Evidence of the Limitations of Water as a Freely Diffusible Tracer in Brain of the Rhesus Monkey

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ABSTRACT

The extraction of $^{18}$O-labeled water by the brain during a single capillary transit was studied in vivo in 20 adult rhesus monkeys by external detection of the time course of the tracer subsequent to the internal carotid injection of 0.2 ml of whole blood labeled with $H_2^{18}$O. The data showed that labeled water does not freely equilibrate with the exchangeable water in the brain when the mean cerebral blood flow exceeds 30 ml/100 g min$^{-1}$. At the normal cerebral blood flow in the rhesus monkey (~50 ml/100 g min$^{-1}$), only 90% of the $H_2^{18}$O is extracted during a single capillary transit. In addition, cerebral blood flow was determined with $H_2^{18}$O and $^{133}$Xe in these monkeys using residue detection and employing the central volume principle. The data supported the hypothesis that a diffusible tracer, $H_2^{18}$O, need not be in complete equilibrium between the phases of a system for the application of the central volume principle to be valid. Finally, the brain capillary permeability-surface area product was computed from these data; it was approximately 0.023 cm$^2$/sec g$^{-1}$.

KEY WORDS blood-brain barrier cerebral blood flow $^{133}$Xe central volume principle capillary permeability $^{18}$O-labeled water

The diffusion of radioisotopically labeled water into tissue is assumed to be rapid enough relative to bulk movement (convection) by capillary blood flow through the tissue to allow complete equilibration during a single capillary transit and subsequent clearance from the tissue. In effect, the movement of water or a similar tracer through a given tissue is assumed to be blood flow limited (1). Evidence for this assumption comes from the work of Johnson et al. (2), who have shown that water is flow limited in its exchange in dog heart and skeletal muscle. In addition, Yipintsoi and Bassingthwaighte (3) have shown that labeled water is flow limited in its capillary exchange in dog heart. Yudilevich and DeRose (4) have concluded that labeled water diffuses freely in dog brain. Others have also assumed that water attains complete equilibrium with the exchangeable water in the brain during a single capillary transit (5-9). As a result, labeled water has been used as a standard for the measurement of cerebral blood flow (10). In the present paper, we will present evidence that labeled water does not freely equilibrate with the exchangeable water in the brain of the rhesus monkey when the mean cerebral blood flow exceeds approximately 30 ml/100 g min$^{-1}$.

Methods

The fraction of labeled water extracted by the brain during a single capillary transit was determined subsequent to the internal carotid injection of 0.2 ml of whole blood labeled with $H_2^{18}$O in 20 adult rhesus monkeys. To facilitate the injection of the radioisotope into the internal carotid artery, all branches of the right external carotid artery were ligated 2 weeks prior to the experiments. The radioisotope was then injected into the common carotid artery through a small (diameter 0.021 cm) catheter positioned there under fluoroscopic control from the femoral artery.

The monkeys were anesthetized with phencyclidine, paralyzed with gallamine, and passively ventilated with 100% O$_2$. End-tidal P$_{CO_2}$, arterial blood pressure, and rectal temperature were continuously monitored. Temperature was maintained between 37°C and 39°C with a heating pad. Arterial pH, P$_{CO_2}$, and P$_O_2$ were measured before and after each injection.

The time course of tracer movement through the injected hemisphere was monitored by a 3 x 2-inch NaI(Tl) scintillation detector appropriately collimated and positioned to ensure essentially uniform detection efficiency for the entire hemisphere. The signal from the detector was processed by a pulse-height analyzer with an energy window of acceptance adjusted symmetrically around the 511-kev photopeak of $H_2^{18}$O (481–541 kev) to eliminate scattered radiation. The accepted events (counts) per time frame were stored in the memory of a.}

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This work was supported by U. S. Public Health Service Grants 5 PO1 HL13581, RR 06396, and 5 PO1 NS0 6833 from the National Institutes of Health and by Teacher-Investigator Award 1 F11 NS11069 (Dr. Raichle) from the National Institute of Neurological Disease and Stroke.

