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On the Theory of the Indicator-Dilution Method for Measurement of Blood Flow and Volume[1]

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ABOUT 60 YEARS AGO Stewart (1) introduced and for the past 25 years Hamilton and his colleagues (2) have developed and extended the indicator-dilution technics for measurement of cardiac output. These methods have more recently been applied to measurement of regional blood flow. Although there has been some criticism of the use of the indicator-dilution technics to measure blood *flow*, its application for this purpose has achieved wide acceptance.

It was also Stewart (3) who first used these technics to measure the *volume* of blood in the heart and lungs, and again it was Hamilton and his colleagues (2) who developed and emphasized the use of mean circulation time to determine the volume of a vascular bed. It is of interest that mean circulation time has also been applied in hydraulic engineering to the measurement of the volume of water conduits (4). No theory was presented and the mean time was only one of several parameters used.

This application of the Stewart-Hamilton methods has been subject to considerable controversy and misinterpretation. The mean time has on occasion been confused with the median time, and several workers have expressed doubt as to the validity or meaning of the measurement of volume as a function of the mean circulation time (5, 6).

The basic relationship, volume = flow times mean circulation time, has been accepted as obvious by proponents of these methods. However, in view of the confusion which has arisen it may be of some value to present a direct proof of its validity under appropriate conditions.

It is the purpose of this paper to present such a proof, and also to consider the

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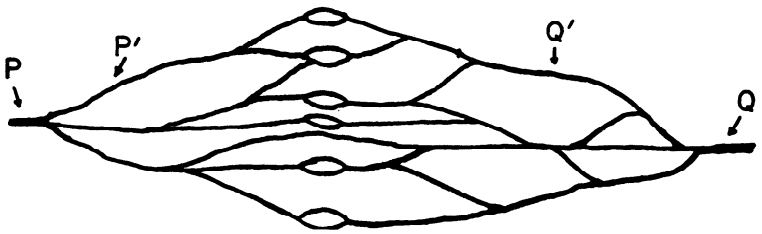

FIG. 1. Schema of vascular bed with injection sites P, P' and sampling sites Q, Q'.

circumstances under which the indicator-dilution technics provide reasonable measures of mean circulation time. In particular, the instantaneous injection and constant injection technics will be shown to share essentially the same advantages and defects. As the latter method has escaped some of the criticism applied to the former, their equivalence may be regarded as additional support for the instantaneous injection technic.

The issues are obscured to a considerable extent by the complex nature of a vascular bed. In order to make clear the rationale of the Stewart-Hamilton methods we will consider first a relatively simple model in which certain complications do not arise. The effects of these complications are considered in SECTION 3.

1. THE MODEL

For simplicity consider a *closed* flow system, that is, one with a single in-flow orifice, P, and a single outflow orifice, Q, (fig. 1). The system contains a volume, V, of fluid which enters and exits at a constant rate of flow, F. In general, the internal structure of the flow system will consist of many branchings and interlacings of blood vessels. As a consequence of this, particles of fluid entering at P at the same instant will require varying amounts of time to reach Q, the time required for any particle depending on the path taken and the velocity with which it travels. Thus one cannot speak of a single traversal time, but must instead consider a distribution of traversal times. No assumptions need be made concerning the relative proportions of particles having long or short traversal times; that is, the distribution of traversal times is determined solely by the experiment and is not made a part of the theoretical structure.

In addition to the above requirements needed to define volume and flow the following assumptions are made. *a*) The distribution of traversal times for entering particles of fluid does not change with time; that is, the particles entering at P at any time are dispersed when they leave at Q in exactly the same manner as the particles entering at P at any other time. This property will be referred to as *stationarity* of flow. *b*) The flow of indicator particles is representative of the flow of total fluid; that is, the distribution of traversal times for indicator particles is the same as that for fluid particles. *c*) The system has no stagnant pools; that is, fluid anywhere in the system is eventually eliminated. (This assumption is needed only for measurement of volume. The existence of stagnant pools does not affect the measurement of flow.) *d*) Recirculation of indicator is not present.

