Cerebral Extraction of N-13 Ammonia: Its Dependence on Cerebral Blood Flow and Capillary Permeability — Surface Area Product

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SUMMARY "N-labeled ammonia was used to investigate 1) the cerebral extraction and clearance of ammonia, 2) the mechanism by which capillaries accommodate changes in cerebral blood flow (CBF) and 3) its use for the measurement of CBF. The unidirectional extraction of "NH, in rhesus monkeys was measured during Paco2-induced changes in CBF and dog studies were performed using in vitro tissue counting techniques to examine "NH2 extraction in gray and white matter, mixed tissue and cerebellum during variations in CBF produced by combinations of embolization, local brain compression, and changes in Paco2. The single pass extraction fraction of "NH2 varied from about 70 to 20% over a CBF range of 12 to 140 cc/min/100 g. Capillary permeability-surface area product (PS) estimates with a Renkin/Crone model show PS increasing with CBF. The magnitude and rate of increase in PS with CBF was highest in gray matter > mixed tissue > white matter. Tissue extraction of "NH2 vs CBF relationship was best described by a unidirectional transport model in which CBF increases by both recruitment of capillaries and by increases of blood velocity in open capillaries. This saturable-recruitment model provides a possible explanation for the mechanism of flow changes at the capillary level. The net "NH2 extraction subsequent to an i.v. injection increases non-linearly with CBF. Doubling or halving basal CBF produced from 35 to 50% changes in the "N tissue concentrations with further increases in CBF associated with progressively smaller changes in "N concentrations.

SINCE IT WAS originally proposed by Hunter et al.1-3 and Harper et al.4 (N-13) ammonia ("NH2) has been used as an imaging agent for the heart and brain with very little information available regarding what factors determine its tissue distribution. The rapid diffusion of "NH2 through cerebral and myocarndal capillaries and its rapid incorporation and trapping in the slowly turning over amino acid pools can potentially allow the use of this labeled substrate for imaging capillary perfusion, the study of the capillary flow mechanisms, or the study of ammonia extraction and amino acid metabolism. However, due to the complexities involved in the extraction and retention of "NH2 in these organs, more extensive studies are required to better understand the potential uses of this tracer.

Phelps et al.,4 using positron computed tomography (PCT) with normal volunteers, showed that the relative "N concentrations in structures of the brain were in good agreement with the relative capillary densities and/or cerebral blood flow (CBF). Subsequently, Phelps et al.6 demonstrated in the monkey that the unidirectional extraction fraction of "NH2 by the brain was 1) less than 100% at normal values of CBF, 2) inversely related to CBF, 3) unaffected by blood ammonia concentrations from 80 to 1400 /ug% and insensitive to changes in arterial blood pH over the range of 7.2 and 7.7, 4) decreased by about 24% during hypoglycemic coma and 5) was limited by capillary permeability. Intravenously injected "NH2 was proposed for estimation of capillary perfusion in brain and heart in analogy to the microsphere model with capillary occlusion by microspheres replaced by metabolic trapping of "NH2.8

Cooper et al.7 found that the brain ammonia pool
turnover rate was rapid (t<sub>1/2</sub> ≤ 1 to 3 secs) and resulted primarily from the reaction of ammonia with glutamate to form glutamine. This reaction has been anatomically localized by Norenberg and Martinez-Hernandez, who found glutamine synthetase almost exclusively confined to astrocytes with equal concentrations in the astrocytes of gray and white matter and the highest concentration in the astrocytic pericapillary end-feet. This work<sup>1,2</sup> provides a mechanism for R NH₃ extraction at appropriate anatomical sites to explain our previous observations.<sup>3,4</sup>

We have further investigated the relationship of net cerebral extraction and the unidirectional extraction fraction of R NH₃ as a function of CBF to gain insight into the rate limiting steps and mechanisms in the cerebral extraction of R NH₃. In order to determine quantitatively the relationship between CBF and R NH₃ extraction by the brain, we have also measured the local CBF and net extraction of R NH₃ by direct tissue sampling.

The tissue uptake and clearance and the equilibrium distribution of R N in the brain of man after i.v. injection of R NH₃ was measured with PCT and compared to the distribution of cerebral metabolic rate for glucose with (F-18) fluordeoxyglucose (FDG). The relationship between the capillary permeability-surface area product and CBF was estimated in terms of a number of unidirectional transport models and a saturable-recruitment model is proposed to describe the mechanism for CBF changes at the capillary level. The use of R NH₃ and other non-flow limited tracers for the measure of capillary blood flow was examined.

Materials and Method

Preparation of R NH₃

Our R NH₃ was produced with the UCLA medical cyclotron as described elsewhere<sup>6</sup> and the R NH₃ was buffered to pH 7.4 in physiologic saline prior to injection. The radiochemical purity was greater than 99% and ammonia concentration was less than 10<sup>-4</sup>M.

Animal Preparation

Twenty-two mongrel dogs, 20 to 30 kg, were anesthetized with sodium pentobarbital (25 mg/kg), paralyzed with gallamine triethiodide and ventilated on room air or mixtures of CO₂ and O₂. A polyethylene cannula in the left atrium was used for injection of labeled microspheres and/or R NH₃. Systemic blood pressure was monitored with a catheter in the aorta connected to a Statham pressure transducer. This catheter was also used for withdrawal of arterial blood samples. End tidal Pco₂ was continuously monitored with a Beckman LB50 capnograph.

