Comparison of Microsphere and Xenon-133 Clearance Method in Measuring Skeletal Muscle and Cerebral Blood Flow

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SUMMARY In this study, we compared the Xenon-133 (133Xe) clearance method with the microsphere method in measurement of skeletal muscle and cerebral blood flow (CBF). Over a wide range of flows (1–47 ml/min × 100 g), we compared these two methods with direct measurements of venous outflow in the gracilis muscle. Regression equations relating the 133Xe (stochastic method) and microsphere measurements of flow to venous outflow were Y = 0.92X - 0.93 (r = 0.81) and Y = 1.01X - 0.42 (r = 0.94), respectively. Thus, both 133Xe clearance and microspheres accurately measure flow in an isolated organ with a simple bloody supply. When CBF was measured with 133Xe, extracerebral contamination and scattered radiation were minimized by ligation of the external carotid artery, removal of the soft tissue of the head, injection of the 133Xe into the internal carotid artery, and use of lead shielding and a collimated detector. In the dog (n = 10), CBF (range, 12–200 ml/min × 100 g) measured with 133Xe was substantially less than that measured with microspheres. In baboons (n = 18), when mean CBF < 120 ml/min × 100 g, there was a good relationship between values obtained with 133Xe (stochastic method) and microspheres. The regression equation relating those two measurements was Y = 0.62X + 16.9 (r = 0.83). At flow rates > 120 ml/min × 100 g, values obtained for mean CBF with 133Xe (stochastic method) and microspheres differed widely. We also compared fast and slow clearance curves obtained with 133Xe (compartmental analysis) with grey and white matter flows measured with microspheres. The two techniques yielded results which differed widely. Although the 133Xe clearance method (stochastic analysis) and microspheres provide similar values for mean CBF in baboons under some experimental conditions, values obtained with the two techniques differ importantly under several conditions.

THIRTEEN years ago, Høedt-Rasmussen et al. (1966) suggested the clearance of xenon-133 (133Xe) determined with an external detector could measure regional cerebral blood flow in humans. Validation of the method was based on findings that: (1) mean values obtained with 133Xe clearance correlated with values obtained with the Kety-Schmidt method (Ingvar et al., 1965 and Høedt-Rasmussen, 1967); (2) estimates of grey and white matter volume determined by a compartmental analysis of the 133Xe curves were similar to anatomical data (Høedt-Rasmussen and Skinhøj, 1966); and (3) regional flows approximated those obtained with autoradiographic techniques (Landau et al., 1955). When the 133Xe method was introduced, repeated measurements of regional cerebral blood flow in the same animal could not be obtained with another method. Also, the range of cerebral blood flow examined seldom exceeded 100 ml/min × 100 g (Ingvar et al., 1965; Høedt-Rasmussen and Skinhøj, 1966; Høedt-Rasmussen, 1967). Measurements of regional cerebral blood flow with the 133Xe clearance method may have serious limitations. The validity of using 133Xe clearance to measure regional renal blood flow using a compartmental analysis (curve-stripping) approach has been challenged (Stein et al., 1973). By implication, compartmental analysis of 133Xe clearance curves to obtain regional cerebral blood flow may not be valid. Also, the assumption that the two exponential decays from 133Xe clearance curves reflect flow from grey and white matter can be challenged on theoretical grounds (Forker and Luxon, 1978). Even when clearance curves are obtained from a relatively homogeneous tissue such as an isolated skeletal muscle, they are frequently bi-exponential.

The present studies were undertaken to reevaluate the 133Xe clearance method of measuring organ blood flow. First we measured blood flow in the isolated gracilis muscle with the 133Xe clearance technique and microspheres to compare the two approaches with a precise method (venous outflow) of measuring organ blood flow. The muscle preparation had a simple blood supply, and could be shielded completely so that the clearance of 133Xe from surrounding tissues would not contaminate the measured clearance curve. In our studies of brain blood flow, the range of flows examined was...
wide, and the stimuli (primarily changes in arterial 
\( \text{PCO}_2 \)) produced stable alterations in cerebral blood 
flow. Dogs and baboons were examined because the 
exclusion of extracerebral contamination is more 
difficult in non-primates. Since the method of ob-
taining and analyzing \( ^{125}\text{Xe} \) clearance curves varies 
(Hoedt-Ramsussen, 1967; Ponte and Purves, 1974; 
Fitch et al., 1975; Bates and Sundt, 1976), we used 
two approaches to obtain the \( ^{133}\text{Xe} \) curves and 
several methods to analyze them.

The studies are unique and extend the work of 
Blomstrand et al. (1978); and Fan et al. (1979) 
because: (1) the range of flows examined was broad, 
and (2) several potentially important variables (or-
gan, species, method of data collection, and method 
of analysis) were examined.

Methods

Animal Preparation

Mongrel dogs \((n = 25)\) of both sexes weighing 15–
25 kg were anesthetized with iv chloralose (50 mg/
kg) and urethane (500 mg/kg), and anti-coagulated 
with heparin (500 U/kg, iv). Ventilation was me-
chanically controlled via an endotracheal tube, and 
the exhaled air was passed through a \( ^{133}\text{Xe} \) trap 
(Nonex; Radiation Medical Products Corp.). A can-
nula was placed in the left atrium via a left thora-
cotomy. Cannulae were placed in both brachial 
arteries and one femoral artery for measurement of 
arterial pressure and withdrawal of two reference 
arterial blood samples. A cannula also was placed 
in a femoral vein for administration of drugs.

Gracilis Muscle Preparation

In the dogs used for the muscle flow studies, the 
gracilis muscle was isolated. The artery, vein, and 
nerve of the muscle were identified and kept intact. 
The muscle was wrapped in Saran to prevent de-
hydration. A cannula (PE60) was placed in a small 
proximal branch of the femoral artery, and the tip 
was positioned about 0.5 cm from the origin of the 
gracilis artery. The gracilis vein was cannulated, 
and the drainage was arranged so that blood could 
be collected for measurement of venous outflow or 
returned to the femoral vein. During each measure-
ment of muscle flow with microspheres and \( ^{133}\text{Xe} \), 
between 5 and 10 measurements of venous outflow 
(30- or 60-second periods) were obtained so that the 
stability of muscle flow could be documented. The 
average variation in venous outflow measurements 
during an intervention was 6.8 \pm 4.9\% (mean \pm sd). 
Thus, during the sequential measurement of blood 
flow with microspheres and \( ^{133}\text{Xe} \) clearance, muscle 
flow was stable.