The technics considered require the injection of indicator at P and the measurement of indicator concentration at Q as a function of time, the indicator being injected either nearly instantaneously or continuously at a constant rate. In the instantaneous

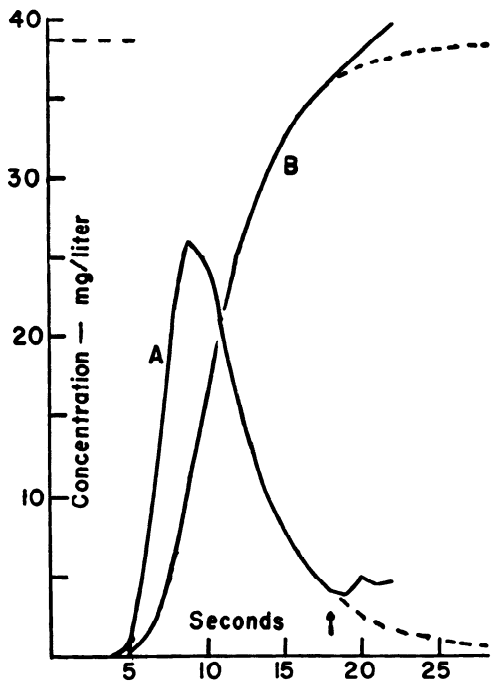

FIG. 2. Concentration of indicator at exit as a function of time. *Curve A* results from single, instantaneous injection and represents a curve obtained when the blue dye, T-1824, is injected into the systemic venous circulation, passes through the pulmonary circulatory tree and the heart, and is sampled from a systemic artery. The continuous line is the curve obtained experimentally, the break in the *smooth curve* at 18 seconds, indicated by the *arrow*, is interpreted to represent the appearance of detectable recirculating dye. The *dashed line* which continues the smooth curve is an extrapolation (see legend to fig. 4) and represents the curve that would obtain if there were no recirculation.

Curve B represents the concentration-time curve to be expected when dye is injected continuously at constant rate. It is the integral of *curve A*, with the concentration units on the ordinate adjusted to give a maximum concentration within a practical range. The break in its asymptotic behavior at 18 seconds has the same significance as the break at that time in *curve A*; recirculating indicator must appear simultaneously in the two curves. The dashed line which extends the smooth curve toward a maximum and constant value, indicated by the dashes at 38.6 mg/l. on the ordinate scale, is an extrapolation (see legend to fig. 4) and represents the curve that would obtain if there were no recirculation.

injection method, q units of indicator are injected at P and the concentration at Q will rise to a maximum and then decrease to zero as in figure 2, *curve A* (recirculation not present). In the continuous injection method indicator is injected at a rate of I units per unit time and the concentration of indicator will rise asymptotically to a constant level as in figure 2, *curve B*. The fact that the curve resulting from constant injection is simply the integral of that resulting from single injection was first pointed out by Hamilton and Remington (7).

2. THEORY OF INDICATOR-DILUTION METHODS

Instantaneous Injection. In the model described above, when indicator is introduced at P (fig. 1), it must all, in due time, leave the system at Q, giving a concentration vs. time curve such as shown in figure 2, *curve A*. If flow is great, the indicator will be mixed with a large amount of blood and the concentration at Q (fig. 1) will be low. This relationship is made the basis of a quantitative method for measuring flow. Thus, let q units of indicator be injected at P at time zero and let the observed concentration at Q, which varies as in figure 2, be denoted by $c(t)$. Then the amount of indicator which leaves the system during a small time interval, t to $t + dt$, is the concentration of indicator at Q multiplied by the volume of fluid which leaves the system during this time interval, or $c(t)$ times $F\,dt$. Since all of the indicator finally

leaves the system, the amount injected, q, equals the sum of the amounts leaving the system during all subsequent time intervals, or

$$q = \int_0^\infty c(t)(F\,dt) = F\int_0^\infty c(t)\,dt. \tag{1}$$

From this it follows that the area under the curve of indicator concentration vs. time, or $\int_0^\infty c(t)\,dt$, is equal to q/F. Solving for F we may write

$$F = \frac{q}{\int_0^\infty c(t)\,dt}. \tag{2}$$

Now $Fc(t)$ is the rate at which indicator is leaving the system at time t. For the discussion which follows it will be convenient to introduce the function

$$h(t) = \frac{Fc(t)}{q}, \tag{3}$$

which is the fraction of injected indicator leaving the system per unit time. Under *assumption b*, the distribution of traversal times for indicator particles is the same as that for all fluid particles. Therefore, of those fluid particles which entered the system at time zero, the fraction leaving per unit time is $h(t)$. Thus *equation 3* frees us of the necessity for considering indicator particles and makes available the distribution of traversal times for all fluid particles. Since all the fluid entering the system at zero time must eventually leave, we have

$$\int_0^\infty h(t)\,dt = 1, \tag{4}$$

as may be verified by combining *equations 2* and *3*; $h(t)$ will be recognized as the frequency function of traversal times.

