Quantification of regional myocardial blood flow in vivo with H$_2^{15}$O*

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ABSTRACT Using H$_2^{15}$O (half-life = 2.1 min) we demonstrated that a modification of the tissue autoradiographic approach permitted quantitation of myocardial blood flow in open-chest dogs by direct assay of myocardial tissue and that noninvasive estimation with positron-emission tomography (PET) delineated relative myocardial blood flow in intact dogs. In open-chest anesthetized dogs, the single-pass extraction fraction of H$_2^{15}$O averaged 96 ± 5% at flows of 80 to 100 ml/100 g/min. This high extraction fraction did not differ significantly over the range of 12 to 238 ml/100 g/min. Myocardial blood flow calculated after a 60 sec intravenous infusion of H$_2^{15}$O and direct analysis of tissue correlated well with results obtained with microspheres (r = .94, n = 9 dogs). Subsequently the approach was adapted for preliminary use with PET. Estimation of myocardial content of radiolabeled H$_2^{15}$O after intravenous infusion of 20 to 30 mCi of H$_2^{15}$O was corrected for vascular pool radioactivity with the use of tomographic data obtained after administration of C$^{14}$O by inhalation to label red blood cells. Tomograms obtained in vivo in six dogs with either normal or reduced regional blood flow correlated closely with the tomographically detectable distribution of $^{68}$Ga-labeled microspheres (r = .93) and with postmortem microsphere distribution (r = .95). The technique accurately reflects myocardial blood flow. With the use of PET, rapid sequential noninvasive estimation of relative regional myocardial blood flow has been demonstrated that should ultimately permit improved objective assessment of nutritional blood flow in patients in response to medical and surgical interventions designed to augment perfusion.

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NONINVASIVE QUANTIFICATION of regional myocardial blood flow is necessary for the assessment of dynamic and fixed coronary vascular obstruction and for objective assessment of therapy designed to augment nutritional perfusion. The microsphere technique is viewed as the “gold standard” since the extraction of radiolabeled microspheres during their first pass through the coronary circulation is virtually 100%.1-4 Hence, deposition is proportional to flow. However, the need to inject spheres into the systemic arterial or coronary circulation and the risk of occlusion of capillary beds in patients with already compromised flow precludes general use of this method in human subjects.

An alternative approach for measurement of blood flow with diffusible tracers derives from the work of Kety and Schmidt,5-7 who used principles governing the exchange of inert gas between blood and tissue. In applications of this approach in which tissue autoradiography is used, a freely diffusible radiotracer is infused intravenously for a short time, followed by direct assay of the concentration of radiotracer in tissue.8-12 Flow is then calculated based on the arterial and tissue concentration of tracer and knowledge of the tissue/blood partition coefficient ($\lambda$).

We have been interested in methods of assessing myocardial perfusion that do not require the intracoronary injection of tracer.13,14 During the past 5 years, advances in the technology of positron-emission tomography (PET) have permitted adaptation of the autoradiographic technique for use in vivo.15-17 We undertook the present study to develop and evaluate a method for measurement of myocardial blood flow

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with H$_2^{15}$O. $^{15}$O is an attractive tracer for this purpose since its short half-life of 2.1 min permits rapid sequential measurements and since water can be labeled easily with $^{15}$O. Both considerations are critical in the clinical application of sequential perfusion measurements.

Although the myocardial cell is highly permeable to H$_2$O, the presence of membranes could, theoretically, limit diffusibility. Rose et al.$^{18}$ and others$^{19}$ have suggested the existence of barriers to free diffusibility at the capillary and sarcomemmal levels.$^{18, 19}$ On the other hand, membrane permeability appears to be high enough in the heart (in contrast to the brain$^{20}$) so that the distribution of H$_2$O at physiologic flows is flow rather than diffusion limited.$^{21-23}$ Results in the present study obtained in open-chest dogs by direct assessment of myocardial radiotracer content, as well as those obtained noninvasively in vivo with PET, demonstrate that even with some restrictions to free diffusion, the permeability of H$_2$O is sufficiently high to permit accurate measurement of myocardial blood flow. Since water injected intravenously circulates in the blood as well as diffuses into tissue, for PET, a method of visualization of H$_2^{15}$O in myocardium was developed whereby amount of labeled water in the region of interest could be corrected for radioactivity in blood within the same region with the use of a second tracer. Red blood cells, labeled in vivo with C$^{18}$O, remain confined to the vascular space. The noninvasive method developed is rapid, can be employed sequentially, and should be suitable for application in patients.

