Selective Alteration of the Blood-Brain Barrier*

C. NORMAN SHEALY, M.D., AND DAVID CRAFTS

Department of Surgery, Division of Neurosurgery, Western Reserve University, University Hospitals, 2065 Adelbert Road, Cleveland, Ohio 44106

While most neurosurgeons have been uneasy at the prospect of possible cerebral or blood-brain barrier damage induced by various arteriographic media and have attempted to decrease this effect, these harmful aspects suggested a potential usefulness. It is well documented that every currently used radiopaque substance may, at certain dosage levels, induce damage to normal brain through alteration of the blood-brain barrier, red blood cell aggregation or toxic effects upon the intima of blood vessels. 1,7,16 Minor arguments exist as to which agent is better or worse; and, using a variety of test situations, investigators have championed one agent or another.

There may be a wide differential in the existing blood-brain barrier in various parts of the brain with significant variations in susceptibility to damage. In this discussion, the expression blood-brain barrier refers to the failure of most substances passing through blood vessels to enter normal brain tissues. Certain areas may not have a blood-brain barrier. 6 The choroid plexus, area postrema, intercolumnar tubercle and the pituitary and pineal glands may be examples; moreover, there is normally a moderate blood-brain barrier difference between white and gray matter. 8

The most important factor is the substance being used to test the barrier; there are differing beliefs in the superiority of one test compound over another. For instance, trypan blue was for many years a favorite tool to differentiate "normal" from impaired blood-brain barrier. Since the advent of radioisotopes, trypan blue has fallen into disfavor and investigators have attempted to discredit those earlier studies with visible dyes. It has become increasingly clear, however, that there are gross differences in the relative barrier to various substances. This depends to a large extent upon the size of the molecule, its attachment to blood proteins and its possible involvement in cellular metabolism.

In all the studies, measurement of the blood-brain barrier has been done in one of two ways:

1) By visual differentiation of either vital dye or radioisotope "staining"; this is a qualitative test whether the "stain" be measured as degree of "blue" or relative differential uptake on a brain scan or radioautograph.

2) The second method is quantitative and involves tissue counting of specimens after cerebral perfusion with radioisotope.

The most important concept in blood-brain barrier evaluation is that each test substance has an inherent capacity for penetration. If a difference is noted qualitatively with brain scan or trypan blue, the degree of barrier can be determined precisely by tissue counts. Conversely, minor differences in the barrier can be determined only by this latter method.

If this is so, potential alteration of the blood-brain barrier by any one substance must also vary. In other words, suppose that a normal blood-brain barrier is invariably damaged by the rapid injection of 15 cc. of 50 per cent Hypaque but that there is no apparent damage after rapid injection of 12 cc. of 50 per cent Hypaque. It is quite conceivable that in a situation of this sort a slightly altered blood-brain barrier, such as that in a low grade astrocytoma, will be further damaged by this same smaller subthreshold volume of Hypaque without damage to normal.
brain. In other words, theoretically, an impaired blood-brain barrier is more susceptible to further damage than a normal blood-brain barrier.

**Experimental Method**

To test this theory, a series of experiments was performed. The first problem was to develop a fairly reproducible blood-brain barrier lesion. After several trials with leukotomes, coagulation, injections of alcohol, etc., we settled upon focused ultrasonic lesions. Using the techniques which have been described by Bakay et al.,\textsuperscript{2,3} identical symmetrical bilateral lesions were placed in the white or gray matter of more than 50 cat brains at depths of 5 to 10 mm. Thus the lesion on one side was used for additional blood-brain barrier damage by intracarotid injections while the other lesion served as a control.

It is known from extensive work previously reported that the blood-brain barrier is altered for trypan blue perfusion testing for about 3 days;\textsuperscript{4} it is altered for \( \text{P}^{32} \) for at least 6 days.\textsuperscript{2} In our studies, barrier damage was measured qualitatively by trypan blue and quantitatively by tissue sample counts after administration of Mercury\textsuperscript{208} (Neohydrin). Four to 6 days after the lesions were made, the right common carotid artery was exposed under Nembutal anesthesia, a \#25 or \#26 needle inserted and the artery injected with 50 per cent Hypaque, distilled water, or 15 per cent ethyl alcohol. Blood flow in the artery was occluded for 20 to 60 seconds during the injection and re-established upon withdrawal of the needle. The right lesion in each animal was subjected to this additional stress and the left served as a control. Five cc. of 5 per cent trypan blue solution were then administered intravenously or intraperitoneally in all animals. In 10 animals a 300 to 500 microcurie dose of Mercury\textsuperscript{208*} was injected intravenously at the same time. The animals were sacrificed 2 to 18 hours later.

\* Supplied by Squibb Pharmaceutical Company.

**Results**

It soon became evident, as seen in Fig. 1, that injection of 15 cc. of 50 per cent Hypaque, 15 per cent alcohol or distilled water usually caused diffuse blood-brain barrier damage in the injected hemisphere and these animals showed neurological damage. But injection of 10–12 cc. of each of these substances did not damage the blood-brain barrier of normal brain. Furthermore, after injection of this smaller volume of any of the 3 substances tested, the animals awoke and in most cases appeared neurologically intact. Animals with asymmetrical lesions were discarded.

In the 19 injected animals with symmetrical lesions the difference in the two 6-day-old lesions was striking (Fig. 2). There was minimal, if any, trypan blue staining on the left side. On the perfused right side the lesion was stained a deep blue exactly as in a 1-day ul-