lactin levels in males was similar to that observed following a hypotensive dose of the dopamine agonist lergotrile mesylate (*2, 12*). These data suggest that endogenous prolactin is involved in blood pressure regulation in the rat.

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Morphologic Effect of Dimethyl Sulfoxide on the Blood-Brain Barrier

Abstract. *Dimethyl sulfoxide (DMSO) opens the blood-brain barrier of mice to the enzymatic tracer horseradish peroxidase. A single injection of horseradish peroxidase in 10 to 15 percent DMSO into the tail vein along with 10 to 15 percent DMSO delivered intraperitoneally allowed horseradish peroxidase to fill the extracellular clefts throughout the brain within 2 hours. In the absence of DMSO, peroxidase failed to enter brain parenchyma except through the circumventricular organs. Opening of the blood-brain barrier by DMSO is reversible. Dimethyl sulfoxide stimulated the pinocytosis of horseradish peroxidase by the cerebral endothelium; the peroxidase was then directed to lysosomal dense bodies for degradation. Vesicular transport of horseradish peroxidase from the luminal to the abluminal wall of the endothelial cell was not observed. Dimethyl sulfoxide did not alter the morphology of endothelial cells or brain parenchyma.*

Dimethyl sulfoxide (DMSO) is an aprotic solvent with widespread laboratory use as a cryopreservative and, at higher concentrations, in the solubilization of biological membranes (*1*). The use of this drug in the treatment of cerebral infarction, brain swelling, and spinal cord injury is controversial (*2*). These conditions often exhibit abnormalities of blood-brain barrier function, but the effect of DMSO on the blood-brain barrier is unknown. The ability of DMSO to open the blood-brain barrier has been the subject of conflicting reports (*3, 4*). We know of no morphologic study of DMSO action on the blood-brain barrier. Although the blood-brain barrier normally prevents many blood-borne, large molecular weight, fat-insoluble substances from entering the cerebral extracellular fluid, opening of the blood-brain barrier can be induced by a number of experimental manipulations and pathological conditions, including seizures, systemic hypertension, hypervolemia, and the intracarotid injection of hyperosmotic substances (*5*). A safe chemical means for reversibly opening the blood-brain barrier would be of value in the treatment of brain tumors and central nervous system infections. We now report the reversible opening of the blood-brain barrier of the mouse to the intravenously administered protein tracer horseradish peroxidase (HRP) when the animals are injected intravenously and intraperitoneally with DMSO.

A total of 80 female white mice weighing 25 to 30 g were used. Injections of HRP (Sigma, type VI) (1 mg per gram of body weight) dissolved in 0.25 ml of saline or in 0.25 ml of DMSO (Tera Pharmaceuticals) at concentrations of 2, 3, 5, 10, 15, 20, or 30 percent were given as a bolus into the tail vein of unanesthetized, but restrained, mice. Some mice were also given intraperitoneal injections of 10 to 30 percent DMSO 5 to 30 minutes before the HRP injection (*6*). The brains from all mice were fixed by perfusion with a mixture of aldehydes (*7*) 2 hours after intravenous injection of HRP (*8–10*).

In mice given an intraperitoneal injection of 0.5 ml of DMSO in combination with an intravenous injection of HRP in 0.25 ml of DMSO, best results without adverse physical or behavioral effects were obtained when 10 to 15 percent DMSO was used. Dense HRP reaction product was distributed homogeneously throughout most of the forebrain, brainstem, and cerebellum (Fig. 1a). In the absence of DMSO, leakage of blood-borne HRP into the brains of control mice occurred only at sites normally containing fenestrated capillaries (*10*), such as the choroid plexus and circumventricular organs (Fig. 1b). At the ultrastructural level in brain sections from mice exposed to 10 to 15 percent DMSO, peroxidase reaction product filled the extracellular clefts of the neuropil. The membranes and general morphology of neurons, glia, and microvascular endothelial cells appeared to be unaltered. Peroxidase was never observed free in the cytoplasm of these cells, an indication that the plasma membranes remained intact. The numbers of HRP-labeled endocytotic vesicles, vacuoles, and elongated tubules in endothelial cells of DMSO-treated mice were greater than the numbers of similarly labeled structures in the cerebral endothelial cells of control animals. No concentration of labeled vesicles or tubules was seen adjacent to the abluminal surface of endothelial cells. In control animals, the HRP-labeled endocytotic structures were channeled to endothelial dense bodies, which were shown by acid-hydrolase cytochemistry to be lysosomes (*7*). Such HRP-labeled dense bodies were also present in endothelial cells of brains exposed to DMSO, but their concentration was not noticeably greater than that in controls. Neither intraperitoneal injection of DMSO with intravenous administration of HRP in saline nor intravenous injection of HRP in 0.25 ml of DMSO alone opened the blood-brain barrier to peroxidase.