The fundamental relationship, volume = flow multiplied by mean circulation time, may be established as follows. Consider the fluid present in the system at any particular instant, say at time zero. The particles of which this fluid is composed may be distinguished by the time each requires to traverse the system from P to Q. To find the volume of the system it is only necessary to find the volume of those particles having traversal times in the vicinity of t, specifically in the interval t to $t + dt$, and to sum these elements of volume, dV, for all such time intervals. The particles of this kind present at time zero will have entered the system at times extending back from zero to a time t units earlier. Those which entered earliest are now ready to leave the system; those which just entered will leave the system at time t. Now, the rate at which fluid enters the system is F, and the fraction of entering particles which require times between t and $t + dt$ to leave is $h(t)\,dt$. Therefore, the rate at which such particles enter the system, and also the rate at which they leave it, is $F\,h(t)\,dt$. Thus, the particles of this kind present in the system at time zero leave at the rate $F\,h(t)\,dt$ and continue to do so until time t, at which instant all such particles will have been eliminated. The volume of such particles is the time required for them to

leave the system multiplied by the rate at which they leave, or, $dV = [t][F\ h(t)\ dt]$. Summing for all such time intervals we get

$$V = \int_0^\infty tFh(t)\ dt \qquad \text{or} \qquad V = F \int_0^\infty th(t)\ dt. \tag{5}$$

Since $h(t)$ is the frequency function of traversal times, $\int_0^\infty t\ h(t)\ dt$ is just the mean of the traversal times, or the mean circulation time, which we will denote by $\bar{t}$.

$$\bar{t} = \int_0^\infty th(t)\ dt. \tag{6}$$

In terms of the observed function, $c(t)$, this is

$$\bar{t} = \frac{\int_0^\infty tc(t)\ dt}{\int_0^\infty c(t)\ dt}. \tag{7}$$

Thus, finally, we may write

$$V = F\ \bar{t}, \tag{8}$$

which is our fundamental relationship: volume = flow multiplied by mean circulation time. As stated earlier this derivation requires no assumptions about the form of the frequency function, $h(t)$, which is determined solely by the observed curve of $c(t)$ vs. time, as indicated by *equation 3*.

Constant Injection. If, instead of a sudden single injection, indicator is introduced at a steady rate, the concentration of indicator in the fluid leaving the system will increase until all of the indicator-free fluid initially in the system is washed out, as shown in figure 2, *curve B*. If indicator is introduced at the rate of I units per unit time, its concentration in the blood entering the system is clearly I divided by the rate at which fluid enters the system; that is, I/F. This is the concentration that will be approached at Q (fig. 1) as indicator-free fluid is washed out of the system. Writing C_{max} for the limiting concentration at Q we have

$$C_{max} = I/F. \tag{9}$$

This equation follows from an important general formula given by Stephenson (8) which is derived below.

If indicator is introduced at P at the rate $i(t)$, not necessarily constant, the concentration of indicator at Q, $C(t)$, will be determined by the rate at which indicator was introduced at all times before t and by the frequency function of traversal times, $h(t)$. Consider the contribution to the rate at which indicator is leaving the system at time t made by indicator introduced in the vicinity of s time units earlier, specifically in the interval s to $s + ds$ time units before t. The amount of indicator introduced during this interval is $i(t - s)\ ds$. The fraction of this amount being eliminated per unit time at time t, s time units later, is $h(s)$, in accordance with the argument following *equation 3*. Therefore, the contribution to the rate at which indicator is leaving the system at time t made by indicator introduced between s and $s + ds$ time units earlier is the product $[h(s)][i(t - s)\ ds]$.

Summing for all such time intervals before t, the rate at which indicator is leaving the system at time t is $\int_0^\infty i(t - s)\, h(s)\, ds$. Since, also, the rate at which indicator is leaving the system is equal to the product of concentration and flow, we have

$$C(t) = \frac{1}{F} \int_0^\infty i(t - s) h(s)\, ds. \tag{10}$$

For the special case of constant injection

$$i(t) = \begin{cases} 0 \text{ for } t < 0 \\ I \text{ for } t \geq 0, \end{cases} \tag{11}$$

where I is the constant rate of injection. *Equation 10* then reduces to

$$C(t) = \frac{I}{F} \int_0^t h(s)\, ds. \tag{12}$$

Now, it will be recalled that $h(s)\, ds$ is just the fraction of entering fluid particles with traversal times between s and $s + ds$. The sum of $h(s)\, ds$ over all time intervals less than t gives the fraction of entering fluid particles with traversal times less than t. This integral defines the distribution function

$$H(t) = \int_0^t h(s)\, ds. \tag{13}$$

Note that since $\int_0^\infty h(s)\, ds = 1$, $\lim_{t \to \infty} H(t) = 1$.

