Journal of Cerebral Blood Flow and Metabolism
7:527–542 © 1987 Raven Press, Ltd., New York

Positron Emission Tomographic Measurement of Cerebral Blood Flow and Permeability–Surface Area Product of Water Using [^{15}O]Water and [^{11}C]Butanol

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Summary: We have previously adapted Kety's tissue autoradiographic method for measuring regional CBF in laboratory animals to the measurement of CBF in humans with positron emission tomography (PET) and $H_2{}^{15}O$. Because this model assumes diffusion equilibrium between tissue and venous blood, the use of a diffusion-limited tracer, such as $H_2{}^{15}O$, may lead to an underestimation of CBF. We therefore validated the use of [^{11}C]butanol as an alternative freely diffusible tracer for PET. We then used it in humans to determine the underestimation of CBF that occurs with $H_2{}^{15}O$, and thereby were able to calculate the extraction E_w and permeability–surface area product PS_w of $H_2{}^{15}O$. Measurements of the permeability of rhesus monkey brain to [^{11}C]butanol, obtained by means of an intracarotid injection, external detection technique, demonstrated that this tracer is freely diffusible up to a CBF of at least 170 ml/min-100 g. CBF measured in baboons with the PET autoradiographic method and [^{11}C]butanol was then compared with CBF measured in the same animals with a standard residue detection method. An excellent correspondence was obtained between both of these measurements. Finally, paired PET measurements of CBF were made with both $H_2{}^{15}O$ and [^{11}C]butanol in 17 normal human subjects. Average global CBF was significantly greater when measured with [^{11}C]butanol (53.1 ml/min-100 g) than with $H_2{}^{15}O$ (44.4 ml/min-100 g). Average global E_w was 0.84 and global PS_w was 104 ml/min-100 g. Regional measurements showed a linear relationship between local PS_w and CBF, while E_w was relatively uniform throughout the brain. Simulations were used to determine the potential error associated with the use of an incorrect value for the brain–blood partition coefficient for [^{11}C]butanol and to calculate the effect of tissue heterogeneity and errors in flow measurement on the calculation of PS_w. **Key Words:** [^{11}C]Butanol—Cerebral blood flow—$H_2{}^{15}O$—Permeability–surface area product—Positron emission tomography.

In 1951 Kety presented a mathematical theory describing the exchange of inert gas between blood and tissue. This theory was later extended by Kety and colleagues in the development of a tissue autoradiographic technique to measure local CBF in laboratory animals (Landau et al., 1955; Kety, 1960, 1985). This technique involves the intravenous infusion of an inert, radiolabeled tracer that diffuses across the blood–brain barrier. After a brief infusion period, the animal is killed and measurements of local radioactivity are subsequently obtained by quantitative tissue autoradiography. These measurements and measurements of the arterial concentration of radiotracer during the infusion period are used to calculate local CBF. In this calculation, it is assumed that the radiotracer is freely diffusible across the blood–brain barrier, so that there is equilibration of tracer between blood and tissue during a single vascular transit. Because the tracer initially used for this application, trifluoroiodomethane, was very volatile, more convenient tracers were subsequently developed. It was realized that unless these tracers were also freely diffusible across the blood–brain barrier, their use could

Received December 22, 1986; accepted March 27, 1987.

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Abbreviations used: IMP, [^{125}I]iodoamphetamine; PET, positron emission tomography.

lead to an underestimation of local CBF with Kety's model. Thus, permeability characteristics were an important consideration in the selection of radiotracers used to measure CBF in laboratory animals (Eklof et al., 1974; Eckman et al., 1975; Sakurada et al., 1978; Goldman et al., 1980; Van Uitert et al., 1981).

The development of positron emission tomography (PET) made possible the in vivo measurement of local tissue radioactivity. Given the ability to obtain data in humans analogous to those provided by tissue autoradiography, we developed an adaptation of Kety's technique to measure regional CBF with PET and $H_2{}^{15}O$ (Herscovitch et al., 1983; Raichle et al., 1983). Several other methods were also described to measure CBF with PET and inert diffusible radiotracers (Subramanyam et al., 1978; Frackowiak et al., 1980; Holden et al., 1981; Huang et al., 1983; Kanno et al., 1984; Jones et al., 1985; Carson et al., 1986). These methods are also based on the principle of equilibration of tracer between blood and tissue and therefore assume that the tracer is freely diffusible. In addition, most of these methods use $H_2{}^{15}O$ as the flow tracer, because of its favorable characteristics with respect to synthesis, administration, and half-life. However, $H_2{}^{15}O$ is not freely diffusible across the blood–brain barrier (Eichling et al., 1974). We have shown that in baboons this diffusion limitation results in an underestimation of CBF as measured with the PET autoradiographic approach (Raichle et al., 1983). This led us to propose that comparative measurements of CBF with both $H_2{}^{15}O$ and a freely diffusible tracer might demonstrate a similar underestimation in humans.

Radiolabeled alcohols have been suggested as diffusible radiotracers because of their lipid solubility (Eklöf et al., 1974; Raichle et al., 1976). Preliminary data in our laboratory suggested that [^{11}C]butanol might be a suitable flow tracer for use in PET (Raichle et al., 1976; Dischino et al., 1983). This hypothesis has been supported by studies of the permeability of rodent brain to [^{14}C]butanol (Sage et al., 1981; Van Uitert et al., 1981). In this communication, we report our observations obtained in primates and humans that validate the use of [^{11}C]butanol as a freely diffusible flow tracer for PET. Measurement of the permeability of rhesus monkey brain showed that [^{11}C]butanol is freely diffusible at flow levels as high as 170 ml/min-100 g. CBF measured in baboons with the PET autoradiographic method and [^{11}C]butanol was then compared with CBF measured in the same animals by means of a standard residue detection method. An excellent correspondence was obtained between these two measurements. Finally, both $H_2{}^{15}O$ and [^{11}C]butanol were used to measure CBF with the PET autoradiographic approach in humans. It was observed that the use of $H_2{}^{15}O$ resulted in an underestimation of CBF in comparison to [^{11}C]butanol. The potential for this underestimation to occur with a diffusion-limited tracer was, in fact, anticipated by Kety in the development of his model. He incorporated an additional parameter m to denote the extent to which diffusion equilibrium is achieved by the tracer during a single vascular transit (Kety, 1951, 1985). In addition, comparative blood flow measurements with $H_2{}^{15}O$ and [^{11}C]butanol permitted us to calculate the extraction fraction E_w and permeability–surface area product PS_w for $H_2{}^{15}O$ with PET. A preliminary report of this work has appeared in abstract form (Herscovitch et al., 1985).

THEORY

Regional CBF f is measured following the intravenous injection of an inert, diffusible radiotracer (Herscovitch et al., 1983; Raichle et al., 1983). The rate of accumulation of radiotracer in a tissue element is given by

$$\frac{dC_b}{dt} = f(C_a - C_v) - \alpha C_b \qquad (1)$$

where C_b is the local brain tissue concentration of radiotracer (counts/s-g), C_a and C_v are the time-dependent arterial and cerebral venous concentrations of radiotracer, respectively (counts/s-ml), and α is the physical decay constant of the tracer (1/s). Equation 1 is based on the standard Fick principle (Kety, 1960), modified to account for physical decay of the radiotracer during the period of blood flow measurement. As $C_v(t)$ is not directly measurable, Kety (1951, 1960, 1985) introduced the following relationships. For a freely diffusible tracer, diffusion equilibrium will be achieved by the time of its exit from the capillary. Thus, $C_v = C_b/\lambda$, where λ is the equilibrium brain–blood partition coefficient for the tracer, and $C_a - C_v$ can be replaced by $C_a - C_b/\lambda$. In the case of a diffusion-limited tracer, a parameter m is introduced into this latter expression for the arterial-venous concentration difference of the tracer. Thus,

$$C_a - C_v = m\left(C_a - \frac{C_b}{\lambda}\right) \qquad (2)$$

where m is a constant between 0 and 1 that denotes the extent to which diffusion equilibrium is achieved between blood and tissue during passage

of the tracer from the arterial to the venous end of the capillary. Combining Eqs. 1 and 2,

$$\frac{dC_b}{dt} = mfC_a - \left(\frac{mf}{\lambda} + \alpha\right)C_b \quad (3)$$

This equation may be integrated between time 0 (the time of radiotracer injection) and time T (the end of the study) to give

$$C_b(T) = mf\int_0^T C_a(t)\exp[-k(T-t)]dt \quad (4)$$

$$k = \frac{mf}{\lambda} + \alpha \quad (5)$$

These equations (without the modification for radioactive decay) have been applied by several investigators to study the behavior of diffusible tracers and to measure local blood flow using tissue autoradiography (Landau et al., 1955; Kety, 1960; Eckman et al., 1975; Sakurada et al., 1978; Lucignani et al., 1985).