Methods

Theoretical and mathematical basis of the method. For an inert, diffusible tracer, conservation of mass exists such that the rate of change in concentration of tracer in the tissue is equal to the rate of tracer entering the tissue minus the rate of tracer leaving the tissue$^5, 7$ with respect to tissue volume. Thus

$$\frac{dC_T}{dt} = F \left(C_a - C_T\right)$$

where $C_T$ = tissue tracer concentration (counts/g); $F$ = flow per unit of tissue (mlg/min); $C_a$ = arterial concentration of tracer (counts/ml); $C_T$ = venous concentration of tracer (counts/ml).

Kety$^7$ described the relationship of venous concentration of tracer to the tissue concentration of tracer by:

$$\left(C_a - C_T\right) = m(Ca - C_T)/\lambda$$

where $\lambda$ = tissue/blood partition coefficient (mlg/ml); $m$ = a diffusion constant reflecting effects of diffusion limitations and all other factors tending to limit equilibration of tracer between tissue and blood and defined by

$$m = 1 - e^{-PS/F}$$

where $PS = \text{product of capillary permeability and surface area (mlg/min), as follows:}$

$$PS = -F \ln(1 - E)$$

where $E = \text{the extraction fraction of tracer.}^{22, 23}$ $E$ can be mathematically defined as $I - e^{-PS/F}$. Thus, $E = m$ (equation 3).$^{16}$

By combining equations, we obtain

$$\frac{dC_T}{dt} = k(\lambda Ca - C_T)$$

where $k$ is defined as

$$k = mF/\lambda$$

By substitution and integration, the tissue concentration of $C_T$ at time $T$ is

$$C_T(T) = \lambda k \left[Ca(t)e^{-kt} - kT - t oh\right]$$

Kety$^7$ modeled the concentration of tracer in a region of tissue by assuming that blood and tissue (including capillaries) act as one compartment, with the concentration of tracer in the tissue at time $T$ given by equation 7. Ca(t) is the concentration of tracer in the arterial blood as a function of time.

Experimental determination of extraction fraction of water by the heart. To determine whether the extraction fraction of H$_2^{15}$O by the myocardium varied with flow, and to concomitantly calculate the permeability–surface area product per unit tissue for H$_2^{15}$O, we measured the myocardial single-pass extraction fraction of water in 15 mongrel dogs that had been anesthetized with thiopental (12.5 mg/kg) and a-chloralose (60 mg/kg) and ventilated with room air. Permeability–surface area product was determined with equation 4.

In each dog, the heart was exposed by a lateral thoracotomy and elevated in a pericardial cradle. The left circumflex coronary artery was isolated near the origin of the left main coronary artery, and a 22-gauge catheter (Quick-Cath) was inserted. In some of the 15 dogs ($n = 8$) the marginal branch of the circumflex coronary artery was isolated and ligated to induce a region of ischemia. In other experiments dogs ($n = 8$) were given 1.0 mg/kg dipyridamole (a gift of Boehringer Ingelheim, Ltd.; Ridgefield, CT) intravenously to augment coronary flow. Some dogs were subjected to both coronary ligation and dipyridamole to create a wide range of flow in a single animal. To determine extraction fraction, H$_2^{15}$O (0.3 ml, 4 mCi/ml) was injected as a bolus (in 1 sec) directly into the coronary artery. The injection was given by preloading the infusion catheter and administering the tracer with the use of a constant-flow roller pump to obviate variations that might be induced with hand injections. Since total circumflex flow was at least 40 ml/min, administration of tracer did not alter total coronary flow or pressure (measured in pilot experiments in which distal coronary arterial pressure was measured during tracer administration) by more than 5%. Radioactivity was assessed with a $\beta$-probe that detected radioactivity in a field of view directly under the probe.$^{24}$ In our previous study$^{24}$ we demonstrated that the contribution of gamma radiation elicited from positron annihilation to the count rate observed by the probe was less than 5%. In the current study, repeat injections of H$_2^{15}$O with lead interposed over the $\beta$-probe reduced count response to less than 4% (10 determinations). Thus, error due to scatter from gamma radiation was low. Since the half-life of H$_2^{15}$O is 2.1 min, background radioactivity was negligible before each administration of tracer in those dogs in which multiple injections were made. The probe was positioned over the distribution of the marginal branch of the circumflex coronary artery. The fractional extraction of water was determined by back-extrapolation of the monoexponential tail of the tracer time-activity curve.$^{20, 25-28}$

