Arch. Psychiatr. Nervenkr. 218, 369—377 (1974)

Transventricular Albumin Absorption in Communicating Hydrocephalus

Semiquantitative Analysis of Periventricular Extracellular Space Utilizing Autoradiography

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Received December 17, 1973

Summary. The movement of radioactive labelled albumin (RISA) after intraventricular or intrathecal injection from the cerebral ventricular system into the brain parenchyma has been observed in six dogs with experimental communicating hydrocephalus as well as in a control group. Autoradiography with determination of grain counts per brain-tissue unit was performed to obtain the relation of radiopharmaceutical tissue-concentration versus distance from the ependymal lining. 4 h after injection there was a significant higher tissue-concentration of RISA in the hydrocephalic than in the control group. The distribution in normal animals appears to be consistent with diffusion into the extracellular space of the brain; whereas in hydrocephalic animals intracerebral bulk flow with alteration of the cerebrospinal fluid-brain barrier is suggested.

Key words: Hydrocephalus — Brain — Cerebrospinal Fluid — Extracellular Space — Radioautography.

Zusammenfassung. Nach intraventrikulärer oder intrathekaler Injektion von radioaktiv markiertem Jodserumalbumin (RISA) wird dieses Radiopharmazeutikum sowohl bei 6 Hunden mit experimentellem, kommunizierendem Hydrocephalus als auch bei einer Kontrollgruppe vom Ventrikelsystem in das Gehirnparenchym aufgenommen.

Das Verhältnis zwischen RISA-Konzentration im Hirngewebe und Entfernung vom Ventrikelependym wurde durch Autoradiographie mit Bestimmung der Silberpunkte pro Gewebseinheit durchgeführt.

Die Gewebskonzentration von RISA ist nach 4 Std in der Hydrocephalusgruppe wesentlich höher als bei den Kontrolltieren. Die Verteilung kann bei normalen Tieren durch Diffusion erklärt werden. Bei Hydrocephalustieren wird ein intracerebraler Transportfluß mit Veränderung der Liquor-Hirn-Schranke angenommen.

Schlüsselwörter: Hydrocephalus — Gehirn — Liquor cerebrospinalis — Extrazellulärer Raum — Autoradiographie.

Introduction

Cisternography has demonstrated altered cerebrospinal fluid (CSF) flow in patients with chronic communicating hydrocephalus and associated symptoms as dementia, gait, problems an mental deterioration [5].

In these individuals high molecular weight radioactively labelled substances injected into the lumbar subarachnoid space concentrate in the lateral ventricles instead of the parasagittal areas over the cerebral hemispheres as is seen in normals. These observations are thought to reflect abnormal cerebrospinal fluid (CSF) flow and absorption, because radiopharmaceutical concentration probably occurs at the site of maximal CSF out-flow and absorption. Transependymal CSF absorption from the ventricles into the parenchyma of the brain is suggested by autoradiographic investigations on animals with experimental communicating hydrocephalus [7, 13]. These experiments clearly show the evidence of radiopharmaceutical in the extra-cellular space (ECS) of the brain parenchyma which was migrated through the ependymal lining into the neurophil. This abnormal intracerebral movement of albumin is probably accompanied by the neurological symptoms seen in patients with communicating hydrocephalus. The concept of transventricular CSF absorption is confirmed by the observation that after a CSF diversionary shunt operation, these patients often regain near normal neurological function.

This study describes the intracerebral movement of CSF by evaluation of the distribution of radiopharmaceutical in control dogs and those with experimental chronic communicating hydrocephalus.

Material and Methods

Six mongrel dogs, weighing 10—16 kg each, were examined by cisternography. No evidence of spontaneously occurring hydrocephalus was seen. Communicating hydrocephalus was then induced in these dogs by injection of a silicone-pantopaque or silicone-tantalum powder mixture into the subarachnoid space as described previously [6]. Within 6 weeks of this procedure, cisternograms indicated the existence of communicating hydrocephalus in all 6 dogs. Images were obtained at 0.5, 4 and 24 h following the injection of radiopharmaceutical into the cisterna magna. The 0.5 h images showed ventricular entry of the radioactivity in all 6 dogs. In 3 of the dogs, radiopharmaceutical was seen outside of the ventricles at 4 h. At 24 h, radioactivity was seen uniformely distributed over the subarachnoid space (communicating hydrocephalus with clearing). In the three remaining animals, radioactivity remained in the ventricles for 24 h (communicating hydrocephalus with stasis).