Positron emission tomographs do not have adequate temporal resolution to measure tissue radioactivity $C_b(T)$ instantaneously. A scan must be performed over many seconds, summing the decay events occurring during the scan. Therefore, we modified Eq. 4 (Herscovitch et al., 1983; Raichle et al., 1983) by an integration over the time of the scan, T_1–T_2, to give

$$C = mf\int_{T_1}^{T_2}\int_0^T C_a(t)\exp[-k(T-t)]dtdT \quad (6)$$

where C is the local number of counts per unit weight recorded by the tomograph from a region of brain during the scan.

Equations 5 and 6 differ from our original adaptation of the tissue autoradiographic method to PET in that physical decay of the radiotracer is explicitly included. In our original formulation, both the arterial time-activity curve $C_a(t)$ and the tissue count measurement C were decay corrected prior to their use in the computation of flow. Because the PETT VI system (Ter-Pogossian et al., 1982) cannot correct for radioactive decay during data collection, an average decay correction technique was applied to the tissue count measurement (Raichle et al., 1983). We have subsequently used data collected with $H_2{}^{15}O$ as the flow tracer to compute CBF using both this average technique and the above model (Eqs. 5 and 6) in which decay correction is explicitly included. The average decay correction technique resulted in a 2–4% underestimation of CBF (unpublished data). This degree of systematic underestimation does not occur with [11C]butanol, owing to the long half-life of 11C (20.38 min) in comparison to the brief scan length (40 s). As one of our goals was to compare CBF measured in the same subjects with both $H_2{}^{15}O$ and [11C]butanol, we accordingly modified our model to include radioactive decay so as to avoid this systematic error.

The parameter m in Eq. 2 denotes the extent to which the flow tracer achieves equilibrium with tissue during a single capillary transit. Using certain simplifying assumptions, Kety (1951, 1985) showed that m could be expressed in terms of flow and permeability–surface area product as

$$m = 1 - \exp\left(\frac{-PS}{f}\right) \quad (7)$$

An equivalent expression was obtained by Renkin (1959) and Crone (1963) for the extraction fraction E of a diffusible tracer:

$$E = 1 - \exp\left(\frac{-PS}{f}\right) \quad (8)$$

Thus, m is equivalent to the extraction E of the Renkin-Crone model. Although the original Renkin-Crone formulation holds strictly only for a single capillary during a constant infusion of radiotracer, Raichle and Larson (1981) showed that their formulation is appropriate to external monitoring of an entire organ or region during finite radiotracer injection of arbitrary time course.

Equations 6–8 provide a strategy for the measurement of the extraction and PS of $H_2{}^{15}O$ (Raichle, 1980). For a freely diffusible tracer, m = 1 and Eq. 6 can be used to calculate flow f from measurements of $C_a(t)$ and C. For $H_2{}^{15}O$, which is not freely diffusible (Eichling et al., 1974), flow will be underestimated by a factor of m if the autoradiographic approach (Eq. 6) is used with m assumed to be equal to 1. Measuring this underestimation, in relation to flow measured with a freely diffusible tracer, permits the calculation of m, i.e., the extraction of water E_w and the PS for water PS_w. Thus, if f_{diff} is the flow measured with a freely diffusible tracer and f_w is flow measured with $H_2{}^{15}O$,

$$m = E_w = \frac{f_w}{f_{diff}} \quad (9)$$

$$PS_w = -f_{diff}\ln\left(1 - \frac{f_w}{f_{diff}}\right) \quad (10)$$

This approach to measure PS_w has been implemented by others using tritiated water and tissue sampling in the rat, with either [14C]iodoantipyrine (McCulloch and Angerson, 1981; Reid et al., 1983) or [14C]butanol (Ginsberg et al., 1985) as the refer-

ence radiotracer. In addition, the above equations have been used to analyze the effect of the permeability limitation of water on techniques for measuring CBF with PET and $H_2{}^{15}O$ (Lammertsma et al., 1981; Raichle et al., 1983; Kanno et al., 1984).

In this communication, we present data that demonstrate that [^{11}C]butanol is a freely diffusible radiotracer, i.e., $E = 1$. We then apply the above theory to calculate E_w and PS_w from paired flow measurements obtained in humans with PET using both [^{11}C]butanol and $H_2{}^{15}O$. For these flow measurements, the value of 0.90 ml/g was used for the partition coefficient of $H_2{}^{15}O$ (Herscovitch and Raichle, 1985), and 0.77 ml/g was used for that of [^{11}C]butanol (Gjedde et al., 1980).

METHODS

Simulation studies

Simulation studies were performed to assess the effect of certain potential sources of error on the measurement of CBF, E_w, and PS_w. A detailed error analysis of the PET autoradiographic method for measuring regional CBF with $H_2{}^{15}O$ has been published (Herscovitch et al., 1983). To use [^{11}C]butanol as a flow tracer, one must specify a value for its brain–blood partition coefficient λ_b in Eq. 6. We used the value 0.77 ml/g that was measured in rat brain (Gjedde et al., 1980). If the actual partition coefficient were different, there would be an error in the calculated value of flow. The magnitude of this error was determined as follows. Equation 6 was used with a representative arterial time-activity curve to calculate the tissue activity corresponding to various values of λ_b and flow. The flow value that would be computed in practice was then determined using this calculated value of tissue activity in Eq. 6 with $\lambda_b = 0.77$ ml/g, and the two flow values were compared.

An error in the measurement of flow with either $H_2{}^{15}O$ (f_w) or [^{11}C]butanol (f_b) will be propagated in the calculation of PS_w. The error in PS_w resulting from specific percentage errors in f_w or f_b measurement was calculated using Eq. 10.

Finally, the effect of tissue heterogeneity on the calculation of PS_w was determined. Because of the limited spatial resolution of PET, a region of interest usually receives tissue count contributions from both gray and white matter (Mazziotta et al., 1981). Because of the nonlinear relationship between flow and PS_w (Eq. 10), the PS_w value computed in such a heterogeneous region will not necessarily equal the true weighted PS_w of the gray and white matter components contributing to the measurement. The magnitude of this error was computed as follows: A region of interest is considered to contain varying fractions w_1 and w_2 of gray and white matter, respectively, where $w_1 + w_2 = 1$ (subscript 1 refers to gray matter, subscript 2 refers to white matter). Values of flow and PS_w are specified for each fraction. The average weighted flow in such a heterogeneous region is

$$\bar{f} = w_1 f_1 + w_2 f_2 \quad (11)$$

For a diffusion-limited tracer, i.e., $H_2{}^{15}O$, the average weighted flow that would be observed is given by

$$\bar{f}_w = w_1 f_1 [1 - \exp(-PS_1/f_1)] + w_2 f_2 [1 - \exp(-PS_2/f_2)] \quad (12)$$

Here, Eqs. 8 and 9 have been used to calculate the gray and white matter flows that would be observed with $H_2{}^{15}O$. The PS_w that would be calculated in the heterogeneous region of interest is given by

$$PS_w = -\bar{f} \ln (1 - \bar{f}_w/\bar{f}) \quad (13)$$

This is to be compared with the actual mean PS_w in the region,

$$\overline{PS}_w = w_1 PS_1 + w_2 PS_2 \quad (14)$$

Any difference between these two values for permeability–surface area product reflects the error due to the specified degree of tissue heterogeneity in the region of interest.

Synthesis of [1-^{11}C]butanol

[1-^{11}C]butanol was prepared in a remote apparatus by procedures similar to those previously reported (Welch et al., 1975; Raichle et al., 1976; Oberdorfer et al., 1982; Dischino et al., 1983). By use of a flow of helium (3 ml/min), $^{11}CO_2$ was bubbled into 1.0 ml of 1.2 *M* propyl magnesium chloride in diethyl ether. After trapping of the $^{11}CO_2$, the helium flow was increased and the diethyl ether evaporated. Then 0.5 ml of 1 *M* lithium aluminum hydride in diethyl ether was added to the residue, and the vessel was shaken for 1 min. The ether was again evaporated, a cold (0–5°C) solution of hydrochloric acid (2.5 ml of 1 N HCl) was added, and the vessel was shaken vigorously. The aqueous mixture was then drawn through two C_{18} Sep-Paks. The reaction vessel and Sep-Paks were washed with three 2-ml portions of water. The product was then eluted with 10 ml of 10% ethanol in saline and collected in a sterile vial. For injection, a portion (1–2 ml) of the product was withdrawn, diluted to 7–10 ml with saline, and sterilized by Millipore filtration.

