Monoexponential Extrapolation of Tracer Clearance Curves in Kinetic Analysis

By Niels A. Lassen and Per Sejersen

ABSTRACT

Kinetic analysis of inert tracers shows that some of the most important parameters such as the average turnover rate and the total volume of distribution can be calculated only if the entire time course of the tracer clearance is known. This means that extrapolation beyond the observation period (to infinity) must necessarily be accomplished. This paper presents arguments to support the monoexponential extrapolating function which often is used without justification. The arguments show that one cannot in the general case assign any clearcut physical value to the intercept or exponential coefficient of the extrapolating function. Theoretically, a monoexponential tail of a tracer clearance curve obtained from a system in a steady state is reached when the slope of the curve is proportional to the curve. Under certain conditions this slope can be measured as an independent observation, and hence the monoexponentiality can be put to a fairly rigorous experimental test. This concept is illustrated by clearance studies of $^{51}$Cr-EDTA and $^{131}$I-thalamate from the isolated cat gastrocnemius muscle. Furthermore, it is demonstrated that monoexponential extrapolation as made before the appearance of the final exponential part of the outflow curve can cause considerable error in determination of the mean transit time and hence of the volume of distribution for the tracer. Even with an apparent recovery of the tracer of about 99.7%, the mean transit time was underestimated by 20%.

KEY WORDS: multicompartamental analysis, cat gastrocnemius muscle, Poiseuille flow, volume of distribution, $^{51}$Cr-EDTA, “black box” analysis, residue detection, $^{131}$I-thalamate, outflow detection, Taylor dispersion

In tracer experiments, one usually encounters the need for extrapolating clearance or disappearance curves beyond the time of observation. Some of the most important parameters can be calculated from clearance studies only when using such extrapolation. This is typical for many biological tracer studies, as in the Stewart-Henriques-Hamilton method for calculation of cardiac output from an estimation of the total area under the clearance curve for a single passage through the central circulation. The same problem is encountered when measuring the mean transit time ($t$)—of albumin molecules in the plasma pool, for example—a transit time equaling the ratio of size of the plasma pool ($V$) and the turnover rate of albumin ($F$), i.e., $t = V/F$. In this case $t$ is calculated as the ratio of the total area ($A$) under the clearance curve and the initial height ($H$) of this curve, i.e., $t = A/H$. The same basic equations are valid for the Kety-Schmidt inert gas clearance method for determining blood flow in various organs: $t = A/H = (V/W)/(F/W)$, where $W$ is the weight of the tissue in grams, $V/W$ is the relative volume of distribution $\lambda$, and $F/W$ is the flow per gram of tissue.

In the examples above and in numerous other cases, one customarily uses monoexponential extrapolating functions. However, no general theory justifying this simple mode of extrapolation has gained acceptance. It is the
purpose of this paper to present such a theory and to illustrate it by experimental observations of the clearance of radioactive tracers from the isolated gastrocnemius muscle of the cat.

**Kinetic Analysis**

In recent years, theoreticians have criticized the compartmental analysis of clearance curves which previously dominated tracer theory. That an arbitrary system (e.g., an entire living organism) should consist of only two or three homogeneous compartments was considered to constitute an unreasonable and unnecessary simplification. It was stressed that a simple kinetic analysis which made no assumptions regarding compartments nevertheless permitted formulation of equations for some of the most important parameters.

The bases of the kinetic analysis are only the law of the conservation of matter (often called the Fick principle in physiological studies) and the principle of linearity, which is assumed to prevail in the condition studied. An important conceptual instrument is the frequency function of transit times of the indicator through an open system, \( h(t) \). This function is defined so that \( h(t) \times dt \) is that fraction of the tracer bolus (admixed with the inflow to the system at time zero) which leaves the system between time \( t \) and time \( t + dt \). Consequently, \( h(t) \times dt \) denotes the fraction of indicator particles having transit times between \( t \) and \( t + dt \), i.e., the outflow tracer concentration curve after impulse injection and with good cross stream mixing, has the shape of \( h(t) \). Eventually when the washout of the tracer is complete,

\[
\int_{0}^{\infty} h(t) \, dt = 1 \quad \text{(conservation of matter)}.
\]

The kinetic analysis has a major defect; it does not give any clear indication of how one determines the decisive parameters from the observed clearance curve. To illustrate this point we may return to the examples given in the first paragraph. Kinetic analysis tells us that the entire area, \( A \), must be determined.

