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Estimation of Blood Flow With Radioactive Tracers

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Abstract

The techniques of tracer dilution in the circulation, and of tracer uptake by and washout from an organ, may be described using expressions that are general and are not dependent on specific models such as exponentials. The expressions have been applied to the measurement of cardiac output using impulse and constant rate injection techniques. Further expressions have been given for estimating organ blood flow from inflow/outflow concentration-time curves, washout curves, and from the distribution of deposited tracer. Some problems with respect to the use of deposition techniques as they are ordinarily applied to the estimation of regional blood flow must be considered, particularly where there are capillary beds in series or where there is countercurrent diffusional shunting of diffusible tracers between inflow and outflow. This review deals with these various aspects of tracer theory as they relate to the measurement of blood flow.

The use of tracers for estimating blood flow should be regarded as simply an extension of standard indicator dilution techniques. The theoretical bases for these techniques have been well defined by Zierler^{1,2} and have been given in particularly nice detail for laminar flow situations by Gonzalez-Fernandez.³ The principle of conservation of mass is the basis: The amount of tracer entering the organ is equal to the amount that will emerge from it. When indicator traverses an organ, the degree of average dilution gives the flow, and the average time for transorgan passage gives a measure of the volume. Those tracers that enter the extravascular space can be expected to take longer to escape from the organ; this will be advantageous in the application of washin and washout techniques where slow exchange makes measurement of uptake or washout rates or of mean transit time easier. In general, intravascular tracers tend to be more useful for estimating absolute flows (ml/min) and diffusible tracers more useful for estimating the flow per unit volume or mass of an organ [ml/(g-min)].

Labeling of compounds with radionuclide has the tremendous advantage that a wide variety of substances can be labeled, and that several tracers can be measured simultaneously. Since different tracers permeate different spaces and since they have differing molecular dimensions or lipid solubility and enter their regions of access at differing rates they can be used for giving information not only on blood flow and volumes of distribution but also on metabolic or other functions of particular organs. In addition, gamma-emitting tracers have the great advantage of being detectable externally.

Flow may be estimated in absolute terms (milliliters/second) and this is commonly what is desired. There is also a need to estimate relative flows such as the fraction of cardiac output

going to individual organs or relative regional flows within an organ. From the functional point of view we are really most interested in the flow per unit mass of tissue, which is useful in discerning whether or not the amount of substrate or oxygen delivered to the tissue is proportional to what one thinks the tissue requires.

Thus we can see that there are a variety of goals associated with the estimation of blood flow and the substances within it, and we shall attempt to give some idea as to which techniques satisfy these different ends.

TRACER DILUTION TERMINOLOGY

The definitions that we will use are those that have been pretty well standardized. 4,5 A simplified viewpoint from which one can look at a biologic system is diagrammed in Fig. 1. The system is presented as having a constant volume, V , and a constant flow, F . Information concerning the system might be gleaned through detectors placed so that signals could be recorded from the system itself, from the outflow from the system, or in the very artificial circumstance where one collects all of the outflow in a bucket. The mathematical relationships between these differing viewpoints are precise and can be expressed in terms of the residue function, $R(t)$, the impulse response, $h(t)$, and the cumulative outflow residence time distribution $H(t)$. In the simplest experiment an amount of tracer, q_0 , is injected into the entrance of the system at time zero.

The residue function, $R(t)$, is defined as that fraction of the tracer that remains within the tissue at time t , that is:

$$R(t) = \frac{q(t)}{q_0} \quad [1]$$

where $q(t)$ is the amount of tracer in the organ at time t . Ordinarily a gamma-emitting tracer is detected externally with some particular efficiency dependent on the geometry and the efficiency of the detector. The efficiency of detection, K , assumed to be uniform for all tracer within the system, does not affect the shape of $R(t)$:

$$R(t) = \frac{Kq(t)}{Kq_0} \quad [2]$$

Since no further tracer is injected after time zero, the residue function $R(t)$ is the usual washout curve obtained by external detection; it has the general form shown in Fig. 2.

The unit impulse response or transport function, $h(t)$, is the probability density function of transit times through the system from the point of injection to the point of detection in the outflow. It has the shape of the intravascular tracer concentration-time curve, in the venous outflow $C_v(t)$ (mg/ml or cpm/ml). The scaling factor is the flow, F , (ml/sec), divided by the injected dose, q_0 (mg or cpm), of either a gamma- or a beta-emitting tracer:

$$h(t) = \frac{F}{q_0} \cdot C_v(t) \quad [3]$$

Therefore $h(t)$ is in units of seconds⁻¹.

