Published in final edited form as:

Circ Res. 1995 December ; 77(6): 1212–1221.

Fractal¹⁵O-Labeled Water Washout From the Heart

James B. Bassingthwaighe and Daniel A. Beard

Center for Bioengineering, University of Washington, Seattle

Abstract

To characterize the washout of water from the heart, we used a flow-limited (not diffusion- or permeability-limited) marker for blood-tissue exchange, namely, tracer-labeled water. Experiments were performed by injecting ¹⁵O-labeled water into the inflow to isolated blood-perfused rabbit hearts and by recording the tracer content in the heart and in the outflow simultaneously for up to 5 minutes. The data exhibit a particular combination of power law forms: (1) The downslopes of the residue and outflow curves were both power law functions, with the residue diminishing as $t^{-\alpha}$ and the outflow as $t^{-\alpha-1}$, where α is interpreted to be the dimensionless exponent of a fractal power law relation characterizing the selfsimilarity inherent in each curve. (2) The fractional escape rate, given by the outflow curve divided by the residue curve, diminished almost exactly as t^{-1} . In 18 sets of curves, α averaged 2.21 ± 0.27 . These results lead to an improved method for extrapolating the downslopes of indicator dilution curves to estimate their areas and therefore the blood flows. The evidence also points strongly to the conclusions that myocardial water washout is a fractal process and that stirred tank models are inappropriate for the heart.

Keywords

flow-limited blood-tissue exchange; power law kinetics; positron emission; oxygen-15; capillary permeability; statistical self-similar processes

Over the last five decades, the washout of tracers from a body or from an individual organ has been used to obtain inferential information about the processes governing the retention of the tracer within the organ. This is important for drug kinetic studies; eg, the retention of drug is usually a measure of the slowness of its dissociation from a receptor site. In other circumstances, however, when chemical binding and unbinding reactions are either very fast or not involved and when there is no limitation to tracer washout because of slow diffusion or retarded permeation of membranes, then the washout from an organ is dominated principally by the flow per unit mass of tissue. A purely intravascular marker that does not stick to or cross the endothelial barrier will be “flow-limited” in its local and global washout. Inert tracers, which distribute rapidly across the membranes of an organ so that there is effectively instantaneous local blood-tissue equilibration of each point along the capillaries, also display flow-limited washout.^{1,2} Whether or not tracer washout is completely flow-limited, its mean transit time, $\bar{t}$, equals V/F , where V (in milliliters per gram) is the volume of distribution of the tracer within the organ, and F is the flow (in milliliters per gram per minute). This is simply a statement of conservation of mass and is true whether or not there are limitations to the exchange rate by membrane barriers or slow diffusion.

Organs are not normally homogeneously perfused. Regional flows in the heart are broadly heterogeneous.³ In both the heart⁴ and the lung,⁵ the spatial variation appears to be fractal. This means that the spatial heterogeneity shows statistical self-similarity. The apparent variance or standard deviation of the regional flows is dependent on spatial resolution: observing smaller regions with higher resolution reveals further variation. Finding that the smaller regions are nonuniform is proof that the overall apparent heterogeneity must increase with higher resolution. In special cases, a proportional increase in resolution reveals a proportional increase in heterogeneity. This relation follows the power law function:

$$\frac{RD(m)}{RD(m_0)} = \left(\frac{m}{m_0} \right)^{1-D} \quad (1)$$

where RD is the standard deviation divided by the mean flow for the organ at a particular resolution level defined by m , the mass of the individual pieces into which the organ has been divided (that is, piece mass) in order to make the observation; m_0 is an arbitrarily chosen reference mass, usually 1 g; and D is the fractal dimension. The spatial fractal relation holds in the hearts of awake baboons and of anesthetized sheep and rabbits over a 100-fold range of piece masses in the heart⁴ and also the lung.⁵

Turn now to the temporal characteristics or kinetics of tracer exchange in the heart. These are not predictable from a knowledge of regional spatial flow distributions. For example, the spatial flow distributions are approximately gaussian: assuming that the local volume of distribution, V_i , for a tracer is the same in all regions, then the time course of tracer transit times for an intravascular tracer traveling between inflow and outflow would be directly calculable. The mean transit time for the i th path, $\bar{t}_i$, is as follows:

$$\bar{t}_i = V_i / F_i \quad (2)$$

where F_i is the i th flow and V_i is the vascular volume along the i th pathway. Even if the V_i s were the same everywhere, which they are not, then the distribution of the $\bar{t}_i$ s would be skewed to the right in a fashion dependent on the distribution of F_i s.

However, there is some heterogeneity of regional blood volumes,⁶⁻⁸ so the analysis of washout times would be further confounded by the volume variation in an unpredictable fashion. The alternative is to seek a flow-limited marker with a relatively uniform distribution space, ie, one with a steady state volume of distribution that has very small regional variation. The fractional water content of the myocardium is remarkably uniform^{7,9,10} at 0.78 ± 0.01 mL/g. The washout curves for water, and for the lipid-soluble substance antipyrine, have been shown to be flow-limited in the heart.¹¹ Therefore, these are the preferred test tracers for examining the shapes of washout curves. These washout curves will not be influenced by variation in V_i , or by any diffusional retardation, nor will they be skewed by slow barrier penetration, such as that which prolongs the tails of dilution curves for potassium or sucrose or larger hydrophilic solutes. Consequently, washout curves for water will be governed by the velocities through the hundreds of thousands of vessels of the arterial and venous networks and scaled through the local equilibration via capillary-tissue exchange by the ratios of blood space to total water space. The washout time course will thus be dominated by pathway transit time distributions; the influence of the blood-tissue exchange process for water is to render all the V_i s uniform, leaving the $\bar{t}_i$ s reciprocal to the local F_i s. The observations of Rose et al¹² suggest a small barrier limitation in the highest flow regions, which might affect the upslope and peak of the curves but would not affect the tails of the curves or our analyses.

This analysis focuses on the question of the nature of the distribution of washout times. The resultant observation that the distribution appears fractal is revealed for the first time in the present study. The result contrasts remarkably with and clearly refutes the long-held perception that washout is an exponential process. This idea was the basis of the Stewart-Hamilton method¹³ for extrapolating the tails of dilution curves mono-exponentially in order to measure the areas under indicator dilution curves for the estimation of organ flow or cardiac output. The results to be shown below will be used to argue that the Stewart-Hamilton method slightly but systematically underestimates the areas and so gives a minor degree of overestimation of flows.