In 5 dogs, the left common carotid artery was isolated for injection of 30 mg of 100–200 micron diameter resin microspheres to embolize portions of the brain. Two dogs were also ventilated with 5% CO₂–95% O₂ and 3 with 10% CO₂–90% O₂. Arterial blood gases, pH and ammonia concentrations were determined by standard techniques.

In 12 dogs a small inflatable balloon was inserted into the subdural space through a burr hole on one side of the calvarium to produce local compressions of the brain. Five dogs were ventilated with 5% CO₂–95% O₂ and seven were ventilated on room air. In 5 other dogs, 2 were ventilated on room air and 3 were ventilated with 10% CO₂–90% O₂ with no other intervention performed.

Seven rhesus monkeys were anesthetized with pentobarbital sodium (5 mg/kg) 2 to 4 weeks prior to experimentation and the right external carotid artery ligated at its origin. At the time of the experiment, the animals were again anesthetized and a 0.2 mm diameter catheter positioned in the right common carotid artery. The animals were paralyzed with gallamine triethiodide and passively ventilated on room air or different CO₂–O₂ admixtures (5, 7 or 10% CO₂) and anticoagulated with heparin. The arterial catheter was used for injection of R NH₃ and ¹⁸Xe, monitoring of arterial blood pressure and sampling of blood for Pco₂, pH, and ammonia analysis.

Measurement of CBF, R NH₃ Unidirectional Extraction Fraction and Net R NH₃ Extraction

Global CBF. Global CBF was measured in rhesus monkeys by the 10-min height/area method<sup>19</sup> from the clearance curve resulting from a 0.2 cc bolus internal carotid artery injection of ¹⁸Xe.<sup>11</sup> The clearance curve was recorded with a shielded and collimated NaI detector which viewed the injected hemisphere. Data were collected at 0.2 sec intervals. The final value of CBF was calculated assuming the ¹⁸Xe 10 min height/area method systematically overestimates flow by 11<sup>%</sup><sup>12</sup> and using a whole brain partition coefficient for Xe of 1.10 ml/g.<sup>17</sup>

Local CBF. Local CBF in the in vitro dog studies was determined by the quantitative microsphere technique.<sup>24</sup> Carbonized polystyrene microspheres of 15 ± 5 microns were suspended in 10% dextran with one drop of Tween 80 to prevent clumping, thoroughly mixed with a vortex shaker, and suspended by ultrasonification just prior to injection. Approximately 2 × 10⁶ microspheres§ labeled with ¹⁴C, ¹⁰³Ce, or ⁶⁶Sr were injected into the left atrium. Just prior to injection of microspheres and R NH₃, arterial blood withdrawal was initiated at a constant rate and continued for 2.5 min. The total microsphere radioactivity in this sample and the microsphere radioactivity from excised samples of known weight were measured in a well counter. The local CBF was then calculated by:

\[
\text{CBF}(\text{cc/min/100 g}) = \frac{(F_p \times C_i \times 100)}{C_b} (1)
\]

Where \(F_p\) is the pump withdrawal rate of arterial blood (cc/min), \(C_i\) is the microsphere tissue radioactivity concentration in region i (counts/min/g) and \(C_b\) is the total microsphere activity in arterial blood sample (counts/min).

Unidirectional Extraction Fraction. The fraction (E) of

<sup>§</sup>3M Company, Nuclear Products, St. Paul, MN.
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$^{13}$NH$_3$ extracted by the brain unidirectionally was determined by extrapolation of the tail of the washout curve, which is the extracted and retained $^{13}$NH$_3$ in the tissue, back to the initial peak of the curve, which is the total $^{13}$NH$_3$ activity input to the tissue. $E$ is then estimated from the ratio of the extrapolated value to the peak value.$^9$ $^{11}$

**Net Extraction.** The net extraction (i.e., $^{15}$N tissue concentration) of $^{13}$NH$_3$ in the *in vitro* dog experiments was determined in the same manner as the CBF in equation 1. The input function (i.e., $C_b$ for $^{13}$NH$_3$ in equation 1) corresponded to only $^{15}$N in the form of $^{13}$NH$_3$ by correcting for the contribution due to metabolized $^{15}$N (Schelbert et al., unpublished work: 90% of $^{15}$N activity is free $^{13}$NH$_3$ from time of injection to 5 min).

**Animal Procedures**

**Measure of $E$ vs CBF.** In the measurements of the unidirectional extraction fraction $E$, the monkey was stabilized at a basal Paco$_2$ a 0.2 cc bolus of $^{133}$Xe injected as a 0.7 sec bolus into the internal carotid and data collected for 11 min for the measurement of CBF. A 0.2 cc bolus of $^{13}$NH$_3$ dissolved in the animals plasma was then injected into the internal carotid and data collected for the next 20 to 30 min. The Paco$_2$ level was then increased or decreased by varying the inspired CO$_2$ concentration and/or respiratory rate. Fifteen minutes was allowed for equilibration at each Paco$_2$ level before $^{133}$Xe and $^{13}$NH$_3$ were injected. End tidal CO$_2$ was monitored to assure constant Paco$_2$ levels. Arterial blood samples were taken before and after each injection for blood gas and pH.

Local net extraction of $^{13}$NH$_3$ vs CBF. In the *in vitro* dog experiments, variations in local CBF were produced by embolism, variation in Paco$_2$, and local compression. Five dogs were subjected to embolism using initial data collection times of 2 to 3 min after the injection of labeled microspheres (labeled with a different radionuclide), and $^{13}$NH$_3$ 5 min after injection of the balloon. In three additional dogs microspheres were injected in the basal state, the balloon was inflated for 30 min, deflated, and 30 min later $^{13}$NH$_3$ and a second set of labeled microspheres were injected. In this series tissue samples were taken without separation into gray and white matter.