$H(t)$ is the cumulative residence time distribution function for the system, and represents the fraction of injectate collected by time t in a bucket into which all of the outflow is emptied. This accumulation can be expressed by the integral of $h(t)$:

$$H(t) = \int_0^t h(\lambda) d\lambda \quad [4]$$

where λ is a variable running from 0 to t used for the purpose of the summation or integration. Since $H(t)$ represents the fraction of tracer that has left the organ, and $R(t)$ represents the fraction of tracer that is retained within the organ, the relationship between the two can be seen to be completely defined:

$$R(t) = 1 - H(t) \quad [5]$$

$R(t)$, the residue function, is particularly useful and provides a rather simple method for estimating the mean transit time, $\bar{t}$, namely the area beneath $R(t)$ is equal to $\bar{t}$:

$$\bar{t} = \int_0^\infty R(t) dt \quad [6]$$

Later, $\bar{t}$ will be shown to be very useful for the estimation of flow per unit volume of distribution of the tracer within the system, since by conservation of mass the volume of the system is simply the flow times the mean transit time through it:

$$V = F\bar{t} \quad [7]$$

Another very useful measure taken from the residue function most often is the fractional escape rate, FER, which is the rate of loss of tracer, $-dq/dt$, divided by the amount of tracer residual in the organ, $q(t)$:

$$\text{FER} = -\frac{dq(t)/dt}{q(t)} \text{ or } -\frac{dq/dt}{q} \quad [8]$$

This will ordinarily be a time-varying function and is not constant except under the particular circumstance that the rate of loss is proportional to the quantity present, as is the case for a first order, or monoexponential system. The definition of FER in Eq. [8] is general; the particular FER that characterizes a system is that which is observed following an impulse injection, and which is called the emergence function, $\eta(t)$:

$$\eta(t) = -\frac{dq(t)/dt}{q(t)} = \frac{h(t)}{R(t)} \quad [9]$$

This can be derived from Eqs. [1] and [3] when it is appreciated that the product $FC_v(t)$ is equal to dq/dt . When an impulse injection has been used, $\eta(t)$ is that fraction of the particles residing in the system, which will exit in the t^{th} second. (In population statistics or renewal theory it is known as the risk function, the death rate of those living at age t .) If we look at it with respect to the residue function alone, then it may be defined as:

$$\eta(t) = \frac{-dR/dt}{R} \quad [10]$$

$$\eta(t) = -d \log_e R(t) / dt \quad [11]$$

Eq. [11] shows that $\eta(t)$ is the logarithmic slope, the slope that would be obtained as the local slope around time t on a semilogarithmic plot. This demonstrates that it is equivalent to the rate constant that one obtains by fitting a straight line to a curve on a semilogarithmic plot. However, in the general expressions given in Eqs. [10] and [11], we have been careful to note that $\eta(t)$ is not necessarily a constant. The fractional escape rate will change with time in any longitudinally dispersed system, and in a system of parallel pathways of differing average transit times; one should not be committed to thinking of the fractional escape rate as a constant, or of $R(t)$ as being monoexponential. Although sometimes the approximation is useful, we like to avoid it on the basis that it is not general, and is sometimes distinctly misleading. On the other hand, however, it is reasonable to argue that many systems become monoexponential in the limits of their washout, and this can be seen even in Fig. 2 where at late times $\eta(t)$ becomes flat, which indicates that the last portions of $h(t)$, and $R(t)$ have become monoexponential.

Given these definitions, we can now go on to their utilization for the measurement of flow and volume within a system of interest. These will simply be variations on the theme of using conservation of mass equations. We will consider three types of illustrative applications: the measurement of cardiac output, the measurement of organ blood flow, and the measurement of regional flows within an organ.

THE MEASUREMENT OF CARDIAC OUTPUT

Sudden Injection Technique

A typical intravascular tracer dilution curve recorded from the arterial circulation following injection into the pulmonary artery, proximal to the lung bed, is seen in Fig. 3. The tracer is so diluted and dispersed during the traversal of the central circulation that some recirculated tracer masks the tail of the primary curve. The technique of semilog replot and monoexponential extrapolation introduced by Hamilton et al.⁶ in 1931 is still considered quite adequate to account reasonably well for the tail of a curve, although it must be recognized that there is some possibility of error here. (For example, when injecting into the left atrium and sampling from the aorta and femoral artery, one may see that there is a long, low tail that is higher than that given by the monoexponential extrapolation and that is clearly separable from recirculation.) Assuming the correctness of the monoexponential extrapolation, one can calculate flow from the first pass or primary curve:

$$F = \frac{q_0}{\int_0^\infty C_v(t) dt} \quad [12]$$

The flow, F (ml/sec), equals the amount of tracer injected divided by the area underneath the concentration time curve. The standard assumptions made in using this equation include (1) stationarity, constant flow and constant distribution of flow and (2) linearity, which states that responses of the system must summate linearly; these have been outlined more fully by Zierler.⁷ There are a few indicators, like temperature, hydrogen, and xenon, whose recirculation is minimal, and for whose curves the extrapolation is not required.