Since

$$MBF = k'\lambda/D$$

where $k' = \text{myocardial turnover rate constant}^{*} \text{ (min}^{-1}); \lambda = kq = e^{-kq}$ where $q = \text{counts/min corrected for physical decay}; k' = \text{the turnover rate constant.}$
tissue/blood partition coefficient (ml/g); D = specific gravity of heart muscle (assumed to be 1.06). Flow in these studies was determined by calculating the monoeponential washout of water, as previously described.24–28

The tissue/blood partition coefficient (λ) is a measurement of the ratio of concentration of tracer in the tissue to concentration of tracer in the blood at equilibrium. It was calculated from the known water contents of left ventricular myocardium (0.78 g H2O/g muscle) and of blood. Plasma water varies with hematocrit. Since measured systemic hematocrit ranged from 0.35 to 0.45, plasma water was taken as 0.80 g H2O/g blood (0.83 g H2O/ml blood with correction for blood density). Thus, a value for λ of 0.92 ml blood/g tissue was used in these studies. No attempt was made to correct for small vessel hematocrit changes. In addition, no correction was made for λ in studies in which dipyrindamole was used since pilot studies indicated no change in H2O content of tissue after administration of this drug.

Measurement of myocardial blood flow with H15O in open-chest dogs. To evaluate the tissue autoradiographic model in vivo with H15O, additional studies were performed. To induce ischemia, the distal portion of the left anterior descending coronary artery was ligated in each of nine open-chest anesthetized dogs. Fifteen to thirty minutes after coronary ligation, approximately 4 × 10^6 15µm 85Sr-labeled microspheres (3M Corp.) were injected into the left atrium while blood was collected from the femoral artery at a rate of 15 ml/min with the use of a constant withdrawal pump. In four of the nine dogs, to augment flow in nonischemic regions, 5 min before injection of microspheres 0.2 mg/kg/min dipyrindamole was administered intravenously for 5 min. Immediately after the withdrawal of blood was completed, approximately 30 to 50 mCi of H15O (specific activity of 3 mCi/ml) was infused intravenously at a constant rate of 7 ml/min. The arterial concentration of tracer as a function of time was determined by collecting samples from a free-flowing catheter positioned in the midhilar area. Samples (approximate volume of 0.5 ml) were collected every 3 to 5 sec. Exactly 1 min after the infusion of tracer was begun (and while it was being continued) the heart was excised rapidly. Six to 15 transmural myocardial samples of approximately 0.9 g each from both normal and ischemic regions were placed in preweighed vials. Radioactivity in tissue and blood samples were assayed in a Beckman gamma well counter within 20 (and usually within 15) min after excision of the hearts. Counts were corrected for physical decay of the H15O. Since the half-life of 15O is short (2.1 min), myocardial samples from the ischemic area were counted first (since they had the lowest expected counts/g), followed by myocardium from normal regions and then blood. Samples were counted for 15 sec. In ischemic regions, counts from H15O before decay and weight correction but after correction for 85Sr ranged from 425 to 12,925 counts/min; in normal regions the range was from 646 to 7,962 counts/min and in blood (at 60 sec of infusion), from 38,518 to 54,007 counts/min.

