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Blood-Brain Barrier: Morphology, Physiology, and Effects of Contrast Media

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The blood-brain barrier (BBB) separates the brain and cerebrospinal fluid from the blood and regulates the exchange of substances between the blood and the brain. It is comprised chiefly of brain capillaries, choroid plexus cuboidal epithelium, and the arachnoid membrane (23). Barrier-type capillaries are located also in the retina, iris, inner ear, and within the endoneurium of peripheral nerves (27). All BBB sites are characterized by the presence of tight junctions (zonulae occludens, less than 2 ram in width) between contiguous cells, the absence of endothelial pores, and a paucity of pinocytic vesicles (24). Further, brain capillaries contain a several-fold increase in the numerical density of endothelial mitochondria as compared with capillaries from other regions of the body (20,21) (Fig. 1). The cells constituting the BBB, connected by tight junctions, lacking endothelial pores, and possessing relatively few pinocytic vesicles act almost like a continuous cell layer, permitting solute exchange primarily by the transcellular route only (23). Thus lipid-soluble solutes easily penetrate the BBB while electrolytes, lipid-insoluble nonelectrolytes, and protein enter the brain from blood more slowly than they enter non-nervous tissues.

EVOLUTION *OF* THE CONCEPT *OF* A BBB

According to Dunn and Wybum (12), the concept of the BBB began to evolve from the studies of Ehrlich in 1882 and Gokhnan in 1913. It had often been observed that in jaundice all tissues of the body except the central nervous system were stained yellowish-green. Ehrlich and Goldman noted that parenteral injection of trypan blue, an acidic dye, resulted in the staining of connective tissues throughout the body with no staining of CNS tissue. It is well known that in kemicterus, a condition with severe neural symptoms and high plasma levels of bilirubin, associated with hemolytic disease of the newborn such as erythroblastosis fetalis, there is a deep yellow staining of the globus pallidus, caudate-putamen, cerebellar and bulbar nuclei, and cerebrum. However, bilirubin will not enter the brain, even in the neonate, unless the plasma level of bilirubin exceeds its capacity for binding biirubin. Thus the primary factor controlling passage of bilirubin (MW 584.65) across the cerebral capillary is in the plasma, not in the BBB per se. This points to the complexity of the BBB, with its numerous