This equation will apply to any tracer whether it penetrates the vascular wall or not, and whether or not it enters erythrocytes; the only requirement is that one be able to obtain all of the tracer in the outflow and that it be included under the "primary curve," i.e., before

recirculation. Furthermore, there is no requirement that this equation be applied to a sudden or slug injection. The injection may be well dispersed or even given as a sequence of short injections, so long as the total amount of the injectate eventually traverses the sampling point and is recognized. We will come back to this point later, since any form of injection can be used including a constant rate injection. However, for practical purposes a short injection is much better, simply because the ability to separate the primary curve from recirculated tracer is most readily done under this circumstance. The reason the conditions are so little restricted for this method is that it is simply dependent on conservation of mass: what was injected, q_0 , comes out as the integral of the product of flow times concentration in the outflow at the sampling point. This conservation of matter approach, what goes in comes out, can be appreciated by substituting in the definition of $C_v(t)$ from Eq. [3] into Eq. [12]. The identity is seen to hold, since the integral of $h(t)$ to infinity is equal to 1.

Radioactive microspheres as well as the complete gamut of diffusible and nondiffusible tracers can be used for measuring cardiac output when the injection is made into left atrium or left ventricle and the sampling is from a peripheral artery. All that is required is that there be good mixing of the tracer in the blood before any branching occurs.

One requirement for the application of Eq. [12] that sometimes fails is that the flow be steady. When it is not, there will be errors in the estimation of flow that may be over- or underestimates, but there will be a tendency toward overestimation.⁸ Since this type of error is somewhat greater when the injection and sampling point are close together, this is a reason for maintaining as much separation as possible between injection and sampling point and thus permit enough accuracy in the monoexponential extrapolation to exclude recirculated tracer. But the separation cannot be too great, since the primary curve will be so dispersed that recirculated tracer summates with the early portion of the down-slope and cannot be distinguished in a semilog replot.

THE ESTIMATION OF MEAN TRANSIT TIME VOLUME

This “volume” is simply the product of flow, F , and the mean transit time, $\bar{t}$, as in Eq. [7]. The estimation of mean transit time is somewhat trickier and more subject to error than the estimation of flow. With an impulse injection, or an extremely short sudden injection at the entrance to the system, the mean transit time can be calculated as:

$$\bar{t} = \frac{\int_0^{\infty} t \cdot C(t) dt}{\int_0^{\infty} C(t) dt} \quad [13]$$

$$\bar{t} = \int_0^{\infty} t \cdot h(t) dt. \quad [14]$$

The relationship between these two forms of the equation can be obtained via Eq. [3]. The denominator is omitted in Eq. [14], since the integral to infinity of $h(t)$ is equal to 1. For practical purposes, Eq. [13] may be expressed as follows:

$$\bar{t} = \sum_{i=1}^{i=N} (t_i \cdot C_i \cdot \Delta t) / \sum_{i=1}^{i=N} (C_i \Delta t). \quad [15]$$

In this expression, the continuous curve, $C(t)$, is treated as a sequence of points along the curve indexed by the subscript i where N equally spaced points in time, t_i , are taken with a time interval, Δt , between each one. The summation in the denominator is simply the area

underneath the primary curve and the summation in the numerator provides the weighting or relative amount of the tracer existing over each particular time interval, t_i , so that the result is the weighted average or mean transit time. As in measuring flow, it is the primary curve that is used as $C(t)$ or as the sequence of concentration values, C_i .

The volume of distribution, or mean transit time volume of the system, can now be estimated, since the mean transit time is by definition:

$$\bar{t} = \frac{V}{F}. \quad [16]$$

One must be somewhat careful in thinking about what is the volume V . It is simply the volume accessible to the tracer during its passage from the injection point to the sampling point; when the tracer is limited to the vascular system such as with an erythrocyte label or a plasma albumin label, the mean transit time volume, V , will simply be the blood volume lying between the injection and sampling points. When the tracer escapes from the vascular system, the calculation of V is equally valid, so long as the primary curve can still be identified before recirculation masks it too severely. Thus, if one injects ^{24}Na as a slug injection into the circulation, the mean transit time volume for sodium would be the blood volume between the injection and sampling points plus that fraction of the extravascular space accessible to the sodium. Since sodium enters cells only very slowly, the additional volume beyond the vascular space is approximately equal to the extracellular, extravascular, or the interstitial space. Thus, the mean transit time volume for sodium would be the vascular plus the interstitial space. This can be useful in circulatory studies, but the problem of excluding recirculation becomes severe because recirculation is ordinarily fairly rapid compared to the time for washout of the sodium from the extravascular space. Of course, if one simultaneously samples at the inflow and outflow from the organ, recirculation can be accounted for and the mean transit time obtained accurately using deconvolution techniques. 19,20

When the tracer penetrates the cells as well as the interstitial space then the volume of distribution includes both these spaces. Again the theory is applicable but the practice becomes more difficult, as recirculation will tend to mask the tail of the curve.

