A quantitative method has been developed to measure the water extraction fraction (WEF) in rat brain after successive intravenous bolus injections of $[^{15}\text{O}]$ water and $[^{14}\text{C}]$ butanol based on a mathematical equation developed by the authors. This new method is noninvasive to the brain or cranio-cervical large vessels and does not require sacrifice of the rats. Arterial concentration curves and total head counts were measured in 8 rats by means of external coincidence detectors. Water extraction fraction in rat brain was $0.67 \pm 0.13$ (mean $\pm$ SD) and permeability–surface product was $1.75 \text{ml/g min}$, where cerebral blood flow and arterial carbon dioxide tension were $1.71 \pm 0.86 \text{ml/g min}$ and $44.8 \pm 14.0 \text{mm Hg}$, respectively. Water extraction fraction was stable with different measurement times from 20 to 80 seconds. (Stroke 1987;18:177–183)

**Materials and Methods**

Eight male Wistar rats were provided with food and water *ad libitum* until the experiment. Anesthesia was induced with ethyl ether. The animals were tracheostomized, paralyzed with tubocurarine (7 mg/kg i.p.), and artificially ventilated (tidal volume 3.1 ml, 58 breaths/min) with 33% oxygen and 67% nitrous oxide using a mechanical ventilator. Body temperature was maintained at 37°C with an automatic heating blanket coupled to a rectal probe (Yellow Springs Instrument Co., Inc.). The right femoral artery was cannulated with polyethylene tubing (PE-50) having an internal diameter of 0.58 mm for measuring arterial radioactivity, for anaerobic removal of blood for carbon dioxide tension (Paco$_2$), oxygen tension (Pao$_2$), and pH determinations (Corning model 165/2 pH/blood gas analyzer) and for blood pressure recording (Micro Switch, 142PC056). Blood drawn for the measurement of radioactivity was reinfused after each study, with a resultant loss of less than 0.28 ml of blood for each arterial gas measurement. In 2 rats, Paco$_2$ was changed by adding carbon dioxide to the inspired air to change CBF. The right femoral vein was also cannulated with PE-50 tubing for the injection of radioactive tracers and other drugs.

A loop was made in the arterial tube, where a pair of detectors was positioned for coincidence counting. The length of the tube between the site of the femoral artery cannulation and the loop was adjusted to 40 cm.

The equipment for fixing the rat brain and the detector system was described in detail by Lockwood and Kenny. A standard stereotactic head holder (David Kopf, Model 900, Tujunga, Calif.) equipped with 3.5-cm offset earbars and a riser block was selected to provide a rugged means for securing each rat. Offset
earbars raise the animal above the structural elements of the frame and simplify placement of the collimators. The skin and temporal muscle of the rat were removed. The coincidence collimation zone with this positioning system was described by Lockwood and Kenny. The rejection rate for events that occurred outside the collimated zone was very high.

**Detectors**

Two cylindrical bismuth-germanium oxide crystals, 2.5 cm in height and diameter, equipped with RCA 4885 photomultiplier tubes and voltage dividers (Harshaw Chemical Co., Solon, Ohio) were used to measure the brain clearance curve. The crystals were shielded with lead (3.7 cm minimum) on all sides. Another 2 NaI crystals, 4.4 cm in height and 2.5 cm in diameter, equipped with RCA 6655A photomultiplier tubes and voltage dividers (Harshaw Chemical Co.) were positioned at the loop of the arterial catheter to measure the arterial curve. These NaI crystals and loop were shielded with 5 cm of lead. A Power Designer model 1570 high-voltage supply (Westbury, N.Y.) was used. Photomultiplier amplifiers (model 612AM), discriminators (model 620BL), and coincidence logic units (model 622) were purchased from LeCroy Research Systems (Spring Valley, N.Y.). Signals from the paired discriminators were processed by the coincidence logic circuit in the AND mode to yield the total coincidence count rate, recorded by an EG&G Ortec model 772 counter (Oak Ridge, Tenn.). The signal from each discriminator was also processed by a circuit and recorded by a counter to yield the single-photon count rate for each crystal. Pulse shaping by the coincidence logic circuit is required to make the output of the discriminator compatible with the counters. An Ortec model 776 timer-counter was used as a master to the model 772 slaves. Data from the counters were acquired automatically via a SYM model 1 microprocessor. A Perkin-Elmer 3220 digital computer (Norwalk, Conn.) was used for data analysis. Our preliminary study revealed no difference in the sensitivity of the arterial or head detectors, whether 14C-tracer or 15O-tracer is used.