Cerebral Blood Flow Measurements in Dogs 
and Baboons

The methods of anesthesia, ventilation, and cath-
eter placement were similar to those described 
above. In addition, the dogs were paralyzed with 
decamethonium bromide (0.3 mg/kg, iv).

Eighteen male baboons (weighing 3–9 kg) were 
sewed with phencyclidine (4–5 mg/kg, im) and 
anesthetized with iv chloralose (50 mg/kg) and ure-
thane (500 mg/kg), paralyzed with decamethonium 
bromide (0.3 mg/kg, iv), and anti-coagulated with 
heparin (500 U/kg, iv). Control of ventilation and cather placement were similar to the above.

In dogs and baboons, the right external carotid 
artery was ligated 0.5 cm distal to the carotid bifur-
cation. A cannula for injection of \( ^{133}\text{Xe} \) was placed 
in the right lingual artery and directed toward the 
internal carotid. Soft tissue on the right side of the 
cranium was removed so that the only tissue sepa-
rating the brain and its coverings from the \( ^{133}\text{Xe} \) 
detector was bone.

Measurements of Hemodynamics, Blood 
Gases, EEG, and Oxygen Consumption

Arterial pressure was monitored continuously 
with a Statham P23B transducer leveled at the mid-
chest position. In studies in which seizures were 
induced, the electroencephalogram was monitored 
using bitemporal leads. All signals were recorded on 
an oscillographic recorder.

Blood gases and pH were monitored frequently. 
Hypocapnia was induced by increasing the rate and 
volume of ventilation, and hypercapnia was induced 
by adding 5% \( \text{CO}_2 \) (1–2 liters/min) to the gas 
mixture the animal was breathing.

In some studies \( \text{O}_2 \) consumption of the gracilis 
muscle was determined. The \( \text{O}_2 \) content was calcu-
lated by measuring \( \text{O}_2 \) saturation and hemoglobin 
concentration in blood from an artery and the gra-
cilis vein. Gracilis blood flow was measured by 
timed venous collection. Oxygen consumption of 
the muscle was calculated using the formula: \( \text{O}_2 \) 
consumption = BF \( _g \) \( \times (A\text{O}_2 - V\text{O}_2) \), where BF \( _g \) = 
blood flow to the gracilis muscle, \( A\text{O}_2 \) = arterial \( \text{O}_2 \) 
content, and \( V\text{O}_2 \) = \( \text{O}_2 \) content of the blood from the 
gracilis vein.

In several baboons, cerebral \( \text{O}_2 \) consumption was 
measured using a similar approach. The venous 
sample was obtained from the superior sagittal 
sinus, and blood flow to the left and right cerebrum 
was measured with microspheres.

Measurement of Blood Flow with 
Microspheres

Microspheres 16.2 \pm 2.6 \( \mu \text{m} \) in diameter and 
abeled with \( ^{14}\text{Ce}, ^{85}\text{Sr}, ^{125}\text{I}, ^{46}\text{Sc}, \) and \( ^{99}\text{Nb} \) were 
used. We previously have described our protocol for 
microsphere injection in detail (Marcus et al., 1976).

Briefly, we injected 0.7 to 3 \( \times 10^8 \) spheres of each 
label suspended in 0.2 to 3 ml of 10% dextran into 
the left atrium for each measurement. Prior to 
injection, the vial containing the microspheres and 
one drop of Tween-80 was agitated vigorously for 4 
minutes. Microscopic examination of the spheres
prepared in this manner showed that 98% of the spheres were dispersed. Occasionally small groups of 3-5 spheres were observed. Starting 1 minute before injection and continuing until 3 minutes after injection, blood was withdrawn simultaneously from two reference arteries at a rate of 2.06 ml/min into glass syringes with Harvard pumps. The microspheres were injected slowly over a 30-second period, and the cannula was flushed over a 60-second period with 5 ml of saline. We always obtained two arterial reference samples. The 10 measurements in which the difference in the reference sample activity exceeded 25% were deleted. In the remaining studies, the average difference in the activity of the two reference samples was 4 ± 3% (mean ± sd).

We have shown that significant shunting of 15-μm microspheres does not occur in the brain of either dogs or monkeys under many conditions (Heistad et al., 1976; Marcus et al., 1976). To determine whether microspheres 15 μm in diameter shunt in skeletal muscle, in five experiments the venous outflow from the gracilis muscle was collected over a 4-minute period starting 30 seconds prior to injection of spheres. Since the radioactivity in the samples was at background level, shunting of 15-μm microspheres in muscle is negligible.

Following the study, the animals were killed with an injection of potassium chloride. The skull was opened, and the brain was excised to the level of C6. The right cerebral hemisphere was separated from the brain, and multiple samples of cortical grey and white matter (0.2-0.6 g) were taken. The remainder of the right cerebral hemisphere and the remaining brain then were divided into small (1- to 3-g) segments. In the gracilis muscle studies, the entire gracilis muscle was divided into 2- to 3-g segments and counted.

The cerebral and muscle segments were weighed, placed in glass tubes and counted for 5 minutes in a well-type γ counter. The reference blood samples were divided into aliquots so that their counting geometry was similar to that of the cerebral samples. The energy windows used were 46Sc, 700-1500 keV; 95Nb, 600-700 keV; 85Sr, 400-600 keV; 141Ce, 125-175 keV; and 125I, 20-50 keV. The isotope separation was performed using standard techniques (Rudolph and Heyman, 1967). When 141Ce and 125I were used, the tissues and blood samples were stored for 1-2 weeks until most of the 133Xe activity had decayed. The remaining 125I was excluded by differential spectroscopy.

The blood flow was calculated using the formula, 

\[ BF = C_b \times 100 \times RBF + C_r \times 100 \times \frac{mL}{min} \times g, \] 

where BF = blood flow in mL/min × 100 g, C_b = counts per gram of brain (or muscle), RBF = reference blood flow in mL/min (rate of withdrawal from the reference arteries), and C_r = total counts in the reference blood. The counts in the paired reference blood samples were averaged.

The blood flow, sample weight, and anatomic site for each segment were punched on computer tape. Subsequent analysis was performed with a PDP/11 computer. Regional blood flows reported are the weighted mean flow of all tissue samples from a given anatomic region.

### Measurement of Blood Flow with 133Xe Clearance Method

#### Acquisition of 133Xe Clearance Curves

The 133Xe was dissolved in 0.2-0.8 ml of saline and injected over a 2 to 3-second period into the appropriate artery and then the catheter was flushed with 2-5 ml of saline. The dose of 133Xe was adjusted so that the resulting peak count rates were between 2 and 10 × 10^4 cps. In most studies, less than 1 mCi of 133Xe was used for each injection. The energy window of the 133Xe detector was 72-88 keV, and the counts were integrated using a rate meter with a 1-second time constant. The dead time of the detector system is approximately 2.75 μsec (<3% at 10^4 cps).