MEAN TRANSIT TIME ESTIMATION BY RESIDUE DETECTION

The detection of tracer within the organ or within the segment of the circulation using an external sodium iodide detector for gamma emitters can be utilized to provide an estimate of the mean transit time of the organ or of the segment of the circulation from which the counts are detected. This holds as long as recirculation either does not occur or can be excluded by some empirical artifice such as was applied to intravascular dilution curves as in Fig. 3. The mean transit time is given by the area underneath any of the three curves of $R(t)$ in Fig. 4:

$$\bar{t} = \int_0^\infty R(t) dt. \quad [17]$$

This is the basis of the "height/area" technique introduced by Zierler 2 for estimating flow per unit volume. Using the definition of $R(t)$ from Eq. [23] in combination with Eq. [16], one obtains

$$\frac{F}{V} = \frac{\bar{t}}{\bar{t}} = \frac{\text{Height}}{\text{Area}} = \frac{Kq_0}{\int_0^\infty Kq(t) dt} = \frac{1}{\int_0^\infty R(t) dt}. \quad [18]$$

In this expression, V is the volume of distribution of the tracer just as in Eq. [16], so that if one wishes to know the flow per unit volume of the organ itself, one has to know something more about the tracer; that is, one should have an estimate of the steady state volume of distribution of the tracer relative to the total organ volume. We shall use an apparent partition coefficient, λ , which is the ratio of the volume of distribution for the tracer within the organ to the total organ volume (V_{org}):

$$\lambda = \frac{V}{V_{\text{org}}} \quad [19]$$

For sodium in heart or skeletal muscle, λ is only about 30%. For sodium in the brain, λ is the blood volume divided by the total brain volume, since the blood-brain barrier is virtually impermeable to sodium. However, for antipyrine, which is more lipid soluble and penetrates both capillary and cell walls, λ is close to 1.0 for most organs.

Because of the problem of recirculation the residue technique can be modified in the fashion illustrated in Fig. 5:

$$\frac{F}{V} = \frac{F}{\lambda V_{\text{org}}} = \frac{1}{\bar{t}} = \frac{1 - R(T)}{\int_0^T R(t) dt} \quad [20]$$

The modification from Eq. [18] to Eq. [20] is designed to avoid having to record the tail of the washout curve for infinite time, since recirculation would make this portion of the curve clearly elevated. The height of the curve at the particular time of stopping the recording, T , is used to provide a measure of the indicator still retained within the organ so that the numerator is reduced by this amount and, similarly, the area under the curve is reduced. The equation is only an approximate expression, but it would be more nearly exact if the residue function itself were close to a monoexponential curve. This expression was used by Hoedt-Rasmussen and Lassen 9 in the brain and by Bassingthwaighe, Strandell, and Donald 10 in the heart, and in the latter study was shown to give estimates comparable to those obtained by measuring the flow and the organ weight.

USE OF THE CONSTANT RATE INJECTION TECHNIQUE

This technique can be used for the estimation of flow either from the plateau concentration during a steady infusion, or from the area under the curve as outlined for the slug injection. Although this technique has proven very useful for the estimation of flow in parts of the arterial system,¹¹ it has the disadvantage, in applications to transorgan blood flow, of increased problems with recirculation.

The simplest system, one in which there is no recirculation, can be diagrammed as in Fig. 6A. At time zero a constant rate injection of dm_i/dt mg or cpm/sec into the flow is begun resulting in an inflow concentration of $C_a(t)$, which is maintained for the duration of the infusion. The concentration in the outflow of this segment of the system, $C_v(t)$, rises after some delay and slowly reaches a plateau, which is then maintained until a short time after the infusion has ceased. It then falls in a fashion which is the complement of the form of the rising curve. In this situation the rising curve of $C_v(t)$ has exactly the form of $H(t)$, the residence time distribution function. The corollary is that the wash-out curve, $C_v(t)$, beyond t_{end} , has the shape of $R(t)$. The flow can be calculated from the venous outflow curve by observing the degree of dilution of the tracer at steady state, given the known infusion rate, dm_i/dt :

$$F = \frac{dm_i/dt}{C_p} \quad [21]$$

where C_p is the concentration of tracer during the plateau of the curve. However, conservation of mass also holds so that when the total amount of tracer injected over the total infusion period, q_0 , is known (it is simply the integral of dm_i/dt over the time period of infusion) then flow can be calculated as before:

$$F = \frac{q_0}{\int_0^\infty C(t) dt} \quad [22]$$

The mean transit time can also be calculated from the same information. The area of the curve between $C_a(t)$ and $C_v(t)$ from time zero up to the time when the plateaus match is $\bar{t}$. Similarly the areas of the curve between $C_a(t)$ when it is turned off and $C_v(t)$ during its washout phase is also $\bar{t}$. Thus such an experiment provides two estimates of $\bar{t}$.

Note that this technique as described here applies to intravascular detection rather than external residue function detection, as the degree of dilution of the tracer cannot be determined knowing only the external counts and the infusion rate (see below).