Quality control analysis was performed as follows. The chemical and radiochemical purity of the [1-^{11}C]butanol was routinely determined using high performance liquid chromatography. Analyses were done using an analytical C_{18} column (0.46 × 25 cm Spherisorb 10 ODS) and radioactivity [NaI(T1)] and refractive index detectors. Radiochemical purity was routinely 99.6% as [1-^{11}C]butanol, with a trace of [1-^{11}C]butanoic acid. Specific activity was estimated at a minimum of 3 Ci/mmol. The preparation of $H_2{}^{15}O$, which was also used in this study, is described in detail elsewhere (Welch and Kilbourn, 1985).

Blood–brain barrier permeability of [^{11}C]butanol

The fraction of [^{11}C]butanol extracted by the brain during a single capillary transit E_b was determined in adult rhesus monkeys by intracarotid injection of radiotracer and external residue detection (Raichle et al., 1976). Accompanying each measurement of E_b was a measurement of CBF obtained by using residue detection of a bolus of $H_2{}^{15}O$ injected into the internal carotid artery.

To facilitate injection of radiopharmaceuticals into the internal carotid artery, at least 2 weeks prior to experimentation the monkeys were anesthetized with phencyclidine (2 mg/kg i.m.) and the right external carotid artery was ligated at its origin. For the measurement of E_b, the monkeys were anesthetized with phencyclidine (2 mg/kg i.m.), paralyzed with gallamine (2–4 mg/kg i.v.), intu-

bated with a soft-cuffed endotracheal tube, and ventilated with 70% nitrous oxide and 30% oxygen. A small catheter (0.021-cm diameter) was inserted percutaneously into the femoral artery and its tip positioned in the right common carotid artery under fluoroscopic control. This catheter was used for intracarotid injection of radiopharmaceuticals, monitoring of arterial blood pressure, and sampling of arterial blood. To prevent clotting in the catheter system, the animals were heparinized at the beginning of each experiment.

Paired measurements of E_b and hemispheric CBF were obtained using the residue curves recorded subsequent to the intracarotid injection of [11C]butanol and $H_2{}^{15}O$, respectively. These residue curves were recorded by a 3 × 2-in NaI(T1) scintillation detector collimated and positioned under each animal's head to ensure uniform detection from a single cerebral hemisphere. The respiratory rate and thus the arterial P_{CO_2} were varied to obtain measurements over a wide range of CBF. At least 15 min was allowed between changes in respiratory rate to reach a new steady state, as confirmed by measurements of arterial blood gasses.

The signal from the NaI(T1) detector was processed by a pulse height discriminator to minimize recording of scattered radiation and by computer to correct the count rate for deadtime losses, physical decay of radiotracer, and background. A plot of count rate as a function of time was obtained and used to calculate CBF and radiotracer extraction. Hemispheric CBF was obtained from the residue curve recorded following intracarotid injection of $H_2{}^{15}O$. The calculation is based on the central volume principle and height/area analysis of the residue curve (Zierler, 1965; Roberts et al., 1973; Eichling et al., 1974; Raichle et al., 1975). The extraction fraction of radiotracer was obtained by graphically extrapolating the relatively slow clearance of tracer from brain back to the abscissa of the curve peak and computing the ratio $E = B/A$, as shown in Fig. 1 (Raichle et al., 1976). Although the goal of these experiments was to measure the extraction of [11C]butanol, we also calculated the extraction of $H_2{}^{15}O$ from the $H_2{}^{15}O$ residue curve.

Validation of CBF measurements obtained with [11C]butanol and PET

We performed an in vivo validation of our method for measuring CBF with [11C]butanol and the PET autoradiographic approach. CBF measured with PET in baboons was compared with that measured in the same animals with residue detection and intracarotid injection of $H_2{}^{15}O$. Animal preparation, including right external carotid artery ligation, anesthesia, and insertion of a right common carotid artery catheter, was as described above. A small catheter was placed percutaneously in the femoral vein for injection of [11C]butanol. The baboons were positioned on a special couch that could be rotated on its base. This permitted placement of the head in the tomograph or over an NaI(T1) scintillation detector, as described above, for residue detection.

PET was performed with the PETT VI system (Ter-Pogossian et al., 1982; Yamamoto et al., 1982). Studies were performed in the low-resolution mode, with a transverse resolution of ~14 mm in the center of the field of view. The head of the baboon was positioned with the aid of a vertical laser line, such that the center of the lowest tomographic slice corresponded to a line running transversely through the center of the cerebral hemispheres. A lateral skull radiograph with this line marked by a vertical radioopaque wire provided a record of the position of this PET slice. Because of the small size of the adult baboon brain (~150 g), only data from this bottom slice and corresponding to the hemisphere studied with residue detection were used. A tomographic transmission scan was obtained with a $^{68}Ge/^{68}Ga$ ring source for each animal to determine the attenuation characteristics of the head required for reconstruction of the emission scans (Ter-Pogossian et al., 1982). To measure CBF with PET, a 40-s emission scan was obtained following intravenous bolus injection of 20–30 mCi of [11C]butanol. Scanning was

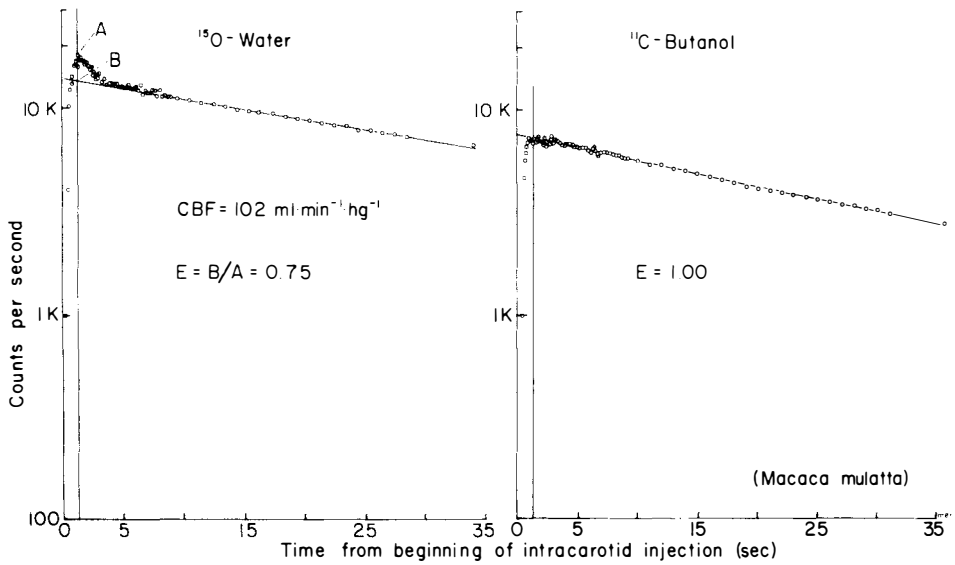

FIG. 1. Brain time-activity curve (semilog) obtained following the intracarotid injection of $H_2{}^{15}O$ **(left)** and [11C]butanol **(right)** in an adult rhesus monkey. Graphical extrapolation of the clearance phase of radiotracer to the abscissa of the perfusion peak allows the calculation of radiotracer extraction, $E = B/A$. Note the lack of an unextracted fraction for [11C]butanol.

begun at the time of arrival of radiotracer in the head, as determined by a sudden increase in the bank pair coincidence counting rate of the tomograph. Arterial blood samples were collected at 4- to 6-s intervals following injection of radiotracer and used to construct a time-activity curve.

Accompanying each measurement of CBF with PET was a CBF measurement made with residue detection and intracarotid injection of $H_2{}^{15}O$, as described above. This latter measurement was called f_{true}, to distinguish it from f_b, the flow measured with PET and [^{11}C]butanol. To obtain these paired measurements over a wide range of flow, the respiratory rate and thus the arterial P_{CO_2} were varied. The tomographic image was processed on a pixel-by-pixel basis by use of Eq. 6 to provide quantitative flow data. The value of f_b was obtained from this flow image using a square region of interest (3.6 cm^2) placed centrally over the right cerebral hemisphere.

