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C. W. Sheppard and A. S. Householder

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THE INTERPRETATION OF EXPERIMENTAL INVESTIGATIONS of TRANSFERS WITHIN A TWO-COMPARTMENT SYSTEM, USING ISOTOPIC TRACERS*

C. W. Sheppard and A. S. Householder

As further biological investigations include the use of isotopic tracers, there will be an increasing tendency for the results in many cases to be interpreted in terms of the behavior of an idealized multicompartment system. With this in mind the theoretical basis of such an interpretation was discussed in rather general terms in an earlier paper (1). The basic postulates were outlined, the necessary measurements were listed and the equations were derived which relate the quantities transferred between compartments to the changes observed within the compartments. A more elegant derivation of these equations is presented here in Appendix A.

There may be instances where systems of several compartments are of sufficient biological interest to justify the increasingly large number of measurements necessary for their description. However, from the point of view of simplicity, the two-compartment case will probably continue to be the most important. A few typical biological systems may be cited where the behavior has been or might be compared with that of a typical ideal system of this type.

Visscher *et al.* have used the concept to interpret their results in the study of the exchange of sodium tagged with Na^{24} between the circulation and isolated chronic Thiry-Vella gut loops in the dog (2). Cohn and Brues (3) have made similar comparisons in interpreting their observations of the exchange of phosphorus tagged with P^{32} and potassium tagged with K^{42} between tissue explants and the surrounding culture medium.

The observations of Chambers *et al.* (4) on potassium and phosphorus exchange between Echinoderm eggs and surrounding sea water might be considered on the basis of an idealized two-compartment system. A typical example in the chemical field is the study of exchange rates between two chemical compounds (5). Other examples such as the theory of surface decontamination may be cited.

It will be shown below that the derivation of the usual equations used

* The writers wish to acknowledge the assistance of Dr. W. A. Arnold with whom the following material was discussed.

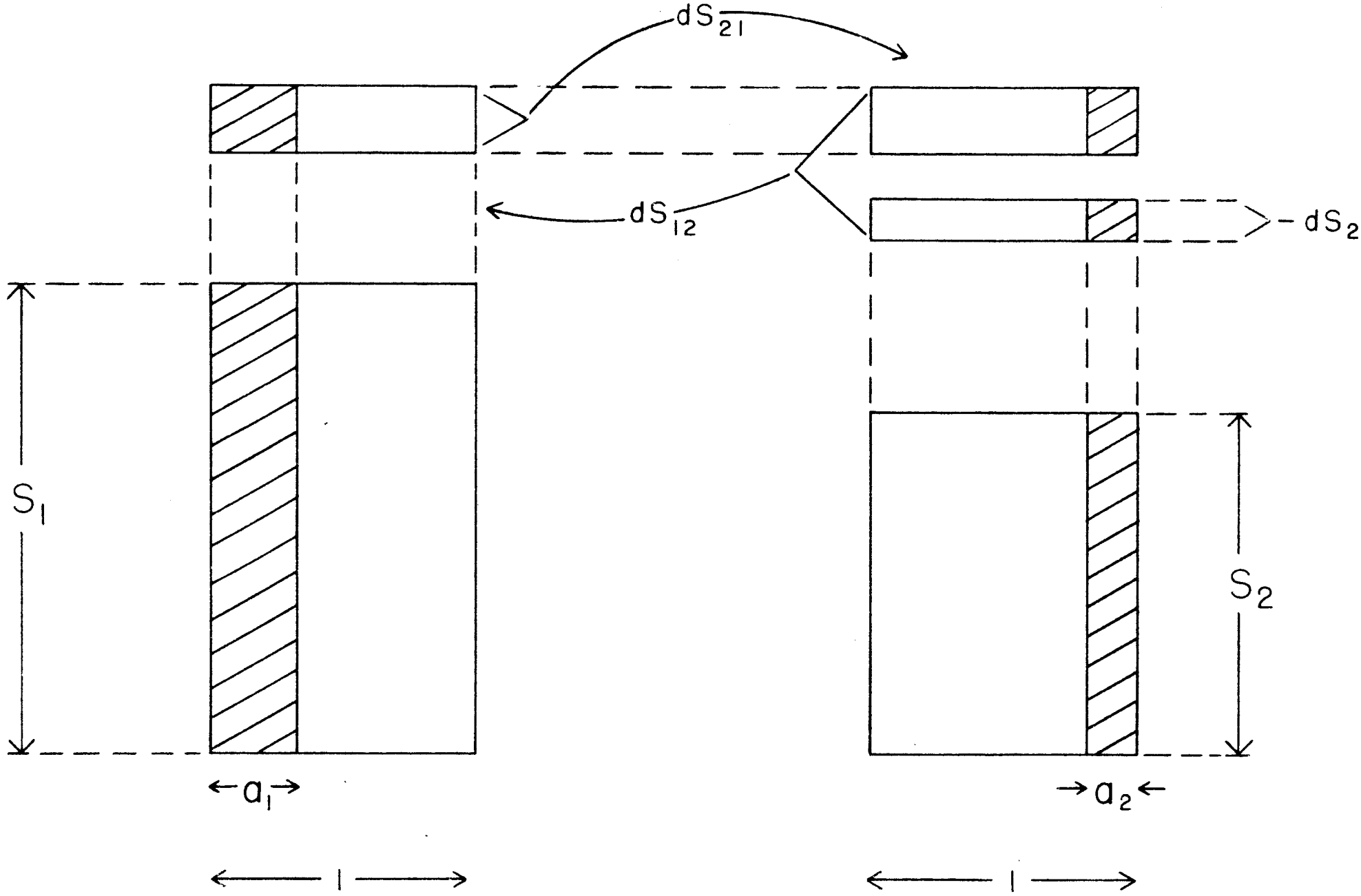
for describing the variations with time of the radioactivity of both elements of a two-compartment system are based on the assumption that the contents of each of the two compartments are uniformly mixed. This assumption limits the utility of these equations in interpreting the general behavior of tagged systems of the above type. In the tissue culture experiments on potassium transport the deviations of the results from the behavior of the idealized system may quite easily be interpreted as a failure of the criterion of uniform mixing within the internal compartment, i.e. the total cellular interior. The same might perhaps be said for the situation in the case of the Echinoderm eggs. It can be shown that phosphorus injected as phosphate tagged with P^{32} does not mix rapidly with the total blood phosphorus in canine loop experiments (6) which leads to the result that the specific activity of the gut perfusate is higher initially than that of the blood. Although the loop experiments with sodium are probably not subject to this objection, the existence of other compartments with which material in the circulation may exchange (such as the extracellular fluid) must be admitted to complicate the simple idealized case.

It is therefore of considerable interest that recent, improved studies of the exchange *in vitro* of potassium between cells and plasma of human blood have been sufficiently precise and controllable to show that the cellular fraction and the plasma form two elements whose behavior in all observed respects is identical with that of the idealized case (7) (8). For this reason it is now pertinent to review the theory of the two-compartment system and to discuss a few additional theoretical aspects of the interpretation of experimental results.

The theory of the two-compartment system. - We consider a system of two compartments denoted respectively by the subscripts 1 and 2, (Fig. 1). Various quantities of a substance S (such as potassium) are moving from one compartment to the other and *vice versa*, and it is required to determine how much of S has moved, say, from compartment 1 to compartment 2 in a given small interval of time. This is defined as dS_{21}. By analysis at a given instant, the amount of S and its change in either compartment may be determined (e.g. dS_1 or dS_2). Similarly, the *total* radioactivity of the contents of either compartment may be determined by familiar methods. This will be called dR_1 or dR_2. We use the

Figure I

Graphical illustration of changes occurring in a two-compartment system during the simultaneous two-way transport of a tagged substance between compartment I (left) and compartment 2 (right). The shaded portions represent the radioactivities of the various fractions. The areas of the rectangles represent the amount of substance in each case. Since unit widths are employed the areas are numerically represented by the heights.


dS_{21}
dS_{12}
dS_2
S_1
S_2
a_1
a_2
1
1

Italic R here, reserving R for radioactivity per cc, as in the earlier communication (1). Finally we define the ratio $R_1/S_1 = a_1$, etc. This is the relative specific activity of the material in compartment 1, etc. It is usually measured in such units as counts-per-minute-per-millimol. It was shown in the earlier communication that to obtain dS_{12} or dS_{21} it is necessary to determine the values of a in both compartments and the values of dR and dS in one. The final equation is obtained by taking the general solution for the n-compartment case and applying it to the case n = 2. In this most simple case it is more easily seen as follows. In figure 1 the amount of S in each compartment is equal to the area of the rectangle in each case. Since unit width is employed, the amount is also given by the height. We assume justifiable license in representing the width of the shaded portion by the quantity a in the interests of simplicity, although strictly it is a quantity proportional to it. The results are not affected by this choice. We consider the most general situation where the exchanges are opposite but not equal. Thus dS_{21} units are transferred from compartment 1 to compartment 2. In the opposite case $dS_{21} - dS_2$ units move from compartment 2 to compartment 1. The dS_2 represents the excess which leaves compartment 2 and is not made up by what arrived from 1. The negative sign is used since compartment 2 suffers a decrease in the transfer process as it is indicated here. Each lot contains the amount of radioactivity indicated in terms of the shading. Thus the lot $-dS_2$ contains $-a_2\ dS_2$. By adding and subtracting the various amounts as shown, the total change in radioactivity in compartment 2 is

$$dR_2 = a_1\ dS_{21} - a_2\ dS_{21} + a_2\ dS_2.$$

We solve for the quantity transported, dS_{21}, and divide by dt to obtain the corresponding rate obtaining