But how is this accomplished when one cannot record the entire clearance curve until infinity? The difficulty here is that the kinetic analysis is a "black-box" analysis, which is so modest in its assumptions regarding the system under study that the results are next to useless. One is forced to make some kind of model regarding the clearance conditions inside the black box.

**Multicompartamental Analysis**

It is the thesis of the present paper that by supplementing the kinetic analysis by "multicompartamental" considerations a useful result is obtained. Actually it is possible to omit the kinetic analysis and use only the multicompartamental approach. That would, however, not be an advantage. It is convenient to utilize the simple integral equations resulting from the kinetic approach to find the basic relations between the clearance curves and the physical (or physiological) parameters to be measured and then supplement these equations by one result of the multicompartamental approach, namely, monoexponential extrapolating functions. The model with an arbitrarily large number of homogeneous compartments (and the corresponding \( n \)-dimensional system of linear differential equations) is much more difficult to conceptualize.

Any one compartment, \( i \), of a multicompartamental system is homogeneous, i.e., all its tracer particles have the same chance for transfer to those other compartments with which \( i \) exchanges tracer. In terms of a radioactive tracer we may say that the specific activity is, at any time, the same all over such a compartment. The system is thought to have constant exchange rates of the "mother substance" of the tracer and to be stationary (with no net changes of mother substance in any compartment).

On this basis, it is easy to set up one first-order differential equation for each of the \( n \) compartments, and the solution of this system is \( n \) equations with \( n \) exponential terms.

Assuming the system is irreducible we have for compartment \( i \):

\[
x_i = x_0 + C_{1i}e^{-k_1t} + \ldots + C_{ni}e^{-k_it}.
\]
where the important points to be learned from this solution are:

1. In all compartments of the system, the tracer concentration, $x_i$, will equal the sum of $n$ exponential functions.

2. These exponential functions have, regardless of the initial conditions, the same time constants in every compartment, $k_1$, $k_2$, ..., $k_n$ are the same in all compartments.

3. As one of the $k$ values is the smallest (let it be $k_\alpha$), then this same term will dominate in every compartment when sufficient time has elapsed. Consequently, we learn that $x_i$ will (after this time) approach the equilibrium concentration according to a monoexponential function with the same time constant, $k_\alpha$, in all compartments. The possibility must be considered of two or more $k$ values all being slower than the others, while differing only minimally from one another. This poses theoretical but not practical problems, as indeed a dominant slowest time constant is the usual experimental finding as referred to below.

4. If the system is open ($x_F = 0$), then the coefficient $C_{i,n}$ is positive and hence a monoexponentially decreasing function with exactly the same time constant is found in every compartment. Thus all the $n$ compartments can be lumped together without implying in any way that they have the same transfer characteristics.

5. $k_\alpha$ is a linear combination of all the fractional transfer rates of the mother substance and $k_\alpha$ is slower than the slowest unidirectional fractional transfer rate, i.e., one cannot in the general case interpret $k_\alpha$ in terms of transfer rates in any single compartment.

6. While $k_1$, $k_2$, ..., $k_n$ are independent of the initial conditions, the coefficients, $C_{i,1}$, $C_{i,2}$, ..., $C_{i,n}$ depend on these conditions. Thus it also follows that the time after which all the curves are monoexponential will depend on the initial conditions.

There is one aspect of this multicompartimental analysis which is of theoretical interest. The solution given in Eq. 1 is not the most general one. In the general case, the coefficients $C_{i,j}$ ($j = 1$, 2, ..., $n$) can be functions of time ($\theta$). But it can be shown that $k_\alpha$, which is the smallest eigenvalue of the characteristic matrix is always single and real and the $C_{i,n}$ consequently is a non-negative constant. This is true only for an “exchanging system” where all compartments are directly or indirectly interconnected (an “irreducible system”). Whether the system is irreducible is, however, not a serious problem, because if it is reducible, it can be partitioned into irreducible subsystems which can be treated separately. The only peculiarity of such a system is that different parts of it will have different smallest $k$, i.e., different compartments would have different final slopes. This comment stresses that the solution here given (Eq. 1) is correct, but much less trivial than suggested in many presentations of the problem.