CBF measurements in humans

We performed paired flow measurements in human subjects using the PET autoradiographic method with both $H_2{}^{15}O$ and [^{11}C]butanol. These data were used to determine the extent, if any, to which flow is underestimated with $H_2{}^{15}O$ and to calculate E_w and PS_w. Seventeen normal subjects, nine men and eight women, with an age range of 18–75 years (mean 34 years), were studied. Fourteen of the subjects were <41 years of age and had no history or evidence of neurologic or psychiatric disease. The other three subjects, all older than 65 years, were participants in the Washington University Memory and Aging Project. These subjects were classified as normal following standard histories and physical examinations, psychometric testing, basic blood and urine tests, and a computerized tomographic scan of the head (Berg et al., 1982). Informed written consent was obtained from each subject prior to the PET studies. All procedures were approved by the Human Studies Committee and the Radioactive Drug Research Committee of Washington University School of Medicine.

PET was performed with the PETT VI tomograph operating in the low-resolution mode. Data were recorded simultaneously for seven contiguous tomographic slices with a center-to-center separation of 14.4 mm and a transverse resolution of ~14 mm. The head was immobilized with an individually molded plastic face mask attached to the headrest of the scanner couch. A lateral skull radiograph was then obtained with the center of each PET slice marked by a radioopaque wire. This record of the PET slices in relation to the bony landmarks of the skull was subsequently used to obtain regional CBF values using a stereotaxic method for anatomic localization (Fox et al., 1985). Venous and radial arterial catheters were placed percutaneously to permit injection of radiotracers and to obtain arterial blood samples. A transmission scan was performed for each subject with a ^{68}Ge/^{68}Ga ring source to determine the attenuation characteristics of the head. All scans were done with the subjects in the resting state with eyes closed and with the room darkened. The ears were not occluded. Ambient noise during the scans was primarily from cooling fans for the electronic equipment.

In each subject, paired CBF measurements were obtained, first with $H_2{}^{15}O$, then, after sufficient time had elapsed for decay of the radioactive background, with [^{11}C]butanol. Radiotracers were administered by bolus intravenous injection, in amounts of 50–75 mCi for $H_2{}^{15}O$ and 30–40 mCi for [^{11}C]butanol. Arterial blood gas measurements were made with each flow determination. Following injection of radiotracer, a 40-s emission scan was performed. Each scan was started at the time of arrival of radioactivity in the brain, as indicated by a sudden increase in the bank pair coincidence counting rate of the tomograph. Arterial blood samples were obtained every 4–6 s, starting at the time of radiotracer injection, and were used to construct a time-activity curve. The curve was shifted to adjust for the delay between arrival of the radioactivity at the radial artery sampling site and its arrival in the head (Raichle et al., 1983). The arterial time-activity curve and the image of regional radioactivity were then used in Eq. 6 on a pixel-by-pixel basis to obtain quantitative images of CBF.

Both global and regional CBF data were obtained. Global CBF was calculated from an average of the pixel values of contiguous supratentorial slices. The border of each PET slice was defined by a threshold routine applied to the corresponding slice of the attenuation scan for each subject (Herscovitch et al., 1986). Global E_w was calculated from the ratio of global flow measured with $H_2{}^{15}O$ to that measured with [^{11}C]butanol, and global PS_w was then calculated from Eq. 10. Regional values for CBF were obtained from the tomographic flow images using a stereotaxic method for anatomical localization (Fox et al., 1985). The stereotaxic coordinates of 17 1.35 × 1.35-cm^2 (5 × 5-pixel) regions were obtained from a standard atlas (Talairach et al., 1967) (Table 1). The location of these regions in each individual scan was then determined and regional CBF data obtained for each subject. In addition, on the tomographic slice containing the white matter regions, a midline region was selected with the anteroposterior position chosen in each subject to obtain the highest CBF value. This region (referred to as central cortex) was included so as to obtain data over a high flow range. Equations 9 and 10 were then used to calculate regional E_w and PS_w for each subject from the values of regional f_w and f_b.

Paired t tests were used to compare global values of f_w and f_b, and also the measurements of arterial P_{CO_2} during the two flow studies in each subject. Mean values for regional measurements of f_w, f_b, E_w, and PS_w were calculated and linear correlations computed between f_b and E_w and f_b and PS_w. An α significance level was taken at $p = 0.05$ and was adjusted to 0.0125 to compensate for the

TABLE 1. *Stereotaxic atlas coordinates of regions in which local physiologic measurements were made*

	Vertical	R-L	A-P
Central white matter	3.4	2.6	0.0
Caudate	0.8	1.0	2.5
Putamen	0.3	2.3	1.5
Globus pallidus	0.0	1.6	1.0
Thalamus	0.8	1.0	−0.5
Sensorimotor cortex	4.9	4.7	0.6
Visual cortex	0.9	0.0	−6.0
Insular cortex	0.3	4.0	2.0
Mediotemporal cortex	−0.3	2.3	−2.3

Coordinates (cm) are for the center of a 1.35 × 1.35-cm^2 (5 × 5-pixel) region in either hemisphere, except for the visual cortex, for which a midline region was used. Coordinates are taken from the stereotaxic atlas of Talairach et al. (1967). R-L, right-left; A-P, posteroanterior.

effect of performing these four separate statistical comparisons (Wallenstein et al., 1980).

RESULTS

Blood–brain barrier permeability of [^{11}C]butanol

Figure 1 provides an example of our residue detection data recorded by the single NaI(T1) scintillation detector following the intracarotid injection of radiotracer. The residue curve obtained with $H_2{}^{15}O$ was used to calculate CBF and the cerebral hemispheric extraction of $H_2{}^{15}O$. The companion residue curve obtained with [^{11}C]butanol shows that during this experiment, the extraction of butanol was 1.0. Thirteen experiments were performed, over a flow range of 70–170 ml/min-100 g. In no case was an unextracted fraction observed with [^{11}C]butanol (Fig. 2). In contrast, there was a progressive decline in the extraction of $H_2{}^{15}O$ with increasing CBF, as has been previously reported (Eichling et al., 1974; Raichle et al., 1974).

Validation of CBF measurements obtained with [^{11}C]butanol and PET

The relationship between f_{true}, measured with residue detection and standard tracer techniques, and f_b, measured with the PET autoradiographic method and intravenous [^{11}C]butanol, is shown in Fig. 3. Data were obtained over a range of true flow of 33.5–124 ml/min-100 g. Linear regression analysis gave the following relationship: $f_b = 1.08 f_{true} - 5.9$, $r = 0.954$, $p < 0.00002$. The line of identity fell within the 95% confidence limits for this relationship. It should be noted that CBF measured with the PET autoradiographic method was based on a single 40-s emission scan following the bolus intravenous administration of [^{11}C]butanol. Preliminary data indicate that increasing the scan duration leads to a progressive underestimation of f_{true}

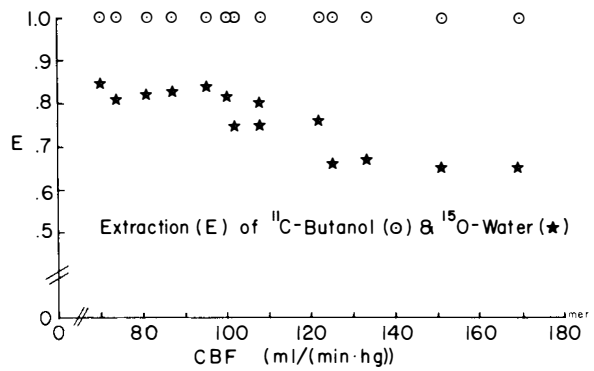

FIG. 2. Fraction of [^{11}C]butanol and $H_2{}^{15}O$ extracted during a single cerebral passage, plotted as a function of blood flow. In contrast to the declining extraction E of $H_2{}^{15}O$ observed with increasing blood flow, in no case was an unextracted fraction observed with [^{11}C]butanol.

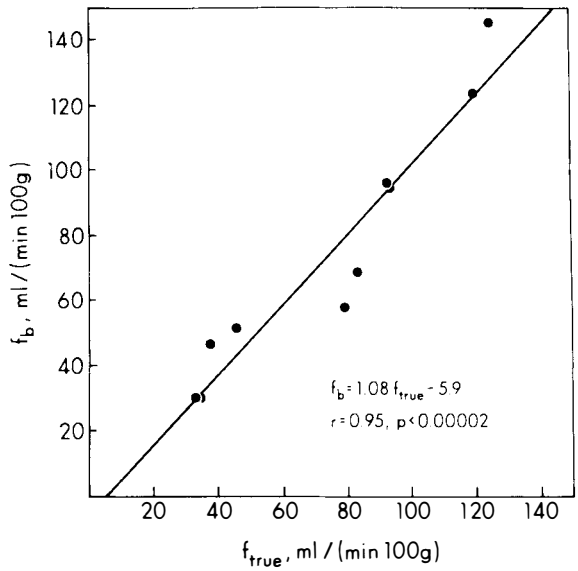

FIG. 3. Comparison between CBF measured in adult baboons using the positron emission tomography (PET) autoradiographic method with [^{11}C]butanol as the flow tracer (f_b) and CBF measured in the same animal using intracarotid injection of $H_2{}^{15}O$ and residue detection (f_{true}). Estimation of CBF using the PET autoradiographic method was based on a single 40-s emission scan obtained following the intravenous bolus injection of 20–30 mCi of [^{11}C]butanol.