After sufficient time had elapsed for complete decay of H15O, samples were recounted to assess activity due to 85Sr and myocardial blood flow was determined by the standard microsphere reference technique. Flow determined from the tissue content of radiolabeled water was calculated with the use of equation 7 with values for the constant, m, determined from the extraction fraction of H15O that had been defined experimentally. Since the results (see below) indicated that permeability–surface area product increased linearly with flow, m, calculated from equation 3, was constant.

Correction was made for timing discrepancies related to flow rate and volume of the catheter used for arterial blood sampling. This was accomplished by predetermining the volume of the catheter and measuring the flow rate of the free-flowing catheter. Thus, the time delay of the appearance of blood from the aorta to the sampling tube was known and the arterial blood curve was corrected for the slight delay in the appearance time. This usually was on the order of 1 to 3 sec. Correction for catheter-induced ‘smearing’ was not performed.

Measurement of relative regional myocardial tissue water with PET. After demonstrating experimentally that myocardial blood flow could be measured with H15O assayed directly in tissue (see Results), we wished to determine whether estimation of regional myocardial H15O content could be accomplished in intact animals. Six conditioned dogs were anesthetized. In three, coronary arterial thrombosis was induced by placing, under fluoroscopic guidance, a copper coil into the left anterior descending coronary artery.50 Dogs were then positioned in PET VI, a positron-emission tomograph designed for clinical neurologic studies. Since PET VI does not provide the temporal resolution required to measure tissue radioactivity instantaneously, 40 sec scans were performed to permit collection of adequate count statistics. For PET studies, the concentration observed is an integral of concentration over the scan interval. Herscovitch et al.17 have recently demonstrated that integration of the operational equation (No. 7) does not impair the validity of the mathematical model.

A bolus of 30 to 50 mCi of H15O was given via a peripheral vein. After a 10 to 20 sec delay to allow for transit to the heart, a 40 sec period of data collection from seven simultaneous transaxial planes was initiated in the high-resolution mode.

Since a fraction of H15O resides in the blood in the field of view, to visualize H15O in the myocardium, radioactivity emanating from the H15O in the blood was corrected for by subtraction. After H15O activity had completely decayed, the vascular space was labeled by administering approximately 30 mCi C15O (which binds avidly to hemoglobin) by inhalation and, after 1 min to allow for equilibration, data were acquired for 300 sec, where the subscripts of ROI, MYO, and BLD represent radioactivity in a myocardial region of interest, in myocardium, and in blood, respectively. Thus:

\[
H_{15}^{O}_{MYO} = H_{15}^{O}_{ROI} - H_{15}^{O}_{BLD}
\]

Since blood labeled with C15O is confined to the vascular space and since the ratio of H15O/C15O in the left ventricular cavity blood (LVBLD) can be measured accurately tomographically (verified by direct counting of blood samples), H15O can be calculated as

\[
H_{15}^{O}_{MYO} = H_{15}^{O}_{ROI} - \left( \frac{H_{15}^{O}_{LVBLD}}{C^{15}O_{LVBLD}} \right) C^{15}O_{ROI}
\]

To obtain the needed H15O/C15O ratio in left ventricular blood, a midventricular slice in the heart was selected and a region of interest in the mid--left ventricular cavity was identified in the C15O scan. The identical region of interest was then identified in the H15O tomogram and the H15O/C15O ratio was calculated. Pilot studies demonstrated a correlation between the H15O ratio obtained in ungated tomograms from the left ventricle and those from the left atria .92 Since the resolution of PET VI measured as the full width at half-maximum is 0.7 cm,31 absolute myocardial radioactivity cannot be determined directly because of effects of cardiac motion and partial volume. Due to spillover from the left ventricular cavity, equation 11 will slightly overestimate C15O ROI and therefore slightly underestimate myocardial H15O.

