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Research Article

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External Quantification of Myocardial Perfusion by Exponential Infusion of Positron-emitting Radionuclides

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ABSTRACT A technique was developed and evaluated using the exponential infusion of positron-emitting diffusible tracers to quantitate myocardial perfusion. The approach employs a parameter that rapidly reaches a constant value as a function of tracer delivery rate, isotope decay constant, and the monotonically increasing tissue radioactivity. Isolated rabbit hearts with controlled flow were used to evaluate the approach, because tracer kinetics in such preparations mimic those in vivo. Accordingly, exponential infusions of $H_2{}^{15}O$ and [^{11}C]butanol were administered to 25 isolated rabbit hearts perfused with Krebs-Henseleit solution (KH) alone or KH enriched with erythrocytes (KH-RBC, hematocrit = 40). With flow varied from 1.2 to 5 ml/g per min in eight KH hearts infused with $H_2{}^{15}O$, actual and estimated flow correlated closely ($r = 0.95$, $n = 52$ determinations). For the KH-RBC hearts, flow was varied from 0.3 to 1.5 ml/g per min. Actual and estimated flow correlated significantly for both the 14 KH-RBC hearts infused with $H_2{}^{15}O$ ($r = 0.90$, $n = 89$ determinations) and the 3 KH-RBC hearts infused with [^{11}C]butanol ($r = 0.93$, $n = 13$ determinations). In addition, the required exponentially increasing arterial tracer concentrations were shown to be attainable in vivo in dogs and rhesus monkeys after intravenous exponential administrations of tracer. The results suggest that the approach developed employing exponential tracer infusion permits accurate measurement of myocardial perfusion and that it should prove useful in the noninvasive measurement of regional myocardial perfusion in vivo by positron emission tomography.

INTRODUCTION

Positron emission tomography (PET),[1] an intrinsically quantitative tool, has been applied rapidly to noninvasive assessment of myocardial metabolism. Its successful application for quantification of regional perfusion is hampered by several serious difficulties, however. Our study was designed to develop and evaluate a novel mathematical approach to quantifying myocardial perfusion, employing exponential infusion of diffusible tracers. The tracers were selected to embody physical characteristics particularly attractive for ultimate quantitative applications in patients. The approach was designed to permit use of radionuclides with relatively long physical half-lives amenable to quantification by PET. An ideal tracer should exhibit several additional characteristics, including free diffusibility and an extraction fraction uninfluenced by either residence time per se or by alterations of myocardial metabolism induced by ischemia. In the initial experiments in our study with isolated perfused hearts, a less-than-ideal but easily prepared tracer, $H_2{}^{15}O$, was used to define the feasibility of the overall concept. Subsequently, studies were performed with [^{11}C]-butanol, synthesized for this purpose because its lipid: aqueous partition coefficient is near unity and because of its high diffusivity in tissue (1). The results obtained in isolated perfused rabbit hearts indicate that myocardial flow can be accurately measured noninvasively by this approach. In addition, results obtained in intact dogs and rhesus monkeys indicate that the required exponentially increasing arterial tracer concentration necessary for in vivo determinations can be obtained by peripheral venous infusion of tracer.

Mathematical basis. Equilibrium methods for measurement of regional perfusion use constant infusion of a diffusible tracer such as krypton-81 (^{81m}Kr) or [^{15}O]water ($H_2{}^{15}O$), so that a constant concentration of tracer is maintained in the arterial blood or perfusate of the organ of interest (2–4). In the case of the heart, regional myocardial tracer activity reaches 98% of the steady-state value after six half-lives, based on the combined physical decay and biological turnover rate constants. Thus, a steady-state value can be considered to have been reached at time t based on the following

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[1] *Abbreviations used in this paper*: KH, Krebs-Henseleit buffer; KH-RBC, Krebs-Henseleit buffer enriched with erythrocytes; PET, positron emission tomography.

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relation:

$$t > 6 \cdot \frac{\ln 2}{\kappa + \alpha} \quad (1)$$

where t = time after commencement of infusion of tracer; κ = biological turnover rate constant within the myocardium; α = isotope physical decay rate constant.

After the steady-state condition is achieved,

$$F = \frac{\alpha}{\frac{C_B}{q} - \frac{1}{\lambda}} \quad (2)$$

where F = regional myocardial blood flow per mass of tissue; α = physical decay rate constant of the tracer; C_B = the concentration of tracer in arterial blood; q = steady-state activity of tracer in myocardium per mass of tissue; λ = tissue:blood partition coefficient of the tracer.

The primary advantage of the constant infusion technique is that it is compatible with steady-state imaging systems and with slow speed scanners. However, the approach is limited for the following reasons. First, a steady supply of constant activity of tracer is required, which may be difficult to achieve for some otherwise attractive tracers and which may lead to a high radiation burden. Second, recirculation artifact may influence markedly the values of both C_B and q. Although this problem may be minimized by employing a tracer with an ultrashort half-life, assurance of a constant coronary arterial concentration of such an agent may be difficult. Third, small measurement errors may result in very large errors in the estimates of flow obtained from Eq. 2, particularly since the quantity C_B/q is often only marginally larger than $1/\lambda$. Further more, because relatively large changes in flow result in only modest changes in q, sensitivity for detection of changes in flow may be low. Fourth, the diffusible tracer employed should ideally partition equally in lipid and aqueous phases so that the blood:tissue partition coefficient will not vary in the face of heterogeneous distribution of tissue lipid content.