When recirculation occurs, and of course there is a greater chance of this occurring when the infusion is long than when it is short, then the situation may be diagrammed as in Fig. 6B. Here the recirculation is diagrammed as starting at t_r and increasing as a slow ramp. When this occurs the plateau level, C_p , tends to last only for a very brief time (or may not be achieved at all) after which there is a rise in concentration. If recirculation is not accounted for, then the denominator of Eq. [21] will be overestimated and the flow underestimated. Thus the best way of accounting for recirculation is to measure the concentration at the inflow, $C_a(t)$, or perhaps better, to measure the concentration of recirculated tracer just upstream from the infusion point, $C_r(t)$. Then the correct estimate of C_p can be obtained by shifting the curve of $C_r(t)$ one mean transit time to the right in time and subtracting from the observed $C_v(t)$. This is suggested for the two points shown on the diagram. Notice that the tails of the curves are more awkward, since both $C_a(t)$ and $C_v(t)$ are changing, and may not reach zero or come together for a long period of time depending on how the tracer is handled in the circulation. Nevertheless, the same principles apply as before, but it will take slightly more complex mathematics to utilize the washout phase of the curves. Eq. [22] can be applied simply by shifting $C_r(t)$ one mean transit time to the right and then subtracting the area under $C_r(t)$ from the area beneath $C_v(t)$. Similarly, the mean transit time can be calculated from the area between $C_a(t)$ and $C_v(t)$ during the washout phase after t_{end} and the approximation is not too bad.

External detection techniques can be used with constant rate injections to some advantage, as has been suggested by Lassen. 12 The theory is shown in Fig. 7 for a constant rate infusion into a system without recirculation. The ordinate is tracer content of the system $q(t)$ divided by the tracer content at steady state, q_∞ . Mean transit time can be obtained by two means: (1) Extrapolation of the initial linear portion of $q(t)$ to its intersection with the plateau, q_∞ , at $t = \bar{t}$; and (2) q_∞ divided by the initial slope also is $\bar{t}$.

$$\bar{t} = \frac{q_\infty}{(dq/dt)_{t=0}} \quad [23]$$

To apply this experimentally, one must have a clearly defined segment of the circulation from which one can detect the inflow and outflow with equal efficiencies and be completely masked from tracer in the neighboring circulation. These requirements are important, since both the initial slope and the steady state need to be estimated in order to make the calculation. Here, as in all constant rate injections, one has to beware of recirculation, which is omitted from the diagram.

External detection when using constant rate injection has an additional advantage: to determine when a plateau has been reached. At that time a sample from the venous outflow from the observed region can be taken to estimate C_p . This illustrates the general principle that a combination of internal and external detection techniques may be valuable experimentally and, of course, may provide a certain amount of redundancy, which will reduce the error of the estimations.

THE ESTIMATION OF ORGAN BLOOD FLOW

Impulse and Constant Rate Injection Techniques Can Be Applied Just as for the Measurement of Cardiac Output

In general, the problems with measuring organ blood flow are the same as in measuring cardiac output; that is, one need to have a constant flow, to obtain mixing between tracer and blood in the inflowing fluid, and to obtain representative samples. The problems of nonmixing at the injection site and non-representative sampling, however, are more severe in most organs than they are for the estimation of cardiac output.

Input/Output Analysis: Application of the Kety-Schmidt Technique 13

This is a technique for measuring the mean transit time across an organ and is essentially identical to that outlined for the constant rate infusion technique. The principle is that one does not need to have a perfect step change in arterial concentration, $C_a(t)$, and therefore can use an injection site more remote from the inflow to the organ and record the concentration time curve at the inflow as well as at the outflow to obtain $C_a(t)$ and $C_v(t)$. The situation is diagrammed in Fig. 8. The mean transit time between the injection point and the arterial inflow point would be given by the area between the dashed line representing C_∞ and the rising slope of $C_a(t)$. This is identical to the situation shown in Fig. 6A. Similarly, the mean transit time from the injection point to the outflow point would be the area lying between the dashed line at C_∞ and the out flow curve of $C_v(t)$. The difference between these two areas is the shaded area on Fig. 8, the area lying between $C_a(t)$ and $C_v(t)$, which is the mean transit time across the organ itself. The interpretation of the mean transit time is the same as that used in Eqs. [18] through [20]; that is, the mean transit time is the volume of distribution of the tracer divided by the flow, or the flow per unit volume of organ is the partition coefficient, λ , divided by the mean transit time:

$$\frac{F}{V_{\text{org}}} = \frac{\lambda}{t} \quad [24]$$

In practice, the way this is calculated is shown in Eq. [25]:

$$F = \frac{V_{\text{org}} \cdot \lambda \cdot C_v(\infty)}{\int_0^\infty (C_a - C_v) dt} \quad [25]$$

where the numerator is the mass (mg or cpm) of the indicator taken up by the organ, and the denominator is the shaded area (mg · min/liter) of Fig. 8. Since the volume of the organ is not usually known, the equation is rewritten:

$$\frac{F}{V_{\text{org}}} = \frac{\lambda C_v(\infty)}{\int_0^{\infty} (C_a - C_v) dt} \quad [26]$$

The relationship to the mean transit time from injection point to arterial inflow point, $\bar{t}_a$, and to venous outflow point, $\bar{t}_v$, is emphasized by dividing numerator and denominator of Eq. [26] by $C_v(\infty)$ and rewriting the denominator as mean transit times:

$$\frac{F}{V_{\text{org}}} = \frac{\lambda}{\bar{t}_v - \bar{t}_a} \quad [27]$$

This has been a very useful method of estimating organ blood flow and was first applied by Kety and Schmidt 13 to the estimation of cerebral flow. Its main problem is that the tail of the curve is long and there is some difficulty in prolonging the experiments long enough to achieve an equilibrium between arterial inflow and venous outflow concentrations. In order to shorten the time required for measurement, Kety and Schmidt proposed a formula that is useful when $C_v(T)$ is very close to C_{∞} .