**Radiopharmaceuticals**

Oxygen-15-labelled water was synthesized by the Cyclotron Unit at Mount Sinai Medical Center. The specific activity of the H215O was 0.75 mCi/μl of H2O when delivered at approximately 120 seconds after the end of bombardment. The synthesis of 1-14C-butanol was described previously. The specific activity of the [14C]butanol was 25 ± 8 mCi/μmol at the time of delivery corrected to the end of bombardment.

**Measurement of WEF and CBF**

In all 8 rats, 2 successive procedures were done — first the injection of H215O (water study), and second the injection of [14C]butanol (reference study) at intervals of 13-48 minutes. After a steady physiological state was attained, 10–20 mCi of radioisotopes were dissolved in 0.5 ml of saline and injected as successive i.v. boluses. Arterial blood samples were drawn into the detectors at a steady rate of 0.74 ml/min by an infusion–withdrawal pump (Harvard Apparatus, model 907) or manually. At a flow of 0.74 ml/min, the response of the detection system to a step function does not vary when hematocrit changes. Arterial and head coincidence count rates were recorded every 2 seconds for 1.5 minutes. Two factors contribute to changes in the measured arterial curve — a smearing effect of the catheter and taking a mean concentration from that portion of the catheter facing the detectors. Both factors can be corrected simultaneously by deconvolution between the measured arterial curve and the known transfer function of the detection system. CBF during the reference study was calculated by the 1-compartment curve-fitting method. CBF at the time of the water study (f) was estimated from CBF in the reference study (f*) and the difference in Paco2 values in the water and reference studies by means of the following equation:

\[ f = f* \times \frac{A \text{Paco}_2 (\text{wat}) + B}{A \text{Paco}_2 (\text{ref}) + B} \]  

(1)

where A and B are 0.0629 ml/g min mm Hg and -0.715 ml/g min, respectively, from the regression of normal CBF vs. Paco2, obtained in our laboratory using the same system. Note that f is the supposed CBF during the water study, and not the CBF measured by the water.

Data from rats with more than 5 mm Hg difference in Paco2 between the water and reference studies were not included to avoid error in the calculated WEF resulting from error in estimating CBF during the water study.

The operational equation to calculate WEF is as follows:

\[
\frac{\int_1^t N (\phi) d\phi}{\int_1^t N^* (\phi) d\phi} = \frac{(1 - \text{WEF}) \int_1^t \frac{\text{ca}* (\phi - \tau)}{t} e^{k_1 t} d\tau d\phi + \text{WEF} \int_1^t \frac{\text{ca} (\phi - \tau)}{t} e^{k_2 t} d\tau d\phi}{f* \int_1^t \frac{\text{ca}* (\phi - \tau)}{t} e^{k_3 t} d\tau d\phi}
\]  