An initial approach to obviating some of these limitations entails an exponential infusion to replace constant infusion of isotope so that the infusion rate constant (β) would be equal to but opposite in sign to the isotope physical decay constant (α). That is, tracer delivery would conform to the function $C_B(t) = C_B' e^{\alpha t}$. With such an infusion, a constant activity source of tracer is not required. Based on mathematical considerations (see Appendix), however, it became apparent that by driving the delivery of tracer so that the exponential constant (β) exceeds the isotope physical decay rate constant (α), the time required for estimation of flow could be decreased, recirculation artifact could be minimized, and the estimate of flow could be made less sensitive to measurement errors. For this case, tracer delivery conforms to the function $C_B(t) = C_B' e^{\beta t}$. The exponential infusion does not result in steady-state activity in the myocardium. However, it does give rise to a derived activity function (q*), which includes as one parameter the actual activity of tracer in myocardium (q) and which reaches a plateau value ("steady state") (Fig. 1). The function q* is computed as follows:

$$q^*(t) = q(t)e^{(\alpha-\beta)t} \quad (3)$$

where q*(t) = derived activity function; q(t) = measured activity of tracer in tissue per mass of tissue; α = the physical decay rate constant of the isotope; β = the exponential infusion rate constant.

A value that is 98% of the steady-state value of the derived activity function (q*) is achieved after six half-lives, based on the combined myocardial (biological) turnover rate constant and the exponential infusion rate

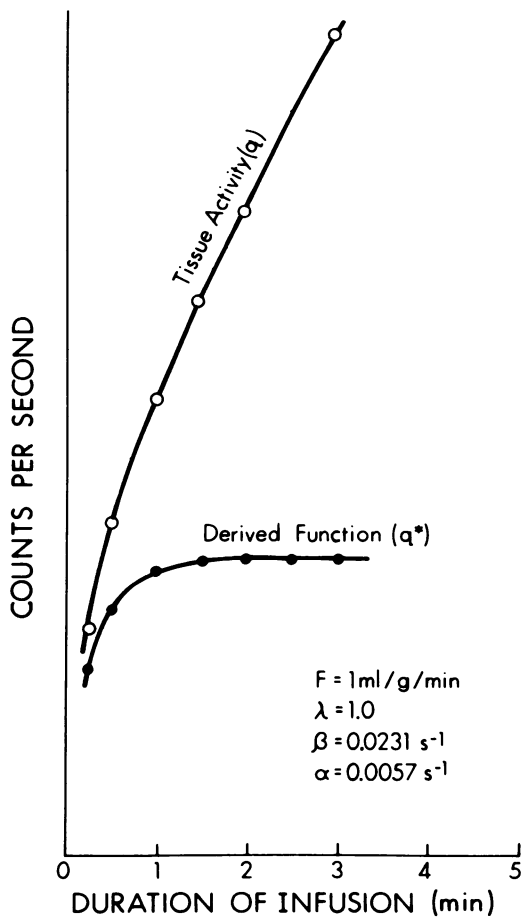

FIGURE 1 Hypothetical relation between myocardial activity (q) and the derived activity function (q*) resulting from an exponential intravenous infusion achieving an arterial blood concentration conforming to an exponential function with rate constant β and the infused tracer being $H_2{}^{15}O$.

constant. That is, the derived function q* reaches a plateau for:

$$t > 6 \cdot \frac{\ln 2}{\kappa + \beta} \quad (4)$$

where t = time after commencement of infusion of tracer; κ = myocardial turnover rate constant; β = exponential infusion rate constant.

After the steady-state value of q* has been achieved,

$$F = \frac{\beta}{\frac{C_B'}{q^*} - \frac{1}{\lambda}} \quad (5)$$

where F = myocardial blood flow per mass of tissue; β = exponential infusion rate constant; α = isotope physical decay constant; C_B' = arterial blood concentration of tracer at time zero; λ = tissue:blood partition coefficient of tracer; q* = steady-state value of the derived activity function.

With the constant infusion technique and use of the tracer $H_2{}^{15}O$, and in the case when coronary flow is 1 ml/min per g, the time required to reach a steady-state value for q (Eq. 1) is ~3 min. When the exponential infusion technique is employed under comparable conditions, however, with an infusion rate half-time of 30 s, the time required to reach a steady-state value for q* (Eq. 4) is only 1.5 min. In addition, exponential infusion helps to reduce the influence of unavoidable measurement errors on estimates of flow. Because the numerator in the equation pertinent to programmed infusion (Eq. 5) exceeds the corresponding term related to the constant infusion approach (Eq. 2), the difference between quantities C_B'/q^* and $1/\lambda$ in the equation employed for estimates of flow with the programmed infusion method is larger than the difference between the quantities C_B/q and $1/\lambda$ in the equation used for the calculation under conditions of constant infusion. Thus, estimates of F are less dependent on variations in C_B' and q* (Table I).

Recirculation artifact is reduced by use of the exponential infusion approach. Because tracer is infused at a progressively increasing rate, the concentration of tracer recirculated from that delivered previously is small compared to tracer administered from the infusion pump. For example, in the case of constant infusion of $H_2{}^{15}O$ ($t_{1/2}$ = 123 s) under conditions in which the mean transit time of the tracer through the circulatory system is 1 min, a conservative estimate of average activity of the recirculating tracer would be 71% of the initial activity. However, in the case of an exponential infusion with an infusion half-time of 30 s, the average activity of tracer due to recirculation after a single pass would be 18%.