$$\frac{F}{V_{\text{org}}} = \frac{\lambda C_v(T)}{\int_0^T [C_a(t) - C_v(t)] dt} \quad [28]$$

The use of Eq. [28] was based on the idea that $\lambda C_v(T)$ should be close to the tissue concentration. This is true if the blood tissue exchange is flow-limited and not limited either by slow permeation of the capillary membrane or slow diffusion through the tissue. 14 However, there is inaccuracy even under this circumstance, which becomes very large if there is not a continuous equilibrium between the tissue and the venous outflow.

Combining Residue Function and Venous Outflow Sampling

Washout techniques have been valuable because they permit the use of external detection of the tracer contained within the organ, which is ordinarily easier to perform than intravascular blood sampling. But they provide estimates only of $\bar{t}$ and of the flow per unit volume and not the actual flow.

Figure 9 demonstrates that one can use a combination of techniques to obtain an estimate of the absolute flow, F , rather than just the relative flow, F/V . The method is to obtain the slope of the residue function at the same instant that one obtains a sample of the actual concentration from the outflowing blood $C_v(t)$. The method applies to the residue function obtained following a slug injection into the inflow at time zero. The derivation is taken from Eq. [3] using the identity:

$$h(t) = -dR/dt \quad [29]$$

and substituting it into Eq. [3], which, with rearrangement, provides an estimate of the absolute flow, F :

$$F = \frac{-q_0 \cdot dR(t_s)/dt}{C_v(t_s)} \quad [30]$$

where t_s is the time of obtaining the sample from the venous outflow and of measuring the local slope of the residue function curve.

Using the integral of the residue function to provide $\bar{t}$ and the combination of the slope of the residue function and the venous outflow concentration, $C_v(t)$, to provide F , we can calculate V , the volume of distribution of tracer in the tissue:

$$V = F \cdot \bar{t} \quad [31]$$

$$V = \frac{-q_0 \cdot dR(t_s)/dt \cdot \int_0^\infty R(t) dt}{C_v(t_s)}. \quad [32]$$

This again demonstrates the virtue of combining intravascular and external detection techniques to obtain the desired information.

Deposition Techniques for the Estimation of Organ Blood Flow

The use of small, tracer-labeled microspheres has been of great benefit for estimating the fraction of cardiac output going to each organ and to estimating regional flows within organs. The techniques are based on the principle that the microspheres flow with the blood and are distributed in proportion to the blood flow both within each organ of the body and within tissue regions composing each organ. Since it has been shown that microspheres do not necessarily distribute in proportion to the flow, one must keep in mind the potential weakness of the technique. However, it appears that the smallest microspheres that are trapped in the organ are distributed very nearly in proportion to regional flows.¹⁵

The total systemic circulation can be regarded as a parallel pathway system as illustrated in Fig. 10. When tracer-labeled microspheres of amount q_0 are injected into the left atrium or left ventricle and mixed thoroughly with the blood, then one might expect their distribution to be proportional to the blood flow. Thus, if $f_1, f_2, \dots, f_i, \dots, f_n$, are the fractions of the cardiac output going to each of the end regions or organs, then the amount of tracer deposited in each of these regions, $q_1, q_2, \dots, q_i, \dots, q_n$ should be distributed proportionately. Subsequently, on making measurements of the amount of tracer in each region, one can estimate the flow to the region as a fraction of the cardiac output:

$$f_i = \frac{q_i}{q_0}. \quad [33]$$

Then, as diagrammed in Fig. 11, by constant rate sampling from the arterial circulation during the time of passage of the microspheres following the injection into the left side of the heart, the amount of tracer in the collected sample should also be in proportion to the flow into the sampled system as a fraction of cardiac output, F_T :

$$f_s = \frac{F_s}{F_T} = \frac{q_s}{q_0}. \quad [34]$$

(Capital F's are used for absolute flows (ml/sec) and small f's for relative or fractional flows.) Since the absolute flow into the syringe, F_s (ml/sec) can be accurately known, then the cardiac output can be calculated directly:

$$\text{Cardiac output} = F_T = F_s \frac{q_0}{q_s} \quad [35]$$

This is similar to Hale's technique of injecting into a peripheral vein and sampling from the pulmonary artery to estimate F_T . Similarly, the flow to the i^{th} organ is given by the analogous expression:

$$F_i = \frac{F_s q_i}{q_s} = \frac{F_T q_i}{q_0}. \quad [36]$$

When using tracers other than microspheres, the extraction during passage through a capillary bed is usually not complete. It is true that the deposition of potassium, rubidium, antipyrine, and other tracers has been used to give estimates of regional blood flow and the distribution of cardiac output, but such techniques are clearly in error theoretically, and the practical question is what is the magnitude of the error. Errors are due to (1) incomplete extraction, (2) recirculation, and (3) back diffusion or tracer washout from the extravascular space.