Arterial blood clearance rates were determined by monitoring the activity with a femoral artery catheter which passed through a NaI well counter to a withdrawal pump. The catheter had a total volume of 2.1 cc and blood was withdrawn at a rate of 4.29 cc/min. The data sampling time (0.1 sec) and catheter produced only a minor distortion of the blood clearance rate (tested with step impulse into the catheter).

**Human Studies**

Six male volunteers (age 21-26) were studied by tomographically measuring the distribution of $^{13}$N in the brain after an i.v. bolus injection of 10 to 15 mCi of $^{13}$NH$_3$. Tomographic scans with the ECAT$^+$ positron tomograph$^9$ were started 4 to 5 min after injection using initial data collection times of 2 to 3 min with the time progressively increased to compensate for $^{15}$N decay. From 4 to 8 scans were made per injection each containing from 700,000 to 1.5 million counts per image. The relative $^{15}$N concentration of various substructures of the brain were measured as previously described$^{13}$ and compared to the distribution of the local cerebral metabolic rate for glucose as measured with (F-18) fluorodeoxyglucose, FDG$^{14,10}$

Three male volunteers (ages 21-23) were injected intravenously with $^{13}$NH$_3$ and a single cross-section of the brain was imaged as a function of time. The $^{15}$N concentration in cortex and white matter in these images was used to determine the uptake and clearance rates of these tissues.

**Unidirectional Transport Model**

A starting point for the description of the cerebral extraction of $^{13}$NH$_3$ is the Renkin/Crone model for the unidirectional transport through a capillary membrane$^{20, 21}$ as initially formulated by Kety.$^{22}$ This model assumes; 1) the capillary is a rigid tube, 2) an
exponential decrease in tracer blood concentrations along the capillary, 3) the intravascular concentration of tracer is much larger than extravascular, 4) the extravascular volume of distribution is infinitely larger than intravascular, and 5) the capillary membrane is the rate limiting step in the extraction of the tracer. This model is described by:

\[ E = 1 - e^{-\frac{PS}{F}} \]  

or

\[ \ln(1-E) = -\frac{PS}{F} \]  

Where \( E \) is the unidirectional extraction fraction for the tracer, \( P \) and \( S \) are the capillary permeability \((\text{cm}/\text{min})\) and surface area \((\text{cm}^2/100 \text{ g})\) and \( F \) is the capillary blood flow \((\text{cc}/\text{min}/100 \text{ g})\).

Strict application of this model to a multi-capillary environment would require that all the capillaries be identical in length and diameter (i.e. \( S \)), \( P \) and \( F \). This assumption is not valid for global measurements of \( E \) in the brain. Since the relationship between \( E \) and \( PS \) is nonlinear, the sum of local values of \( E \) for the whole brain cannot be used to calculate the sum of local \( PS \) values from equation 2. It has been shown\(^2\) that the \( PS \) from equation 2 underestimates the sum of \( PS \) values in heterogeneous tissues. The result is an "effective" \( PS \) value which is an underestimation of the true value.\(^2\) This model also assumes that the value of \( PS \) is constant (i.e. capillaries are rigid tubes) and that capillary flow increases by velocity and not by changes in the number of open capillaries (i.e. recruitment), vasodilatation or frequency of opening and closing of capillaries.

By changing these assumptions, various extended forms of the Renkin/Crone model can be derived to describe the relationship between \( E \) and \( F \). For example, by assuming the tissue consists of various sizes of capillaries each with a different \( PS \) value but obeying the original Renkin/Crone assumptions, \( E \) will have the following functional form.

\[ E = 1 - \left( \sum A_i e^{-\frac{PS}{F}} \right) + A_2 e^{-\frac{PS_2}{F}} + \ldots + A_n e^{-\frac{PS_n}{F}} \]  

Where \( \sum A_i = 1 \). In this paper a model with 2 components was tested.

Alternatively, if capillary recruitment is included in the model (i.e., as flow is increased, more capillaries become active) and all capillaries are identical and behave like rigid tubes, then \( E \) would have the same functional form as the Renkin/Crone model (equation 2), except \( PS \) is not constant. Since \( PS \) will not increase indefinitely, a non-divergent function for \( PS \) was examined. That is,

\[ E = (1 - e^{-\frac{PS}{F}}) \]  

with

\[ PS = A + B(1-e^{-CF}) \]

We refer to this model as the saturable-recruitment (SR) model. At saturation (i.e. all capillaries are open and active) the sum of \( A \) and \( B \) will be equal to the \( PS \) of all the anatomical capillaries in a given type of tissue. The value of \( C \) indicates the rate at which any given tissue will approach saturation as flow increases.

The extraction of \(^{13}\text{NH}_3\) as a function of \( CBF \) was analyzed with each of the above forms (equations 2, 4 and 5) to determine which provided the best fit to our data.

**Results**

**Human Studies**

Figure 1 illustrates the cerebral distribution of \(^{13}\text{NH}_4\) subsequent to an i.v. injection as compared to cerebral metabolic rate for glucose (CMRGlc) in normal human volunteers. The average \(^{13}\text{NH}_4\) tissue concentration ratio of gray (frontal, temporal, parietal, occipital and visual cortex) to white matter (frontal, parietal and occipital) was found to be 1.77 ± 0.18 (sd) as compared to 1.91 ± 0.23 for the CMRGlc in 6 normal volunteers (20 cross sectional images with an average of 12, 1.0 to 1.5 cm\(^2\) regions/image). These values are in good agreement with those of Kuhl et al.,\(^2\) who found values of 1.69 ± 0.15 (sd) for \(^{13}\text{NH}_4\) and 1.88 ± 0.17 for CMRGlc in normal man. These data are consistent with the fact that normally the blood flow and metabolic distribution in the brain are the same.