Equation 12 may now be written

$$C(t) = \frac{I}{F} H(t). \tag{14}$$

(Hereafter $C(t)$ will be used solely to denote the concentration resulting from a constant injection at the rate of I units per unit time.) As t gets large $C(t)$ approaches C_{max}, and since $\lim_{t \to \infty} H(t) = 1$, we get *equation 9*, $C_{max} = I/F$.

The volume of the system is now easily determined from the fact that at any time, t, the amount of indicator in the system, say $Q(t)$, is the difference between the amount introduced and the amount which has left the system. That is,

$$\begin{aligned} Q(t) &= (\text{input up to time } t) - (\text{output up to time } t) \\ &= It - \int_0^t FC(t)\, dt \\ &= \int_0^t [I - FC(t)]\, dt \end{aligned}$$

or,

$$Q(t) = I \int_0^t [1 - H(t)]\, dt, \tag{15}$$

which follows from *equation 14*. For large t the average concentration throughout the system, that is $Q(t)/V$, approaches C_{max}, so

$$\lim_{t\to\infty} \frac{Q(t)}{V} = \frac{I}{V}\int_0^\infty [1 - H(t)]\,dt = C_{max} = \frac{I}{F},$$

or,

$$V = F\int_0^\infty [1 - H(t)]\,dt. \qquad (16)$$

Written in terms of observed concentrations of indicator this becomes

$$V = \frac{F}{C_{max}}\int_0^\infty [C_{max} - C(t)]\,dt. \qquad (17)$$

($\int_0^\infty [C_{max} - C(t)]\,dt$ may be determined as the area between the *curve* C(t) vs. t and the line C_{max} vs. t; see fig. 2, *curve B*.)

Relationship Between Formulas for Single and Constant Injection. The two methods, single and constant injection, must lead to the same estimate of volume, although the identity of *equations 8* and *16* is not self-evident. To prove the identity we must show that the factor $\int_0^\infty [1 - H(t)]\,dt$ in *equation 16* is, in fact, the mean circulation time, $\bar{t}$, which appears in *equation 8*. This may be seen by an integration by parts; thus

$$\int_0^t [1 - H(s)]\,ds = t[1 - H(t)] + \int_0^t sh(s)\,ds$$

or,

$$\int_0^\infty [1 - H(s)]\,ds = \lim_{t\to\infty} t[1 - H(t)] + \int_0^\infty sh(s)\,ds.$$

It is evident from figure 3 that the integrals must both be finite or both infinite (i.e. infinite volume) and that in the former case $\lim_{t\to\infty} t\,[1 - H(t)] = 0$. Since the volume must be finite, it follows that

$$\int_0^\infty [1 - H(s)]\,ds = \int_0^\infty sh(s)\,ds = \bar{t}, \qquad (18)$$

which shows the equivalence of *equations 8* and *16*. Since the derivation of *equation 16* is independent of *equation 8*, this identity provides an independent demonstration of the basic relationship, volume = flow multiplied by mean circulation time.

As we have seen, the height of the curve resulting from a constant injection is proportional to the area under the curve resulting from a single injection. It is important to recognize that the sole condition required for this relationship to hold is that the flow be stationary. Even in the presence of recirculation this relationship is maintained. This was demonstrated experimentally by Howard, Hamilton and Dow (9). Thus, the argument sometimes offered that one or the other method is less subject to the difficulties arising from recirculation appears to be without foundation.

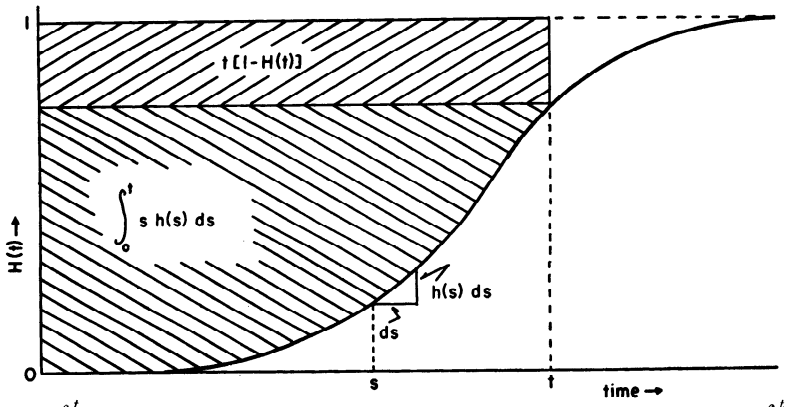

FIG. 3. $\int_0^t [1 - H(s)]\,ds$ is represented by the sum of the two shaded areas, and $\int_0^t sh(s)\,ds$ by the lower shaded area. For the limit of this area as $t \to \infty$ to remain finite, it is intuitively clear that the area of the upper rectangle must approach zero, and this can easily be proved.