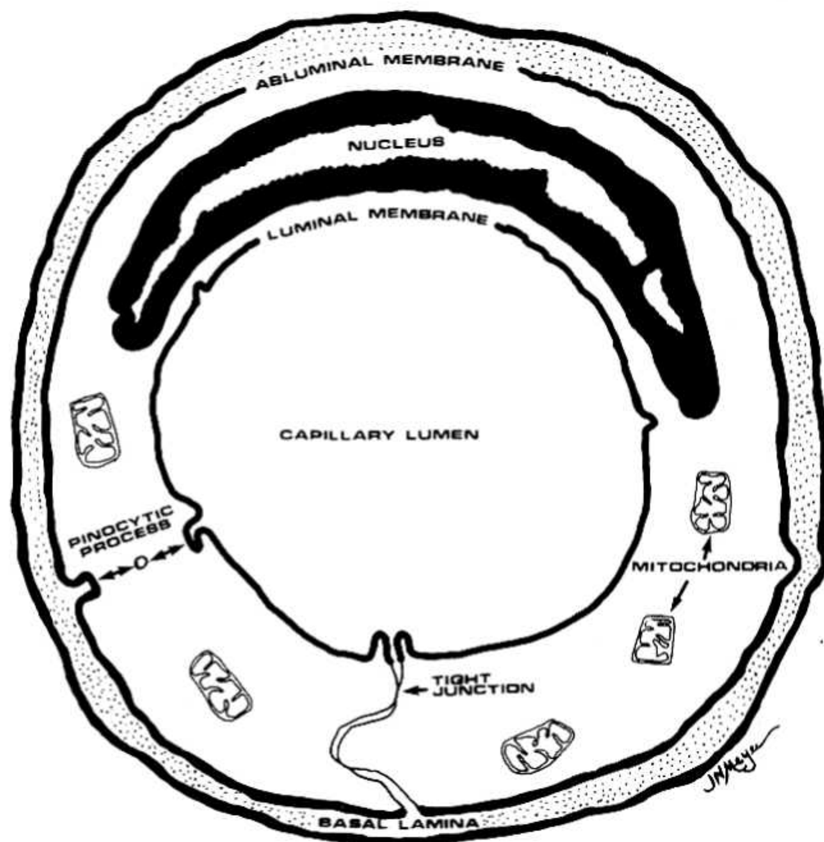

FIG. 1. Schematic representation of a cerebral capillary illustrating characteristic features of the blood-brain barrier: tight junctions in the interendothelial cleft, absence of endothelial pores, paucity of pinocytic vesicles, and a high numerical density of mitochondria as compared with capillaries from other regions of the body.

physiological as well as morphological aspects. Kernicterus does not occur in adults since high levels of unconjugated bilirubin are extremely rare in adult life. The concept of the BBB, however, remained vague until the mid 1950s. These early experiments were subject to criticism since the dyes used were found to bind strongly to serum albumin, thus it was the large dye-protein complex, not simply the dye molecule, that was restrained by the barrier. In 1961 Dobbing (11) concluded that all of the previous morphological studies only provided a historical reason for the assumption that there existed a structural basis for the mechanisms ascribed to the BBB. His plea at that time was for a reconsideration of the concept from basic physiological principles.

It is apparent that whatever dissolved material enters the CNS, extracellular fluid (ECF) from the blood must enter either from CNS capillaries or via the CSF as it is elaborated from blood circulating through the choroid plexuses in the ventricles of the brain. Thus if the rate of passage of a substance is restrained in its transit from blood to brain, the site of the rate-limiting barrier must be the capillary endothelium, the basal lamina between the capillary and the surrounding astrocytic sheath, the astrocytic end-feet, or some combination thereof. In the case of the blood-CSF barrier (B-CSF-

B), **the site of the rate-limiting barrier must be either the choroid plexus capillary endothelium or the choroid plexus cuboidal epithelium.**

ELUCIDATION OF THE BBB BY ELECTRON MICROSCOPY

Early electron microscopic studies by Dempsey and Wislocki (9) and Van Breemen and Clemente (26) revealed the absence of a restraining barrier in the pineal body (attached to the roof of the third ventricle), the neurohypophysis and adjacent ventral portion of the median eminence (wherein the hypothalamo-hypophyseal-portal system originates), the area postrema (an emetic center, immediately rostral to the obex, on each side of the fourth ventricle), and the intercolumnar tubercle (located inferiorly to the two choroid plexuses of the lateral ventricles). Brightman in 1965 and 1967 (2-4) showed that either ferritin or horseradish peroxidase (HRP) injected intraventricularly passed between ependymal cells into brain ECF, through the astrocytic end-feet gap junctions, and into the basal lamina of the cerebral capillary; however, these substances did not pass through the capillary endothelium into the capillary lumen. Thus it was clear that no barrier existed between the CSF and brain ECF. Likewise, the barrier mechanism clearly was not due to the absence of interstitial space as claimed by Wyckoff and Young (28) and Phelps et al. (22). According to Fenstermacher and Rall (14), the extracellular space of the brain equals approximately 18% of brain wet weight. Clearly, the above-mentioned work of Brightman showed that the site of the barrier mechanism was neither the astrocytic end-feet nor the basal lamina of capillaries, but rather the capillary endothelium itself. In 1967 Reese and Karnovsky (24) injected HRP intravenously and showed that it did not pass through cerebral capillary endothelium. The HRP reaction product was found in only a few pinocytic vesicles within capillary endothelial cytoplasm, none of which appeared to be discharging at the abluminal cytoplasmic membrane. In the case of cardiac and skeletal muscle, however, the endothelial intercellular gap junctions allowed the passage of HRP. In 1968 and 1969 Brightman (5) and Brightman and Reese (6) showed that HRP was restrained from passage from the blood to the CSF and vice versa by the tight junctions connecting the apical regions of the choroid plexus epithelium, whereas HRP passed freely out of the fenestrated capillaries in the choroid plexus.