Materials and Methods

Experiments were performed on seven isolated blood-Perfused rabbit hearts (New Zealand White rabbits with hearts weighing 6 to 8 g) by using $^{15}\text{OH}_2$ water as the indicator. (^{15}O is a positron emitter with a half-life of ≈ 121 seconds.) The perfusate was Krebs-Ringer bicarbonate solution with 1% albumin, to which was added triply washed beef red blood cells to a hematocrit of 20%. The partial Pressure of oxygen was 145 to 180 mm Hg. The isolated heart was mounted on a perfusion apparatus. The residual tracer activity within the heart, $R(t)$, was obtained via a pair of NaI crystal gamma detectors situated on opposite sides of the heart and used in coincidence mode to provide a Measure of the two simultaneous gamma emissions resulting from positron annihilation events occurring in the heart. The coincidence method reduces the counting of emissions from other sources, including scatter from outside the cylindrical region in which the heart was situated. The coronary sinus and right ventricular thebesian veins drained into an outflow cannula inserted through the pulmonary valve. The weight of blood in the outflow cannula, positioned below heart level, holds the pressure in the right ventricle at a negative pressure; therefore, the ventricle stays empty. ^{15}O detectors sensitive directly to the positron (positive beta) particles were set up on the inflow tubing and outflow tubing, giving the inflow, $C_{\text{in}}(t)$, and outflow concentration-time curves, $C(t)$. The structure of the detectors (made in our laboratory) was similar in design to that described by Lerch et al.¹⁴ All three curves were corrected for the isotopic decay of ^{15}O , which has a half-life of 121 seconds.

By this experimental approach, we attempted to minimize error in the data acquisition. Because the outflow curve is a slightly dispersed and delayed measure of the derivative of the residue curve and because both outflow and residue curves must conserve mass (account for the amount of tracer in the inflow curve), analyzing the set of curves together minimizes error due to incompleteness of outflow collection because of leaks or contamination of $R(t)$ by any accumulations of tracer leaked from the heart and included in the signal because of the poor collimation.

Analytical Methods and Indicator Dilution Theory

Basic conservation theory, as summarized by Zierler,¹⁵ maintains that after an impulse injection at $t=0$ into the entrance to an organ, the fractional residual content $R(t)$ at the next instant is 100% of the injectate. $R(t)$ remains at 1.0 until there is an appearance of tracer in the outflow. The fraction of the injectate appearing in the outflow per unit time is $h(t)$ (fraction per second). The accumulated outflow is the integral of the outflow response and is the total dose, unity, minus the fraction of dose still retained within the organ, $R(t)$:

$$\int_0^t h(\lambda) d\lambda = 1.0 - R(t) \quad (3)$$

where λ is a dummy variable used in the integration. Further, in the ideal situation, in the absence of error in the data acquisition, the outflow fraction of the dose appearing per second is the derivative of the organ content:

$$h(t) = -dR/dt \quad (4)$$

In practice, one records neither $R(t)$ nor $h(t)$ because injecting an impulse input is impossible. Instead, the signal recorded from the heart, $Q(t)$, is a convolution of $R(t)$ with the input:

$$Q(t) = \bar{F} \cdot C_{in}(t) * R(t) \quad (5)$$

where $\bar{F}$ is the mean flow, and the asterisk denotes the convolution integration, more formally written as $Q(t) = \bar{F} \int_0^t C_{in}(\lambda) \cdot R(t - \lambda) d\lambda$

Likewise, the outflow concentration-time curve, $C(t)$, is as follows:

$$C(t) = C_{in}(t) * h(t) \quad (6)$$

When the input curve, $C_{in}(t)$, is completed within a period that is short compared with the mean retention time within the organ, then the tail portion of $h(t)$ has a form influenced almost solely by the network characteristics and scarcely at all by the form of C_{in} . (For these experiments, the input was 99% complete in 18 to 22 seconds, which was short compared with washout, which took 3 to 6 minutes.) Given these conditions, a good approximation for the residue function is as follows:

$$R(t) \simeq \frac{Q(t)}{Q_{max}} \quad (7)$$

where Q_{max} is the maximum value of the curve recorded via the coincidence counters. Although the approximation is poor in the initial seconds, it is good thereafter. Likewise, the approximation for the tail of $h(t)$ is the normalized outflow concentration-time curve:

$$h(t) = \frac{C(t)}{\int_0^\infty C(t) dt} \quad (8)$$

where the denominator is the area under the curve $C(t)$. Alternatively, $h(t)$ can be calculated by using the injected dose, q_0 , and the measured flow, $\bar{F}$, so that $h(t) = \bar{F}C(t)/q_0$, illustrating that $h(t)$ is the fraction of the dose reaching the outflow per unit time.

The fractional escape rate, $\eta(t)$, is defined as the fraction of residual tracer escaping per unit time, and is therefore given directly by the amount of tracer appearing in the outflow per unit time, $\bar{F}C(t)$, where $\bar{F}$ is the total flow, divided by the amount of tracer retained within the organ at each time t :

$$\eta(t) = \frac{h(t)}{R(t)} \simeq \frac{\bar{F}C(t)}{Q(t)} \quad (9)$$

This review of standard indicator dilution theory and of the secondary approximations sets the stage for the special approach to the analysis using fractals. Note that there are two assumptions in the normalization of Equation 9 in going from the middle to the right

expression: (1) $Q_{\max}$ in Equation 7 is obtained at a time when all of the tracer is in view of the coincidence detectors, with all of the tracer having entered and none having left. (2) The area of the outflow washout is essentially complete. Assumption 1 seems reasonable in view of the fact that the input curves, C_{in} , are 99% complete by 18 to 22 seconds (not illustrated), although $R(t)$ does have a measurable plateau since the washout begins at about this time. Assumption 2 is valid within <2%, as can be seen from the final values of $R(t)$ at $t=300$ seconds in Fig 1.