Cerebral tissue \(^{13}\text{NH}_4\) concentration was found to increase rapidly for 2 to 3 mins, slowly increase for the next 50 min (15% increase), and then slowly decrease with about a 2.3 h half time (fig. 2). Gray matter appeared to clear \(^{13}\text{NH}_4\) at a faster rate than white matter but the difference in clearance rates was not statistically significant. The time variation of the \(^{13}\text{N}\) concentration is consistent with the rapid blood clearance of \(^{13}\text{NH}_3\) (5% and 1 to 2% of the peak value at 2 and 10 min after injection), its rapid diffusion into cerebral tissue and the slow turnover rate of the tissue \(^{13}\text{NH}_3\) activity.\(^3\)

**Unidirectional Extraction Fraction Studies**

The results of whole brain unidirectional extraction fraction (E) measurements from carotid bolus studies in monkeys shown in figure 3, demonstrate that E decreases as CBF increases. However, net extraction of \(^{13}\text{NH}_4\) (E times CBF) or tissue \(^{13}\text{NH}_4\) concentration increases monotonically though nonlinearly with CBF (fig. 5A). The increase in net extraction with CBF is due to the fact that the rate of increase in CBF (i.e. amount of \(^{13}\text{NH}_4\) delivered to tissue) more than compensates for the decrease in E.

Figure 4 is a plot of the unidirectional extraction fraction data assuming a Renkin/Crone model (equation 2). If a Renkin/Crone model adequately described the data, a linear relationship would exist between \( \ln(1-E) \) and \( CBF \) with a slope of \(-PS\). This is clearly not the case.

The average tissue clearance of the extracted portions of \(^{13}\text{NH}_4\) subsequent to the intracarotid in-
FIGURE 1. Comparison of the cross-sectional distribution of the cerebral metabolic rate for glucose measured with FDG and the distribution of intravenously injected $^{13}\text{NH}_3$ in 2 different normal volunteers. Notation of O.M. refers to the cm above the orbital meatal plane. Brain slices at top are at levels similar to the three right hand FDG and $^{13}\text{NH}_3$ images and are shown for anatomical comparison. Note similarity in the distribution of metabolism and $^{13}\text{NH}_3$ distribution.

The net extraction of $^{13}\text{NH}_3$ (i.e. tissue $^{13}N$ concentration and equivalent to $E$ times CBF from single pass studies) was found to have a half time of $1.4 \pm 0.5$ hours (SD).

Net Extraction Studies

The net extraction of $^{13}\text{NH}_3$ (i.e. tissue $^{13}N$ concentration and equivalent to $E$ times CBF from single pass studies) vs CBF for mixed gray-white matter and cerebellum (mixed gray-white matter) are shown in figures 5B and 5C. The local changes in CBF in these studies were produced by combinations of embolization, variations in Paco$_2$ and local brain compression. A plot of net extraction vs CBF for mixed gray/white matter for animals subjected to only local compressions of the brain is shown in figure 5D. Figures 5E and 5F show the net extractions for gray and white matter as a function of CBF for CBF changes induced by alterations in Paco$_2$, embolization and local compression. No apparent difference in the net extraction vs CBF was noted when different maneuvers were used to produce changes in the local CBF values. To estimate the amount of admixture in samples classified as gray or white matter, we calculated the gray/white matter CBF ratio. The average ratio for the samples shown in Figures 5E and

FIGURE 2. Example of the gray and white matter uptake and clearance of $^{13}N$ activity subsequent to an intravenous bolus injection of $^{13}\text{NH}_3$. $^{13}\text{NH}_3$ is rapidly accumulated into the brain due to its relatively free diffusion, rapid clearance from the blood, and its rapid incorporation into glutamine (see fig. 9). From 3 to 50 minutes, there is a very gradual increase in gray and white matter tissue $^{13}N$ concentration (15%) due to the very low levels of circulating $^{13}\text{NH}_3$ 3 mins after injection. Subsequent to 60 mins, there is a slow removal of $^{13}N$ activity from the tissue which probably results from back diffusion of free $^{13}\text{NH}_3$, $^{13}N$-amino acids and from deamination of $^{13}N$-amino acids.

FIGURE 3. Whole brain unidirectional extraction fraction from intra-carotid bolus injection of $^{13}\text{NH}_3$ as a function of CBF in rhesus monkeys. The solid line is a least squares fit to data by equation 5.
The equations from the least square fits to SR model (equation 5) for the global single pass study, CBF (1U microspheres) to the CBF values (14Ce microspheres) and 15N tissue concentrations measured during local compression. The 15N tissue concentrations follow the general trend of the CBF variation. However, as would be expected from the results shown in figure 5, the magnitude of the variation are less than the observed CBF variations.

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limited BBB permeability 2) that the metabolic trapping occurs in the small glutamate-glutamine synthetase pool located in astrocytes, 3) the general large distribution of astrocytes in brain and their specific proximity to capillaries (astrocytic pericapillary end-feet) provide an efficient mechanism for trapping of $^{15}$NH$_3$ diffusing from both gray and white matter capillaries, and 4) the net extraction of $^{15}$NH$_3$ depends primarily upon CBF, capillary PS product and integrity of the glutamate-glutamine synthetase reaction.

