Stochastic Analysis of $^{133}$Xe Clearance for Determining Regional Cerebral Blood Flow in a Primate Model

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SUMMARY A technique for stochastic analysis of $^{133}$Xe washout using a computer-assisted Anger camera is described. The technique produces functional maps of both regional cerebral blood flow (rCBF) and error in rCBF. The calculational limitations of the technique are discussed and an experimental estimate made of the physical limitations associated with the low energy photon emitted by $^{133}$Xe. The method is demonstrated in a rhesus monkey model before and after surgical occlusion of the middle cerebral artery.

ANALYSIS OF $^{133}$Xe washout is currently an important technique for determining cerebral blood flow. Recent improvements in the collection and analysis of these data have been reported by Verbist et al. We have independently developed a similar method for application in a primate model of experimentally induced anemic infarction.

This paper describes the method by which a functional map of regional cerebral blood flow (rCBF) is constructed by stochastic analysis of $^{133}$Xe washout from the brain. The calculational limitations of the technique are discussed, and an experimental estimate is made of the physical limitations associated with the low energy photon emitted by $^{133}$Xe.

Methods

All data are collected on an Anger camera equipped with a low energy converging collimator (Searle Div-Con) and interfaced to a minicomputer. A 20% window was placed around the 81 KeV photopeak of $^{133}$Xe. The method assumes a bolus injection of $^{133}$Xe into the internal carotid artery. Immediately prior to bolus injection, data collection is begun in histogram mode on the minicomputer system. The histogram intervals are preset at one second for the first 90 seconds and at 10 seconds for an additional 10 minutes. This high initial framing rate is necessary to determine accurately the time of occurrence of the maximum count rate and to allow evaluation of the bolus nature of the injection.

Immediately after each study, the first 30 images, each of 1-second duration, are sequentially displayed by the minicomputer system. Maximum count rates between 13,000 and 20,000 count/s are considered reliable for rCBF calculations. Count rates which exceed 20,000 count/s are considered too high to be reliably corrected for dead time, while rates less than 13,000 count/s are felt to be too low to allow statistically reliable regional flow calculations. If maximum count rates are outside of this optimum range, the study is repeated as soon as the $^{133}$Xe is cleared from the cerebral tissue.

Regional cerebral blood flow is calculated on a $32 \times 32$ matrix basis using an approximation of the stochastic method described by Zierler as shown in figure 1. The maximum height of the curve, $H_{\text{max}}$, is determined at each matrix element by selecting the maximum count rate from one of the initial 90 one-second images. $H_{\text{m}}$ represents the count rate at the corresponding point 10 minutes after the time of occurrence of $H_{\text{max}}$. $A_{\mu}$ is the integral at the same point of counts from $H_{\text{max}}$ to $H_{\text{m}}$. $\lambda$ is the coefficient of partition of xenon between brain and blood tissue. It is assumed that there is no regional variation in $\lambda$. A single value of $\lambda$, averaged over gray and white matter, is used for the entire brain. This value is corrected for variations in hematocrit during the course of the study.

A functional map of the whole brain is created by computer analysis of approximately 200 regional counts vs. time curves over the region of the brain. Electronic amplification of the gamma camera analog signals and use of the converging collimator result in a pixel density of approximately 20 pixels/cm². Each of the 200 or more curves is derived from four of these pixels. After each curve is corrected for dead time, the regional flow value is calculated and placed in the image element from which the curve is derived. In this manner approximately 200 values of flow per unit volume are calculated and placed in their appropriate image elements, thus creating a functional map which is either displayed as a shades-of-gray image or printed as numerical values. In addition to the functional flow map, an "error" map is also generated. In the error image, focal values represent one standard deviation in the calculated value of flow per unit volume, based solely on the errors in $H_{\text{max}}$, $H_{\text{t}}$, and $A_{\mu}$ due to counting statistics at each point. In both the flow and the error maps, the intensity of an image element is directly proportional to the value of the flow per unit volume or to the error (due to counting statistics) in the flow per unit volume, respectively (i.e., the brighter a point on either map, the greater the numerical value of flow per unit volume or $\sigma$ at that particular point). These errors are felt to be the primary source of random error at each point. There are other very significant sources of error in the values of flow per unit volume — systematic errors, whose origins are the subject of the discussion below.