3. Relation of the Model to Real Vascular Systems

Formal treatment of the sort undertaken here is only as good as the model which it analyzes. Since vascular systems, in general, are not apt to be represented accurately by a stationary system with a single input and single output, it is important to consider the effect of relaxing some of the restrictions on the original model to approach more closely the real situation, with the understanding that no thoroughly defined system can be expected to mimic completely all the phenomena of physiological importance.

Finite Injection Time. In the instantaneous injection method it was assumed that q units of indicator could be injected at the instant $t = 0$. In fact some possibly non-negligible time is required to accomplish the injection. This in no way affects the determination of flow, but it does require a slight modification in the determination of volume. This is merely to shift the zero point from the start of injection to the average injection time. If the rate at which indicator is injected varies symmetrically (e.g. a constant rate for a short interval) the appropriate zero is midway between the start and finish of the injection.

Structure of the System. A potentially serious weakness of the model lies in the condition that it be closed, i.e. that it have a single input and single output. However, the results of the analysis can usually be interpreted when this restriction is removed. Consider now the situations illustrated in figure 1. A single artery, P, divides into a number of branches, one of which is P', and these subdivide, ultimately to terminate at capillaries. Venous channels coadunate progressively, one of many being represented at Q', and ultimately all venous drainage flows out through the single channel, Q. Injection at P and sampling at Q are the case of the original model.

If injection occurs at P' and sampling at Q (many inputs, single output), the equations developed for the closed system still permit measurement of flow, but the volume measured by application of these equations will include some portion of other input channels. From the argument leading to *equation 5* it is clear that the portion of each uninjected input channel included properly in the measured volume begins at a site into which injection would produce the same mean time as was measured from the site actually used.

If injection occurs at P and sampling at Q' (single input, many outputs), the equations developed for flow again hold. The volume includes a portion of each output channel up to that site at which the mean time is the same as that determined for the sampling site actually used.

In either of these cases, $P' \rightarrow Q$ or $P \rightarrow Q'$, the total flow passes either entrance or exit and, in the case of continuous injection, flow can be determined by the limiting concentration of the indicator. However, if injection occurs at P' and sampling at Q' (many inputs, many outputs) the interpretation is more difficult. If both injection and sampling sites are minor branches of the system, determination of flow and volume may bear little relation to the quantities of interest. For a reasonable interpretation of the measurements further assumptions are necessary. For example, if there is reason to believe that the concentration vs. time curve is essentially the same in all output branches, the situation reduces to that of many inputs, single output. A weaker assumption is that the flow from the site of injection becomes mixed in the sense that the fraction of it leaving through any output channel is proportional to the flow in that channel. In the case of constant injection, this assumption is equivalent to saying that the limiting concentration in each channel is the same. It is important to note that for certain vascular beds these assumptions can be and have been put to experimental test (10). If this latter assumption holds, flow can be determined by the equations established. The volume can be interpreted as follows. Include each output channel up to the point at which the mean time is the same as that at the actual sampling point, and include each input channel up to the point at which the average mean time to all the output points just determined, weighted by the flow through each output channel, is the same as that at the actual sampling site.

In general, then, the volume determined by application of these equations is constructed by joining all inputs at an appropriate point and all outputs at an appropriate point so that the limiting concentration, in the case of constant injection, and the mean time are the same as in the actual experiment. (It is not necessary that the shape of the concentration curve be the same in each channel.)

This description of the volume measured may seem artificial and indeterminate. Nevertheless, it is of assistance in conjuring up an image of the volume and in deciding whether a particular experiment is likely or not to give reasonable results. If the system being measured is well understood, it may be possible to decide that the contributions to the measured volume of accessory input and output channels are negligible and can be ignored.