(2)

where

\[ k_1 = \frac{f}{\text{CBV}} + \alpha \text{ (min}^{-1}) \]  
\[ k_2 = \frac{\text{WEF} f}{\lambda} + \alpha \text{ (min}^{-1}) \]  
\[ k_3* = \frac{f*}{\lambda*} + \alpha* \text{ (min}^{-1}) \]
Table 1. Variables Used in the Equations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$ and $N^*$</td>
<td>Respective count rates (not absolute concentrations) of water and butanol from the brain (count/min)</td>
</tr>
<tr>
<td>$ca$ and $ca^*$</td>
<td>Respective count rates of water and butanol from the arterial catheter (count/min)</td>
</tr>
<tr>
<td>$f$ and $f^*$</td>
<td>Respective CBF values during water and butanol studies (ml/g min)</td>
</tr>
<tr>
<td>WEF</td>
<td>Water extraction fraction</td>
</tr>
<tr>
<td>$t_1$ and $t_1^*$</td>
<td>Respective start measurement times of water and butanol studies (min)</td>
</tr>
<tr>
<td>$t_2$ and $t_2^*$</td>
<td>Respective end measurement times of water and butanol studies (min)</td>
</tr>
<tr>
<td>$\lambda$ and $\lambda^*$</td>
<td>Respective partition coefficients of water and butanol (ml/g)</td>
</tr>
<tr>
<td>$\alpha$ and $\alpha^*$</td>
<td>Respective decay constants of $^{15}$O (0.3354 min$^{-1}$) and $^{12}$C (0.03381 min$^{-1}$)</td>
</tr>
<tr>
<td>CBV</td>
<td>Cerebral blood volume (ml/g)</td>
</tr>
<tr>
<td>$\phi$ and $\tau$</td>
<td>Integration variables</td>
</tr>
</tbody>
</table>

Variables used in the equations are shown in Table 1. WEF is calculated by preparing a look-up table. Because of the technical difficulty in measuring CBV after the introduction of $^{12}$C-tracer, a notional value of 0.03 ml/ml was used for CBV.

To examine the effect of changing the measurement time on WEF, WEF was calculated with end measurement times ($t_1$ and $t_2^*$) from 20 to 80 seconds. Start measurement times ($t_1$ and $t_2^*$) were always 0 seconds. To calculate the permeability-surface product (PS) the following equation was used$^{13-15}$:

$$ WEF = 1 - e^{-PS/CBF} $$

where $P$ is the permeability to water and $S$ is the surface area of the capillary. PS was determined by the least-squares fitting method, by which the sum of squares of the differences between observed WEF and expected WEF is minimized.

**Results**

Figure 1 shows the actual arterial and head curves for $^{15}$O-water (left) and $^{12}$C-butanol (right). The difference in height between the arterial and head curves for water is greater than that for butanol, indicating that the extracted portion of water is smaller than that of butanol. Calculated WEF in this rat is 0.62.

WEF from all the rat studies were 0.67 ± 0.13 (mean ± SD) with measurement times from 0 to 60 seconds. There was no change in WEF with different end measurement times between 20 and 80 seconds. CBF and $Paco_2$, were 1.71 ± 0.86 ml/g min and 44.8 ± 14.0 mm Hg, respectively.

Figure 2 shows the relation between WEF and CBF in all studies. WEF decreases with increasing CBF, theoretically expressed by Equation 3. The best fit was obtained when PS was 1.75 ml/g min; the correlation coefficient between observed and estimated WEF was 0.87. The function

$$ WEF = 1 - e^{-PS/CBF} $$

is also shown in Figure 2.

The data from 8 rats were divided into two groups according to CBF values. Table 2 shows the mean CBF, WEF, and PS values in each group along with the previously reported values. In the group of lower CBF (0.9–1.6 ml/g min), WEF was 0.75 ± 0.12, and PS was 1.57 ml/g min. In the group of higher CBF...
Figure 2. Relation between WEF and CBF in 8 rats. WEF values are plotted against CBF values (ml/g min). WEF $= 1 - e^{-1.75/CBF}$ is the line of best fit.

(1.8-3.6 ml/g min), WEF was 0.58 ± 0.09, and PS was 2.09.

Discussion

The method described enables measurement of the physiological WEF in rats noninvasively and without sacrifice. We define “noninvasive” as not invasive to the brain, cerebral sinus, or cervical large vessels. The new method has clear advantages over previously developed methods, which are discussed below.

