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THE FAILURE OF BUFFERED 5-HYDROXYTRYPTAMINE TO INCREASE BRAIN CAPILLARY PERMEABILITY TO ALBUMIN

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FOREWORD
(Nontechnical summary)

The brain swells following a wide variety of injuries such as strokes, head traumas, and intracranial tumors.

It has been suggested that brain swelling (brain edema) may be related to the release of 5-hydroxytryptamine (5-HT) from the site of injury. It is the purpose of the experiments described herein to test this hypothesis under carefully controlled conditions.

Rhesus monkeys were anesthetized and a 5-HT solution introduced into the ventricular system by appropriately placed needles. It was found that the 5-HT diffused into the periventricular brain tissue as expected but did not alter the blood-brain barrier in this region. Since an increase in brain capillary permeability is usually associated with the types of brain edema in question, this is evidence against an etiologic role for 5-HT.

ABSTRACT

The effect of buffered 5-hydroxytryptamine (5-HT) on the blood-brain barrier to ^{125}I-albumin was studied in 14 rhesus monkeys. 5-HT was introduced into the lateral ventricle by ventriculocisternal perfusion resulting in a concentration gradient in the periventricular tissue from 1 to 800 times endogenous levels. Buffered 5-HT failed to induce an increase in periventricular vascular permeability to ^{125}I-albumin. Earlier reports to the contrary may have resulted from species differences, as well as failure to control physiologic parameters and the use of highly acidic 5-HT preparations.

I. INTRODUCTION

The role of 5-hydroxytryptamine (5-HT) in brain edema has been studied by several investigators[4,8,10] but the results have been conflicting, in part reflecting a wide range of variability in experimental design. To resolve these conflicts, experiments should fulfill the following criteria: (1) 5-HT should be introduced at physiological pH, (2) tissue levels of 5-HT should be determined, (3) the gray and white matter should be studied separately, (4) the blood gases, pH and vital signs of the experimental animals should be maintained within normal limits,[3] (5) the effect of complexing ions present in commercial preparations and metabolites of 5-HT should be determined and (6) effects should be verified in primates.

The purpose of the present study was to measure the effect of 5-HT on the blood-brain barrier to albumin while adhering to the above criteria.

II. METHOD

General. Rall et al.[18] have developed a modification of Pappenheimer and Heisey's ventriculocisternal perfusion technique which allows sampling of the periventricular gray and white matter. 5-HT was introduced into the periventricular tissue by this method and the permeability of the blood-brain barrier to intravascular ^{125}I-albumin was then determined.

Rhesus monkeys weighing from 2.8 to 4.3 kg were premedicated with 0.5 ml atropine, anesthetized with intravenous pentobarbital, intubated, and maintained on a volume respirator. Rectal temperature was monitored continuously with a thermistor probe (Yellow Springs) and cooling prevented with an electric heating pad. Blood pressure and pulse were monitored through an arterial catheter and arterial pH, PCO_2

and PO_2 followed with a blood gas analyzer (Radiometer). The pH was maintained at 7.35-7.45, PCO_2 35-45 and PO_2 70-150. The monkey's head was secured in a stereotactic holder and a 22-gauge spinal needle was placed into the body of the lateral ventricle.[14] The extracranial portion of the needle was surrounded by a 38°C brass block which warmed the perfusion fluid before it entered the skull. An outflow path was provided by a needle passed percutaneously into the cisterna magna.

Tissue levels of 5-HT. Two monkeys were perfused with a balanced salt solution (BSS)[14] containing 1 mg/ml ^{14}C-5-HT creatinine sulfate (Amersham/Searle). The pH was adjusted to 7.26-7.36 and the solution sealed in a 50-ml lubricated glass syringe to prevent CO_2 loss. The syringe was placed in a Harvard pump and the solution infused at 0.764 ml/min. The perfusion was slowed to 0.382 ml/min after 10 minutes and continued at this rate until the experiment was terminated. The intracerebral path of the perfusion fluid was marked by a final 1-minute perfusion with pH adjusted BSS containing 4 mg/ml Evans blue.