TABLE I
Error Propagation

Constant infusion		Programmed infusion	
C_B/q	F	C_B'/q^*	F
%	%	%	%
1	5	1	2
2	11	2	4
5	31	5	10
10	92	10	23
λ	F	λ	F
%	%	%	%
1	4	1	1
2	7	2	2
5	16	5	5
10	27	10	9

Values derived for $H_2{}^{15}O$: decay constant, 0.006568 s^{-1}; infusion constant, 0.0231 s^{-1}. Percentage errors in estimates of C_B/q and λ lead to percentage errors in the estimates of flow indicated. F = estimated flow per unit mass of tissue; C_B and C_B' = concentration and initial concentration of tracer in the perfusate; q and q* = actual activity of tracer in the heart and the derived function described in Eq. 3 per unit mass of tissue; and λ = tracer tissue:perfusate (blood) partition coefficient.

METHODS

Exponential infusion in vivo. Applications in vivo of the approach developed would obviously require a systemic arterial blood tracer time-activity curve that exhibits an exponentially increasing concentration when a peripheral, intravenous infusion of tracer is used. In view of the potential difficulties from mixing in vivo, it was necessary to determine whether this condition could be met easily in large experimental animals. Experiments were therefore performed with rhesus monkeys and dogs anesthetized with phencyclidine and pentobarbital, respectively. Exponential intravenous infusions of $H_2{}^{15}O$ and of ^{11}C-tracers were implemented via the femoral vein with a stepping motor-driven infusion pump. The speed of the motor and hence the delivery rate were microcomputer controlled such that the desired delivery rate constant for each infusion could be selected by the operator. Determinations of the arterial concentration of tracer as a function of time during the femoral vein infusions in six monkeys were based on activity measured in a well counter from serial samples (10-s intervals) of blood from a cannulated carotid artery. Similar experiments were performed in two dogs with carotid arterial blood withdrawn with a constant withdrawal pump at 20 ml/min. Continuous time-activity curves were obtained from the cannula activity in the coincidence mode by the NaI(T1) system described below. Arterial blood concentrations were corrected for isotope physical decay. Selected exponential infusion pump rate constants (β) ranged from 0.069 to 0.017 s^{-1}, corresponding to infusion half-times of 10 to 40 s, respectively.

Isolated perfused hearts. The exponential infusion approach for delivery of tracer and estimation of coronary flow by external detection of a position-emitting radionuclide was evaluated with the use of isolated perfused rabbit hearts.

We have previously shown that this preparation is particularly useful in characterizing behavior of tracers with potential applicability for imaging in vivo, because it permits assessment of tracer kinetics under conditions in which flow and metabolic behavior of the heart can be controlled and monitored independently and precisely. In addition, such preparations permit evaluation of behavior of the tracer under conditions in which concentrations in the perfusate of substrate, protein, hormones, and other moieties potentially present in vivo can be controlled, and in which recirculation can be excluded, included, or simulated to permit assessment of its possible effects on accumulation of tracer in vivo. By inclusion of washed sheep erythrocytes in the perfusion medium to enhance oxygen-carrying ability, perfusion can be performed under conditions in which the absolute magnitude of flow simulates flow in vivo, a particularly important consideration in this study since the kinetics of a tracer with buffer-perfused hearts may be influenced by the shorter residence time associated with the high flow rates conventionally required to provide adequate oxygenation of buffer-perfused hearts.

Hearts were isolated from New Zealand white rabbits weighing 2–3 kg and perfused retrograde through the aorta as described previously in detail (5). Briefly, hearts were perfused with or without washed sheep erythrocytes at a hematocrit of 40, suspended in modified Krebs-Henseleit solution containing 0.4 mM (27.6 g/liter) bovine serum albumin (at levels approximating those in plasma). The endogenous albumin-bound fatty acid content was 0.1±0.01 mM, (SD), based on direct assays of the albumin solutions. Albumin was obtained as Cohn fraction V from the Sigma Chemical Co., St. Louis, Mo. The Krebs-Henseleit buffer (with 5 mM glucose and 70 mU/ml insulin) contained the following ions in milliequivalents per liter: Na^+, 143; Cl^-, 128; K^+, 6.1; Ca^{++}, 2.5; Mg^{++}, 1.2; $SO_4^=$, 1.2; H_2PO^-, 1.4. All buffer solutions were dialyzed extensively before use and filtered through a 0.45 μm filter. Sheep erythrocytes were used because of their small size and because their affinity for oxygen is less dependent on the concentration of 2,3-diphosphoglycerate in contrast to cells from many other species. All hearts were flushed initially with modified Krebs-Henseleit solution (KH) to wash out platelets and other blood elements that might lead to microaggregation and impair perfusion later. Within 2 min after excision of the heart, the pulmonary artery was cannulated and the caval and pulmonary vessels ligated. 90–95% of total flow and virtually all nutritional flow (excluding flow through the Thebesian system) was collected via the pulmonary artery. Isovolumically beating hearts were prepared by passing a fluid-filled latex balloon into the left ventricle via the left atrium. With the balloon connected to a Gould P23Db pressure transducer (Gould, Inc., Instruments Division, Cleveland, Ohio), left ventricular pressure and *dP/dt* (obtained by electronic differentiation) were recorded continuously. Perfusion pressure was monitored with the use of a side-arm cannula immediately distal to the aortic valve. Coronary flow was monitored by timed collections of effluent from the pulmonary artery. Drainage from Thebesian flow and any retrograde ventricular filling resulting from occult aortic insufficiency were monitored via a left ventricular stab wound; both were found to comprise <10% of pulmonary outflow in all hearts. Left ventricular end-diastolic pressure was adjusted by changing the volume of the balloon and was maintained at 10 mm Hg. The isolated heart was enclosed in a water jacket to maintain myocardial temperature at 37°C (verified by monitoring with a thermistor in some experiments) and paced at a rate of 180 beats/min with the use of pacing electrodes sutured onto the left ventricle.

