*Annals of Biomedical Engineering*, Vol. 28, pp. 836–848, 2000
Printed in the USA. 

0090-6964/2000/28(8)/836/13/$15.00


# Indicator Dilution Methods for Measuring Blood Flow, Volume, and Other Properties of Biological Systems: A Brief History and Memoir

KENNETH ZIERLER

Professor Emeritus, Medicine and Physiology, The Johns Hopkins University School of Medicine, Baltimore, MD

*(Received 13 October 1999; accepted 31 March 2000)*

**Abstract—**In 1824 Hering introduced an indicator-dilution method for measuring blood velocity. Not until 1897 was the method extended by Stewart to measure blood (volume) flow. For more than two decades, beginning in 1928, Hamilton and colleagues measured blood flow, including cardiac output. They proposed that the first-passsage indicator concentration-time curve could be recovered from observed curves that included recirculation by semilogarithmic extrapolation of the early downslope. Others followed with attempts to fit the complete first-passage curve by various forms, such as by the sum of three exponential terms (three well-stirred compartments in series). Stephenson (1948) thought of looking at indicator-dilution curves as convolutions of indicator input with a probability density function of traversal times through the system. Meier and I reached a similar conclusion, and extended it. The fundamental notion is that there exists a probability density function of transit times, $h(t)$, through the system. We proved that mean transit time $t = V/F$, where $V$ is volume in which the indicator is distributed. Thus, $V$, $F$, and $t$ might all be calculated, or $t$ alone might suffice if one wanted only to know relative blood flow. I extended the analysis to include residue detection of indicator remaining in the system, so that $V$, $F$, and $t$ could be calculated by external monitoring. Chinard demonstrated the value of simultaneous multiple indicator-dilution curves with various volumes of distribution. Goresky extended the technique to study cell uptake and metabolism. He also found a transform of indicator-dilution output curves (equivalent to multiplying the ordinate by $t$ and dividing the time by $t$) which made congruent the family of unalike curves obtained by simultaneous injection of indicators with different volumes of distribution. Bassingthwaighte showed the same congruency with the transform of outputs of a single indicator introduced into a system with experimentally varied blood flows. We showed the same congruency for the pulmonary circulation, adding a correction for delays. Success of these transforms suggests that the architecture of the vascular network is a major determinant of the shape of density functions of transit times through the system, and that there is in this architecture, a high degree of self-similarity, implying that the fractal power function is a component in shaping the observed density of transit times. I proposed that the distribution of capillary critical opening pressures, which describes recruitment of vascular paths, may be important in shaping indicator-dilution curves, and that $h(t)$ may be derived from flow-pressure and volume-pressure curves under some circumstances. © *2000 Biomedical Engineering Society.* [S0090-6964(00)00708-6]

**Keywords—**Indicator-dilution, Tracer-dilution, Blood flow, Blood volume, Distribution volume, History, Metabolism.

Indicator- or tracer-dilution methods have been useful in metabolic and circulatory studies. This article sketches the first 150, more or less, of the 175 years evolution of some ideas behind indicator-dilution methods for measurement of blood flow, volume of distribution, translocation across barriers, etc. It is also a memoir because it emphasizes my own relation to the field, not that my contributions were particularly meritorious but because I think I know where my ideas came from, which may have some interest as an illustration of development of a science.

Hering, professor in the Royal Veterinary School in Stuttgart, was the first to use an indicator—that is, a substance introduced into blood circulation and easily identified—to measure some hemodynamic property. [Indicator can be introduced into blood circulation according to one of several time programs. For example, it can be injected instantaneously (bolus, impulsive, or delta-function injection are synonyms), or by continuous intravascular infusion at constant rate (a pulse). In this article, if the manner of indicator injection is not otherwise specified, assume that the indicator injection was instantaneous, or as nearly so as the experimenter could make it.] I wish Hering had told us how the idea came to him. Between 1824 and 1826 he carried out experiments which he published in 1829.[18] He injected K ferrocyanide into a jugular vein on 14 horses, and sampled from other parts of the vascular system. Although the title of Hering's paper was ''Experiments to measure velocity of blood circulation,'' he did not measure velocity, but rather the time required for the indicator, injected instantaneously into one vein to be first detected in another vein by addition of ferric chloride to the blood sample to

Address correspondence to Dr. Kenneth Zierler, Endocrinology and Metabolism, Johns Hopkins Medical School, 1830 E. Monument Street, Suite 333, Baltimore, MD 21287.

form Prussian blue. He called this the *circulation time*, which we call, more accurately, the *appearance time* or the *delay* because there is an entire family of circulation times. The *appearance* time is not a reliable substitute for the *fastest transit time* through the system because our measure of appearance time depends upon the sensitivity and resolution of the analytical method to detect the indicator.

Fast-forward about 65 years. In 1890 G. N. Stewart, then at Cambridge, published an abstract in the November 8, 1890 Proceedings of the Physiological Society of Great Britain describing his use of Hering's method to measure appearance time, but with NaCl as the indicator. An exhaustive report of the method and its applications appeared in the first 89 pages of the 1893 Journal of Physiology.[27] He could record continuously the increased conductivity imposed by NaCl, so he knew that there was not only delay but also dispersion of indicator.

It was not until 1897, 70 years after Hering, that Stewart,[28] then in Cleveland at Western Reserve, suggested indicator dilution could be used to measure blood flow. He argued that the volume of solution necessary to dilute indicator injectate to equal the observed mean concentration of indicator output was exactly the volume of blood that had diluted the injectate (hence the name, indicator dilution) over the time interval in which indicator was recovered. The suggestion has intuitive appeal, but it is not quite correct. The volume calculated by Stewart's method is not necessarily the volume of blood in the vascular bed under study, and the time interval Stewart used is not, in general, the time that expresses the exact relationship between blood or plasma volume and flow, as we shall see. Nevertheless, Stewart's formula was an important evolutionary step because, eventually (it took about 30 years before Stewart's idea was picked up), it pointed people's minds toward the possibility of using indicator-dilution curves to calculate flow, and because it suggested that the area under the indicator output concentration curve might have something to do with it.

The dominant figure in the indicator-dilution field from the late 1920's through the 1940's was William F. Hamilton, with his many colleagues, first in Louisville, then in Georgia. Hamilton used the Hering–Stewart technique, with a dye as the indicator. At first he measured only appearance time. Then, in 1928, nearly 30 years after Stewart's proposal and 100 years after Hering, he published a paper titled modestly ''Simultaneous Determination of the Pulmonary and Systemic Circulation Times in Man and a Figure Related to the Cardiac Output''[16] in which he resurrected Stewart's formula. (It is interesting that, although the last part of this article does report efforts to measure cardiac output, the bulk of the article is still focused on appearance times.)

Hamilton recognized that Stewart's calculation only worked if indicator particles were counted only once, a point Stewart had not made. Hamilton wondered what to do about recirculation of indicator. In his first effort, he pointed out that recirculation became obvious when there was an upward bump during the downlimb of the tracer-dilution curve. But, he said, recirculation had actually begun some time before its presence became obvious. Hamilton guessed at the onset of recirculation and extended the first passage curve in his imagination.[16] But, Hamilton realized, clearly, a guess is not reliable.

Hamilton and his colleagues, along with other groups, were interested in finding if early portions of the indicator-dilution concentration curve predicted the entire course of first circulation. Hamilton was inspired to plot indicator-dilution curves semilogarithmically. He claimed that, after some initial variation following the peak, the curve of first circulation fell as a single exponential line on the semilog plot. Lulled by its simplicity, investigators eagerly adopted Hamilton's extrapolation, even though many of the downlimbs had fallen by less than 70%, not enough to justify any extrapolation. Furthermore, there were circumstances, some of which are referred to in the last three illustrations in this article, in which first passage indicator-dilution curves were complete before recirculation occurred; the downlimb fell more slowly or more rapidly than Hamilton's exponential, depending on how early in the downlimb the exponential was drawn.

