). BBB integrity varies with the type of infective agent; viruses cause minimal BBB damage, making delivery of antiviral agents a greater challenge for physicians. In general, BBB integrity may be better correlated with CSF protein levels than with CSF white blood cell counts, and CSF analyses may facilitate selection of appropriate doses of therapeutic agents. Monitoring of the CSF may also be prudent, because the BBB may be quickly reestablished as the infection subsides, reducing the permeability to antiinfective agents severalfold. The keys to the treatment of CNS bacterial infections are rapid establishment of bactericidal antibiotic levels and maintenance of such levels throughout the course of therapy (62).

## Inflammatory CNS Disorders and MS

Lymphoid cells normally circulate in the vasculature, extravasate into tissues (including brain tissue), traffic through the lymphatic vessels (via arachnoid granulations or the olfactory nerves [32] in the CNS), and return to the circulation. Extravasation into the brain through the BBB may be influenced by weak inflammatory stimuli such as pain (27) and can be accentuated by stronger stimuli such as complete Freund's adjuvant, which can open the BBB to albumin, immunoglobulin G, and immunoglobulin M, without concomitant extravasation of lymphoid cells and without gliosis (63). Such extravasation may underlie CNS lupus, in which there is usually minor vasculopathy despite profound encephalopathy.

Infections and other immunological stimuli, such as Freund's adjuvant plus myelin basic protein, can cause experimental allergic encephalomyelitis (EAE) in animals (48). Experimental allergic encephalomyelitis is widely, but not uniformly, considered to be a model of MS. In both MS and experimental allergic encephalomyelitis, increased BBB permeability and lymphoid cell extravasation (59) across the BBB (*Fig. 5*) are early events (52). Extravasation of $CD4^+$ T cells sensitized to several self-myelin and nonmyelin MS antigens mediate the hallmark of MS, namely, demyelination. Lymphoid cells may transverse the cerebral capillaries through and/or between endothelial cells; the exact route is not clear. Locally produced antibodies are also involved and result in

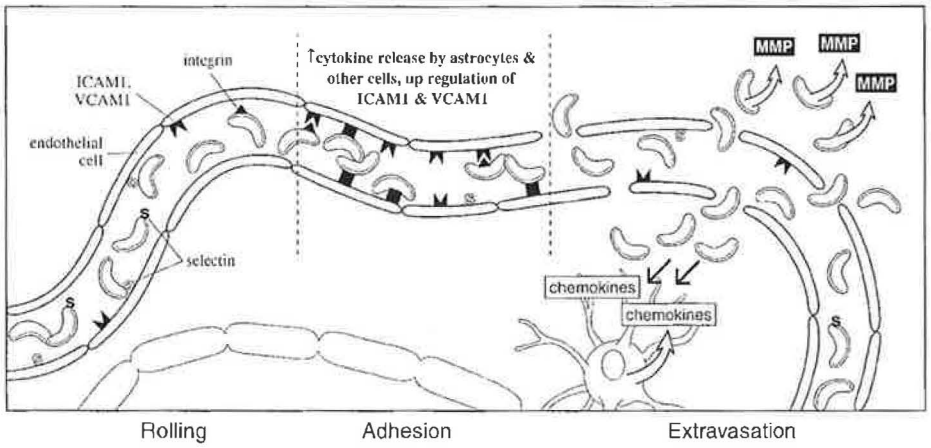


**FIGURE 5.** *Diagram of the extravasation process. Lymphoid cells roll along the endothelium in the body because of binding between selectins and their carbohydrate ligands, leading to firm adhesion of integrins to an immunoglobulin G-like superfamily, including intercellular adhesion molecule-1* (ICAM1) *and vascular cell adhesion molecule-1* (VCAM1). *Molecules such as intercellular adhesion molecule-1 are normally only barely detectable on the cerebral endothelial surface, but their expression is up-regulated in response to proinflammatory cytokines released by astrocytes and other cells (2). Such cytokines include tumor necrosis factor-α, interleukin-1β, and interferon-γ. Lymphoid cell adhesion results in rearrangement of the actin cytoskeleton of endothelial cells via a number of mediators, opening the BBB to lymphoid cell extravasation. Lymphoid cells and macrophages are attracted to the CNS by low-molecular weight chemokines (chemotactic cytokines), some of which are released by astrocytes. As the lymphoid cells ($CD4^+$ T cells) and macrophages extravasate, they release matrix metalloproteinases* (MMP), *which digest the extracellular matrix (31). Inhibitors of metalloproteinases represent an effective treatment for experimental allergic encephalomyelitis. Recent studies emphasized integrin binding to adhesion molecules but questioned the role of selectins in the brain (3, 85).*

oligoclonal bands with CSF electrophoresis, which represents an important diagnostic finding (48). Corticosteroids decrease the BBB leakiness in MS (20), whereas the three major therapeutic agents (15) (interferon-1$\beta$, interferon-1$\alpha$, and glatiramer acetate) all decrease the passage of immune cells across the BBB. BBB compromise and extravasation of lymphoid cells (59) via adhesion molecules, with modulation by cytokines and chemokines, are key events early in the pathogenesis of MS and autoimmune inflammatory diseases.