### TABLE

**Estimated Values for Variables of Saturable-recruitment Model, $E = (1 - e^{-PS/F})$ where $PS = A + B (1 - e^{-CF})$**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CBF range (cc/min/100 gm)</th>
<th>A (cc/min/100 gm)</th>
<th>B (cc/min/100 gm)</th>
<th>C (cc/min/100 gm)$^{-1}$</th>
<th>Basal PS* (cc/min/100 gm)</th>
<th>A + B* (cc/min/100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray matter</td>
<td>0-250</td>
<td>10.4 ± 2.9</td>
<td>49.9 ± 3.2</td>
<td>0.0096 ± 0.0021</td>
<td>32.2 (F = 60)</td>
<td>60.3 ± 4.3</td>
</tr>
<tr>
<td>Mixed gray/white matter</td>
<td>0.1-160</td>
<td>7.0 ± 1.8</td>
<td>39.6 ± 3.2</td>
<td>0.0118 ± 0.0027</td>
<td>21.9 (F = 40)</td>
<td>46.6 ± 3.7</td>
</tr>
<tr>
<td>White matter</td>
<td>0-130</td>
<td>6.2 ± 2.4</td>
<td>24.4 ± 1.9</td>
<td>0.0192 ± 0.0050</td>
<td>14.0 (F = 20)</td>
<td>30.6 ± 3.1</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>8-170</td>
<td>5.8 ± 2.7</td>
<td>52.3 ± 3.9</td>
<td>0.0115 ± 0.0028</td>
<td>31.4 (F = 60)</td>
<td>58.1 ± 4.7</td>
</tr>
<tr>
<td>Compression (mixed gray/white matter)</td>
<td>1-135</td>
<td>11.8 ± 1.9</td>
<td>27.0 ± 2.7</td>
<td>0.0137 ± 0.0043</td>
<td>23.2 (F = 40)</td>
<td>38.8 ± 3.3</td>
</tr>
<tr>
<td>Single pass (mixed gray/white matter)</td>
<td>12-140</td>
<td>9.4 ± 4.5</td>
<td>34.6 ± 5.4</td>
<td>0.0119 ± 0.0065</td>
<td>22.5 (F = 40)</td>
<td>44.0 ± 7.0</td>
</tr>
</tbody>
</table>

± values are standard errors from least squares fit of data to equation 5.

*Estimates of basal PS values assuming basal blood flow, F, for gray matter and cerebellum of 60 cc/min/100 g, white matter of 20 cc/min/100 g and mixed gray/white matter of 40 cc/min/100 g.

*A + B is equal to the total PS of all anatomical capillaries in a given type of tissue.
**Figure 6.** Variations in local values of CBF from a basal state to that of a local compression of a section of one hemisphere. The variations in $^3$H tissue concentration follow the variations in flow in both the hemisphere with compression and the contralateral hemisphere, although the increases and decreases in tissue $^3$H concentration tend to be less than the changes in CBF as would be expected from Fig. 5. All samples contained admixtures of gray and white matter.

**Figure 7.** Combined plot of equations shown in table and fig. 5. The differences between the equations of CBF values less than about 40 are not statistically significant. Note similarity in response of gray matter with the high capillary density tissue of cerebellum, similarities of mixed gray and white matter from in vivo single pass and in vitro data, and the lower response and rapid flattening of the curve for white matter due to its low CBF reserve and low inherent capillary density (see Discussion Section on SR model).

**Figure 8.** Combined plots of PS product as a function of CBF from fits of the data in fig. 5 to the SR model of equation 5.

**$^3$NH$_3$ Extraction vs CBF**

Phelps et al. demonstrated from unidirectional extraction studies that the extraction fraction, E, of $^3$H$^3$NH$_3$ was inversely related to CBF. A linear relationship was reported between ln(1-E) vs 1/CBF (equation 2) and the Renkin/Crone model was used to estimate the value for whole brain PS product. Now with many more unidirectional extraction studies (fig. 3), it is clear that the relationship between ln(1-E) and 1/CBF is not linear (fig. 5). This indicates that the simple Renkin/Crone model for capillaries cannot describe the CBF vs E relationship for $^3$H$^3$NH$_3$ unless the PS product increases as CBF increases.

Phelps et al. used a combination of intracarotid and intravenous injections of $^3$H$^3$NH$_3$, and a vascular tracer to determine the unidirectional transport of $^3$H$^3$NH$_3$ and assess the amount of back diffusion. It was concluded that the size of the extravascular space for $^3$H$^3$NH$_3$ diffusion and the rapid incorporation of $^3$H$^3$NH$_3$ into amino acids normally prevents significant back diffusion. We reexamined this by the same technique and further support this conclusion. Thus E is assumed to be the unidirectional extraction fraction.