A Fractal Theory To Be Tested

The fundamental feature of a fractal is self-similarity or self-affinity. A fractal washout process is therefore one for which the rate of washout decreases by some exact proportion for some chosen proportional increase in time; the self-affinity requirement is fulfilled whenever the “exact proportion” remains unchanged, independent of the moment or the segment of the data set selected to measure the proportionality constant. The length of time or the portion of the washout curve for which this relation holds is known as the “scaling region” and is necessarily finite, as is the case for all natural, nonmathematical, fractals. Fractal washout behavior can begin after the input to the organ is complete. If complete, then one would expect the late portion of $R(t)$ to have the shape of a power law relation defining the proportionality relation:

$$R(t) = At^{-\alpha} \quad (10)$$

where α is the power law exponent. (The observation of a power law relation is not an explanation of the phenomenon but provides an incentive for a search for an explanation.) This power law relation defines the log-log slope $d \log R / d \log t = -\alpha$. The corollary of this is that the outflow tracer concentration-time curve should also be fractal and since $h(t) = -dR/dt$, then taking the derivative of the right side of Equation 10 yields

$$h(t) = A\alpha t^{-\alpha-1} \quad (11)$$

and the slope on the log-log plot is $d \log h(t) / d \log t = -\alpha - 1$.

According to the hypothesis, expressed in Equations 10 and 11, $\eta(t)$ should be a power law function with an exponent equal to -1 :

$$\eta(t) = \frac{h(t)}{R(t)} = \frac{A\alpha t^{-\alpha-1}}{At^{-\alpha}} = \alpha t^{-1} \quad (12)$$

Three equations pose the tests of the fractal washout hypothesis: Over some substantial period of time late in the washout, (1) does Equation 10 provide a good fit to $R(t)$, (2) does Equation 11 provide a good fit to $h(t)$, and (3) can one value for α be used to fit Equations 10 and 11 to the data simultaneously obtained for $R(t)$ and $h(t)$? If so, then does Equation 12 provide a good fit to the data for the fractional escape rate $\eta(t)$, with the specific power law slope of -1 ; ie, does $\eta(t)$ decay at a rate proportional to $1/\text{time}$?

Results

Eighteen sets of experimental data were recorded from seven rabbit hearts weighing 8.76 ± 0.78 g (mean $\pm$ SD) with perfusate hematocrits of $20.3 \pm 0.01\%$. Data from inflow and outflow and from the heart itself were recorded for 140 to 440 seconds after injection. In the subsequent analysis, the figures have been chosen to give an impression of the average result

rather than “best” results and to show as many different data sets as possible. Results are expressed as mean $\pm$ 1 SD for the 18 data sets from these seven hearts.

Fig 1 shows the type of curves obtained. Both the residue and the outflow curves are continuously concave upward on the semilogarithmic plot (left); therefore, these curves are not monoexponential. On the log-log plot, both $R(t)$ and $h(t)$ are apparently straight for times $>\approx 1$ minute. The slope of the outflow tracer concentration-time curve is steeper than that of the residue function, as was seen for all of the sets of data; this is a major point, for it denies the suitability of a stirred tank or mixing chamber model where $R(t)$ and $h(t)$ would have the same slope fitted by a single exponential.

Because the input to the system is somewhat dispersed rather than being an ideal instantaneous impulse, the values of $R(t)$ do not peak at $t=0$; the peaks of $R(t)$ occurred at 8 to 30 seconds, averaging 19 ± 8 seconds, whereas the peaks of $h(t)$ occurred at 14 to 47 seconds, averaging 29 ± 12 seconds. The delays in the tubing from the heart to the outflow detector are on the order of 1 to 3 seconds. (The volume was 0.12 mL, and the flows were 5 to 30 mL/min.) The dispersion due to this tubing has no detectable influence on the shape or slope of the outflow curves.

The data of highest relevance to our analysis are the tails of the curves beyond the first minute, where the washout is clearly not significantly influenced by the form of the input, because all of the transit times are long compared with the duration of the input. The early parts of $R(t)$ and of $h(t)$ are therefore clearly influenced by the form of the input function, but the later portions of the curves are not, since the input was complete in 10 to 15 seconds. The subsequent analysis is performed on the data beyond, where $R(t)/R_{\max} < 0.2$; these are the data beyond the first 50 to 80 seconds after the injection, and the data in most runs extend to ≈ 300 seconds.

Unconstrained Fitting of R , h , and η

The tails of the residue $R(t)$, outflow $h(t)$, and the escape rate $\eta(t)$ for two experiments are plotted on log-log scales in Fig 2. Each curve was fit with the log-log regression to give best estimates of the power law exponent. The values from 18 experiments for α from the residue functions, fitting Equation 10, were 2.12 ± 0.33 (mean $\pm$ 1 SD); the values for α from the outflow curves, fitting Equation 11, were 2.13 ± 0.34 . These values were obtained independently from residue and outflow data. The coefficients of variation for the fits to the $R(t)$ s averaged 0.073 ± 0.042 ($N=18$); the coefficients of variation for the fits to the outflow curves averaged 0.074 ± 0.070 ($N=18$). Thus, the independently estimated values of α from residue and outflow were close to each other (2.12 and 2.13). The paired differences averaged 0.005, with a standard deviation of 0.43 for the 18 pairs. Thus, the first two tests listed below Equation 12 are satisfied and do not cause us to reject the fractal relation, since the two independent estimates of α are not statistically different, as tested by either unpaired or paired differences by Student's t test.

The curves for the fractional escape rate $\eta(t)$ are necessarily noisier than those for either $h(t)$ or $R(t)$, since $\eta(t)$ is calculated by dividing one curve by the other (Equation 9). Because of the noise, the coefficients or Ration for the power law regression fitting are larger (0.21 ± 0.16 , $N=18$). The power law exponents obtained for unconstrained fitting of the equation $\eta(t)=kt^{-\beta}$ gave estimates of $\beta=1.12\pm 0.47$ ($N=18$), so that the average of the unconstrained estimates of β did not differ statistically from 1.0, the value theoretically anticipated in Equation 12. Again, the fractal hypothesis cannot be rejected.