## Cerebrovascular Disease

The integrity of the BBB in cerebrovascular disease attributable to hypertension (29) or cardiac bypass (72) is variable, because the underlying cerebral ischemia varies with respect to mechanism, severity, and duration (11, 66). During ischemia, decreasing nutrient levels can lead to endothelial membrane failure, as can nitric oxide, which may be produced by the free radicals formed as a result of oxygen deprivation. Ischemia can also have effects similar to those of inflammatory disorders, leading, as noted, to the activation of cytokines such as tumor necrosis factor and interleukin-1 and the upregulation of cell adhesion molecules. White blood cells can then penetrate the BBB, releasing proteases (particularly metalloproteinases) and resulting in both cytotoxic and vasogenic edema; the latter is attributable to BBB opening, which can be detected on enhanced CT and/or MRI scans. Unlike with vasogenic edema observed in CNS tumors, corticosteroids have little effect on ischemia-induced edema, just as they have little effect on edema produced by trauma. Ischemia may also compromise the BBB by increasing vesicular transport across the BBB and by opening tight junctions. Transient ischemia is normally worse than permanent occlusion attributable to reperfusion injury (66). Hyponatremia, hyperglycemia, and fever can all exacerbate the process.

Cerebral insults such as stroke and trauma illustrate two basic concepts of BBB biological processes (6, 39, 66). The first is that BBB opening in and around the stroke or trauma can be biphasic, particularly after reperfusion injury (37). The second is that BBB opening is different for small versus large molecules. Among patients with ischemic infarctions, technetium-labeled diethylenetriamine penta-acetic acid ($M_r$ 398) can penetrate the BBB, whereas technetium-labeled albumin (approximately $M_r$ 68,000) cannot (24). More severe infarctions, however, can induce opening to both large and small molecules (48), suggesting that ischemic patients with more BBB damage may have greater risks of hemorrhage after fibrinolysis (i.e., reperfusion). In such cases, the administration of neurotropins with genetic delivery vectors may be neuroprotective if performed within 60 minutes (79).

Finally, the location of the BBB damage may depend on the type of cerebrovascular disease (48). Openings at capillaries are more likely with ischemia, whereas openings at arterioles are more often produced by diabetic vasculopathy, emboli, and hypertension and openings at venules can be induced by subarachnoid hemorrhage.

## CNS Tumors

Therapeutic options for the treatment of primary and metastatic tumors have been limited because of the BBB. Perioperative corticosteroid administration has reduced the mortality rates associated with brain tumor surgery from more than 50% to less than 5%, by rapidly restoring BBB integrity (12) and thus decreasing vasogenic cerebral edema and contrast agent enhancement. However, chemotherapy has proven only marginally successful (75, 87); for sensitive tumors such as small cell lung cancers, breast cancers, lymphomas, and germ cell tumors, there may be complete systemic responses to chemotherapy concomitant with tumor progression in the CNS (16).

There are several reasons for this difference in systemic versus CNS responses (36). The BBB is extremely heterogeneous and is frequently more permeable in the center of a malignant tumor, whereas the well-vascularized, actively proliferating, infiltrating edge, which is sometimes referred to as the brain tissue adjacent to the tumor, exhibits a variable degree of BBB integrity (*Fig. 6*) (75). These different permeabilities result in sharply reduced concentrations of chemotherapeutic agents at the rapidly growing periphery, because of limited diffusion from the central leaky hypoxic tumor (88). This is termed the sink effect, and it can contribute to chemotherapy failure.

In addition, because the bulk of a tumor gradually decreases with treatment, BBB integrity often recovers. In the case of primary CNS lymphomas, positron emission tomographic scans demonstrated that BBB integrity may be reestablished after 6 weeks of chemotherapy (53), but responses are short-lived unless high-dose chemotherapy and/or BBB modification techniques are used to enhance delivery as the BBB recovers. Indeed, BBBD-enhanced delivery of chemotherapeutic agents was observed to increase survival rates for patients with primary CNS lymphomas, with outcomes being statistically correlated with the number and degree of BBBDs (33).