Received February 13, 1974. Accepted for publication May 15, 1974.
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small laboratory computer (LINC). Appropriate data processing including conversion to count rate (counts/sec) as a function of time and corrections of the count rate for electronic dead time loss, physical decay, and background were performed by the computer. Routine data retrieval was in the form of processed count rate as a function of time plotted by an x-y plotter. Optimal temporal resolution was achieved in the initial portion of each recording by utilizing sampling integration times of 0.1 seconds. Statistically smooth recordings were ensured by injecting enough labeled water (~200 μC/0.2 ml) to achieve a peak counting rate of 10,000-20,000 counts/sec. Radioactive water labeled with ^18O (half-life 123 seconds) was produced for these studies in the Washington University Medical School Cyclotron by deuteron bombardment of nitrogen gas (11).

The fraction of labeled water extracted by the brain during a single capillary transit (E) was determined by graphically extrapolating the relatively slow clearance of labeled tissue water back to the abscissa of the maximum of the perfusion peak and computing the ratio

\[ E = \frac{B}{A}, \]

as shown in Figure 1.

Cerebral blood flow was determined by utilizing residue detection (12) of the bolus of labeled water injected into the internal carotid artery. The time-activity curve for the washout of H_2^18O from the brain was used to calculate the water mean transit time (T_{H^18O}), which is defined as

\[ T = \int_0^T q(t)dt, \]

where \( q(t) \) is the radioactivity level in the region under study as a function of time and \( q_0 \) is the dose of radioactivity in the injected bolus. The computed value of \( T_{H^18O} \) was combined with the central volume principle (12),

\[ T = V/F, \]

where \( F \) is the volumetric flow rate of vascular fluid and \( V \) is the "volume of distribution" of the tracer, and the mean equilibrium brain-blood partition coefficient of water (\( \lambda_{H^18O} \)) to yield cerebral blood flow (CBF) in ml/100 g min^-1:

\[ CBF = \frac{\lambda_{H^18O} \times 100}{T}. \]

A value of 0.95 ml/g was used for \( \lambda_{H^18O} \).

The extraction fraction (E) of labeled water and the cerebral blood flow were measured at different levels of arterial CO_2 tension. Arterial PCO_2 was lowered by passive hyperventilation and raised by ventilation with a gas mixture of 90% O_2-10% CO_2 (arterial PCO_2 range 14 to 98 torr). A period of at least 15 minutes was allowed for the establishment of a steady state at each level of arterial PCO_2.

Results

An example of the data obtained is shown in Figure 1. The early portion of the data (0-5 seconds) reveals the rapid clearance of a fraction of the injected water from the field of view of the detector. The half-time for the clearance of this rapidly egressing fraction during the first several seconds following injection was compared with that of the vascular indicator C^14O-labeled hemoglobin. C^14O-labeled hemoglobin and water were injected in sequence; arterial blood gas measurements bridged the injections to ensure a steady state. The half-times were obtained by conventional curve stripping. The computed half-times for the rapid component of the water clearance ranged from 0.5 to 2.5 seconds; in all instances, they were similar to those of the corresponding vascular tracer. An example of such a comparison is shown in Figure 1.

The relationship between E and cerebral blood flow is shown in Figure 2. These data demonstrate a highly significant relationship between cerebral blood flow and E (P < 0.001). The equation for the linear regression line is \( E = 1.11 - 0.0037CBF \) with a correlation coefficient of \( r = -0.97 \). From these data it can be seen that complete extraction

\[ \text{Semilogarithmic recordings obtained subsequent to serial intracarotid injections of H}_2^{18}\text{O (top) and C}^{14}\text{O-labeled hemoglobin (bottom). Extrapolation of the relatively slow clearance of labeled tissue water back to the abscissa of the maximum of the perfusion peak allows the computation of the extraction fraction: } E = \frac{B}{A}. \]

Resolution of the water clearance into two components shows that the half-time (T60) of the fast component is essentially that of labeled red cells.

Circulation Research, Vol. 35, September 1974
of water is achieved only for cerebral blood flows less than approximately 30 ml/100 g min⁻¹; this value is considerably less than the normal cerebral blood flow for the rhesus monkey (~50 ml/100 g min⁻¹) (13).

**Discussion**

The rapid egress of an appreciable fraction of the injected labeled water from the field of view of the detector could be interpreted in several ways. It could represent (1) an artifact of the experimental procedure, (2) anatomical arteriovenous shunting in the brain, (3) a permeability restriction imposed on the labeled erythrocyte water, or (4) a brain-capillary permeability limitation of the water exchange. We have established to our satisfaction that an appreciable brain-capillary permeability limitation to water is the only plausible explanation. Negation of the other interpretations was achieved in the following manner.