Comparing Power Law With Monoexponential Fits

Fig 3 shows optimized best fits using both the power law expressions, $\hat{R}(t)=a_1 t^{-a_1}$ and $\hat{h}(t)=a_2 a_2 t^{-a_2-1}$, and the exponential expressions, $\hat{R}(t)=A_1 e^{-k_1 t}$ and $\hat{h}(t)=A_2 e^{-k_2 t}$. The test was designed as a test of appropriateness of extrapolation to predict the shape of the last parts of the tails of $R(t)$ and $h(t)$; to do this, the model functions were fit to only a limited segment of the data: for $R(t)$, from 0.2 to 0.1 only; for $h(t)$, over exactly the same time period as for $R(t)$. For this test, the power law fits were not constrained to use the same α for $R(t)$ and $h(t)$ but were best-fitting regressions, log-log for the power law and log-linear for the exponential. The rate constants for the exponential fits represent best fits over the same segments of $R(t)$ and $h(t)$ as were used for the power law fitting.

The two sets of data shown in Fig 3 on semilogarithmic, not log-log, plots are representative of the predictive capacity of the two extrapolation methods. The power law extrapolation beyond the time where $R(t)=0.1$ always lies above the exponential best fit for both $R(t)$ and $h(t)$. For 15 of 18 $R(t)$ s, the power law extrapolation predicted and fit the data for $R(t)$ 0.1 better by far than did the exponential extrapolation; in two sets, the data sets were not long enough beyond $R(t)=0.1$ to make the distinction, and in one set, $R(t)$ was better fit by the exponential, but $h(t)$ for the same set was much better fitted by the power law. For $h(t)$ the results are less secure, because the choice of the region fitted, being defined by $R(t)$ and not by $h(t)$, means that the curves are relatively noisy. For 12 curves, the power law fit was better by far; for 4 curves, the exponential fit was as good; and for 2 curves, the exponential fit was distinctly better. Of the 4 intermediate results, the data in 2 cases lay clearly in between the 2 extrapolations (power law and exponential) and did not fit either, and in 2 cases, the data points were too scattered to make the distinction.

The areas under the tail of $R(t)$ were underestimated by the monoexponential extrapolation, with the ratio to that estimated by the power law being 0.64 ± 0.12 ($N=18$). For $h(t)$, the ratio of areas by exponential fit over that for power law was 0.63 ± 0.08 ($N=18$).

From the point of view of estimation of total areas of curves, this difference is not great, a 3% to 4% under-estimation for $R(t)$ and less than that for $h(t)$. The errors are of course greater for mean transit times, where the tails have more influence on the estimate.

Using One α for Fitting R , h , and η

The most stringent test of the hypothesis is test 3. In this approach to obtaining the best estimate of α for each experiment, the data for $R(t)$ and $h(t)$ were fitted simultaneously by using $\hat{R}(t)=a_1 t^{-\alpha}$ and $\hat{h}(t)=a_2 a_1 t^{-\alpha-1}$. By this approach, the hypothetical fractional escape rate must have the form $\eta(t)=a_3(a_2/a_1)a_1 t^{-1}$; the exponent is forced to be -1 . The deviations of a_3 from 1.0 represent the difference in the sensitivities of the outflow detector (for positron emissions) and the residue detector (for 511-keV coincident gamma emissions). The best estimates of the a_i values and α were found by nonlinear optimization using SENSOP, a sensitivity function-based modified Levenberg-Marquardt type routine (Chan et al 16). In this approach, the fractal hypothesis of a difference of 1.0 between the power law exponents for $R(t)$ and $h(t)$ is assumed, and with this assumption having been made, the test lies in the goodness of fit to the data for the set of three curves. Two examples are shown in Fig 4. The values of α so estimated are reported in the Table. The values of α range widely around 2.0, averaging 2.2 ± 0.27 (mean $\pm$ SD, $N=18$). The constrained theoretical lines appear to be close enough to the three data sets so that the hypothesis cannot be rejected. The inference from both the slopes of $R(t)$ and of $h(t)$ and of the relation between their slopes is that the tails of the transit time distributions are approximately fractal in these isolated perfused rabbit hearts.

Generally, myocardial residue curves exhibit similarity upon scaling time by dividing by mean transit time.² This would be the same as scaling by a flow-to-volume ratio or simply by multiplying time by F when V is constant. One might intuitively expect a higher value of α at higher flows, and this is suggested by the positive slope of α versus F in Fig 5, but it is not definitive.

In Fig 6, a more specific test is performed with respect to the question of whether α is related to flow. Three sets of curves were obtained from one heart at flows of 0.76, 1.34, and 3.44 mL · g⁻¹ · min⁻¹. The three residue curves have been superimposed on each other by scaling time with respect to mean transit time (Fig 6, upper curves). The three outflow curves were treated likewise (Fig 6, lower curves). The close juxtaposition of the curves upon each other shows that their shapes are similar, meaning that their higher moments (variance and the skewness and kurtosis obtained from the third and fourth moments) are close to being the same. In Appendix A, this time scaling is applied to the function $h(t) = Aa\tau^{-\alpha-1}$, and it is shown that the exponent α is the same on the time-scaled $h(t/t)$ as on the original $h(t)$. Since scaling by flow should be equivalent to scaling by time, the theory would predict that in one heart at three different flows, the residue and outflow curves should exhibit the same power law exponent α . In this experiment (the one with the widest range of flows in the present study), the α values were 1.82, 1.91, and 2.03; ie, there is a 10% difference between the lowest and the highest. The trend for each individual heart in which there is a range of flows is like that for the whole data set in Fig 5. Thus, although the idea that α is related to flow is neither refuted nor supported by such data, there is room for suspicion despite the theory in Appendix A. Both Fig 5 and the analysis of the similarity-scaled data sets of Fig 6 leave open to further study the question of whether or not α increases with flow.

The absolute levels of myocardial blood flow influence the washout curve, $h(t)$, with higher flows giving an earlier peak and a shorter mean transit time. The fractional escape rate of tracer from the organ is higher at high flows. What is interesting about the fractional escape rate is the exponent: the power law slope of -1 is independent of the flow and of the shapes of $R(t)$ and $h(t)$. This universality is striking: whenever the system is fractal, the relation between $R(t)$ and $h(t)$ gives rise to $\eta(t) = at^{-1}$, a power law fractal such that in all situations the escape rate diminishes as t^{-1} . This is a strong statement because $\eta(t)$ is equal to the derivative of $R(t)$ and also to $h(t)$ divided by 1.0 minus its integral. What this means is that when $\eta(t)$ has an exponent of -1 , both $R(t)$ and $h(t)$ must be power law functions if either one is. The provocation provided by this observation is to find the physical explanation, as discussed below, and to ask whether it is necessarily fractal or allows some other descriptor.