The radiopharmaceutical employed in the imaging portion of this study was ^{99m}Tc serum albumin. The short half-life and lack of β-radiation ^{99m}Tc make it unsuitable for autoradiographic studies, so ^{131}I serum albumin (RISA) was employed. Unbound iodine was measured by paper electrophoresis and preparations of RISA with more than 2% unbound ^{131}I were excluded from the study.

Each of the 7 animals (6 hydrocephalic and 1 control) was given a 0.2 ml injection of RISA (500 μCi/ml). The injections were made into the cisterna magna of 5 of the hydrocephalic dogs and infused by slow injection into the ventricles of the other 2 (1 hydrocephalic and 1 control). 4 h after radiopharmaceutical placement the animals were sacrificed by perfusion with 10% formalin (approximately 500 ml) through both carotid arteries. Attempt was made to do this at as physiological pressures as possible. The brain with meninges was removed immediately and cut into 10 mm thick coronal slices. The tissue was fixed for 12—24 h in 10% formalin

and embedded in paraffin. Four micron thick coronal sections were made through the lateral ventricles, placed on slides and coated with liquid emulsion (Kodak NTB²)[1]. The slides were stored at 4° C. for 24 h, developed with D-19[2] and fixed. The tissue was stained with hematoxylin and eosin, and cover slips were mounted over tissue and emulsion.

The relative tissue concentration of labelled albumin was determined by serial grain counts along a straight line transiting the brain tissue starting at the ventricular ependyma and ending at the subarachnoid space. Grains were counted in areas defined by a 6×6 ocular reticle at a magnification of $1000\times$. The slides were moved one reticle width at a time until the cerebral surface was reached. The relationship between reticle width and tissue distance was such that 10 reticle widths = 1 mm tissue distance. Three non-overlapping, parallel, replicate counts were obtained from each slide. These three counts were combined and the serial counts were averaged for 1 mm of tissue length (i.E. 10 reticle counts). Thus, each grain count/mm is the average of 30 counts. In order to allow pooling of counts from different animals, the counts from each were normalized to the count at the ventricular ependymal surface for that dog.

In all animals, radiopharmaceutical concentration was determined from the superior surface of the lateral ventricle through the corpus callosum and white matter to the cerebral cortical surface. Careful attention was paid to avoid the ventricular angles because total loss of the cuboidal ventricular ependyma and frank tears in the lining occur here.

Results

The data is presented as relative concentration (the ratio of the amount of radiopharmaceutical in the parenchyma to the amount of radiopharmaceutical at the ependyma) plotted against distance from the ependyma in millimeters. Fig. 1 demonstrates the pattern noted in the control dogs. In these animals, significant tissue levels of albumin are present at a distance of 2.5 mm from the ventricular ependyma. Between 3.5—14.5 mm the levels of radioactivity are so low that they are indistinguishable from zero. At 15.5 mm distal to the ventricular ependyma there is a small rise in concentration which correlates with the location of this point at the cerebral surface of the subarachnoid space reflecting normal flow of radiopharmaceutical. This portion of the curve is considered to represent movement of albumin from the cortical subarachnoid space into the extra-cellular space (ECS) of the brain tissue.

In the animals whose CSF images demonstrate communicating hydrocephalus and "clearing" of the ventricles on later views, there is radiopharmaceutical in the ventricular system and cerebral subarachnoid space simultaneously at 4 h after injection. The radiopharmaceutical appears to penetrate from both the ventricles and cortical subarachnoid space into the brain parenchyma. However, periventricular radioactivity is greater than in normals with intraventricular infusion especially at depth in the neurophil.