At 110 minutes the animal was sacrificed with intravenous pentobarbital. Within 2 minutes the skull was opened with a Stryker saw, the brain removed, bisected, and immersed in liquid nitrogen until firm. Two 0.5-cm thick coronal sections were cut, one on each side of the anterior commissure. The tissue was kept firm by reimmersing it frequently in liquid nitrogen.

Slabs were cut from the corpus callosum and caudate nucleus with the long axis of the slab perpendicular to the ependymal surface (Figure 1). Only areas showing dark blue staining were sampled. Each slab was then cut into 0.5-mm slices beginning with the ependymal surface using either a multibladed knife or a specially

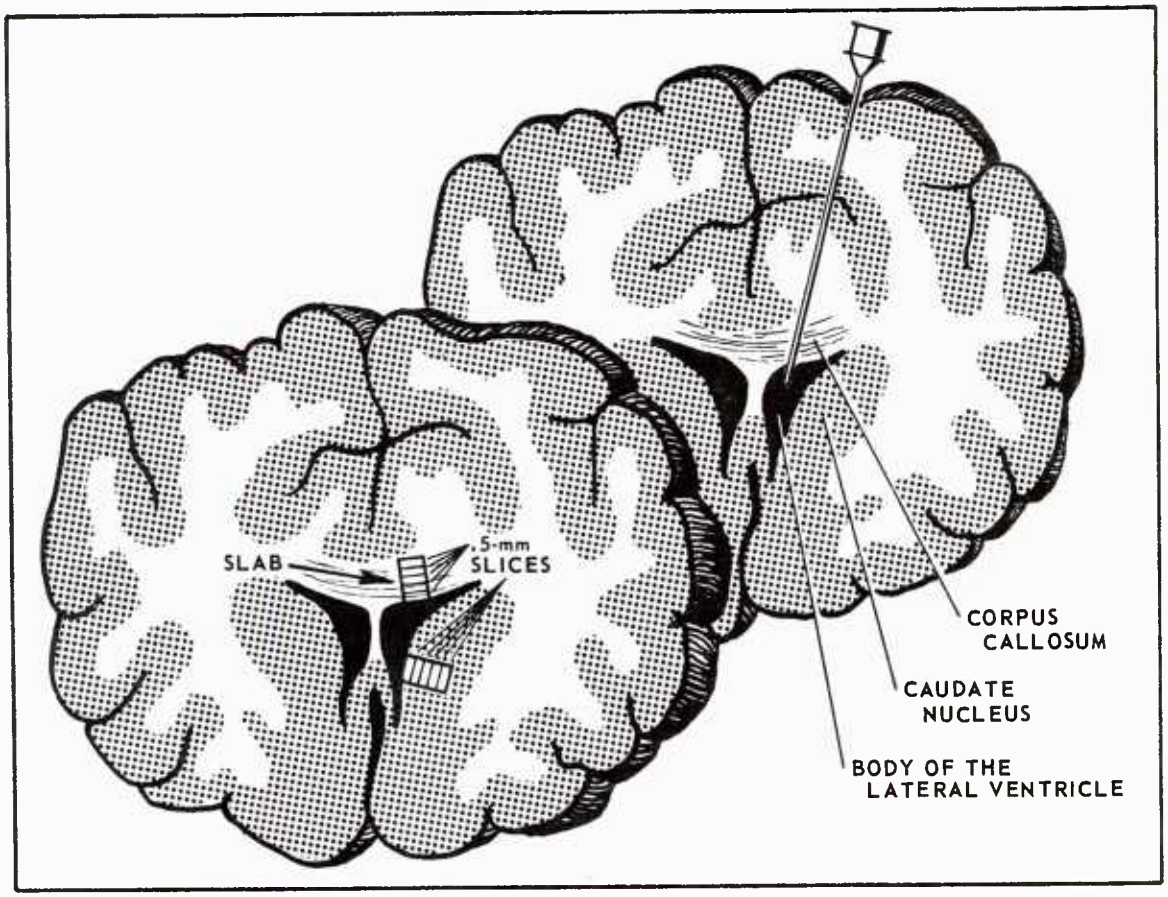

Figure 1. Coronal sections through monkey brain demonstrating location of ventricular needle and tissue sampling sites. Long axis of slab was cut perpendicular to ependymal surface. (Redrawn from Levin et al.[14])

fabricated micrometer driven slicer. Duplicate slabs were cut from each site. Slices from one caudate nucleus and one of the corpus callosum blocks were placed directly into individual tared counting vials for determination of ^{14}C activity per milligram of tissue. These vials were reweighed and the tissue shaken with 1 ml of NCS solubilizer (Nuclear-Chicago) for 1 hour at 50°C. Ten milliliters of scintillant (Liquiflor, New England Nuclear) were added and the radioactivity determined with a Nuclear-Chicago Model Mark II liquid scintillation counter. Samples of the inflowing CSF were similarly processed.

