Selective Alteration of the Blood-Brain Barrier*

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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.\textsuperscript{1,7,10} 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.\textsuperscript{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.\textsuperscript{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

Received for publication November 30, 1964.
* This paper was presented at meeting of the Academy of Neurological Surgeons, Key Biscayne, Florida, on November 13, 1964.
This research was supported by Grant \#779 from the National Institutes of Health.
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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 leucotomes, 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 P\textsuperscript{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{209} was injected intravenously at the same time. The animals were sacrificed 2 to 18 hours later.

* Supplied by Squibb Pharmaceutical Company.

![Fig. 1. There is diffuse staining of the brain on the right with some spread to the medial portion of the contralateral hemisphere.](image)

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-
trasonic lesion. There was, however, no apparent edema or staining of the surrounding brain. Furthermore, if the lesions and adjacent areas of normal brain were carefully excised, tissue counts showed significant changes. The left lesion and left and right normal brain showed similar counts. The right lesion, which had been challenged with a subthreshold blood-brain barrier damaging dose of 12 cc. of 50 per cent Hypaque, now showed a striking increase in its permeability both to trypan blue and to Mercury^{203}. The differential uptake was 7 to 10 times that in normal brain or the control lesion. Choroid plexus, said to have no blood-brain barrier, showed counts ranging from 10 to 50 times greater than those in normal brain. In two animals, injection of 10 cc. of 10 per cent glucose prior to the Hypaque did not prevent the differential damage in the perfused right-sided lesion.

Discussion

The exact point at which Hypaque further damages an already impaired blood-brain barrier (as can occur in tumor or infarct) has not yet been defined. Our study suggests that this level may be well within the limits which can be tolerated in normal brains. This may explain the occasional undesirable changes in patients during or shortly after cerebral arteriography. As Ballantine\textsuperscript{4} has noted, complications of arteriography are exceedingly rare in patients with no cerebral lesion; theoretically, the worse the cerebral lesion the greater the risk of arteriography. Many brain scanning laboratories suggest that scans not be done within 24 hours of cerebral arteriography. On the other hand, our experiments suggest that increased uptake of isotopes should occur only in pathological areas of damaged blood-brain barrier. Possibly post-arteriography scans would prove more accurate, especially in the low grade tumors which are notoriously difficult to diagnose.

Brain tumor chemotherapy has to date been unsuccessful, largely because an adequate ratio between tumor and normal brain uptake has not been achieved. Ojemann \textit{et al.}\textsuperscript{5} showed a differential uptake of 15 to 1 for Dysprosium^{165} versenate in mice brain tumors when compared to that in normal brain. Sweet\textsuperscript{9} in an attempt to eradicate human brain tumors has reported the use of a 10 to 1 tumor to normal brain boron com-

![Fig. 2. Symmetrical deep lesions in gray matter; the right hemisphere has been perfused with 50 per cent Hypaque prior to administration of trypan blue and Mercury^{203}.](image-url)
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pound combined with neutron capture therapy. But both of these possibilities appear to have practical limitations. Frigerio\textsuperscript{5} was able to eradicate mouse brain tumors completely with neutron capture by uranium phthalocyanate, which gave a 50 to 1 differential of tumor to normal brain. This suggests that a tumor to brain differential of 10 to 1 is not sufficient for radiation therapy to eliminate a brain tumor, although this level is certainly quite satisfactory for the qualitative differences needed in diagnostic brain scans. A tumor to brain differential of 50 to 1 may be the level needed for therapy. Most human brain tumors have tumor to brain differentials of less than 15 to 1 for most substances. If some method can selectively and safely multiply this differential to a more useful therapeutic level, both chemotherapy and chemoradiation might be more successful.

Further efforts should be directed at finding a safe, simple, and effective method of selective alteration of the blood-brain barrier. Intracarotid injections have obvious disadvantages. Other stresses which may be useful are low-level radiation or unfocused ultrasound.

Summary

We have reported animal experiments demonstrating that the pathologically altered blood-brain barrier of a focused ultrasonic lesion is further damaged by intracarotid injection of Hypaque, ethyl alcohol or distilled water in concentrations which do not damage the normal blood-brain barrier. A 7 to 10 fold increase of Mercury\textsuperscript{203} in perfused lesions occurred without apparent neurological impairment or gross edema. This type of selective modification of the blood-brain barrier may offer a method for increasing effectiveness of brain tumor diagnosis and therapy.

References

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