Estimates of back diffusion at low CBF are most important since PS estimates are very sensitive to errors in E when the value of E is high. Also at low CBF, back diffusion is most difficult to resolve from...
the vascular and the trapped components of the curve. Since local back diffusion cannot be determined from the dog studies employing in vitro tissue counting in this work, we compared average values of E from the single pass carotid studies with the mixed gray/white matter tissue values from the in vitro tissue sampling studies (figs. 3, 5 and the table). At CBV values of 20 and 40 cc/min/100 g, single pass values of E were 0.57 ± 0.05 and 0.43 ± 0.05 and values from mixed gray/white matter in the dog studies were 0.54 ± 0.11 and 0.42 ± 0.05. This agreement occurred down to the lower limit in CBV values of 12 cc/min/100 g in the single pass studies. Our data provide no direct way to estimate back diffusion at CBV values ≤12 cc/min/100 g in the in vitro dog experiments so inferences from the data in this range must be made with some caution (i.e. if back diffusion does occur to a significant degree then the degree and rate of capillary recruitment may be over-estimated). Since the single pass and in vitro results are in good agreement, back diffusion was assumed to be the same for both techniques for mean CBV values of ≥12 cc/min/100 g. This is consistent with the fact that the tissue 15N clearance rate is 1.4 ± 0.5 h from the intracarotid studies and would predict that ≤6% of the 15N would clear from the tissue during the 5 min period of the intravenous studies. The rapid blood clearance of i.v. injected 15NH4+ probably accounts for this fact.8,9 Cooper et al.7 found that constant 15NH4+ levels in blood for 10 min in the rat allowed back diffusion and equilibration of the intra- and extravascular compartments which in turn led to reduced values of E.

At the extreme low values of local CBF produced by embolization and compression, 15N tissue concentrations were disproportionately higher than the microsphere tissue concentration. This may be due to high extraction of 15NH4+ at low CBF and/or delivery of 15NH3 in plasma via collaterals or partially occluded vessels which exclude microspheres. In the latter cases 15NH3 may be more representative of nutrient flow than microspheres. This is particularly evident in regions that had positive 15N concentrations and zero values for microspheres.

Since CBF increases at a faster rate than E decreases, the net extraction (i.e. E times CBF) or tissue 15N concentration (fig. 5 and the table) increases monotonically, though nonlinearly, with CBF.

**pH, Ammonia Concentration and Metabolism**

Phelps et al.8 found no change in E when the pH of the circulating blood was varied from 7.1 to 7.7. This disagrees with results of Carter et al.9 in dogs and Lockwood et al.10 in rats. Carter et al.9 did not control respiration or Paco2 and consequently CBF was allowed to vary. Since the average Paco2 at the elevated pH was 8 torr higher than at the low pH, the resulting CBF increase of about 11 cc/min/100 g11 could have caused the small reported increase in uptake of 15N with increased pH. In addition, the data of Carter et al.9 are not very specific to the brain since intravenous injections were used and the detector viewed the whole head of the dog, which has a small ratio of intra- to extracerebral tissue. Lockwood et al.10 using the Oldendorf technique12 in rats, reported that E for 15NH3 decreased as the pH of the injected solution (5% equilibrated Ringer-Hepes buffer, 30 mM, 0.2 mM ammonium acetate and H2O) was decreased from 8.0 to 6.5. Since the brain is suddenly exposed to anoxia and to changes in osmolarity and pressure which are known to open the blood-brain barrier,13 it is not clear that the observed changes can be attributed solely to changes in pH. Hardebo et al.14 have reported that hydrostatic pressure of only 200 mm at the carotid artery will open the blood-brain barrier in rats. If this occurred in the Lockwood et al. studies and H+ penetrated the regions of pH sensitive flow regulation, the decreased extraction of low pH might have resulted from increased CBF that was not measured in their experiments.

One effect of changing the vascular pH is a change in the 15NH4+/15NH3+ ratio. Although steady state distributions of total ammonia (NH3 and NH4+) in the different compartments of the brain will clearly be altered by pH, the effect on unidirectional transport of an altered 15NH4+/15NH3+ ratio is unclear. The 15NH4+ is expected to have an extremely low extraction (i.e. similar to Na+ and K+ which have zero or near zero single pass extraction).4 and 15NH3 should be the form extracted to yield large values of E. As 15NH3 leaves the vascular compartment it is immediately replaced by conversion of 15NH3+ to 15NH4+. This process is so rapid that it is difficult to see how it could be rate limiting. It should also be remembered that ammonia in water does not exist as simply NH3 and NH4+, but rather as a hydrated molecule and the charged and uncharged forms occur by proton exchange with water (i.e. H2O+ + NH4+(H2O) = NH4+(X+1)(H2O), where X is between 1 and 3). However, there may be...
some pH effects that were not large enough to be observed in the pH range of 7.2 and 7.7 reported by Phelps et al. In any case, extreme changes in pH are limited in vivo due to hemolysis. Phelps et al. and Cooper et al. found that E was unaffected by 17 and 1000 fold increases in blood ammonia levels, indicating that it is unlikely that ammonia diffusion occurs by a saturable carrier. Phelps et al. found E reduced by 24% when glucose and oxygen cerebral metabolism was decreased by a factor of 2.2 in hypoglycemic coma in monkeys. However, in hypoglycemia with elevated ammonia the value of E was unchanged even though oxygen metabolism remained low and glucose metabolism doubled. These data indicate a sensitivity of E to metabolic rate in brain and hypoglycemia-hyperammonia data are indicative of detoxification shunt for ammonia. Cooper et al. found that when methionine sulfoximine (MSO) produced an 86% deactivation of glutamine synthetase in rat brain, 14N was still rapidly incorporated into glutamine. However, significant changes in the distribution of labeled amino acids occurred; free 14NH3 increased and net extraction of 14NH3 by brain decreased (the actual amount extracted may not have been reduced since the reduction could have occurred only in the amount trapped due to deactivation of glutamine synthetase). These data are for prolonged infusions. The single pass bolus technique would clearly delineate the effects of MSO at the point of forward and back diffusion and metabolic trapping.