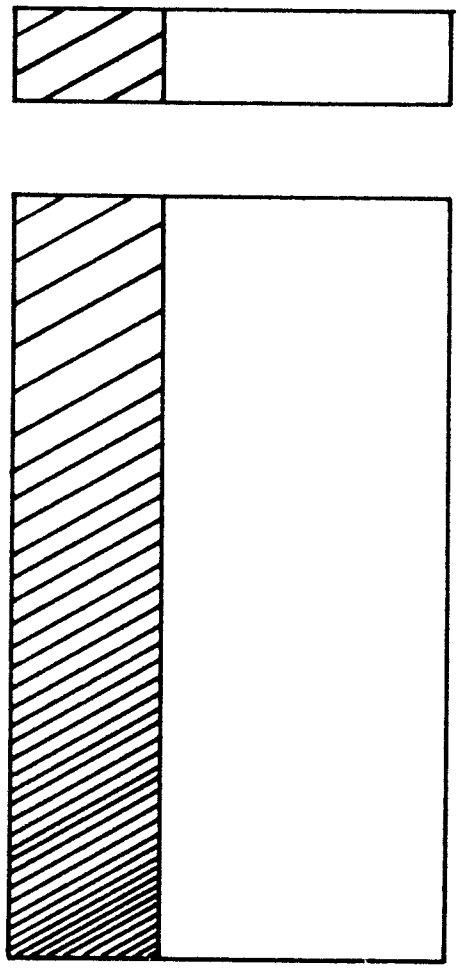
$$dS_{21}/dt = \frac{a_2\ dS_2/dt - dR_2/dt}{a_2 - a_1} = \frac{S_2 da_2/dt}{a_1 - a_2} \qquad (1)$$

The return rate dS_{12}/dt is obtained similarly. As already pointed out the two basic postulates of uniform mixing and identical behavior must be satisfied. Effect of the failure of either criterion is shown graphically in figure 2.

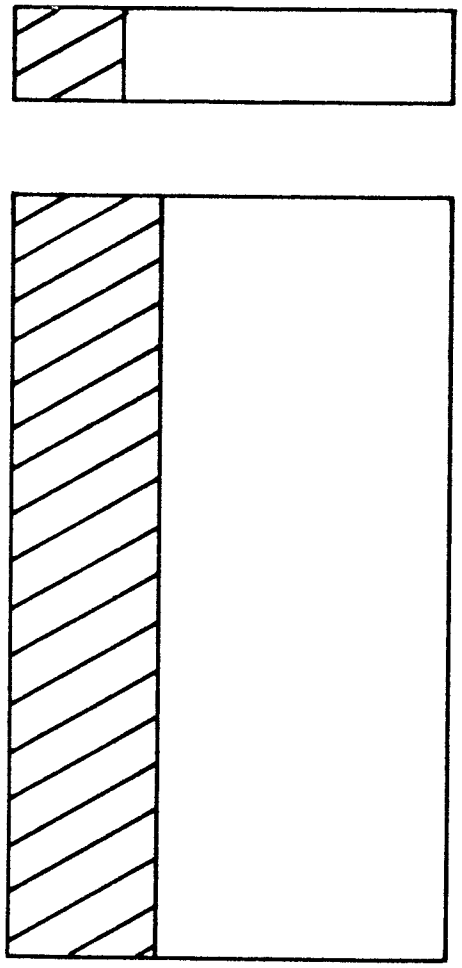
Up to this point no law of transfer of S between the two compartments has been postulated. As in the earlier communication the observations of S and a in

Figure 2

Graphical illustration of the failure of either of the basic postulates on which the present isotopic method is based.



NOT UNIFORMLY MIXED



DO NOT BEHAVE IDENTICALLY

the compartments represent the means whereby the laws of transfer are to be elucidated. We now consider the predictions which can be made of the variation with time of a_1 and a_2 when subject to a known law of exchange.

The most important situation biologically is that where there is no disturbance of the equilibrium and thus $dS_1 = dS_2 = 0$. Then $dS_{12} = dS_{21}$. In this case the substance S is exchanged at equal and opposite rates for which we use the symbol ρ. Thus

$$dS_{12}/dt = dS_{21}/dt = \rho = \frac{dR_1/dt}{a_2 - a_1} \qquad (2)$$

Or alternatively

$$da_1/dt = \frac{\rho}{S_1}(a_2 - a_1) \qquad (3)$$

Similarly

$$da_2/dt = \frac{\rho}{S_2}(a_1 - a_2) \qquad (3)$$

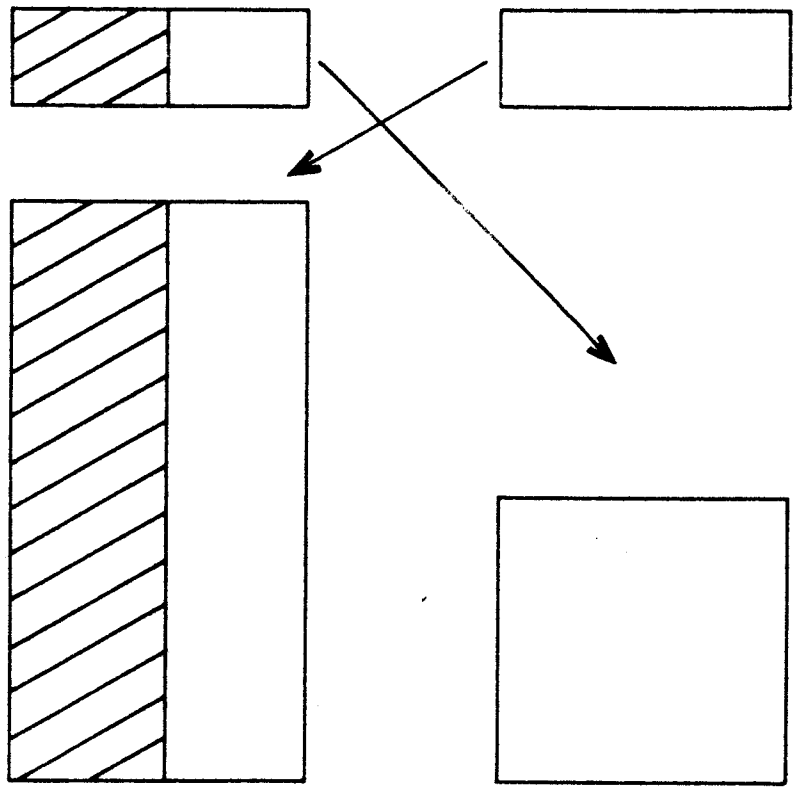
As pointed out by earlier investigators (9), this equation is identical in form to that describing the changes in concentration in a system of two compartments separated by a thin membrane where Fick's law of diffusion applies. This principle will be considered further below.

It is supposed for the moment that initially all activity is in one compartment only. The initial behavior of such a system can be analyzed in a rather simple manner and the results will be sufficient to establish the exchange rate ρ from the initial changes. Detailed study of the variations in specific activity with time at later times will be required only if doubt exists as to the validity of the idealized model of the two-compartment system for the description of the actual system under study. The initial situation is shown in figure 3. It is seen that the radioactivity is going one way only and in direct proportion to the exchange rate ρ. Thus the specific activity in compartment 1 decreases linearly with time and the exchange rate is obtained from the initial slope of the curve of a_1 or R_1 as a function of time using the expression

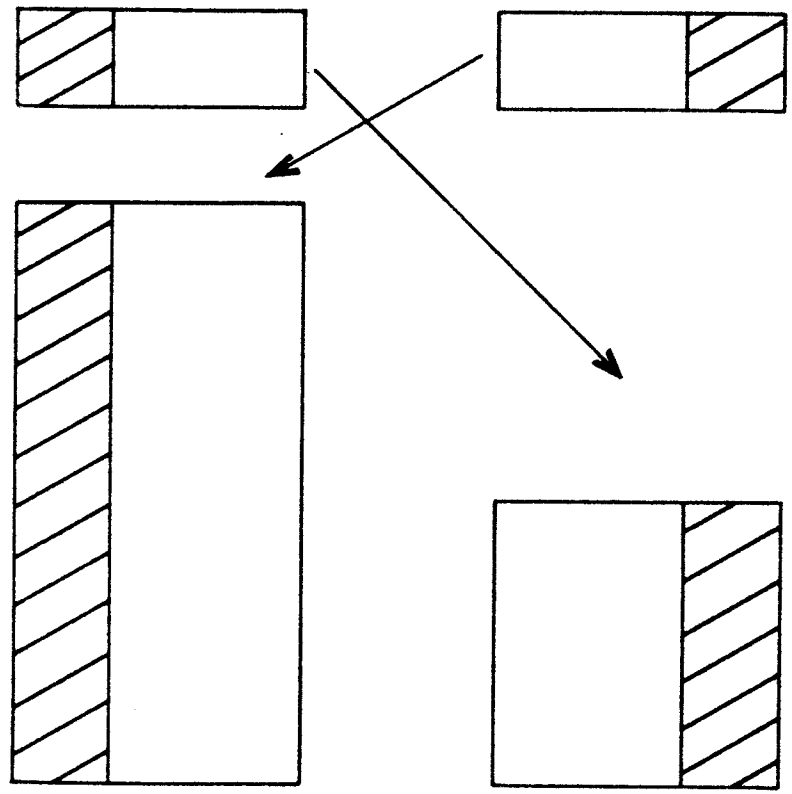
$$\rho = \frac{-1}{a_1}\frac{dR_1}{dt} = \frac{-S_1}{a_1}\frac{da_1}{dt} \qquad (4)$$

Figure 3

Illustration of a system where exchange occurs at equal and opposite rates and where initially all of the activity is in one compartment (t = 0). At final equilibrium (t = 00) the activity is shared in equal proportion between the compartments.



