Theoretical Basis of Indicator-Dilution Methods
For Measuring Flow and Volume

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The purpose here is to inquire into the justification of the use of the indicator-dilution principle for measurement of fluid flow and volume. What is the validity of the formal expressions? Upon what assumptions are they based? What are the effects of specified violations of these assumptions?

An indicator, in the sense used here, is a substance that permits observations of some element of volume of the fluid under study. The indicator shows the position of the element of volume in space and with respect to time, and distinguishes the indicated element from all other elements of volume.

In practice, a known quantity of indicator is introduced into a fluid flowing at unknown rate through a system of unknown volume. Fluid is sampled or monitored at one or more points downstream from the plane of introduction and the concentration of indicator, diluted by the parent fluid, is measured as a function of time. Indicator may be introduced into the system in any of a number of ways, usually either only once as rapidly as possible (sudden injection) or continuously at constant rate (constant injection). It is claimed that from a knowledge only of the quantity of indicator injected (or of the rate of its injection for the case of constant injection) and of the observed concentration of diluted indicator at the sampling site, over appropriate time intervals, both flow and volume can be calculated. The validity of this statement has been argued effectively by Stewart, Hamilton and his colleagues, Sheppard, Meier and Zierler, and Burger and colleagues. The argument that follows is adapted from one given previously.

The Assumptions of the Ideal System

Consider a system with a single inflow and a single outflow orifice. The internal structure of the system is of no concern for the present. Recirculation does not occur; that is, once a unit of fluid leaves the system it does not re-enter. In order to measure flow through and volume of a system it is necessary that flow and volume be constant during the period of measurement. Constant volume implies that every unit of fluid entering the system must eventually leave the system. One other stipulation must be made, but this one requires some exposition. The system must exhibit stationarity. To understand stationarity we must look for a moment at a real system, say a vascular bed.

When an indicator is injected suddenly into some portion of a vascular bed it does not all appear suddenly at a sampling site but it is dispersed with respect to time. The time required for a given indicator particle to flow from entrance to exit through the system, by whatever path, is its transit time. No one transit time applies to all indicator particles; rather, there is a family of transit times. Stationarity demands that the frequency with which each transit time occurs, that is, the distribution of transit times, must remain constant during the experiment.

Finally, it is essential that the distribution of transit times of indicator particles be identical with the distribution of transit times of the native fluid; that is, indicator and native fluid must mix thoroughly at the entrance to the system.

Measurement of Flow and Volume by Sudden Injection

We now consider measurement of flow and

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Original studies described here have been aided by a contract between the Office of Naval Research, Department of the Navy, and The Johns Hopkins University (NR 101-241), and by a grant from the National Institute of Arthritis and Metabolic Disease (A-750).

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Circulation Research, Volume X, March 1968
volume by means of sudden injection of an indicator. To measure flow, we rely on the assumption that all the indicator must leave the system sooner or later.

Let $m_1$ units of indicator be injected at time zero into the entrance to the system, and measure the concentration of indicator at exit as a function of time, $c(t)$. The amount of indicator, $dm$, leaving the system during a small time interval between time $t$ and time $t + dt$ is the concentration of indicator, $c(t)$, multiplied by the volume of fluid leaving the system during this time interval, and this is the flow, $Q$, in units of volume/time, multiplied by the time interval, $dt$; that is, $dm = c(t) Q dt$.

Because all indicator, $m_1$, must leave the system, $m_1$ equals the sum of the amounts leaving the system during all such time intervals, or

$$m_1 = \int_0^\infty c(t) Q dt = Q \int_0^\infty c(t) dt,$$

whence

$$Q = \frac{m_1}{\int_0^\infty c(t) dt}. \quad (1)$$

The unknown flow, $Q$, through the system is determined by measuring the area under the observed indicator concentration-time curve and dividing it into the known quantity of injected indicator.

We now introduce the distribution, or frequency, function, $h(t)$, which describes the fraction of injected indicator leaving the system per unit time at time $t$; that is,

$$h(t) = \frac{Q c(t)}{m_1} \quad (2)$$

where $Q c(t)$ is the rate at which indicator leaves the system at time $t$.

We must bear in mind that the area under a frequency function is unity and specifically

$$\int_0^\infty h(t) dt = \frac{Q}{m_1} \int_0^\infty c(t) dt/m_1 = 1.$$

Combining equations (1) and (2),

$$h(t) = c(t) \int_0^\infty \frac{c(t)}{Q} dt. \quad (3)$$

Thus, to determine $h(t)$ for fluid particles, each experimentally observed $c(t)$ for indicator is divided by the area under the curve $c(t)$ versus $t$.

Consider, for example, a system in which indicator is diluted so that its concentration at outflow appears as illustrated in figure 1. Then, the frequency function, $h(t)$, is as illustrated by the histogram in figure 2, in which two twelfths of the fluid have transit times occurring during the third unit of time, four twelfths of the fluid have transit times during the fourth unit time, three twelfths during the fifth, two twelfths during the sixth, and the remaining one twelfth during the seventh.

To find the volume of fluid present in the system at time zero, imagine that the particles of fluid that compose the volume can be distinguished by their transit times. An element of volume, $dV$, is made up of all those particles which, initially present at time zero, have transit times between $t$ and $t + dt$. The fraction of particles that require times between $t$ and $t + dt$ to leave is $h(t) dt$. Some of these particles have just entered the system at time zero. Others entered the system $t$ units before time zero and are therefore just ready to leave the system at time zero. The rest of the particles making up $dV$ entered the system during all times between zero and $t$ units before zero.
The rate at which all fluid particles enter the system and leave it is \( Q \). The rate at which particles making up \( dV \) leave the system is therefore \( Q \cdot h(t) \, dt \). Some of these particles leave at time zero, and particles of this transit time continue to leave the system until time \( t \), at which instant all such particles, originally present in the system at time zero, will have been eliminated.