Perfusion was initiated with either KH alone or with KH enhanced with erythrocytes (KH-RBC). The perfusion rate in hearts perfused with buffer alone was adjusted to 4.2 ml/g wet wt per min with a constant flow roller pump (a value obtained in a previous study with hearts perfused with erythrocyte-free buffer at a constant pressure of 60 mm Hg and found to provide adequate oxygenation based on stability of the preparation and ventricular performance). In hearts perfused with KH-RBC, the initial flow rate was 1.4 ml/g per min, since under these conditions, ventricular, mechanical, and metabolic performance were stable, reflecting adequate oxygenation. When hearts were perfused with buffer alone (without erythrocytes), the perfusate was equilibrated with 95% oxygen and 5% CO_2 through a Silastic membrane as previously described (5). When erythrocytes were used in the buffer, equilibration was accomplished with room air and sufficient CO_2 to maintain pCO_2 in the perfusate (monitored with an IL-213 blood gas meter [Instrumentation Laboratory, Inc., Watertown, Mass.]) within the physiological range. The salutary effects of erythrocyte-enhanced buffer perfusion on physiological and metabolic parameters in this preparation have been documented previously (5).

Preparation of radioactive tracers. $H_2{}^{15}O$ was prepared with the Washington University Allis Chalmers Cyclotron as previously described (6). [^{11}C]butanol was prepared from $^{11}CO_2$ by the *N*-propyl magnesium chloride reaction (1).

Detection of tracer activity. Cardiac radioactivity time-activity curves were obtained generally as previously described (7). 50–100 μCi of $H_2{}^{15}O$ (physical $t_{1/2}$ = 123 s) or [^{11}C]-butanol (physical $t_{1/2}$ = 20.4 min) was infused at an exponentially increasing rate with a half-time of 30 s into the perfusate 1 cm distal to the aortic valve, through a side arm in the aortic cannula to assure adequate mixing. The infusion pump was controlled by a MITs microcomputer (Pertec Computer Corp., Chatsworth, Calif.) used also for data collection and analysis. Time-activity curves were recorded with two NaI(T1) detectors placed 180° apart, ~3 cm from the surface of the heart at the midventricular level. Simultaneous detection with each of the two detectors of activity within their colinear fields of view permits coincidence detection of pairs of 511 keV photons emitted as a result of positron annihilation, with measured background activity of virtually zero. The detectors were aligned so that the percentage of coincidence counts represented ~10% of singles, verified before each experiment with the use of a standard germanium-68 source inserted in the field of view. Events were recorded with Ortec model 485 amplifiers, model 488 single channel analyzers, and a model 414A fast coincidence unit (Ortec Inc., EG&G, Inc., Oak Ridge, Tenn.) as previously described (7). Output was monitored on-line and collected with a microcomputer system to correct residue detection curves for physical decay of the tracer and to provide the mathematical analysis used to obtain estimates of flow.

Exponential infusion in isolated perfused hearts. 25 isolated hearts were randomized after initial equilibration, and perfusion was maintained with either KH alone or KH-RBC. Hearts were then equilibrated for an additional 20 min, during which period hearts were discarded that did not generate developed left ventricular systolic pressure >60 mm Hg (a total of six). After the second equilibration, exponential infusion of tracer was initiated under conditions of control flow (4.2 ml/g per min in KH hearts and 1.4 ml/g per min in KH-RBC hearts). After a 10-min interval, during which the myocardial time-activity curves were obtained, and at a time when background activity had declined to preinfusion values, the perfusion rate was reduced in a stepwise fashion, first to 50% and then to 25% of control flow; repeat exponential in-

fusions of tracers were implemented, and tissue time-activity curves were obtained.

Two tracers were used for this purpose. In 22 hearts, $H_2{}^{15}O$ was used because its short physical half-life ($t_{1/2}$ = 123 s) permitted multiple injections within a short time interval while avoiding accumulation of background activity from previous injections. In three hearts, [^{11}C]butanol was employed because its lipid:aqueous partition coefficient is virtually one. This characteristic results in a distribution of tracer that is essentially invariant despite heterogeneous distribution of tissue lipid (1).

Quantitation of tissue activity. Analysis of data with the exponential infusion method entails measurements of two parameters: the tissue activity of tracer and the concentration of tracer in the perfusate. Because the activity in tissue is measured with two scintillation probes, results expressed as counts per second are dependent on geometry. Initial attempts were made to calibrate the NaI(Tl) probe systems with a well counter; however, the geometrical dependence of the probe system was too extensive to permit reproducible calibrations.

Because of the calibration limitations, we linearized Eq. 5 to obtain

$$\frac{\beta}{F} = \frac{C_B'}{q^*} - \frac{1}{\lambda}. \qquad (6)$$

With this linearized form, results from a series of exponential infusions from each heart were employed to provide a calibration that does not require definition of the relative counting efficiencies of the two systems (i.e., the system used to detect counts in the perfusate and the one used to detect counts in the heart), as shown in Appendix 1. Results of one group of experiments from the same heart can then be graphically represented as a plot of C_B'/q^* vs. β/F. The Y-intercept of the plot provides an estimate of $-1/\lambda$, and the slope of the plot provides a measure of the relative counting efficiencies of the two systems (Fig. 2).