The biological kinetics of C15O and H15O in blood and heart muscle are different. The C15O scan delineates intravascular radioactivity. At equilibrium, the blood/tissue counts due to

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C\(^{15}\)O are constant. Dynamic tomographic studies with H\(^{15}\)O (data acquisition every 10 sec after intravenous administration of the tracer) have demonstrated that the PET integral of blood/tissue H\(^{15}\)O is also constant over the 40 sec scan interval.* Thus, there is little error in the subtraction technique as the result of different kinetics of C\(^{15}\)O and H\(^{15}\)O.

To compare the myocardial water counts obtained with results obtained with microspheres acquired in the same nongated tomographic system under identical geometric considerations, approximately 4.0 × 10\(^6\) \(^{68}\)Ga-labeled macroaggregated albumin microspheres were injected into the left ventricle through a pigtail catheter immediately after completion of the C\(^{15}\)O scan. After 30 sec had elapsed, a 15 min tomographic scan was obtained to visualize the distribution of microspheres.

Human albumin microspheres (10 to 35 \(\mu\)m in diameter) were labeled with \(^{68}\)Ga obtained in ionic form from a tin dioxide/1M HCl \(^{68}\)Ge/\(^{68}\)Ga generator (New England Nuclear Corp.). One milliliter of 0.5M sodium acetate was added to the generator eluate (3 to 6 ml) and the pH was adjusted to 5.0 with 3M NaOH. The contents of three vials of tin-soaked human serum albumin microspheres (approximately 15 mg, containing 4.0 × 10\(^6\) spheres) (3M Corp.) were reconstituted and mixed with 1 ml H\(_2\)O. After incubation with \(^{68}\)Ga for 10 min at 37°C the microspheres were sonified for 2 min. The \(^{68}\)Ga-labeled microspheres were separated by centrifugation and washed with plasma in 0.9% NaCl to remove all traces of unbound \(^{68}\)Ga. resuspended in 0.9 NaCl, and sonified again before injection. After administration, 5% to 10% of injected \(^{68}\)Ga dissociated from the albumin microspheres and appeared as counts in the blood. Accordingly, regional tomographic measurements were corrected for \(^{68}\)Ga bound to transferrin and remaining in the vascular space with the use of the subtraction technique with C\(^{15}\)O analogous to the technique used for correction of H\(^{15}\)O tomograms.

To verify that the distribution of \(^{68}\)Ga-microspheres detectable tomographically reflected flow accurately, 4.0 × 10\(^6\) \(^{85}\)Sr-microspheres (15 \(\mu\)m) were injected into the left ventricle simultaneously with the \(^{68}\)Ga-microspheres while blood was withdrawn at a constant rate through a femoral artery.

For analysis of PET data, after reconstruction of the myocardial distribution of H\(^{15}\)O, transmural regions of interest representing a volume of 1 cm\(^3\) (at least 1 cm in the long \(z\) axis × 1 cm wide × 1 cm high) were placed on the images in areas corresponding to the anterior, septal, posterior, and lateral left ventricular myocardium. Relative regional data were calculated for each slice as the anterior/normal (the mean of septal, posterior, and lateral) count ratio. The precisely defined regions were then placed on the reconstructed \(^{68}\)Ga images, and the anterior/normal count ratio for \(^{68}\)Ga was calculated. After the animals had been killed, myocardial blood flow was measured by assessing the myocardial distributions of \(^{68}\)Ga- and \(^{85}\)Sr-microspheres by well counting with the use of appropriate decay and count spillover corrections by the standard reference technique. Assessment of the relative (i.e., anterior/normal) myocardial blood flow was made with myocardial samples obtained at approximately the same levels as were the tomographic slices that were analyzed.