Peroxidase staining of brain sections from mice given HRP in saline intravenously 2 to 24 hours after being given 10 to 15 percent DMSO intravenously and intraperitoneally was identical to that seen in brain sections from control mice. Opening of the mouse blood-brain barrier by DMSO, therefore, may be a transient event.

All mice given DMSO intravenously exhibited brief hind-limb muscle twitching and hematuria. No morphological changes were observed on gross or mi-

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croscopic examination in brain parenchyma from mice exposed to DMSO concentrations of up to 15 percent. Intraperitoneal injection of 0.5 to 1 ml of 20 to 30 percent DMSO coupled with a 0.25-ml intravenous injection of DMSO at the same concentration was tolerated by the animals; however, many exhibited anterior pituitary and superficial cortical hemorrhages and poorly preserved cell and organelle membranes. Brains and pituitaries from animals given 0.5 ml of DMSO intraperitoneally and 0.25 ml of DMSO intravenously at concentrations of up to 15 percent did not exhibit hemorrhage. Regardless of the volume, concentration, and route of delivery of DMSO, the corneas, lungs, heart, kidneys, liver, and intestines of all DMSO-injected mice appeared normal on gross examination at autopsy.

Previous studies with peroxidase have demonstrated that the normal blood-brain barrier to protein consists of circumferential belts of tight junctions between adjacent capillary endothelial cells and a relative absence of vesicular transport of endocytosed protein from the luminal to the abluminal surface of these cells (*11*). More recently, blood-borne HRP has been shown to be taken into cerebral capillaries by pinocytosis and channeled to lysosomes for degradation (*7*). Possible mechanisms of action for the opening of the blood-brain barrier by DMSO include (i) stimulation of pinocytosis and vesicular transport through the microvasculature, (ii) opening of tight junctions between contiguous endothelial cells, (iii) formation of transcellular channels, and (iv) rupture of the endothelial cell membrane both luminally and abluminally. The last two possibilities are unlikely since 10 to 15 percent DMSO does not appear to alter the ultrastructural morphology of brain vasculature and parenchyma, and no transcellular endothelial channels were identified. Vesicular transport of tracer substances across the cerebral capillary endothelium under experimental conditions is highly controversial (*12*). Although DMSO does appear to stimulate pinocytosis of HRP, we have yet to identify a demonstrable transport and buildup of HRP-containing vesicles on the abluminal side of the endothelial cytoplasm.

Transient opening of the tight junctions may result from systemic hypertension, expansion of the intravascular volume, or hyperosmotic stimulation (*5*). Because HRP entry into the brain parenchyma of our mice occurred after only 0.25 ml of DMSO was injected intravenously in combination with DMSO injected intraperitoneally, the possibility

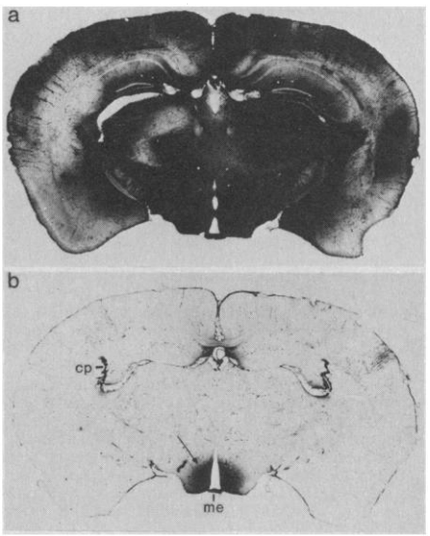

Fig. 1. Frontal sections through the forebrain from mice given (a) an intraperitoneal injection of 10 to 15 percent DMSO plus an intravenous injection of HRP in 10 to 15 percent DMSO, or (b) an intravenous injection of HRP in saline. The sections, fixed 2 hours after injection, demonstrate that, in the company of DMSO, peroxidase passes through the blood-brain barrier to enter the brain. In the absence of DMSO, peroxidase (arrow) enters the brain only at sites where the blood-brain barrier is absent, such as the choroid plexus (*cp*) and the median eminence (*me*) (×40).