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The technique employed here for estimating the residual area contains regions of both low and normal flow. This underestimate A in the case in which the volume element employed to estimate this residual area. For example, the area under the washout curve, A, is then underestimated. Techniques can be accomplished by a technician. The flow and error maps from the image data are obtained within five minutes by the minicomputer.

Discussion and Results

Despite the widespread use of the $^{133}$Xe washout technique for determination of rCBF, the method has several significant limitations. Interpretation of the flow values depends on the adequacy of the calculational model, assumptions about the behavior of xenon in tissue, and on the ability of the scintillation camera to resolve regions of low count rate in a high activity absorbing and scattering medium. These limitations set an upper limit to the sensitivity and accuracy of the procedure.

Since the values of flow per unit volume are obtained from two-dimensional images of three-dimensional objects, three factors must be considered. The H/A calculation produces a value of flow per unit volume at each point in the image. Since each image point is a cylindrical volume element in the brain, this value of flow per unit volume is a weighted average over the depth of this cylinder. Such averaging will partially mask the effects of focal areas of low flow which might occur within the cylinder. Secondly, if the flow to a volume element within the cylinder falls to zero, its volume may no longer contribute to the volume of distribution of the isotope. In this case F/V would remain constant, the decrease in V compensating for the decrease in flow to the cylindrical volume element. Thirdly, the experiment must be terminated after a finite time. The area under the washout curve, A, is then underestimated. Techniques can be employed to estimate this residual area. For example, the last portion of the data could be fit to a mono-exponential and extrapolated accordingly. Such techniques, however, tend to underestimate A in the case in which the volume element contains regions of both low and normal flow. This underestimate in A causes an overestimate in the value of F/V.

The technique employed here for estimating the residual area (subtracting $H_{th}$ from $H_{max}$ as in fig. 1) is subject to this error and is strictly valid only for the case of a mono-exponential washout curve.

The second limitation of the $^{133}$Xe washout method is due to the assumption that the partition coefficient is the same for both normal and infarcted brain tissue. Apparent changes in flow per unit volume after infarction may be due in part to changes in the partition coefficient.

Finally, because of photon attenuation, scatter, and the finite spatial resolution of the collimator-detector system, small regions of low activity embedded in a high activity background will be undetected. The effect of attenuation of photons by the brain tissue tends to produce a flow map which preferentially samples surface tissue. The problem is less significant in studying the brains of monkeys because of the smaller brain mass than it would be for the brains of humans. The effect of scatter, on the other hand, is to reduce the spatial resolution of the flow map, possibly merging normal and infarcted regions. The net effect of scatter or attenuation is to place a lower limit on the size of the infarcted region which can be discerned.

The limitations discussed above all serve to introduce uncertainties in the calculated values of F/V. In addition, these limitations can cause a reduction in the sensitivity of the technique. Incorrect estimation of washout area, photon scatter and attenuation, and changes in the volume of perfused tissue—all of these factors may tend to make it difficult to distinguish regions containing ischemic tissue from normal regions. One of these limitations, that imposed by the scattering and attenuation of the low energy photon emitted by $^{133}$Xe, is studied further in this paper. These scattering, averaging, and attenuation effects serve to make two laterally adjacent regions—one possessing normal flow, the other containing a focal region of reduced flow—have nearly the same calculated flow per unit volume. In order for these two regions to be perceived as different, counting statistical uncertainties in the flow values must be smaller than such differences. The counting statistical uncertainties in F/V are primarily due to those in $H_{max}$. To quantitate this effect, a phantom study was performed and the following assumptions made: First, it was assumed that all the errors in F/V associated with counting statistics, that due to $H_{max}$ was the largest. Thus, the influence of counting statistics on $H_{max}$ alone was investigated. This is reasonable because although the error maps include errors (from counting statistics) from $H_{max}$, $A_{10}$, and $H_{10}$, uncertainties in $H_{max}$ account for most of the counting statistical uncertainties in F/V. The second assumption used to interpret the phantom study was that the area under the washout curve was invariant—that is, that the perfused volume was not a function of flow. This is probably true except for regions of truly zero flow. In this latter case no change in the calculated value of F/V would occur (although $H_{max}$ would drop). Under these assumptions the following phantom study was undertaken in order to quantitate the effect that uncertainties in $H_{max}$ would have on the ability of the method to detect small volumes of ischemic tissue.