**Results**

Myocardial extraction fraction of H\(^{15}\)O. The determination of the extraction fraction of H\(^{15}\)O during a single pass was performed in open-chest dogs after bolus injections of tracer directly into the coronary artery (n = 15). Figure 1 depicts representative time-activity curves from one dog after injections under control conditions, after flow had been augmented by infusion of dipyridamole, and after coronary flow had been reduced by ligation of the marginal branch of the circumflex coronary artery. Under the conditions of the study, H\(^{15}\)O was highly extracted (extraction fraction averaging 96 ± 5% at flows of 80 to 100 ml/100 g/min)


FIGURE 1. Time-activity curves obtained in an individual dog after bolus injection of H\(^{15}\)O into the left circumflex coronary artery under control conditions (top), after augmentation of flow with dipyridamole (middle), and after ligation of the marginal branch (bottom). Fractional extraction was determined by back-extrapolation of the tail of the time-activity curve to the time of peak counts.
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FIGURE 2. A, The single-pass extraction fraction of $H_2^{15}O$ obtained after bolus injection of tracer into the left circumflex coronary artery over a range of flows ($n = 15$ dogs). For statistical analysis, flows were grouped into 40 ml/100 g/min groups. Values are mean ± SD for each group. B, Permeability-surface area (PS) product vs flow. PS product was determined experimentally from extraction data and equation 4. PS product varied directionally with flow. Values are mean ± SD.

during a single pass. This high level of extraction was seen over the entire range of flows evaluated (12 to 238 ml/100 g/min) (figure 2, A).

With the measured values for extraction fraction, permeability–surface area product was determined from equation 4. For this calculation, values of extraction fraction of 1.0 (100%) were set to 0.99 since the natural log of 0 is indeterminant. Permeability–surface area product was found to approximate $3.8 \times$ myocardial blood flow – 28 and to vary directionally with flow (figure 2, B).

Application of the Kety-Schmidt model to $H_2^{15}O$. In nine experiments, $H_2^{15}O$ was infused at a constant rate for 1 min and hearts were excised rapidly. Myocardial blood flow was calculated from equation 7. Results were compared with those obtained with microspheres. The one-compartment Kety model slightly underestimated flow ($r = .95$, $y = 0.83x + 6.6$, $n = 95$ comparisons) (figure 3, A). Some of the scatter in the data may have resulted from the fact that although radioactivity in myocardial samples was assayed promptly (within 10 min) after excision of the heart, the interval represented up to five half-lives. Thus, a large correction factor was required to correct for physical decay of the isotope. However, despite the scatter in the data in aggregate, results from each individual animal were quite linear, with little scatter about the best-fit line (figure 3, B).

Applications to PET. The feasibility of measuring regional myocardial blood flow with $H_2^{15}O$ and PET was tested in six dogs. Figure 4 depicts tomographic reconstructions from a single midventricular slice obtained from a normal dog. Figure 4, top, depicts the tomogram obtained after intravenous injection of $H_2^{15}O$; the middle panel depicts the tomogram after inhalation of
C\textsuperscript{15}O, and the bottom panel, the tomogram after subtraction of the vascular pool H\textsubscript{2}\textsuperscript{15}O activity from the initial water tomogram. Representative counts in a midventricular slice after injection of H\textsubscript{2}\textsuperscript{15}O were \(5.39 \times 10^5\) counts/slice; after inhalation of C\textsuperscript{15}O they averaged \(3.09 \times 10^6\) counts/slice and after administration of \(^{68}\text{Ga}\). \(2.68 \times 10^6\) counts/slice. After correction for activity emanating from blood pool–associated tracer, the values for H\textsubscript{2}\textsuperscript{15}O tomograms averaged \(3.55 \times 10^5\) counts/slice. Under conditions of normal flow (figure 5) or coronary thrombosis (figure 6) the relative tomographic distribution of H\textsubscript{2}\textsuperscript{15}O correlated closely with the tomographic distribution of gallium-labeled microspheres \((r = 0.93, y = 0.91x + 11.3, n = 92\) comparisons) (figure 7. A). Relative regional myocardial blood flow obtained by analysis of tomographic distribution (anterior/normal) of H\textsubscript{2}\textsuperscript{15}O also correlated well with relative myocardial blood flow determined by postmortem analysis with microspheres (figure 7. B). The correlation between the measurements of flow obtained by postmortem direct tissue counting of \(^{68}\text{Ga}\)-labeled and \(^{85}\text{Sr}\)-labeled microspheres was close \((r = 0.98;\) figure 8), indicating that the distribution of the \(^{68}\text{Ga}\)-spheres accurately reflected the distribution of blood flow.