In the intracarotid injection method of Bolwig and Lassen and Go et al., CBF may be increased temporarily by the large volume of the intracarotid injection itself, as pointed out by Clark et al. Hence, WEF may be decreased, resulting in underestimation of PS since they used reported CBF values or weighted mean CBF measured by the height/area method. Their WEF and PS are lower than our data. Moreover, CBF might be disturbed by the surgery or the catheter, resulting in a possible deviation from the normal physiological state.

Methodological improvements were suggested by Clark et al., who used a narrow (33 gauge) needle and small injection volume of 5 μl. Although WEF can be measured accurately, PS cannot be calculated because of the inability to measure CBF in the same rat. Their WEF in the normocapnic group (0.69 ± 0.07) is almost the same as ours (0.73 ± 0.12).

Clark et al. measured the extraction fraction of [H]ethanol and [H]water after i.v. injection of each tracer with [14C]butanol. Although this is a noninvasive method, the rats must be sacrificed after each study. The results are accurate only if the extraction of ethanol in the normal flow state is measured within 10 seconds. Progressive overestimation was observed with increasing measurement time, because washouts of the test and reference tracers were not incorporated in the equation. The difference in washout rates between the test and reference tracers increased when the test tracer was water instead of ethanol and if CBF was high. Hence, a 10-second measurement time might still be long enough to overestimate WEF, especially in high flow states. From their Figure 2 and the equation,

$$PS / F = 16.7 Paco_2^{-0.60}$$

WEF can be calculated as 0.94, 0.84, and 0.76 when Paco$_2$ is 20, 40, and 60 mm Hg, respectively. These values are higher than our data as well as of their previous work. This overestimation might be explained by the factors suggested above.

WEF could be estimated as the ratio of CBF measured by water and the reference tracer. McCulloch and Angerson and Reid et al. measured WEF in various regions after continuous i.v. injection of [H]water and [14C]iodoantipyrine as a reference tracer for 1 minute. WEF can be calculated accurately only if tissue concentration is measured separately from the intravascular concentration. Since water is a diffusion-limited tracer, a high concentration can be expected in the intravascular space after continuous injection of water. This results in overestimation of CBF measured.
Table 2. Reported Water Extraction Fraction Values in Rats

<table>
<thead>
<tr>
<th>Authors</th>
<th>Injection site</th>
<th>Region</th>
<th>Counting method</th>
<th>$\text{Paco}_2$ (mm Hg)</th>
<th>CBF (ml/g min)</th>
<th>WEF (ml/g min)</th>
<th>PS (ml/g min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolwig and Lassen\textsuperscript{1}</td>
<td>N\textsubscript{2}O</td>
<td>IC Whole brain</td>
<td>Venous sampling</td>
<td>15</td>
<td>0.74</td>
<td>0.71</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(12–18)</td>
<td>(0.70–0.79)</td>
<td>(0.61–0.74)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>38.5</td>
<td>0.47</td>
<td>0.53</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(37.5–40)</td>
<td>(0.43–0.50)</td>
<td>(0.51–0.55)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.0</td>
<td>0.33</td>
<td>1.50</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(80.0–90.0)</td>
<td>(0.32–0.35)</td>
<td>(1.40–1.52)</td>
<td></td>
</tr>
<tr>
<td>Go et al\textsuperscript{2}</td>
<td>Urethan</td>
<td>IC Whole brain</td>
<td>External counting</td>
<td>20 ± 1\textsuperscript{*}</td>
<td>0.77 ± 0.10\textsuperscript{*}</td>
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<td></td>
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<td></td>
<td>32 ± 4\textsuperscript{*}</td>
<td>0.69 ± 0.07\textsuperscript{*}</td>
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<tr>
<td></td>
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<td></td>
<td>95 ± 4\textsuperscript{*}</td>
<td>0.58 ± 0.04\textsuperscript{*}</td>
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<tr>
<td>McCulloch and Angerson\textsuperscript{4}</td>
<td>Conscious</td>
<td>IV Frontal cortex</td>
<td>Sacrifice</td>
<td>37.3 ± 1.3</td>
<td>1.23 ± 0.06</td>
<td>2.31 ± 0.18</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55.9 ± 1.7</td>
<td>1.77 ± 0.14</td>
<td>4.06 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>Clark et al\textsuperscript{3}</td>
<td>N\textsubscript{2}O</td>
<td>IV Total brain</td>
<td>Sacrifice</td>
<td>20</td>
<td>0.94\textsuperscript{†}</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>0.84\textsuperscript{†}</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>0.76\textsuperscript{†}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reid et al\textsuperscript{6}</td>
<td>Conscious</td>
<td>IV Cerebral cortex</td>
<td>Sacrifice</td>
<td>36.9 ± 4.3\textsuperscript{*}</td>
<td>0.90–1.60</td>
<td>0.73 ± 0.12\textsuperscript{*}</td>
<td>1.57</td>
</tr>
<tr>
<td>Present paper</td>
<td>N\textsubscript{2}O</td>
<td>IV Whole brain</td>
<td>External counting</td>
<td>57.9 ± 15.4\textsuperscript{*}</td>
<td>1.80–3.60</td>
<td>0.58 ± 0.09\textsuperscript{*}</td>
<td>2.09</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Means ± SD. Otherwise, values are means ± SEM.
\textsuperscript{†}Calculated from regression curves.