The clearance curves usually were collected for 10-15 minutes after each 133Xe injection or until at least 90% of the peak activity had cleared from the tissue. The analog curves were digitized at 3- to 30-second intervals and the results processed with a PDP/11 computer. Computer programs adapted for the PDP/11 computer, but identical to those suggested by Høedt-Rasmussen (1967), were used. The computer results also were plotted and inspected to verify the accuracy of the curve-fitting procedure.

#### 133Xe Curves from the Gracilis Muscle

The gracilis muscle was surrounded completely with lead shielding with the exception of small openings to admit the gracilis artery, vein, and nerve. In addition, a 5-cm aperture in the lead shielding was placed over the center of the muscle, and the 133Xe detector was positioned 3-5 mm above the opening. The 133Xe was injected into a catheter directed toward the gracilis artery as described above. The detecting crystal was collimated with an opening 2.5 cm in diameter.

#### 133Xe Curves from the Dog Brain

The arrangement of the head, lead shielding, and the 133Xe detector are shown in Figure 1. The 133Xe was injected into the right lingual artery. The detecting crystal was collimated, and the opening was 5 cm in diameter. The detecting crystal was placed over the right cerebral hemisphere and directed so as to minimize extracerebral contamination.

### 133Xe Curves from the Baboon Brain (Group I)

The preparation was identical to that used in the dog except that the opening used to collimate the detecting crystal was 2.5 cm in diameter.

### 133Xe Curves from the Baboon Brain (Group II)

The major difference between the group I and group II baboons relate to the protocol used to acquire the 133Xe clearance curves. The 133Xe used in these studies was more concentrated (5-10 mCi/ml), and therefore the volume to be injected (usu-
ally 0.1–0.2 ml) could be loaded into the catheter in the right lingual artery (volume = 0.9 ml) and then flushed into the internal carotid in less than 1 second with 1 ml of saline. Thus the theoretical requirement of “instantaneous injection” could be met within practical limits. The dose of 133Xe was adjusted to yield peak count rates of $3 \times 10^3$ cps.

The crystal was collimated with an opening 18 mm in diameter.

**Analysis of 133Xe Clearance Curves**

**Stochastic Analysis**

Zierler (1965) has employed stochastic theory to show that mean blood flow in either brain or muscle is given by:

$$f = \lambda \cdot (H/A),$$

where $\lambda$ is the partition coefficient in ml/g, $H$ is the peak count rate, and $A$ is the total area under the clearance curve. Since it is not possible to measure $A$ for an infinite time, two alternative approaches have been employed. The simplest approach is to estimate $f$ from the formula:

$$f = \lambda \cdot \frac{H}{A(10)},$$

where $H(10)$ is the difference between the peak count rate and that at 10 minutes, and $A(10)$ is the area under the curve between time zero and 10 minutes. This approach results in a systematic overestimation of cerebral blood flow (Olesen et al., 1971). Another approach is to assume that the tail of the clearance curve can be represented by a simple exponential and extrapolate the curve to infinity. Both approaches were compared in this study.

The partition coefficients used for grey and white matter were 0.80 and 1.5, respectively; the partition coefficient used for the brain was 1.07 (Veall and Mallett, 1965). The muscle partition coefficient was taken to be 0.70 (Conn, 1961). These partition coefficients were adjusted for hemoglobin concentration and recirculation as recommended by Hoedt-Rasmussen et al. (1966).

**Compartmental Analysis**

A compartmental analysis assumes the clearance curves are the result of diffusion equilibrium of two separate parallel compartments. The curves are therefore a sum of two exponential functions, a fast component and a slow component. The contribution of each component is determined by performing a least squares fit of the sum of two exponential functions to the experimental data points. Each exponential function yields a straight line when plotted vs. time on a semilogarithmic scale. The slope of this line, $K$, yields the flow in this compartment according to the equation (Kety, 1951)

$$f = \lambda \cdot K.$$

The mean flow can then be calculated from the relative weight ($W$) and flow ($f$) in each compartment, or

$$f = W(1) f(1) + W(2) f(2).$$

A more detailed description is provided in the Appendix. Partition coefficients were corrected for hemoglobin concentration and recirculation, and mean flow values were compared with those obtained by stochastic analysis.

**Protocols**

**Gracilis Muscle Studies**

Muscle flows in 15 dogs were obtained under the following conditions: (1) control ($n = 15$); (2) gracilis nerve stimulation [6–8 Hz, 6–8 V, and 8 msec ($n = 8$)]; (3) infusion of adenosine (6 $\mu$M/kg per min) into the gracilis artery ($n = 5$); (4) infusion of papaverine (0.6 mg/min) into the gracilis artery, and gracilis nerve stimulation during hypertension produced by iv infusion of norepinephrine (200 $\mu$g/min) ($n = 6$).

After muscle flows were stable, as assessed by venous outflow, microspheres were injected into the left atrium, and 3 minutes later $^{133}$Xe was injected into gracilis artery. The arterial pressure, blood gases, and hemoglobin concentration were measured with each flow. Flow measurements under different conditions usually were separated by 30 minutes. Venous outflow was measured in all 34 studies; microsphere measurements were obtained in 27, and $^{133}$Xe clearance curves in 28.

**Studies in Dogs**

In 10 dogs, brain blood flow was measured under the following conditions: normocapnia ($n = 10$), hypercapnia ($n = 6$), and hypocapnia ($n = 8$). Each condition was maintained for 10–20 minutes prior to measurement of flow, and the order of interventions was varied. For each determination, microspheres were injected 3 minutes prior to injection of $^{133}$Xe. Cerebral blood flow measurements usually were separated by 30 minutes. Arterial blood gases and pH were measured prior to and following each determination, and hemoglobin was measured after each determination.

**Studies in Baboons (Group I)**

In 11 baboons, brain blood flow was measured under the following conditions: normocapnia ($n = 11$), hypercapnia ($n = 5$), hypocapnia ($n = 4$), and hypertension ($n = 4$) induced with an iv infusion of angiotensin (12.3 $\mu$g/min). The protocol employed was similar to that used to study the dogs. In four
of the baboons, measurements of cerebral blood flow were also obtained 2–5 minutes after induction of seizures with bicuculline (1–6 mg/kg, iv) when blood pressure and the electroencephalographic responses had stabilized. The injection of bicuculline was always the last intervention in an experiment. In two of these baboons, a second seizure was induced with bicuculline (3 mg/kg, iv), and flow measurements were repeated.