Water-filled plastic spheres of various sizes were placed in a water medium in which $^{133}$Xe was dissolved. The water medium was 28 cm by 16.5 cm in area parallel to the face of a 140 keV converging collimator and 7.6 cm in depth measured perpendicular to the collimator face (inset, fig. 2). The “cold” spheres were placed at various depths in the water, including one depth at which the spheres were tangential to the surface closest to the collimator (referred to as a “surface infarction”) and another depth at which the spheres were tangential to the surface farthest from the collimator.
Farction (Zero Flow) at Two Levels of Statistical Uncertainty

**Table 1**

Furthermore, if the ischemic region does not have can be detected, as influenced only by counting statistical different levels of statistical uncertainty in $H_{\text{mal}}$, a standard brain of either monkey or man, it allows an estimate to be made as to the magnitude of the effects of scattering and attenuation. Figure 2 demonstrates how the image contrast (defined as the difference between maximum and minimum count rate divided by the maximum count rate) of the surface and deep infarctions varies with the diameter of the spherical infarction. The data of figure 2, along with similar data at other depths, were used to estimate the minimum size region of zero flow which the $^{133}$Xe flow measurement could detect. In these estimates $H_{\text{max}}$ was the only factor considered to vary with flow. The area under the washout curve (and hence the perfused volume) was taken as constant. Table 1 summarizes the results of this study for regions of zero flow at the surface, at a depth of 3.2 cm (approximately the thickness of a monkey hemisphere), and at a depth of 7.6 cm (approximately the thickness of a human cerebral hemisphere). Table 1 lists minimum detectable sizes for two different levels of statistical uncertainty in $H_{\text{max}}$, a standard deviation in $H_{\text{max}}$ of 5% or 10%. Table 1 shows that even if the perfused volume (washout area) and $\lambda$ can be determined without error, a 5% or 10% standard deviation in $H_{\text{max}}$ (due to poor counting statistics or any other reason) will result in an inability to discern focal regions of near zero flow if the dimensions of such regions are smaller than those shown in table 1. Furthermore, if the ischemic region does not have zero flow but possesses instead a finite but reduced flow, a significantly larger volume would be necessary before such a region would be discernible as being different from surrounding tissue. Table 1, then, lists minimum sizes of infarcted tissue which can be detected, as influenced only by counting statistical errors in $H_{\text{max}}$. Although such errors in $H_{\text{max}}$ are felt to be the most significant source of random error in the calculated value of $F/V$, other errors (both random and systematic) will cause further difficulty in detecting ischemic regions.

As noted above, these calculations assume that the perfused volume remains constant regardless of changes in flow. This assumption is true as long as some small number of xenon atoms flow into the volume in question. Thus, the washout area to a volume element should remain constant as the flow to that volume element approaches zero. However, it may be quite difficult to measure the washout area accurately for small values of flow because of the slow clearance rate. If, on the other hand, the flow to one small volume element of a larger volume were truly zero and no xenon distributed itself in that small volume element (either by diffusion or bulk flow), that small volume element would no longer contribute to the average flow of the larger volume. The contribution to $H_{\text{max}}$ and to the washout area from the smaller volume would be zero, and the calculated flow per unit volume to the larger volume would be as though the smaller volume had been excised. It should be noted that an "$H_{\text{max}}$ map" would detect such a zero flow region, even though the $F/V$ map would not.