Effect of a Collecting Catheter. The concentration of indicator is not always measured directly at the exit of the system, Q. Often a sampling device, such as a catheter, is interposed between Q and the site at which concentration is measured. The mean time determined in such an experiment is the sum of the mean time required to traverse the system and the mean time required to traverse the catheter. In effect the catheter acts as another outflow branch and its effect may be analyzed as in the preceding section. If, however, the blood sampled by the catheter is representative of the total outflow, the error in volume contributed by it is simply the mean time required to traverse the catheter, say $\bar{t}_c$, times the total flow through the system, F. If $\bar{t}_c$ is known, the volume of the system may be determined from the formula

$$V_{\text{system}} = F(\bar{t} - \bar{t}_c). \qquad (19)$$

Stationarity. The stationarity condition will be violated particularly by any system including the heart, because both volume and flow change phasicly. It might be pointed out, however, that the constant-injection method is relatively insensitive to violation of stationarity since the input-output relationship used to derive *equations 10* and *12* remains valid. To the extent that a steady-state output concentration is reached, the results apply to determination of average flow and average volume. On the other hand, the results of a single injection may depend greatly on the relation of the time of injection to the phase of the cardiac cycle and may be unsatisfactory. The more cycles occurring during the evolution of the indicator-dilution curve, the less important this violation is likely to be. A careful treatment of nonstationary systems is somewhat complex and will not be pursued further.

Representative Behavior of Indicator Particles. *a) Inhomogeneity of blood and significance of the venous hematocrit.* It is well known that the mean velocity of erythrocytes is greater than that of plasma in flowing blood. Consequently, the distribution of traversal times determined from, for example, T-1824-labeled plasma differs from the distribution of erythrocyte traversal times (11, 12). Determination of indicator concentration, then, permits a direct estimate of volume and flow of plasma, but not of whole blood. It is customary to compute whole blood flow or volume by dividing the measured plasma flow or volume by a factor (100 − venous hematocrit). It is the purpose of this section to show that such a computation is valid only with respect to estimation of flow and to introduce what appears to be a new concept concerning the physiological significance of the venous hematocrit.

Let the rate at which erythrocytes flow through a vein be F_E and let the rate at which plasma flows through the same vein be F_P. If sampling of venous blood, as ordinarily performed, is representative of the total flow through the vein, then we may consider the hypothetical case in which the vein is cannulated and its entire flow collected. In one unit of time the collecting vessel will contain a volume F_E of erythrocytes and a volume F_P of plasma, a total volume of blood, $F_E + F_P$. When this collecting vessel is centrifuged it will be found, therefore, that the hematocrit (as %) is

$$Ht = [F_E/(F_E + F_P)]100 = (F_E/F_B)100$$

$$\text{and } 100 - Ht = (F_P/F_B)\ 100,$$

where $F_B = F_E + F_P$. Thus the hematocrit measures the ratio of erythrocyte flow to total blood flow and not the ratio of erythrocyte volume to total blood volume. Blood flow, therefore, can be calculated from plasma flow by a simple hematocrit factor.

The calculation of blood volume from a knowledge of either plasma volume or of erythrocyte volume is more complicated. Since erythrocytes travel faster than does plasma the mean traversal time for erythrocytes will be less than that for plasma. Consequently a given flow of erythrocytes corresponds to less volume than does the same flow of plasma, as is evident from the general relation $V = F\bar{t}$. Note first that $F_E = F_P\, Ht/(100 - Ht)$. Then, substituting $V/\bar{t}$ for F we have

$$\frac{V_E}{\bar{t}_E} = \frac{V_P}{\bar{t}_P}\left(\frac{Ht}{100 - Ht}\right)$$

and

$$V_E = V_P\left(\frac{\bar{t}_E}{\bar{t}_P}\right)\left(\frac{Ht}{100 - Ht}\right).$$

The volume of blood $V_B = V_E + V_P$, or

$$V_B = V_P \left[\left(\frac{\bar{t}_E}{\bar{t}_P} \right) \left(\frac{Ht}{100 - Ht} \right) + 1 \right],$$

where subscripts B, P and E refer to values for whole blood, plasma, and erythrocytes, respectively. Thus a solution for blood volume from a knowledge of plasma volume requires measurement not only of the venous hematocrit but also of the ratio of mean traversal times of plasma and erythrocytes through the system for which the volume is estimated; a single hematocrit correction factor is insufficient.

Indeed it is well established that estimation of blood volume by multiplying plasma volume by a venous hematocrit factor yields a value which is in disagreement with the sum of values obtained by direct estimation of plasma volume and of erythrocyte volume, measured by the use of two appropriate indicators (13, 14). For example, in man, the so-called total body hematocrit, $V_E/(V_E + V_P)$, obtained by measurement of both V_E and V_P, has been found to be less than the venous hematocrit by a factor which is rather consistently equal to 0.91 (13). This finding and similar observations in experimental animals (14) have led to a search for excess plasma in the body, but the discrepancy has remained incompletely explained. It is proposed here that the discrepancy can be explained by recognizing the venous hematocrit as a ratio of flows, as we have described it above. Since the venous hematocrit is not a ratio of volumes there is no a priori reason for demanding that it agree with the total body hematocrit.