This brings me to my entry into the field. In 1946 I was studying skeletal muscle from a number of aspects—organic metabolism, salt and water metabolism, mechanical properties, electrical properties. Some metabolic experiments were on fragments of rat diaphragm in the Warburg apparatus. I became dissatisfied with such experiments; what we found with them was only what pieces of dead or dying meat were capable of doing under the abnormal circumstances of *in vitro* experiment, not what muscle really does in life. I wanted to study muscle metabolism *in situ* and *in vivo*. The idea for how to do this came to me from a study I had been conducting on renal handling of creatine. Creatinuria was a hallmark of muscle disease, because muscle is by far the largest reservoir of creatine and diseased muscle is apt to leak creatine into the circulation, when it appears in the urine. My hypothesis was that some cases of creatinuria might not be due to muscle disease but to failure to reabsorb creatine. (This turned out to be the case, for example, post-partum creatinuria was attributed to involution of the pregnant uterus, but it would require extraction of all the creatine in a room full of pregnant uteri to supply the creatine one woman excretes in her urine in one day post partum. This creatinuria is due to failure of tubular reabsorption.) What is a renal clearance except a straightforward application of the mass balance equation?

I conceived of experiments in which we calculated net

uptake or output of a metabolite by muscles of the human forearm as the difference between input in arterial blood, flow times arterial concentration, and output in venous blood, flow times venous concentrations of substances of interest; that is, $q=F(c_a-c_v)$. So, I needed to measure forearm blood flow.

There were several choices of methods. I had heard about an indicator-dilution method to measure blood flow from my next door lab neighbor, E. Newman.[23] I did not know whether it was the most reliable method, but we were going to have needles or catheters in forearm artery and veins to measure metabolite concentrations, it seemed convenient to use them to measure blood flow by indicator dilution. I was not attracted to Newman's model, three well-stirred bottles in series. But it did not matter about the model for purposes of measuring blood flow because the formula for measuring blood flow appeared to me to be model independent. Yet there were nagging questions about theory and uses of indicator-dilution which made me uncomfortable. We began using indicator dilution in 1950 and published a report in 1954 in the *Journal of Clinical Investigation*, on measurement of blood flow and volume in the forearm, to which I added a naïve and conceptually incorrect mathematical appendix. At least I had sense enough to be dissatisfied with it.

In the autumn of 1953 opportunity to concentrate on the indicator-dilution theory was enhanced by an accident for which the orthopedic surgeon put me to bed, flat on my back, for ten days, during which I used reams of paper, preoccupied with the problems. Newman's paper claimed to be ''an analysis of factors shaping the curve.'' The analysis seemed to me completely unphysiological, but it focused my attention on the question of what might be the mechanism shaping the curve. Most of my effort was directed toward trying out random walk schemes, but none was satisfactory. When I went back to work, I went over the problem with Bill Cochran, chairman of our Biostatistics Department, and showed him my scribbling. He suggested that I present the problem and my tentative approaches to his Department as a seminar. I did. Discussion was lively. A young faculty member, Paul Meier, made some astute criticisms, and, luckily, we agreed to collaborate.

We decided that I had been spending enough time in a fruitless search for a mechanism. Maybe we did not need to know the mechanism of delay and distribution. In the absence of understanding the delay and distributing system that generated the indicator-dilution curves, we decided to treat the system as a black box, with an accessible input and accessible output, but its insides invisible to us. We might still be able to interpret the dilution curve for certain purposes.

We began by setting up the following equation:

$$q_0 h(t)dt = Fc(t)dt.$$

The right-hand side of the equation, blood or plasma flow, $F$, in dimension of volume per time, multiplied by $c(t)$, the concentration of indicator in effluent blood or plasma during the interval between $t$ and $(t+dt)$ time units after instantaneous injection of indicator at time zero, multiplied by the time interval, $dt$, this product gives the quantity of indicator that exits the system during that time interval. This quantity can also be expressed by multiplying the quantity of indicator injected at time zero, $q_0$, by some function representing the fraction of original injectate that exits between time $t$ and $(t+dt)$. This function is characterized by the fact that it can never be less than zero and never greater than 1 (because the quantity of indicator leaving the system during any time interval can never be less than zero and never more than all of it that entered the system) and the sum of these fractions over all time must be 1 because all the injected indicator must be recovered at the exit. We called this function $h(t)$, in dimension of reciprocal time, and the dimensionless fraction during the time interval is $h(t)dt$. The equation then is a definition of $h(t)$. We recognized that the qualities we imposed on $h(t)$ are those of a frequency function or probability density function of transit times from entrance to exit through the vascular bed. It does not matter what the mechanism is that produces $h(t)$, the first-passage indicator-dilution outflow curve, in response to an instantaneous injection, is an amplitude-scaled probability density function, a frequency function of transit times through the system. Paul Meier must be given the major credit for this insight.

Because the integral over all time of a frequency function is unity, by definition, integration of both sides of the equation over all time gives the equation by which the unknown flow is determined by indicator-dilution:

$$F = q_0 \Big/ \int_0^\infty c(t)dt.$$

There was, at the time, a choice of two procedures for introducing indicator into the circulatory system, either instantaneous injection or a continuous infusion at constant rate. I planned to assess forearm metabolism over a period of several hours, for which continuous infusion at constant rate seemed more suitable than a series of repeated impulsive injections. It was conceivable that at some time we, or someone, might be interested in other forms of indicator injection, such as a ramp. We had developed an equation for measuring blood flow only in response to an impulsive injection. What about other forms? Accordingly, we generalized the input function. Whatever it is, call it the input function, $i(t)$, it is con-

volved with $h(t)$ so that the output function, $o(t)$, is the convolution integral:

$$o(t)=\int_0^t i(t-\tau)h(\tau)d\tau.$$

So we could describe the output for any known indicator input. Because $i(t)$ is entirely general, it includes the case in which indicator recirculates; input concentration is, say, in arterial blood, $c_a(t)$, and output concentration is $c_v(t)$.

We thought we had devised a novel way of looking at the system until we searched the literature while we were preparing the paper for publication, not as easy to do then as it is now, and discovered that six years earlier Stephenson[26] had already thought of indicator-dilution curves as the convolution integral of the indicator input function with a probability density of travel times.

Stephenson pointed out, and Meier and I echoed and enlarged, that convolution of input or recirculating indicator concentration with $h(t)$ suggests that, instead of imagining what the first passage curve looks like, as Hamilton did, we make recirculation part of the experiment, say, by measuring input concentration. Stephenson suggested accomplishing this by measuring indicator concentration at two sites, entrance to the vascular net under study and its exit, following injection of one indicator into the entrance. A more general statement is that in order to account for recirculation one additional piece of information is needed. This can be gotten as Stephenson suggested or it can be gotten by injecting two distinguishable indicators with the same volume of distribution, one into the entrance to the system, the other into the exit, measuring both indicator concentrations at the exit. Deconvolve the curve of the one injected into the exit from the curve injected into the entrance to obtain the desired first passage curve. We used this method to obtain the frequency function of transit times through canine pulmonary circulation.[21] We compared this method to Hamilton's exponential extrapolation; only if the curve had fallen to less than 10% of peak before recirculation appeared did it make no difference in calculated mean transit time. Better still, of course, if possible, is to use an indicator that does not recirculate; e.g., a gas removed quantitatively in its passage through the lungs, or study a part of the vascular system through which the first passage curve is complete before recirculation occurs. Paul Meier and I regarded Stephenson's work highly, particularly because there was only one of him while there were two of us.

The only joint paper by Paul Meier and me was published in 1954.[22] It went considerably beyond the areas covered in Stephenson's paper. We proceeded to answer some additional questions I had asked about the method.

First, we made explicit all assumptions inherent in application of the equations, especially the assumption of stationarity; $h(t)$ must not change while we try to reveal it.