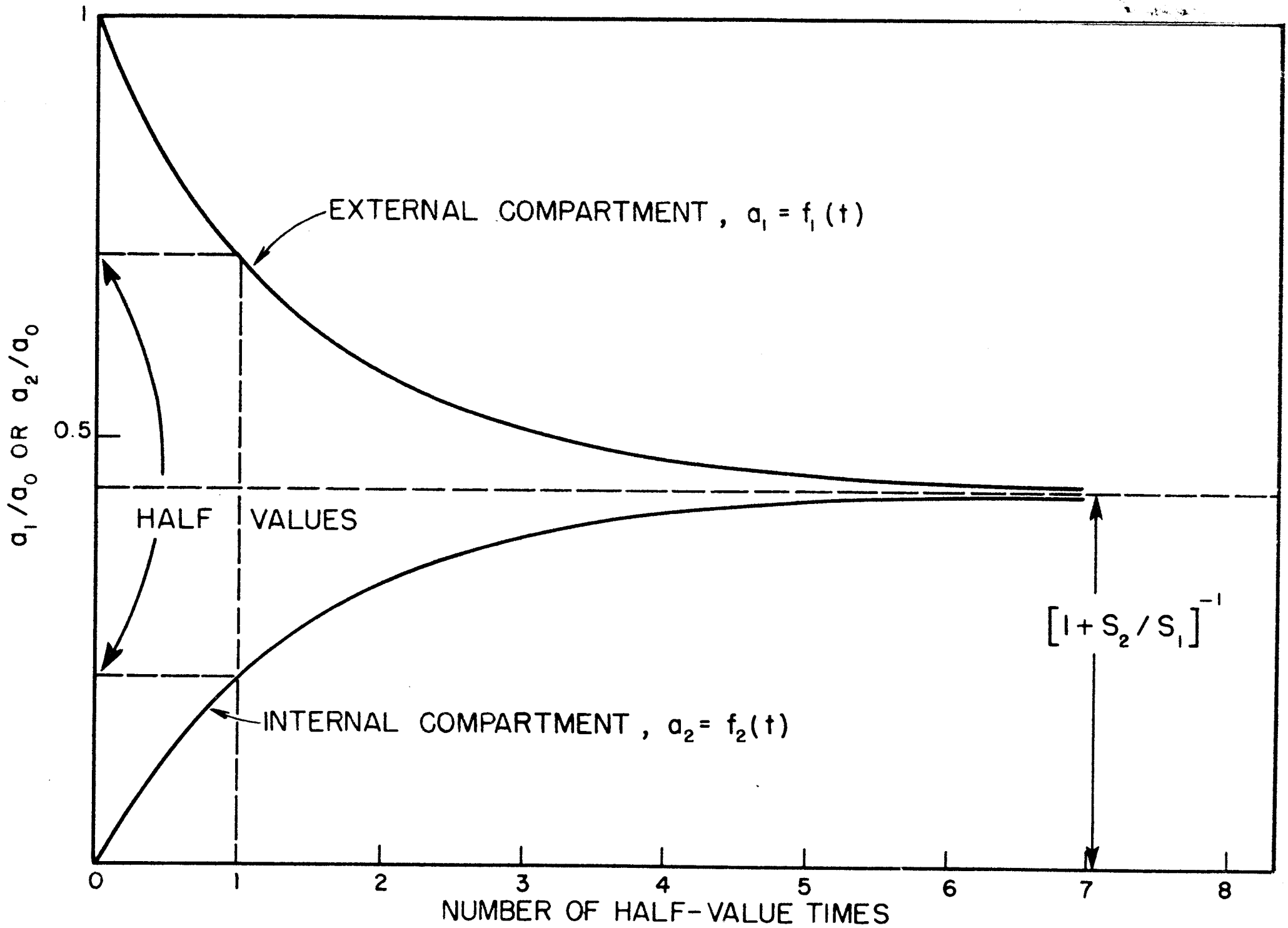
$t = 0$



$t = \infty$

Figure 4

The theoretical variation of specific activities (ordinates) with time (abscissas) for the two compartments when the exchange occurs at equal and opposite rates. The curves vary linearly over any small period of time and approach the same asymptote after sufficient time has elapsed. At any point the difference between the value of a_1 or a_2 and the asymptotic value (i.e. at t = 00) decreases by 1/2 during one half-value time interval.


1
0.5
a_1/a_0 OR a_2/a_0
EXTERNAL COMPARTMENT , $a_1 = f_1(t)$
HALF VALUES
INTERNAL COMPARTMENT , $a_2 = f_2(t)$
$[1 + S_2/S_1]^{-1}$
0
1
2
3
4
5
6
7
8
NUMBER OF HALF-VALUE TIMES

At this stage, the criterion of uniform mixing in compartment 2 can be relaxed, since it applies in the strict sense only.

As time progresses and the activity in the second compartment increases, some of it begins to return, and the decrease in activity in compartment 1 is less rapid. Finally after a sufficiently long time the specific activities approach one another; thus the two compartments contain the same fraction of activity and no further changes occur. This is also shown in figure 3. The typical graph showing the variations in specific activity in the two compartments is shown in figure 4. The general equations describing these variations for all values of time will be discussed in the next section. Often the curves for the variation of R in each compartment under conditions of equal and opposite exchange at constant rate are of interest. On a relative basis (i.e. if R/R_0 is plotted) the curve for compartment 1 will be the same as that for a_1 (Fig. 4). Since the total activity of the system is constant, the curve for compartment 2 will be its mirror image.

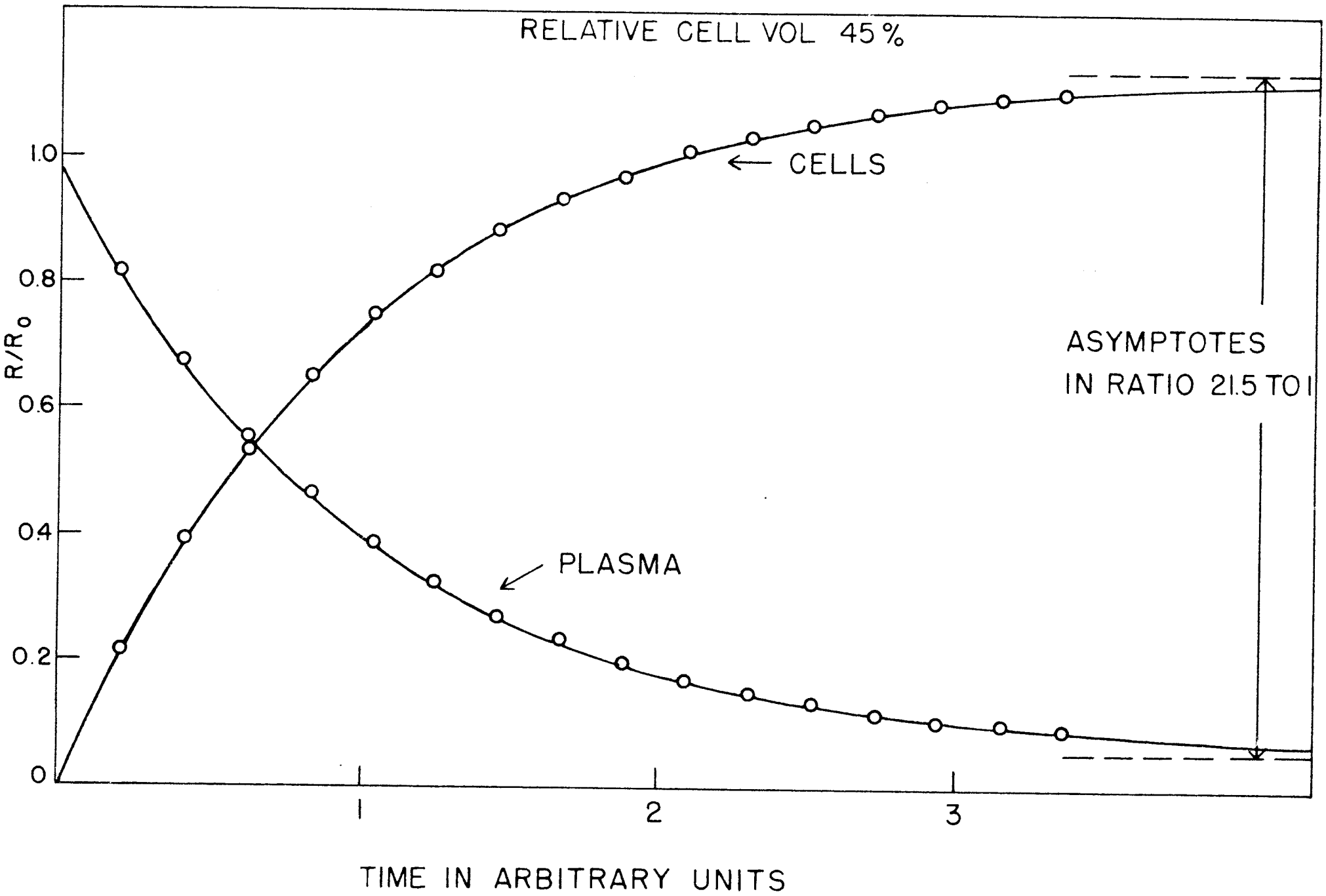
If the volumes of both compartments were nearly the same (e.g., cells and plasma of a typical healthy dog), then the curves of the radioactivity per cc (R_1 and R_2) would be nearly identical with those for total radioactivity. Since R is a commonly observed quantity, typical theoretical curves of this type are shown in figure 5 for activity of K^{42} in the cells and plasma of human blood on the assumption of an ideal two-compartment system. Here a typical ratio of concentrations of intracellular and extracellular potassium is about 21, and the cells occupy about 45 per cent of the total blood volume under normal conditions. The curve for the cellular activity per cc is obtained by taking the mirror image of that for the plasma and multiplying by the ratio of the relative volume of the plasma per cc of blood to that of the cells (i.e., 55/45).