that hypertension or hypervolemia was induced is remote. The possibility that DMSO exerts an osmotic effect on endothelial cells, with concomitant opening of tight junctions, cannot be ruled out even though DMSO is lipid-soluble and may equilibrate rapidly across cell membranes. The molar concentration of 15 percent DMSO is 2.1, a value that exceeds the molar threshold for osmotic opening of the blood-brain barrier with intracarotid administration of mannitol (*13*). Nevertheless, after systemic dilution, the concentration of DMSO reaching the brain is probably substantially less than that injected initially into the tail vein. Exactly how DMSO serves to open the blood-brain barrier is still unclear (*14*).

Dimethyl sulfoxide has been reported to increase brain levels of intravenously administered pemoline and L-dopa in the rat and of adrenaline and noradrenaline in the neonatal chick (*4*, *15*). Quantitative measures of rat brain capillary permeability are increased by DMSO for vincristine, bleomycin, [^{14}C]sucrose, and [^{3}H]inulin, although these drugs may be sequestered in endothelial cells without an actual increase in their concentrations in the brain (*16*). This finding may be explained by our observation that DMSO stimulates pinocytosis. The effectiveness of Cytoxan against meningeal implants of lymphoma in the rat is potentiated by DMSO, which increases brain concentrations of Cytoxan in comparison with those in water-fed controls (*17*). Other pharmacologic studies with DMSO have not shown increased entry of radiolabeled *p*-aminohippuric acid in the mouse, or of methionine, albumin, glucose, and tryptophan in the rat (*3*, *4*). Conceivably, opening of the blood-brain barrier by DMSO has a differential effect on compounds, depending upon their molecular weight, effective diameter, lipid solubility, degree of polarity, or other factors. We have not been able to demonstrate reliably the effect of DMSO on the blood-brain barrier when using radiographic contrast agents or intravital dyes tightly bound to circulating albumin. These findings suggest that an upper limit (molecular weight, 70,000) exists for the size of substances delivered across the blood-brain barrier on exposure to DMSO.

The search for a safe and reliable approach for promoting the entry to the brain of blood-borne chemotherapeutic agents and antibiotics may depend on an increased understanding of the mechanisms of blood-brain barrier function. Whether or not DMSO can safely and effectively open the blood-brain barrier in vivo to chemotherapeutic drugs and antibiotics requires further investigation.

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6. There were five treatment groups: (i) 14 animals received HRP in saline and served as controls; (ii) 23 received HRP in DMSO; (iii) 12 received 0.5 to 1 ml of DMSO intraperitoneally plus 0.25 ml of HRP in saline intravenously; (iv) 24 received 0.5 ml of 10 to 30 percent DMSO intraperitoneally plus 0.25 ml of HRP in 10 to 30 percent DMSO intravenously; (v) 7 received 0.5 ml of 15 percent DMSO intraperitoneally plus 0.25 ml of 15 percent DMSO intravenously, and 2 to 24 hours later, HRP in saline intravenously.
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14. Additional studies in our laboratory are being conducted on larger animals to determine if DMSO does indeed exert an effect on tight junctions. In these animals HRP in DMSO is injected into the femoral vein, and carotid blood is sampled for its osmolarity. Because the DMSO effect on the barrier appears to be reversed within 2 hours, opening of the blood-brain barrier has been looked for in mice in which DMSO/HRP has been permitted to circulate through the brain for 1½ to 5 minutes. At 5 minutes, numerous focal exudates of HRP surround blood vessels throughout the brain. These sites are currently being viewed ultrastructurally to determine if the leaks are attributed to open tight junctions or vesicular transport. In other animals DMSO is injected into the lateral cerebral ventricle and HRP is administered intravenously to determine whether the DMSO effect on the abluminal surface of the blood-brain barrier is similar to that observed on the luminal surface.
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Boradeption: A New Procedure for Transferring Water-Insoluble Agents Across Cell Membranes