Second, we were able to prove the relationship between volume and flow. Both Stewart and Hamilton had pointed out that, if one collected in a bucket all the blood flowing out of a vascular bed for some specified time, the volume of blood in the bucket would exactly equal the volume of blood in the vascular bed. But there was no agreement about what that time had to be, and no proof. Stewart, despite the fact that his formula for calculating blood flow depended upon dividing an estimated dilution volume by the elapsed time during which there was a nonzero indicator concentration, thought it was the appearance time, others thought it was the peak time. Hamilton at first thought it was the median time, then the mean time.

I had been getting nowhere thinking about elements of volume of a vascular bed as being in series, which is how you view it when you analyze indicator-dilution as a random walk. What happens if we put volume elements in parallel? We said that the dynamic volume, $V$, of the circulatory system is composed of elements, $dV$, each of which is the collection of tracks of all those indicator particles that require a specified time, $t$ = distance/velocity, to traverse the system from entrance to exit. The length of a volume element is the traversal time, $t$. Its cross-sectional area is the fraction of the total flow through the system that exits within time $t$ and $(t+dt)$; that is, it is $Fh(t)dt$. The element of volume, then, is the product

$$dV=tFh(t)dt.$$

The sum of all these parallel volume elements is the total volume in which the indicator is distributed:

$$V=F\int_0^\infty th(t)dt=F\overline{t},$$

and which equals the total flow multiplied by the mean transit time ($t$ bar), or the ratio $F/V=1/\overline{t}$.

This has turned out to be quite a useful result. One use of this result is for investigators who wish only to know *relative* blood flow, flow per unit weight (obtained easily from flow per unit volume), rather than *absolute* blood flow, in which case knowledge of the $t$ bar suffices. The relationship $V=F\overline{t}$ has also been useful in calculating volumes of distribution of tracers nor confined to the bloodstream. For example, volume, $V_T$, in which, say, sucrose (or some other extracellular tracer) is distributed is the product of plasma flow, $F$, and mean transit time for sucrose, $\overline{t_T}$. When a nondiffusible tracer

(one confined to the bloodstream) is administered simultaneously with sucrose, its volume of distribution is plasma, $V_P$. The difference between total extracellular volume and plasma volume is interstitial fluid volume, $V_E = V_T - V_P$. A correction needs to be made if the tracer, in this case, sucrose, is not distributed in the same concentration in plasma and interstitial fluid. Similarly, one can find other volumes of distribution. I call the volume calculated as the product of flow and mean transit time the ''dynamic volume'' because it measures only the volume into which the indicator is distributed; it does not include stagnant pools not fed directly by capillaries through which blood is flowing. This caution arose from studies of lung water, in which water determined by difference between wet and dry weight exceeded water volume determined by use of the $Ft$ formula. We make use of this difference later to infer some interesting things about pulmonary circulation. Another interesting thing about our proof that $V = F\bar{t}$ is that analogous relations that had been assumed (perhaps as intuitively true) without proof could now be proven. For example, multiply both sides of the equation by the concentration of the indicator in the system to obtain $Vc = Fc\bar{t}$, or the mass oe quantity of indicator in the system is the product of its flux into the system and its mean transit time.

Third, there was an unresolved question in the literature as to which of two methods, single injection, or constant infusion, is more reliable. Refer to Fig. 1. The top curve is $h(t)$. Its integral is the distribution function $H(t)$, the middle curve. It is the fraction of input at time zero that has left the system by time $t$. But this is also the output curve obtained with constant infusion of indicator, as can be shown by the convolution integral:

$$Fc_v(t) = \int_0^t m(t-\tau)h(\tau)d\tau = mH(t),$$

where $c_v(t)$ is output concentration, $m$ is the continuous constant indicator input rate. This resolved the question. There is exactly the same information in the indicator-dilution curve in response to single injection as there is in response to continuous constant injection.

Fourth, in a later paper[34] I identified the fraction of material still remaining in the system, the residue function, $R(t) = 1 - H(t)$, shown as the bottom curve. This was useful for interpreting experiments in which the residue was measured, as by external monitoring. In response to instantaneous injection of a quantity, $q_0$, of indicator, the quantity of it remaining in the system at elapsed time $t$ is $q(t) = q_0 R(t) = q_0[1 - H(t)]$. The area under the observed external monitoring curve is

$$\int_0^\infty q(t)dt = q_0 \int_0^\infty [1 - H(t)]dt = q_0\bar{t} = q_0 V/F.$$

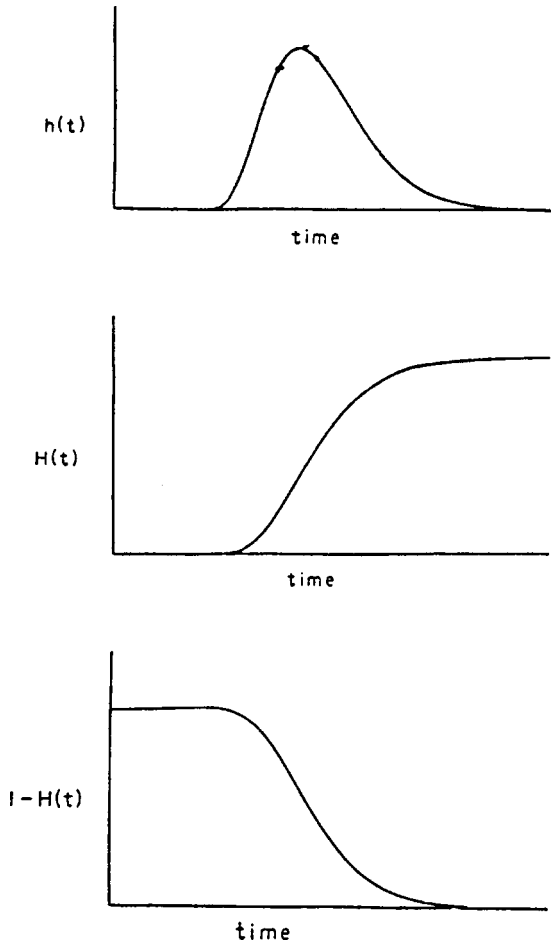


**FIGURE 1. Top: $h(t)$, a frequency, or probability density, function of transit times through a vascular bed. Middle: $H(t)$, the distribution of transit times, the integral of $h(t)$. Bottom: $R(t) = 1 - H(t)$, the residue function. The total area under $R(t)$ is the mean transit time.**

Since the initial height of the curve is $q_0$, relative blood flow, $F/V$, or flow per unit weight calculated from it, is determined as initial height divided by the area under the residue curve.

Although these analyses of indicator-dilution curves provided a basis for measuring absolute or relative blood or plasma flow and volumes of distribution, I still had a gnawing desire to know what was in the black box. In the late 1960's two contacts rekindled hopes that I might get some insights: (a) I served on a Study Section with A. C. Burton, physiologist and biophysicist from Western Ontario, who explained and popularized the Law of Laplace, that capillary critical opening (or closing) pressure was the point at which transmural pressure just exceeded (or was less than) circumferential tension in the vessel wall, and (b) my lab and Solbert Permutt's respiratory physiology lab at Hopkins began an association because we had some common interests. Influenced by Burton, we[29] hypothesized that details of the shape of an

indicator-dilution curve through a vascular bed are determined in large part by the distribution of critical opening pressures among capillaries. We knew that an increase in blood flow, produced by an increase in arterial pressure, caused an increase in blood volume. This increase in blood volume might be due to distension of vessels or to recruitment of new paths by opening capillaries, or by some combination of the two. I[35,36] proposed a mathematical model based on the hypothesis that the major factor in the increase in volume with increased flow is recruitment of channels as their critical opening pressures are reached. I hypothesized that the frequency function of transit times may be predicted from the flow-pressure and volume-pressure curves, a subject taken up later in this essay. In order to test these questions experimentally, we resorted, in part, to techniques by which others had extended the usefulness of indicator-dilution methods.