The ratio, K, of the total body hematocrit to the venous hematocrit, Ht, can be expressed by

$$K = \left(\frac{V_E}{V_E + V_P} \right) \frac{100}{Ht} = \left(\frac{F_E \bar{t}_E}{F_E \bar{t}_E + F_P \bar{t}_P} \right) \frac{100}{Ht}.$$

Since the venous hematocrit is a ratio of flows and $(Ht/100) = (F_E)/(F_E + F_P)$ then $F_P = F_E \dfrac{(100 - Ht)}{Ht.}$. Substitution of this value for F_P leads to

$$K = \frac{\bar{t}_E}{\bar{t}_E Ht + \bar{t}_P(100 - Ht)} 100.$$

It can be estimated from Fries' observation on traversal of plasma and of erythrocytes through the forearm in man (11) that $\bar{t}_P$ is approximately in the range of 1.1 $\bar{t}_E$ to 1.2 $\bar{t}_E$. Substitution of 1.15 $\bar{t}_E$ for $\bar{t}_P$ and of a venous hematocrit equal to 40 yields $K = 0.92$, in agreement with experimental estimates (13).

b) Nonrepresentative behavior within plasma. The possibility of discrepancy in the measurement of plasma flow and volume arises from the fact that plasma itself is composed of many molecular species, only one of which, in usual practice, is tagged by an indicator. However, most molecular species in plasma may be expected to follow essentially identical paths and to have essentially identical distributions of traversal times.

A different type of nonrepresentative behavior arises from the fact that some substances leave the capillary bed by exchanging across capillaries. For example, according to the Starling hypothesis net loss of water occurs over one segment of the capillary bed and net gain over another. In the case of T-1824, which is essentially bound to plasma albumin and so confined to the vascular bed, transcapillary exchange of plasma water will produce concentrations of T-1824 (or albumin) in the

capillary in the region of water exchange which will exceed the limiting concentration at exit. Under these circumstances, although analysis of the concentration curve still leads to a proper estimate of the quantity of indicator captured within the system, it is no longer true that the volume of the system is this quantity divided by the limiting concentration at exit, C_{max}, and estimation of volume from this ratio, *equation 12*, leads to a falsely high value. The magnitude of the error depends upon many factors, but it is limited by the fraction of the flow which crosses the capillary wall (whether or not this fraction is returned to the blood in another portion of the capillary) and also by the portion of the volume to be measured which is concerned with transcapillary exchange. In skeletal muscle, for example, based on estimates obtained in the isolated hind leg of the cat (15), about 5% of the flow is exchanged across the capillary. If the volume in the capillaries is as much as 30% of the vascular volume in the limb, the error of estimate of volume should be no greater than 30% of 5%, or 1.5%.

Recirculation. Since recirculation has been permitted in the usual practice of indicator-dilution experiments, it is essential that its presence be treated. In experiments in which a single rapid injection of indicator has been used, recirculation has been eliminated from the calculations by extrapolation of the down-limb of the concentration vs. time curve on the assumption that the decay in concentration would have been exponential in the absence of recirculation (see fig. 4, *curve A*). A similar

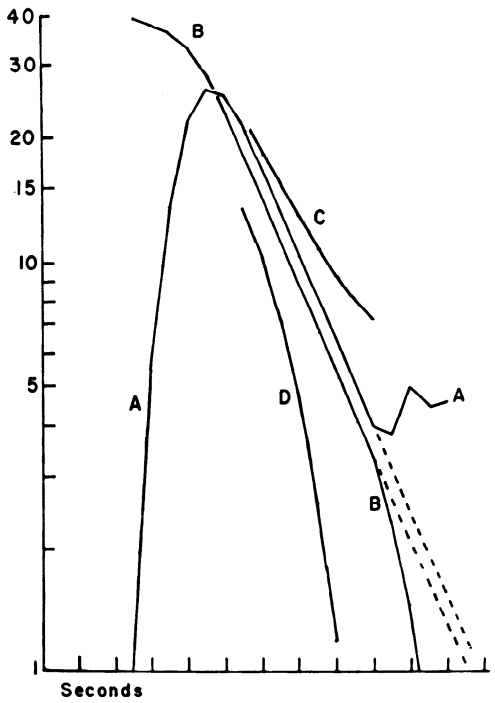

FIG. 4. Extrapolation of indicator-dilution curves. These curves are derived from the same data used to plot the curves in figure 2. *Curve A* is the same as the instantaneous injection curve in figure 2, plotted on a semi-logarithmic scale. If late exponential decay does occur and its presence is established prior to the contribution of recirculating indicator, the curve to be expected in the absence of recirculation can be constructed by extrapolation of the straight line, as indicated by the *dashed line*; that is, it is assumed that after some time, t_1, $c(t)$ takes the form $c(t) = Ke^{-kt}$. This extrapolation has been used widely since it was first proposed by Hamilton *et al.* (2).