BBBD has led to responses that are durable for primary CNS lymphomas and, although the approach is invasive, it has been used with relatively little toxicity. Kraemer et al. (33) reported an association between total dose intensity and survival times among 74 patients with primary CNS lymphomas (highly chemosensitive brain tumors) treated with BBBD (42). Using the total number of disruptions as a surrogate measure of total dose intensity (a weighted quality of disruption score is also discussed [33]), those authors demonstrated a statistically significant association between the number of disruptions (as a time-dependent covariate) and overall survival times for the patients, after adjustment for age, performance status, sex, and prior chemotherapy. *Figure 7* presents a Kaplan-Meier plot of overall survival times among patients grouped according to the number of BBBDs (corresponding to approximately 3-mo intervals). After accounting for survival bias, this analysis confirms the importance of total dose intensity (delivered in many courses, rather than in a short period) in the treatment of primary CNS lymphomas with BBBD.

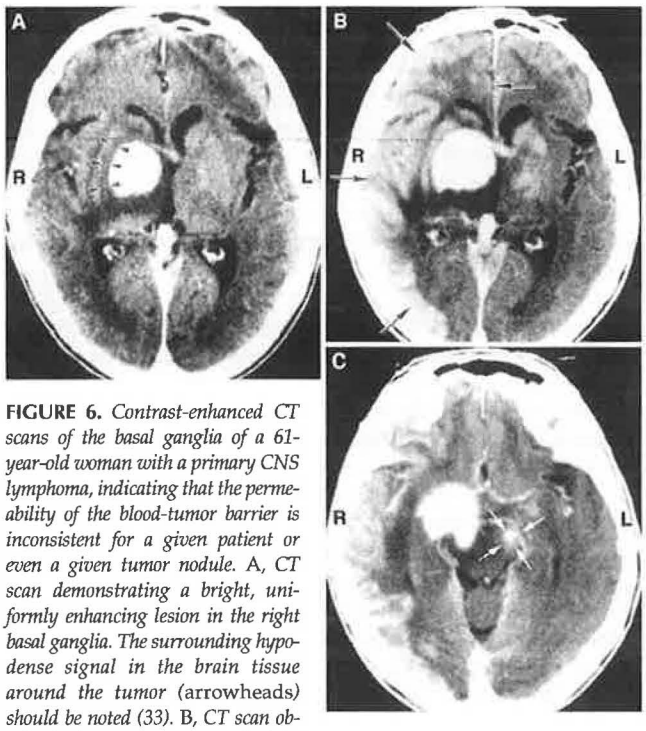


**FIGURE 6.** *Contrast-enhanced CT scans of the basal ganglia of a 61-year-old woman with a primary CNS lymphoma, indicating that the permeability of the blood-tumor barrier is inconsistent for a given patient or even a given tumor nodule. A, CT scan demonstrating a bright, uniformly enhancing lesion in the right basal ganglia. The surrounding hypodense signal in the brain tissue around the tumor* (arrowheads) *should be noted (33). B, CT scan obtained after contrast agent administration. Contrast material was administered immediately after osmotic BBBD and CT scans were obtained 30 minutes after the first BBBD treatment, to confirm and assess the grade of BBBD. The patient underwent right internal carotid artery disruption in the anterior and middle cerebral artery distributions* (arrows). *Opening of the brain tissue around the tumor in the area of the peritumoral hypodense signal evident in the CT scan in A should be noted (33). C, CT scan obtained after BBBD in a patient with a right hemiparesis that was unexplained, because the only visible tumor was in the right cerebrum (A). BBBD the day after the CT scan in A extended into the posterior circulation via the posterior communicating artery. A left-side brainstem lesion not apparent in pre-BBBD imaging studies was noted. The right hemiparesis was thus attributable to a brainstem tumor* (arrows) *on the left that was not apparent on pre-BBBD MRI scans (intact BBB and no edema). Silbergeld and Chicoine (76) demonstrated tumor cells 4 cm from T2-weighted abnormalities among patients undergoing stereotactic biopsies.*

Other BBB-bypass methods that may help patients with tumors include stem cell rescue in the treatment of oligodendrogliomas, as a means to avoid radiotherapy, which too often impairs cognitive function (17), and dendritic cell vaccines, which induce T cells to cross the BBB to the brain (91).