Hamilton and colleagues distinguished between an indicator that was confined to blood or plasma (nondiffusible) and one that could be distributed in some larger volume by crossing at least capillary barriers into interstitial fluid (diffusible). However, Hamilton's interest seems to have been largely in warning against use of diffusible indicators on the grounds that their larger volume of distribution so altered the indicator output concentration curve as to jeopardize calculation of cardiac output, which was his major concern. Francis Chinard first reported simultaneous injection of nondiffusible and diffusible indicators in 1954.[5] Over the next decade he was first to demonstrate the enormous power of simultaneous injection of multiple indicators to measure volumes of distribution, permeant properties of biomembranes, metabolism, and to surmise paths taken by metabolites, which he summarized in 1962.[6] Figure 2 illustrates Chinard's use of indicator dilution in a metabolic study, in this case, the effect of a carbonic anhydrase inhibitor. Note that the curves in Fig. 2 are estimated first passage curves. The actual indicator-dilution curves were first plotted semilogarithmically, the Hamilton exponential extrapolation was made in an effort to eliminate the impact of recirculation, the resulting curves were then replotted on Cartesian coordinates, as in Fig. 2.

Goresky, who learned multiple indicator-dilution technique from Chinard, made a remarkable discovery.[14] He sought to distinguish between two models of hepatic blood flow and distribution into extravascular space by analysis of sets of dilution curves for labeled red blood cells, tagged plasma, and small molecules that diffuses back and forth between intra- and extravascular space. The model that fit the data was one in which the diffusible indicator diffused so rapidly into its interstitial space that the time required for diffusion was negligible compared to the time required for blood flow. Goresky predicted that, if this model was appropriate, a transform of the family of first passage curves obtained by multiplying all first passage output concentrations by the ratio of the indicator's distribution volume to blood volume and dividing the time axis by the same ratio must move all the curves into congruity with that of the nondiffusible indicator. The transform worked, as dramatically illustrated by Fig. 3, which is a composite of three of his illustrations. The top curves are semilogarithmic plots of indicator output, with Hamilton's exponential extrapolation in hopes of eliminating the contribution of recirculation. The putative first passage curves are then replotted on Cartesian coordinates, the middle set of curves. Each indicator has a different volume of distribution. Curves are more spread out, the peak concentrations are lower, and, of course, the mean transit times are longer, the larger the volume of distribution. The congruency is particularly amazing in view of the fact that onset of observed deviation from the assumed exponential fall; onset of recirculation occurred when the labeled water curve had fallen only to about 90% of peak, and even the labeled red cell curve had fallen to only about 30% of peak. The extrapolation under such conditions may not be an accurate representation of the first passage curve.

Nevertheless, the congruency was very impressive. It seemed to be telling us something new about what is inside the black box. Goresky normalized by distribution

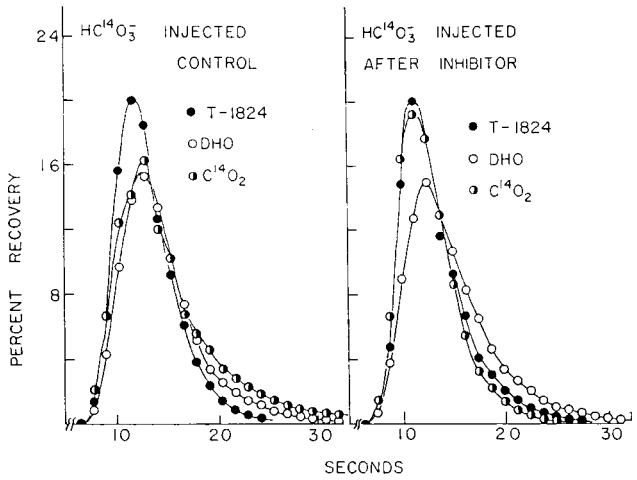


**FIGURE 2. Output curves following simultaneous instantaneous injection of nondiffusible (T-1824) and diffusible (labeled water) indicators into a vascular bed, along with carbon-labeled carbonic acid from which labeled $CO_2$ output is measured. All curves are Cartesian replots of semilogarithmic plots of original data, with Hamilton's extrapolation to remove the effect of recirculation. In both frames water distribution is in a larger volume than that of the reference T-1824 (which is bound to serum albumin). On the left, before injection of carbonic anhydrase inhibitor, labeled $CO_2$ is distributed in a volume similar to that of water. On the right, in the presence of the inhibitor, labeled $CO_2$ is distributed like the reference indicator (constructed from two figures in Ref. 6).**

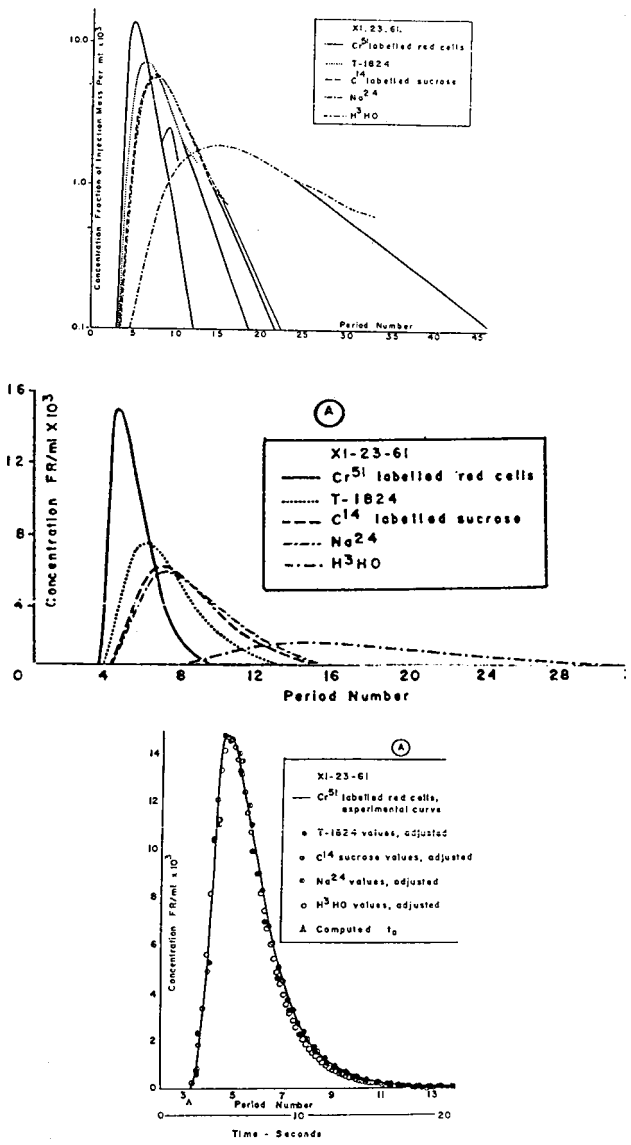


**FIGURE 3. Simultaneous multiple indicator-dilution curves through hepatic circulation to show congruency of curves transformed according to each indicator's distribution volume. Top: Semilogarithmic plot of observed data weighted for injectate quantity, to show extrapolation intended to remove effect of recirculation. Middle: Cartesian coordinate replots of the assumed first-passage curves. Bottom: Congruency of the middle set of curves, transformed according to distribution volume (from Ref. 14).**

volume, but all his indicators, diffusible and nondiffusible, were carried through the system at the same flow. This meant to me that he was in fact normalizing by mean transit time. I anticipated that indicator-dilution curves in response to repeated injections of a single indicator under different flows should be made congruent by a mean transit time transform.

Bassingthwaighte had the same notion.[1] He applied the mean transit time transform to a family of curves

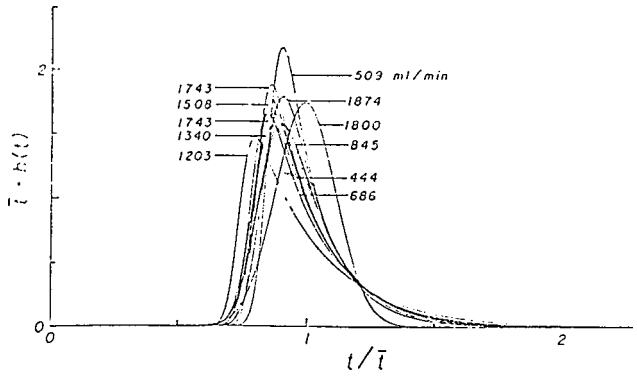


**FIGURE 4. Mean transit time transform of indicator-dilution curves from the internal iliac artery through the femoral circulation. The same nondiffusible indicator in all the curves, but each at the indicated different blood flow. Although the untransformed curves were not published in this case, it is accepted that the transformed curves resemble one another much more than do the untransformed curves (from Ref. 1).**

through the femoral circulation to obtain the degree of congruency shown in Fig. 4.