The volume of such particles, \( dV \), is the time required for them to leave, \( t \), multiplied by the rate at which they leave, \( Q \cdot h(t) \, dt \), or

\[
dV = t \cdot Q \cdot h(t) \, dt.
\]  

(4)

If, at time zero, indicator is introduced into the system in such a way as to be mixed thoroughly with inflowing native fluid (where mixing is defined by the fact that the distribution of transit times of indicator particles is the same as that of native fluid), those indicator particles requiring times between \( t \) and \( t + dt \) to leave the system can be regarded as pushing out ahead of them all fluid particles characterized by the same transit time. The element of volume, \( dV \), can be regarded as a bubble flowmeter, in which the indicator is the bubble. When indicator appears at exit, exactly one element of volume has been washed out.

The process is illustrated by figures 3, 4 and 5, in which the distribution of transit times is the same as that shown in figures 1 and 2. The vertical axis is \( Q \cdot h(t) \), that is, the fraction of the flow with transit times between \( t \) and \( t + dt \) leaving the system per unit time at any time. The horizontal axis from left to right is the transit time characterizing each element of volume. The horizontal axis projected toward the observer is experimental time elapsed after addition of indicator to the inflow.

During the first experimental time unit, no indicator appears at outflow (fig. 3). The 12 white cubes illustrate that during the first time unit, native fluid appears at exit with varying transit times; two twelfths of the outflow entered the system 3 time units earlier, four twelfths entered 4 time units earlier, three twelfths entered 5 time units earlier, and so on. Indicator does not appear until the third time interval (fig. 4), when all those particles with the shortest transit time, originally present in the system at time zero, have now left the system. Indicator particles requiring longer than 3 time units have not yet appeared, so that only the fastest two twelfths of the outflow is marked by the indicator, illustrated by shaded cubes. However, we now have information about that element of volume, \( dV \), characterized by those particles whose transit times occupy the third time interval. The volume of this element is the
experimental elapsed time (3 time units) multiplied by \( Q \cdot h(t) \) (2 units) multiplied by the time interval \( dt \) (in this case, 1 unit, that is, the third time unit) or \( t \cdot Q \cdot h(t) \cdot dt \) equals 6 volume units.

During the fourth time interval, the fraction of the outflow that has transit times during the third time unit is of no interest to us because it entered the system after indicator was introduced and was therefore not part of the volume of the system at time zero. During the fourth time interval all indicator particles with transit times greater than 3 and as large as 4 time units will appear at the outflow, so that a second element of volume will have been displaced from the system.

And so the process continues until all indicator has left the system, in this example, at the end of the seventh time unit (fig. 5). We now have five elements of volume, all characterized by their transit times, all marked by the appearance of indicator at outflow. The distribution of indicator over the experimental elapsed time is exactly the same as the distribution of transit times of native fluid particles at the outflow from the system at any time.

We can now determine the total volume of the system simply by adding together all the elements of volume in figure 5. Formally, we integrate equation (4) to find

\[
V = Q \int_0^\infty t \cdot h(t) \cdot dt.
\]

Because \( h(t) \) is the frequency function of transit times, \( \int_0^\infty t \cdot h(t) \cdot dt \) is by definition the mean of all transit times or the mean transit time, \( \bar{t} \). Therefore

\[
V = Q \cdot \bar{t}.
\]

which states the fundamental fact that volume equals flow multiplied by mean transit time.

In terms of the observed concentration of indicator, from equation (3) and equation (5),

\[
V = Q \left( \int_0^\infty t \cdot c(t) \cdot dt \right) / \left( \int_0^\infty c(t) \cdot dt \right),
\]

where the ratio of integrals is nothing more than instructions for finding \( t \).

### Measurement of Flow and Volume by Constant Injection

When indicator is introduced continuously into the entrance to the system at constant rate, \( \dot{n}_i \), in units of mass per time, if mixing at inflow is complete, the concentration of indicator admitted to the system is \( \dot{n}_i / Q \) (fig. 3, 4 and 5). The system is unaware of whether the indicator diluter is using sudden or constant injection. In the illustrated case, no indicator appears at exit until the third time interval when the element of volume characterized by the briefest transit times will display the first indicator particles. In this element of volume the concentration of indicator is \( \dot{n}_i / Q \) during the third time interval. The fraction of outflowing particles with this transit time is \( h(t) \cdot dt \). Therefore, the concentration of indicator in the total outflow at this time is \( h(t) \cdot dt \cdot \dot{n}_i / Q \). Unlike the case of sudden injection, when indicator is injected constantly, once it has appeared at exit in a given element of volume it will continue to appear, as illustrated in figure 6. During the next, or fourth, time interval, indicator is contributed to the outflow by the element of volume with the next most rapid transit times, as well as by the element of
volume that has already contributed indicator. The fraction of outflowing particles with
transit times from zero to t is \( \int_0^t h(s) \, ds \).