1 Eastman Kodak Corp., Rochester, N. Y.

2 Standard X-Ray Co., Chicago, Ill.

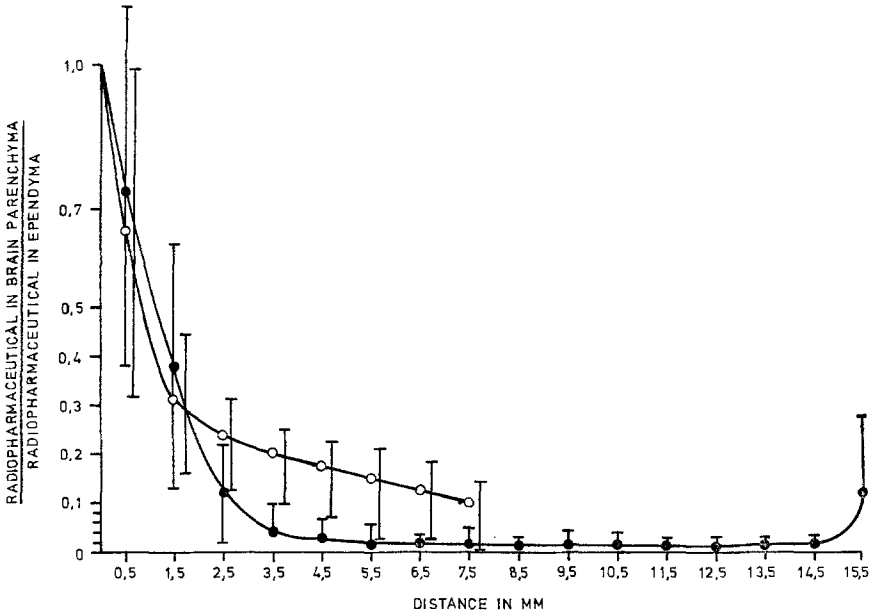

Fig. 1. The lower line which extends to 15.5 mm represents the distribution of radioactivity from ependymal surface in the control animal with ventricular injection of radiopharmaceutical (arithmetical paper). At depth of greater than 3.5 mm the amount of radioactivity approaches zero. The other line which reaches to 7.5 mm reflects the distribution of radioactivity from ependyma to cortex in animals with chronic communicating hydrocephalus. The distance from ependymal surface to cortex in this group measures only 7.5 mm because of the considerable ventricular enlargement and decrease of brain volume

The data for the hydrocephalic dogs with "stasis" is also shown in Fig. 1. The initial slope is different to that for normal animals. In the hydrocephalic group the initial slope is greater than in the control. However, in the animals with ventricular "stasis" there are significant levels of albumin at distances as fare as 7.5 mm from the ependyma. The only apparent source of radiopharmaceutical in chronic communicating hydrocephalus is the ventricle.

In order to characterize the movement of radiopharmaceutical into the brain parenchyma, the data from the control and hydrocephalic dogs was further considered. This analysis of the data utilized the modified method of Schwartz and Lauffer [12] as applied by Fenstermacher *et al.* [4] who performed studies of this type. The method consists of replotting the data on complementary error function paper[3] (modified normal probability paper).