Discussion

These experiments provide data covering about two orders of magnitude in $R(t)$ and three in $h(t)$. The experiment is made particularly powerful by virtue of having data for both residue and outflow. The experimental resolution obtained here has been made possible by using radiotracers, using high time-resolution counting systems on outflow and residues simultaneously over long times, and having low background radioactivity. These conditions are only obtainable in a nonrecirculating system and are most readily obtained with short-lived positron-emitting tracers. Although the 2-minute half-life of ¹⁵O has the disadvantage that the tails of the curves become rather noisy after three half-lives, correction for the decay is still adequate even when the concentrations are low, because it is practical to inject high doses of radioactivity without fear of contaminating the laboratory or risking significant radiation exposure.

It is not possible to do such experiments with standard dye dilution methods. The densitometers for indocyanine green, for example, suffer background drift of up to 2% in 1 minute with changes in blood optical density or other sources of variation, whereas with the ^{15}O -labeled water one can measure accurately down to 10^{-4} times the peak values, as is seen for the outflow curves.

The interpretation of the curves is that both the residue and outflow curves demonstrate self-similarity, in the sense that for each proportional increase in time (eg, twice as long), there is a constant proportional diminution in signal (eg, one quarter as great). In the parlance of the field of nonlinear dynamics, this is termed power law behavior. Such behavior is the hallmark of fractals—the self-similarity means that the apparent behavior (the scaling relation that says when time is twice as long, the signal is one quarter as great) is independent of the magnitude of the time unit considered.

The observation of the fractal time course of washout newly demonstrated in the present study is made secure by the simultaneous and coordinated measures of the three signals $R(t)$, $h(t)$, and $\eta(t)$. The observation that indocyanine green or ^{131}I -albumin dilution curves were not monoexponential but diminished more and more slowly as time progressed has been made when recirculation was sufficiently delayed¹⁷ or absent.¹⁸ When characterization of washout slopes, which were concave upward on semilog plots (as in Fig 1, left), was important, multiexponential fits were used, for example, for xenon washout from the brain by Hoedt-Rasmussen et al¹⁹; although this was expedient and gave good estimates of mean transit times, it also encouraged the misinterpretation that there were regions within the organ having two or three separate flows and inhibited the understanding that there was a broad heterogeneity of regional flows.

Fractal Extrapolation

For the practical purpose of measuring cardiac output, Hamilton et al¹³ proposed using monoexponential extrapolation of the downslope of dilution curves to obtain an estimate of the area under the primary first-pass indicator uncontaminated with recirculated indicator. This was an excellent and powerful suggestion and has been the method used ever since, even by those who recognized that it resulted in a minor underestimation of the area under the curve when the tails deviated (always upward) from monoexponential. Their technique is simple and allows an analytical calculation of the area under the extrapolated tail beyond the section of the down-slope used to estimate the exponential rate constant. Now we propose an equally simple method of extrapolation, but we use the power law expression to exclude the recirculation while accounting for all the first-pass indicator and avoiding the systematic underestimation of the exponential extrapolation.

The method is applied to either outflow curves, $h(t)$, or to residue curves, $R(t)$. The power law exponent is the negative of the slope of the regression of $\log h(t)$ versus $\log t$ for the tail region where the relation is a straight line: $\log h(t) = \log A - \beta \log t$. (For the regression between times t_1 and t_2 , an estimate of β is $\log [h(t_1)/h(t_2)]/\log [t_1/t_2]$.) The area, area 1, up to the time t_2 at which the extrapolation begins, can be obtained directly from the data. Area 2, under the extrapolated continuation of the observed data beyond t_2 , is calculated analytically:

$$\text{Area 2} = \int_{t_2}^{\infty} h(t) dt = \int_{t_2}^{\infty} h_2 \cdot (t/t_2)^{-\beta} d(t/t_2) = \frac{h_2 t_2^{1-\beta}}{\beta - 1} \text{ for } \beta > 1 \quad (13)$$

[The condition, $\beta > 1$, obviously holds for both $R(t)$ and $h(t)$ in the data of these experiments. The integral does not converge when $\beta = 1$, which means the area extrapolated would not be

finite.] The estimate of cardiac output or flow, F , for a single path system into which a bolus of indicator of amount q_0 is injected is $F=q_0/(\text{area 1}+\text{area 2})$.

For the residue and outflow curves in the present study, one can calculate the degree of underestimation of the areas that would result from using a monoexponential extrapolation. The calculation is based on using t_2 as the time where $R(t_2)/R_{\text{peak}}=0.1$, and for the purpose of estimating β , the beginning of the region fitted was t_1 the time at which $R(t_1)/R_{\text{peak}}=0.2$. The outflow $h(t)$ was fitted over the identical time period. For the 18 data sets, area 2 was calculated two ways: first as Equation 13, the fractal extrapolation, and second by monoexponential extrapolation. The monoexponential rate constant was determined from the best fitting linear regression of $\log h(t)$ or $\log R(t)$ versus t (linear). The results of the two extrapolation techniques give necessarily smaller values for the monoexponential approach: For $h(t)$, area 2 (monoexponential)/area 2 (fractal)= 0.637 ± 0.12 (N=18), and for $R(t)$, area 2 (monoexponential)/area 2 (fractal)= 0.630 ± 0.08 (N=18), where ± 1 SD is given. Naturally these errors in area due to the use of monoexponential extrapolation are much smaller than are errors in estimated mean transit times.

Why Is Washout Fractal?

The observed fractal washout may be explicable on the basis that regional flows per gram of tissue have fractal spatial distributions, as is well documented.^{4,5} The link between the spatial and temporal events is not yet defined by any theory, but it makes sense that transit times through a network with fractal flow distributions should be fractal, since regional transit times are the local volumes of distribution divided by the local flow.

It is interesting that the observed fractal exponents are possibly dependent on flow. A theory for thinking that α and F should be independent when washout curves can be superimposed on each other by proportional time scaling^{2,20} is given in Appendix A. The upward trend of α versus F in Fig 5 may be a hint that the fractal model is imperfect, which is no doubt the case, and some deviation toward multiexponential form is causing some degree of flow dependency.