**Artifact of the Experimental Procedure.**—A rapid redistribution of the labeled water within the brain could perhaps produce the observed phenomenon if the detection efficiency of our detector varied appreciably within the monitored field or if the redistribution of the tracer included tissues not in the view of the collimated detector. Both possibilities were eliminated by moving the detector 100 cm from the brain, thus radically changing the detection geometry; no change in E was observed following this maneuver.

Neither the monkey preparation nor the injection procedure is responsible for our observations. The observed E was unchanged whether the labeled water was injected into the common carotid artery of the model hemisphere with the external carotid artery ligated or into the common carotid artery of the contralateral hemisphere. Perturbation of cerebral blood flow due to our standard injection procedure (0.2 ml, 0.5 seconds) seems unlikely, since 0.05 ml of labeled water injected in approximately 2 seconds also yielded similar extraction fractions.

**Arteriovenous Shunts.**—The presence of arteriovenous shunts within the brain could account for the nonextracted fraction of the injected water. Evidence suggesting their presence has been reported (14-17). To exclude the presence of arteriovenous shunts within the brain as a possible explanation for our finding, monkeys were injected with labeled water and labeled microspheres (¹⁸⁸Sr, 514 keV, 15 ± 5 μ in diameter) (3M Company) under identical flow conditions. In addition to monitoring the brain activity with an external detector, a small catheter (diameter 0.021 cm) was operatively placed in the superior sagittal sinus. A second detector was placed over the external extension of this indwelling catheter, and blood was drawn through it at a rate of 2.5 ml/min to record any radioactivity leaving the brain following the injection of the microspheres. These experiments revealed total extraction of the microspheres by the brain when the value of E for water was significantly reduced. An example of this observation is shown in Figure 3.

**Erythrocyte Permeability Limitation.**—The injected labeled water contained in the whole blood could be considered to be partitioned into plasma water and erythrocyte water. The diffusion of the erythrocyte water into brain tissue could be restricted by the permeability of the erythrocyte membrane. Two observations in our study fail to support this hypothesis. First, the systemic hematocrit was reduced in one monkey from 0.41 to 0.20 by the acute removal of blood and its replacement with saline. The observed values of E in this monkey were those predicted by the values of cerebral blood flow, as shown in Figure 2. Second, several injections in additional monkeys of labeled plasma water, labeled erythrocyte water (packed cells), and saline containing labeled water resulted in identical values of E at a given value of cerebral blood flow. These observations coupled with the known very high water diffusion permeability of the erythrocyte (18) make it unlikely that re-
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Restricted diffusion of water across the erythrocyte membrane is responsible for our observations.

Thus, it appears reasonable to assume that the capillary wall is the limiting structure to the diffusion of the labeled water into the brain. The injected bolus, in effect, divides into two fractions, a nonextracted or transmitted fraction which is confined to the vascular volume and an extracted fraction which passes through the capillary wall, diffuses through the tissue space, and eventually returns to the blood. The extracted fraction depends on the permeability of the membrane to the water, the flow rate of the labeled water within the capillary, and the surface area available for transcapillary exchange of the water.

Measurement of the Extraction Fraction of Water

The method we employed to measure the fraction of a labeled substance extracted by the brain during a single capillary transit used external detection of a gamma-emitting isotope; this single-injection method was first proposed by Sejrsen (19). He demonstrated in isolated cat muscle that the estimate of the fraction of $^{51}$Cr-labeled ethylenediaminetetraacetic acid (EDTA) extracted on a single capillary transit determined by this technique was accurate compared with the estimate determined by the standard venous sampling technique which uses labeled albumin as the reference (20). In fact, the accuracy of the single-injection technique is probably greater than that of the venous sampling technique because of its experimental simplicity. The perturbing effects of the venous sampling catheter system are eliminated, and, since the tracer serves as its own intravascular reference, there is no Taylor effect (9).

The accuracy of the single-injection technique of assessing the extraction fraction when it is employed in vivo does require that the peak height of the radioactivity observed over the organ of interest be proportional to the quantity of material injected and that recirculation of the labeled material be negligible during the time over which the extrapolation is made. The first requirement was met in part by the rapid intra-arterial injection of a small volume (~0.2 ml/0.5 seconds) and the use of short sampling times (100 msec). Moreover, injections of labeled water into the internal jugular vein demonstrated that the amount of labeled water recirculating to the monitored tissue during the first 30 seconds was approximately 0.05 (1 - E). Hence, the overestimation of the extraction fraction due to recirculation should be limited to about 2%.