DIAMETERS OF PARTICLES EXCLUDED FROM THE CNS BY THE BBB AND THE B-CSF B

Electron-dense markers indicate the possible dimensions of pores that may be located within tight junctions in the BBB. Ferritin (2-4) has a molecular weight (M.W.) of 900,000 and a diameter of 10 nm, and HRP an M.W. of 40,000 and a diameter ranging from 2 to 6 nm depending on the form used, the colloidal form having the smallest diameter. In 1971 Feder (13) showed that a heme-peptide, microperoxidase, with an M.W. of 1,900 and a particle diameter of 1.7 to 2.0 nor, was distributed in a manner identical to the distribution of HRP with one exception, microperoxidase penetrated into the periaxonal space of myelinated nerve fibers when administered intraventricularly. It is therefore clear from these studies that any pores or channels within endothelial tight junctions are less than 2.0 our in diameter. More recently, Richards (25) has shown that 5-hydroxydopamine hydrobromide (5-OH-DA-HBr) does not cross the B-CSF-B or the BBB. The molecular weight of 5-OH-DA-HBr is 256 and the diameter approximately 0.5 to 0.7 am. Therefore, the diameters of any pores present in tight junctions located in the BBB and B-CSF-B are less than the size needed to accommodate the passage of particles having a hydrated diameter greater than 0.5 to 0.7 run.

PHYSIOLOGICAL MECHANISMS OF THE BBB

The primary function of the morphological stmctures characteristic of BBB and B-CSF-B

is **the exclusion from the CNS of particles larger** in size **than 0.5 to 0.7 ran in diameter. Thus the transit rate of molecules of numerous lipid-insoluble nonelectrolytes and proteins** is regulated at this crucially important blood-brain interface. Differences in molecular structure or ionic charge affect transit rate. **Further, various physiological transport mechanisms are coupled with the morphological substrate of the barrier systems and likewise affect blood-brain exchange.**

Diffusion of some solute particles occurs across the endothelial and epithelial components of the barrier system. This is the major mechanism for the movement of water, urea, and gases into and out of the CNS (19).

Pinocytosis, a process by which large molecules are engulfed by invaginating luminal endothelial membranes which form vesicles that separate and move toward the abluminal membrane, is involved in the transport of protein (18). **Pinocytic vesicles are rare in cerebral capillary endothelial cytoplasm as compared with capillaries in regions other than the** CNS (7). Enhanced pinocytosis occurs in brain capillaries in response to some exogenous substances introduced into the vascular system and is considered a sensitive index of the cytotoxicity of these substances (21).

Mediated transport, via carrier *molecules,* has been hypothesized to explain the transfer of *hexoses* and amino acids across the BBB and/ or the B-CSF-B (15). *Carrier-mediated* transport is dependent on enzymatic mechanisms within endothelial cytoplasm. A number of carrier transport systems have *been* clearly defined and characteristically show affinity, saturability, and stereospecificity.

Enzymatic degradation occurs within endothelial cytoplasm restricting entry of various compounds, particularly monoamine neurotransmitters, into the CNS (15). Examples of this type of metabolic barrier mechanism are the decarboxylation of either t.-3,4-dihydroxyphenylalanine (t--DOPA) or L-5-hy-droxytryptophan (t.-HTP) to the corresponding amines, dopamine (DA) or 5-hydroxytryptamine (5-HT), which are then degraded by monoamine oxidase (MAO) also *present* in cerebral endothelial cytoplasm. Therefore, appreciable amounts of **these monoamines and/or their precursors are prevented from entry into the brain unless** *either* MAO inhibitors or very large doses of the precursors, t,-DOPA or t.-HTP, are *administered.* A similar metabolic barrier restricts entry of gamma-aminobutyric acid (GABA) from blood to brain.