by water, causing WEF and PS to be overestimated, especially in the high CBF state where WEF is low and concentration of the intravascular tracer is high. Studies in conscious instead of anesthetized rats and data from the cortex vs. data from the whole brain might be other reasons for differences between their reported values for WEF and PS and ours.

In our method, blood flow is not disturbed because there is no manipulation of cervical or intracranial large vessels. Also, blood flow and WEF are not changed because there is no intracarotid injection. Washout of test and reference tracers from the brain is incorporated into the equation. CBF can be measured in the same rat using the reference tracer.\textsuperscript{8} These advantages over the previously reported methods contribute to obtaining more accurate WEF and PS values.

Our results for WEF agree well with the data reported by Clark et al.\textsuperscript{2} which, for the above reasons, are considered to be accurate. Our PS values are higher than the possibly underestimated data of Bolwig and Lassen\textsuperscript{1} and Go et al.\textsuperscript{3} and lower than the possibly overestimated data of McCulloch and Angerson.\textsuperscript{4} Clark et al.\textsuperscript{2} and Reid et al.\textsuperscript{6} These considerations indicate that WEF and PS may be measured more accurately by our method.

To test the new method's ability to assess expected changes from a stimulus, we used a physiological stimulus, change in CBF, the quantitative effect of which is predictable and expressed by Equation 3.\textsuperscript{13–15} The high correlation coefficient ($r = 0.87$) between observed WEF and WEF estimated by Equation 4 indicates the ability of the new method to measure the expected change in WEF.

PS tends to be high if CBF is high in our study. This tendency might reflect the increased surface area of the capillaries due to the increased blood flow.\textsuperscript{4}

A few further points should be considered. First, we used a notional value of 0.03 for CBV instead of the actual CBV for each rat because of the difficulty of measuring CBV after introduction of $^{14}C\text{butanol}$ in the same rat. This value of CBV agrees with CBV reported for rats,\textsuperscript{17} and also with that for normal rats in our laboratory (unpublished data). As discussed in our previous paper,\textsuperscript{8} it is important to perform the CBV correction. However, the effect of an error in CBV on calculated WEF is not large. A 10% error in CBV causes less than 3.6% error in WEF if WEF is larger than 0.6.