## CONCLUSION

Endothelial cells, in addition to glia and neurons, should be considered integral components of the CNS. Indeed, in both health and disease and extending to old age (69, 74, 80), these three cell types should be studied mechanistically as a neurovascular unit. Attention currently directed toward delivery of neurotherapeutic agents across the cerebral endothelium is modest, compared with studies of the other two components of the neurovascular unit. More research is needed to allow us to better understand and exploit the endothelium and to develop desperately needed therapeutic agents.

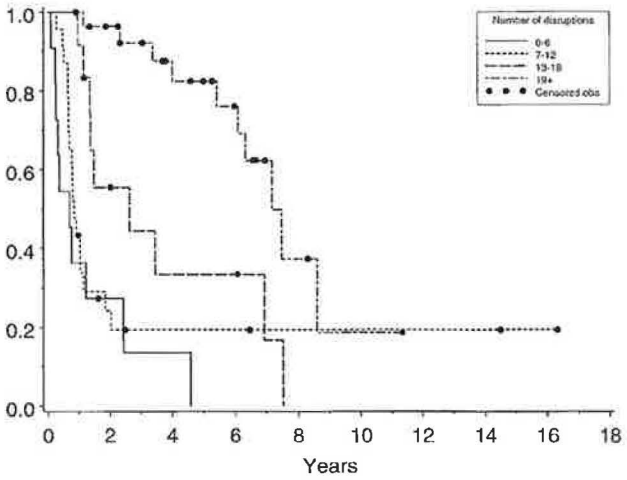


**FIGURE 7.** *Kaplan-Meier plot of overall survival rates for patients with non-AIDS primary CNS lymphomas treated with BBBD-delivered methotrexate. Patients were grouped according to the total number of courses; the groups represent approximate treatment periods of less than 3 months, 3 to 6 months, 6 to 9 months, or 9 to 12 months (33).* Circles, *censored observations. These results confirm the importance of dose intensity in the treatment of CNS neoplasms.*

## DISCLOSURE

EAN and the Oregon Health & Science University have significant financial interests in Adherex, a company that has a commercial interest in the results of this research and technology. This potential conflict of interest has been reviewed and managed by the Oregon Health & Science University Conflict of Interest in Research Committee. For more information, contact the Oregon Health & Science University Research Integrity Office (telephone: 503/494-7887).

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## COMMENTS

This is a superb review of the current status of the blood-brain barrier (BBB) in health and disease. It is packed with the most up-to-date information and is both well written and extremely easy to read. Basic research findings are translated into clinical relevance, and the article provides the reader an in-depth understanding of the physiology and pharmacology of how solutes (nutrients, endogenous peptides, proteins, and immune cells as well as exogenous drugs) either gain access to or are limited from the brain and cerebrospinal fluid in both health and disease. The status of the barrier changes vastly depending on the disease state, as critically reviewed by the author. Nevertheless, this physiological, pharmacological, and enzymatic barrier remains a nemesis in effective treatment of numerous neurological diseases. In many cases, effective drugs have been designed that incorporate the appropriate physicochemical features to maintain efficacious concentrations at the disease target within either the brain or cerebrospinal fluid; for other diseases, such drugs are not available and may never be developed. Entrepreneurial strategies to outwit the BBB have become crucial, and the most interesting have been critically reviewed. Such techniques have allowed innovative clinicians and scientists, as exemplified by the author, to make better use of currently available drugs whose conventional use in the treatment of brain-sequestered diseases is associated with poor outcome.

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In this excellent review, Neuwelt has described the BBB in terms of both anatomy and physiology. He has also described techniques used to break down the BBB to allow for passage of large-molecular-weight compounds. Neuwelt has published extensively on this subject and has presented a large body of data suggesting that BBB breakdown can include the efficacy of therapy using large-molecular-weight compounds, especially for the treatment of central nervous system (CNS) lymphoma.

I believe this review is useful in bringing a reader up to date in a concise fashion. One can only hope that these techniques can be applied to the treatment of other diseases with the goal of increasing efficacy.

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In this insightful review, Neuwelt presents current knowledge about the BBB and its role in the pathogenesis of various disorders of the CNS. Clearly, the physiological activity of the BBB affects virtually all aspects of brain function in both health and disease. Awareness of this fact compels us to revise previous concepts about the CNS and embrace a more encompassing notion of the neurovascular unit, which considers the endothelial cell of brain capillaries to be just as integral to neurophysiology as the neuron and glial cell.

As the author shows, the BBB has traditionally been an impediment to treatment of CNS disorders. It is estimated that more than 98% of all potential CNS drugs do not cross this partition (2). In a previous article, however, he labeled the BBB the "Achilles' heel of CNS therapeutics" and presaged potential opportunities for outwitting it (3).

