



## Blood-Brain Barrier (BBB)



### Porcine cerebral capillary endothelial cells to study Blood-Brain Barrier Permeability

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#### Summary

In many cases a drug molecule that is of potential benefit in treating brain pathologies must first penetrate the blood-brain-barrier. Surmounting this barriers is therefore a crucial factor in developing effective drug therapies for cerebral disorders.

Also it is often useful before conducting full toxicological studies to investigate the permeation behaviour of drugs and their metabolites.

For that the control over drug passage across the complex barriers between the blood and the brain is a challenging goal in drug discovery. The predictive approaches for BBB passage range from

theoretical models based on calculated physico-chemical parameters to in vivo animal experiments. Generally theoretical models statistically predict BBB passage and fail if specific transport mechanisms are involved in permeation. For this reason in vitro biological models are employed for the prediction of brain uptake. These models occupy an important position between the computational approach and animal experiments.

In response to this need Across Barriers has established and standardized an in vitro model of the blood-brain-barrier which is based on cerebral capillary endothelial cells.

## The blood-brain barrier

In 1885, Paul Ehrlich observed that intravenously administering dyes into experimental animals caused staining of all organs with the exception of the brain. In 1913, Edwin Goldmann put forward the hypothesis that the cerebral capillaries provide the anatomical basis for a physiological barrier between the brain and the rest of the body. The hypothesis received confirmation with the development of the electron microscope in the nineteen fifties when it was demonstrated that the outermost layers of the endothelial cells in the brain capillaries are fused together. The formation of these so-called tight junctions at the points of contact between neighboring endothelial cells has far-reaching consequences for the transport processes in the brain and the tissue of the central nervous system.

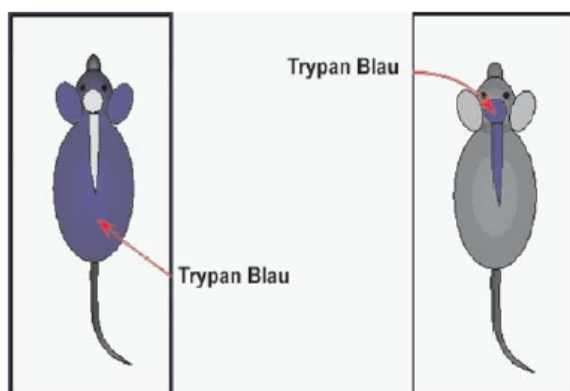


Figure 1: Goldmann's staining experiment. The intravenous injection of the dye trypan blue readily stained all tissues except the brain and spinal column. However, when injected intracerebrally, the dye stained only the central nervous system.

In general, the transport of substances from the blood into the different tissues of an organism occurs either through the endothelial cells (transcellular transport) or via gaps between the endothelial cells (paracellular transport). In the former case, vesicles or thin endothelial cell membranes (fenestrations) are responsible for the transport process. The paracellular pathway involves the gap junctions and thus permits the permeation of larger molecules. In contrast, the tight junctions form an impassable barrier making passage almost impossible for even the smallest, water-soluble molecules.

The structure of the endothelial cells of the brain capillaries severely restrict both transcellular and paracellular transport. Compared to the endothelial cells in the capillary vessels of, for example, the liver, the number of transport vesicles is relatively low and there is no fenestration whatsoever. Furthermore, the gap junctions, which are important in the paracellular transport mechanism, are absent in many regions of the brain. It follows from this, that the transport processes occurring in the endothelial cells of the brain capillaries must be of a different kind.

## Transport processes in cerebral endothelial cells

Investigations of endothelial cells in brain capillaries were able to demonstrate at an early stage that lipophilic substances such as nicotine or ethanol were able to permeate into the brain interstitium in a relatively facile manner, whereas water-soluble compounds were inhibited. This observation can be explained by the composition of the outermost endothelial cell membrane. Like other cell membranes, its structure is that of a two-dimensional lipid layer with the hydrophilic heads directed outwards. Only fat-soluble substances are able to penetrate this lipid membrane and thus move from the blood into the brain. However, the brain also needs certain water-soluble nutrients such as glucose or particular essential amino acids. As the hydrophilic nature of these molecules prevents them from diffusing through the lipid layer, some other specific transport system must be in operation that actively transports these substances across the endothelial cell barrier. These carrier systems show pronounced substrate specificity. Muscle tissue, for example, can absorb D- and L-glucose at the same rate, but the carrier systems active in the endothelial cells of the cerebral capillaries only accept D-glucose and do not transport the L-enantiomer. The specificity exhibited by the brain transport systems towards amino acids is even more striking. Large neutral amino acids, which act as precursors for neurotransmitter substances in the brain, are transported both on the luminal and abluminal sides of the endothelial cells. In contrast, no transport systems exist for smaller neutral amino acids as these can be synthesized by the brain cells themselves. However, glycine, which can block the transmission of nerve signals, is a special case. On the abluminal membrane of the cerebral endothelial cells, there is a carrier which ensures that glycine can be removed from the brain. Apart from its function in regulating inter metabolic products, the blood-brain barrier also is