The over-all time variation of the specific activities. - The set of equa- 3 and 3' can be integrated by standard mathematical procedures. For the case where initially all of the activity is in compartment 1 whose initial specific activity is thus a_0 the results are

$$a_1/a_0 = \frac{S_1 + S_2\, \epsilon^{-\rho t(1/S_1 + 1/S_2)}}{S_1 + S_2}$$

Figure 5

Theoretical prediction of the variation with time (abscissas) of the radioactivity per cc (ordinates) of plasma and cells of human whole blood. Typical values were assumed for the relative cell volume and the extracellular and intracellular potassium concentrations. The circles represent calculated theoretical points. The time scale is arbitrary since this depends on the exchange rate which is to be obtained from experimental rather than theoretical results.


RELATIVE CELL VOL 45%
CELLS
PLASMA
ASYMPTOTES
IN RATIO 21.5 TO 1
R/R₀
1.0
0.8
0.6
0.4
0.2
0
1
2
3
TIME IN ARBITRARY UNITS

$$a_2/a_0 = \frac{S_1 \left[1-e^{-\rho t(1/S_1 + 1/S_2)}\right]}{S_1 + S_2} \tag{5}$$

The second equation is identical to that given by Cohn and Brues (3), when appropriate changes in notation have been made. The expression given by Mullins *et al.* (their equation 3) (10) is dimensionally incorrect.

In Appendix B the analogy between Fick's law and the mixing of isotopes in a system is shown in the general case; this provides an alternative method of obtaining the solution of equations 3 and 3'. Suppose we have two compartments 1 and 2 separated by a membrane and containing concentrations of material c_1 and c_2. The constant volumes are V_1 and V_2. In terms of the familiar membrane constant K, the variation in the c's with time is given from Fick's law by

$$d(c_1 V_1)/dt = -d(c_2 V_2)/dt = K(c_2 - c_1) \tag{6}$$

It is seen that we obtain equations 3 and 3' by substituting

ρ for K, S_1 (i.e. $c_1 V_1$) for V_1, a_1 for c_1, etc.

The solution of equations 6 is familiar in the field of physical chemistry (11). After the necessary substitutions, equations 5 result.

Semilogarithmic plots. – Although the initial slope of the specific activity curves (Fig. 4) would be sufficient to establish the value of ρ in a given system, nevertheless in comparing experimental data with the theoretical results a plot on semilogarithmic coordinates is useful, and the slope of this plot will also establish the value of ρ. By transposing terms in equations 5 and taking logarithms of both sides,

$$\log[a_1/a_0 - S_1(S_1 + S_2)] = \log [S_2/(S_1 + S_2)] - \rho t(1/S_1 + 1/S_2)$$

and

$$\log[S_1/(S_1 + S_2) - a_2/a_0] = \log [S_1/(S_1 + S_2)] - \rho t(1/S_1 + 1/S_2). \tag{7}$$

It is seen that in each case the logarithm of the difference between a_1/a_0 or a_2/a_0 and the base line value (see Fig. 4), taken always with a plus sign is proportional to the time t. When this quantity is plotted on semilog coordinates, the result is linear. The reciprocal of the proportionality constant can be

treated similarly to the mean life of a radioactive isotope. It is the time required for the quantity (minus base line) on the ordinate scale to decline to 1/e of its initial value. The abscissa scale in figure 5 is taken in units of this time interval. More familiar is the "half-value time" (Fig. 4). This is the time during which the quantity changes by one-half. In the present case it is

$$T_{1/2} = 0.69\ S_1\ S_2/\rho(S_1 + S_2). \tag{8}$$

Another method of plotting is sometimes found; since

$$\log\ (a_1/a_0 - a_2/a_0) = -\rho t(1/S_1 + 1/S_2), \tag{9}$$

a plot of the difference of the a's will also be linear with the same slope but with a different intercept.

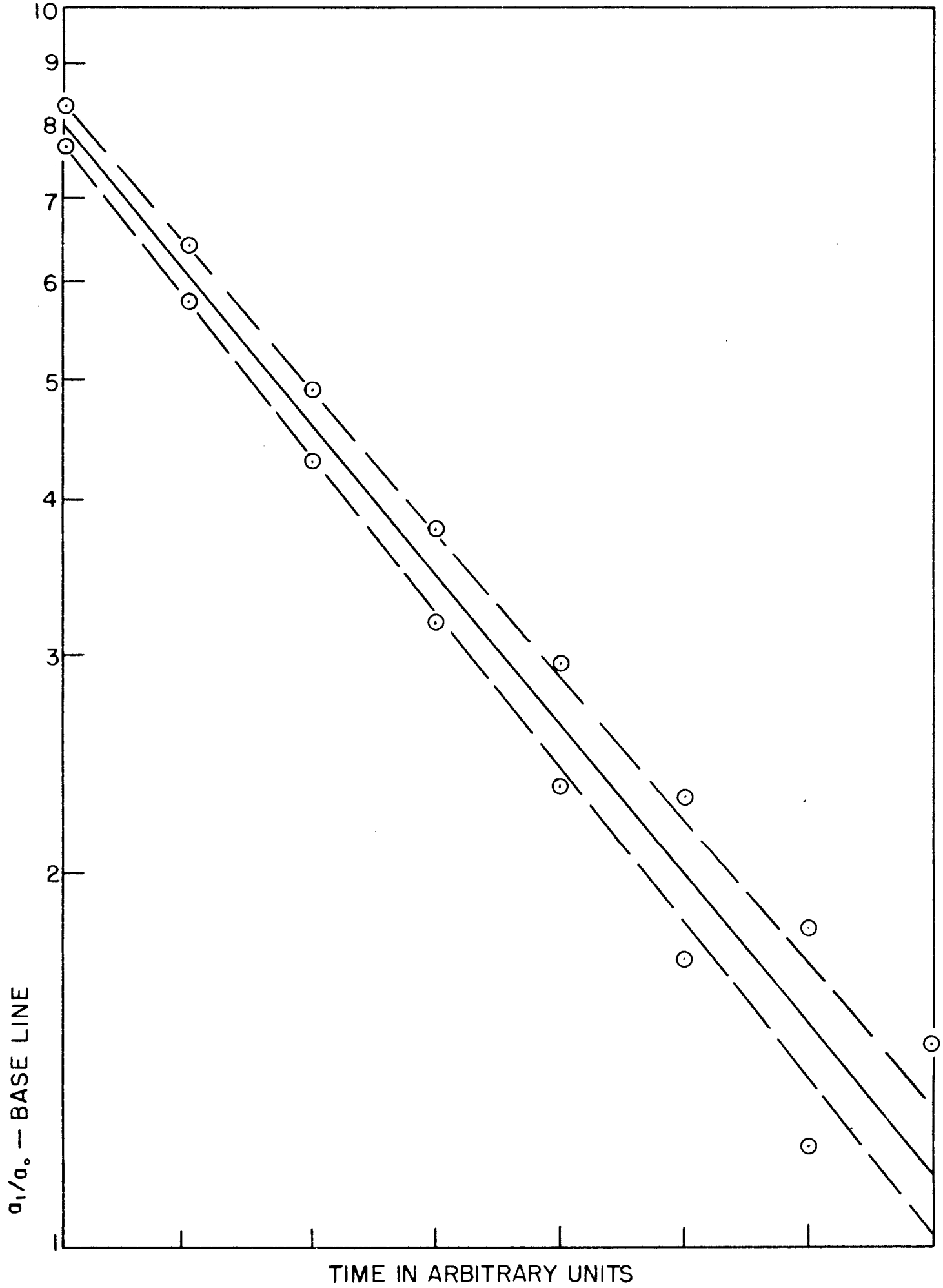
Some practical points should be considered in the construction of semilog plots of this type from experimental data. In the first place, the results become increasingly inaccurate as time progresses since the ordinate values are established from increasingly small differences whose relative experimental uncertainty increases as the values decrease. Unfortunately, the values at increasing time are often of considerable interest, a point which will be further discussed in a consideration of inhomogeneity of exchange rates.

If the experimental data are carried sufficiently far out in time, it may be possible to determine with fair accuracy from the curve alone the base line amount to be subtracted in order to construct the semilog plot. However one may introduce curvature in the results or make a curved result erroneously straight by incorrect choice of this quantity (Fig. 6). Furthermore, changes in the slope occur.

Exchange processes during centrifugation. – The literature contains numerous examples of the practical use of equations 5 in interpreting experimental results. In addition we consider the application of these equations to the correction of data for exchange during centrifugation. If this method be used for removing cells for assay, the cells are often incompletely packed, particularly when facilities are not available for centrifugation at high speeds or when high accelerations produce cell injury. Under these conditions, it is not correct to assume that the exchange processes are arrested at the beginning of

Figure 6

Effect of poor choice of the constant term (base line value) in making semi-logarithmic plots of experimental data. If too small a value is taken the resulting curve becomes convex to the axis and the initial slope is decreased. The reverse occurs for too large a choice.


10
9
8
7
6
5
4
3
2
1
a_1/a_0 — BASE LINE
TIME IN ARBITRARY UNITS

the centrifugation period. The cells are still surrounded by the same medium as before, and until the composition of this medium is changed by local depletion the exchange processes will continue unchecked. Correction can be made by adding to the time at which centrifugation is begun an amount $\Delta t'$ to obtain an effective separation time. This can be expressed in terms of the amount of material xS_1 surrounding the packed cells, the exchange rate ρ and the time interval of centrifugation Δt.

Since the exchange proceeds unchecked for a short time after the cells are thrown down, if Δt is sufficiently short then the ratio $\Delta t'/\Delta t = 1$. If, on the other hand, centrifugation is continued for a very long time, the cells will come into equilibrium with the surrounding occluded suspension material.