The mathematical language here chosen may appear rather abstruse. For this reason a simpler formulation of the basic idea may be relevant. Consider that each point $i$ in the system (each compartment) has a certain probability $P_i$ that a tracer particle located in $i$ will leave the entire system in a given time unit. From some points $i$, tracer is more readily lost (“good sites”) than from others (“bad sites”). What then happens is that gradually tracers will, relatively speaking, appear to accumulate in the bad sites simply because the clearance therefrom is difficult. But, at a certain time the worst constellation of probabilities of clearance has become established, and now the relative concentration is just so much higher in the bad sites than in the good sites that the fractional loss becomes constant throughout.

This limiting distribution of the tracer (“stationary state”) constitutes a “niveau map” of specific activity, the pattern of which is constant with time, i.e., at all sites it will show the same fractional reductions of concentration with time and hence a monoexponential function must result.

**KINETIC VERSUS COMPARTMENTAL ANALYSIS**

We have described above the model of the black box which can be used to supplement the kinetic analysis. In two different ways we...
expressed that all sites (of mother substance) must exchange molecules with the exterior with a finite rate, however slow this may be. This strikes one as being a very modest assumption, since for a tracer to reach any site, this site must be exchanging and hence, sooner or later, the tracer molecule must again be cleared away. This concept of exchangeability underlies the kinetic analysis. Hence one may choose to consider the exponential tail as an unavoidable consequence of the exchangeability, i.e., we do not have to make any supplement by using a complicated n-compartmental theory.

In that case one may simply note that, due to the assumed exchangeability of the inert tracer, \( h(t) \) always ends sooner or later by a monoexponential tail. But in this formulation, the conceptual background explaining why this might be true is not at all explicit.

When Does a Clearance Curve Reach Its Monoexponential Tail?—The problem of finding the "final slope" is of both theoretical and practical interest. The conclusion reached theoretically conforms to the results of a great variety of tracer studies. Indeed, one could omit the theory and just note that a monoexponential clearance curve is usually found after sufficient time has elapsed and that consequently one should use this function to extrapolate beyond the duration of the time of observation.

This simplified approach is, after all, the hard core of the matter. We use this mode of extrapolation because the experiments show us such curves and not because of the multicompartamental theory. Nevertheless, in the authors' opinion, the theory is valuable. It tells us that we should look for the monoexponential tail. But it also tells us always to be aware of an "even slower component," i.e., to continue clearance studies for a very long time to make reasonably sure that the final slope (on semilogarithmic plotting) has been found (Eq. 6), or even better, to compare the final slope of the curve to that of the simultaneously measured derivative of the curve (Eq. 8).

Radioactive \( \gamma \)-emitting tracers offer a convenient means of realizing this possibility by recording the clearance of the tracer from the entire system (residue counting, e.g., whole body counting in human studies) as well as the disappearance curve at the outflow site (outflow counting). Since the latter curve is proportional to the first derivative of the former, it is readily seen that the two curves can be parallel in a logarithmic scale only if the clearance function of the entire system has become monoexponential.

In conventional notation of kinetic analysis, the fraction of the injected dose remaining inside the organ (1 minus the cumulative outflow) is written

\[
1 - H(t) = 1 - \int_0^t h(t) \, dt.
\]

The equation shows that \( \frac{d[1 - H(t)]}{dt} = -h(t) \). Both functions are monoexponential for \( t \geq T \) if, and only if, their relative slopes \( y \) and \( z \) are constant after \( t = T \):

\[
\frac{d[1 - H(t)]}{1 - H(t)} = \frac{d \ln[1 - H(t)]}{dt} = y, \quad t \geq T, \quad (3)
\]

and

\[
\frac{d h(t)}{h(t)} = \frac{d \ln h(t)}{dt} = z, \quad t \geq T. \quad (4)
\]