We assumed that the field observed consisted of homogeneous tissue in regard to CBF and WEF, although there must be a fast flow and low WEF compartment (usually gray matter) and a slow flow and high WEF compartment (usually white matter). In rats, the brain consists mainly of gray matter. The relative lack of white matter makes estimated WEF values close to those for gray matter. To measure regional WEF, rats must still be sacrificed and the isotope concentration must still be measured in each tissue. Since CBF determination requires the relative concentrations of the reference tracer in blood and tissue rather than the count rates as when the tissue time-course is known, relative concentrations of radio-
active water in blood and tissue can usually be measured in the same way. The following operational equation should be applicable, since washout of the tracer is incorporated:

\[
C(\phi) = (1 - \text{WEF}) \int_{t_1}^{t_2} \text{Ca}(\phi - \tau) e^{-k\tau} d\tau + \text{WEF} \int_{t_1}^{t_2} \text{Ca}(\phi - \tau) e^{-k\tau} d\tau
\]

Equation 5 differs from the numerator of Equation 2 only in that concentrations of radioactivity in tissue \(C(\phi)\) and blood \(\text{Ca}\) are used instead of count rates from tissue \(N(\phi)\) and blood \(\text{ca}\), respectively, and in that integration from time \(t_1\) to \(t_2\) was not done, making Equation 5 applicable to tissue sampling techniques where brain concentrations can be measured only at the time of sacrifice. In this case, one or more of the tracers should be replaced by non-positron-emitter-labelled isotopes for technical reasons.

We used a CBF value during the water study \(f\) that is calculated from the reference study \(P\). The effect of a change in \(P\) that has the largest influence on CBF with the contribution factor of 0.88, 2, 9 is incorporated into Equation 1. Changes in physiological condition, however, should still be avoided to keep this assumption valid.

Another point concerns extracerebral contamination. To minimize extracerebral contamination, we removed the scalp, masseter, and occipital muscle, and used narrowly collimated coincidence detectors, which were already reported in detail by Lockwood and Kenny. 11 They found that more than 90% of the total activity counted by the coincidence detectors comes from the cerebral tissue after i.v. injection of \[^{15}\text{N}\]ammonia. This means that extracerebral contamination is less than 10% in our study. The major source of this small contamination may be the cranium, where CBF is low and WEF is presumed to be high. This results in a slight overestimation of WEF, which must be taken into account in evaluating WEF with drugs or procedures that may affect extracerebral circulation.

A prime advantage of our method is that it provides a noninvasive way to measure WEF in the same rat without sacrifice. If the measurement were to be repeated, an interval of about 80 minutes would be required to allow radioactivity to decay before a second study was done. 9 Use of a gaseous reference tracer, such as \[^{13}\text{C}\]methyl fluoride, might shorten the interval required for a repeated measurement. By using a steady state as its own control, the interindividual variability could be eliminated, allowing more subtle changes to be detected.

This method could also be applied for the measurement of the extraction fraction of compounds that are flow- and diffusion-limited, such as ethanol or antipyrene.

Finally, we would like to discuss errors that may ensue if water is used as a tracer for CBF using a positron emission tomograph (PET). It is usually assumed that WEF is 1.0 — in other words, that water is not diffusion-limited. There will be 2 kinds of errors, underestimation and overestimation, in CBF measured by PET using \[^{15}\text{O}\]water. Underestimation simply results from neglecting the diffusion limitation of the tracer. Since WEF in humans may be higher than that in rats because of lower CBF values in humans, underestimation should be on the order of several percent or less. After correction of CBF for WEF, there will be another source of error resulting in an overestimation: that portion of the nonextracted intravascular tracer is included in the total concentration of \[^{15}\text{O}\]water measured by PET. CBF study by PET using \[^{15}\text{O}\]water will ultimately result in a small underestimation in CBF.

Acknowledgment

We are most grateful to Dr. Myron D. Ginsberg for his suggestions and discussions, to the Mount Sinai Medical Center Cyclotron staff for the syntheses of pharmacological agents, and to Ms. Ana Abreu for her secretarial assistance.

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Key Words • water extraction fraction • permeability-surface product • [15O]water • noninvasive method • rat
Water extraction fraction and permeability-surface product after intravenous injection in rats.

S Takagi, K Ehara and R D Finn

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