involved in cerebral metabolic processes. A well-known example of this is the metabolism of L-dopa (levodopa), a precursor of the neurotransmitters dopamine and noradrenaline. L-dopa enters the endothelial cells from the blood either by an active carrier system on the luminal membrane or via a passive transport channel located on the abluminal side. Once in the endothelial cells, the L-dopa is decarboxylated by amino acid decarboxylase to form dopamine. This is followed by a monoamine-oxidase-catalyzed reaction to form dihydroxyphenyl acetic acid. As neither of these metabolic products can enter the brain, these metabolic processes help to regulate the concentration of L-dopa in the brain.

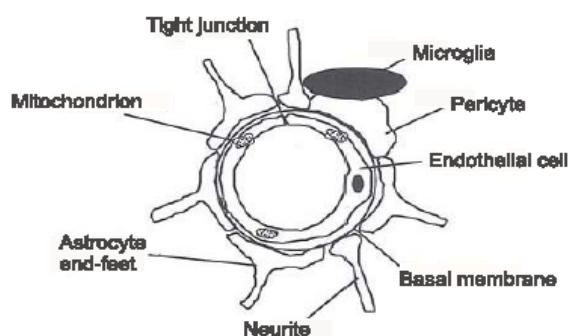


Figure 2: The blood-brain barrier: Morphology of the capillary epithelium in the region of the central nervous system and surrounding tissue.

### The inhibition of drug transport by the blood-brain barrier

The relative impermeability of the brain's endothelial cells underlies the stability of the internal cerebral milieu. The resulting homeostatic regulation ensures that fluctuations in the concentration of nutrients, hormones or ions cannot directly affect the synaptic functions of the nerve cells. Thus, while the blood-brain barrier is beneficial in helping to maintain regular cerebral functions, it represents an obstacle to drug therapies designed to tackle disorders of the central nervous system. Obviously, the criteria that distinguish whether a substance can cross the blood-brain barrier apply equally to drug compounds. The lipophilic antibiotic chloramphenicol can cross the blood-brain barrier without hindrance, whereas passage of the more hydrophilic penicillin is blocked. Therefore, in any potential treatment of encephalopathies or disorders of the CNS tissue, the question arises as to how pharmaceutically

active substances can be effectively transferred across the blood-brain barrier.



Figure 3: A "classical" means of opening the blood-brain barrier.

One possibility involves temporarily opening the blood-brain barrier by injecting hyperosmotic sugar solutions into the carotid artery. This technique is used in the treatment of cerebral tumors where the temporary opening is used to apply tumor suppressant drugs. A further means of overcoming the blood-brain barrier is to administer very high doses of a drug in an effort to overcome the poor membrane permeability (resulting from e.g. insufficient lipophilicity). However, this approach is only feasible in exceptional cases, as many drugs are toxic at high dosage levels. Similarly, the temporary opening of the blood-brain barrier is only applicable to disorders which do not require permanent treatment. A third possibility is to inject the drug directly into the spinal cord. However this approach is controversial as both the type and quantity of the substance that can be injected is limited and the technique requires personnel who have received specific training. It is thus highly desirable that drugs are developed which can either directly or indirectly bypass the blood-brain barrier.

## Overcoming the blood-brain barrier

There have been two main approaches used in developing drugs that can surmount the blood-brain barrier without the need for a temporary opening:

- Structural variation of drug molecules with the aim of increasing their lipophilicity, thus enabling direct permeation of the cerebral endothelial membrane.

- Development of shuttle systems which utilize the transport mechanisms specific to the endothelial cells. In this case, the drug molecule enters the endothelial cells “on the back of” another compound. After separating from the shuttle the drug can then be transferred to the brain via the abluminal membrane.

In both approaches, studies of drug permeation across the blood-brain barrier are an indispensable part of the drug development strategy. Across Barriers has developed and standardized a model of the blood-brain barrier based on porcine cerebral endothelial cells with which it can perform transport experiments of this kind. Using this model, drug permeation of the blood-brain barrier can be simulated at much higher sampling rates and at significantly lower cost than is possible by animal experiments. Tests have confirmed that the outstanding tightness of the cell layer in the Across Barriers' model guarantees the highest possible compatibility with *in vivo* results.