The observation that washout is fractal does fit with the fractal paradigm newly recognized to apply to many aspects of biology. Self-similarity over a wide range of scales is found in time-dependent functions of many sorts. As in all physical systems, the summarizing descriptor, "fractal," applies over only a finite range. Fractals are not forever, except in the mathematics of the ideal, for every real fractal relation fails at both the large and small ends of the scale. The text of Bassingthwaighte et al²¹ presents many examples of these self-similar scaling relations, all of them limited to finite ranges. Fractal power law scaling can emerge from any set of processes repeated multiple times with appropriate scaling between members of the set. See Appendix B for the general theory and an example using multiple exponentials.

It is intriguing that the coronary vascular network has so many fractal characteristics. The anatomy itself is fractal in terms of segment diameters and lengths,²² the number of branches is a fractal function of the diameters of the vessel from which the branches derive,²³ the ratios of diameters of parent to daughter branches follow log-log scaling, and the flow distributions are spatial fractals.^{4,24} The degree of heterogeneity of regional flow distributions has been approximated by those calculated from artificial fractal branching patterns by using an overly simple dichotomous branching.²⁵ Both Gan et al²⁶ and VanBavel and Spaan⁸ estimate spatial distributions of flows from anatomic information; such calculations performed in our laboratory on artificial coronary systems give similar results and exhibit the same fractal correlation structure in regional flows in the heart as was observed in nature.²⁷ The present study adds to the story but uses the completely

independent evidence on washout kinetics *in vivo*, as opposed to structural considerations or spatial distributions of flow at a given moment of observation. Washout kinetics, quite independently of spatial patterns or branching network structures, drive us to the same conclusion that the system is fractal.

Acknowledgments

This study was supported by National Institutes of Health grants HL-50238 and RR-1243. The author greatly appreciates the efforts of James Ploger in analyzing these data and of Andreas Deussen (Department of Physiology, Düsseldorf), Thomas Bukowski, James Revenaugh, and James Ploger in the experimentation. *fortran* code is available by ftp to nsr.bio-eng.washington.edu. (The linear regression program *linreg* allows weighting and provides statistics: the program can be found at the ftp site in *pub/NSR_linreg.tar.Z*. The nonlinear optimizer is in *pub/SENSOP/sensop.tar.Z*.)

References

1. Bassingthwaighte JB. Blood flow and diffusion through mammalian organs. *Science*. 1970; 167:1347–1353. [PubMed: 4904998]
2. Bassingthwaighte JB. Physiology and theory of tracer washout techniques for the estimation of myocardial blood flow: flow estimation from tracer washout. *Prog Cardiovasc Dis*. 1977; 20:165–189. [PubMed: 335437]
3. Yipintsoi T, Dobbs WA Jr, Scanlon PD, Knopp TJ, Bassingthwaighte JB. Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts. *Circ Res*. 1973; 33:573–587. [PubMed: 4752857]
4. Bassingthwaighte JB, King RB, Roger SA. Fractal nature of regional myocardial blood flow heterogeneity. *Circ Res*. 1989; 65:578–590. [PubMed: 2766485]
5. Glenny R, Robertson HT, Yamashiro S, Bassingthwaighte JB. Applications of fractal analysis to physiology. *J Appl Physiol*. 1991; 70:2351–2367. [PubMed: 1885430]
6. Tomanek RJ, Searls JC, Lachenbruch PA. Quantitative changes in the capillary bed during developing peak and stabilized hypertrophy in the spontaneously hypertensive rat. *Circ Res*. 1982; 51:295–304. [PubMed: 6214331]
7. Gonzalez F, Bassingthwaighte JB. Heterogeneities in regional volumes of distribution and flows in the rabbit heart. *Am J Physiol*. 1990; 258:Heart Circ Physiol. 27:H1012–H1024.
8. VanBavel E, Spaan JAE. Branching patterns in the porcine coronary arterial tree: estimation of flow heterogeneity. *Circ Res*. 1992; 71:1200–1212. [PubMed: 1394880]
9. Richmond DR, Yipintsoi T, Coulam CM, Titus JL, Bassingthwaighte JB. Macroaggregated albumin studies of the coronary circulation in the dog. *J Nucl Med*. 1973; 14:129–134. [PubMed: 4685409]
10. Yipintsoi T, Scanlon PD, Bassingthwaighte JB. Density and water content of dog ventricular myocardium. *Proc Soc Exp Biol Med*. 1972; 141:1032–1035. [PubMed: 4645751]
11. Yipintsoi, T.; Tancredi, R.; Richmond, D.; Bassingthwaighte, JB. Myocardial extractions of sucrose, glucose, and potassium. In: Crone, C.; Lassen, NA., editors. *Capillary Permeability (Alfred Benzon Symposium II)*. Copenhagen, Denmark: Munksgaard; 1970. p. 153–156.
12. Rose CP, Goresky CA, Bach GG. The capillary and sarcolemmal barriers in the heart: an exploration of labeled water permeability. *Circ Res*. 1977; 41:515–533. [PubMed: 902358]
13. Hamilton WF, Moore JW, Kinsman JM, Spurling RG. Studies on the circulation, IV: further analysis of the injection method, and of changes in hemodynamics under physiological and pathological conditions. *Am J Physiol*. 1932; 99:534–551.
14. Lerch RA, Ambos HD, Bergmann SR, Sobel BE, Ter-Pogossian MM. Kinetics of positron emitters *in vivo* characterized with a beta probe. *Am J Physiol*. 1982; 242:Heart Circ Physiol. 11:H62–H67.
15. Zierler KL. Theoretical basis of indicator-dilution methods for measuring flow and volume. *Circ Res*. 1962; 10:393–407.
16. Chan IS, Goldstein AA, Bassingthwaighte JB. SENSOP: A derivative-free solver for non-linear least squares with sensitivity scaling. *Ann Biomed Eng*. 1993; 21:621–631. [PubMed: 8116914]
17. Bassingthwaighte JB, Ackerman FH. Mathematical linearity of circulatory transport. *J Appl Physiol*. 1967; 22:879–888. [PubMed: 5337940]