Equation 3 gives the ratio of two functions which both decrease toward zero as \( t \) approaches infinity. Hence this ratio must also equal the ratio of the limit of the first derivative of the same functions (l'Hopital's rule). Thus Eq. 3 leads to

\[
y = \lim_{t \to \infty} \frac{d[1 - H(t)]}{dt} = \lim_{t \to \infty} \frac{d^2[1 - H(t)]}{dt^2} \left/ \frac{\lim_{t \to \infty} df[1 - H(t)]}{dt} \right.
\]

\[
= \left[ \lim_{t \to \infty} \frac{dh(t)}{dt} \right] \left/ \lim_{t \to \infty} h(t) \right. . \quad (5)
\]
It can be seen that both Eq. 4 and Eq. 5 can be satisfied simultaneously only if \( y = z \). The slopes of the curves in a semilogarithmic system are \( y \) and \( z \). In such a system both \( 1 - H(t) \) and \( h(t) \) must be **straight lines with the same slope** for \( t \geq T \).

In the multicompartimental notation we obtain from Eq. 1,

\[
1 - H(t) = W \left( \sum_{i=1}^{n} C_{i,1} e^{-k_{i}t} + \ldots + \sum_{i=1}^{m} C_{i,a} e^{-k_{i}t} \right),
\]

where \( W \) is a weighing factor which must be used in order to normalize so that the function equals 1 for \( t = 0 \):

\[
W = \frac{1}{\left( \sum_{i=1}^{n} C_{i,1} + \ldots + \sum_{i=1}^{m} C_{i,a} \right)}. \tag{7}
\]

By differentiation,

\[
h(t) = W \left( k_{1} \sum_{i=1}^{n} C_{i,1} e^{-k_{i}t} + \ldots + k_{a} \sum_{i=1}^{m} C_{i,a} e^{-k_{i}t} \right). \tag{8}
\]

By comparing Eq. 6 and Eq. 8 it can be shown that the relative intercept (intercept divided by sum of intercepts) of the term with slowest time constant \( (k_{a}) \) is smaller for the \( h(t) \) function than for the \( 1 - H(t) \) function. Hence it will take longer for \( h(t) \) to appear as a monoexponential function, i.e., for the last exponential term to dominate to such an extent that all the other terms may be neglected. For all shorter times, the faster components will dominate \( h(t) \) more than \( 1 - H(t) \), i.e., is a semilogarithmic system \( h(t) \) must have a steeper slope than \( 1 - H(t) \) for \( t < T \).

This result is of some importance since it is necessary to continue the experimental study only until the relative slope of the derivative appears to reach the same low value as that of the curve. We will not attempt to define \( T \) in precise terms. It is apparent from Eq. 6 and Eq. 8 that, strictly speaking, this time cannot be finite.

The experimental data to be given below illustrate that one may nevertheless find a finite time for \( T \), which is very satisfactory. We shall return to this point in the discussion.

It is interesting to apply the above concept to one of the simplest cases where an explicit proposal for the form of the tracer transit function is available, viz., laminar flow in a tube of volume \( V \) (ml) and with a flow \( Q \) (ml/sec) (Poiseuille flow) \( \frac{Q}{V/O} \):

\[
h(t) = \begin{cases} \frac{Q}{V/O} t^2 & t \geq \frac{Q}{V/O} \\ 0 & t < \frac{Q}{V/O} \end{cases} \tag{6}
\]

\[
1 - H(t) = \begin{cases} \frac{Q}{V/O} t^{-1} & t \geq \frac{Q}{V/O} \\ 1 & t < \frac{Q}{V/O} \end{cases} \tag{9}
\]

Whence \( \frac{d}{dt} \ln[h(t)] = \frac{d}{dt} \ln[1 - H(t)] \), since \( \frac{d}{dt} \ln X(t)/dt = \frac{dX(t)/dt}{X(t)} \), \( \frac{d}{dt} h(t) = -2x t^{-1} \), and \( \frac{d}{dt} [1 - H(t)]/dt = -1 x t^{-1} \).