Studies in Baboons (Group II)

In seven baboons, brain blood flow was measured under the following conditions: normocapnia (n = 7), hypercapnia (n = 6), and seizures (n = 7). The protocol employed was similar to that described above with two exceptions: (1) cerebral oxygen consumption was measured with each flow measurement, and (2) during the seizures, two batches of differently labeled microspheres were injected—one immediately before 133Xe injection and the other when the 133Xe activity had fallen to 10–25% of the peak count rate. The average of the two microsphere measurements of brain blood flow was compared with the 133Xe estimate of cerebral flow.

Statistical Analysis

Differences between group means were determined with an analysis of variance, and intergroup differences were assessed with Duncan's test (Miller, 1966). Differences between interventions were not tested statistically. Correlation coefficients and regression equations were calculated to define the relationship between the different methods of measuring brain and muscle blood flow. In addition, the 95% confidence limits of the slope and intercept of these equations were calculated so that the slopes and intercepts of the equations could be evaluated statistically.

Results

Studies of Blood Flow in Gracilis Muscle

Values for blood flow measured by timed venous outflow and microspheres were similar (see Fig. 2 and Table 1).

In 25 of 28 133Xe clearance curves, two acceptable exponentials could be calculated (Table 1). In comparing 133Xe clearance estimates of flow with timed venous outflow, the fast component of the clearance curve overestimated mean flow, and the slow component underestimated mean flow (Table 1). In contrast, the stochastic method analysis of 133Xe clearance curves yielded values that were similar to timed venous outflow measurements (Fig. 3). The relatively close agreement between 133Xe and measurements of timed venous outflow was independent of the method used to increase gracilis muscle flow (see Table 1).

Studies of Brain Blood Flow in the Dog

In 22 of 24 133Xe clearance curves, two acceptable exponentials could be calculated. The slow component of the 133Xe clearance curve underestimated white matter flow, and the fast component underestimated grey matter flow determined with microspheres (see Fig. 4, A and B, and Table 2).

When 133Xe clearance curves were analyzed with the stochastic method and compared with microsphere measurements, the 133Xe values underestimated flow compared with microsphere measurements (see Fig. 5 and Table 2). This underestimate was most prominent at high flow rates.

Studies of Brain Blood Flow in the Baboon (Group I)

In 23 of 30 studies, the 133Xe clearance curves could be described by two exponentials (see Fig. 6 and Table 3). The slow and fast components of the 133Xe clearance curves tended to underestimate blood flow to white and grey matter as determined with microspheres during seizures and significantly overestimated microsphere flow during hypocapnia (see Fig. 7, A and B, and Table 3). Consequently, the slopes of the regression lines that describe these relationships were significantly <1.0.

When 133Xe curves were analyzed by the stochastic method, the values obtained for mean cerebral flow underestimated blood flow determined with microspheres, particularly at high flow rates (see Fig. 8 and Table 3). When mean cerebral flow measured with microspheres was less than 120 ml/min per 100 g, the correlation between the two
COMPARISON OF MICROSFERES AND 133Xe/Marcus et al.

### Table 1

Timed Venous Collection, Microsphere and 133Xe Clearance Measurements of Muscle Blood Flow

<table>
<thead>
<tr>
<th>Method</th>
<th>Control</th>
<th>Adenosine</th>
<th>Nerve stimulation</th>
<th>Papaverine, nerve stimulation, norepinephrine</th>
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<tr>
<td>Number</td>
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<td>5</td>
<td>8</td>
<td>6</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<td>Blood flow (ml/min × 100 g)</td>
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<td>Timed venous outflow</td>
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<td>Slow component</td>
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<tr>
<td>O2 consumption (ml/min)</td>
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<tr>
<th>Value</th>
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<th>Adenosine</th>
<th>Nerve stimulation</th>
<th>Papaverine, nerve stimulation, norepinephrine</th>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>103 ± 3</td>
<td>94 ± 6</td>
<td>104 ± 4</td>
<td>202 ± 1</td>
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<td>Blood flow (ml/min × 100 g)</td>
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<td>Timed venous outflow</td>
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<td>Slow component</td>
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<tr>
<td>O2 consumption (ml/min)</td>
<td>0.25 ± 0.1</td>
<td>0.52 ± 0.1</td>
<td>1.93 ± 0.1</td>
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### Notes

- Adenosine = infusion of adenosine (6 μg/kg per min) into the gracilis artery; Nerve stimulation = stimulation of the gracilis nerve at 6-8 Hz, 6-8 V, and 8 msec; Papaverine, nerve stimulation, norepinephrine = infusion of papaverine (0.2 mg/min) into the gracilis artery, stimulation of the gracilis nerve as indicated above, and iv infusion of norepinephrine (200 μg/min); Stochasticio = stochastic analysis of the 133Xe clearance curves truncated at 10 minutes; Stochastic = stochastic analysis of the 133Xe clearance curves extrapolated to infinity; 2-Component mean = mean blood flow calculated from analysis of the fast and slow slopes of the 133Xe clearance curves; O2 consumption = oxygen consumption of the gracilis muscle in ml/min.

- All values are mean ± 1 SE.

- + Significantly different from timed venous outflow (P < 0.05).

- † 133Xe stochasticio flow was significantly greater (P < 0.05) than 133Xe stochastic or 2-component mean flows.

Methods of measurement was γ = 0.69, and the slope of the relationship (0.86) was not significantly different from one (see Fig. 8). At flow rates greater than 120 ml/min × 100 g, the correlation between the two methods of measuring cerebral flow was γ = 0.28 and the slope of the relationship (0.17) was significantly less than one. When the stochastic method was applied to clearance curves that were extrapolated to infinity, instead of 10 minutes, similar results were obtained (see Table 3).

### Studies of Brain Blood Flow in the Baboon (Group II)

Cerebral oxygen consumption was unchanged during hypercapnia and increased by 2-fold during seizures (see Table 4). Measurements of cerebral blood flow with microspheres, before and after the 133Xe clearance curve obtained during seizures, were almost identical. This suggests that cerebral blood flow was stable during the time required to obtain the 133Xe clearance curves during seizures.