An application of the technique developed is shown in figure 9, which depicts three midventricular tomograms from a single dog before and after thrombolysis was induced with intravenously administered tissue-type plasminogen activator.\textsuperscript{32}

**Discussion**

Quantitative noninvasive measurement of regional myocardial blood flow is necessary to facilitate detection and evaluation of cardiac pathophysiology. Sequential measurements would permit improved objective assessment of medical and surgical interventions designed to ameliorate myocardial ischemia. Water labeled with \(\textsuperscript{15}O\) is a particularly attractive tracer for this purpose. Its short half-life minimizes the potential radiation burden to the subject and facilitates sequential measurements. Although H\textsubscript{2}\textsuperscript{15}O is not totally freely diffusible,\textsuperscript{18, 19} studies of the heart by others\textsuperscript{21, 22} and results of the present study indicate that limitations to free diffusion are modest under conditions of normal and low flow. The first-pass extraction fraction of water measured in this study is sufficiently high at physiologic rates of flow to allow free diffusibility to be approximated in canine hearts. The calculated permeability–surface area product, approximating \(3.8 \times\) myocardial blood flow -- 28 ml/100 g/min, is greater

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**FIGURE 3.** A. The correlation between myocardial blood flow determined with microspheres and flow determined with H\textsubscript{2}\textsuperscript{15}O calculated with the one-compartment model. The results were obtained from nine open-chest dogs after a 60 sec intravenous infusion of H\textsubscript{2}\textsuperscript{15}O and from direct assay of radioactivity in tissue. B. The correlation between blood flow determined with microspheres and with H\textsubscript{2}\textsuperscript{15}O in an individual dog. Linearity of the relationship is evident.

**FIGURE 4.** Midventricular tomographic reconstructions obtained from a normal, closed-chest dog. Top. The tomogram obtained after a 40 sec period of data collection that followed a 30 mCi bolus injection of H\textsubscript{2}\textsuperscript{15}O. Middle. The tomogram obtained after labeling of red blood cells with C\textsuperscript{15}O to define the vascular space. Bottom. Subtraction of H\textsubscript{2}\textsuperscript{15}O emanating from vascular activity yielded the distribution of H\textsubscript{2}\textsuperscript{15}O in the myocardium.
FIGURE 5. Three contiguous midventricular tomograms obtained from a normal dog with the H$_2$O subtraction technique described (left), and after ventricular injection of $^{60}$Ga-albumin microspheres (right). The regional distributions of H$_2$O and microspheres correlate closely (see figure 7).

FIGURE 6. Three midventricular tomograms obtained with H$_2$O (left) and $^{60}$Ga-microspheres (right) from a closed-chest dog with occlusion of the left anterior descending coronary artery. Coronary thrombosis was induced by placement of a copper coil into the artery. The distribution of H$_2$O correlates closely with that of microspheres.
apply in tomographic studies. Tripp et al.,34 in studies with ¹⁵O-water (H₂O) demonstrated very close correlations between measurements of myocardial blood flow with tritiated H₂O (using a one-compartment model) and microspheres over a wide flow range. We chose to use H₂¹⁵O rather than H₂O since H₂¹⁵O permits analysis by external detection.

We have previously demonstrated that myocardial perfusion can be quantified accurately with either H₂¹⁵O or ¹³C-butanol in isolated hearts and intact dogs with a modification of the Kety-Schmidt model, in which exponential infusion is used.¹³,¹⁴ Butanol has the potential advantage of having a tissue/blood partition coefficient close to unity and may be even more freely diffusible than H₂O. However, use of H₂¹⁵O entails a lower radiation burden because its half-life is shorter than that of ¹³C (20.4 min). Furthermore, H₂¹⁵O is considerably easier than ¹³C-butanol to produce and its shorter half-life permits rapid sequential studies.