Poiseuille flow does not, consequently, obey the theorem of monoexponential tail, as can also be seen because \( \frac{d}{dt} \ln[h(t)/dt] \rightarrow 0 \) as \( t \rightarrow \infty \).

As the mean transit time, \( t = \int_{0}^{\infty} t \times h(t) \, dt \),

then \( t \) will be infinite in this system, which is in contrast to the definition of an open system where \( t \) is finite (and equal to \( V/O \)).

Thus the most classic theory of fluid movement is basically wrong when applied to the tracer molecules. The error consists in assuming zero flow and hence zero clearance of the wall-near fluid layer. Due to tracer diffusion between the laminar streams this completely stagnant layer has, nevertheless, a finite clearance rate of tracer molecules as pointed out by Taylor \( \text{\cite{Taylor}} \).

If the Taylor dispersion is taken into account for such a tube and the time to maximum of the curve at the outflow site \( t_{1} \) is much longer than \( \frac{a^{2}/3.82}{D} \), where \( a \) is radius of the tube, and \( D \) is the diffusion coefficient for the tracer in the fluid, then

\[
h(t) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left[ -\frac{(t - t_{1})^{2}}{2\sigma^{2}} \right], \tag{10}
\]

\[Circulation Research, Vol. XXIX, July 1971\]
EXponential EXtrapolation

\[
\sigma^2 = \left( \frac{a^2}{24D} \right) t. \text{ For } t \gg t_r, \text{ the exponential becomes } \approx t^2/2\sigma^2 \approx -t/\tau, \text{ where } \tau = a^2/12D.
\]

In equation 10, \( t \) in the exponential expression will predominate over \( t \) in \( \sigma \) in the preceding coefficient, i.e., \( h(t) \) will, for Taylor dispersion in a tube, become monoexponential in the later part of the curve.

Equations 9 and 10, respectively, give the transit functions for a tube in the limiting cases of zero or of dominant interlaminar diffusion. In the general case a blunt but skew curve is obtained. However, even in the general case a monoexponential tail is obtained, as illustrated by the solution presented by Perl and Chinard (8) to a closely related system.

Experimental Technique and Results

Seven cats weighing 3.0 to 4.5 kg were used in this study. Chloralose, (70 mg/kg iv) was given after ether induction. Spontaneous respiration via a tracheostomy was maintained, and the rectal temperature was kept at 37°C by heating lamps. The right gastrocnemius muscle was isolated as described previously (9). Only the vascular connection to the body was maintained. The side branches on the distal two-thirds of the femoral artery were ligated, except for one branch just proximal to the muscle; in this branch a fine polyethylene catheter was inserted so that the tip reached the level of the wall of the femoral artery. The femoral vein with all side branches ligated was cannulated by a short glass catheter with a polyethylene tip (1.5 mm i.d.). Before the actual study, the venous blood was returned to the contralateral femoral vein via a reservoir. During the study no recirculation was allowed; the entire venous outflow was collected. Venous samples were taken from the outflow catheter for isotope analysis. A graduated cylinder and a stopwatch were used for direct measurement of blood flow. The arterial blood pressure was measured by an electromanometer and blood from 3 to 5 donor cats was used in each experiment to compensate for the venous outflow, so that the blood pressure could be maintained fairly constant during the experiment.

The isolated muscle was wrapped in moist gauze and covered with a film of polyethylene. A heating lamp kept the muscle at 37°C. The sciatic nerve was stimulated with about 1 impulse/sec with a voltage of 7 v and a duration of 1 msec. During the clearance study, the steady state with respect to muscle blood flow and the hematocrit were controlled every 5 minutes. It proved possible to avoid systematical change in flow and to keep the standard deviation of the plasma flow between 4.1 and 12.7% of the mean blood flow values.

In five studies, 0.05 ml ⁵¹Cr-labeled EDTA (approximately 150 µc tracer) dissolved in saline was injected. In two studies, 0.05 ml ¹³¹I-labeled iodothalamate (approximately 25 µc tracer) was injected. The specific activity of the ⁵¹Cr is 40 mc/mg and that of the labeled iodothalamate is 0.04 mc/mg. Both tracers are hydrophilic and extracellular, dispersing only in the plasma and interstitial volume of the muscle.