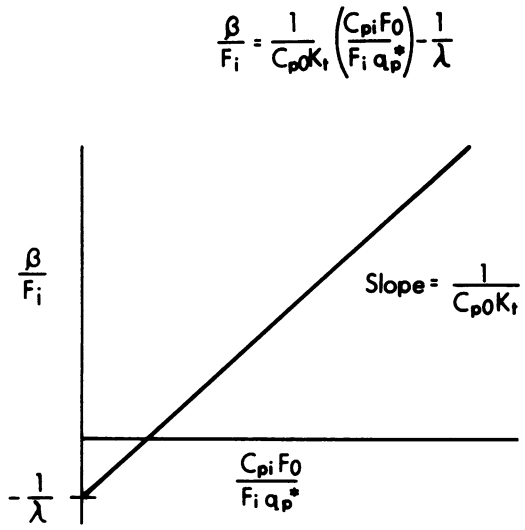

FIGURE 2 The graphic approach employed for parameter estimates of probe calibration factors and arterial blood tracer concentration with terms and data acquisition as described in Appendix 1.

RESULTS

Feasibility of obtaining required exponentially increasing concentrations of tracer in arterial blood in vivo. As shown in Table II, the observed blood time-activity curve in dogs and rhesus monkeys after peripheral intravenous exponential infusions exhibited the desired monoexponential behavior, with rate constants of the observed curves directly related to the rate constants selected for the exponential infusions. Thus, the fit of the arterial blood time-activity data to a monoexponential function was consistently close ($r > 0.95$; $n = 24$ experiments). However, the observed rate constant was not equal to the infusion rate constant. This implies that the arterial blood concentration must be monitored. For applications in vivo with PET, monitoring could be achieved conveniently by analysis of activity in ventricular cavitary blood from the images themselves. These results indicate that even with tracers with relatively long physical half-lives, there is minimal recirculation artifact causing deviation of the arterial tracer time-activity curve from the forcing function delivered by the intravenous infusion pump. Accordingly, application in vivo of this intravenous exponential infusion approach to large animals and ultimately to patients should not be impaired by recirculating tracer.

Results in isolated perfused hearts. Exponential infusions of $H_2{}^{15}O$ were administered in 8 hearts perfused with KH and 14 perfused with KH-RBC. Three additional hearts perfused with KH-RBC were studied with [^{11}C]butanol. Fig. 3 depicts the normalized plot of β/F_i vs. C_B'/q^* (i.e., in which the term plotted on the abscissa represents an adjusted form of C_B'/q^* after parameter estimation for C_B [to avoid calibration artifacts as outlined in Appendix 1] and represents the value of C_B'/q^* normalized by the value of the slope obtained from the individual plots) for KH-perfused hearts studied with $H_2{}^{15}O$. The correlation coefficients for each of the three normalized groups of data obtained (KH-$H_2{}^{15}O$, KH-RBC-$H_2{}^{15}O$, and [^{11}C]butanol-KH-RBC) were 0.95 (n = 52 determinations), 0.90 (n = 89), and 0.93 (n = 13). The closeness of the correlations indicates that the estimates of flow obtained with both tracers and with both types of buffer-perfused hearts conform closely to actual flow.

Refinements of data analysis. Although the correlation coefficients indicate a high degree of linearity for all hearts, the estimated tracer blood:tissue partition coefficients (λ) varied considerably among hearts from the predicted values of 0.77 for the $H_2{}^{15}O$ group in KH-perfused hearts, 0.90 for the $H_2{}^{15}O$ and KH-RBC perfused group, and 1.0 for [^{11}C]butanol-KH-RBC perfused hearts. Accordingly, we considered the likelihood that our measured tissue tracer activity may have been affected by some tracer retained in the cardiac chambers. Such retention is characteristic of the exper-

TABLE II

*Observed Arterial Tracer Curves of the Exponential Intravenous Infusions**

Dog	Tracer	Sample site	Exponential infusion pump rate constant	Arterial blood exponential time-activity rate constant	r‡
			s^{-1}	s^{-1}	
1					
Run 1	$H_2{}^{15}O$	Carotid	0.0347	0.0680	0.98
Run 2	$H_2{}^{15}O$	Carotid	0.0231	0.0673	0.98
Run 3	$H_2{}^{15}O$	Carotid	0.0231	0.0698	0.99
2					
Run 1	$H_2{}^{15}O$	LV§	0.0347	0.0655	0.97
Run 2	$H_2{}^{15}O$	Aorta	0.0231	0.0546	0.96
Run 3	$H_2{}^{15}O$	Aorta	0.0231	0.0539	0.97
Run 4	$H_2{}^{15}O$	LV	0.0347	0.0623	0.98
Run 5	[^{11}C]butanol	LV	0.0347	0.1645	0.98
Run 6	[^{11}C]butanol	LV	0.0173	0.0502	0.95
Run 7	[^{11}C]butanol	LV	0.0173	0.0497	0.98

* Data from experiments with dogs are shown. Analogous results (not shown) were obtained with rhesus monkeys.

‡ Correlation coefficient between observed values and the best fit monoexponential curve (least squares method).