Measurement of Cerebral Blood Flow With Labeled Water

Our calculation of cerebral blood flow, based on the central volume principle, employed the mean brain-blood equilibrium partition coefficient for water. It is clear from our observations that water is not in equilibrium with the interior phases of the brain tissue during the time of our measurement. It has been argued (21) that under these circumstances it is invalid to employ the equilibrium value for the partition coefficient. However, Roberts et al. (22) have recently shown in a rigorous mathematical proof of the central volume principle that equilibrium between phases as a whole is not a necessary condition for the valid application of the central volume principle; only the boundary between two phases must be in equilibrium. Intuitively it seems paradoxical that $T_H$ should not depend on the diffusion coefficients of water in various phases of brain tissue. Roberts et al. (22) rationalized their observation by concluding that the diffusion coefficient and the average depth of penetration of tracer into the tissue phases are coupled. In other words, if the diffusion coefficient is low the average depth of penetration will be
small, whereas if the diffusion coefficient is high the penetration depth will be large. The time required for tracer to enter and then be cleared from the tissue is, however, the same in both cases, because the faster diffusing tracer has to travel correspondingly longer distances.

To test the hypothesis of Roberts et al. (22) experimentally and to verify our method of computing cerebral blood flow with H$_2$O, we compared computed flow values obtained with H$_2$O with those obtained with the standard $^{133}$Xe 10-minute height-over-area technique (23). This comparison was accomplished by sequentially injecting H$_2$O and then $^{133}$Xe. The time course of each tracer was monitored and processed as described earlier in this paper, except that a separate energy window of acceptance was adjusted symmetrically around the 81-kev photopeak of $^{133}$Xe. Suitable correction was applied to subtract the contribution of the $^{15}$O activity from the $^{133}$Xe signal. A value of 1.15 ml/ml or 1.10 ml/g (24) was used for the equilibrium brain-blood partition coefficient for $^{133}$Xe. Cerebral blood flow was manipulated, as described earlier, by changing arterial Pco$_2$ (see Methods); measurements of arterial blood gases bridged each measurement to ensure a steady state. Thirteen paired observations were made over the cerebral blood flow range of 20 to 160 ml/100 g min$^{-1}$. Cerebral blood flow for $^{133}$Xe was calculated assuming that the 10-minute height-over-area method systematically overestimates flow by 11% (23, 25). The resultant line of regression was CBF (labeled H$_2$O) = 0.97CBF (labeled Xe) + 0.7 with a correlation coefficient of $r = 0.96$ ($P \ll 0.001$). On no occasion was a nonextracted fraction observed in the $^{133}$Xe data. The correlation between the two methods of flow determination, practically one of identity for the blood flow range of 20 to 160 ml/100 g min$^{-1}$, verifies our method of computing cerebral blood flow employing H$_2$O. Furthermore, it provides experimental support for the hypothesis that equilibrium between the interior of the phases of a system, in this case the brain, is not a necessary condition for the valid application of the central volume principle.

**MEASUREMENT OF BRAIN-CAPILLARY WATER PERMEABILITY**

Our data provide the opportunity for a more detailed examination of the brain-capillary permeability of water. Renkin (26) and Crone (20) have modeled the loss of diffusible substances from a single capillary. They assumed that diffusible material leaving the capillary is promptly sequestered with none of it returning to the capillary and that the rate of loss of material is proportional to the remaining concentration at each point. From these observations they developed the relationship

$$C_u = C_a e^{-P S/F},$$

where $P$ is the capillary permeability, $S$ is the capillary surface area, $C_u$ and $C_a$ are the concentrations of the material at the arterial and venous ends of the capillary, respectively, and $F$ is the blood flow. Crone applied Eq. 5 to data obtained from whole organs with the implicit assumption that the transit time through each capillary is the same. To use the relationship, Crone set the outflow concentration of the reference vascular tracer equal to $C_a$ and the outflow concentration of the diffusible tracer equal to $C_u$ and defined the extraction fraction of the diffusible material $E$ as $(C_u - C_v)/C_a$. Hence, equivalent forms of Eq. 5 can be expressed as:

$$1 - E = e^{-P S/F}$$

or

$$\ln(1 - E) = -PS/F.$$  

Despite its limitations, the single capillary model of Renkin and Crone can be used for the calculation of the brain $PS$ (capillary permeability–surface area product) for water from our data. Figure 4 shows the result of 47 studies in which the natural logarithm of $(1 - E)$ was plotted vs. the reciprocal of cerebral blood flow. These data demonstrate a significant linear relationship ($P \ll 0.001$). The equation for the regression line is:

$$\ln(1 - E) = 0.04 - 138/\text{CBF},$$

with a correlation coefficient of $r = -0.97$. Thus, the relationship predicts that the capillary $PS$ in the brain is approximately 138 ml/100 g min$^{-1}$ or 0.023 cm$^2$/sec g$^{-1}$.