The concentration of indicator at outflow at time t is therefore

\[
C(t) = \frac{\dot{m}_1}{Q} \int_0^t h(s) \, ds.
\]  (8)

We have already seen that \( \int_0^\infty h(t) \, dt = 1 \), therefore

\[
\lim_{t \to \infty} C(t) = \frac{\dot{m}_1}{Q} = C_{\text{max}}.
\]  (9)

Equation (9) reminds us that \( C(t) \) will increase to a maximal and constant value, and
that we make no new assumptions to be sure that such is the case. It also of course states
that once \( C_{\text{max}} \) is reached, the unknown flow can be measured:

\[
Q = \frac{\dot{m}_1}{C_{\text{max}}}.  \]  (10)

The volume of the system can be determined by one of two equivalent methods.

When the concentration of indicator at outflow finally reaches \( C_{\text{max}} = \frac{\dot{m}_1}{Q} \), the concentration of indicator everywhere within the system must also be \( \frac{\dot{m}_1}{Q} \). This means that there is within the system a mass of indicator, \( m_v \), distributed over \( V \) at concentration \( \frac{\dot{m}_1}{Q} \), or

\[
m_v/V = \frac{\dot{m}_1}{Q} = C_{\text{max}}.
\]

Since \( C_{\text{max}} \) is measurable, \( V \) becomes known if we can determine \( m_v \). \( m_v \) is determined as follows:

The amount of indicator in the system at any time, \( t \), is

\[
m_v(t) = (\text{input up to time } t) - (\text{output up to time } t).
\]

Input up to time \( t \) is simply the known constant rate of injection, \( \dot{m}_1 \), multiplied by the
value, \( t \). The output of indicator between time \( t \) and \( t + dt \) is the concentration of indicator
at time \( t \), \( C(t) \), multiplied by the volume of fluid leaving the system between \( t \) and \( t + dt \), which is \( Q \, dt \), or the output is \( C(t) \cdot Q \, dt \). The
output of indicator up to time \( t \) is the sum of all such outputs or

\[
m_v(t) = \dot{m}_1 \cdot t - \int_0^t Q \cdot C(s) \, ds.
\]

\[
= \int_0^t \left[ \dot{m}_1 - Q \, C(s) \right] \, ds
\]

\[
= Q \int_0^t \left[ \frac{\dot{m}_1}{Q} - C(s) \right] \, ds
\]

\[
= Q \int_0^t \left[ C_{\text{max}} - C(s) \right] \, ds.
\]

The concentration of indicator within the system at time \( t \) is

\[
\frac{m_v(t)}{V} = \frac{Q}{V} \int_0^t \left[ C_{\text{max}} - C(s) \right] \, ds.
\]
The limit of this concentration is, as we have seen, $C_{\text{max}}$. Therefore,

$$\lim_{t \to \infty} \frac{n_i(t)}{V} = \frac{V}{Q} \int_0^\infty [C_{\text{max}} - C(t)] \, dt = C_{\text{max}}.$$  

Whence,

$$V = \frac{Q}{C_{\text{max}}} \int_0^\infty [C_{\text{max}} - C(t)] \, dt. \quad (11)$$

The integral in equation (11) is the area between the line $C_{\text{max}}$, extrapolated back to zero time, and the curve $C(t)$, that is, it is the area above the build-up of indicator concentration.

We know from equation (6) that $V = Q \bar{t}$. Comparison of equation (6) and equation (11) states that

$$\bar{t} = -\frac{1}{C_{\text{max}}} \int_0^\infty [C_{\text{max}} - C(t)] \, dt.$$  

It is of interest to prove this identity in another way.

We begin by redeveloping equation (8), which evolved from inspection of figure 6, in a way that lends itself to further generalization that will be useful later when we examine recirculation.

Consider the contribution to the rate at which indicator leaves the system at time $t$ made by indicator introduced into the system during the time interval between $s$ and $s + ds$ time units before $t$, that is, in the vicinity of time $t - s$. The amount of indicator introduced during this time interval is $n_i ds$. Of that indicator, the fraction eliminated per unit time at time $t$ is $h(s)$. Therefore, of the indicator, $n_i ds$, introduced between $s$ and $s + ds$ time units before $t$, the amount leaving per unit time at $t$ is $h(s)$ $n_i ds$. Summing for all such time intervals before $t$, the rate at which indicator, injected from time zero to time $t$, leaves the system at time $t$ is

$$\int_0^t h(s) \, ds.$$  

But the rate at which indicator leaves the system is also $Q C(t)$. Therefore,

$$C(t) = \frac{j_i}{Q} \int_0^t h(s) \, ds. \quad (8)$$

We introduce the cumulative distribution function, $H(t)$, which is the integral of the distribution function, $h(t)$, or

$$H(t) = \int_0^t h(s) \, ds. \quad (12)$$

Combining equations (8) and (12),

$$C(t) = \frac{j_i}{Q} H(t),$$  

or,

$$H(t) = \frac{C(t)}{C_{\text{max}}}. \quad (13)$$

Returning to equation (11), which we wish to identify with equation (6), we substitute $H(t)$ for $C(t)/C_{\text{max}}$:

$$V = Q \int_0^\infty [1 - H(t)] \, dt. \quad (13)$$

The problem now is to prove the identity of

$$\int_0^\infty [1 - H(t)] \, dt$$  

with

$$\int_0^\infty t \, h(t) \, dt.$$  

This is seen by integrating by parts.