(M. E. Raichle, unpublished observation) in a manner similar to that previously observed for $H_2{}^{15}O$ (Raichle et al., 1983).

Human studies

Paired flow measurements were made in 17 normal subjects with the PET autoradiographic approach using both $H_2{}^{15}O$ and [^{11}C]butanol. Mean global CBF measured with [^{11}C]butanol was 53.1 ± 8.8 (SD) ml/min-100 g, significantly greater than that with $H_2{}^{15}O$, 44.4 ± 7.4 ml/min-100 g ($p < 0.000002$). There was no significant difference between mean arterial P_{CO_2} during the $H_2{}^{15}O$ studies, 37.8 ± 3.9 mm Hg, and that during the [^{11}C]butanol studies, 37.0 ± 2.7 mm Hg ($p = 0.34$). The average global extraction fraction for $H_2{}^{15}O$ was 0.84 ± 0.07, and the average global PS for $H_2{}^{15}O$ was 104 ± 32 ml/min-100 g.

Our regional measurements are presented in Table 2. The relationships between mean values of f_b and PS_w, and f_b and E_w are illustrated in Fig. 4. A significant correlation was found between f_b and PS_w: $PS_w = 1.50 f_b + 20.6$, $r = 0.88$, $p < 0.000002$. The relationship between f_b and E_w, $E_w = -0.00066 f_b + 0.865$, $r = -0.36$, $p = 0.15$, was not significantly different from the line of zero slope.

Simulation studies

The error in flow measurement that would result from the use of an incorrect value for λ_b was com-

TABLE 2. *Regional measurements of* f_w, f_b, E_w, *and* PS_w

Region	f_w (ml/min-100 g)	f_b (ml/min-100 g)	E_w	PS_w (ml/min-100 g)
Sensorimotor, left	42.9 (10.0)	53.9 (10.8)	0.80 (0.10)	97 (52)
Sensorimotor, right	51.5 (10.2)	64.6 (11.2)	0.80 (0.09)	109 (37)
Insula, left	52.3 (8.7)	66.3 (14.0)	0.80 (0.08)	107 (22)
Insula, right	59.5 (10.7)	73.3 (14.0)	0.82 (0.08)	132 (46)
Mediotemporal, left	46.5 (9.1)	53.8 (10.5)	0.87 (0.06)	114 (33)
Mediotemporal, right	49.3 (9.4)	58.4 (9.4)	0.84 (0.07)	117 (45)
Visual cortex	52.5 (9.2)	61.8 (11.5)	0.85 (0.07)	127 (41)
Central cortex	62.9 (11.6)	74.8 (13.6)	0.84 (0.08)	152 (58)
Caudate, left	42.7 (10.6)	51.6 (12.1)	0.83 (0.08)	100 (44)
Caudate, right	42.0 (11.5)	51.3 (15.4)	0.83 (0.07)	91 (29)
Putamen, left	57.2 (11.1)	70.1 (13.3)	0.82 (0.07)	123 (31)
Putamen, right	54.8 (10.3)	67.1 (12.9)	0.82 (0.08)	123 (38)
Pallidum, left	52.6 (10.0)	65.1 (12.5)	0.81 (0.07)	111 (32)
Pallidum, right	51.2 (10.0)	63.1 (15.1)	0.82 (0.09)	112 (33)
Thalamus, left	49.4 (8.1)	60.7 (10.8)	0.82 (0.07)	108 (27)
Thalamus, right	52.2 (10.1)	64.6 (12.9)	0.81 (0.07)	113 (32)
White matter, left	32.7 (8.3)	38.8 (9.3)	0.85 (0.09)	78 (25)
White matter, right	31.8 (7.2)	38.5 (8.3)	0.83 (0.10)	81 (42)

Values are means (SD). f_w, CBF measured with $H_2{}^{15}O$; f_b, CBF measured with [^{11}C]butanol; E_w, extraction fraction of $H_2{}^{15}O$ (dimensionless); PS_w, permeability–surface area product for $H_2{}^{15}O$.

puted for two flow values, 80 and 20 ml/min-100 g (Table 3). If λ_b were actually 0.5 ml/g rather than the value of 0.77 ml/g used in Eq. 6, flow would be underestimated by [^{11}C]butanol in comparison to $H_2{}^{15}O$. This is opposite to our experimental observations. More relevant to our findings is the situation in which the value of λ_b was actually greater than that used. However, even if λ_b were as great as 1.25 ml/g, flow would be overestimated by only 10% in gray matter and 2% in white matter, not enough to account for our experimental results.

The effect of error in the measurement of f_w or f_b on the calculated value of PS_w is shown in Table 4. At higher values of E_w, the calculation of PS_w is more sensitive to errors in f_b or f_w. This is because the value of the natural logarithm is very sensitive to changes in the argument $(1 - E_w)$ when this argument is small. For any given value of E_w, the error in PS_w does not depend on the value of flow. For example, if $E_w = 0.80$, a 5% overestimation of f_w produces a 5% overestimation of E_w (Eq. 9), a 13.9% variation in $\ln(1 - E_w)$, and thus a 13.9% overestimation of PS_w (Eq. 10), independent of the actual values of f_w and f_b. The errors in PS_w measurement due to tissue heterogeneity are given in Table 5. In general, tissue heterogeneity results in an underestimation of PS_w.

DISCUSSION

We have investigated several aspects of the use of [^{11}C]butanol as a blood flow tracer for PET. The cerebral hemispheric extraction of [^{11}C]butanol in the rhesus monkey was shown to be complete over a wide range of CBF. Validation experiments in baboons showed an excellent correlation between flow measured with [^{11}C]butanol and PET and flow measured with a standard residue detection method. Finally, paired PET studies in humans showed an underestimation of flow measured with $H_2{}^{15}O$ in comparison with that measured with [^{11}C]butanol. The measurement of this underestimation permitted the calculation of the extraction fraction and permeability–surface area product for water on a regional basis.

Blood–brain barrier permeability of [^{11}C]butanol

We measured the fraction of [^{11}C]butanol extracted by the brain during a single capillary transit in rhesus monkeys using intracarotid injection of tracer and external residue detection. The validity of this technique has been previously discussed in detail (Eichling et al., 1974; Raichle et al., 1976; Raichle and Larson, 1981) and it has been used to measure the extraction fraction and permeability of radiopharmaceuticals in several species (Raichle et al., 1976; Go et al., 1981; Kuhl et al., 1982; Dischino et al., 1983). Using this approach, we found that there was no diffusion limitation for [^{11}C]butanol in rhesus monkey brain up to a blood flow of 170 ml/min-100 g.

Several other investigators have also examined the blood–brain barrier permeability of butanol (Crone, 1965; Van Uitert and Levy, 1978; Goldman et al., 1980; Sage et al., 1981; Van Uitert et al., 1981; Irwin and Preskorn, 1982; Pardridge and