The above data imply that severe reductions in the metabolic trapping mechanism for NH3 may be required before E is significantly reduced. However, interpretation of data at very low CBF where reductions in metabolism may also be occurring (i.e. gray, white, and mixed values below about 40, 10 and 20 cc/min/100 g, respectively) must be done with caution.

Unidirectional Transport/Trapping Model

The Renkin/Crone model* is considered to give good estimates of the PS products for stationary CBF states but does not describe variations of 15NH3 extraction versus CBF. It is also of limited validity to apply this model to heterogenous tissue with wide ranges of PS and/or CBF. It has been shown that the total PS determined with this model underestimates the sum of PS values in heterogenous tissue. The "effective" PS values for whole brain and mixed tissue, shown in figure 8 and the table, therefore, underestimate the true values. Basal (i.e. CBF = 40 cc/min/100 g) PS values of 22.5 for the unidirectional transport data and 21.9 and 23.2 for in vitro mixed tissues agree with the previous estimate of 24 cc/min/100 g in the monkey from Phelps et al. Tissue sampling experiments to separate gray and white matter (fig. 5) were performed to resolve the discrepancies of heterogenous tissue. The data demonstrate the differences in net extraction (E times CBF) vs CBF of relatively pure tissue to that of mixed tissue (fig. 5).

The model description that best fits the data is a "Saturable Recruitment" (SR) model. In this model all capillaries in the brain are assumed to be identical. At very low CBF, few capillaries are open. Increases in CBF occur through recruitment of additional capillaries and increases in the blood velocity through open capillaries (i.e. changes in capillary perfusion pressure). When 100% recruitment has occurred, further increases in CBF are accomplished by increases in blood velocity. According to the functional form (equation 5) of the SR model PS = A + B(1-e-CB) PS is equal to A at zero flow and as flow increases PS increases to a maximum of A + B (i.e. A + B is equal to the PS of all the anatomical capillaries). The rate of increase and the flow value at which the maximum PS is approached are determined by the value of C.

The SR model allows the capillary PS product to increase with CBF (fig. 8) by an increase in S through recruitment. The model gives good fits to E vs CBF and E times CBF vs CBF data in figures 3-5. The white and gray matter data in figures 5 and 7 illustrate that net extraction of 14NH3 at low CBF are similar. However, as CBF increases and white matter capillaries are almost completely recruited, the response of the net extraction of 14NH3 (fig. 7) and PS (fig. 8) flattens. On the other hand, gray matter's higher inherent capillary density or greater flow reserve allows further net extraction of 14NH3 (fig. 7) and increases in PS (fig. 8) at the high values of CBF. At saturation, the ratio of PS value (i.e. A + B in the table) for gray and white matter is 60.3/30.6 = 1.97 which should be equal to the anatomical ratio of capillary densities of gray to white matter. In man, the anatomical gray/white capillary density ratio has been reported to be about 3, considerably higher than our value of 2. However, the 10 min half life of 15N did not allow sufficient time to completely separate gray and white matter structures. Since the gray/white matter CBF ratio in our experiments of 2.2 and close to the PS saturation ratio of 2, we conclude that the SR model predicts saturation values in good agreement with anatomical capillary ratios of these tissues. The ratio of the rates of PS increase of gray to white matter (i.e. C in equation 5 and the table) is also 2.

Increases in PS and CBF were also observed in the unidirectional transport and extraction fraction measurements of Bolwig et al. for 14C-urea, 15S-thiourea and 14C-glucose in the brain of man. These authors report that PS for 14C-glucose increased by 56 and 90% when CBF was increased by 100 and 200% by induced seizure and hypercapnia. Similar trends for 14C-urea, 15S-thiourea (even larger increases in PS) were observed but their lower values of E may have limited the accuracy of the PS estimates. Exact values
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E vs CBF is not known. These investigators state that their data indicate that some capillaries might be unperfused at normal flows and open up to accommodate increases in flow.41 Pollay and Stevens,42 using rats, measured the single pass unidirectional extraction of 14C-glucose by the brain as a function of CBF in mixed gray and white samples. Using their data we estimated values of effective PS at discrete values of CBF with the Renkin/Crone and the SR model. These data, although statistically limited, indicate that PS for glucose increases by 42% from a CBF of 60 to 120 cc/min/100 g. At higher CBF, the value of PS did not seem to change. We also used the unidirectional transport data for 14C-glucose of Betz et al.43 in the isolated dog brain to estimate PS as a function of CBF. These data exhibited less statistical scatter than that of Pollay and Stevens and also showed increasing PS with CBF that was similar to ours in figure 8 (i.e. gradual increase to a plateau). The data of Betz et al.43 yielded a 52% increase in PS from a CBF of 20 to 40 cc/min/100 g and a 34% increase from CBF of 40 to 80 cc/min/100 g. All the above data are in reasonably good agreement with our 18NH3 data that also indicate that PS changes by about 40% when CBF is doubled or halved from the basal flow.

The above studies and our data indicate that the PS for a number of substances increases as CBF increases, and that it reaches a constant value at high flows. Since this trend occurs for substances that cross the BBB by either passive or facilitated diffusion, it may be the result of changes in capillary surface area and permeability.

Several investigators have reported similar increases in PS with blood flow in heart and skeletal muscle that are similar to our results in the brain with 18NH3. Yipintsoi et al.44 found a near linear increase in PS for 14K, 14C-glucose and 14C-sucrose over a myocardial flow range in the dog of about 40 to 150 cc/min/100 g. Crone45 and Renkin46 found similar results in skeletal muscle. Yudilevich47 found that the PS product for sucrose in the heart increased with blood flow until at higher flow it became relatively constant and the PS for 32Cl continually increased and did not plateau in the flow range studied (maximum myocardial flow of only 80 cc/min/100 g).