Tancredi and this author[30] used excised lobes of canine lung to test the hypothesis that the shape of indicator-dilution curves through the pulmonary vascular bed is predictable from flow-pressure and volume-pressure plots. Data from six dogs were consistent with the hypothesis but insufficient to prove it.

We applied the modified Goresky transform to generate the frequency function:

$$\phi(u) = \bar{t}h(t/\bar{t}).$$

Remember that there was no recirculation of indicator during the first passage time, so that the output curves were truly amplitude-scaled $h(t)$s. At high pulmonary venous pressure, at which the constant venous pressure was the back pressure except at the highest values of pulmonary artery pressure, the family of $\phi(u)$ was nearly perfectly congruent. At low venous pressure, at which the family of capillary critical opening pressure progressively became back pressure as arterial pressure was raised, congruency of the $\phi(u)$ was still quite impressive, but the variance among this set of curves was greater than among the set at high venous pressure (Fig. 5). We think that the superior congruency at high venous pressure occurred because in those experiments venous pressure was high enough to exceed nearly all critical opening pressures, so that back pressure was constant, whereas at low venous pressure the back pressure was determined by the distribution of critical opening pressures. If our hypothesis about the major mechanism of increasing blood volume in response to increased arterial pressure is correct (and we test it below), then high venous pressure means that the entire vascular bed is

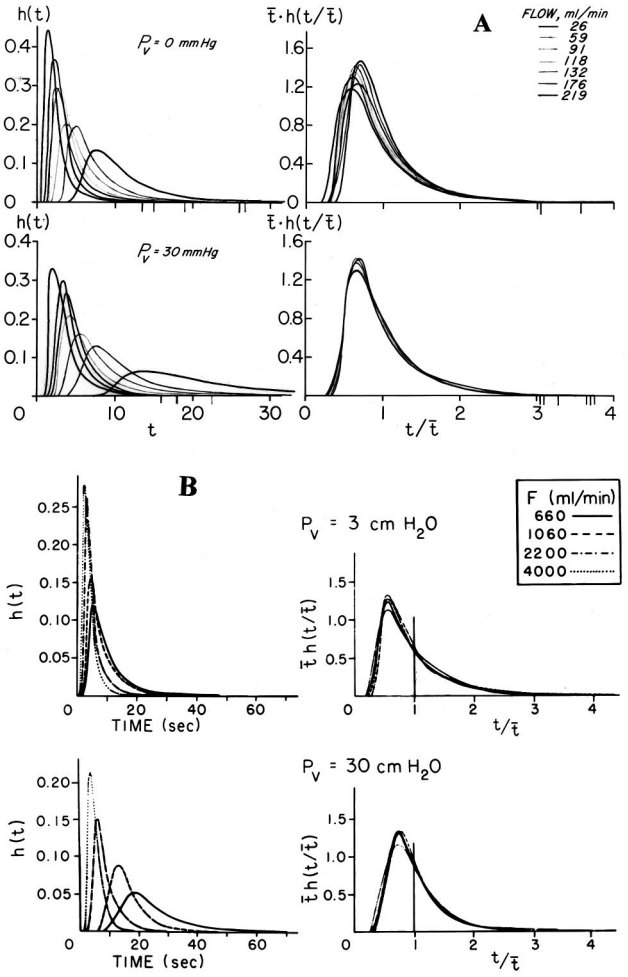


**FIGURE 5. Indicator-dilution curves through canine pulmonary circuit. Nondiffusible reference indicator (labeled albumin) at different blood flows, showing congruency of the transformed curves. There was no recirculation. A: Blood flow range below normal resting; B: Blood flow range from resting to exercise levels. In each set, panels on the left are $h(t)$ at various flows, on the right are the $\phi(u)$ [where $u = t/\bar{t}$ and $\phi = \bar{t}h(u)$] transforms, showing congruency. Upper panels are at near zero venous pressure, so that back pressure is capillary critical opening pressure, and capillaries are recruited as pump flow and pulmonary artery pressure are increased. Lower panels are at high venous pressure, probably the nearly constant back pressure; congruency of transforms is near perfect (unpublished data).**

open, or nearly so, hence nearly constant in volume, whereas low venous pressure means that vascular volume is increased by recruitment of parallel channels as arterial pressure exceeds critical opening pressure.

To test this hypothesis we collaborated with Permutt's lab. Maseri and co-workers[20] prepared anesthetized, open chest, respired dogs, in which the experimenter controlled pulmonary artery blood flow (hence, pulmonary artery pressure), pulmonary venous pressure, and alveolar pressure. Recirculation of indicators injected into the pulmonary artery was not permitted before the first passage curve was complete. Results of this first series of experiments were ambiguous, consistent with the hypothesis that recruitment was the major process, rather than distention, but not definitive proof.

We sought definitive proof based on the following hypothesis. Discrepancy had been reported between the volume of water in the lung measured by difference between wet and dry weight and the lesser volume determined by calculation of lung water from mean transit time of labeled water or similar marker. We attributed the discrepancy to the fact that indicator-dilution measures a dynamic volume, the volume of water the indicator can reach by diffusing across capillaries in the time during which the indicator-dilution curve is written. This explanation implies that, when a capillary bed is opened by an increase on arterial pressure, the diffusible indicator can now diffuse into an extravascular volume previously unavailable to it in the time required. Therefore, when venous pressure is low, increases in arterial pressure should recruit extravascular volume as well as vascular volume. Furthermore, if the recruited extravascular volume is randomly distributed among recruited capillaries, the ratio of capillary volume to extravascular volume should remain relatively independent of arterial pressure.

This hypothesis was tested both in the double bypass preparation[29] and in the isolated lobe preparation.[30] Results led to the conclusion that both distention and recruitment occurred but that recruitment accounted for most of the blood volume increase with increased pressure. In these cases the density function of relative transit times through small vessels was taken to be $\phi(u)$, which implies that flow through large pulmonary vessels is treated as delay with negligible distribution compared to distribution through small vessels.

A few years later Rogus and I[25] reexamined Tancredi's data on double bypass preparation. She noted that delays were not all the same either at different flows or at the same flow for nondiffusible and diffusible indicators, and that if one wanted to obtain the small vessel volume one should correct for delay. She introduced the variable

$$z = (t - \alpha)/(\bar{t} - \alpha),$$

where $\alpha$ is the delay. She then obtained the transform of each $h(t)$ as

$$\Psi(z) = (\bar{t} - \alpha)h(z).$$

We found that, with this transform, the ratio of extravascular to vascular volume was constant, independent of pulmonary artery pressure (Fig. 6). This is consistent with recruitment of parallel microvascular elements, each with its own region of extravascular water. If increased