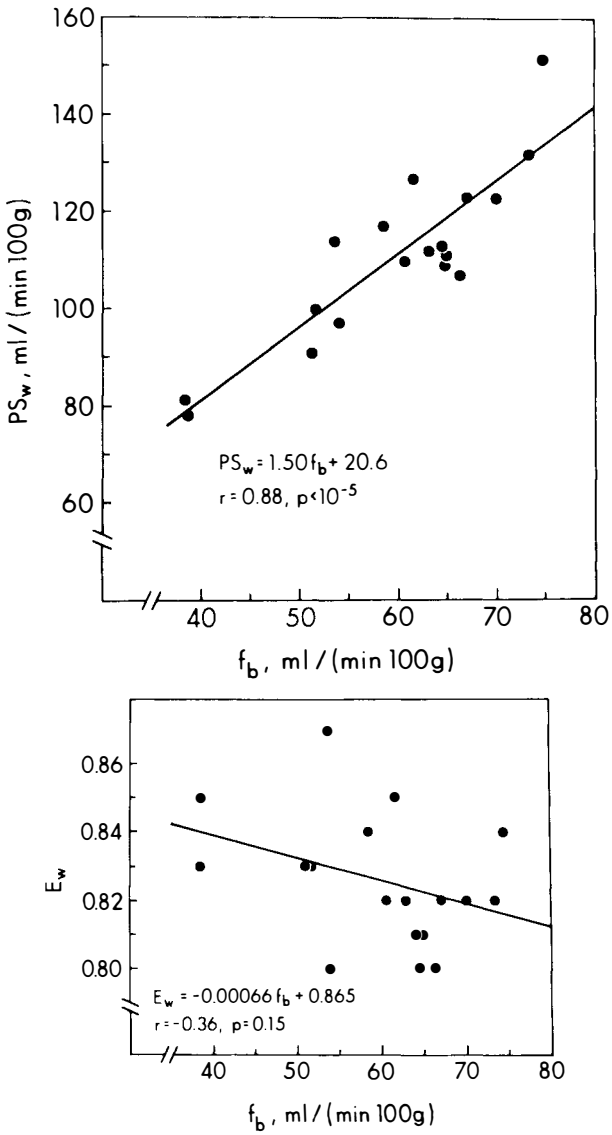

FIG. 4. Upper: Relationship between mean regional CBF measured with positron emission tomography (PET) and [^{11}C]butanol (f_b) and mean regional permeability–surface area produced for water (PS_w) for the brain regions listed in Table 2. **Lower:** Relationship between mean regional CBF measured with PET and [^{11}C]butanol (f_b) and mean regional extraction fraction for water (E_w) for the brain regions listed in Table 2.

Fierer, 1985). They measured the extraction of butanol using either tissue sampling or outflow sampling of cerebral venous blood following injection of tracer. Before reviewing their results, we will discuss certain aspects of the techniques used to measure extraction fraction.

The extraction fraction measured by our intracarotid injection, residue detection method is the quantity of tracer equilibrating with brain tissue in a single vascular transit divided by the quantity of tracer that is available for equilibration. Thus, for a freely diffusible tracer that equilibrates instantaneously across the blood–brain barrier, $E = 1.0$.

TABLE 3. *Error in measurement of CBF due to use of incorrect value of* λ_b

"True" λ_b (ml/g)	CBF (ml/min-100 g) True	CBF (ml/min-100 g) Measured	% error
0.50	80	70.0	−12.5
	20	19.4	−3.1
1.00	80	84.7	+5.8
	20	20.3	+1.4
1.25	80	88.6	+10.7
	20	20.5	+2.4

Tabulation of the flow that would be measured in practice, and the percentage error in flow measurement, if the true value of the partition coefficient for [^{11}C]butanol λ_b were different from the value of 0.77 ml/g used in Eq. 6.

However, even in the case of complete diffusion equilibrium (i.e., $E = 1.0$), there will be tracer remaining in blood after a single capillary passage. With outflow-sampling methods for determining extraction, the concentration of tracer in cerebral venous blood is measured over time. Extraction is calculated in relation to the amount of tracer presented to the brain (Van Uitert and Levy, 1978; Van Uitert et al., 1981) or in relation to the amount exiting the brain of a nondiffusible reference tracer coinjected with the test tracer (Crone, 1965). Because a certain portion of test tracer will always remain in blood, the extraction fraction measured by outflow detection will be <1, even with a freely diffusible tracer. The fraction of a freely diffusible tracer remaining in blood after a single vascular transit depends on the cerebral blood volume and on the equilibrium brain–blood partition coefficient for the tracer. This fraction is given by CBV/[λ d (1 − CBV) + CBV], where d is density of brain (g/ml) and CBV is the cerebral blood volume expressed as a fraction of total brain volume. For butanol, the fraction of tracer remaining in blood after a single vascular transit is ~5%, so that butanol extraction will be underestimated by this amount.

Another factor to be considered is washout from

TABLE 4. *Percentage error in calculation of* PS_w *due to measurement error in* f_b *or* f_w

E_w	% error in f_b +5%	% error in f_b −5%	% error in f_w +5%	% error in f_w −5%
0.80	−6.4	9.0	13.9	−11.3
0.85	−8.2	12.7	17.6	−13.2
0.90	−11.3	21.5	25.9	−16.1
0.95	−17.6	[a]	100.2	−22.3

Abbreviations as in Table 2. Note that for a given value of E_w, the percentage error in PS_w measurement is independent of the actual values of f_w and f_b (see text).

[a] *PS* value is undefined, because $E_w = 1.0$ for the specified values of true E_w and error in f_b.

TABLE 5. *Percentage error in* PS_w *measurement due to tissue heterogeneity*

PS_w (ml/min-100 g)		% gray matter in region		
Gray matter	White matter	30	50	70
150	50	−2.0	−1.4	−0.7
	75	−12.4	−8.4	−4.7
	100	−23.7	−16.5	−9.4
200	50	0	0	0
	75	−5.3	−3.5	−1.9
	100	−14.9	−9.9	−5.4
300	50	−5.8	−4.4	−2.6
	75	0.0	0.0	0.0
	100	−3.7	−2.4	−1.3

Percentage error in calculation of PS_w due to tissue heterogeneity in a region of interest of varying proportions of gray and white matter. Errors are calculated for several combinations of gray and white matter PS_w values, with gray matter flow = 80 ml/min-100 g and white matter flow = 20 ml/min-100 g.

brain tissue of extracted tracer prior to the measurement of extraction. This occurs with both outflow-sampling methods (Sage et al., 1981; Van Uitert et al., 1981) and tissue-sampling techniques (Irwin and Preskorn, 1982). Thus, the measured value of extraction may decrease with increasing time following the administration of radiotracer. The terms apparent or net extraction have been applied to such measurements (Gjedde et al., 1980; Sage et al., 1981; Irwin and Preskorn, 1982).

Outflow sampling has been used in several studies to measure E_b. Crone (1965) obtained a value of 0.91 in anesthetized dogs with Evans blue dye used as the nondiffusible reference tracer. Schaefer et al. (1976), also using an indicator dilution approach, found butanol to be freely diffusible in rat brain. Van Uitert and Levy (1978), Van Uitert et al. (1981), and Sage et al. (1981) calculated E_b from cerebral venous samples in relation to the amount of butanol presented to the brain. In awake gerbils with hemispheric CBF of 102 ml/min-100 g, E_b was 0.96 (Van Uitert and Levy, 1978). In awake normocapnic rats with hemispheric CBF ranging from 180 to 310 ml/min-100 g, E_b was 0.95 (Van Uitert et al., 1981). Sage et al. (1981) obtained similar results in the rat cerebral hemisphere with blood flows of 50–249 ml/min-100 g. At higher flows, the net E_b decreased slightly owing to elution of extracted butanol from brain. These observations are consistent with butanol being freely diffusible across the blood–brain barrier, given the modest underestimation of E_b that occurs using outflow detection methods as discussed above. Van Uitert et al. (1981) also used an alternative outflow-sampling method to demonstrate the complete extraction of butanol. Cerebral venous outflow curves were obtained following the administration of [^{11}C]butanol and [^{14}C]inulin, an intravascular reference tracer. Inulin was observed to pass completely through the brain vasculature and appeared as a single peak of radioactivity in torcular blood. This peak appeared earlier at higher flows. As would be expected for a freely diffusible tracer, there was no corresponding distinct peak in torcular butanol concentration with flows as high as 450 ml/min-100 g. Irwin and Preskorn (1982) obtained lower values of E_b in rat, using tissue sampling with microspheres as the reference tracer. They speculated that this result, as well as an observed underestimation of CBF with butanol in relation to microspheres using the indicator fractionation method, was due to washout of extracted butanol from brain. This hypothesis was subsequently confirmed by experiments in which the tissue-sampling time was adjusted so as to prevent washout (Kent and Preskorn, 1985). The resultant measurements of CBF obtained with butanol and indicator fractionation agreed with those previously reported with microspheres.