The intraarterial tracer injection lasted about 1 second. The amount of tracer in the muscle was followed by external detection using a NaI (Tl) scintillation crystal carefully collimated to register only from the muscle and placed 10 cm from the muscle. The crystal was coupled to a digital ratemeter printing out on tape the total count number in suitable time intervals (½-second periods in the first minute and increasing to 1-minute periods). The venous blood samples were counted in a well type of scintillation counter until about 5000 counts or more had been recorded.

Background counting rate was recorded with special care after finishing the study and arresting the blood flow. As background we used the counting rate obtained at that time minus the counting rate of the muscle itself. The counting rate of the muscle was obtained as the difference in counting rate recorded before and after removing the muscle. Before the last two counting procedures, the surface of the muscle was gently washed with soap and water to remove tracer deposited on the surface. For the well counter, the mean value...
Comparison of Washout Rates of the Monoexponential Tail Part of the External and Venous Curves

<table>
<thead>
<tr>
<th>Exp no.</th>
<th>Flow (ml/100 g/min)</th>
<th>SD of flow</th>
<th>Flow in % of flow</th>
<th>N*</th>
<th>Duration of monoexp (min)</th>
<th>k</th>
<th>S_k</th>
<th>S_k in % of k</th>
<th>N*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.3</td>
<td>1.4</td>
<td>12.4</td>
<td>18</td>
<td>14 - 90</td>
<td>0.0308</td>
<td>0.0028</td>
<td>9.1</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>14.1</td>
<td>0.5</td>
<td>3.5</td>
<td>24</td>
<td>18 - 120</td>
<td>0.0302</td>
<td>0.0005</td>
<td>1.7</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>12.2</td>
<td>1.0</td>
<td>8.2</td>
<td>18</td>
<td>24 - 90</td>
<td>0.0338</td>
<td>0.0007</td>
<td>2.1</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>20.4</td>
<td>0.9</td>
<td>4.4</td>
<td>27</td>
<td>24 - 135</td>
<td>0.0346</td>
<td>0.0004</td>
<td>1.1</td>
<td>18</td>
</tr>
<tr>
<td>5†</td>
<td>11.3</td>
<td>0.5</td>
<td>4.4</td>
<td>25</td>
<td>24 - 130</td>
<td>0.0260</td>
<td>0.0003</td>
<td>1.1</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>14.2</td>
<td>0.8</td>
<td>5.6</td>
<td>20</td>
<td>20 - 100</td>
<td>0.0332</td>
<td>0.0005</td>
<td>1.5</td>
<td>24</td>
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<tr>
<td>7‡</td>
<td>15.5</td>
<td>1.8</td>
<td>11.6</td>
<td>19</td>
<td>18 - 95</td>
<td>0.0303</td>
<td>0.0008</td>
<td>2.6</td>
<td>10</td>
</tr>
</tbody>
</table>

*Number of observations used for calculating the standard deviation $SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$; $k$ = the slope (fractional escape rate) of the final monoexponential tail part of the curves calculated by the method of least squares, and $S_k$ the standard deviation of $k$.
†Curves shown in Figures 1 and 3; ‡Curves shown in Figure 2.

The duration of the studies was 1.5 to 2.3 hours, which, at the blood flow level studied, sufficed to reduce the externally recorded counting rate to 0.12-0.02% of the maximal rate. Two typical studies are shown in Figures 1 and 2, and the value of the time constants of the tail parts calculated by the method of least squares is given in Table 1. No significant difference was noted in these values for monoexponentially appearing final parts of the externally recorded curve and of the venous outflow curve for the seven studies taken as a group. However, in two of the studies, the agreement was not quite satisfactory. Because

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**FIGURE 1**

*Bolus injection of $51^\text{Cr}}$-EDTA in the arterial inflow of an isolated, autoperfused, rhythmically contracting cat gastrocnemius muscle. (Expt. 5.)