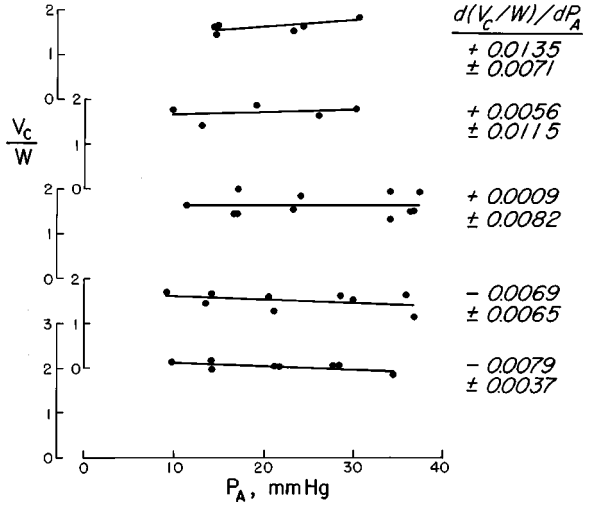


**FIGURE 6. Constancy of ratio of microvascular ($V_c$) to extravascular ($W$) water volume as pulmonary artery pressure is increased in each of five dog lungs. Data obtained from measured blood flows and mean transit times of first-passage curves (there was no recirculation) of nondiffusible (labeled albumin) and diffusible (labeled antipyrene) indicators. Right-hand side columns: mean and SE of slope. No slope is significantly different from zero. This constancy argues that increased vascular volume in response to increased arterial pressure is due to recruitment (opening) of parallel capillaries, thus increasing the regions available for transcapillary diffusion of antipyrine (=water) (unpublished data).**

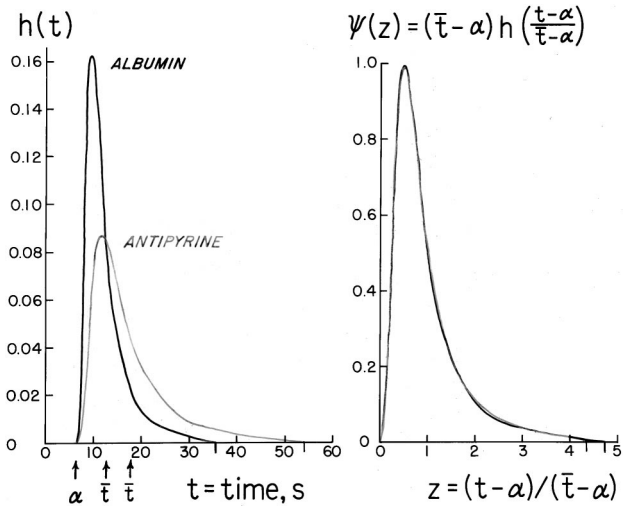


**FIGURE 7. Congruency of transformed indicator-dilution curves through canine pulmonary circuit. Nondiffusible (reference, labeled albumin) and difffusible (labeled antipyrine) curves; $h(t)$ to show gross differences between the two, and the transforms, $\psi(z)$ [where $z=(t-\alpha)/\bar{t}-\alpha$], to show nearly complete congruency. $\alpha$, appearance time; $\bar{t}$, mean transit time, smaller for albumin than for antipyrene. There was no recirculation (unpublished data).**

blood volume were due to distention there would have been little or no increase in extravascular water. We calculated from the data that no less than 80% of the increase in blood volume, and as much as 100%, was due to recruitment.

Furthermore, the transform gave near-perfect congruency to the reference indicator-dilution curve (labeled albumin) and the indicator for water distribution (labeled antipyrene) (Fig. 7).

Taken together, the results of these experiments with nondiffusible and diffusible indicators at varying blood flows and pressures lead to two inferences. First, they demonstrate that in the canine circulation, under our experimental conditions, recruitment of previously closed channels accounts for nearly all the increased volume as blood flow is increased. Second, they suggest that something invariant plays the major role in determining the shape of indicator dilution curves.

With regard to the first inference, concerning recruitment versus distention, early background is given by Maseri *et al.*[20] A number of laboratories found that although pulmonary vessels are distensible, especially at low pressure (only slightly for pressures greater than only 5 cm/$H_2O$), but this distention accounts for only a minor increase in volume defined by the flow-volume or pressure-volume curve of pulmonary circulation. West and his colleagues[32] had previously examined the question in the pulmonary circulation, and concluded from morphologic studies that recruitment occurred at the level of vessels that behaved as a Starling resistor (a tube through which flow is a function of transmural pressure difference rather than inflow−outflow pressure difference). This mechanism accounted for most, but not all of the increased volume, the rest attributed to vasodilatation.[12] Goresky and his colleagues[9,10] published a series of studies on cardiac circulation in which, by simultaneous multiple indicator-dilution studies, they calculated that an increase in blood flow, produced by a variety of means, increased capillary surface area available for solute exchange; that is, there was recruitment of previously closed capillaries. The same conclusion was reached by Overholser *et al.*[24] and by Caruthers *et al.*[4] for the pulmonary circulation. This conclusion is entirely consistent with our own, although Overholser *et al.*[24] refer to us as interpreting our data to mean that arterioles are the site of vascular opening by which volume recruitment occurs. That is not and was not my own interpretation; regrettably, some statement in our papers may have misled them. It is likely that among various vascular beds, both dilatation and recruitment contribute to increased volume in response to increased flow, but most of the increase in cardiac and pulmonary blood volume is due to recruitment. It may be different for hepatic circulation. Cousineau *et al.*[8] reported that hepatic blood flow was the most important factor in determining hepatic blood volume, but they attributed increased volume to vascular distention, although sucrose space (a measure

of extracellular space) also increased, which suggests recruitment. The mix of the two factors probably depends upon the manner in which blood flow is increased, the nature of the given vascular bed, and the range over which pressure and flow are imposed.

With regard to the second inference, that congruency of the transforms of $h(t)$ implies that there is a constant factor despite changes in flow and pressure (and volumes of distribution), it seemed to me that the constant factor was likely to be the architecture of the vascular system. In general, in each organ or tissue the vascular system has a pattern of layers of increasing numbers of vessels due to dichotomous branching from the largest arteries down to capillaries, followed by layers of decreasing numbers of vessels due to paired coadunation from capillaries to final venous drainage. In our study of the pulmonary circulation most of the increase in blood volume (and probably all the increase in extravascular volume) in response to increasing blood flow is accounted for by recruitment of previously closed blood vessels. These vessels must be capillaries if recruitment is a purely passive phenomenon governed by law of critical opening pressure. That capillaries are recruited was demonstrated by Warrel *et al.*[31] in 1972, and by Hanson *et al.*[17] in 1989. Further evidence that recruitment is due to increased numbers of capillaries through which blood flows has accrued from studies in which the PS product (permeability times surface area available for permeation) increased with blood flow to lungs, heart, and liver.[10,15,24,30] Congruency of the transforms of $h(t)$ requires that dispersion of the indicator within a newly recruited group of capillaries be pretty much the same as dispersion among those previously open. By this statement I mean that coefficient of variance, coefficient of skewness, etc., of the probability density of transit times through the newly recruited part of the system are about the same as those through parts already open before flow or arterial pressure is increased. Presently, we recognize this as a description of self-similarity, a concept Bassingthwaighte and colleagues[3,13] introduced into the blood flow and tracer dilution literature, In such a system, as Bassingthwaighte and colleagues[2] have suggested that some portion of the density function of transit times reflects the influence of the fractal power law, of the form $At^{-\alpha}$, where $A$ and $\alpha$ are constants, both$>0$.

This returns us to the question, What mechanism shapes $h(t)$?

To say that architecture of the vascular system plays a major role in inscribing $h(t)$ is to deal with it largely as a structure of noncompliant tubes with no flow regulators, which it is not. The structure of the vascular system is altered *passively* by distension of blood vessels and by recruitment of channels as transmural pressure exceeds critical opening pressure. The structure of the system is altered *actively* by vasodilatation and vasoconstriction. Analysis is complicated further by the fact that pressure changes, as by altered blood flow, can stimulate release of vasoactive agents. In the interest of getting started with analysis of the possible nature of the distributing system I began by dealing only with the passive effects.

The analysis and synthesis which I attempted in 1970 and 1971 were, and remain, incomplete. For that reason I was unhappy with them and never submitted them for publication in a critically reviewed journal, However, they were published as talks I gave in symposia in 1970 (Ref. 35) and 1971,[36] in which I hoped to stimulate discussion and even stir someone to complete the effort. The title of the second, and more complete, talk was ''Why tracer dilution curves through a vascular system have the shape they do.'' The daring title was a promise unfulfilled. The value of the attempted model, as is true for such attempts, lies chiefly in that it makes it clear what information, not yet available, needs to be collected if the approach is to be pursued.