Data on the value of $PS$ for water in the brain are not, to our knowledge, available in the literature. The only comparable data are those recently reported by Eckman et al. (27) for the diffusible indicator antipyrine. Utilizing an autoradiographic technique, they estimated a regional cerebral cortical capillary $PS$ value for $^{14}$C-antipyrine of 0.019–0.027 cm$^2$/sec g$^{-1}$.

To express $P$ as the conventional literature permeability based on indicator concentrations per milliliter of solvent water on both sides of the barrier, the cerebral blood flow should be converted to flow rate of blood water, $F_w$. The water content of blood varies only slightly with changing hemato-
Natural logarithm of the nonextracted fraction of H\textsubscript{2}O during a single cerebral passage, \(\ln(1 - E)\), plotted as a function of the reciprocal of the cerebral blood flow (1/CBF). The equation of the linear regression line is \(\ln(1 - E) = 0.05 - \frac{138}{\text{CBF}}\) \((r = -0.97, P < 0.001)\).

Critical and is approximately 0.81 ml blood water/ml blood for most mammals (28). Thus, Eq. 8 becomes

\[
\ln(1 - E) = 0.04 - \frac{112}{F_w},
\]

predicting a value of \(PS\) of 0.019 cm\textsuperscript{2}/sec g\textsuperscript{-1}.

Assuming an average capillary surface area on the order of 100 cm\textsuperscript{2}/cm\textsuperscript{3} (cerebral cortex [human] 190 cm\textsuperscript{2}/cm\textsuperscript{3} and white matter [human] 57 cm\textsuperscript{2}/ cm\textsuperscript{3} [29]) permits an estimate of \(P\).

\[
P \approx \frac{0.019 \text{ cm}^2/\text{sec cm}^{-3}}{100 \text{ cm}^2/\text{cm}^3} \approx 1.9 \times 10^{-4} \text{ cm/sec}.
\]

This value can be compared with water permeabilities of 33 \(\times 10^{-4}\) cm/sec for the human red cell membrane (30) and 20 \(\times 10^{-4}\) cm/sec for artificial lipid films (31).

The reason brain is apparently less permeable to water than is myocardium or skeletal muscle is not clear. Two factors might be responsible. First, the capillary density in the brain is considerably less than that in the myocardium (1), thus reducing the capillary surface area available for exchange. Second, the capillaries in the brain are structurally unique; they have no apparent openings between constituent endothelial cells (32).

In conclusion, labeled water cannot be viewed as a freely diffusible internal standard when the blood-brain barrier passage of other substances is being evaluated. At normal cerebral blood flow for the rhesus monkey (~50 ml/100 g min\textsuperscript{-1}), only about 90% of the injected bolus of labeled water freely exchanges with brain; this value decreases progressively with higher cerebral blood flows. For smaller animals in which cerebral blood flow is considerably higher (33), this problem is probably magnified.

Our findings confirm experimentally the hypothesis that the valid application of the central volume principle does not depend on the achievement of tracer equilibrium within the interiors of various phases of a system (22) but rather only requires equilibrium at the boundary between phases. As such this observation justifies the use of H\textsubscript{2}O for the measurement of cerebral mean transit time and cerebral blood flow.

Finally, the feasibility of measuring the fractional extraction of labeled substances during a single capillary transit in vivo using external detection of a positron-emitting isotope has been demonstrated. This approach provides not only a means of evaluating the blood-brain permeability to water under a variety of experimental conditions but also of evaluating the blood-brain barrier permeability and transport characteristics of other similarly labeled substances.

**Acknowledgment**

The authors wish to thank Mr. Julius Hecht, Mr. Robert A. Feldhaus, Ms. Gail Batek, and the staff of the Washington University Medical School cyclotron for their invaluable technical assistance in these experiments. We also thank Dr. K. B. Larson of the Biomedical Computer Laboratory, Washington University, for his valuable suggestions concerning data analysis.

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doi: 10.1161/01.RES.35.3.358

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1974 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

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