3 Biometry Chart-MH-276 (7-70) was kindly made available to us by J. D. Fenstermacher.

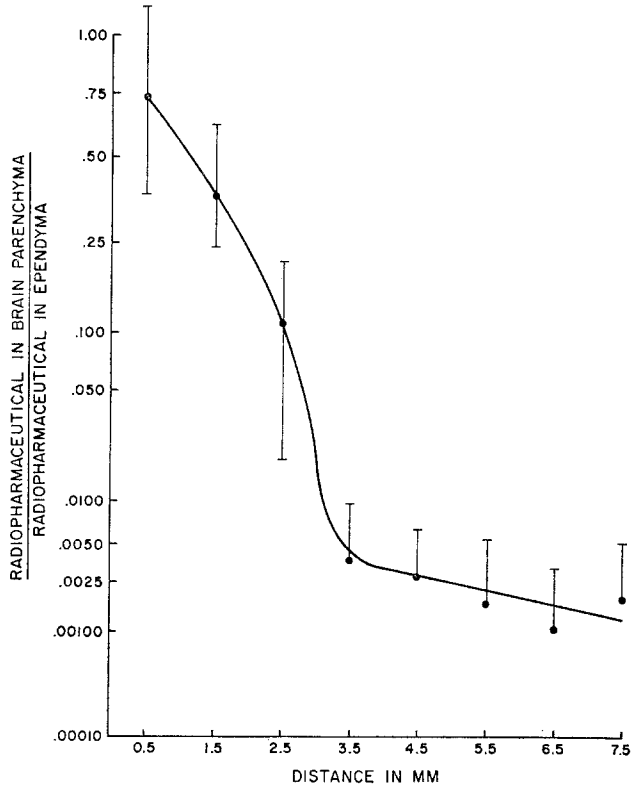

Fig. 2. Plot on complimentary error function paper of the control animal with intraventricular radiopharmaceutical infusion. The radioactivity curve is linear between 0.5 and 2.5 mm suggesting a single process (such as diffusion). The downward deflection of the curve between 2.5 and 3.5 mm may be due to a process acting in the opposite direction (such as passage from the cerebral veins into the parencyma) or minimal periventricular edema due to the infusion technique

The value of this type of evaluation is that if a substance moves into a medium according to a simple diffusion process, a plot of relative concentration vs. distance into the medium characteristically gives a straight line over the measured concentration gradient. Processes of facilitated diffusion such as bulk flow will show data points (and a connecting line) with a less steep slope than in simple diffusion.

Fig. 2 is a plot of the control data on this type of probability paper. The curve does not indicate a simple diffusion process from ventricle into brain parenchyma. There is an increasing slope to a distance 3.5 mm from the ventricular surface. Beyond that point there is a flat linear portion. However, the concentration levels from 3.5—8.5 mm were not

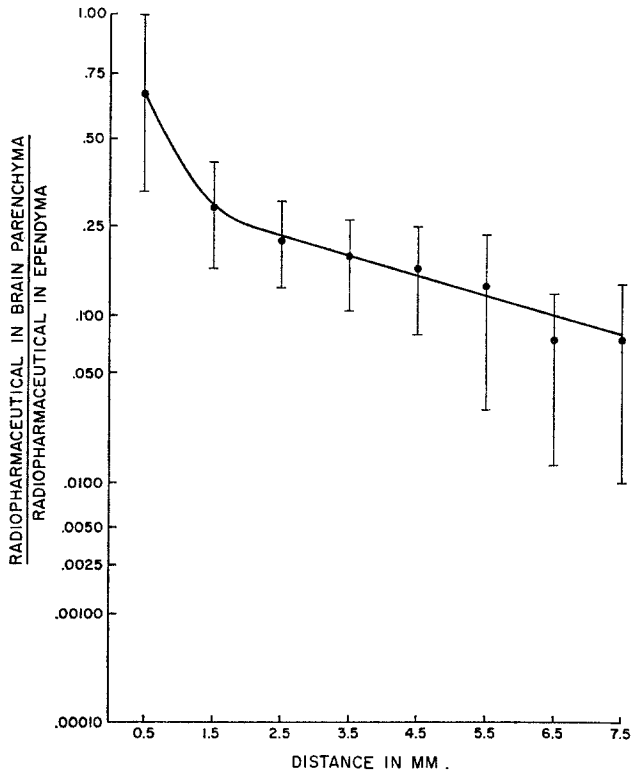

Fig. 3. Plot of radioactivity on complimentary error function paper Biometry Chart MH 276 (7-70) of animals with communicating hydrocephalus and "stasis". Upward curve beginning at 0.5 (50%) demonstrates that another process other than diffusion (such as bulk flow) is present between 0.5 and 2.5 mm

considered significant. In Fig. 3, the data for the hydrocephalic animals with "stasis" are plotted. In these animals, the concentrations of albumin are much higher than would result from diffusion alone; the curvature of the distal portion of the plot is upward. When the primary pathway for CSF clearing of protein through the arachnoid villi is altered (diminished) there is a large increase in the amount of albumin which moves into the parenchyma of the brain by this method.