In contrast to the above experiments, two laboratories have reported considerably lower values for E_b in rat cerebral hemisphere. Goldman et al. (1980) used outflow sampling with inulin as the reference tracer. E_b was 0.74 in awake normocapnic rats and 0.88 in rats under pentobarbital anesthesia. Pardridge and Fierer (1985) used a new tissue-sampling approach to calculate E_b from values for the brain uptake index of [^{125}I]iodoamphetamine (IMP) relative to [^{14}C]butanol measured over time. They obtained a value of 0.87, which, in contrast to the observation of Goldman et al. (1980), decreased with pentobarbital anesthesia to 0.77. There is no immediate explanation for the discrepancy between these measurements of E_b and those reported by several other laboratories as described above. One possibility is a lack of radiochemical purity of the labeled butanol used. Clark et al. (1982), using gas chromatographic analysis, observed that commercially obtained [^{14}C]butanol was subject to contamination with other ^{14}C-labeled entities that appeared to be less permeable than butanol. Such a contamination would result in an underestimation of E_b. Furthermore, Pardridge and Fierer (1985) assume that IMP is completely extracted and retained by rat brain in a single capillary passage. However, residue detection measurements of the single-pass extraction of IMP in monkey brain contradict this assumption (Kuhl et al., 1982). At a hemispheric CBF of 33 ml/min-100 g, considerably lower than that of rat brain, IMP extraction was 0.92, with 8% of the trapped activity having a washout half-time

of 1.8 min, while at a CBF of 66 ml/min-100 g, the extraction fell to 0.74, with a washout half-time of 4.2 min for 16% of the trapped activity. These data might suggest that the use of IMP as a reference tracer would in fact lead to an overestimation of E_b. However, the complex in vivo behavior of IMP precludes its use as a true "fluid microsphere" reference tracer. For example, the observation that E_b decreases with anesthesia (Pardridge and Fierer, 1985) could be explained alternatively by an increase in IMP extraction at the lower flows found in the anesthetized animal.

In summary, then, the bulk of experimental data, including our own, supports of the use of butanol as a freely diffusible tracer suitable for the measurement of CBF. This hypothesis was further demonstrated in our validation experiments discussed below.

[11C]Butanol as a flow tracer for PET

Experiments were performed in baboons to validate our method for measuring CBF with the PET autoradiographic approach and [11C]butanol. Flow measured with this method was compared with flow measured with an established residue detection technique based on the central volume principle (Zierler, 1965; Roberts et al., 1973; Eichling et al., 1974; Raichle et al., 1975). Paired measurements were obtained over a flow range of 33–124 ml/min-100 g. An excellent agreement was observed between flow values obtained with these two techniques (Fig. 3). This observation should be compared with data obtained in similar experiments in which $H_2{}^{15}O$ was the PET flow tracer. At high flow levels, the use of $H_2{}^{15}O$ resulted in an underestimation of CBF that could be completely accounted for by the permeability limitation of water. No such underestimation occurred with [11C]butanol. This latter observation provides further evidence that [11C]butanol is a freely diffusible, flow-limited tracer.

In the use of either [11C]butanol or $H_2{}^{15}O$ as a flow tracer with the PET autoradiographic method, we caution potential users that total data collection time must be $\leq$1 min as originally prescribed by Kety (1960). As we have previously demonstrated for $H_2{}^{15}O$, CBF is progressively underestimated as data collection time is increased beyond 1 min (Raichle et al., 1983) owing, we believe, to an inherent limitation in the one-compartment flow model (Larson et al., 1985). Preliminary observations (M. E. Raichle, unpublished) indicate that [11C]butanol behaves in a similar fashion, thus supporting an interpretation of the phenomenon.

Paired flow measurements were obtained in normal human subjects with PET using both $H_2{}^{15}O$ and [11C]butanol. Mean global CBF measured with [11C]butanol, 53.1 ml/min-100 g, was found to be significantly greater than that obtained with $H_2{}^{15}O$, 44.4 ml/min-100 g. Similarly, regional blood flow measurements were higher with [11C]butanol (Table 2). Physiological differences in the state of our subjects during the two flow determinations do not explain our observations. There was no difference in arterial P_{CO_2} during the two flow measurements. In addition, in other studies in our laboratory, paired flow measurements performed in the same subjects using $H_2{}^{15}O$ as the tracer each time showed no significant difference in global flow between the first and second determinations (Fox and Raichle, 1984). Thus, it is unlikely that a consistent change in mood, attention, or thought content in the subjects between the two scans led to our observations.

If the value for λ_b used in our operational equation (Eq. 6) were too low, flow measured with butanol would be erroneously elevated. We used the value of 0.77 ml/g that was measured in whole rat brain (Gjedde et al., 1980). Similar values have been reported by other investigators (Pardridge et al., 1980; Betz and Ennis, 1985). In addition, λ_b does not vary significantly between brain regions in the rat (Gjedde et al., 1980; Betz and Ennis, 1985). Because both the water and the lipid contents of rat and human brain are similar (Altman and Dittmer, 1973; Herscovitch et al., 1985), it is unlikely that λ_b is substantially different in human brain. Even if λ_b were considerably greater in human brain, i.e., 1.0–1.25 ml/g, our simulations showed that this would not account for all of the difference in CBF measurement with [11C]butanol in comparison to $H_2{}^{15}O$. Therefore, we attribute the observed differences between these flow measurements to the permeability limitation of $H_2{}^{15}O$.

Iida and colleagues (1986) have proposed that failure to account for the dispersion or smearing of the time-activity curve $C_a(t)$ in the arterial system could lead to an overestimation of CBF with the PET autoradiographic method. They modeled the impulse response of intraarterial dispersion by a decaying monoexponential function. For dispersion occurring between the left ventricle and the radial artery, the measured time constant for this function was 4–6 s. It was calculated that this degree of dispersion could result in a 15% overestimation of CBF for a 40-s scan duration. However, in this calculation, it was assumed that dispersion does not occur between the left ventricle and the brain. It is most unlikely, however, that this is the case. Thus, the relative degree of dispersion between the radial

and cerebral arterial input functions should be considerably less than the 4- to 6-s value used in the calculation. As a result, any error in CBF determination due to failure to account for dispersion would be much less than the value of 15% calculated by Iida et al. Furthermore, for our comparative measurements of CBF with $H_2{}^{15}O$ and [^{11}C]butanol, the effect of dispersion would be similar for both tracers. Thus, dispersion would have minimal effect on the relative values of flow obtained with these tracers and on the calculated value of E_w.

In spite of the underestimation of CBF that occurs with $H_2{}^{15}O$, this radiopharmaceutical has several advantages in comparison with more diffusible tracers, such as butanol or derivatives of antipyrine (Ginsberg et al., 1981; Stone-Elander et al., 1985) labeled with ^{11}C. The longer half-life of ^{11}C (20.38 min), in comparison with that of ^{15}O, results in an appreciably higher radiation dose to experimental subjects, even if less radioactivity is administered, as was done in our studies. In addition, much more time is subsequently required for decay of the radioactivity background. Thus, the number and frequency of CBF measurements that can be made in a single subject are reduced. In contrast, in studies of cerebral function with $H_2{}^{15}O$, up to eight CBF measurements can be made within 2 h (Fox and Raichle, 1984). The more prolonged residual background and greater radiation exposure with [^{11}C]butanol also complicate its use in combined experiments with other long-lived radiopharmaceuticals labeled with ^{11}C, ^{13}N, or ^{18}F. An alternative approach of labeling butanol with ^{15}O rather than ^{11}C has been suggested. The synthesis of this compound has been described (Kabalka et al., 1985; Berridge et al., 1986), although its use as a flow tracer for PET has not yet been reported.

Measurement of E_w and PS_w

Several approaches have been implemented to measure the single-pass extraction fraction and permeability–surface area product for water in laboratory animals. The methods used to measure E_w include outflow sampling of cerebral venous blood following intracarotid injection of tritiated water and an intravascular reference tracer (Bolwig and Lassen, 1975); residue detection of the time course of radioactivity in the brain with an external detector following intracarotid injection of $H_2{}^{15}O$ (Eichling et al., 1974; Go et al., 1981; Raichle et al., 1983); and tissue sampling following intravenous or intracarotid injection of tritiated water and a reference tracer that is assumed to be completely extracted (Gjedde and Rasmussen, 1980; Clark et al., 1982; Irwin and Preskorn, 1982; Kent and Preskorn, 1985; Pardridge and Fierer, 1985). Paulson et al. (1977) extended the outflow-sampling method to measure E_w in humans following intracarotid injection of tritiated water. All of these investigators then applied the Renkin-Crone formulation (Eq. 8) (Renkin, 1959; Crone, 1963) to calculate PS_w from measurements of E_w and CBF.

None of these techniques can be routinely implemented for studies in humans, even with the availability of PET to measure regional tissue radioactivity. This is because they involve invasive intracarotid injection or cerebral venous sampling, and also require measurements of tissue radioactivity that are beyond the temporal resolution of current positron emission tomographs. Raichle (1980) proposed an alternative approach for measuring E_w and PS_w, based on Kety's model for diffusible tracers (1951, 1960, 1985) and the Renkin-Crone formulation. This approach is applicable to tissue autoradiographic or sampling experiments (McCulloch and Angerson, 1981; Reid et al., 1983; Ginsberg et al., 1985), and also to data obtained with PET, as described in this communication. The theory underlying an alternative PET method for measuring E_w has recently been described by Takagi et al. (1984).