In these studies, the 133Xe clearance curves were qualitatively similar to those obtained in the group I baboons. In 15 of 20 studies, the curves could be described by two exponentials. Quantitatively, the data obtained from the analysis of the 133Xe clearance curves in the group II baboons were different from that obtained from the group I baboon studies, even though blood flows measured with microspheres in the two studies were similar (see Tables 3 and 4). The major differences between the results obtained in the group I and group II baboons were:

1. The fast component of the 133Xe clearance curves overestimated flow to grey matter in the group II baboons (slope = 1.8 which is significantly greater than 1) (Fig. 9), whereas, in the group I baboons, the fast component significantly underestimated grey matter flow (slope = 0.6 which is significantly less than 1) (Fig. 7B); (2) in the group I baboons, the slow component of the 133Xe clearance curve significantly underestimated white matter flow as determined with microspheres (slope = 0.53 which is significantly less than 1) (Fig. 7A), but this was...
**TABLE 2** Microsphere and 133Xe Measurements of Cerebral Blood Flow in Dogs

<table>
<thead>
<tr>
<th>Number</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Hypocapnia</th>
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<tbody>
<tr>
<td></td>
<td>Mean cerebral blood flow (ml/min x 100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microspheres</td>
<td>37.3 ± 6.22*</td>
<td>114.3 ± 19.6</td>
<td>18.8 ± 2.05</td>
</tr>
<tr>
<td>133Xe compartmental</td>
<td>13.96 ± 1.54↑</td>
<td>28.7 ± 5.9↑</td>
<td>10.21 ± 1.8↑</td>
</tr>
<tr>
<td>133Xe stochastic10</td>
<td>16.94 ± 1.14↑↑</td>
<td>39.3 ± 6.6↑↑</td>
<td>13.47 ± 1.9↑↑</td>
</tr>
<tr>
<td>133Xe stochastic∞</td>
<td>11.20 ± 1.18↑↑</td>
<td>23.1 ± 4.2↑↑</td>
<td>7.99 ± 1.4↑↑</td>
</tr>
</tbody>
</table>

| Grey matter flow (ml/min x 100 g) | | |
| Microspheres | 44.6 ± 6.11 | 181.4 ± 27.6 | 24.8 ± 2.2 |
| 133Xe fast component | 56.8 ± 6.9 | 115.5 ± 16.3§ | 57.5 ± 10.2‡ |

| White matter flow (ml/min x 100 g) | | |
| Microspheres | 23.96 ± 3.7 | 49.5 ± 2.35 | 13.9 ± 2.7 |
| 133Xe slow component | 7.7 ± 1.01§ | 8.5 ± 1.38 | 5.8 ± 0.94↑ |

| Mean arterial pressure (mm Hg) | Before | 112 ± 8 | 107 ± 8 | 95 ± 5 |

| Blood gases and pH | | | |
| PO2 Before | 118 ± 10 | 136 ± 10 | 139 ± 9 |
| PCO2 Before | 37 ± 0.7 | 64 ± 2.4 | 19.2 ± 0.8 |
| pH Before | 7.4 ± 0.01 | 7.22 ± 0.01 | 7.6 ± 0.03 |
| 133Xe compartmental = compartmental analysis of mean cerebral flow using the fast and slow components of the 133Xe clearance curves; 133Xe stochastic10 = stochastic analysis of the 133Xe clearance curves truncated at 10 minutes; 133Xe stochastic∞ = stochastic analysis of the 133Xe clearance curves extrapolated to infinity; 133Xe fast component = fast component of the 133Xe clearance curves; 133Xe slow component = slow component of the 133Xe clearance curves.

* All values are mean ± 1 SE.
† Significantly different from microspheres at 0.05 level of confidence.
‡ Significantly different from microspheres at 0.02 level of confidence.
§ Significantly different from microspheres at 0.01 level of confidence.

**TABLE 3** Microspheres and 133Xe Measurements of Cerebral Blood Flow in Baboons (Group I)

<table>
<thead>
<tr>
<th>Number</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Hypocapnia</th>
<th>Hypertension</th>
<th>Seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean cerebral blood flow (ml/min x 100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microspheres</td>
<td>49.1 ± 7.8</td>
<td>135.7 ± 12</td>
<td>23.3 ± 8.8</td>
<td>47.5 ± 8.7</td>
<td>195.8 ± 16.2</td>
</tr>
<tr>
<td>133Xe compartmental</td>
<td>39.3 ± 2.2*</td>
<td>117.4 ± 11.2</td>
<td>22.5 ± 2.8</td>
<td>54.1 ± 11.9</td>
<td>91.7 ± 19.0*</td>
</tr>
<tr>
<td>133Xe stochastic10</td>
<td>40.6 ± 3.3↑</td>
<td>126.4 ± 5.8↑</td>
<td>22.5 ± 2.8↑</td>
<td>56.6 ± 16.9↑</td>
<td>113.3 ± 11.1↑</td>
</tr>
<tr>
<td>133Xe stochastic∞</td>
<td>36.5 ± 2.8*</td>
<td>125.1 ± 7.4</td>
<td>18.8 ± 1.3</td>
<td>54.9 ± 17.7</td>
<td>105.8 ± 14.2*</td>
</tr>
</tbody>
</table>

| Grey matter flow (ml/min x 100 g) | | | | |
| Microspheres | 55.2 ± 7.7 | 207.3 ± 19.5 | 30.63 ± 14.6 | 68.3 ± 15.2 | 187.49 ± 24.19 |
| 133Xe fast component | 59.4 ± 3.9 | 185.9 ± 17.9 | 58.7 ± 11.7* | 78.4 ± 16.3 | 157.0 ± 22.5 |

| White matter flow (ml/min x 100 g) | | | |
| Microspheres | 16.4 ± 2.2 | 35.45 ± 2.8 | 8.4 ± 1.7 | 18.11 ± 2.6 | 36.2 ± 4.4 |
| 133Xe slow component | 23.9 ± 2.3 | 36.2 ± 6.3 | 17.0 ± 2.2* | 30.0 ± 7.5 | 26.0 ± 6.32 |

| Blood gases and pH | | | |
| PO2 | 108.7 ± 6.7 | 122.6 ± 17 | 107.8 ± 10.9 | 120.2 ± 4.4 | 93 ± 3.3 |
| PCO2 | 38.4 ± 0.6 | 83.0 ± 1.8 | 17.0 ± 1.2 | 40 ± 1.3 | 36.7 ± 0.6 |
| pH | 7.35 ± 0.01 | 7.18 ± 0.03 | 7.56 ± 0.03 | 7.35 ± 0.01 | 7.33 ± 0.01 |
| 133Xe stochastic10 = stochastic analysis of 133Xe data truncated at 10 minutes; 133Xe stochastic∞ = stochastic analysis of 133Xe data extrapolated to infinity; 133Xe compartmental = mean flow calculated from weighted flows of grey and white matter.
* Significantly different from microspheres at the 0.05 level of confidence.
† 133Xe stochastic10 flow was significantly greater (P < 0.05) than 133Xe stochastic∞ or 2-component mean flows.
‡ 133Xe stochastic∞ flow was significantly greater than 133Xe stochastic10.
COMPARISON OF MICROSPHERES AND $^{133}$Xe/Marcus et al.