Our model of the blood-brain barrier is equally suitable for testing substances whose permeation into the brain via the cerebral endothelial cells

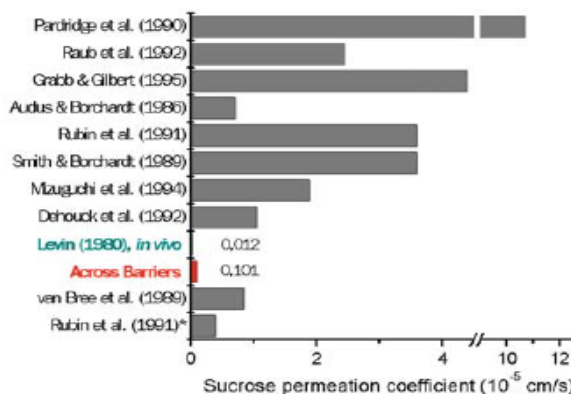


Figure 4: Comparison of sucrose permeability values from the Across Barriers model, *in vivo* experiments and literature data.

is undesirable. In the case of substances or formulations for which there are reasonable grounds for assuming that they are neurotoxicologically active, pre-screening programs offer a fast, cost-effective alternative to what can be lengthy and expensive neurotoxicological studies.

## Transport studies using the blood-brain barrier model

The model system used by Across Barriers to study transport across the blood-brain barrier is a Transwell system with a polycarbonate membrane. The concentration of the test substance is typically 10 mM, with ethanol or DMSO being added in cases of low water solubility. Experiments are normally conducted seven days after preparation. Analysis is by high performance liquid chromatography (HPLC) using UV, EC or MS detection. Scintillation

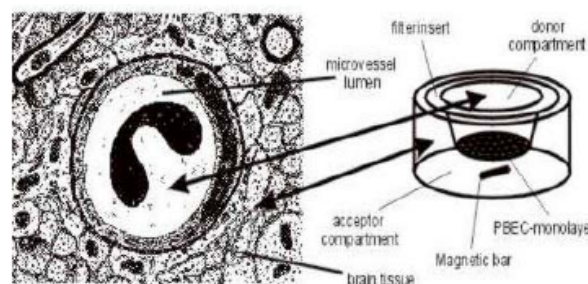


Figure 5: Comparison of the *in vivo* situation and the experimental set-up used at Across Barriers.

counting is also available as an alternative detection method.

An important variant of standard permeability studies is the investigation of the preferred direction of transport (luminal > abluminal or abluminal > luminal). If the apparent permeability coefficient  $P_{app}$  of a substance in the abluminal > luminal direction is higher than in the reverse direction, it is likely that efflux proteins such as P-glycoproteins are involved. By varying the transport conditions (temperature, inhibitors), our model of the blood-brain barrier enables the influence of active carrier systems to be predicted reliably.

**Digoxin transport across the BBB in vitro.  
Inhibition of P-glycoprotein mediated efflux by Verapamil**

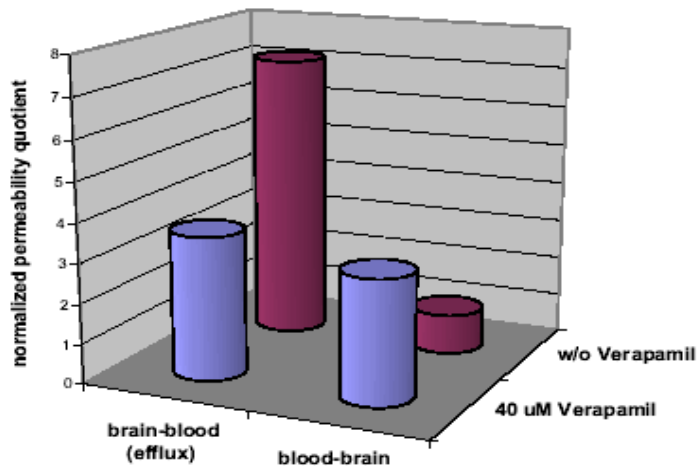


Figure 6: Digoxin transport (luminal <> abluminal) with and without P-glycoprotein blockage by verapamil.

### Quality control

- Routine measurement of the transepithelial electric resistance (TEER) before and after every transport process to check that TEER > 300 Ohm cm<sup>2</sup>.
- Determination of monolayer integrity using <sup>14</sup>C-labelled sucrose. The measurement with the low permeability marker is carried out after each transport measurement and the permeability coefficient P<sub>app</sub> must be less than 2·10<sup>-6</sup> cm·s<sup>-1</sup>.
- The tightness of the cell layer is checked prior to every experiment using the quality assurance markers (<sup>14</sup>C-sucrose (low permeability) and propranolol (high permeability)).