The occurrence of active transport across the BBB has not been clearly established in vivo, although it has been recently shown to occur in isolated capillaries (17). Halides and some small organic *molecules* are actively transported from CSF against a concentration gradient or an electrochemical gradient (l9). A greater work capability is indicated by the markedly higher numerical density of endothelial mitochondria in cerebral capillaries as compared with the nonbarrier-type of capillary. Ionic differentials are **maintained between blood plasma and brain** ECF, e.g., K' concentration in plasma is 4.63 mEq/kg H2O and in CSF is 2.86, in spite of large variations in plasma K' induced experimentally and sustained over long time periods (1). The exclusion of K' implicates an energy-dependent mechanism; however, it is not possible at this time to state definitively whether K' efflux occurs at both the B-CSF-B and BBB or only at the B-CSF-B **and not at the** BBB. Further research is required to clearly elucidate active transport mechanisms at the level of the cerebral capillary.

BLOOD-BRAIN BARRIER MODIFIABILITY

Morphologically, the BBB can be modified as has been shown by the opening of tight junc**tions in response to hyperosmolar insults (23), the thinning of capillary walls, and the decline in numbers of endothelial mitochondria with increasing age (8), and the enhancement of pinocytosis by toxic chemicals (18).** This is to mention only a few of the ways in which BBB modifiability is manifested. Physiological mechanisms will be altered if the morphological

substrates in which they reside are modified. Not only will water, urea, and gases diffuse into and out of the CNS more readily if the BBB becomes leaky, but also, for example, protein may diffuse into brain ECF giving rise to brain edema. Similarly, enhanced pinocytosis is likely to lead to increased transport of protein into brain ECF along with the involved cytotoxic agent resulting in edema due to the increase in brain ECF protein and/or a neurotoxic response to the cytotoxic agent.

EFFECTS OF CONTRAST MEDIA ON THE *BBB*

The toxicity of radiographic contrast agents continues to be of concern particularly to the neuroradiologist who is dependent on these media for radiographic enhancement in computed tomographic (CT) brain scanning for evaluation of brain circulation. Intravenous (i.v.) administration of contrast media reduces the risk of cytotoxicity or neurotoxicity as compared with intra-arterial (i.a.) administration; nevertheless, some degree of toxicity is associated with contrast media currently available, regardless of the route of administration. It is therefore of interest to examine the effects of these compounds on the BBB.

Recent studies (7) of the effects of Isopaque, 3 - acetamido-2,6-triiodo-5-(N-methylacetamido) benzoic acid, Amipaque, metrizamide, 2-f3 - acetamido-2,4,6-triiodo-5-(N - methylacetamido) benzomido)-2-deoxy-D-glucopyranose (Winthrop Laboratories, New York, N.Y.), Reno-M-60, 3,5-diacetamido-2,4,6-triiodobenzoic acid methylglucamine salt, and Renografin-76, 3,5-diacetamido-2,4,6-triiodobenzoic acid methylglucamine salt 66% with 3,5-diacetamido-2,4,6-triiodobenzoic acid sodium salt 10% (E. R. Squibb & Sons, Inc., Princeton, N.J.) were performed in female Sprague-Dawley rats ranging in weight from 300 to 350 g. The dosage of contrast medium (0.45 ml/kg body weight) as well as the route of administration (i.v.) simulate current practice in CT scanning for evaluation of brain circulation (10). Four minutes after the injection of contrast medium, HRP (Sigma Type VI, Sigma Chemical Co., St. Louis, MO) I mg/6 g body weight was given via the common carotid artery, and 3 min later the animal was decapitated, Samples were obtained from the striate cortex and from the choroid plexus of the fourth ventricle and prepared for transmission electron microscopy.