Abstract. *A new process has been developed which is called "Boradeption" to signify boronic acid–dependent phase transfer of water-insoluble agents. Highly fluorescent boronic acid derivatives, FluoroBoras, are solubilized with a physiologically compatible carrier buffer containing a receptor group for boronate adduct formation. The system can be used to stain living cells. In another variation of the Boradeption concept, an insoluble reporter molecule containing a boronate receptor is solubilized with a carrier buffer containing a boronic acid functional group. The boronate-receptor complexes, which are in dynamic equilibrium, can be designed as vital stains and reagents for a variety of biological and medical applications.*

We have developed new reagents and procedures for transferring water-insoluble agents across membranes into living cells. The principles involved are applicable to phase transfer in a variety of biomedically and chemically significant situations. We call the process "Boradeption" to indicate boronic acid–dependent phase transfer.

Aryl and alkyl boronic acids react with a variety of compounds having amino and hydroxyl groups and form five- or six-membered cyclic complexes of varying stability. The ability of such complexes to resist hydrolysis depends on steric factors and ring size, such that the boronate adducts are either triagonal or tetragonal complexes. This is related to the effective Lewis acid strength of the boronic acid at the *p*H of the buffers employed.

We coupled certain reporter groups to boronic acids to form ReportaBoras. Starting with a 1,3-diaryl-Δ^2-pyrazoline [useful as an optical brightener (*1*)], 1-(phenyl-*p*-sulfonic acid),3-phenyl-Δ^2-pyrazoline sulfonyl chloride was coupled to *m*-aminophenylboronic acid (*2*). This highly fluorescent pyrazoline-sulfamido-phenylboronic acid is very insoluble in water and thus would seem to be useless as a stain in living tissue. However, a variety of carrying buffers with receptor groups able to form complexes effectively with boronic acids can be used to solubilize this and other insoluble boronic acids at physiological *p*H values (see scheme 1 in Fig. 1A).

The extent of the dynamic equilibria in water between the complexing carrying buffer containing the receptor for the boronic acid and the normally water-insoluble reportabora can be used to great advantage to carry the latter into aqueous solution as a complex, then as the free compound through hydrophobic zones on the cell membranes, and finally into cellular components. In living cells, the transferred reportabora can be dynamically distributed into both hydrophobic zones and into areas where relatively tight boronate complexes can be formed. One can vary the hydrophobicity and net charge of the reporter group, the *p*K and Lewis acid complexing strength of the selected boronic acid, and the complexing potential of the carrying buffer so that selective cellular uptake, transport, and binding of the reporter in the cell give staining patterns reflecting important structural and metabolic cell components. Among the reporter groups can be various fluorophores (FluoroBoras), chromophores (ChromoBoras), heavy metal compounds (MetalloBoras), and drugs (TheraBoras). Zwitterion buffers that contain appropriate hydroxyl and amine functions (*3*) are particularly useful as boronate-carrying buffers. Such buffers are generally nontoxic to cells, and those which also contain sulfonates are generally not taken up by cells.

In a variation of scheme 1, it is possible by scheme 2 to design reporter groups attached to boronate receptors (reportareceptors) that react with boronic acids, and then to use as carrying agents or buffers various soluble boronic acids (carrierboras) (Fig. 1B). For example, one attaches *N*-(3-aminopropyl)-diethanolamine to various reporter agents and then carries these reportareceptor compounds as soluble complexes of certain aryl sulfonic acids containing a boronic acid.

Certain FluoroBoras, such as *m*-dansylamidophenylboronic acid (FBI) and *m*-darpsylamidophenylboronic acid (FBII), have the additional valuable feature of extensive enhancement of their fluorescence when placed in hydrophobic regions, as in certain proteins. There usually is a shift in fluorescent emission wavelength.

The new boronate adducts that form with the cellular components can have a wide range of stabilities by virtue of the nature of the complexing cellular receptor components and the relative lack of a competing hydrolytic solvent, such as water, in various hydrophobic cellular regions. By manipulation of (i) the charge and other chemical features of the

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