Since 5-HT is metabolized mainly to 5-hydroxy indoleacetic acid (5-HIAA),[16] the fraction of the total ^{14}C activity in a slice due to ^{14}C-5-HT was also determined utilizing the remaining slabs from the caudate nucleus and corpus callosum. Slices from these slabs were placed in individual 0.4 polyethylene test tubes containing 0.1 ml 0.4 normal perchloric acid and homogenized. 5-HIAA was extracted from the acidified homogenate into butyl acetate by the method of Perez-Cruet et al.[17] After alkalinizing the remaining solution, the 5-HT was extracted with n-butanol. Each fraction was placed in a counting vial, mixed with 10 ml of dioxane base scintillant and counted for 10 minutes. Control tubes to which either ^{14}C-5-HT (Amersham/Searle) or ^{14}C-5-HIAA (New England Nuclear) had been added were included with every run to determine the efficiency of the extraction procedure. It was found that 92-95 percent of the ^{14}C-5-HT was extracted with the n-butanol phase and 98-99 percent of the ^{14}C-5-HIAA was extracted with the butyl acetate phase.

Once the total ^{14}C activity in a slice is separated into that due to 5-HT and that due to 5-HIAA a ratio, r, can be defined as:

$$r = \frac{^{14}C\text{-5-HT}}{\text{Total } ^{14}C} .$$

The ^{14}C-space and 5-HT-space in each slice are computed as follows:

$$^{14}C\text{-space} = \frac{^{14}C \text{ activity/g tissue}}{^{14}C \text{ activity/g CSF}}$$

$$\text{5-HT-space} = ^{14}C\text{-space} \times r .$$

Tissue 5-HT and 5-HIAA concentrations were calculated from the formulas:

$$\text{5-HT (mg free base/g tissue)} = r \times \frac{\text{(MW 5-HT base)}}{\text{(MW 5-HT creatinine sulfate)}} \times {}^{14}\text{C-space} \times \text{mg 5-HT}$$

$$\text{5-HIAA (mg/g tissue)} = (1-r) \times \frac{\text{(MW 5-HIAA)}}{\text{(MW 5-HT creatinine sulfate)}} \times {}^{14}\text{C-space} \times \mu\text{g 5-HT}$$

where MW denotes the molecular weight.

Blood-brain barrier studies. The permeability of the periventricular capillaries to albumin after ventriculocisternal perfusion was determined in 12 monkeys. Four were perfused with BSS alone, two with creatinine sulfate, four with 5-HT creatinine sulfate, one with 5 M urea and one with 10^{-3} M $HgCl_2$. Both urea and $HgCl_2$ are known to injure the blood-brain barrier and were included to provide positive controls.[6,19]

Experimental details are the same as in the preceding section except that ^{125}I-albumin (Albumotope-Squibb) was injected intravenously at the onset of the perfusion. Two hours later the animal was sacrificed. Sequential slices from the caudate nucleus and corpus callosum were taken, weighed, dissolved in NCS solubilizer and the ^{125}I activity measured in a gamma counter Nuclear-Chicago Model 4333. Simultaneous counting of plasma samples allowed the albumin space to be calculated:

$$\text{albumin space} = \frac{^{125}\text{I activity/mg tissue}}{^{125}\text{I activity/mg plasma}} \times 100 .$$

III. RESULTS

5-HT levels. Tissue concentrations of ^{14}C-5-HT established by this technique range from 1 to 800 times endogenous levels (Figure 2) in both white and gray matter.