There was no evidence of opening the BBB or B-CSF-B following any of the contrast media used in this study under the conditions in which these experiments were conducted. This negative finding is in keeping with Rapoport's hypothesis (23) that opening of the BBB tight junctions usually is due to osmotic shrinkage of capillary endothelial cells. Dilution of contrast media solutions upon i.v. injection, as in our experiments, would have prevented significant alteration of plasma osmolality; therefore, damage to tight junctions would be unanticipated.

The nature of the cytotoxic effect of iodinated compounds has not yet been clearly elucidated. However, a sensitive measure of cytotoxicity is the effect of a compound on the process of pinocytosis. As previously mentioned, pinocytic vesicles are rare in cerebral capillary endothelial cytoplasm. Godeau and Robert (16) reported a significant increase in pinocytic vesicles in cerebral capillaries following i.v. injection of either collagenase or pronase in rats, and Joo (18) noted a similar significant increase in pinocytic vesicles following i.v. injection of either mercuric or nickel chloride, also in rats.

In our study, we found a significantly greater mean number (p 5 0.001) of pinocytic vesicles in cerebral capillary profiles from animals injected either with Renografin-76 or Amipaque than from animals injected with Isopaque or Reno-M-60 as shown in Fig. 2. We attribute this enhanced formation of pinocytic vesicles to a direct cytotoxic action of these compounds.

It is imperative, therefore, that additional studies of the effects on the BBB of these and other radiographic contrast media be performed with a view toward determining which of the available compounds provides adequate contrast enhancement coupled with the lowest possible level of toxicity. Further, it is essential

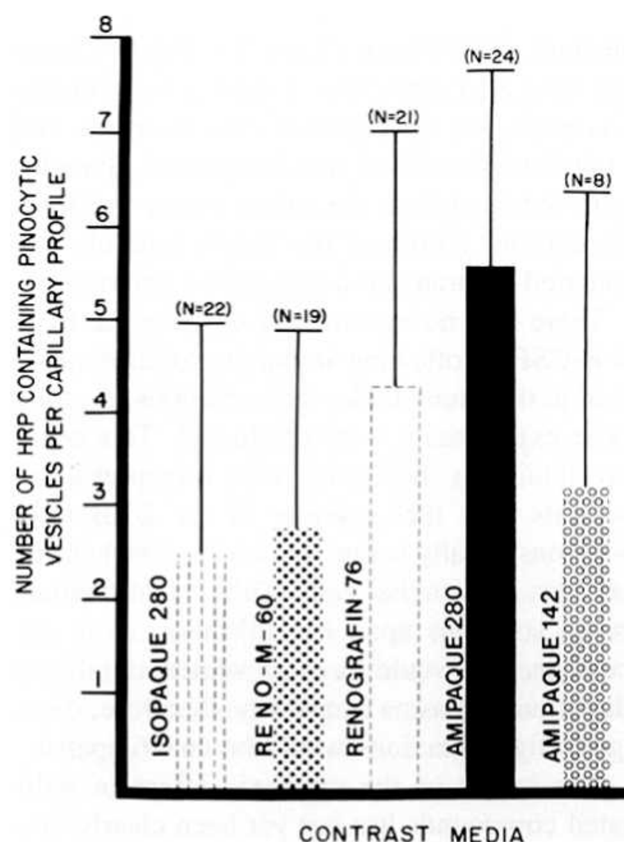

FIG. 2. Effect of contrast media on endothelial pinocytic activity.

that changes occurring with time in the effects of these compounds on BBB integrity be investigated. For example, electron microscopic studies should be performed on brain samples obtained within 30 sec after the injection of contrast media, with HRP having been injected prior to the i.v. administration of the iodinated compound, as well as additional studies at longer time intervals post-injection.

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