Briefly, I imagined that $h(t)$ could be constructed from flow-pressure and volume-pressure curves such as we had obtained for canine pulmonary circulation. I began with the fact that blood flow, $F$, is just the product of conductance to blood flow, $\gamma$, and a pressure difference, $P'_a - P_B$, where $P'_a$ is transmural pressure, $P_a - P_E$, arterial minus extravascular pressure, and back pressure, $P_B$, is either venous pressure, $P_V$, or critical opening pressure, $P_c$, whichever is higher. When one increases arterial pressure from some $P_a$ to $(P_a + dP_a)$, one opens those vessels whose critical opening pressure is in the interval $P_c$ to $(Pc + dP_c)$, thereby recruiting some number of channels, $dN$, whose volume is $dV(P_c)$. There must exist some function $g(P_c)$, the probability density function of critical opening pressures in the system. Then, $dV(P_c) = V_m g(P_c) dP_c$, where $V_m$ is the maximum volume of the system accounted for by opening all channels. There may or may not be additional volume due to distention. If there were only negligible contributions to volume from distension, the volume-pressure curve would be simply the integral of $dV$, $V(P'_a) = V_m G(P'_a)$, where $G(P'_a)$ is the distribution of critical opening pressures. Thus, if the conditions are met, a plot of $V(P'_a)/V_m$ gives empirical $G(P'_a)$, from which the density function may be obtained, if needed. With increased pulmonary artery pressure there is an increment in blood flow, $dF(P_c) = \gamma(P'_a - P_c) \times g(P_c) dP_c$. This is an oversimplification; $\gamma$ should not be a constant, but a function of $P_c$. However, to make it a variable function of $P_c$ makes $dF$ not integrable analytically. This is a cowardly reason to oversimplify, but I did in order to see if the result might still be reasonable.

Note that if $g(P_c)$ is the normal density $dV(P_c)$ has the shape of the normal density, scaled by $V_m$, but $dF(P_c)$ is skewed so that its shape resembles the familiar indicator-dilution curve. This occurs because as chan-

nels are added at each higher critical opening pressure, the driving force for blood flow through the new channel, $(P'_a - P_c)$, decreases, so that the increment in flow is less for any value of $g(P_c)$. Since the higher values of $P_c$ are on the downlimb of $g(P_c)$, the curve is skewed, with the mean falling to the right of peak $g(P_c)$.

Integrals of $dV(P_c)$ and $dF(P_c)$ give volume-pressure and flow-pressure curves that represent the contributions of flow and volume associated with channels through which critical opening pressure is the back pressure. Despite the oversimplifications, the curves have the shape of familiar volume-pressure, flow-pressure, and volume-flow curves.

The ratio of $dV(P_c)$ to $dF(P_c)$ gives a time, $a(P_c)$, which is the time required for the indicator to traverse the newly recruited $dN$ paths from entrance to exit. $dF(P_c)$ is converted to $dF(a)$ by plotting it against $a(P_c)$. $dF(P_c)/F(P'_a)$ is the fraction of the total flow through that part of the vascular system under study for which $P_c$ is the back pressure and that requires time between $a$ and $(a+da)$ to traverse the system. We recognize this statement as a definition of $h(t)dt$; that is, we now have an expression for that part of $h(t)$ contributed by transit times through paths for which $P_c$ is the back pressure in the pressure gradient driving blood flow.

But, while it is true that recruitment was by far the major factor to volume increase with flow under conditions of our experiment, it is not necessarily so under other conditions and in other vascular beds so I had to consider the contribution of distensibility. I let the contribution of distensibility to increased volume appear as increased conductance, $\gamma$, to blood flow, where $\gamma$ is a function of some power of the volume. The relationship depends upon the distribution of cross sectional and longitudinal resistances, as well as the character of blood flow—organized or chaotic. So we need one or more additional probability density functions to define the elements of flow and volume when $P_V$ is the back pressure. In the published version of my 1971 talk,[36] I let the distensibility term be a delta function, which cannot be correct. We need to find out what the correct relation is between conductance and volume elements. Furthermore, I simply added the two terms, distention and recruitment, as though they were only parallel. That is unjustified; paths in which volume is increased by vessel distension may be in parallel or in series with paths in which volume is increased by recruitment, and we have no idea yet of how much is series and how much is parallel. Of course, if we set $P_V$ sufficiently high, it can become the only back pressure, in which case, we would need to include a density function of conductance through all the paths of the system.

In this early attempt to define factors shaping $h(t)$ I identified those properties of the system we need to know if we are to use this sort of approach. The density I have called $g(t)$ is obviously a detailed accounting of the architecture of the vascular system. The system is of course even more complex; I have been considering only passive factors, vessel distention, and pressure-induced capillary opening. To that must be added vasoactive controls. An enormous amount of detail is lacking. If obtained in a few cases, there is no warranty that it would apply to all. The task is somewhat more complicated than tracking a hurricane, but it is likely to be ''doable.'' The question is, ''Is it worth doing?''

Perhaps the most interesting thing about my 1971 effort to express $h(t)$ in terms of a density of $P_c$ and a density of $\gamma$ is that I was in fact only substituting some functions of unknown, or only empirically determinable, densities for $h(t)$. In other words, I opened the black box and what I found in it was just $h(t)$.

Of course, in the years since my adventure inside the black box there have been other efforts. Domenech *et al.*[11] reported that there were large regional differences in myocardial blood flow in dogs, measured by radioactive microspheres. The observation was confirmed in 1973 by Ypintsoi *et al.*[33] in isolated dog hearts and by several subsequent reports from Bassingthwaighte's laboratory in other species, including baboons. By heterogeneity, these investigators meant that the scatter among regional measurements at any given time was large, with standard deviation from the mean of about 35%. This geographical heterogeneity was distinguished from the familiar heterogeneity of transit times through any vascular bed in which $h(t)$ is not a delta function. Knowledge of geographical heterogeneity is important for such considerations as permeability surface product for the entire region. It also tells us is that our overall $h(t)$ is a weighted sum of individual regional $h(t)$s in parallel; it leaves us still not knowing the details by which $h(t)$ is generated. These observations of geographic heterogeneity stimulated more curiosity about what factors in the black box were generating the details of $h(t)$.

The next step was accumulation of evidence that time spent in passage through the capillary network accounts for most of the dispersion of indicator, something that many had assumed, but for which evidence had to be amassed. Clough *et al.*,[7] by means of x-ray angiography of isolated dog lung circulation, concluded that most of the transit time and most of the dispersion of transit times through the lung occurred in passage through capillaries, not in conducting arteries and veins.

I referred earlier to contributions by Bassingthwaighte and colleagues, looking inside the black box, particularly their report of fractal washout of tracer from the heart.[3] Earlier and related efforts are reviewed by King *et al.*[19] Bassingthwaighte and Beard[3] found that, beginning only at some point on the down slope of the washout curve, there was an excellent fit to the fractal equation $At^{-\alpha}$,

which always fell more slowly than the exponential most commonly used to extrapolate an outflow tracer curve for the purpose of eliminating the effect of recirculation. As Bassingthwaighte and his colleagues point out, this may not be simply curve fitting; the self-similarity produced by dichotomous vascular branching predicts such a relationship. But, again, as they remind us, this fractal hyperbola cannot explain the entire observed $h(t)$; it cannot fit or explain the upslope, the peak, and some undefined early part of the downslope. The fractal hyperbola, if it is to be used at all as part of a theory of $h(t)$, needs to be combined with, perhaps convolved with, something else that will probably look like $h(t)$, as is implied by King *et al.*[19] Of course, if the fractal is convolved with something that looks like our usual $h(t)$ the downslope cannot be an exact fit to the fractal hyperbola, although, in the examples reported, it has been quite close. We might note that the fractal hyperbola can never entirely be our familiar observed $h(t)$ because the fractal, a distant, nonsymmetrical cousin of the Cauchy density function, has no moments; its area is indeterminate.