The tissue ^{14}C activity presented as ^{14}C-space decreases as the distance between the slice and the ependymal surface increases (Figure 3). The 5-HT-space is also

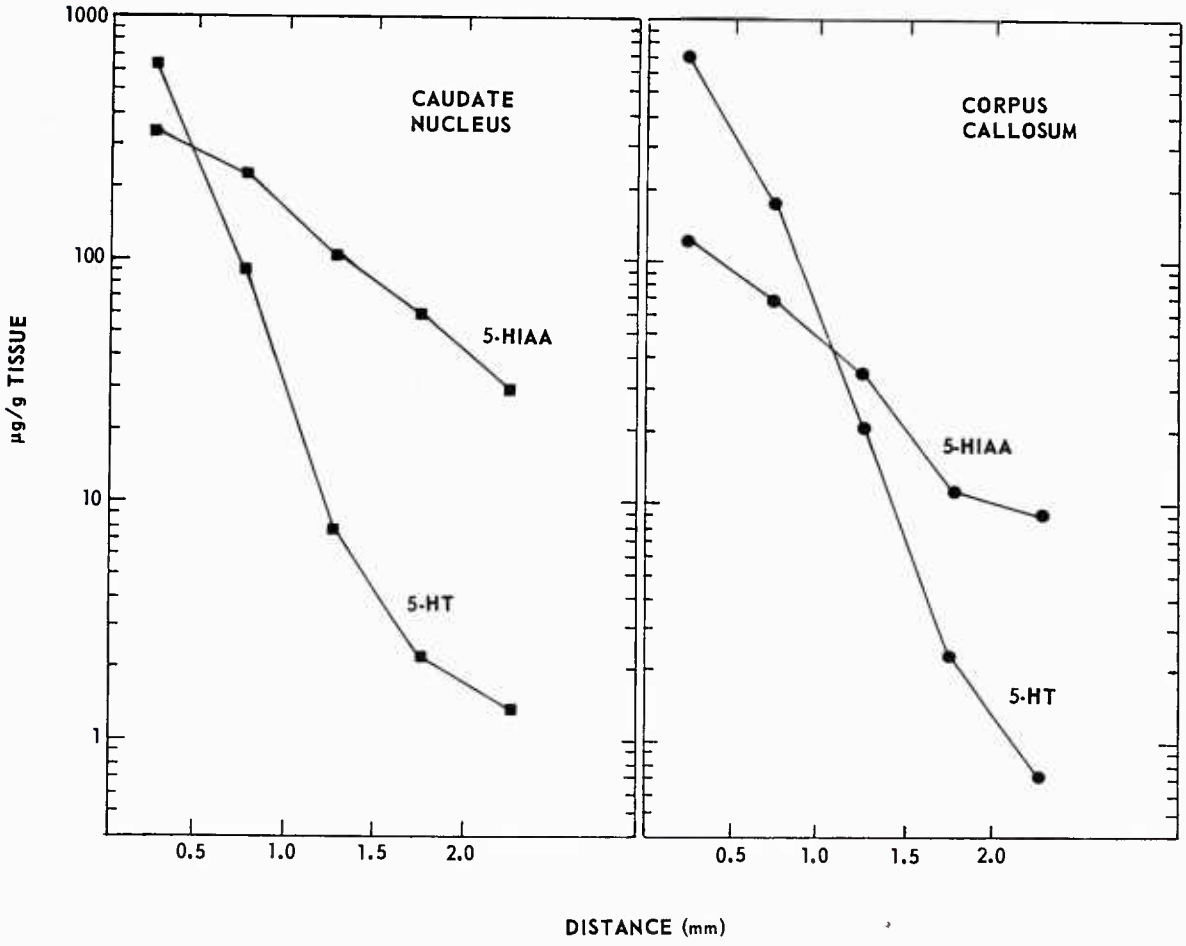

Figure 2. Caudate nucleus and corpus callosum tissue levels of exogenous 5-HT and 5-HIAA after ventriculocisternal perfusion. Micrograms free base or acid in each slice are plotted against the distance between the center of the slice and the ependymal surface.

plotted (Figure 3). In both the corpus callosum and caudate nucleus it initially exceeds 100 percent and then falls to less than 1 percent by the fifth slice. The ^{14}C-5-HT does not remain a fixed proportion of the total ^{14}C but decreases due to oxidation of 5-HT to 5-HIAA (Figure 4), in both the white and gray matter. No 5-HIAA was detected in the effluent CSF.