18. Kuikka J, Levin M, Bassingthwaighte JB. Multiple tracer dilution estimates of D- and 2-deoxy-D-glucose uptake by the heart. *Am J Physiol.* 1986; 250:Heart Circ Physiol. 19:H29–H42.
19. Hoedt-Rasmussen K, Sveinsdottir E, Lassen NA. Regional cerebral blood flow in man determined by intra-arterial injection of radioactive inert gas. *Circ Res.* 1966; 18:237–247. [PubMed: 5323856]
20. Yipintsoi T, Bassingthwaighte JB. Circulatory transport of iodoantipyrine and water in the isolated dog heart. *Circ Res.* 1970; 27:461–477. [PubMed: 5452741]
21. Bassingthwaighte, JB.; Liebovitch, LS.; West, BJ. *Fractal Physiology.* New York, NY/London, England: Oxford University Press; 1994.
22. Kassab GS, Rider CA, Tang NJ, Fung YB. Morphometry of pig coronary arterial trees. *Am J Physiol.* 1993; 265:Heart Circ Physiol. 34:H350–H365.
23. Arts T, Kruger RT, van Gerven W, Libregts JA, Reneman RS. Propagation velocity and reflection of pressure waves in the canine coronary artery. *Am J Physiol.* 1979; 237:H469–H474. [PubMed: 495732]
24. Bassingthwaighte JB. Physiological heterogeneity: fractals link determinism and randomness in structures and functions. *News Physiol Sci.* 1988; 3:5–10. [PubMed: 20871797]
25. van Beek JHGM, Roger SA, Bassingthwaighte JB. Regional myocardial flow heterogeneity explained with fractal networks. *Am J Physiol.* 1989; 257:Heart Circ Physiol. 26:H1670–H1680.
26. Gan RZ, Tian Y, Yen RT, Kassab GS. Morphometry of the dog pulmonary venous tree. *J Appl Physiol.* 1993; 75:432–440. [PubMed: 8376295]
27. Bassingthwaighte JB, Beyer RP. Fractal correlation in heterogeneous systems. *Physica D.* 1991; 53:71–84.
28. Knopp TJ, Dobbs WA, Greenleaf JF, Bassingthwaighte JB. Trans-coronary intravascular transport functions obtained via a stable deconvolution technique. *Ann Biomed Eng.* 1976; 4:44–59. [PubMed: 779536]

Appendix A: Similarity of Time Scaling Means That the Fractal Exponent Should Not Be Influenced by Flow

The question of why the power law exponent α of the transport functions obtained at different flows is independent of flow can be explained mathematically on the basis of previous observations. Similarity on scaling of the time axis by the mean transit time is usually observed for the coronary system.^{2:20-28} The test of “similarity” is a statistical one; namely, can the shape of an impulse response $h_1(t)$ obtained at flow F_1 , be considered similar to the response $h_2(t)$ obtained at flow F_2 ? Because there may be volume changes between two different physiological states, rather than scaling by F_1/F_2 , one uses the more general scaling factor, the mean transit time or the ratio of mean transit times, $\bar{t}_1/\bar{t}_2$, where $\bar{t}_i = V_i/F_i$ and where the i indicates a particular state or condition or time of day when the observation of $h_i(t)$ was made. When similarity holds, then all time-normalized impulse responses have the same shape and are superimposed on each other on a plot of $\bar{t}_i h(t/\bar{t}_i)$ versus $t/\bar{t}_i$.

For the special situation where $h(t)$ is a power law function, as in Equation 11 in the text, the following equation applies:

$$h(t) = A\alpha t^{-\alpha-1} \quad (14)$$

Then we can use $\bar{t}_i = V_i/F_i$ for clarity:

$$\begin{aligned}
 \bar{t}_i h(t/\bar{t}_i) &= \frac{V_i}{F_i} \cdot A\alpha \left(\frac{t}{V_i/F_i} \right)^{-\alpha-1} \\
 &= \frac{V_i}{F_i} \cdot A\alpha \left(\frac{F_i}{V_i} \right)^{-\alpha-1} \cdot t^{-\alpha-1} \\
 &= \left(\frac{V_i}{F_i} \right)^{-\alpha} \cdot A\alpha t^{-\alpha-1} \\
 &= (\bar{t}_i)^{-\alpha} \cdot h(t)
 \end{aligned} \tag{15}$$

Thus, when similarity scaling holds and $h(t)$ is a fractal power law function, then the similarity scaling by mean transit time results merely in a scaling of the observed $h(t)$ by $(\bar{t})^{-\alpha}$, and the power law exponent α is unaffected by transformation. Since the scaling transformation can be performed either by using $\bar{t}_i$, generally or by using F_i when V_i is constant, then it follows that α should be unaffected by flow, as observed in Fig 5.

The same logic holds for $R(t)$ and $\eta(t)$, which have absolute values influenced by flow but have power law exponents that are not influenced by flow.

Appendix B: Sums of Scaled Functions Can Give Power Law Behavior

A power law function can be represented as the sum of a finite number of fractal-scaled basis functions. Consider approximating of the power law function of Equation 16 with the weighted sum of basis functions $f(t)$ in Equation 17:

$$F = t^{-\beta} \tag{16}$$

$$F \approx \sum_{i=1}^N a_i f(k_i t) \tag{17}$$

where a_i is the amplitude scalar and k_i is the time scalar for the i th member. Since the basis functions are not necessarily orthogonal, a finite sum of N scaled basis function is considered.

The minimum mean-squared error between $F(t)$ and a particular $f(k_i t)$ over the interval from $t=0$ to $t=\infty$ is found by calculating a_i :

$$a_i = \frac{\int_0^\infty f(k_i t) t^{-\beta} dt}{\int_0^\infty f^2(k_i t) dt} \tag{18}$$

From this, one can solve the relation between a_i and k_i by using a dummy variable, $\tau = k_i t$, substituted into Equation 18:

$$a_i = \frac{k_i^{\beta-1} \int_0^\infty f(\tau) \tau^{-\beta} d\tau}{\frac{1}{k_i} \int_0^\infty f^2(\tau) d\tau} = \left(\frac{\int_0^\infty f(\tau) \tau^{-\beta} d\tau}{\int_0^\infty f^2(\tau) d\tau} \right) k_i^\beta \tag{19}$$

or

$$a_i = C k_i^\beta \tag{20}$$

where C is a constant that does not depend on k_i .