For the purposes of the arguments to follow, let us define "extraction" as the maximum value, E , of an instantaneous apparent extraction, $E(t)$, obtainable from paired, simultaneous indicator dilution curves for a permeant and a nonpermeant tracer:

$$E(t) = 1 - h(t)/h_R(t). \quad [37]$$

Here, $h(t)$ is the dilution curve for the permeating or extracted tracer, and $h_R(t)$ is that for a nonpermeating reference tracer such as albumin, both defined as in Eq. [3].

When a tracer such as potassium passes through a capillary bed, the extraction is dependent on the flow through the organ, on the permeability-surface area product of the capillary network within the organ, and on the rate of cellular uptake of potassium by the cells. In the heart, where the vascular network is rich and the capillary density is almost the highest in the body, the extraction of potassium ranges from less than 70% at low coronary blood flows to about 50% at high flows. If one knew the instantaneous extraction value in each of the organs or regions, then one could make corrections for this incomplete extraction. The next problem is recirculation; the indicator traversing each of the organs and passing into their venous outflow recirculates and subsequently passes through each organ again. The recirculation from organs with high flow such as the heart, kidney, and liver occurs very early, but the fractional extraction is different in each. The recirculation from low flow organs with longer arterial and venous systems occurs later. The complex concentration-time curve of the recirculated indicator reenters each of the organs, exposing each organ to further tracer. At the same time, a third feature complicates the analysis yet further: the tracer initially having entered the organ and having been extracted from the blood now tends to diffuse back from the organ into the bloodstream. This back diffusion is smaller and later in organs with large extravascular volumes of distribution for the tracer being used and is higher and earlier in organs with small extravascular volumes of distribution. Thus, the effort to account for incompletely extracted and back-diffusing tracer from each organ and each region of the body, these together comprising the recirculated tracer, is sufficiently

complex that no simple mathematical expression can be laid down as being universally useful. Suffice it to say that the less the instantaneous extraction and the less the extravascular volume of distribution, the more complex is the problem and the more inaccurate the estimates obtained from the relative abundances of the residual tracer sequestered in individual organs or regions.

An approximate expression may be useful if there is a time when the bolus of nonextracted tracer has passed through an organ but back diffusion has not begun. If the circulation could be stopped at this time, then the flow, F_i , through the i^{th} organ could be estimated using division by the instantaneous single pass extraction, E_i :

$$F_i = \frac{F_s q_i}{E_i q_s} = \frac{F_T q_i}{E_i q_0} \quad [38]$$

To apply this equation it is not necessary to know the instantaneous extractions for all the organs of the body, but only for those in which one has measured E_i experimentally over a variety of flows. Since E_i is flow dependent, and it is F_i that is desired from the calculation, it is worth remembering that their estimations are interdependent, and that the experiments should really be regarded as only providing an estimate of their product:

$$E_i F_i = \frac{q_i}{q_s} \cdot F_s \quad [39]$$

In this expression q_i , q_s , and F_s are all known, but there is in theory no way of separating E_i from F_i with this experimental technique. Nevertheless, this is still of some value, since E_i will tend to fall within a 30%–40% range, leaving the estimate of F_i somewhat questionable, but perhaps reasonably useful.

ESTIMATION OF REGIONAL FLOWS WITHIN AN ORGAN

The measurement of relative densities of deposition of specific diffusible tracers in different regions within an organ is a commonly used method of estimating the regional flows in *parallel capillary beds*. The assumptions are just as those described above for the estimation of the fractionation of cardiac output. Again, the problem of incomplete extraction is a major one, but in this case the problem of recirculation is lessened. The reasons for this are as follows: (1) If the tracer has been injected into the arterial inflow to the organ, then the nonextracted tracer is diluted in the total body circulation before recirculating to this and the other organs, thus reducing the absolute amount returning to the organ of interest. (However, it should be remembered that intraarterial injection at the entrance to an organ seldom provides for good mixing across the whole arterial inflow, and very commonly results in abnormal distributions of tracer that are not indications of distributions of flow, but only of streaming in the inflow.) (2) When tracer does recirculate, then it can be expected to be distributed within the organ in the same proportion as the first pass tracer and therefore is mainly a scaling factor, so that when one is estimating the relative regional flows to different organs the presence of recirculated tracer entering the organ is not very important. However, neither of these factors reduces the problem introduced by incomplete extraction in the first place, nor of differences in washout rate or back diffusion of tracer from tissue into the bloodstream.

For such reasons, microspheres have been the indicator of choice. The main problem with microspheres as mentioned earlier is that they do not necessarily distribute in proportion to flow, but distribute in a manner influenced by the microsphere size and by the geometry of

the microvasculature. The experiments of Yipintsoi et al. 15 have shown, for example, that large microspheres go preferentially to the endocardium of the heart, this differentiation between large and small microspheres being similar to that shown by Utley et al. 16 It appears that the maldistribution of microspheres is due in part to the geometry of the branching of the penetrating arterioles causing a “skimming” effect and in part due to a tendency for spheres, at a branch point, to enter the stream with the higher flow, as observed by Fung.¹⁸

The problems in measuring flow through *serial capillary beds* are formidable. Deposited tracers are of little value, since the major extraction will occur in the first capillary bed, through which the tracer passes, leaving only the unextracted tracer to be deposited in the second bed. Obviously, microspheres are useless if they are completely extracted for they will be deposited only in the first region in the series. The problem of *countercurrent diffusional exchange* is also one that applies to many organs, especially the renal medulla. These problems should not be neglected in choosing techniques for estimating regional flows.