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

$$+ \int_0^t s \, h(s) \, ds$$  

or,

$$\int_0^\infty [1 - H(t)] \, dt = \lim_{t \to \infty} t [1 - H(t)]$$  

$$+ \int_0^\infty t \, h(t) \, dt.$$  

Figure 7 illustrates the graphic meaning of the parts of the integral and shows that the integrals must both be finite or both be infinite. They cannot both be infinite since this would yield infinite volume. For the case of finite integrals,

$$\lim_{t \to \infty} t \, [1 - H(t)] = 0. \quad \text{Therefore,}$$  

$$\int_0^\infty [1 - H(t)] \, dt = \int_0^\infty t \, h(t) \, dt = \bar{t}. \quad (14)$$

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Relation Between Single and Constant Injection

Differentiation of equation (8) with respect to time yields
\[
\frac{dC(t)}{dt} = \frac{\overline{ni}}{Q} h(t).
\]

But for the case of sudden injection, \(h(t) = Q \epsilon(t)/m\). Combining the above two equations,
\[
\frac{dC(t)}{dt} = \frac{\overline{ni}}{m} \epsilon(t) \quad (15a)
\]
and
\[
C(t) = \frac{\overline{ni}}{m} \int \epsilon(s) \, ds. \quad (15b)
\]

Equation (15) states that the concentration at outflow following sudden injection is simply some multiple of the derivative of the concentration at outflow during constant injection. This means that the time course of the concentration curve following sudden injection coincides with the time course during constant injection. The instant some indicator appears following sudden injection so that \(c(t)\) just assumes a nonzero value, \(C(t)\) also just assumes a nonzero value, that is, appearance time is the same for both. When \(c(t)\) reaches its maximal value, that is, when its first derivative is zero, \(C(t)\) will simultaneously have a flex point. When \(c(t)\) returns to zero, \(dC(t)/dt\) becomes zero and \(C(t)\) just reaches its maximal value, \(C_{\text{max}}\). Irregularities in one curve will appear simultaneously in the other. For example, when recirculation is apparent in \(c(t)\) it will also be apparent in \(C(t)\). Manipulation of one curve, for example, in an effort to exclude the effect of recirculation, is as simple or as difficult for the other curve. The choice between sudden and constant injection techniques lies not in the formal treatment of the data but in the individual experiment.

Effects of Violation of Assumptions, or Real Vascular Systems

1. The System Does Not Have a Single Inflow and a Single Outflow Orifice.—If the system has either a single inflow or a single outflow or if somewhere within the system there is a single channel through which all flow must pass and in which mixing occurs

\[
\int_0^\infty [1 - H(t)] \, dt \text{ is mean transit time. } \int_0^t [1 - H(s)] \, ds \text{ is the sum of the two shaded areas, of which } \int_0^t \epsilon h(s) \, ds \text{ is the lower.}
\]

(as in systems that include the heart, provided it can be shown that mixing of indicator and blood is complete), then flow can be measured by either sudden or constant injection by the equations developed earlier, equations (1) and (10). This is because equation (1) depends only on the fact that all indicator must eventually leave the system and that at some time between \(t\) and \(t + dt\) prior to sampling, all indicator has been diluted by a volume equal to \(Q \, dt\), and because equation (10) depends only on the fact that once injected indicator has been diluted so that its concentration is \(\frac{\overline{ni}}{m}\) it cannot be diluted further nor can it be concentrated, and outflow concentration must sooner or later become constant at \(\frac{\overline{ni}}{Q}\).

However, it is not even essential that all fluid pass through a single channel. If fluid from each input channel mixes with that from every other input channel before leaving the system, it may still be possible to measure flow. This thesis is developed in reference 11. The criterion for satisfactory intermingling is simply that, for the case of constant injection, the limiting concentration of indicator in every output channel must be the same. The volume measured by the equation
V = Q t may or may not have little relation to some anatomic volume of interest.

Consider many inputs, \( A_1, A_2, A_3 \), and so on, and a single output, \( B \). Indicator is injected suddenly into one input, say \( A_1 \). Let the flow past \( A_1 \) be \( Q_1 \), past \( A_2 \) be \( Q_2 \), and so on, where \( Q_1 + Q_2 + \ldots = Q \), the total flow past \( B \). There is an experimentally determined mean transit time, \( \bar{t} \). Then there is a volume beginning at \( A_1 \) and proceeding to \( B \) such that \( V_1 = Q_1 \bar{t} \). There is also a volume beginning at \( A_2 \) such that \( V_2 = Q_2 \bar{t} \), and so on. Summing all these volumes, the volume of the system is

\[
V = V_1 + V_2 + \ldots + V_n = Q_1 \bar{t} + Q_2 \bar{t} + \ldots + Q_n \bar{t} = Q \bar{t}.
\]

In this case, then, the relation \( V = Q \bar{t} \) is used to define a volume and it is up to the investigator to decide whether or not he needs to identify anatomically the points \( A_2, A_3 \), and so on defined by the relation given above. The same argument applies to the case of a single input, many outputs in which only one output is sampled. The volume in either of these cases includes a portion of each input or each output channel up to that site at which the mean transit time is the same as that determined for the injection-to-sampling site actually used.

2. The System Is Nonstationary.—The stationarity condition, that is, that the distribution of transit times for entering particles does not change with time, is violated in real vascular systems. It is certainly violated in pulsatile systems, particularly those that include the heart, and it is apt to be violated by vasomotor activity. If phasic, not necessarily regular, alterations in distribution of transit times fluctuate rapidly about some central value and if the periods of the phases are brief compared to the time required for evolution of the sudden-injection indicator concentration-time curve, then the violation of stationarity may not be important.