Discussion

It has been established that CSF is absorbed from the ventricular system in animals and patients with noncommunicating hydrocephalus [1, 9, 11, 13]. Although is has been documented that radioactive-labelled albumin appears in the circulation in patients with chronic communicat-

ing hydrocephalus, the pathways from the ventricle into the brain parenchyma and from there into the circulation are still unknown. Ventriculocisternal perfusion studies on "normal" animals [2, 4], as well as light and ultrastructural studies in both normal and hydrocephalic animals [3, 7] indicate that the ventricular ependyma is only a partial barrier to the movement of molecules ranging in size from sucrose to ferritin. Based on these observations, it is not surprising that some RISA were found in the brain parenchyma following ventricular infusion. An alternative explanation for the observed difference is that the radiopharmaceutical remains in contact with the ventricular wall in animals with "stasis" longer than in normals or animals with ventricular entry and "clearing". For this reason slow intraventricular infusion was utilized to minimize this effect.

The distribution pattern of RISA in the normal animal appears to be consistent with diffusion into the ECS and transfer to the circulation. There is free movement of the albumin out of the ventricle into the subarachnoid space which is seen cisternographically and is indicated autoradioagraphically by presence of significant concentrations of RISA in brain parenchyma adjacent to the ependymal surface. At the time of sacrifice (4 h), the paraventricular concentration of RISA was greater than in other areas containing CSF, thus, movement across ventricular ependyma appears to be the dominant feature of the distribution pattern. When the diffusion profile is plotted (Fig. 2), a curve with downward deflection is seen until it reaches trace amounts of RISA at distances greater than 3.5 mm from the ependymal surface.

Rall discusses departures from linearity in this type of analysis [10]. The occurrence of back diffusion from tissue to ventricle associated with diffusion of solute from the ventricle into the tissue would generate a curve of the type seen in normals. This circumstance may exist when the marker is inulin; in the case of albumin which crosses the blood-brain barrier, there is the additional possibility that the tissue concentrations are being lowered by transfer of the radiopharmaceutical into the blood [10]. This effect of transfer across the bloodbrain barrier has been reported in the diffusion profile of H^3OH [4]. Because H^3OH diffuses rapidly into the parenchyma and is rapidly transferred from CSF to blood, there is no significant activity beyond 1 mm distance to the ventricular ependyma.

In animals with chronic communicating hydrocephalus and stasis (Fig. 3) the diffusion profile demonstrates an initial slope similar to the control. However, the slope rapidly begins to decrease suggesting that albumin accumulates against a gradient. Rall points out that bulk flow would produce a curve of this type with a decreasing slope [10]. This appearance can also result from a blockage or resistance to transfer through

the tissue. Since the albumin is being transferred to the blood, a result of this type could be explained if the transfer rate from the parenchyma into the blood is the limiting factor. If the albumin is moving into the ECS faster than it is being transferred across the barrier, it will accumulate in the brain parenchyma. It seems unlikely that simple enlargement of the ECS would be a sufficient explanation alone. These may contribute to the observations reported but are not suggested either by the temporal sequence or the histological appearance from that present in normals. Other processes such as reversible absorption to cells or pinocytosis were not evaluated in the present study but again could seem insufficient to explain the dynamic changes observed.

It should be noted that all animals in this study had normal cisternal CSF pressures at the time of sacrifice. The greatly increased intracerebral bulk flow of RISA in animals with communicating hydrocephalus observed by us, suggests alteration of the CSF brain barrier. Studies of transfer rates of albumin from CSF to blood support the suggestion by Levin *et al.* [8] that hydrocephalus causes some changes in brain capillary permeability. This alteration of the blood-brain barrier system with a resultant increase in intracerebral bulk flow might disturb the function of the central nervous system and underly the observed clinical symptoms.

We wish to acknowledge inspiration from Giovanni DiChiro, Guy McKhann and Martin W. Donner. The resources of the Department of Radiology and Radiological Sciences (M. W. Donner, Chairman) were utilized. Gary Novak, Carol Digel, Jim Larzan and Henry Wagner assisted in many ways.

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