If, at the beginning of centrifugation, the specific activity of cells and suspension medium are a_2' and a_1', respectively, it is easily shown that the final equilibrium specific activity of the cells is obtained by adding to a_2' the amount

$$\Delta a_2' = (xS_1/S_2)(a_1' - a_2'). \qquad (10)$$

The time correction for prolonged centrifugation is thus

$$\Delta t_\infty' = xS_1/\rho.$$

In general if $xS_1/S_2 \ll 1$ then $$\Delta t' = \frac{xS_1}{\rho}(1-e^{-\rho\Delta t/xS_1}) \qquad (11)$$

The correction is plotted in figure 7. Figure 8 shows the effect of applying this correction to actual experimental data.

Swelling and leakage of cells. - It is of interest that equations 5 are independent of the volume of either compartment. Often during *in vitro* experiments the system is in good condition in the earlier stages but gradually deteriorates. This may be manifested by gradual swelling of the cells and gradual changes in the concentration of S in the two "compartments" of the system. Although unfortunate in other respects, these changes in no way invalidate the applicability of equation 1. Changes in the volume of either compartment do not affect the result as long as determinations of S and a are obtained, since the volumes do not enter into the equation at all. Thus if the

Figure 7

Correction $\Delta t'$ (ordinates) as a function of Δt (abscissas). Both quantities are in units of $X\ S_1/\rho$.

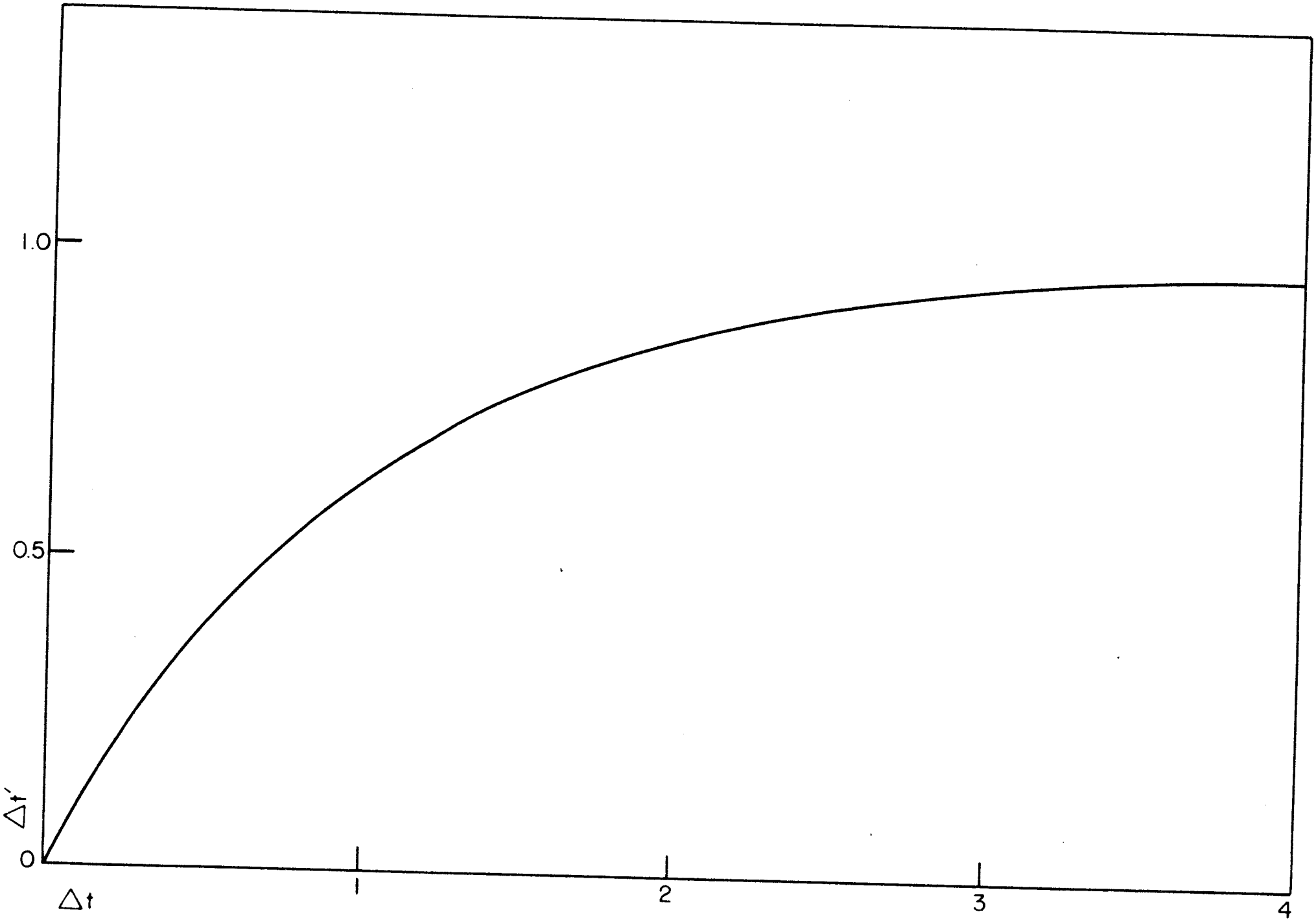
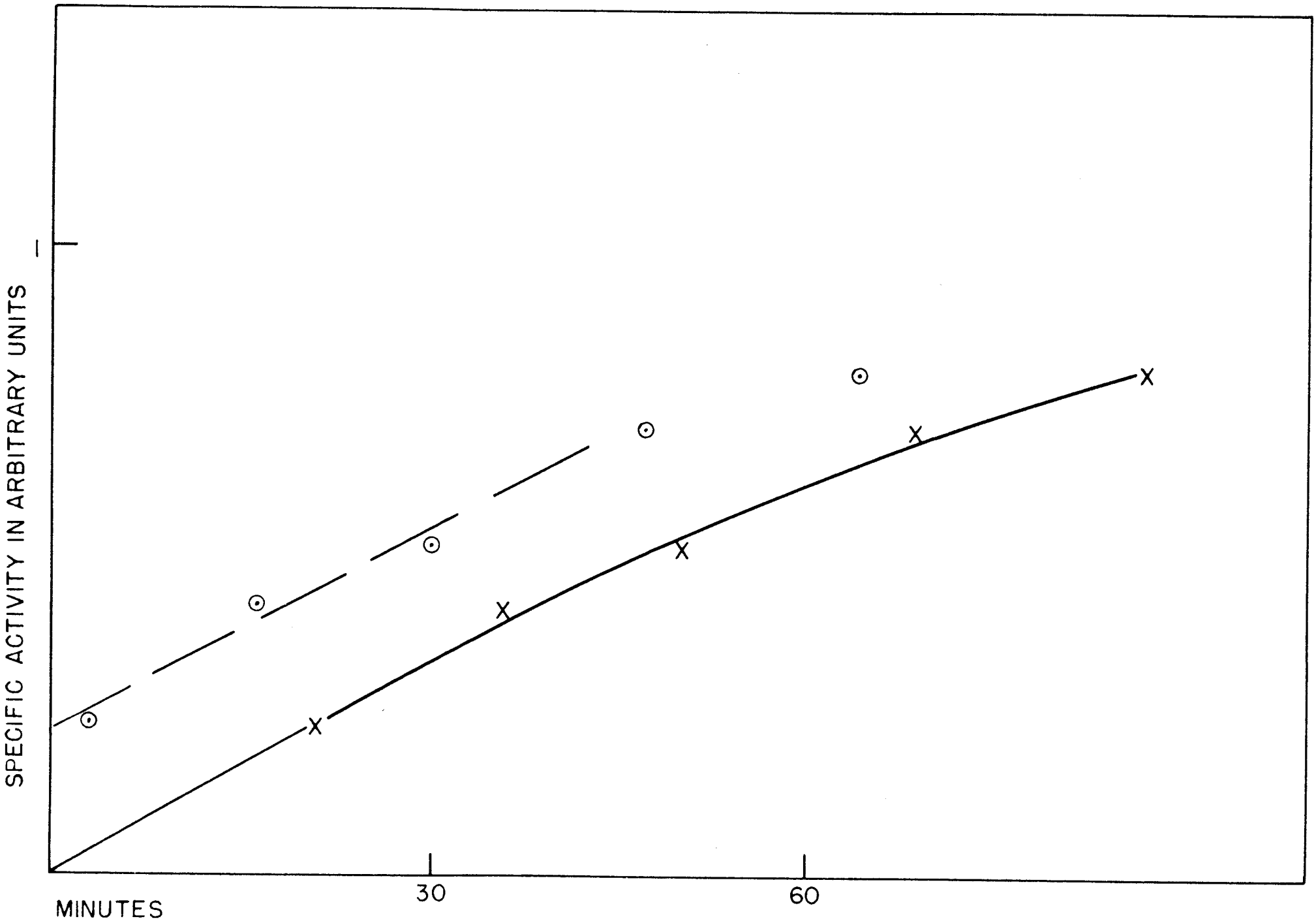

1.0
0.5
0
Δt'
Δt
1
2
3
4

Figure 8

Effect of exchange of potassium between cells and layers of trapped plasma and correction of experimental data. The circles represent the specific activities of canine erythrocytes as experimentally determined. The crosses are corrected points. The values of $\frac{X\ S_1}{\rho}$ was 40 minutes, taken from typical experimental data for the dog.


SPECIFIC ACTIVITY IN ARBITRARY UNITS
MINUTES
30
60

specific activity in either compartment is changing exponentially according to equations 5 the exchange rate is still constant even though the cells are swelling. Nevertheless, the progressive disturbance which ensues will in all likelihood soon result in changes in S_1 and S_2. If these quantities are no longer constant then the exchange rates will no longer be equal and in general they may also vary with time. Equation 1 still applies and if the nature of the disturbance were such that the ratios $(dS_{21}/dt)/S_2$ and $(dS_{12}/dt)/S_1$ were constant then the changes in specific activity would still be exponential in character.