FIGURE 4 A: Relationship between $^{133}$Xe clearance (slow slope) and measurements of blood flow to cerebral white matter in the dog. The correlation between the blood flow estimates by the two techniques is poor. The slope of the regression line is significantly less than 1, and the intercept is significantly greater than zero. *Significant at 0.05 level of confidence. B: Relationship between $^{133}$Xe clearance (fast slope) and measurements of flow to cortical grey matter in the dog. The correlation between the two methods is high. The slope of the regression line, however, is significantly less than 1, and the intercept is significantly greater than zero. * Significant at 0.05 level of confidence.

not the case in the group II baboon studies (Fig. 9a). In both groups of baboons, at mean cerebral flows measured with microspheres of less than 120 ml/min × 100 g, the correlations between the $^{133}$Xe clearance curves and microsphere estimates of cerebral flow were relatively high, and the slope of the relationship between the two methods did not differ significantly from one. In contrast, with both approaches, when mean cerebral flow was greater than 120 ml/min × 100 g, as measured with microspheres, the two techniques yielded widely differing results and the correlation coefficients were low (see Figs. 8 and 10).

Discussion

The new observations in this study are: (1) in both the baboon and dog brain, there is not a close relationship between microsphere and $^{133}$Xe estimates (compartmental analysis) of blood flow to grey and white matter; (2) in the baboon, modest changes in the application of the $^{133}$Xe method can alter estimated flows to grey and white matter; (3) in the dog, the $^{133}$Xe method (stochastic analysis) underestimates microsphere measurements of cerebral blood flow; (4) in the baboon, mean cerebral flows assessed with microspheres or $^{133}$Xe clearance are similar at flow rates less than 120 ml/min × 100 g; at flows greater than 120 ml/min × 100 g, values obtained with $^{133}$Xe clearance and microspheres vary widely.

The remaining portion of the Discussion section will focus on five areas: (1) the microsphere technique (technical considerations), (2) the $^{133}$Xe clearance technique (technical considerations), (3) difficulties in comparing the cerebral blood flow measurements with microspheres and $^{133}$Xe clearance, (4) validity of the microsphere technique, and (5) validity of the $^{133}$Xe clearance technique.

FIGURE 5 Relationship between mean cerebral flow estimated from $^{133}$Xe clearance curves and microspheres. The $^{133}$Xe values were calculated from stochastic analysis of the clearance curves truncated at 10 minutes. The $^{133}$Xe clearance curves substantially underestimate mean cerebral flow (slope of the regression line is significantly less than 1), but the correlation between the two methods is good. * Significant at 0.05 level of confidence.

FIGURE 6 Original data and a logarithmic transformation of the $^{133}$Xe clearance curve obtained from a baboon during hypercapnia. The curve can be described by two exponentials. It has been suggested that the fast component represents flow to grey matter and the slow component represents flow to white matter in the brain.
Baboons (Group I)

Xenon (slow slope) 30 ml/mm x 100g

Microspheres (cerebral white) ml/min x 100g

40 80 120 160 200 240 280 320

Microspheres (cortical grey) ml/mm x 100g

FIGURE 7
A: Relationship between $^{133}$Xe clearance (slow slope) and microsphere measurements of flow to white matter in the group I baboons. The correlation between these two methods of measuring white matter flow is weak. Furthermore, the slope of the regression line is significantly less than 1, and the intercept is significantly greater than zero. * Significant at 0.05 level of confidence.

B: Relationship between $^{133}$Xe and microsphere estimates of grey matter flow. The correlation between these two estimates of grey matter is high. The slope of the regression line, however, is significantly less than 1, and the intercept is significantly greater than zero. * Significant at 0.05 level of confidence.

Microsphere Technique (Technical Considerations)

We have had extensive experience with the microsphere method (Heistad et al., 1976, 1977; Marcus et al., 1976, 1977). Since shunting of 15-μm spheres in the brain is insignificant, the accuracy of measuring flow with 15-μm spheres depends on the site of injection, the number of spheres administered, and the difference in radioactivity of the reference arterial samples. To ensure adequate mixing, microspheres were injected into the left atrium (Marcus et al., 1976). The number of spheres injected was sufficient to deliver at least 400 spheres to each brain region (Buckberg et al., 1971). In the reported studies, the average difference in radioactivity between the paired reference samples was 4%.

The accuracy of measuring flow to grey and white matter with microspheres depends on the precision with which the samples are removed from the brain. Pure samples of cerebral white are obtained easily. In contrast, if the cortical grey matter is not dissected careful, it can easily be contaminated with a significant amount of white matter. To determine the homogeneity of our grey matter samples, we obtained histological sections (hematoxylin and eosin stain) of random grey matter samples from three baboons. At least 95% of the tissue in each section was grey matter.

Thus, the microsphere technique was applied properly in these studies.

$^{133}$Xe Clearance Technique (Technical Considerations)

We have not used the $^{133}$Xe clearance method previously, and, consequently, particular care was taken in its application. Four major factors that can markedly affect the accuracy of this method are: (1) the site of injection of $^{133}$Xe, (2) the amount of