3. Flow or Volume or Both Not Constant.—The sudden-injection method fails completely in this case. The constant-injection method cannot measure a changing volume but it may measure flow. If the rate at which flow changes is slow (time constant of the change is long compared to mean transit time) or if the change is from one steady flow to another, the limiting concentration, \( \bar{m}_i/Q \), will reflect altered flow appropriately.

4. Flow of Indicator Particles Is Not Representative of Native Fluid.—Obviously, the investigator must accept responsibility for selection of an appropriate indicator. In the case of a heterogeneous fluid, such as blood, coursing through a leaky system, such as the cardiovascular net, it may be difficult to find an indicator for which the distribution of transit times is that of the component of the native fluid under investigation.

As an example of the difficulty, consider the case in which the indicator is tagged serum albumin and the native fluid is blood plasma flowing through some portion of the vascular system in which there are capillaries. Insofar as there is net water loss from plasma at the arteriolar end of capillaries, indicator protein will be concentrated. For the case of constant injection, indicator leaves arterioles at concentration \( \bar{m}_i/Q \). If all water returns to capillaries at venular ends, concentration of indicator entering venules is again \( \bar{m}_i/Q \). If the water does not return completely to venular ends of capillaries, as in edema formation, in prominent lymphatic flow or in the elaboration of urine, concentration of indicator in veins will exceed \( \bar{m}_i/Q \), where \( Q \) is arterial inflow, but will equal \( \bar{m}_i \) divided by venous outflow. The latter is, therefore, the flow measured.

Even if water simply exchanges across capillaries with no net loss from the vascular system, the methods must underestimate the volume. By the sudden-injection method, the mean transit time of indicator is less than that of water that has an extravascular circuit. By the constant-injection method, the mass of indicator in the system is estimated correctly from the equation \( m_v = Q \int_0^\infty [C_{\text{max}} - C(t)] \, dt \), but it is no longer true that this \( m_v \), divided by \( V \) is \( C_{\text{max}} \) because some portion of \( m_v \) exists at a concentration greater than \( C_{\text{max}} \). Some
bound on the error may be calculable if there is sufficient information about the system.

If the indicator diffuses out of capillaries and does not all return to the vascular system under study, flow cannot be measured by the sudden-injection method (because the true value of $m_i$ is unknown, unless the appropriate quantity of indicator can be measured by some independent means). Flow can, however, be measured by the constant-injection method. If indicator escapes from capillaries at concentration $i\overline{h}/Q$, the measured flow is the true inflow that originally diluted the indicator. Otherwise the asymptotic concentration of indicator at outflow will depend upon the outflow. The volume measured, however, is the volume in which indicator is distributed and therefore exceeds that of plasma. The mean transit time, $\bar{t} = V/Q$, calculated from transients of the constant-injection curve is that of the indicator and not of plasma.

An interesting error, discussed elsewhere, occurs when an indicator that tags plasma is used to estimate whole-blood flow and volume.

5. Recirculation Occurs.—Recirculation of indicator is of practical importance when it occurs before all indicator particles have completed the first transit. Recirculation is a nuisance which confuses interpretation of the primary circulation curve, making it difficult to calculate $Q$ and $V$. On the other hand, the concentration-time curve during recirculation may give some information about the channels through which recirculation occurs, as in the case of shunts.

There are two ways to handle problems created by recirculation. One is to extend the treatment of the concentration of indicator as a function of flow and volume to include recirculation; the other is to treat formally the concentration of indicator during the first circulation and by some means extract from the over-all concentration-time curve the concentration-time curve that applies only to the first circulation.

Let us first examine the possibility of including concentration due to recirculation in our fundamental equations for flow and volume.

We extend the second argument by which equation (8) was developed. The important equation that follows was first produced by Stephenson.

Indicator is introduced into the inflow of the system at rate $m_i(t)$, where the function is unspecified for the moment. During the interval $s$ to $s + ds$ time units before $t$, that is, in the vicinity of time $(t - s)$, the amount of indicator introduced is $m_i(t - s) ds$. The fraction of this amount eliminated per unit of time at time $t$, or $s$ time units later, is $h(s)$. The contribution to the rate at which indicator leaves the system at time $t$ made by indicator introduced between $s$ and $s + ds$ time units earlier is the product $[h(s)] [m_i(t - s) ds]$. Summing for all such time intervals before $t$, the rate at which indicator leaves at time $t$ is

$$\int_0^t [m_i(t - s)] h(s) ds.$$

But this rate is also equal to $Q C(t)$, where $C(t)$ is concentration at outflow. Therefore,

$$C(t) = \int_0^t \frac{1}{Q} m_i(t - s) h(s) ds. \quad (16)$$

The concentration of indicator at input to the system is $C_i(t) = m_i(t)/Q$, so that equation (16) may be written

$$C(t) = \int_0^t C_i(t - s) h(s) ds. \quad (17)$$

With the aid of figure 8 we shall now develop an expression for $C_i(t)$ in terms of a distribution function. Imagine a flow system shaped like a doughnut. One plane normal to the axis of flow is selected arbitrarily as the input. A quantity of indicator, $m_i$, is injected suddenly into the input. The concentration of indicator is then monitored constantly at the input. Indicator is distributed through the flow system so that the fraction of it making the complete circuit per unit of time is described by the frequency function $g(t)$.