Blood-brain barrier studies. Initial experiments showed that ^{125}I-albumin space in the effluent CSF sampled at the cistern was less than 0.03 percent. After perfusion with BSS alone neither the gray nor white matter albumin space exceeded 1 percent (Figure 5). Two monkeys were perfused with creatinine sulfate because 5-HT is usually sold as the creatinine sulfate complex. The albumin space in these animals was similar to that found in controls perfused with BSS. The space in the first slice from the caudate nucleus was slightly larger than that in the corresponding slice from the BSS perfused animals; however, the significance of this small difference cannot be assessed due to the sample size (Figure 5).

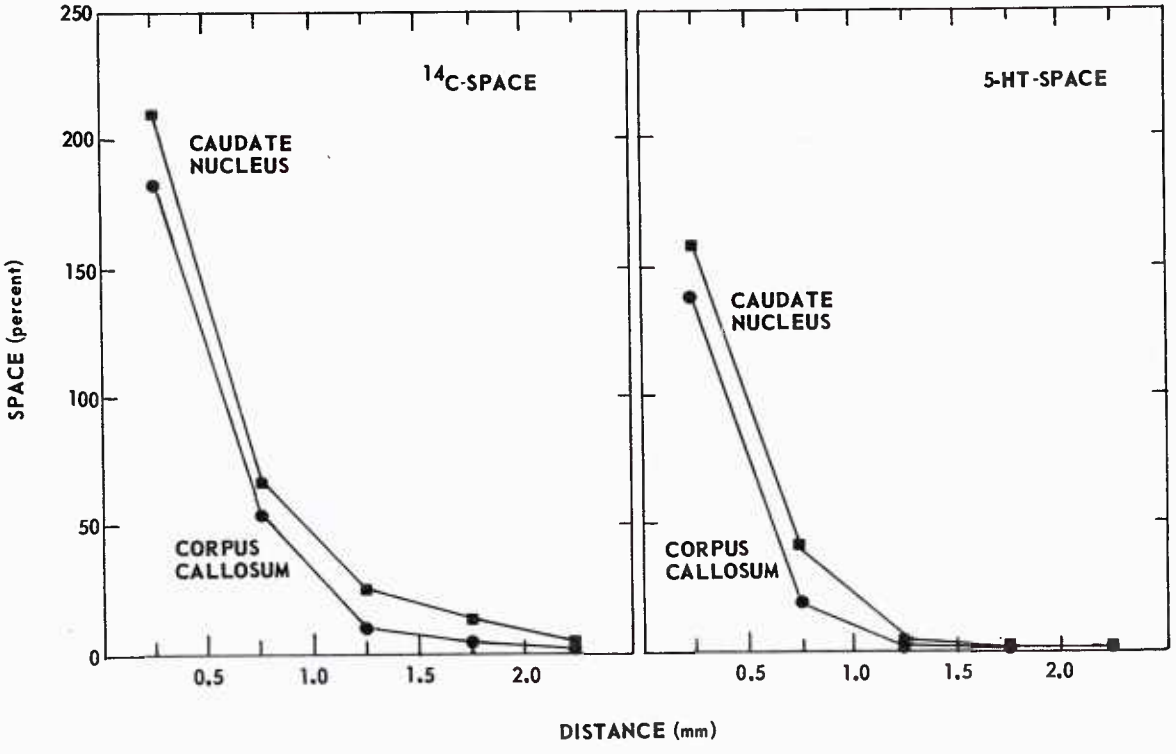

Figure 3. ^{14}C-space versus distance away from ependymal surface and 5-HT-space versus distance. Space initially exceeds 100 percent in both corpus callosum and caudate nucleus.