FIGURE 8
Comparison of $^{133}$Xe clearance and microsphere estimates of cerebral blood flow in group II baboons. The $^{133}$Xe curves were calculated using the stochastic method, and the data were truncated at 10 minutes. At mean cerebral flow rates less than 120 ml/min × 100 g, there was a good correlation between measurements of mean cerebral flow with these two methods ($r = 0.69$). The slope and the intercept of the regression line were not significantly different from one and zero, respectively. At flow rates greater than 120 ml/min × 100 g, the correlation between these two methods of measuring cerebral flow was poor ($r = 0.27$). At these high flow rates, $^{133}$Xe clearance consistently underestimated mean cerebral flow measured with spheres. If all the data points are combined, the correlation coefficient between the two methods is $r = 0.82$. The slope of the regression line (0.82) is significantly less than one, and the intercept (21.7) is significantly greater than zero. * Significant at 0.05 level of confidence.
extracerebral contamination, (3) the characteristics of the $^{133}$Xe detector system, and (4) the method of analyzing the curves. In all studies, the $^{133}$Xe was injected into the internal carotid artery. This eliminates the recirculation that occurs when $^{133}$Xe is administered intravenously or by inhalation (Mallett and Veall, 1965; Austin et al., 1972; Obrist et al., 1975). The methods used to exclude extracerebral contamination vary widely (Heistad and Marcus, 1978). We employed several approaches simultaneously: (1) the external carotid artery was ligated on the side in which the $^{133}$Xe was injected; (2) the soft tissue was removed from that side of the head; (3) the head was shielded with lead; and (4) we used a heavily collimated detector with a ±10% energy window to minimize scattered radiation and the volume of noncerebral tissue in the field. The characteristics of the $^{133}$Xe detector were similar to the specifications used in other laboratories (Høedt-Rasmussen, 1966). The $^{133}$Xe curves usually were collected for 15 minutes and repeated determinations were not made until 85–95% of the $^{133}$Xe activity had been eliminated. The effect of the remaining activity was minimized by subtraction from subsequent curves. The analysis of the curves was based on a computer program recommended by Høedt-Rasmussen et al. (1966). Logarithmic plots of the curves and the exponentials chosen by the computer were printed and reviewed. We used standard values for partition coefficients and correction factors for recirculation and hemoglobin concentration. Furthermore, values obtained with several analytical approaches were compared with the microsphere measurements. As expected, we found that when the $^{133}$Xe curves were truncated at 10 minutes, the calculated flows to the baboon brain were about 10% higher than flows calculated from $^{133}$Xe curves extrapolated to infinity. The mean flow rates obtained with $^{133}$Xe were similar to those reported by laboratories that have used the method extensively (Heistad and Marcus, 1978). With the group II baboons, the collimation of the crystal was increased to minimize further extracerebral contamination. In addition, the $^{133}$Xe was injected in less than 1 second to satisfy the theoretical requirement of an "instantaneous bolus."
In our experiments, the distribution of microspheres probably was complete in less than 30 seconds, whereas the average half-time of our $^{133}$Xe curves was greater than 2 minutes. Many of the $^{133}$Xe measurements included data collected more than 10 minutes after injection. Thus, for the data to be comparable, cerebral blood flow must have been stable during that time period. We usually studied chemical stimuli, since they have been shown to produce stable changes in cerebral blood flow (Purves, 1972). The measurements were not taken until 10–15 minutes after the change in gases was introduced because responses to these stimuli are not maximal during the first few minutes after stimulation (Purves, 1972). In the studies during seizures, we demonstrated with microspheres that cerebral blood flow was stable. Thus, blood flow to the brain was not changing importantly during the time required to complete measurements with both techniques.

Another assumption is that the cone of cerebral tissue in the field of the $^{133}$Xe detector is similar to the tissue used to determine flow by the microsphere method. It is impractical to determine precisely the cone of tissue that contributed to the $^{133}$Xe measured activity. Furthermore, even if anatomic area could be delineated, the contribution of tissue at different distances from the crystal would be difficult to quantify because the tissue attenuates the $\gamma$ emissions. Therefore, we compared

**Difficulties in Comparing Cerebral Blood Flow Measurements with the $^{133}$Xe Clearance Method and Microspheres**

Even if two techniques for measuring cerebral blood flow are applied properly, the results might differ if the methods are not measuring the same parameters. Two potential discrepancies relevant to this study relate to temporal and spatial factors.
the $^{133}$Xe values (stochastic analysis) with mean flows measured in the same cerebral hemisphere with microspheres. Likewise, the fast and slow components of the $^{133}$Xe curves were compared with mixed cortical grey matter and cerebral white matter from the same cerebral hemisphere. Several points suggest that these comparisons are valid: (1) neither the detector nor the animal's head was moved during the experiment; (2) the variability of flow to small segments of cortical grey and cerebral white matter is minimized during anesthesia (Landauf et al., 1955); (3) in several experiments, we compared microsphere flows obtained from cortical grey samples in the area of the cerebrum below the detector with $^{125}$Xe flow estimates, and the results were similar to those obtained from mixed samples of grey matter; (4) in theory, with the compartmental analysis of $^{133}$Xe curves, the percent of white and grey matter contributing to the curve can be calculated and weighted appropriately in the mean flow; this calculation was performed and the values obtained are similar to those from the stochastic analysis; and (5) the major difference that we observed between the techniques could not be explained by a "constant error" resulting from fixed differences in geometry.

Thus, it is likely that both techniques were measuring similar responses, and spatial differences could not account for our findings. Consequently, the differences that we found can best be explained by inherent limitations in one or both of these techniques.

Validity of the Microsphere Technique

In the heart (Utley et al., 1974), kidney (Stein et al., 1973), and skeletal muscle, there is a close relationship between measurements of blood flow with timed venous collection and microsphere distribution over a wide range of flows. Similar comparisons have not been made in brain because of the complexity of the venous bed.

The accuracy of values obtained with microspheres for flow to small regions of the brain, such as cortical white and cortical grey matter, is less certain. However, several lines of evidence suggest that microsphere measurements of flow to small regions within an organ can be accomplished with reasonable accuracy. Tripp et al. (1977) have shown that, in the heart, the transmural distribution of $^3$H$_2$O and spheres is similar under many conditions. In a preliminary report, Meyer et al. (1975) have shown that $^3$H$_2$O and spheres distribute in a similar fashion in small regions of the brain. Also, rheological characteristics of 15 $\mu$m microspheres do not markedly distort the distribution of flow within small regions of the brain (Marcus et al., 1976).

Validity of the $^{133}$Xe Clearance Technique

In a simple vascular bed, such as muscle, we and others have shown that the $^{133}$Xe clearance method measures mean blood flow accurately (Kjellmer et al., 1967; Tönnesen and Sejrsen, 1970). These data support the theoretical basis of the $^{133}$Xe method put forth by Zierler (1965). In a complex vascular bed such as brain, $^{133}$Xe clearance will estimate mean flow accurately with certain limitations: in the dog and possibly other non-primates, the position of the brain increases the magnitude of extracerebral contamination and cerebral blood flow is underestimated; at high flow rates (>120 ml/min x 100 g) changes in cerebral blood flow measured with microspheres and $^{133}$Xe clearance correlate poorly; and in the baboons, microspheres and $^{133}$Xe measurements of cerebral flow yield similar results at flow rates less than 120 ml/min x 100 g.