For instance, adsorptive endocytosis allows the ingress of specific peptides and proteins across the BBB. Approximately 15 transporters have been characterized so far, and more than 50 may exist (2). Drug conjugation to ligands or antibodies directed against these receptors can enhance entry into the brain via transcytosis. This "Trojan horse" strategy has successfully escorted therapeutic molecules across the BBB in animal models of stroke and brain tumors. The BBB can also be transiently opened with brief intra-arterial infusions of hypertonic solutions, such as mannitol or arabinose, that produce osmotic shrinkage of the endothelial cells and mechanical separation of the tight junctions, or with the infusion of various inflammatory mediators.

When coupled with these techniques of permeabilizing the BBB, selective intra-arterial delivery of genetic or cellular therapeutic agents to the CNS may circumvent many of the limitations imposed by conventional routes of access (1). The advantages of this strategy over craniotomy or stereotactic instillation include the potential for widespread distribution, the ability to deliver large volumes, limited perturbation of neural tissue, and the feasibility of repeated administration. Therapeutic agents may be injected into the CNS arterial system as liquid suspensions or may be integrated into mechanical scaffolds (such as stents or coils) that are deposited intravascularly, allowing release of the biological mediator with regulated temporal and spatial profiles. Polymers such as poly-L-lactic acid and polyglycolic acid have been engineered with mechanical properties, porosity, and degradation rates favorable for their use as reservoirs for intravascular delivery of therapeutic agents. Biodegradable polymeric coils and stents have provided the platforms for local delivery of recombinant growth factors, cell cytokines, recombinant viruses, and other gene therapy vectors after endovascular placement. Incorporation of gene therapy vectors into resorbable endoluminal stents and coils has allowed site-specific, sustained transduction of cells of vessel walls, including the adventitia. Impregnating these devices with more mobile vehicles, such as neural progenitor cells, may allow gene transfer into the surrounding parenchyma as well.

Therefore, it is reasonable to envision catheter-based deposition of molecular, genetic, and cellular therapies that treat both the arterial wall and neighboring tissues of the brain and that are based on emerging understanding of the physiology of the BBB (1). Thus, the review that Neuwelt has provided is both timely and vital.

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3. Neuwelt EA, Abbott NJ, Drewes L, Smith QR, Couraud PO, Chiocca EA, Audus KL, Greig NH, Doolittle ND: Cerebrovascular biology and the various neural barriers: Challenges and future directions. **Neurosurgery** 44:604–609, 1999.

This authoritative review covers a broad range of issues related to the BBB, ranging from basic principles of function under physiological conditions to disrupted or altered function in selected disease states. Some of the highlights of the section on physiological function include the description of the multiple contributors that determine the extent of movement of soluble molecules into and out of the CNS. These include passive diffusion and active influx and efflux transport mechanisms that are operative at the level of the BBB, the choroid plexus, and the selected CNS regions that lack a BBB (circumventricular organs of the hypothalamus). These are all of central importance for the issue of drug delivery into the CNS. The review describes the challenges of how to "outwit" the BBB by manipulating transport systems and using novel vector delivery systems and/or direct intrathecal injections. The latter bypasses the restrictions imposed by the BBB but not the problem of diffusion within the CNS tissue. Illustrated in the review are emerging neuroimaging techniques that will enhance our capacity to evaluate the status of the BBB in vivo.

The section on disease includes consideration of infectious, inflammatory, vascular, and neoplastic disorders, each of which is affected by function and dysfunction of the BBB. Important issues raised include how infections access the CNS, the regulation of autoreactive immune cell trafficking across the BBB, the effect of ischemia on the BBB, and how to sustain tumor-directed chemotherapy responses. As the review concludes, an enhanced understanding of the biology of the BBB will enhance our opportunities to manipulate its properties for therapeutic purposes.

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There is a resurgence of interest in the biology of the BBB. Disruption of the BBB is an early event in many neuroinflammatory conditions, including bacterial meningitis and multiple sclerosis, and occurs secondary to cell damage in ischemia and trauma. Astrocytes, neurons, and pericytes around the endothelial cells form a neurovascular unit. Between the endothelial cells and the astrocytic end feet is a basal lamina. Damage to any of the components affects the function of the entire unit. Many laboratories are attempting to discover novel ways to alter BBB permeability transiently to allow drugs to pass from the blood into the brain. In this review, Neuwelt describes a wide range of experimental approaches. Some are based on the action of drugs, whereas others use changes in serum osmolality.

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