Many potential sources of error must be considered in autoradiographic approaches to measurement of blood flow, including inhomogeneity of flow, uncertainty concerning the choice of λ, differences in tissue H₂O content (which may be especially important in evaluations of ischemia or reperfusion), and the timing and "smearing" of the arterial time-activity curve nec-

FIGURE 7. A. Correlation between regional H₂¹⁵O counts (expressed regionally as anterior/normal counts) and ⁶⁸Ga-microsphere counts obtained noninvasively with PET in six dogs. The data indicate good agreement between relative regional flow estimated with H₂¹⁵O and with the microsphere technique. B. Correlation between H₂¹⁵O tissue counts obtained tomographically and myocardial tissue counts (expressed as the anterior/normal ratio) attributable to microspheres assayed postmortem in corresponding slices of the heart studied.

than the theoretical value of 3.0 promulgated by Ginsburg³³ as characteristic of a flow-limited tracer and is consistent with observations by Yudilevich and Alvarez²².

The results of the present study indicate that single-pass extraction of H₂¹⁵O is high over the entire range of flows studied. The correlation between flow determined with the microsphere technique and that determined with H₂¹⁵O after a 1 min constant infusion was close (figure 3, A). Some scatter in the data is not surprising since a large decay correction is necessary due to the logistics of the experiments and the short half-life of ¹⁵O. Obviously, this limitation would not

FIGURE 8. Correlation between myocardial blood flow measured with ⁶⁸Ga-albumin microspheres and ⁸⁵Sr-microspheres in the postmortem assay. The excellent correlation between the two measurements indicates that the ⁶⁸Ga-spheres are distributed in the heart in direct relationship to flow.
necessary for quantification. Limitations of tomographic resolution, especially when the tomographic system is not gated, must be considered. Their effect and strategies for correction have been presented.

Despite these limitations, results of the present study show that the technique using $^3$H$^2$O yields results that correlate well with results obtained with microspheres. In a computer analysis of the use of the autoradiographic technique for measurement of cerebral blood flow, Herscovitch et al. demonstrated the relative insensitivity of the mathematical model to inhomogeneity with respect to tissue flow and $\lambda$. On the other hand, it is clear that for quantification, timing of blood sampling is critical. As flows become supraphysiologically high, estimations become more sensitive to the mathematical model employed as well as to the $\lambda$ and $m$ chosen. For reasons yet unclear, extending the time of the infusion of tracer to more than 60 sec results in measurement error. Although the operational equation (No. 7) is nonlinear, as pointed out by Herscovitch et al. and Raichle et al., if only relative flow measurements are required, arterial sampling is unnecessary since the tomographically obtained integral of tissue counts of $^3$H$^2$O is nearly linearly related to flow over short scan intervals. This relationship permits the results of the present study to be presented in terms of relative regional flow measurements.

As shown in figure 7, the correlation between the tomographic distribution of $^3$H$^2$O and that of $^{68}$Ga-labeled microspheres is a close one. In this study, microspheres were injected in the ventricle with a pigtail catheter. Buckberg et al. demonstrated that mixing of microspheres is equivalent with left ventricular injections and left atrial administration. The close correlation between the flows measured after simultaneous administration of $^{85}$Sr- and $^{68}$Ga-microspheres (figure 8) corroborates this observation, since incomplete microsphere mixing would have resulted in greater discrepancy in the two measurements. Correction for cardiac motion and partial volume effects should permit quantification of tissue radioactivity and estimation of flow in absolute terms.

Optimal application this approach to PET requires instrumentation that permits gating with respect to both the cardiac and respiratory cycles for correction of motion artifact, count spillover, and partial volume effects. Super PETT I, a fast scanning tomograph developed to employ time-of-flight measurements and currently undergoing clinical testing at our institution should meet these requirements. The implementation of the method developed in this study in patients should facilitate objective, noninvasive, sequential

**FIGURE 9.** Three midventricular tomograms obtained with the $^3$H$^2$O technique obtained from a dog with myocardial ischemia due to thrombosis (left) and after thrombolysis (right) induced by intravenous administration of tissue-type plasminogen activator. Results demonstrate the potential utility of the technique for sequential analyses of myocardial blood flow.
evaluation of myocardial blood flow and its response to medical or surgical interventions designed to favorably augment nutritional perfusion.

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