Eichling et al.48 and Raichle et al.49 measured the global single pass extraction of H218O and labeled alcohols by the brain as a function of CBF in rhesus monkeys. They did not detect increases in PS (analyzed with the Renkin/Crone model) with increasing CBF. However, increases in PS might have been missed because 1) the increase with CBF is small (fig. 8), 2) the near flow limited nature of H218O and these labeled alcohols make the measurements less sensitive to PS and 3) the small flow range (less than or equal to 30 to 100 cc/min/100 g) reduces the detectability of a non-linear relationship between ln(1-E) and 1/CFB (i.e. see fig. 3).

The SR model is a possible explanation of the observations of the relationship between 18NH3 extraction, PS and CBF. As to whether the concept of changes in capillary flow in the brain requires additional investigative work. Our investigative approach is limited because the results are average values that do not allow inspection or determination of a number of specific variables for the prediction of a detailed mechanism. For example, our method can not directly measure and the model ignores variable capillary lengths and diameters, topology of the vascular bed, upstream and downstream pressures, variable velocity, mechanical properties of blood and vessels, pulsatile flow, and other factors.

Tissue 15N Concentrations as an Indicator of Local CBF

Phelps et al.4 and8 proposed that 15NH4 uptake in brain and heart from i.v. injections could be understood in terms of a unidirectional transport and trapping model and in this respect 15NH4 was considered to be analogous to microspheres for capillary blood flow measurements. 15NH4 is, however, not strictly flow limited in brain and heart and its distribution is dependent upon blood flow, PS, and integrity of the glutamine metabolic trapping mechanism. Recent work of Norenberg and Martinez-Hernandez22 demonstrates that glutamine synthetase exists in equal concentrations in both gray and white matter astrocytes with highest concentrations in the astrocytic pericapillary end-feet in direct contact with capillaries. Thus the principal components of the trapping mechanism have the same distribution as the cerebral capillaries.

While the 15N tissue concentration increases monotonically with CBF, (fig. 5) the response is non-linear and the rate of increase diminishes at higher CBF. Doubling or halving CBF from the basal state produces about a 35 to 50% change, depending on the specific tissue and CBF values, with further increases producing even smaller changes (fig. 7, table). This can be attributed to ammonia's moderate BBB permeability. Its better flow response in heart is probably due to a factor of 4 higher capillary density and PS product in the heart. Figure 10 shows the calculated response of hypothetical tracers with different PS values assuming a Renkin/Crone capillary model. As PS increases, the response is higher (i.e. the more flow limited the tracer, the more linear the response). While the Renkin/Crone model does not quantitatively predict the flow response, it can be used to qualitatively evaluate potential flow tracers. For example, it can be used to determine how large the PS of the tracer must be to yield an acceptable flow response.

The low PS of 15NH4 in brain and the normal range of CBF are such that 15NH4 is adequate to measure flow reductions but is of limited value at higher values of CBF (fig. 5).

Increases in BBB permeability accompanying disease may also produce anomalous changes in 15N tissue concentrations. However, indirect evidence suggests this may be less detrimental than expected. Kuhl et al.38 and Phelps et al.40 have reported positron tomography studies in patients with demonstrated
The determination of absolute CBF requires the measurement of the arterial $^{14}$NH$_3$ input function ($C_b$ in equation 1) and a calibration factor from figure 7. This was tested on a preliminary basis using the ECAT and a rhesus monkey, giving a global CBF of 45 cc/mm/100 g at a PacO$_2$ of 39 mm Hg which is in good agreement with reported basal CBF in monkeys. $^{14}$ The arterial $^{14}$NH$_3$ concentration could possibly be approximated by a blood sample from an "arterialized" vein from a heated hand.

Traditional methods of measuring CBF with external detection of tracers requires high temporal resolution to adequately define the tracer washout curve. $^{10, 12}$ Since high count densities are required to provide adequate tomographic images, tomographic washout measurements of CBF will suffer from either poor spatial resolution or poor temporal resolution. Although $^{14}$NH$_3$ has limitations as an indicator of CBF, all other known flow tracers are also limited and their application in pathology usually amplifies their weaknesses.

Acknowledgment

We would like to thank Ms. Joann Miller, Ms. Francine Aguilar, for their technical assistance, Mrs. Lee Grieswold for the illustration work and Drs. H. R. Schelbert and William Partridge for their many helpful discussions. Dr. N. S. MacDonald and his cyclotron staff and Dr. Gerald Robinson and his chemistry staff's efforts and help are deeply appreciated.

References


![Figure 10. Plot of the flow response (i.e. net tissue extraction or concentration of tracer) for tracers with different PS products using Renkin/Crone model. Although the rigid tube Renkin/Crone model does not predict the exact flow response, it can be used for guidance in the selection of non-flow limited tracers for their potential use as indicators of blood flow in a unidirectional transport and trapping approach. Although, the highest possible PS product is desirable, it is quite apparent that acceptable flow responses over a given flow range can be achieved with tracers with less than ideal values of PS. However, a lower limit in the value of PS produces unacceptable flow response. It should be noted that any mechanism which increases capillary surface area as a flow increases (i.e. SR model) will improve the flow response as compared to the rigid tube Renkin/Crone prediction (see fig. 7).](image-url)


Cerebral extraction of N-13 ammonia: its dependence on cerebral blood flow and capillary permeability -- surface area product.

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doi: 10.1161/01.STR.12.5.607

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