*Circulation Research, Vol. XXIX, July 1971*
EXPONENTIAL EXTRAPOLATION

<table>
<thead>
<tr>
<th>Duration of monocycle (min)</th>
<th>k</th>
<th>S0</th>
<th>S0 in % of k</th>
<th>n*</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 - 90</td>
<td>0.0331</td>
<td>0.0008</td>
<td>2.4</td>
<td>11</td>
</tr>
<tr>
<td>45 - 120</td>
<td>0.0287</td>
<td>0.0012</td>
<td>4.3</td>
<td>17</td>
</tr>
<tr>
<td>45 - 90</td>
<td>0.0336</td>
<td>0.0007</td>
<td>2.1</td>
<td>10</td>
</tr>
<tr>
<td>50 - 135</td>
<td>0.0252</td>
<td>0.0008</td>
<td>3.2</td>
<td>18</td>
</tr>
<tr>
<td>55 - 130</td>
<td>0.0261</td>
<td>0.0006</td>
<td>2.3</td>
<td>16</td>
</tr>
<tr>
<td>40 - 100</td>
<td>0.0242</td>
<td>0.0007</td>
<td>4.1</td>
<td>13</td>
</tr>
<tr>
<td>40 - 95</td>
<td>0.0296</td>
<td>0.0013</td>
<td>4.4</td>
<td>12</td>
</tr>
</tbody>
</table>

Duration of monocycle:
- 40 - 90 min
- 45 - 120 min
- 45 - 90 min
- 50 - 135 min
- 55 - 130 min
- 40 - 100 min
- 40 - 95 min

The importance of measuring the outflow curve long enough to determine the final monoeponential part of the curve correctly can be demonstrated by calculation of the interstitial volume of distribution. This calculation is based on determining the mean transit time, $t$, at different times of observation of the same experimental curve, i.e., by extrapolating to infinity with different monoeponential functions. Such extrapolations are demonstrated for experiment 5 in Figure 3 for the outflow curve, where the same experimental results are presented with four

[Diagram]

FIGURE 2

Bolus injection of $^{131}$I-thalamate as in Figure 1. (Expt. 7.)

TABLE 2

Dependency of the Calculated Interstitial Volume of Distribution on the Time of Observation Used in the Calculation

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Time of observation (min)</th>
<th>Final concn as fraction of peak concn</th>
<th>Recovery (%)</th>
<th>t (%)</th>
<th>Vol distribution (ml/100 g tissue)</th>
<th>Interstitial vol distribution (ml/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>6.6 \times 10^{-4}</td>
<td>90.0</td>
<td>25</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2.8 \times 10^{-2}</td>
<td>82.0</td>
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different time scales in a semilogarithmic plot. For the four curves the time from the left to the right border in the diagram is about 1, 10, 25, and 130 minutes. In all four curves it is very easy to fit the right half by a monoexponential curve, as demonstrated on the graph. For the lower curve, the results of these four different monoexponential extrapolations are shown on the same curve. The $t$ calculated from these four curves is presented in Table 2, together with values for experiments 1 and 3 obtained in the same way. These three experiments were used because the curves were well described in full length and the experiments were mutually comparable ($^{51}$Cr-EDTA) in all cases.

In Table 2 the following data are listed: the final concentration as fraction of the peak concentration, the apparent recovery and the $t$ of the tracer. The apparent recovery and the $t$ values were calculated for various times of observation of the curve, including the corresponding monoexponential extrapolation to infinity. The data are given in percent of the total tracer amount as calculated using the longest time of observation of the curve (90 and 130 minutes) with corresponding monoexponential extrapolations to infinity. All values of $t$ are corrected for the mean transit time in the sampling catheter. The volume of distribution for the tracer was calculated from the equation $V = t \times F$ (ml/100 g tissue), where $F$ is the average plasma flow obtained from the directly measured blood flow and the hematocrit value, both measured every 5 minutes. Simultaneous measurements of the time-concentration outflow curve for the intravascular tracer T-1824-albumin were performed. From these results the intravascular mean transit times were obtained. The interstitial volumes of distribution (ml/100 g tissue) were then computed as the total volume of distribution minus the intravascular volume of distribution.

**Discussion**

The experiments showed that the externally recorded clearance curves of both extracellular tracers ended as a well-defined monoexponential slope. The corresponding venous outflow curves, while initially decreasing at a steeper rate on the semilogarithmic plot, ended as a monoexponential tail having practically the same slope.