Nonuniform mixing and multiple exchange rates. - The postulate of uniform mixing must be satisfied if the unknown processes occurring in a given arbitrary system are to be determined by external observation. Nevertheless, the behavior of a known system may be predicted even though uniform mixing is not fulfilled provided that the nature of the distribution of the different species of S in a given compartment is known. One example of the failure of uniform mixing occurs when a two-compartment system contains two species of S which have different exchange rates. Anomalous exchange curves in experiments with radioactive isotopes (3) have led to the suggestion that in some cases there may be more than one species of S exchanging at different rates, causing the semilogarithmic plots of the results to deviate from linearity.

This situation is represented by a two-compartment system containing two pairs of subcompartments. It is assumed that the two species of S do not interact. Thus exchange between the two subcompartments of a given compartment is prevented. (This assumption may at times prove too stringent.) One subcompartment is distinguished from the other by the use of primed symbols. As before, the total amount of S in the entire cellular compartment is S_1. Of this one subcompartment contains the fraction F' and the other the fraction F'' (F' + F'' = 1). Each subcompartment contains $F'S_2$, and $F''S_2$ units, and the amount of radioactivity in each case will be $F'S_2\ a_2$ and $F''S_2\ a_2$. Adding and dividing by the total amount of S in the compartment,

$$a_2/a_0 = F'\ a_2' + F''\ a_2''. \qquad (12)$$

Thus the over-all specific activity relative to a_0 is the weighted mean of the

specific activities of the subcompartments, the weighting factor being determined by the fractional amount of S available in each case for exchange. If the exchange rates of the two forms of S are ρ' and ρ'' then

$$a_2/a_0 = \frac{S_1}{S_1 + S_2}\left[1 - F' e^{-(\rho'/F')(1/S_1 + 1/S_2)t} - F'' e^{-(\rho''/F'')(1/S_1 + 1/S_2)t}\right] \quad (13)$$

in which two different exponential terms appear. In this case, the semilogarithmic plot will not be linear.

This is the most simple example of multiple exchange. Here, although there are two exponentials, the initial portion of the curve of equation 13 is still nearly a straight line. This can be shown as follows. Consider a series of any number of exponentials:

$$Y = A + Be^{-kt} + Ce^{-lt} + De^{-mt} + \ldots \text{ etc.}$$

Using the series expansions:

$$Y \simeq A + B(1 - kt + \ldots) + C(1 - lt + \ldots) + D(1 - mt + \ldots) \text{ etc.}$$

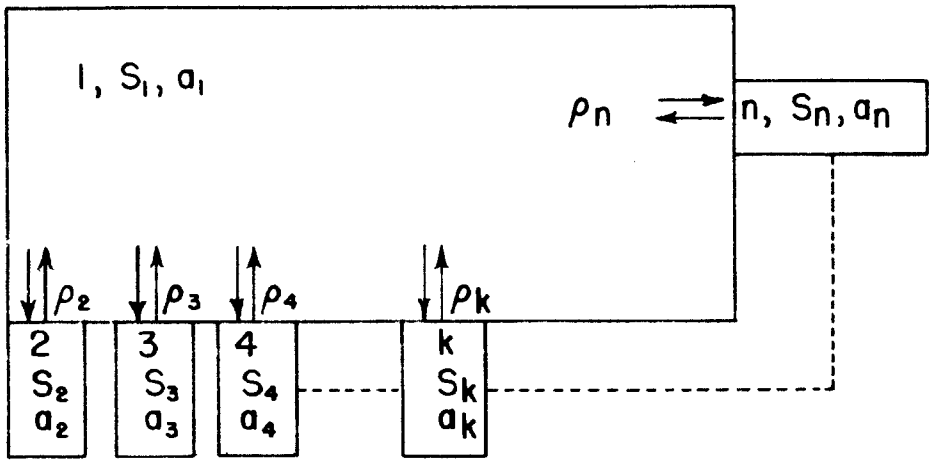
$$Y \simeq (A + B + C + D + \ldots) - (kB + lc + mD + \ldots)\, t + \ldots \text{ etc.} \quad (14)$$

For small values of time this approximation is very good and the variation with time is essentially linear. Initially, when all of the activity is still in a single compartment, it is lost to the other compartments at a net rate determined by the arithmetic mean of the various exchange rates. Since the initial slope gives only the arithmetic mean of the exchange rates in the case of multiple exchange processes, it tells nothing about their individual variation.

Compartment within compartment. - Often a multicompartment system is simplified because exchange between a certain fraction of the compartments is prevented. Compartments may be arranged in a line so that each member of the system can communicate only with the two neighbors on either side. Such is the case in certain types of metabolic cycles which might be studied by the isotope method. In another typical case a series of compartments are separated from one another but can all communicate with one central compartment. Consider the system shown in figure 9. Here a system of n-1 individual compartments communicates with one central one, but not with one another. If, in such a system, one had access to

Figure 9

A multicompartment system in which a series of compartments (numbered from 2 to n) communicate with a central one (number one). S is exchanged at equal and opposite rates (ρ_2 to ρ_n) with the central compartment only.


1, S1, a1
ρn
n, Sn, an
ρ2
ρ3
ρ4
ρk
2
S2
a2
3
S3
a3
4
S4
a4
k
Sk
ak

all compartments for analysis, then the amounts transferred between any side compartment and the central one could be determined using only two species of S. Such is the case in the *in vivo* measurements of exchange between cells and plasma of mammalian blood. Here, although the system is complex, the plasma is correctly represented by the central compartment and the cells by one of the side compartments. However, the use of the simple two-compartment analysis in interpreting the results is incorrect since the time variation of the a's will not be truly exponential.

The case of n compartments is treated in Appendix C. It requires the solution of a system of n linear first order differential equations. The solution for the plasma specific activity is obtained from equations 10', 18' and 19' in the appendix with proper changes in notation:

$$a_1/a_0 = S_1/S + \Sigma x_i \, e^{-\lambda_i t},$$

where the λ_i are the roots of

$$g(\lambda) = 1 - \sum_2^n \frac{\rho_j/S_1}{(\lambda - \rho_j/S_j)} = 0$$

and the x_i are obtained from an expansion of $[\lambda g(\lambda)]^{-1}$ in partial fractions. They are thus defined by the identity

$$\frac{1}{\lambda g(\lambda)} \equiv \frac{S_1}{S\lambda} + \sum_1^{n-1} \frac{X_i}{(\lambda - \lambda_i)}. \qquad (15)$$

The three-compartment case. - As an illustrative application of equations 15, the case of three compartments (n = 3) will be considered. The equation for λ yields two roots. Although there are only two exponentials in the result, the half-value times are not expressible simply in terms of the exchange rates of contents of the two pairs of compartments. The situation is thus one degree more complicated than that of equation 13. In general the semilogarithmic plot will not yield two exponents which can be expressed in terms of independent exchange rates. The situation is best seen in the case where the values of ρ_k/S_k are close to one another. Here we have as a good approximation

$$\lambda_1 = \frac{\rho_2/S_2 + \rho_3/S_3}{2} \text{ and } \lambda_2 = \lambda_1 + \frac{\rho_2 + \rho_3}{S_1},$$

and the effect of the exchange between each pair of compartments upon the processes occurring in the other pair is evident.

One case can be found where the interpretation of the results is straightforward. If one exchange is much more rapid than the other, the effect will be nearly as though one compartment is not exchanging at all initially. After the initial rapid exchange is completed between one pair of compartments, the slowly exchanging process will proceed as though the rapidly exchanging compartment is always of the same composition as the central one. These two compartments then become a single larger compartment.

We suppose that exchange proceeds rapidly at first between compartment 2 and the central one (compartment 1). Then

$$a_1/a_0 = \frac{S_1 + S_2\, e^{-\rho_2 + (\frac{1}{S_1} + \frac{1}{S_2})t}}{S_1 + S_2} .$$

When the exponential term has declined to a negligible value the exchange between the additional compartment (number 3) has been negligible as yet because of the slow rate. The specific activity of the first two compartments is now $a_0\, S_1/(S_1 + S_2)$. This is now taken as a new initial specific activity a_0' and the same equations applied as before. Considering the exchange between compartment 3 and compartments 1 and 2 together,

$$a_1/a_0' = \frac{(S_1 + S_2) + S_3\, e^{-\rho_3 (\frac{1}{S_1 + S_2} + \frac{1}{S_3})t}}{S} \quad \text{where } S = S_1 + S_2 + S_3 . \quad (16)$$