§ LV, left ventricle.

imental preparation used in this study, but would not be included in measurements by PET in vivo. To quantify and account for the potential contributions of the factor to the variance in estimates of partition coefficients in isolated hearts, we injected boluses (0.1 ml each) of tracer (50–100 μCi) into a cannula sutured either into the right atrium, for the assessment of right heart tracer turnover rate (K_R), or into a cannula passed into the balloon-filled left ventricle via the left atrium, for the assessment of the left ventricular chamber tracer turnover rate (K_L). The values obtained for K_R and K_L were linear functions of pulmonary outflow and left ventricular leakage flow, respectively, with an average correlation coefficient of 0.91. Using experimentally determined values for K_R and K_L, we performed an additional series of experiments in which we corrected the measured tissue tracer activity for activity found in the chambers, as outlined in Appendix 2. Six hearts perfused with KH-RBC were studied with $H_2{}^{15}O$, and three hearts perfused with KH-RBC were studied with [^{11}C]-butanol. The recorded time-activity data was transformed to reflect only the myocardial tracer activity, as outlined in Appendix 2, and utilized to obtain plots of β/F_i vs. C_B'/q^*. The correlation coefficients and estimated partition coefficients obtained in this fashion are shown in Table III. As shown in Fig. 4, plots made for all hearts studied with $H_2{}^{15}O$ and with [^{11}C]butanol exhibited composite correlation coefficients of 0.99 (n = 41 determinations) and 0.92 (n = 6 determinations), respectively. Furthermore, the estimated partition coefficients did not differ significantly, at the P = 0.005 level, from the known partition coefficient (λ) of each tracer. These results indicate that the scatter in the estimates of λ evident in the earlier results with isolated hearts was attributable to contributions from tracer retained in the cardiac chambers in such preparations. Furthermore, by incorporating the slopes of the

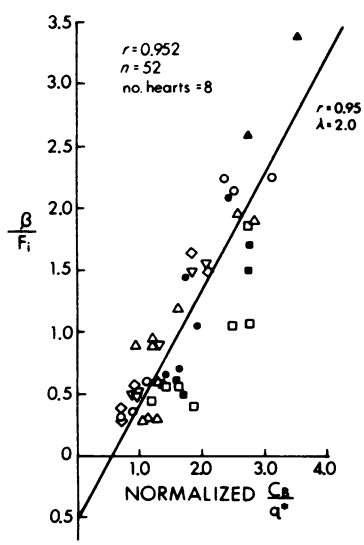

FIGURE 3 Results obtained from exponential infusions of $H_2{}^{15}O$ in eight KH-perfused hearts. β and Fi are the exponential infusion rate constant and estimated flow: C_B and q* are the normalized concentration of tracer in the perfusate and the derived activity function from Eq. 3. Each type of symbol refers to data from the same heart.

TABLE III

Correlation Coefficient (r) of Parameters Used to Estimate Flow and the Tissue:Perfusate Partition Coefficient (λ) in Experiments from Nine Perfused Hearts with Data Corrected for Chamber Activity

Heart	Tracer	r	Estimated λ
1	$H_2{}^{15}O$	0.99	0.72
2	$H_2{}^{15}O$	0.99	0.91
3	$H_2{}^{15}O$	0.99	0.95
4	$H_2{}^{15}O$	0.99	0.94
5	$H_2{}^{15}O$	0.99	0.86
6	$H_2{}^{15}O$	0.99	0.91
7	$H_2{}^{15}O$	0.92	1.01
8	[^{11}C]Butanol	0.96	0.95
9	[^{11}C]Butanol	0.88	0.76

All hearts were perfused with KH-RBC.

plots of each heart as a calibration factor in Eq. 5, plots of estimated flow vs. measured flow can be obtained. Fig. 5 depicts such a plot for the $H_2{}^{15}O$ hearts.

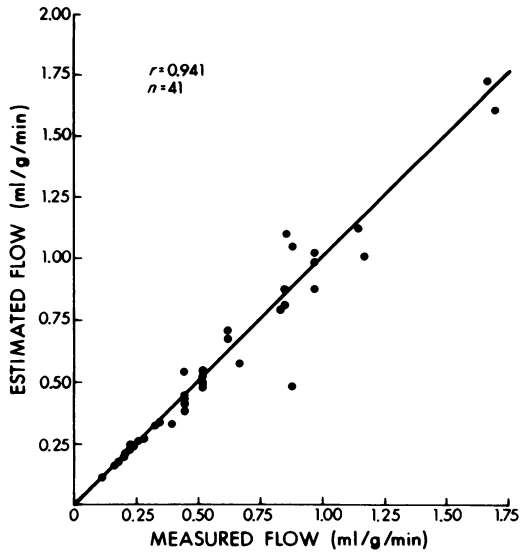

FIGURE 5 Results with exponential infusions of $H_2{}^{15}O$ in six KH-RBC perfused hearts. Estimated flow is compared with flow measured by collection of effluent with tracer data corrected for chamber activity.

DISCUSSION

Noninvasive, quantitative assessment of myocardial perfusion is of considerable potential importance. Delineation of the distribution and extent of impaired regional perfusion at rest or with stress could provide valuable diagnostic information for the characterization of coronary artery disease and objective assessment of therapy designed to augment or favorably redistribute perfusion. Although several approaches have been developed to assess regional myocardial perfusion externally (8–18), each entails some unavoidable limitations. One approach that has been employed, namely constant infusion of a diffusible tracer, suffers from potential constraints discussed in the Introduction.

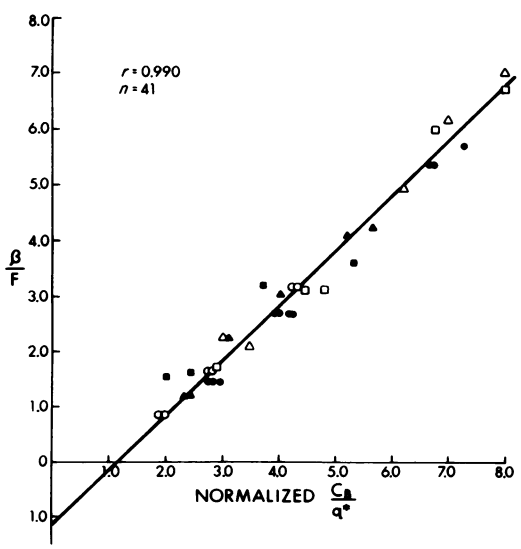

FIGURE 4 Results with exponential infusions with $H_2{}^{15}O$ in six KH-RBC perfused hearts with data corrected for chamber activity. Axes and symbols are defined as in Fig. 3.