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Fig. 1.

Diagram of experimental approach to measuring the response of a system to a slug or impulse injection of tracer of dose q_0 . The striped cylinders represent isotope detectors: The signal provided by the left-most detector is proportional to the amount of indicator $q(t)$ contained in the volume V and is proportional to the residue function $R(t)$. The upper detector, on the outflow, provides the impulse response $h(t)$. In the idealized nonrecirculating system diagrammed here, the accumulated outflow is estimated via the right-most detector, and gives a signal proportional to $H(t)$, the cumulative residence time distribution function.

Fig. 2.

Relationships between $h(t)$, $H(t)$, $R(t)$, and $\eta(t)$. These curves have shapes representative of those obtained across organ beds or through particular segments of the arterial or venous circulation. Note that $h(t)$ and $R(t)$ are not monoexponential. The abscissa is the time relative to the mean transit time through the system. The ordinate of the left-lower panel for $\eta(t)$ is scaled up by the mean transit time so that it has become dimensionless on this plot. During the level portion of the curve of $\eta(t)$, the value for $\bar{t} \cdot \eta(t)$ is 0.34, showing that approximately one-third of the tracer will leave per mean transit time.

Fig. 3.

Tracer-dilution curve with monoexponential extrapolation. The curve of tracer passing the sampling site for the first time (not recirculating) is termed the primary curve, the tail of which is approximated by a monoexponential extrapolation of the downslope, as was used by Hamilton et al. 6 in 1931. The equation for flow uses the area under the primary curve.

Fig. 4.

The area under the residue function is the mean transit time. Curves A, B, and C have the same area and same $\bar{t}$. Curve C represents a system having all transit times the same, as in piston flow.

Fig. 5. Modification of the residue function technique for obtaining an approximate estimate of mean transit time, using early truncation of the curve to avoid recirculation. $\bar{t} = [\text{area up to time } T]/[1 - R(T)]$.

Fig. 6.

(A) Constant rate infusion technique for the estimation of flow. The infusion starts at time zero and ends at t_{end} . Flow can be estimated either from the concentration at the plateau, C_p , and a known constant rate of injection, dm_i/dt or by the single injection formula, Eqs. [12] or [22]; q_0 is the total amount of tracer injected during the constant rate injection, and is t_{end} times dm_i/dt . The diagram is for a nonrecirculating system. (B) The presence of recirculated tracer during the constant rate injection deforms the curve and reduces the accuracy of the technique. $C_r(t)$ is the concentration of recirculated tracer just upstream from the site of the constant rate injection, $C_a(t)$ is the concentration at the inflow which is composed of the injected tracer plus the recirculated tracer so that it rises slowly, more or less as a ramp. The concentration-time curve in the venous outflow, $C_v(t)$, reaches a plateau, C_p , for a very brief period indicated by the bar, and then rises slowly from the plateau as a ramp delayed from $C_a(t)$ by one mean transit time. The correct estimate of C_p during the period of recirculation is the difference between $C_v(t)$ and $C_r(t - \bar{t})$, which is $C_r(t)$ at the injection point delayed by one mean transit time. Calculating the area under $C_v(t)$ is difficult, but if the recirculated curve, $C_r(t)$, is known it can be shifted one transit time to the right and subtracted from the area under $C_v(t)$.

Fig. 7.

External detection with constant rate injection into a nonrecirculating system. The intersection of the initial slope (dashed line) with the plateau, q_{∞} , is at $t = \bar{t}$. The curved portion of $q(t)/q_{\infty}$ (thick line) gives information on the shape of $h(t)$ and of the dispersion of transit times through the system. (This is analogous to the frequency distribution of ratios of renal filtration of glucose to its tubular reabsorption maximum, T_m , as shown by Smith et al. in 1943.17)

Fig. 8.
Flow estimation from analysis of recorded input and output following step injection. At steady state, $C_\infty = C_v(\infty) = C_a(\infty)$.

Fig. 9.

Combined intravascular and external detection. External detection provides $\bar{t}$ and the slope dR/dt at a particular time t_s . Sampling from the venous outflow from the organ at t_s provides the concentration $C_v(t_s)$. The combination allows estimation of F , F/V , and therefore of V .

Fig. 10. Parallel pathway system, f_i is the fraction of the total flow going through the i^{th} pathway, q_i is the amount of tracer deposited in region i where extraction is complete.

Fig. 11. Injection of completely extracted tracers into left ventricle during constant rate sampling from an artery. The quantity of microspheres in the syringe, q_s , is the same fraction of the total injectate, q_0 , as is the syringe flow F_s of the cardiac output, F_T .