Because of the large dose and small size of the brain, a 5% standard deviation in $H_{\text{max}}$ (table 1) corresponds to the uncertainty in $H_{\text{max}}$ found at each point in the monkey studies described below. From table 1 a perfused region of nearly zero flow which is 1 cm in diameter could probably be detected, providing the washout area was accurately determined. If the volume element had reduced flow rather than nearly zero flow, the minimum detectable size would be correspondingly larger. The effects of scattering from the skull are not included in this estimate. The estimates in table 1 are the minimum diameters of infarcted but perfused regions which could be detected as being different from the surrounding normal tissue. It should be emphasized that the ability to detect the presence of such a region does not imply the ability to determine accurately the flow to that region because of all the limitations mentioned previously. In fact, it is probable that quantitating flow to the ischemic region is impossible. The best one can do is to determine an average flow per unit perfused volume to each cylinder seen by the gamma camera, with the understanding that the average is significantly affected by attenuation and scattering.

**Application to Primates**

The techniques described above were initially developed to allow serial daily rCBF measurements to be made before and after surgical occlusion of the MCA in rhesus monkeys. Occlusion of the MCA was carried out by previously described methods. To illustrate the results obtainable with the method, the results of two typical studies are presented, one made prior to surgical occlusion, one after.

Microsurgical techniques were employed to insert a permanent indwelling catheter (polyethylene PE-10, i.d. = 0.028 cm) into the right internal carotid artery of the rhesus monkey. This technique has been previously described in detail by Hammock et al. After the catheter was firmly secured in place, the monkey was placed in a specially constructed holder designed to restrain head and body motion. The monkey and holder were placed directly against the con-

<table>
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<tr>
<th>Diameter of Minimum Detectable Spherical Infarction (Zero Flow) at Two Levels of Statistical Uncertainty in $H_{\text{max}}$</th>
<th>SD in $H_{\text{max}}$</th>
<th>At surface</th>
<th>At 3.2 cm depth</th>
<th>At 7.6 cm depth</th>
</tr>
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<tbody>
<tr>
<td>5%</td>
<td>0.8 cm</td>
<td>1.1 cm</td>
<td>1.9 cm</td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td>1.3 cm</td>
<td>1.9 cm</td>
<td>3.2 cm</td>
<td></td>
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vergeng collimator (Searle 140KeV Div-Con) of a computer-assisted Anger system in the right lateral position (fig. 3). The exact location of the holder was marked on the collimator surface, such that the monkey could be easily repositioned for subsequent studies. Light anesthesia was provided with intraperitoneal pentobarbital (10 to 15 mg/kg). During periods of study, the animal moved its extremities spontaneously and maintained normal respiration.

The $^{133}Xe$, dissolved in saline, was then injected as a bolus into the internal carotid artery catheter. The injection time was typically three to four seconds. In six animals, each studied for up to six weeks after infarction, the dose for the preinfarction studies ranged from 10 to 14 mCi. This large dose was necessary to maintain adequate counting statistics when the brain was divided into 200 or more regions of interest. On the day after infarction, the dose frequently had to be increased by 20 to 30% in order to provide adequate regional counting statistics. This requirement for an increased dose following MCA occlusion was felt to be a consequence of early edema and/or ischemia. On subsequent days the dose required for adequate counting statistics again approached the preinfarction dose. Because of this day-to-day variation in the required dose, it was often necessary to repeat a given study. Therefore, the initial postinjection data were analyzed immediately after each study to determine whether adequate counting statistics had been obtained.

In order to prevent exhaled $^{133}Xe$ from appearing in the field of view of the camera and to prevent room contamination, a plastic bag was placed over the monkey's head prior to each study. Room air was allowed to enter the loose-fitting bag freely. The air in the bag was continually removed by a vacuum exhaust tube positioned near and below the nose and mouth.

To correlate anatomy from one study to the next, a system of $^{60}Co$ point source and line source markers was used. The xenon images themselves were not useful for anatomical correlation because they changed in size and shape as the distribution of cerebral blood flow changed. Prior to a xenon flow study, $^{60}Co$ point source markers were placed on the nasion, inion, bregma, and external auditory meatus adjacent to the camera surface. In addition, a thin, $^{60}Co$-filled, polyethylene tube was placed along the entire sagittal suture and midsagittal line of the occipit. The data from these markers were recorded and stored by the computer for later anatomical correlation with the xenon flow studies. Marker data were collected both before and after each study to determine if any significant head movement had occurred during the procedure.