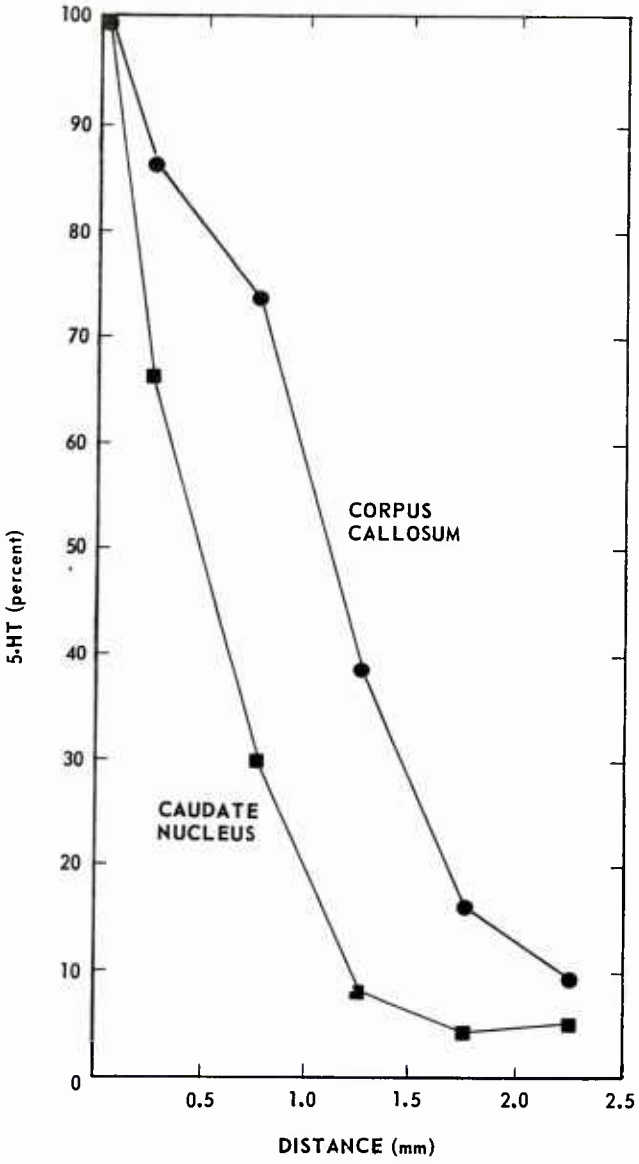

Figure 4.
^{14}C-5-HT percent of total ^{14}C activity (r x 100) plotted against distance of slice from ependymal surface

One monkey was perfused with 5 M urea and another with 10^{-3} M $HgCl_2$. Both substances are known to increase the permeability of the BBB to albumin[6,19] (Figure 5). No attempts were made to determine the tissue concentration of these substances as was done for 5-HT. The 5 M urea produced a maximum albumin space of 19.5 percent in the caudate and 4.7 percent in the corpus callosum. After perfusion