In the illustration, no indicator has returned until the fifth time interval, and all of it has completed the first circuit by the end of the eighth time interval, shown by the first set of four white bars. We already know that this event is described by $C_i(t) = m_i g(t)/Q$, and
where \( m_1g(0)/Q = C_i(0) \). Those particles that had the shortest transit times during the first circuit re-enter the system and are distributed in accordance with \( g(t) \), so that the first of them emerge during the ninth time interval, and by the twelfth time interval, as shown by the second set of four white bars, they have all completed the second circuit. The area of the second set of four white bars is, of course, the same as that of the first white bar appearing during the fifth time interval. Meanwhile, those indicator particles that appeared initially during the sixth time interval, that is, those with the second shortest transit times on the initial circuit, have re-entered the system and been distributed in accordance with \( g(t) \), as shown by the first set of four shaded bars. Their contribution to \( C_i(t) \) is additive to that of particles that entered earlier but that now take paths with greater than the shortest transit times. The white bars added to the shaded bars represent the contribution of indicator particles that initially traversed the system with the next longest transit times. The distribution of successive re-entering particles is marked by alternate white and shaded bars.

By analogy with equation (17),

\[
C_i(t) = \int_0^t C_i(t - s) g(s) \, ds
\]

or, since \( C_i(t) = m_1g(t)/Q \) for the initial circuit,

\[
C_i(t) = \frac{m_1}{Q}\int_0^t g(t - s) \, g(s) \, ds \tag{18}
\]

In this case, the distribution function, \( g(t) \), is redistributed, or convoluted, upon itself. Integrals of the type found in equation (18) are called convolution integrals. They can be manipulated through their Laplace transforms, and they are in fact transformable, although frequently there is insufficient knowledge about the functions to make their transforms useful. More practically, they lend themselves to analysis by computer technics so that the distribution function may be retrieved from the integral.

Before leaving figure 8 we should point out that with each recirculation, indicator particles are dispersed more and more with respect to time, so that as \( t \) grows large, \( C_i(t) \) tends to approach the average concentration in the system, which of course is the mass of indicator, \( m_i \), divided by the total volume, \( V_T \). Therefore,

\[
\lim_{t \to \infty} C_i(t) = \int_0^\infty C_i(t - s) \, g(s) \, ds = m_i/V_T \tag{19}
\]

which provides a measure of volume of the full recirculating system.

Now let us say that we are interested in obtaining the flow and volume through only a portion of the recirculating system. Stephenson\(^6\) was the first to show that this could be done by the use of two indicators injected continuously at constant rate. If \( h(t) \) is the frequency function through the fraction of the system under study and \( C_o(t) \) is concentration at output, we already know that

\[
C_o(t) = \int_0^t C_i(t - s) \, h(s) \, ds,
\]

and if \( C_i(t) \) is concentration at input and \( g(t) \) is the frequency function through the whole recirculating system, we already know that

\[
C_i(t) = \int_0^t C_i(t - s) \, g(s) \, ds.
\]
Combining the two equations,

\[ C_v(t) = \int_0^t h(s) \int_0^{t-s} C_i(t-s-r) g(r) \, dr \, ds. \]  \hspace{1cm} (20)

In our discussion of figure 8, we saw that, the concentration function in a recirculating system behaves like a damped oscillation and at any point in the system approaches \( m_i \), \( t/V_T \), where \( m_i \) is the rate of constant injection. Therefore, the asymptotic behavior of concentration at outflow is

\[
\lim_{t \to \infty} C_v(t) = \frac{m_i}{Q} + \int_0^\infty h(s) \frac{m_i \cdot (t-s)}{V_T} \, ds
\]

\[
= \frac{m_i}{Q} + \frac{m_i \cdot t}{V_T} \int_0^\infty h(s) \, ds - \frac{m_i}{V_T} \int_0^\infty s \cdot h(s) \, ds
\]

where \( t \) is mean transit time through the desired fraction of the system.

Now, if a second indicator is injected at constant rate into some point of the recirculating system, outside the primary system whose \( Q \) and \( V \) are desired, the concentration of the second indicator at the outflow from the primary system (the same point at which \( C_v \) is measured) will have as its limit for large \( t \)

\[
\lim_{t \to \infty} K_0(t) = K_1(t) = \frac{m_i \cdot t}{V_T}
\]  \hspace{1cm} (22)

where \( K_0 \) and \( K_1 \) are concentrations of the second indicator.

Equations (21) and (22) determine \( Q \) and \( t \), and therefore \( V \). \( V_T \), the total volume, which appears in both equations, is estimated independently, or simply by the limit equation (19). Thus, recirculation is an essential part of the scheme and is not corrected for. Other methods, exploiting the convolution integral, have been discussed and some will be presented elsewhere in this symposium.

We turn now to consider the possibility of correction for recirculation without actually measuring it. Hamilton and colleagues discovered that, when an indicator was injected suddenly intravenously in the dog and the concentration measured in arterial blood, the down limb of the concentration-time curve sooner or later fitted an exponential of the form \( ae^{-st} \), where \( a \) is any arbitrarily selected concentration on the proper part of the down limb, until it was obviously interrupted by an increase in concentration attributed to the first recirculation of indicator. This means that, after an appropriate time, a plot of the logarithm of indicator concentration versus linear time yields a reasonably straight line until recirculation appears. When recirculation does appear, the logarithmic down slope is simply extrapolated to very small concentrations. The extrapolated concentration-time curve is then replotted on linear coordinates and, with recirculation thus eliminated, the area and the mean transit time of the primary curve are calculated.