It is a natural human desire to seek a synthesis of $h(t)$. At one level of inquiry it has been fruitful, and enough, just to obtain reliable measurement of $h(t)$ or its integral, and from their appropriate moments to obtain flow and volume of distribution. Whatever success students may have in the future, when they open the black box they will find that it contains $h(t)$.

## REFERENCES


[1] Bassingthwaighte, J. Blood flow and diffusion through mammalian organs. *Science* 167:1347–1353, 1970.

[2] Bassingthwaighte, J. B., and D. A. Beard. Fractal $^{15}$O-labeled water washout from the heart. *Circ. Res.* 77:1212–1221, 1995.

[3] Bassingthwaighte, J. B., R. B. King, and S. A. Roger. Fractal nature of regional myocardial blood heterogeneity. *Circ. Res.* 65:578–590, 1989.

[4] Caruthers, S. D., T. R. Harris, K. A. Overholser, N. A. Pou, and R. E. Parker. Effects of flow heterogeneity on the measurement of capillary exchange in the lung. *J. Appl. Physiol.* 79:1449–1460, 1995.

[5] Chinard, F. P., and T. Enns. Transcapillary pulmonary exchange of water in the dog. *Am. J. Physiol.* 178:197–202, 1954.

[6] Chinard, F. P., T. Enns, and M. F. Nolan. Indicator-dilution studies with ''diffusible'' indicators. *Circ. Res.* 10:473–490, 1962.

[7] Clough, A. V., S. T. Haworth, C. C. Hanger, J. Wang, D. L. Roerig, J. H. Linehan, and C. A. Dawson. Transit time dispersion in the pulmonary arterial tree. *J. Appl. Physiol.* 85:565–574, 1998.

[8] Cousineau, D., C. A. Goresky, and C. P. Rose. Blood flow and norepinephrine effects on liver vascular and extravascular volumes. *Am. J. Physiol.* 244:H495–H504, 1983.

[9] Cousineau, D., C. P. Rose, D. Lamoureux, and C. A. Goresky. Changes in cardiac transcapillary exchange with metabolic coronary vasodilation in the intact dog. *Circ. Res.* 53:719–730, 1983.

[10] Cousineau, D., C. A. Goresky, C. P. Rose, A. Simard, and A. J. Schwab. Effects of flow, perfusion pressure, and oxygen consumption on cardiac capillary exchange. *J. Appl. Physiol.* 78:1350–1359, 1995.

[11] Domenech, R. J., J. I. E. Hoffman, M. I. M. Noble, and K. B. Saunders. Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs. *Circ. Res.* 25:581–597, 1969.

[12] Glazier, J. B., J. M. Hughes, J. E. Maloney, and J. B. West. Measurement of capillary dimensions and blood volume in rapidly frozen lungs. *J. Appl. Physiol.* 26:65–76, 1968.

[13] Glenny, R., H. T. Robertson, S. Yamashiro, and J. B. Bassingthwaighte. Applications of fractal analysis to physiology. *J. Appl. Physiol.* 70:2351–2367, 1991.

[14] Goresky, C. A. A linear method for determining liver sinusoidal and extravasular volume. *Am. J. Physiol.* 204:626–640, 1963.

[15] Goresky, C. A., A. Simard, and A. J. Schwab. Increased hepatic permeability surface area product for 86RB with increase in blood flow. *Circ. Res.* 80:645–654, 1997.

[16] Hamilton, W. F., J. W. Moore, J. M. Kinsman, and R. G. Spurling. Simultaneous determination of the pulmonary and systemic circulation times in man and of a figure related to cardiac output. *Am. J. Physiol.* 84:338–344, 1928.

[17] Hanson, W. L., J. D. Emhardt, J. P. Barket, L. P. Latham, L. L. Checkley, R. L. Capen, and W. W. Wagner, Jr. Site of recruitment in the pulmonary microcirculation. *J. Appl. Physiol.* 66:2079–2083, 1989.

[18] Hering, E. Versuche, die Schnelligkeit des Blutlaufs und der Absonderung zu Bestimmen. Z. Phys. 3:85–126, 1829.

[19] King, R. B., G. M. Raymond, and J. B. Bassingthwaighte. Modeling blood flow heterogeneity. *Ann. Biomed. Eng.* 24:352–372, 1996.

[20] Maseri, A., P. Caldini, P. Harward, R. C. Joshi, S. Permutt, and K. L. Zierler. Determinants of pulmonary vascular volume. Recruitment versus distensibility. *Circ. Res.* 31:218–228, 1972.

[21] Maseri, A., P. Caldini, S. Permutt, and K. L. Zierler. Frequency function of transit times through dog pulmonary circulation. *Circ. Res.* 26:527–543, 1970.

[22] Meier, P. and K. L. Zierler. On the theory of indicator-dilution method for measurement of blood flow and volume. *J. Appl. Physiol.* 6:731–744, 1954.

[23] Newman, E. V., M. Merrell, A. Genecin, C. Monge, W. R. Milnor, and W. P. McKeever. The dye dilution method for describing central circulation. An analysis of factors shaping the time-concentration curve. *Circulation* 4:735–746, 1951.

[24] Overholser, K. A., N. A. Lomangino, R. E. Parker, N. A. Pou, and T. R. Harris. Pulmonary vascular resistance distribution and recruitment of microvascular surface area. *J. Appl. Physiol.* 77:845–855, 1994.

[25] Rogus, E., R. Tancredi, K. Zierler. Capillary recruitment in pulmonary vascular bed. *Fed. Proc.* 36:535, 1977.

[26] Stephenson, J. L. Theory of measurement of blood flow by the dilution of an indicator. *Bull. Math. Biophys.* 10:117–121, 1948.

[27] Stewart, G. N. Researches on the circulation time in organs and on the influences which affect it. *J. Physiol. (London)* 15:1–89, 1893.

[28] Stewart, G. N. Researches on the circulation time and on the influences which affect it. IV. The output of the heart. *J. Physiol. (London)* 22:159–183, 1897.

[29] Tancredi, R., Caldini, P., Shanoff, M., Permutt, S., and Zierler, K. The pulmonary microcirculation evaluated by tracer

dilution techniques. In: Cardiovascular Nuclear Medicine, edited by H. W. Strauss, B. Pitt, A. E. James, Jr., St. Louis: C. V. Mosby, 1974, pp. 255–260.
[30] Tancredi, R., and K. L. Zierler. Indicator-dilution, flow-pressure and volume-pressure curves in excised dog lung. *Fed. Proc.* 30:380, 1971.
[31] Warrel, D. A., J. W. Evans, R. O. Clarke, G. P. Kingaby, and J. B. West. Patterns of filling in the pulmonary capillary bed. *J. Appl. Physiol.* 32:346–356, 1972.
[32] West, J. B., C. T. Dollery, and A. Naimark. Distribution of blood flow in isolated lung: relation to vascular and alveolar pressures. *J. Appl. Physiol.* 19:713–724, 1964.
[33] Ypintsoi, T., W. A. Dobbs, Jr., P. D. Scanlon, T. J. Knopf, and J. B Bassingthwaighte. Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts. *Circ. Res.* 33:573–587, 1973.
[34] Zierler, K. L. Equations for measuring blood flow by external monitoring of radioisotopes. *Circ. Res.* 16:309–321, 1965.
[35] Zierler, K. L. Measurement of the volume of extravascular water in the lungs in intact animals and man. A review of tracer dilution principles, of some reported results, and a new hypothesis to explain the shape of tracer-dilution curves and certain other interesting relationships. In: Central Hemodynamics and Gas Exchange, edited by C. Giuntini. Torino: Minerva Medica, 1970, pp. 3–18.
[36] Zierler, K. L. Why tracer dilution curves through a vascular system have the shape they do. In: Computer Processing of Dynamic Images, edited by K. B. Larson and J. R. Cox, Jr., New York: Society of Nuclear Medicine, 1974, Chap. 8, pp. 95–107.