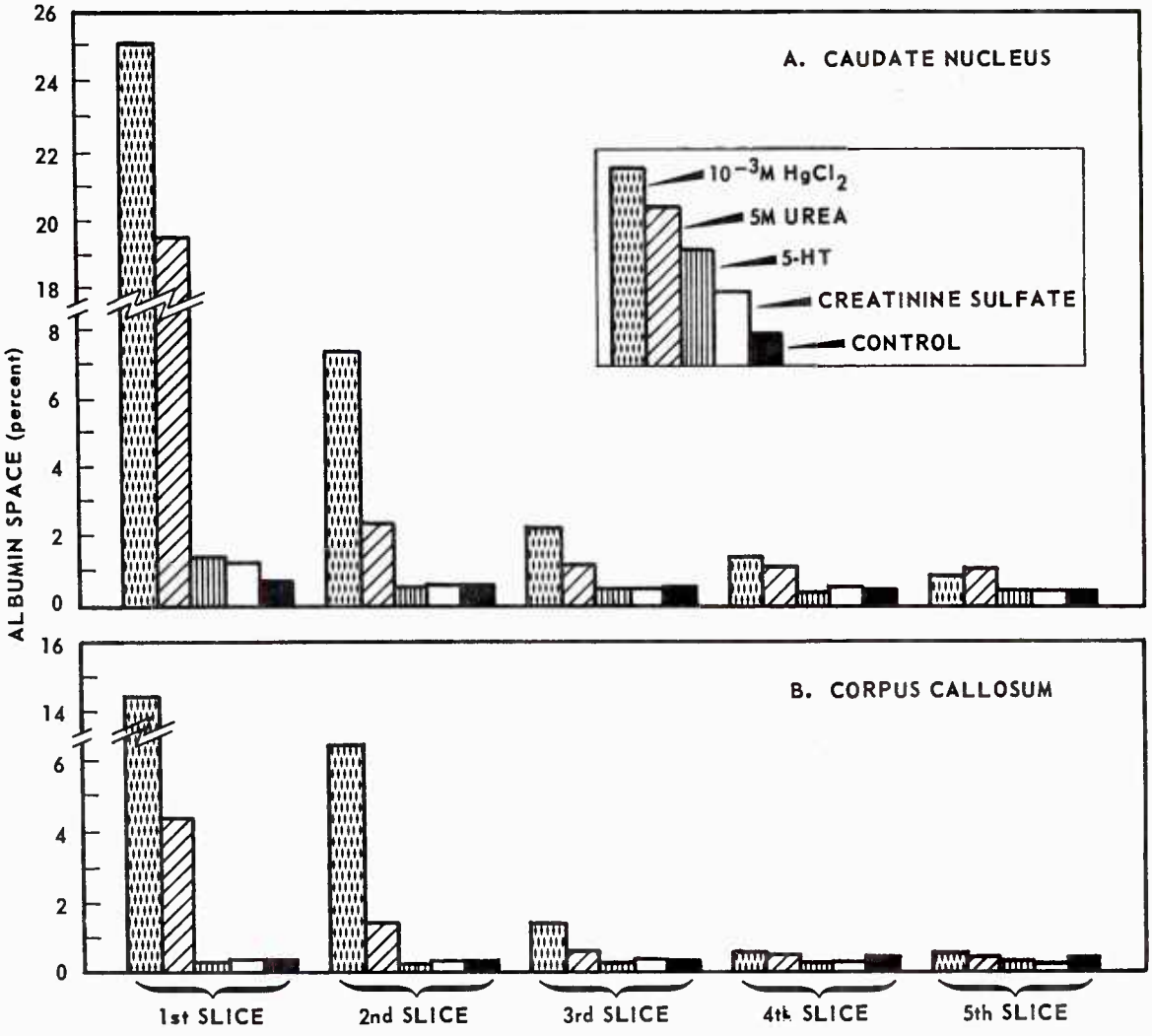

Figure 5. ^{125}I-albumin space for caudate nucleus and corpus callosum in successive slices after ventriculocisternal perfusion with various substances

with 10^{-3} M $HgCl_2$ the first periventricular slices from the caudate nucleus and corpus callosum had, respectively, spaces of 25.2 and 14.6 percent (Figure 5).

Four monkeys were perfused with 5-HT. As was the case for creatinine sulfate, there was a slight increase in the albumin space to 1.4 percent in the first slice taken

from the caudate nucleus (Figure 5). This was significantly different ($p < 0.01$) from controls perfused with BSS but not from those perfused with equimolar creatinine sulfate. Apart from this small difference in the first slice from the caudate nucleus the albumin space in the remaining slices did not differ significantly from control values.

IV. DISCUSSION

No significant increase in blood-brain barrier permeability was found following ventriculocisternal perfusion with buffered 5-HT, although the 5-HT penetrated the ependymal barrier and permeated the periventricular brain parenchyma. The latter observation is in agreement with the results of other laboratories.[1,7]

Osterholm et al.[15] have reported endogenous brain 5-HT concentrations between 0.1 and 1.0 μg/g in normal cats and noted that these levels approximately double after head trauma. Following ventriculocisternal perfusion with 5-HT, tissue levels reach approximately 800 times the endogenous concentration (Figure 2).

Since 5-HT is metabolized to 5-HIAA in both the gray and white matter by monoamine oxidase which is bound to the outer mitochondrial wall[12] conversion of exogenous ^{14}C-5-HT suggests that some of the amine gains access to the intracellular compartment.

The concentration of 5-HT in the first periventricular slice from both gray and white matter exceeds that of the perfusion fluid, but nothing can be concluded about the specificity of the concentrating mechanism.