Although the isolated perfused heart preparation is a simple preparation in contrast to the use of intact experimental animals and patients, it permits control of flow and exclusion of recirculation under conditions in which the metabolic environment and physiological demands of the heart can be controlled and monitored. Analysis of behavior of tracer in such preparations permits delineation of the relative quantitative importance of specific, potentially influential, biological factors. Accordingly, the approach developed and evaluated in this study for external assessment of regional coronary perfusion was tested initially in isolated perfused hearts and applied to global perfusion only.

Results obtained indicate that the programmed exponential infusion technique provides a promising approach for externally assessing myocardial perfusion with positron radionuclides, with results of estimated flow conforming to actual flow over a wide range despite marked variation of myocardial metabolism induced by low flow.

Clinical implications. Results of this study indicate that the exponential infusion technique is potentially applicable to external assessment of regional myocardial blood flow in vivo in experimental animals and ultimately in patients. The results show that the required coronary artery tracer concentration can be achieved in vivo by exponential intravenous adminis-

tration of isotope in dogs, and that close correlations are observed between estimated and independently measured flow in isolated rabbit hearts. [^{11}C]Butanol was selected because its lipid:aqueous partition coefficient should avoid potential artifacts from heterogeneous distribution of cardiac lipid constituents in vivo. It has been synthesized successfully (1). Calculations indicate that the exponential infusion required will entail lower organ radiation burdens than encountered with the constant infusion method (e.g., 93% less for the case of [^{11}C]butanol).

In the current study with isolated hearts, C_B' had to be calculated (Appendix 1), and corrections had to be made for tracer in the right and left heart chambers. These methodological considerations apply only for the particular preparation used, the isolated perfused heart. For patient studies, a gated tomographic scan will be performed with either [^{11}C]palmitate or ^{11}CO-hemoglobin to outline the ventricular myocardium and the heart chambers. The tracer used for measurement of myocardial blood flow will be infused exponentially via a peripheral vein and gated tomographic scans will be obtained. Gating will facilitate definition and differentiation of tracer in the blood from that in the myocardium, and will reduce partial volume effects of the beating heart. Myocardial activity (q[t]) and ventricular chamber blood activity (C_B[t]) will be obtained simultaneously from each tomographic slice.

C_B' (blood activity at time 0) will be calculated by fitting a monoexponential function to chamber blood activity ($C_B[t]$) obtained from serial scans and extrapolating the exponential to time 0. The derived steady-state function (q*) will be calculated based on myocardial activity (q[t]) (Eq. 3) after the pseudosteady-state level has been achieved (Eq. 4).

Although gating will decrease the number of counts recorded per second, counting statistics for the myocardial tissue activity can be controlled by the total scan time. Because the technique provides a value of the derived myocardial tracer activity function (q*) that remains constant once it plateaus, the total scan time can be manipulated to ensure adequate counting statistics. Counting statistics for ventricular cavity blood activity can be optimized by integrating counts from all positions of the chamber common to systole and diastole. Thus, the model parameters C_B' and β can be determined from the serial gated scans of the ventricular blood activity, and the model parameter q* can be determined regionally from the gated scan of the myocardium, based on consideration of the elapsed time from the onset of the exponential infusion (Eq. 3). The tissue:blood partition coefficient (λ) is a previously experimentally determined quantity of the tracer. Regional myocardial mass and, by calculation, flow/mass, can be determined from definition of pixel size and depth. We have shown that the resolution of the PET instrument is sufficient to permit quantification of the regional distribution of tracer within hearts of patients during imaging, and that regional tissue mass can be determined accurately and reproducibly by knowing pixel size and depth (19–21). Independent assessment of regional left ventricular mass and wall thickness can be utilized if necessary with techniques such as echocardiography. Before patient studies are initiated, experiments in animals will be performed to validate the method by comparing calculated myocardial flows with flow values obtained with microspheres. It appears likely that the technique developed and evaluated in this study will be readily applicable to large experimental animals and ultimately to patients, and that it will facilitate accurate, noninvasive, external quantification of regional coronary perfusion.

APPENDICES

Appendix 1: exponential infusion model. For an exponential delivery of tracer to the coronary arteries, $C_B'e^{\beta t}$, the exponential infusion model yields the following differential equation:

$$\frac{dq}{dt} + (\kappa + \alpha)q - FC_B'e^{(\beta-\alpha)t} = 0 \qquad (7)$$

where q = myocardial tissue activity per unit mass of tissue; κ = myocardial perfusion turnover rate; α = isotope physical decay constant; F = myocardial blood flow per unit mass of tissue; C_B' = arterial tracer concentration; and β = exponential infusion rate constant.