A power law function can therefore be represented by a finite sum of the scaled basis functions, where the weight of each basis function is determined by the scale factor raised to the power law exponent:

$$F \approx C \sum_{i=1}^N k_i^\beta f(k_i t) \quad (21)$$

In general, the k_i , can be chosen on the basis of the interval over which the power law slope is fit. If the interval is defined by $t=t_a$ to $t=t_b$, then k_1 can be chosen by $k_1=1/t_a$ or a conveniently chosen value. In order to evenly distribute all of the k_i in the log-time domain, the rest of the k_i can be calculated over the range chosen:

$$k_i = \left(\frac{k_N}{k_1} \right)^{\frac{i}{N-1}} = \left(\frac{t_a}{t_b} \right)^{\frac{i-1}{N-1}} k_1 \quad (22)$$

An example using exponentials as the basis function is demonstrated in Fig 7. F and f are given as follows:

$$F = t^{-2} \quad (23)$$

$$f(t) = e^{-t} \quad (24)$$

The finite-sum approximation is shown for $N=2, 3$, and 4 exponentials. An approximate fit is achieved using only four exponentials over the interval of $t_a=1$ to $t_b=100$. Making t_a and t_b outside of the desired region to be fitted and increasing N allows one to approach exact power law behavior arbitrarily closely.

Fig 1.

Residue curves, $R(t)$, and normalized outflow concentration-time curves, $h(t)$, from a rabbit heart after injection of ^{15}O -labeled water into the coronary inflow. Both $R(t)$ and $h(t)$ have tails that are power law functions of time; ie, they are straight on the log-log plots (right) and clearly not monoexponential, being concave upward on semilog plots (left)

Fig 2.

Fitting power law curves of general form $kt^{-\beta}$ to residue and outflow curves, $R(t)$ and $h(t)$, respectively, and to the fractional escape rate, $\eta(t)$, for two separate experiments. These are unconstrained best power law regressions fitted to the individual curves for $R(t)$, $h(t)$, and $\eta(t)$.

Fig 3.

Extrapolation of residue and outflow curves, $R(t)$ and $h(t)$, respectively, with power law versus exponential, fitting. Fitting was done over the time period where $0.2 > R(t) > 0.1$ for both $R(t)$ and $h(t)$. The power law and exponential curves beyond $R(t)=0.1$ represent the extrapolated predictions beyond 60 seconds (top) and 50 seconds (bottom).

Fig 4.

Optimized best fit of combined fractal power law relation for residue $R(t)=a_1t^{-\alpha}$ and outflow $h(t)=a_2a_1^{-\alpha}$ for the same value of α , forcing the fractional escape rate $\eta(t)=a_3(a_2/a_1)a_1^{-\alpha}t^{-1}$; Top, The exponent α was 2.22, and the parameters a_1 , a_2 , and a_3 were 13485, 9396, and 1.01, respectively. The 95% confidence range on α was 2.22 ± 0.01 . Bottom, α was 2.41 ± 0.03 (95% confidence limits), and a_1 , a_2 , and a_3 were 1342, 1178, and 1.15, respectively.

Fig.5.
Fractal exponent (α) versus myocardial blood flow (F).

Fig 6. Residue (upper three) and outflow (lower three) curves, $R(t)$ and $h(t)$, respectively, for ^{15}O -labeled water from one heart superimposed by scaling the time axis by individual mean transit time, $\bar{t}$. There is close similarity in the shapes of the curves obtained at 0.76, 1.34, and $3.44 \text{ mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$.

Fig 7.

Log-log plot showing multiexponential fits to $F=t^{-2}$, where F is flow and t is transit time, using two, three, and four exponentials with $t_a=1$, $t_b=100$, $k_2/k_1=100$ for $N=2$, $k_2/k_1=10$ for $N=3$, and $k_2/k_1=4.642$ for $N=4$, where k is the time scalar, and N is the number of exponentials.

Table

Fractal Exponent α for ^{15}O -Labeled Water Washout From $R(t)$, $h(t)$, and η Simultaneously

Heart	Experiment	F , $\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$	α	Confidence Limits*	CV		
					$R(t)$	$h(t)$	$\eta(t)$
1	02121107	1.65	2.10	± 0.036	0.06	0.03	0.12
1	02121112	3.32	2.59	± 0.011	0.16	0.15	0.38
1	02121115	1.22	1.58	± 0.050	0.04	0.03	0.11
2	02121505	3.44	2.03	± 0.061	0.12	0.16	0.33
2	02121508	1.34	1.91	± 0.046	0.04	0.05	0.11
2	02121513	0.76	1.82	± 0.115	0.12	0.06	0.34
3	12111308	2.91	2.34	± 0.034	0.06	0.05	0.17
3	12111313	2.91	2.72	± 0.008	0.08	0.09	0.18
4	12111533	2.19	2.26	± 0.032	0.07	0.05	0.20
4	12111545	2.19	2.29	± 0.031	0.18	0.28	0.51
4	12111548	2.19	2.20	± 0.074	0.20	0.30	0.62
5	19111150	1.26	2.49	± 0.006	0.05	0.05	0.12
5	19111157	1.26	2.05	± 0.050	0.06	0.07	0.18
6	19111342	1.10	2.33	± 0.026	0.08	0.04	0.22
6	19111349	3.31	2.41	± 0.035	0.09	0.10	0.19
7	29041356	0.97	2.22	± 0.010	0.03	0.03	0.11
7	29041405	0.97	2.23	± 0.033	0.09	0.05	0.30
7	29041411	0.97	2.14	± 0.144	0.11	0.08	0.42
Mean $\pm$ SD		...	2.21 $\pm$ 0.27	...	0.09 $\pm$ 0.05	0.09 $\pm$ 0.08	0.26 $\pm$ 0.15

F indicates flow; CV, coefficient of variation; $R(t)$, residual tracer activity; $h(t)$, outflow per unit time; and $\eta(t)$, fractional escape rate.

* Confidence limits for α at the 95% level for a single value of α in Equation 10 for $R(t)$, Equation 11 for $h(t)$, and Equation 12 for $\eta(t)$ a priori without any freedom.