Figure 4 shows a typical flow map obtained from a normal monkey (4A) and the corresponding error map (4B). Both images have been masked so that data outside the brain (as determined by the $^{60}Co$ markers) are set to zero intensity. The flow image is relatively homogeneous with slightly greater flow values in the periphery. In the corresponding error map the distribution of standard deviations of flow are homogeneous and low (usually 5% or 10%), except at the superior aspect of the image. The increased error in the superior aspect of the image is a result of the low count rates due to the small depth of brain tissue along that region. Within this error the apparent increase in flow superiorly could be attributed to the sagittal sinus.

Figure 4C shows a typical postinfarction flow map and its associated error map (4D) obtained 24 hours after surgical clipping of the right MCA. A large area of decreased flow is readily identified on the postinfarction image in the distribution territory of the MCA. In addition, there is increased perfusion anterior, posterior, and, to a lesser extent, superior to this territory relative to the preinfarction image.

**Conclusion**

A method has been described which allows rapid determination of rCBF at a large number of points over the cerebral hemisphere. Occlusion of the middle cerebral artery produces regions of infarction larger than the experimentally measured resolution limits of the technique. Quantitative interpretation of regional flow values is complicated by the several experimental and calculational problems discussed above.

In the serial studies for which the method was developed, however, the changes in flow with time are of primary interest, not the absolute flow. Under these circumstances the method appears capable of assessing the temporal evolution of rCBF following cerebral infarction in the primate.
Local Mechanism of CO₂ Action on Cat Pial Arterioles

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SUMMARY The effects of local hypercapnic acidosis or local hypocapnic alkalosis on pial arterioles were studied in anesthetized cats equipped with a cranial window for the direct observation of the pial microcirculation of the parietal cortex. Changes in Pco₂ and pH of the extracellular fluid were induced by perfusing the space under the cranial window with artificial cerebrospinal fluid equilibrated with different concentrations of CO₂ while Paco₂ was maintained constant. Hypercapnic acidosis dilated and hypocapnic alkalosis constricted pial arterioles markedly. The results indicate that a basis exists for considering CO₂ as a mediator for local regulation of brain blood flow. The vasodilation associated with arterial hypercapnia was abolished by a reduction in CSF PCO₂ equal in magnitude to the rise in arterial blood PCO₂, suggesting that the action of CO₂ is entirely local.

Methods

Twenty-four cats were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The animals were paralyzed with intravenous decamethonium (1 mg/kg) and ventilated with a positive pressure respirator connected to a tracheostomy tube. Expired CO₂ concentration was monitored continuously with a CO₂ analyzer. End-expiratory PCO₂ was maintained constant throughout the experiment, except when gases containing CO₂ were breathed. Arterial blood gas tensions and pH were determined periodically by Radiometer electrodes. The PCO₂, PO₂, and pH of artificial cerebrospinal fluid (CSF) used to perfuse the space under the cranial window were similarly determined. Arterial blood pressure was monitored continuously with a Statham strain-gauge connected to a catheter placed into the aorta via the femoral artery. The brain vessels were visualized through a cranial window which was located on the vertex of the skull to show arterioles in the parietal cortex. The cranial window technique has been described in detail previously. During the experiment the outlet of the cranial window was connected to plastic tubing whose end was set at a predetermined level to give a constant intracranial pressure of 5 mm Hg. The other two openings of the window were used as inlet and outlet for perfusing the space under the window with artificial CSF. The composition of the fluid is identical to that of CSF of the cat and its composition has been given previously. The application of this fluid, when equilibrated with 6.5% CO₂, 6% O₂, and 87.5%
Stochastic analysis of 133xe clearance for determining regional cerebral blood flow in a primate model.
H Agress, Jr, S L Bacharach, M K Hammock, M V Green, G S Johnston and G Di Chiro

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