The fraction of total ^{14}C activity due to 5-HT decreases with distance away from the ependymal surface (Figure 4). This observation may be the result of the more rapid diffusion of 5-HIAA due to its smaller size and negative charge, rather than the

greater rate of 5-HT oxidation in the deeper periventricular tissue. Conversely, estimates of the half-life of 5-HT based on single blocks of periventricular tissue as done by Aghajanian et al.[1] are likely to be too large, since the 5-HT in the sample is constantly diffusing into the periventricular tissue and is not present for the entire time interval.

Significant breakdown of the blood-brain barrier to albumin did not occur following ventriculocisternal perfusion with 5-HT at physiologic pH. Bakay and Haque[2] found that 1 hour after a cortical freezing lesion the albumin space was increased in the lesion to 26-39 percent and to 40-60 percent in the adjacent subcortical white as compared to control values of 2.8-3.2 and 0.3-0.7 percent respectively. We have shown that ventriculocisternal perfusion with 10^{-3} M $HgCl_2$ or 5 M urea produces considerable expansion of the albumin space in the caudate nucleus and corpus callosum (Figure 5). 5-HT perfusion did not result in a physiologically significant expansion of the albumin space even though its presence in the tissue was confirmed.

The 0.5 percent increase in the albumin space of the first slice from the caudate nucleus noted in both 5-HT and creatinine sulfate perfused animals is at the limits of resolution of the technique and is 10 to 15 times smaller than spaces associated with agents known to open the blood-brain barrier to albumin.

Our results do not support the conclusions of several earlier reports. Bulle[8] gave mice an intravenous infusion of Evans blue then injected 0.05 ml of a 5-HT solution into one cerebral hemisphere. A 2-mm blue globule was found in each injected hemisphere when his animals were sacrificed after 24 hours. This edematous response did not develop in mice pretreated with chlorpromazine or reserpine.

Approximately two-thirds of all animals died and about one-third of the survivors had intracerebral hemorrhages possibly due to the injection technique, making it difficult to evaluate the role of 5-HT in the production of edema. Second, Bulle used an unbuffered 1 mg/ml saline solution of 5-HT creatinine sulfate which has a pH of 3.9 at 38°C (personal observation); this in itself may open the blood-brain barrier to albumin (see below). Third, Klatzo et al.[13] have shown that the amount of brain edema which surrounds a freezing lesion is proportionate to the blood pressure. Both reserpine and chlorpromazine can produce severe hypotension.[11]

Gomirata and Zanalda[10] have demonstrated increased tissue water content in rabbit cortex slices bathed in Locke-Ringer solution containing 5-HT but Bourke[5] has demonstrated that in vitro swelling of cortical slices occurs by a chloride dependent electrolyte shift which is not analogous to extracellular edema occurring in vivo in the white matter.

Clasen et al.[9] were unable to demonstrate that reserpine diminished the edema resulting from cortical freezing in the monkey, but since none of their animals were pretreated with reserpine to deplete the 5-HT their negative results do not support or refute a role for 5-HT in brain edema.

Osterholm et al.[15] injected 5-HT creatinine sulfate into the periventricular white matter of cats and 24 hours later found that the injected hemispheres weighed 0.36 g more than the contralateral control side and had a 0.5 percent higher water content. Such small changes are difficult to interpret since intrahemisphere differences in weight and water content for saline injected controls were not published. Furthermore, it is not clear that all residual CSF was removed. As was the case for

Bulle's report, these authors used an unbuffered solution of 5-HT creatinine sulfate with pH 3.5-3.9. There is conflicting data about the pial blood-brain barrier after topical application of acids and its integrity appears to depend on the type of acid and duration of the application.[6] Possibly, an acidic substance injected into the cortex may be far more damaging since it is isolated from the buffering action of the CSF. Finally, the difference in species response to vasoactive drugs is well known, and any attempt to implicate 5-HT in the pathogenesis of brain edema in man based on results obtained in nonprimates may not be justified.

On the basis of our results the role of 5-HT in the pathogenesis of brain edema appears questionable. While our results meet the criteria outlined above, they pertain only to the state of the blood-brain barrier in an acute experiment. The experimental design does not allow inference regarding the effects of 5-HT on the blood-brain barrier after longer intervals, nor does it exclude the possibility that 5-HT may induce an increase in tissue water without altering the blood-brain barrier.

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13. ABSTRACT

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