This is not a unique finding. An analogous result was obtained with $^{131}$I-labeled human
The $^{51}$Cr-EDTA outflow curve presented in Figure 1, here plotted with four different time scales. The curves and the corresponding time axis are placed in the same succession from above to below. For further explanation of the figure see text.

The $^{51}$Cr-EDTA outflow curve presented in Figure 1, here plotted with four different time scales. The curves and the corresponding time axis are placed in the same succession from above to below. For further explanation of the figure see text.

We shall not at this point attempt to review the literature in which many similar results are reported. What is important is to stress that the observation of seemingly monoexponential final slopes for tracers of different types in various tissues is a general finding, also when an experimental test for monoexponentiality as that used in the present study is performed.

However, the experimental test given here may exceed what is necessary for using monoexponential extrapolation in practice in some cases. Suppose one wants to measure blood flow by the Stewart-Henriques-Hamilton principle, i.e., as the ratio of dose to total area. If, then, the blood flow can be measured by an independent technique (e.g., directly at the outflow) then total area = dose/flow. Consequently, we can compare the extrapolated area by the indirect measure thereof. Because of the many variables involved it is not so very important if the $t^*$ of the supposed final slope is take to be 10–15% smaller than the true one.

Customarily the extrapolation of the indicator dilution curves to infinity is made by using the first steep part of the downslope of the curve. And, according to the accuracy needed in such studies, this may be entirely adequate even though a slower final slope can often (always?) be recorded if the recirculation free curve can be followed until very low relative tracer concentrations are reached (11).

The problem of finding the true final slope is of much greater importance when the abscissa of the point of gravity of the curve is...
used as in calculations of the ratio of volume to flow from $t = \int_0^\infty t \times h(t) \, dt$. Due to the linear increase in the weighting factor, $t$, then the contribution of the tail is much greater than in the area calculation. In this situation it is appropriate to use a fairly rigorous test indicating that the final slope has actually been recorded. This is stressed by the results presented in Table 2. Even when the outflow curves were followed to $10^{-4}$ of the peak concentrations, and the cumulative recovery of the tracer to 99.7%, the mean transit time was underestimated by about 20% and the interstitial volume of distribution by about 21% of the maximum value.

It can be concluded from our findings that $t$ can be determined more easily and maybe more correctly from the externally recorded curve as the monoexponential tail part of the curve appears earlier and has a higher relative intercept than the outflow curve. Furthermore, it shall be stressed that saturation experiments with hydrophilic tracers such as those used in the present study will need a long time, similar to that of the washout after a bolus injection, before the saturation condition can be obtained by extrapolation.

The interstitial volume of distribution as determined in the present study using the whole measured curve with extrapolation to infinity was about 15% of the tissue mass. This figure is in good agreement with those obtained for other extracellular tracers, and it does not indicate that $^{51}$Cr released from EDTA enters the cells. This would involve a much higher value for the interstitial volume of distribution.

The present discussion has been limited to linear systems. However, under certain conditions it may even be relevant to nonlinear systems. Consider, for example, the clearance of a substance in the mammalian liver: if the plasma concentration is high, then a constant maximal uptake is often found, whereas at lower concentrations the uptake decreases proportionately to the plasma concentration. In this case the system becomes linear after some time, and again a monoexponential clearance function may evolve.

A living cell or organism may be said to constitute a nonlinear tracer. The red blood cells do not have a constant chance of clearance from the blood independent of their age. And one finds that an exponential clearance function has no meaning in describing the survival of a cohort of such cells.

Actually, one here encounters a characteristic separating the living from the dead matter: it is precisely because a $^{131}$I atom or an albumin molecule does not age that exponential functions come into use: because they are not subject to aging, their chance of disappearance is constant. Not so with the living matter. Nevertheless, even for living organisms one might find monoexponential functions of some use, namely, in instances in which randomly operating modes of destruction override the individual variations in viability. This function is, however, likely not to be valid for extrapolation, as is well known, e.g., in microbial survival in face of toxic agents.

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References


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Niels A. Lassen and Per Sejrsen

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