In specific instances it may be possible to find other useful approximations and, indeed, others exist. If recirculation is a late event, no great error is introduced by any reasonable extrapolation, and it is far simpler to extrapolate. However, if recirculation occurs early so that the down limb following sudden injection is obscured, then no correction for recirculation can be made with confidence. It is then necessary to use one of the other methods that include recirculation as part of the analysis, such as the procedure proposed by Stephen- son. These methods, although complex, have the important advantage that they make no assumptions about the form of the distribution function.

**Effect of Injection Which Is Neither Sudden Nor Constant**

1. **Sudden Injection That Is Not Truly In-**
stantaneous.—Obviously it is impossible to inject literally instantaneously. If the mean transit time through the system is long compared to the mean time of injection it is usually sufficiently accurate to ignore the fact that the injection is not really instantaneous. However, if the mean time of the distribution of the injection process is significant compared to the mean transit time through the system, then the equations for measurement of volume following sudden injection do not hold because the mean transit time determined from the measured output concentration will exceed the true mean transit time through the system, described by \( h(t) \). The correct equation is the convolution,

\[
C(t) = \frac{m_1}{Q} \int_0^t h_1(t - s) h(s) \, ds,
\]

where \( h_1(t) \) is the fraction of injectate entering the system per unit of time at time \( t \), that is, it is the frequency function of injection.

Calculation of flow from the area under the concentration-time curve is still correct because, as was stated previously,

\[
\int_0^\infty \int_0^t h_1(t - s) h(s) \, ds \, dt = \int_0^\infty h_1(t) \, dt = \int_0^\infty h(t) \, dt = 1,
\]

so that it is still true that

\[
Q = \frac{m_1}{\int_0^\infty C(t) \, dt}.
\]

To calculate the true mean time,

\[
\int_0^\infty t \, h(t) \, dt,
\]

advantage is taken of an important property of frequency functions. Given the frequency function

\[
f_1(t) = \int_0^t f_2(t - s) f_3(s) \, ds,
\]

then \( \bar{t}_1 = \bar{t}_2 + \bar{t}_3 \), where \( \bar{t}_1 \) is mean transit time for distribution \( f_1(t) \), that is,

\[
\bar{t}_1 = \int_0^\infty f_1(t) \, dt,
\]

\( \bar{t}_2 \) is mean transit time for \( f_2 \), and \( \bar{t}_3 \) is mean time for \( f_3 \). Thus, mean transit times are additive.

Therefore, to find the mean time through the system, \( \int_0^\infty t \, h(t) \, dt \), we determine the mean time described by the measured concentration of indicator at outflow, \( \int_0^\infty t \, C(t) \, dt \), and subtract from it the mean time of the injection process,

\[
\int_0^\infty t \, h_1(t) \, dt,
\]

which must be measured independently. If the injection is very rapid, it may be sufficiently accurate to consider the mid-point in time of injection as its mean time.

2. **Other Forms of Injection.**—These are not used widely but may have some theoretical interest or practical value in special cases. The ease of injection at constant acceleration yields a very simple method for determination of mean transit time.\textsuperscript{11}

**Effect of Collecting Catheter**

If blood is led from the system under study through a device such as a catheter, the distribution of transit times through the system, \( h(t) \), is convoluted upon the distribution of transit times through the catheter, \( h_o(t) \). If the sudden-injection technique is used and the injection itself is sufficiently rapid to neglect its mean time, then the observed concentration at outflow from the catheter is

\[
C(t) = \frac{m_1}{Q} \int_0^t h(t - s) h_o(s) \, ds,
\]

which is identical to the case in equation (23) in which an inflow distribution is convoluted through \( h(t) \), because the sequence in which the convolution occurs makes no difference, that is,

\[
\int_0^t f_1(t - s) f_2(s) \, ds = \int_0^t f_2(t - s) f_3(s) \, ds.
\]

Therefore, all the arguments used with reference to convolution of injection upon the
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distribution function, \( h(t) \), apply equally to convolution of the outflow through the catheter upon \( h(t) \). Flow is measured correctly from the usual equation, and the true mean transit time through the system (excluding the catheter) is the difference between the over-all mean transit time (determined from the observed concentration-time curve) and the mean time through the catheter, measured independently by some in vitro method from the relation \( t = V/Q \), where \( V \) is catheter volume and \( Q \) is some experimentally produced flow.

Several investigators have examined the problem of the effect of the catheter on the shape of the indicator concentration-time curve. For calculation of flow we are concerned only with the area of the curve. For calculation of volume we need only the mean transit time and the flow. The shape of the outflow curve is immaterial.

**Formal Expressions for the Distribution Function**

In the previous sections we have not cared what form the distribution function of transit times, \( h(t) \), might take. It has been simplest and completely accurate to let the flow system determine the function for us by delivering an indicator at a concentration that is measurable experimentally as a function of time. However, it would be nice to know whether \( h(t) \) could be placed in some other form in which it might be operated upon if occasion demanded. There are two ways to approach the problem. One is to examine the concentration-time curves obtained experimentally and, by curve fitting or otherwise, derive an empirical expression for concentration as a function of time or some other useful relation. The other is to assume that certain given laws govern the distribution, predict the distribution function from these laws and test the observed concentration-time curve for goodness of fit.

1. **Empirical Expressions.**—The earliest of these is attributable to Allen and Taylor\(^{12} \) who treated the sudden-injection indicator concentration-time curve as a triangle. Hamilton's extrapolation of the down limb as an exponential, to which reference has already been made, was of course also an empirical fit, although it has since formed the basis for development of a model system.