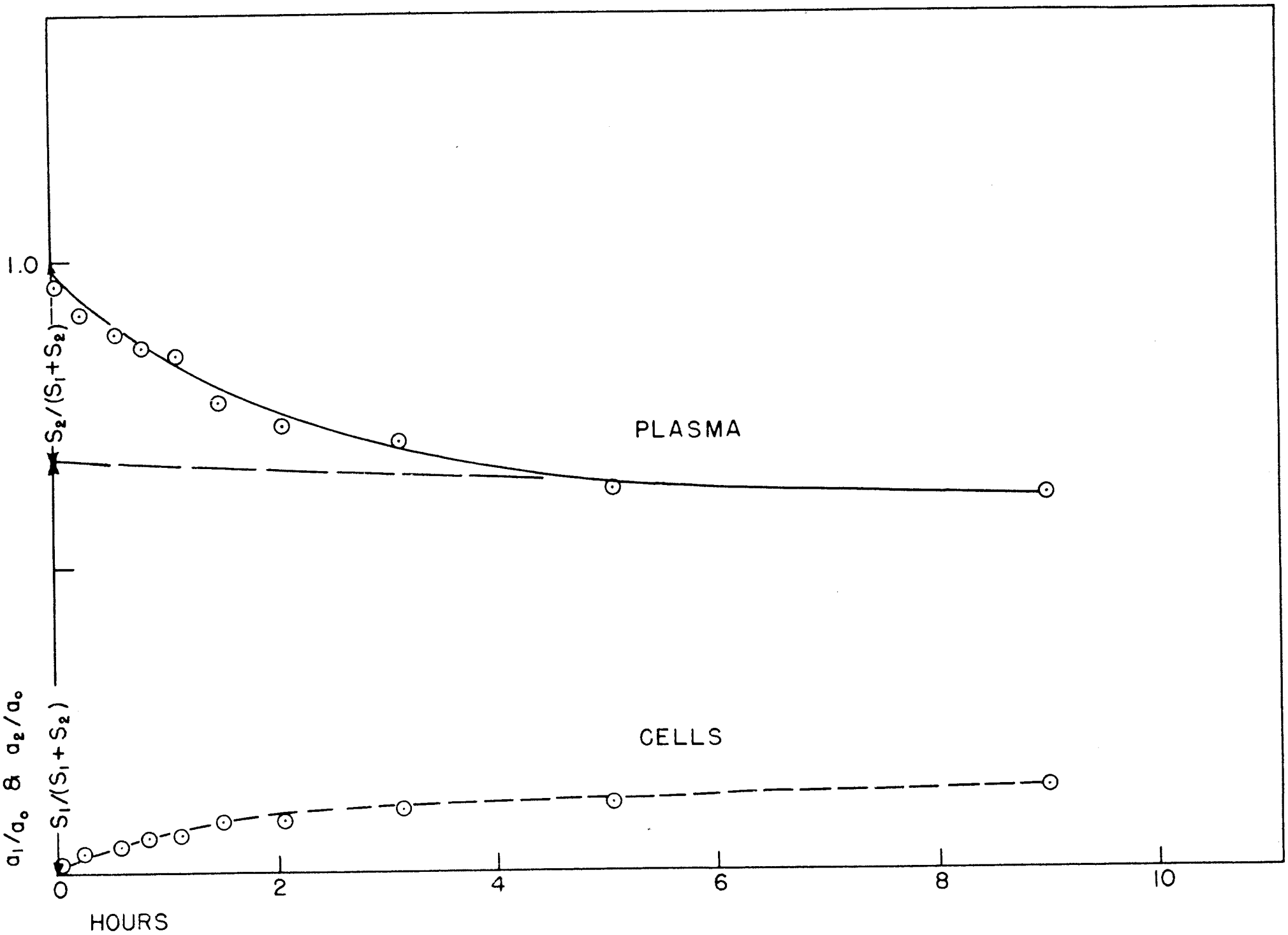
If this is now referred to the original specific activity a_0 then

$$a_1/a_0 = S_1/S \left[1 + S_3/(S_1 + S_2)\, e^{-t(\frac{\rho_3}{S_3} + \frac{\rho_3}{S_1 + S_2})} \right] + \frac{S_2\, e^{-(\frac{\rho_2}{S_2} + \frac{\rho_2}{S_1})t}}{S_1 + S_2} \quad (17)$$

An example (12) of this type of process in the exchange of potassium in dog blood is shown in figure 10. From the value of $S_2/(S_1 + S_2)$ obtained by extrapolation, the rapidly exchanging fraction represents about one-half the plasma potassium in this case. Although the existence of more than one type of S (say two different molecules) whose transfer properties were not the same would appear in the results as a superposition of the equations obtained for each type of S separately, this is not the case when only one type of S is involved in a

Figure 10

Application of equation 17 to actual experimental data. The points on the upper curve were obtained from determinations of radioactivity and potassium concentrations in samples of plasma separated from canine whole blood after varying periods of equilibration *in vitro* following addition of a radioactive tracer. The curve for cells is indicated below for comparison. It is seen that the data for the plasma can be reduced to two exponentials whose individual half-value times differ by a large factor. It is evident that equal specific activity will not be reached for many times the duration of the present experiment. From the zero intercepts it is seen that S_1/S_2 is about 0.5. Thus the easily exchangeable fraction of potassium (S_2) is about one-half the total plasma potassium (S_1).


1.0
$S_2/(S_1+S_2)$
$S_1/(S_1+S_2)$
a_1/a_o & a_2/a_o
PLASMA
CELLS
0
2
4
6
8
10
HOURS

system of several compartments. Here, in general, the exchange of S between one pair of compartments affects the exchange between the other pairs as well. The case just cited, where the exchange rates were widely different, represents a particular exception.

Statistical distribution of exchange rates. - In general, a biological system such as that of the cells and plasma of mammalian blood should be inhomogeneous. Thus at least to some extent the exchange rates among cells will vary statistically about a mean value. The effect of this variation on the change of the plasma specific activity with time can be analyzed by considering the individual cells. Since in a typical case there are roughly 5×10^9 cells in 1 cc we will obtain a very close approximation for the results by replacing the sums in equations 15 by integrals. As indicated in the appendix, the general solution is difficult. Nevertheless an approximate treatment is satisfactory for describing the initial phases of the exchange process. As a representative example we will consider a symmetrical distribution. Under these conditions the deviation from linearity of the semilog plot of the specific activity of the central compartment is (equation 42' with appropriate change of notation)

$$C(t) \simeq \frac{x^2 \, d^2 \, t^2 \, S_1}{2S} \left[1 + X \frac{(S - S_1)t}{3S} \right]$$

where 0.69/x is the effective half-value time and d is the standard deviation, relative to the mean of the distribution of exchange rates taken about the mean. This approximation will be sufficiently accurate at least up to the first half-time value. During this interval, an upper limit may be set

$$C(t) < d^2/3.$$

Since the relative standard deviation is squared and usually small, the departure from an exponential during the initial phases of the exchange will be small. Even for $d \sim 0.3$ the net effect will be less than three per cent.

This analysis does not rule out the possibility of determining the distribution of exchange rates by analysis of the curve as it approaches the base line. However, considerable precision will be required, including an accurate determination of the base line value. Unfortunately, as time progresses, the experimental system will be more likely to deteriorate.

SUMMARY

The theory of the movement of a radioactive tracer from one compartment to another of a two-compartment equilibrium system containing a tagged substance S is reviewed. If a_1 and a_2 are the specific activities, and S_1 and S_2 the total amounts of material in compartments 1 and 2, respectively, and if at $t = 0$ $a_1 = a_0$ and $a_2 = 0$, the values of a at any later time t are related to the exchange rate ρ by the equations

$$a_1/a_0 = \frac{S_1 + S_2\, e^{-\rho t\left(\frac{1}{S_1} + \frac{1}{S_2}\right)}}{S_1 + S_2}$$

$$a_2/a_0 = \frac{S_1\left(1 - e^{-\rho t\left(\frac{1}{S_1} + \frac{1}{S_2}\right)}\right)}{S_1 + S_2}.$$

Methods are described for determining ρ from measurements of the S's and the a(t)'s. It is shown that the results are not affected by volume changes providing the S's remain constant. An approximate expression is derived from the equations with which experimental results in cellular systems may be corrected for isotope exchange during the centrifugal separation of the cells. Equations are derived for the case where two kinds of S are being exchanged.

An n-compartment system is discussed in which a central compartment can exchange with n − 1 peripheral compartments and equations are derived which describe the specific activity changes. These are particularized to the case where n = 3. It is shown that if the two exchange rates differ widely then the results may be expressed in terms of two separate exponentials characteristic of the two separate exchange rates. In the more general case each exchange process affects the other. The analysis of the n-compartment system is applied to the study of the exchange of S between cells and plasma of mammalian blood *in vitro* where the exchange rates between the two fractions are statistically distributed. In a typical case, even though the spread is extreme, the departure of the results from the pure exponential behavior of a two-compartment system is less than three per cent during the initial phase where t is less than one half-time value.

An analogy with Fick's law of diffusion is applied to the determination of the radioactivity changes in a tagged system where the concentration of S is held constant in time but not in space.

REFERENCES

1. Sheppard, C. W. - The theory of the study of transfers within a multicompartment system using isotopic tracers. *J. Applied Physics*, 19:70-76, 1948.

2. Visscher, M. B., R. H. Varco, C. W. Carr, R. B. Dean and D. Erickson - Sodium ion movement between the intestinal lumen and the blood. *Am. J. Physiol.*, 141:488-505, 1944.

3. Cohn, W. E. and A. M. Brues - Metabolism of tissue cultures. III. A method for measuring the permeability of tissue cells to solutes. *J. Gen. Physiol.*, 28:449-461, 1945.

4. Chambers, E. L., W. White, N. Jeung and S. C. Brooks - Penetration and effects of low temperature and cyanide on penetration of radioactive potassium into the eggs of Strongylocentrotus purpuratus and Arbacia punctulata, *Biol. Bull.*, 95:252-253, 1948.

5. Duffield, R. B. and M. Calvin - The stability of chelate compounds. III. Exchange reactions of copper chelate compounds, *J. Am. Chem. Soc.*, 68:557-561, 1946.

6. Robinson, C. S., L. Fox and D. Dziewiatkowski - Private Communication.

7. Hastings, A. B., J. W. Raker, I. M. Taylor and J. M. Weller - In preparation.

8. Sheppard, C. W. and W. R. Martin - Isotopic studies of potassium exchange rates between cellular elements and plasma of human and canine blood *in vitro*, *Federation Proceedings*, 8:145, 1949.

9. Dean, R. B., T. R. Noonan, L. Haege and W. O. Fenn - Permeability of erythrocytes to radioactive potassium, *J. Gen. Physiol.*, 24:353-365, 1941.

10. Mullins, L. J., W. O. Fenn, T. R. Noonan and L. Haege - Permeability of erythrocytes to radioactive potassium, *Am. J. Physiol.*, 135:93-101, 1941.

11. Jacobs, M. H. - Diffusion processes, *Ergenbnisse der Biol.*, 12:1-160, 1935.

12. Sheppard, C. W. and W. R. Martin - Studies of the kinetics of potassium exchange between cells and plasma of canine blood *in vitro* using K^{42}, Atomic Energy Commission Declassified Report, AECD-2249.