Curves B, C and *D* are constructed from the data used in plotting *curve B* of figure 2, which is $C(t)$, the concentration of dye resulting from constant injection. Since $C(t) = (1/q)\int_0^t c(s)\,ds$, the assumption of exponential decay for $c(t)$ after some time, t_1, leads to the form $C(t) = C_{max}(1 - e^{-kt})$ for $C(t)$ after t_1. C_{max} may be established graphically by choosing trial values of C_{max} and plotting $C_{max} - C(t)$ on semi-logarithmic paper as shown. The value of C_{max} giving the best straight line for $C_{max} - C(t)$ is the value chosen. *Curve B* shows the results using C_{max} = 38.6 mg/l. *Curves C* and *D* show the results using values approximately 10% higher and lower than 38.6. It will be seen that *curves C* and *D* show marked curvature and that *curve B* is linear over the same range in which *curve A* is linear and has the same slope. The *dashed line* extending the linear portion of *curve B* represents the values of $C_{max} - C(t)$ to be expected in the absence of recirculation.

extrapolation may be made in the case of constant injection (see fig. 4, *curve B*). In either case, when recirculation occurs early, there may be doubt that sufficient data have been obtained to warrant the extrapolation and large errors are possible. Alternative analyses, based on consideration of recirculation as an essential event, have been formulated by others (see next section), and experimental methods based on one of these may be useful in the case of uncertain correction for recirculation.

4. Alternative Approaches

The analysis given herein has formalized the methods developed by Stewart (1), Hamilton and his colleagues (2), and others. The variation introduced by Lewis (6) amounts to a special choice of quadrature formula for determining mean circulation time; that is, Lewis has in fact calculated $(q/F)h(t)$ due to a single injection, then evaluated $\int_0^\infty [1 - H(s)]\, ds$, which is, as shown above, exactly the mean circulation time.

A different method of using data from a single injection was proposed by Newman *et al.* (16). They propose a model in which portions of the vascular system are likened to mixing chambers. If indicator is instantly and thoroughly mixed with the fluid in a single chamber, the concentration of indicator leaving the system will be proportional to the mean concentration throughout the system; that is, concentration at outflow will exhibit exponential decay, the rate of decay being equal to flow divided by the volume of the chamber. If a number of such chambers of different volume are connected in series, the dominant rate of decay will be determined by the largest volume in the series. Since the rate of decay is measured experimentally, and flow can be calculated by the usual means, this largest volume can be estimated. The aim of this method, therefore, differs from the aim of the mean circulation time method, the former claiming to estimate only a portion of the volume estimated by the latter. However, it should be pointed out that in the case of a single mixing chamber model, assuming for the moment that the model applies, the two methods need not give identical answers. According to Newman *et al.*, the single mixing chamber produces a concentration vs. time curve described by a lag exponential of the form $c_0\, e^{-k(t-t_a)}$ where t_a represents the least time required for indicator to travel from the site of injection to the site of sampling, c_0 is the maximum concentration, and k is a constant. They equate k to F/V, or $V = (F/k)$. However, the mean time of an exponential is $1/k$, and the mean time of the lag exponential is $(1/k) + t_a$. Therefore, volume calculated by the mean time method is $V = F(1/k + t_a)$. Since t_a may be appreciable compared to $1/k$, the two estimates of volume may differ considerably.

Using the same basic formulation as in this paper Stephenson has given a more highly developed mathematical approach (8) in which recirculation is made the central feature. Estimates of flow and volume are derived from the asymptotic form of concentration curves approached under two types of constant injection, one being injection within V and the other injection outside V.

An independent analysis appropriate to constant injection at a single site is given by Zierler (10).

An extension of Stephenson's mathematical methods is employed by Sheppard *et al.* (17) for the case of indicator which diffuses out of the flow system. Experimental trials on dogs gave volumes which were smaller than those determined by Stewart's traditional method.

The comparative usefulness of the asymptotic approach and the traditional methods introduced by Stewart and by Hamilton and co-workers will depend upon

the system under study. If recirculation begins so early that it is impossible to get an adequate picture of $h(t)$, the traditional methods fail. However, if a simple correction for recirculation is adequate, flow and volume can be determined much more simply and perhaps more accurately by the traditional methods discussed in this paper.

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