Our method for measuring E_w is based on the observation that when a nonfreely diffusible tracer is assumed to be freely diffusible in the application of the Kety autoradiographic method, CBF is underestimated (Eckman et al., 1975; Raichle et al., 1983). The potential for this underestimation to occur was anticipated by Kety by the incorporation of the diffusion factor m into his tracer kinetic model (1951, 1960, 1985). This parameter denotes the extent to which diffusion equilibrium is achieved during a single vascular transit by the tracer. Kety further derived an expression for m in terms of CBF and PS (Eq. 7) that is equivalent to the expression for extraction fraction later derived by Crone and by Renkin. Thus, measurement of the underestimation of flow with the Kety autoradiographic approach and a nonfreely diffusible tracer, in relation to flow measured with a freely diffusible tracer, permits the calculation of E for the less diffusible tracer, and thence PS (Eqs. 7–10).

The derivation of the expression for m in terms of CBF and PS was based on two simplifying assumptions (Kety, 1951, 1985; Eckman et al., 1975). These were that the tissue could be represented by a single, well-mixed compartment and that the change with time in tracer concentration at a point in the capillary $\partial C/dt$ was small in comparison with the change along the length of the capillary $\partial C/dx$. Thus, $\partial C/dt$ was not included in the formulation. It

was subsequently shown that this latter assumption represents the asymptotic solution of the more complete model when capillary blood volume is small with respect to tissue volume (Johnson and Wilson, 1966; Eckman et al., 1975). In the case of antipyrine, a diffusion-limited tracer, simulation studies have shown that this assumption results in a modest underestimation of m (Eckman et al., 1975). Alternative approaches involving distributed models to describe more completely the in vivo behavior of diffusible radiotracers are being developed for potential use in PET (Larson et al., 1985). However, the greater mathematical complexity of distributed models precludes their routine use at this time. In addition, we have obtained experimental evidence to support the use of the less complex Kety model to determine E_w (Raichle et al., 1983). We measured CBF in baboons with $H_2{}^{15}O$ and the Kety autoradiographic method adapted to PET, and also with a standard intracarotid injection, residue detection method. The latter method allowed us to compute not only the true hemispheric CBF, but also hemispheric E_w at each flow level. At high flow values, CBF was underestimated with PET and $H_2{}^{15}O$. However, when each of these PET measurements of CBF was divided by the corresponding value of E_w, the result agreed with the true hemispheric CBF as measured by residue detection. This was in accordance with the theory presented in Eqs. 6–10. It follows, then, that when both $H_2{}^{15}O$ and a freely diffusible tracer, such as [^{11}C]butanol, are used with the PET autoradiographic method, the ratio of the measured flow values should equal E_w.

We obtained both global and regional measurements of E_w and PS_w. The global E_w was 0.84 and the PS_w 104 ml/min-100 g. The only comparable data from human studies are those of Paulson and colleagues (Paulson et al., 1977; Friis et al., 1980), although several investigators have measured either global or regional PS_w in experimental animals. A summary of the PS_w measurements that have been reported for rat, primate, and human brain is given in Table 6. Although these measurements of PS_w do vary, most likely owing to differences in both species and experimental technique, these data all are in agreement with the existence of a permeability limitation for water as originally observed in rhesus monkey brain (Raichle et al., 1974).

Regional measurements for E_w and PS_w are given in Table 2 and Fig. 4. There was a linear relationship between PS_w and regional CBF, while E_w did not show this variation over the brain regions studied. To our knowledge, these are the first such measurements to be reported in humans. However, an approach similar to ours has been used to mea-

TABLE 6. *Measurements of* PS_w *in different species*

Species	Anesthesia	Brain region[a]	PS_w (ml/min-100 g)	CBF (ml/min-100 g)	Method	Author
Rhesus monkey	Phencyclidine	Hemisphere	138	—	i.c. $H_2{}^{15}O$; external residue detection	Eichling et al. (1974)
Baboon	70% NO_2	Hemisphere	104	—	i.c. $H_2{}^{15}O$; external residue detection	Raichle et al. (1983)
Rat	70% NO_2	Hemisphere	53	100	i.c. ^{3}HOH, [^{14}C]sucrose; outflow sampling	Bolwig and Lassen (1975)
Rat	Conscious	Cortex	158	186	i.c. ^{3}HOH, [^{14}C]butanol; BUI measured over time	Gjedde and Rasmussen (1980)
Rat	66% NO_2	Hemisphere	217[b]	—	LV ^{3}HOH, ^{141}Ce-microspheres; BUI	Irwin and Preskorn (1982)
Rat	Conscious	Hemisphere	131	164	i.c. ^{3}HOH, [^{125}I]IMP; BUI measured over time	Pardridge and Fierer (1985)
		Cortex	138	199		
Rat	Urethane	Hemisphere	117	—	i.c. $H_2{}^{15}O$; external residue detection	Go et al. (1981)
Rat	Conscious	Cortex	231	123	i.v. ^{3}HOH, [^{14}C]IAP; tissue sampling	McCulloch and Angerson (1981)
Rat	Conscious	Hemisphere	235	92	i.v. ^{3}HOH, [^{14}C]IAP; tissue sampling	Reid et al. (1983)
		Cortex	262	111		
Rat	Conscious	Cortex	319	199	i.v. ^{3}HOH, [^{14}C]butanol; tissue sampling	Ginsberg et al. (1985)
Human	Conscious	Hemisphere	169	60	i.c. ^{3}HOH, $^{36}Cl^-$; outflow sampling	Paulson et al. (1977)
Human	Conscious	Hemisphere	90[c]	44	i.c. ^{3}HOH, $^{36}Cl^-$; outflow sampling	Friis et al. (1980)
Human	Conscious	Hemisphere	104	53	i.v. $H_2{}^{15}O$, [^{11}C]butanol; PET autoradiography	This work
		Cortex	127	62		

i.c., intracarotid; i.v., intravenous; LV, left ventricular; IAP, iodoantipyrine; IMP, iodoamphetamine; BUI, brain uptake index; PET, positron emission tomography.

[a] Cerebral hemisphere or cerebral cortex.

[b] Calculated from regression equation relating PS_w and P_{CO_2} for P_{CO_2} = 38 mm Hg.

[c] Calculated from mean values of CBF and extraction of water.

sure regional PS_w in rat brain with tissue sampling (McCulloch and Angerson, 1981; Reid et al., 1983; Ginsberg et al., 1985). These data also showed regional variation, with PS_w tending to be higher in those brain regions with higher local flow. If the water permeability P of the human blood–brain barrier is uniform throughout the brain regions studied, our regional measurements of PS_w indicate that the capillary surface area S increases with flow. This is consistent with more direct measurements that have shown vascular density to be greater in brain regions with higher levels of flow (Lierse and Horstmann, 1965).

We did not observe E_w to be lower in regions with higher local flow. This may appear to contradict studies that show E_w to vary as a result of experimental manipulations in CBF (Eichling et al., 1974; Raichle et al., 1976; Kent and Preskorn, 1985). However, these latter studies examine a different physiological process, i.e., the change in E_w in the same brain under different flow conditions. Declining E_w with P_{CO_2}-induced increases in CBF indicates that there is not a proportional increase in PS_w to match the increase in local flow that occurs. In contrast, in the resting state, PS_w and CBF are relatively matched throughout the brain, with little regional variation in E_w.

Certain methodological factors may result in an underestimation of both global and regional PS_w as measured with PET. Global f_w and f_b were obtained by averaging over several PET slices without a correction for the inclusion of metabolically inactive CSF spaces (Herscovitch et al., 1986). Thus, for any given experimental subject, f_w and f_b are both underestimated by the same fraction. E_w, the ratio of f_w and f_b, will be unaffected. However, PS_w will be underestimated by the same fraction that flow is underestimated, i.e., by ~5%. Similarly, regional PS_w measurements will be artifactually reduced because of contamination by CSF spaces due to partial volume averaging with a nearby sulcus or ventricle. In addition, our simulation studies showed that tissue heterogeneity will also cause a modest underestimation of regional PS_w (Table 5).

Our experimental data demonstrate the potential of PET to measure regional PS in vivo in humans. The ability to perform this measurement will be of importance in the study of blood–brain barrier function in humans, both in disease states and in response to specific interventions.

Acknowledgment: This work was supported by NIH grant nos. NS06833, AG03991, and HL13851. We would like to thank the staff of the Division of Radiation Sciences, Mallinckrodt Institute of Radiology, for their assistance.

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