We found that blood flow obtained with $^{133}$Xe was higher when calculated with stochastic$_{	ext{io}}$ analysis than calculated with stochastic$_{	ext{a}}$ analysis (Tables 2-4). It is possible that this discrepancy is related to redistribution of $^{133}$Xe within the brain. During the washout period, the $^{133}$Xe in the brain could redistribute from areas of high concentration (grey matter) to areas of low concentration (white matter). If this occurred, white matter would be reflected disproportionately in the latter phase of the $^{133}$Xe clearance curve. If redistribution of $^{133}$Xe within the brain actually occurs during the washout period, this violates one of the basic assumptions of the $^{133}$Xe method. Furthermore, if the $^{133}$Xe partition coefficients are very dissimilar in different tissue compartments (grey and white matter), it would be impossible to compensate quantitatively for redistribution with calculations based on externally recorded washout curves. At present, these inferences have not been tested directly.

The theoretical validity of the compartmental analysis of $^{133}$Xe clearance curves is less secure. Major problems with this analysis are: (1) the slow components do not correlate closely with white matter flows measured with microspheres and the relationship between the fast components and grey matter flows measured with microspheres is markedly altered by most changes in the protocol used to collect the xenon data; (2) a third component in the xenon clearance curve, which has a slow clearance and probably represents washout from muscle, may be an important confounding variable (Fan et al., 1979); (3) clearance curves obtained from relatively homogeneous tissues such as skeletal muscle are often biexponential; and (4) clearance curves are complex (Glass and DeGarreta, 1971), and compartmental analysis can be influenced by subjective judgments unless stringent measures are taken to avoid this source of error. We suggest that the compartmental analysis of the $^{133}$Xe clearance curves be abandoned. Furthermore, recent modifications of the $^{133}$Xe clearance method (inhalation and iv infusion of $^{133}$Xe) should be viewed critically since they are dependent upon compartmental analysis (Mallett and Veall, 1965; Austin et al., 1972; Obrist et al., 1975).

Studies related to neural control of cerebral ves-
sels which measured blood flow with $^{133}$Xe clearance frequently obtained results that were very different from those with microspheres (Heistad and Marcus, 1978). The differences between these two techniques observed in this study are not sufficient to explain this controversy. However, one additional point deserves emphasis. The time required to measure cerebral flow with the $^{133}$Xe method is usually 4-10 minutes. In contrast, with the microsphere method, the measurement represents integrated blood flow over a 10- to 30-second interval (Bache and Lambert, 1977). Because cerebral vessels rapidly escape from the constrictor effects of peripheral sympathetic stimulation (Lacombe et al., 1977; Marcus et al., 1978), it is not surprising that, in this area of research, the $^{133}$Xe and microsphere methods yield very different results. The divergent results obtained when $^{133}$Xe clearance and microspheres are used to measure cerebral blood flow in neural control studies probably are related to the temporal characteristics of the methods and to species differences (Heistad et al., 1978). When transient changes in cerebral blood flow are likely to occur (such as with sympathetic nerve stimulation), the $^{133}$Xe clearance method should not be used.

Appendix

For the compartmental analysis, a least squares fit to the sum of two exponential functions is performed with an algorithm similar to that reported by Høedt-Rasmussen et al. (1966). The curve is assumed to have the functional form: $y(t) = a_3 \exp(-a_1 t) + a_4 \exp(-a_2 t)$, where $y(t) =$ count rate at any time $t$; $-a_1 =$ slope of the fast component on a semi-log plot; $-a_2 =$ slope of the slow component on a semi-log plot; $a_3 =$ initial count rate due to the fast component; and $a_4 =$ initial count rate due to the slow component.

Initial estimates for the slopes and intercepts are calculated by means of a curve-peeling technique. These estimates are incremented by the small quantities $a_i = 1,4$ and the function is expanded in a Taylor series keeping only lowest order terms. Thus

$$y(t) = y_0(t) + \sum_{i=1}^{4} \frac{\partial y_0(t)}{\partial a_i} \delta a_i$$

where the zero subscript indicates evaluation of the function and its partial derivatives, using the initial estimates for the parameters $a_i$. The least squares approach minimizes the sum

$$S = \sum_{j=1}^{n} \left[ y_j - y_0(t_j) - \sum_{i=1}^{4} \frac{y_0(t_j)}{\partial a_i} \delta a_i \right]^2$$

where the quantity in square brackets is the difference between the $n$ data points $y_j$ and the value of the function $y(t_j)$. We minimize $S$ with respect to the parameter increments by requiring that the partial derivatives satisfy $\delta S/\delta a_i = 0$ for $k = 1,4$.

Calculating the derivatives and writing in matrix form, we find $\beta = \alpha \cdot \delta a$, where

$$k = \sum_{i=1}^{n} \left[ y_j - y_0(t_j) \right] \frac{\partial y_0(t_j)}{\partial a_i}$$

$$\delta a_i = \sum_{i=1}^{n} \frac{\partial y_0(t_j)}{\partial a_i} \cdot \frac{\partial y_0(t_j)}{\partial a_i}.$$ 

Inverting the matrix, $a$, we can find the incremental values from the matrix equation, $\delta a = \alpha \cdot \beta$. In our program, this process is repeated in iterative fashion until

$$\left| \delta a_1 + \delta a_2 + \frac{\delta a_3}{a_3} + \frac{\delta a_4}{a_4} \right| < 10^{-4}.$$ 

The program also terminates the iterative process if the matrix $\alpha$ is singular. This least squares approach converges rapidly if the initial estimates are fairly close to a minimum point but can diverge if this is not the case. To prevent uncontrolled divergence, the program contains upper and lower limits for the slopes and intercepts which, when exceeded, result in termination of the iterative process.

After the final values for $a_i, i = 1,4$ are determined, the flow ($f$) in each component is calculated using the equation, $f_i = a_i \cdot a_i i = 1,2$ where $a_i$ is the partition coefficient for that component. The relative weight ($W$) of the fast component is calculated using the equation,

$$W_1 = \frac{a_1/\beta}{a_2/\beta + a_1/\beta}$$

while the relative weight of the slow component is given by $W_2 = 1 - W_1$. The mean flow is then calculated, using $\bar{f} = W_1 \bar{f}_1 + W_2 \bar{f}_2$.

There are a few $^{133}$Xe curves in each of the studies mentioned in this report for which the computer program was unable to calculate a least squares fit for a clearance curve. In all cases, this is due to the singularity of the matrix $\alpha$ resulting from the inability of the program to distinguish between two separate components.

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