Solution:

$$q = \frac{FC_B'}{\kappa + \beta}(e^{(\beta-\alpha)t} - e^{-(\kappa+\alpha)t}) \qquad (8)$$

let $q^* = qe^{(\alpha-\beta)t}$; then

$$q^* = \frac{FC_B'}{\kappa + \beta}(1 - e^{-(\kappa+\beta)t}). \qquad (9)$$

As t increases without bound,

$$q^* = \frac{FC_B'}{\kappa + \beta},$$

but

$$\kappa = \frac{F}{\lambda} \qquad (10)$$

(Central Volume Principle). Therefore,

$$F = \frac{\beta}{\frac{C_B'}{q^*} - \frac{1}{\lambda}}; \qquad (11)$$

and by linearization,

$$\frac{\beta}{F} = \frac{C_B'}{q^*} - \frac{1}{\lambda}. \qquad (12)$$

However, for the isolated heart preparation using a pair of coincident scintillation probes, the parameters C_B and q can be measured only indirectly. Because of the geometrical

dependence of the response of the probes, it is impossible to calibrate directly an independent arterial blood tracer concentration measuring device with the probe system. In addition, the scintillation detectors do not record all of the positron emissions from the isolated heart, but only ~10% under the conditions employed. Therefore, we devised a parameter estimation scheme in which a series of exponential infusions are performed on each heart at selected flows.

The parameter C_B is derived as a ratio from each infusion referenced to the first infusion as follows:

$$\frac{Cpi(t)}{Cpo(t)} = \frac{Ai \cdot Fo \cdot K}{Ao \cdot Fi \cdot K} = \frac{Ai \cdot Fo}{Ao \cdot Fi} \tag{13}$$

where Cpi(t), Cpo(t) = arterial tracer concentration for experiment i and experiment o (first experiment) in counts per second per milliliter as recorded by probe system; Ai, Ao = infused tracer activity measured by an independent detector system in counts per second per milliliter for experiments i and o; Fi, Fo = perfusate flow to the aorta in milliliters per second for experiments i and o; and K = probe efficiency/calibration factor.

In addition,

$$q^* = K_T \cdot q^*_p \tag{14}$$

where $q^*_p = q_p e^{(\alpha-\beta)t}$ and q^* = derived pseudosteady-state quantity of tracer in myocardium in disintegrations per second per gram; q^*_p = derived pseudosteady-state activity of tracer in myocardium as recorded by the probe system in counts per second per gram; and K_T = scintillation detector calibration/efficiency factor in disintegrations per second per counts per second. Therefore, substituting Eqs. 13 and 14 yields:

$$\frac{\beta}{Fi} = \frac{C_{po}}{AoK_T}\left(\frac{AiFo}{Fiq^*_p}\right) - \frac{1}{\lambda}. \tag{15}$$

By performing several experiments on the same isolated heart at selected flow rates and plotting the results as a graph of β/Fi vs. Ai Fo/Fi q^*_p, the unknown parameters, K_T and Ao, become the slope of a straight line fitted to the data [slope = Cpo/(AoK$_T$)]. Additionally, the Y-intercept of the fitted straight line estimates $-1/\lambda$ (Fig. 2).

Appendix 2: three-compartment heart model. With consideration of activity within the isolated heart chambers, the exponential infusion model can be expanded with a model that assumes some of the flow entering the heart via the aorta leaks into the left ventricle. Additionally, the model assumes that the perfusate exiting the myocardium via the coronary veins enters the right atrium and then leaves the heart via the pulmonary artery. This three-compartment model yields the following series of equations:

$$q_{TOT} = q_M + q_L + q_R \tag{16}$$

$$\frac{dq_M}{dt} + (K_M + \alpha)q_M - FC_B'e^{(\beta-\alpha)t} = 0 \tag{17}$$

$$\frac{dq_L}{dt} + (K_L + \alpha)q_L - F_LC_B'e^{(\beta-\alpha)t} = 0 \tag{18}$$

$$\frac{dq_R}{dt} + (K_R + \alpha)q_R - (K_M + \alpha)q_M = 0 \tag{19}$$

with q_M, q_L, and q_R = myocardial, left ventricular, and right atrial chamber activity.

Solution:

Let $q^* = qe^{(\alpha-\beta)t}$.

As t increases without bound,

$$q^* = \frac{F_MC_B'}{\dfrac{F_M}{\lambda V_M}}\left(1 + \frac{\dfrac{F_M}{\lambda V_M} + \alpha}{K_R + \beta}\right) + \frac{F_LC_B'}{K_L + \beta}. \tag{20}$$

The time constants for the three compartments are:

$$(a)\ \text{myocardium} = K_M + \beta; \tag{21}$$

$$(b)\ \text{left ventricle} = K_L + \beta; \tag{22}$$

and

$$(c)\ \text{right atrium} = K_R + \beta. \tag{23}$$

To avoid long delays to steady state due to K_R and K_L, we used the three-compartment model to estimate the myocardial tissue tracer activity from the measured total heart tracer activity as follows:

$$q_M(T) = q_T(T) - \frac{F_A - F_P}{F_A}\int_0^T \dot{q}_T(t)e^{-(K_L+\alpha)(T-t)}dt - \int_0^{T-\bar{t}} \dot{q}_T(t)e^{-(K_R+\alpha)(T-t)}dt \tag{24}$$

Where $q_M(T)$, $q_T(T)$ = myocardial and total heart tracer activity for time T per unit mass of tissue; T = time after commencement of infusion of tracer; t = dummy time variable of integration; and F_A, F_P = flow of aortic and pulmonary perfusate; K_L, K_R, K_M = left ventricular, right atrial, and myocardial turnover rate of tracer; α = isotope decay constant; and $\bar{t}$ = mean transit time of tracer through myocardium. The computed myocardial tracer activity, $q_M(T)$, can then be used to find the steady-state derived tissue activity parameter, q^*, which is subsequently used in the linearized flow Eq. 6.

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