Several investigators have asked whether or not indicator-dilution curves commit themselves as soon as they have written the rising limb and reached peak concentration. To this end Dow\(^{13} \) examined a large number of curves obtained by sudden injection into the cardiopulmonary circuit and obtained an expression for the area under the curve (which equals \( \bar{n}_i/Q \)) from a knowledge only of the time at which indicator just appeared at outflow (the shortest transit time or appearance time), the time at which peak concentration was reached (the time at which the mode of the distribution function occurred) and the peak concentration. Keys and associates\(^{14} \) found that, in man, Dow's formula systematically underestimated flow and they applied a correction factor.

A closely related approach was taken by Hetzel and colleagues\(^{15} \) who compared the area of what they called the forward triangle to the total area of the curve corrected for recirculation. The forward triangle is the area under the rising concentration curve, from appearance time to time of peak concentration (modal time) following sudden injection.

These methods have been reviewed in greater detail elsewhere.\(^{11} \) Their development was stimulated by the need to handle the problem of early appearance of recirculation. The empirical correlations were prepared from curves in which recirculation was necessarily late, so that it could be eliminated by Hamilton's exponential extrapolation. There is no information as to whether or not correlations from the one set of experiments, from which the constants of the equations were obtained, can be applied to other sets of experiments. Furthermore, these empirical formulas yield approximations only of the area of the curve. They can be used only for estimates of flow, not of volume.

2. **Theoretical Approaches.**—When a Newtonian fluid flows through a long straight tube of uniform bore, providing the Reynolds number is below a certain critical value, its be-
behavior is described as laminar and it obeys Poiseuille's law. Several treatments of a system in which indicator is distributed through a laminar flow system have been given. Of course, the resulting curves do not in themselves apply to a branching vascular system, although verisimilitude is improved by connecting laminar flow systems in series and in parallel.

Sheppard and Savage suggested a probabilistic “random walk” approach to the question of curve shape. From the empirical point of view it was noted that the relation was approximated by a normal distribution if a log scale was used on the horizontal rather than the vertical axis. Stow and Hetzel reported a reasonable fit. Although such curves do arise in the theory of random walks, the agreement seems purely fortuitous so far as basic theoretical significance may be concerned. Skewed probability distributions often become more like the normal after a log transformation of the abscissas. The down limb of the normal distribution falls more rapidly than Hamilton's simple exponential, so that the two methods do not yield the same flow.

Sheppard later extended the random-walk concept and indicated its validity in relation to experiments with dye in long glass-bead columns. Vascular beds are considered to be randomizing nets, but the random progress of dye in a more general sense may also be included in the concept. Experiments were performed on glass-bead systems contained in spherical flasks. Here, where the system deviated to a great extent from the ideal long-bead column, a small but definite amount of horizontal shifting became necessary in obtaining a satisfactory fit.

Another specific form, suggested originally by Hamilton and his colleagues, was adopted by Newman and his colleagues. The exponential down limb of Hamilton and his colleagues suggested that indicator was washed out of a chamber in which it had been mixed thoroughly. The general equations for such a system were given by Sheppard. Newman and co-workers claimed that the exponential washout was \( Q/V \) and that it could therefore be used to measure volume. In general, this has not been correct; at least the magnitude of whatever has been determined has not agreed with volume determined from the relation \( V = Q \). It will be noted that if the slope is \( Q/V \) it must be \( t \). It has been shown elsewhere that this cannot be the case. Newman and colleagues have also considered the effect of placing mixing chambers in series. I obtain a slightly different equation, by plugging exponential functions into convolution integrals, owing chiefly to having set different boundary conditions, but I conclude that in general the slope of the falling concentration curve cannot be used to measure volume.

If indicator distribution is limited to the chambers of the heart, then Newman’s model becomes more plausible, and may do very well as a first approximation despite evidence that mixing of blood is indeed not complete and immediate in the right ventricle. The equations are step functions, owing to the discrete nature of cardiac systole and diastole. They assume that there is some volume or volumes in the cardiac chambers in which mixing of indicator and blood is complete and instantaneous.

For the simplest case, consider that an amount of indicator, \( m_i \), is introduced into a ventricle during diastole. End-diastolic volume, with which indicator is assumed to be mixed, is \( V_a \). During systole a quantity of blood, \( V_s \), is ejected forward and there is no regurgitation. At the end of systole there remains in the ventricle a volume \( V_r = V_a - V_s \). During diastole a volume equal to \( V_a \), containing no indicator, flows from atrium to ventricle. From these assumptions it is easy to show that

\[
e(t) = \frac{m_i}{V_a} \left( \frac{V_r}{V_a} \right)^t F,
\]

where \( F \) is beats per unit of time (heart rate), and where for \( n F < t < (n+1) F \) and \( n \) is an integer, \( t \) assumes the value of \( n \). Since flow can be determined by the usual equations, and so \( V_a \), the residual volume can be estimated from the above equation.

Because flow equals stroke volume multi-
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plied by heart rate, or Q = F V s, F = Q/V s = 1/t; that is, heart rate is the reciprocal of the mean transit of the cardiac output through a volume equal to that ejected with each systole.

More complicated systems have been examined. A solution for a two-chambered system, including regurgitant flow, is given by McClure and colleagues.23

With regard to all of the various formal expressions for the distribution function of transit times, it is not likely that any arbitrary distribution function will describe the real distribution function as well as the properly obtained indicator concentration-time curve.

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Circ Res. 1962;10:393-407
doi: 10.1161/01.RES.10.3.393

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