13. Widder, D. V., The Laplace transform, Princeton University Press, Princeton, N. J., 1946.

APPENDIX A: Derivation of the Equations for the Multicompartment System

The original theoretical treatment of transfers within a multicompartment system used mathematical methods which, although cumbersome, are more likely to be familiar to the average reader. We now present a more concise derivation of equation 10 of the earlier report.

Consider a system of n compartments containing n individual species of a substance S uniformly mixed, and although individually identifiable, otherwise behaving identically. In compartment j there will be ${}^rS_j$ units of species r and its fractional amount will be ${}^rA_j$. If transfers occur such that dS_{jk} units are delivered from compartment k to compartment j these quantities may be experimentally determined by finding the ${}^rA_k$ in all compartments and the changes of all species $d{}^rS_j$ in compartment j only.

The equation relating them is obtained as follows. In a given compartment (say k) the amount of a given species leaving it and going to compartment j is ${}^rA_k\, dS_{jk}$. The total loss in species r is obtained by summing over all other compartments. Similarly one obtains the total gain, so that the net change in compartment j is

$$d{}^rS_j = \sum_{k=1}^{k=n} ({}^rA_k\, dS_{jk} - {}^rA_j\, dS_{kj}).$$

We define the reciprocal determinant α_{jr} such that

$$\sum_r \alpha_{jr}\, {}^rA_k = \delta_{jk}, \quad \sum_j {}^rA_j\, \alpha_{jp} = \delta_{rp},$$

where $\delta_{jk} = 0$ if $j \neq k$ and $\delta_{jj} = 1$.

Multiply both sides of the original equation by α_{ir} and sum over r giving

$$\sum_r \alpha_{ir}\, d{}^rS_j = \sum_r \alpha_{ir} \sum_k ({}^rA_k\, dS_{jk} - {}^rA_j\, dS_{kj})$$

$$= \sum_k (\delta_{ik}\, dS_{jk} - \delta_{ij}\, dS_{kj})$$

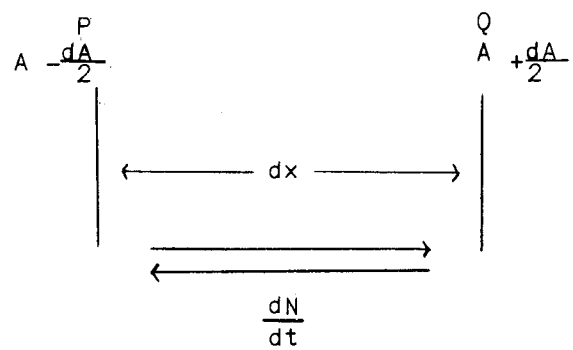
$$= dS_{ji}.$$

But from the known properties of a reciprocal determinant each term α_{kr} is equal to the cofactor of the corresponding ${}^rA_k$ divided by the determinant of all the ${}^rA_k$'s, giving the same solution as equation 10 in the earlier paper.

APPENDIX B: The Fick Law Analogy

Consider a system in which molecules of a given substance S are distributed in space.

The distribution is such that the concentration of S varies from point to point but S is so constrained that the concentration does not vary with time. However the molecules can move freely by processes of exchange. We will consider only the case where the exchange proceeds with equal ease in all directions and at all points in space. The molecules of S include some containing a labeling isotope so that at some point the absolute specific activity is A. Two planes P and Q are passed perpendicularly to the direction of maximum rate of change of A and separated a distance dx. Let the specific activity at P be $A - \frac{dA}{2}$ and at Q, $A + \frac{dA}{2}$.



The intervening layer will be bombarded by molecules from either side. Whatever their origin, the fraction of tagged molecules in plane P at any instant is A-dA, and this is the probability that a given molecule leaving this plane and traversing the intervening layer will be tagged. In dt seconds dN total molecule will traverse the layer from either side. Those going from Q to P will carry with them (A+dA)dN, and those in the opposite direction (A-dA)dN. The net rate of transfer of tagged molecules is thus dA dN/dt, and

$$-dN^*/dt = \underset{x}{A} \frac{dN}{dt} dx = \frac{dN}{dt} dx \overline{\text{grad } A}$$

We take the sign with the same convention as used in Fick's Law. The equation of Fick's first law may be written

$$dS/dt = -D \overline{\text{grad } c}.$$

We see that in the two equations equivalent quantities are N^* and S, A and c, and finally dN/dt dx and D. As a result for any system which satisfies the above assumptions we can immediately take over bodily the results of the analogous calculations of formal diffusion theory.

APPENDIX C: The Multicompartment Case

The equations--solution for a finite number of compartments. - We consider here the case of a single "outer" compartment, possibly large by comparison with the others, within which or attached to which are a number of others which do not exchange directly with one another. We let the subscript "o" refer to the outer compartment. We shall come to the case of many "inner" compartments (e.g. erythrocytes within the plasma), but we first consider the simpler case where these are distinguishable each one from the others. As a matter of convenience we adopt a simpler notation.

Let

$$A_k = \rho_k/S_o, \ \alpha_j = \rho_j/S_j, \ (j, k = 1, 2, \ldots, n) \tag{1'}$$

where the ρ's and the S's represent, as before, exchange rates and masses respectively. Thus the A_k and the α_j represent relative exchange rates, referred to the total contents of the outer compartment and of the inner compartments, respectively. Let $a_o(t)$ and $a_j(t)$ represent the specific activity in the outer compartment and the inner compartments. (The $a_o(t)$ here is the same as $a_1(t)/a_o$ in the original equations 5.) Then for equal and opposite exchange between each compartment j and the central one when all exchanges are equal in both directions

$$da_o/dt = \Sigma(a_k - a_o)A_k, \tag{2'}$$

$$da_j/dt = (a_o - a_j)\alpha_j. \tag{3'}$$

Although we are concerned with a multicompartment system a simplification exists because the exchange can only occur between the jth compartment and the central one. Thus equation 3 permits the jth exchange rate to be determined even though only two species of S are available, provided access can be gained to both compartments to determine a_j and a_o at any time. The variation of a_j will not be exponential in this case as in the simple two-compartment system since the a_o is affected by exchange both with compartment j and with all others as well.

We are interested in particular in the case where initially all radioactive material is introduced into the outer compartment, but not in such amounts as to

disturb the equilibrium sensibly. In this event a_0 has some known value initially, which may be taken as unity, and all the a_j are zero:

$$a_0(0) = 1, \qquad a_j(0) = 0. \tag{4'}$$

As will be shown later it is no restriction to suppose that no two α_j are equal. We may then suppose the compartments numbered in such a way that

$$\alpha_1 < \alpha_2 < \ldots < \alpha_n. \tag{5'}$$

By standard theorems it is known that there are solutions of the differential equations that can be written in the form

$$a_0 = \beta_0\, e^{-\lambda t}, \qquad a_j = \beta_j\, e^{-\lambda t}, \tag{6'}$$

with properly chosen λ. In fact, any λ for which the determinant

$$\Delta(\lambda) \equiv \begin{vmatrix} \lambda - \Sigma A_i & A_1 & \cdots & A_n \\ \alpha_1 & \lambda - \alpha_1 & \cdot & 0 \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \alpha_n & 0 & \cdot & \lambda - \alpha_n \end{vmatrix} \tag{7'}$$

vanishes will provide such a solution. Moreover, since it will be shown presently that the equation $\Delta = 0$ has $n + 1$ distinct roots, every one providing a distinct solution of the form (6'), it follows that the general solution is a linear combination of these.

In order to demonstrate these assertions, one substitutes the expressions (6') into the equations, leaving the β's and the λ undetermined. The exponentials cancel out leaving $n + 1$ linear homogeneous equations in the $n + 1$ coefficients β, with $\Delta(\lambda)$ as the determinant of these equations. There exist β's not all zero satisfying these homogeneous equations, and hence providing a solution (6'), if and only if this determinant vanishes. Hence any λ satisfying $\Delta = 0$ can be substituted into the homogeneous equations, and a set of β's determined therefrom to provide a solution (6').

An expansion of the determinant $\Delta(\lambda)$ can be effected as follows. First subtract the 2nd, 3rd, ..., and last column successively from the first. The

resulting first column has a λ in every position and this is therefore removable as a factor, leaving a column of ones. Now divide column $(j + 1)$ by $(\lambda - \alpha_j)$, placing it outside as a factor. In the column $(j + 1)$ one has then $A_j/(\lambda - \alpha_j)$ in the first position, 1 in the diagonal position, and 0 elsewhere. The resulting determinant is now readily expanded, and one can write

$$\Delta(\lambda) = \lambda\varphi(\lambda)\, g(\lambda), \tag{8'}$$

where

$$\varphi(\lambda) = (\lambda - \alpha_1)(\lambda - \alpha_2) \ldots (\lambda - \alpha_n), \tag{9'}$$

$$g(\lambda) = 1 - \Sigma A_i/(\lambda - \alpha_i). \tag{10'}$$

It is now possible to show that the equation $g = 0$ has n roots, $\lambda_1, \lambda_2, \ldots, \lambda_n$ satisfying

$$\alpha_1 < \lambda_1 < \alpha_2 < \lambda_2 < \ldots < \alpha_n < \lambda_n. \tag{11'}$$

These must be roots also of $\Delta = 0$, and since this has the obvious root $\lambda_0 = 0$ in addition, we have accounted for all $n + 1$ roots of $\Delta = 0$. To verify (11') we have only to observe that $g(\lambda)$ is everywhere monotonically increasing in λ except at the points $\lambda = \alpha_j$ where it becomes infinite, and that

$$g(0) > 0, \quad g(+\infty) = 1.$$

Thus we have accounted for $n + 1$ linearly independent solutions of the form (6') for the differential equations, and since these are of order $n + 1$ every solution is a linear combination of these.

We proceed now to construct the particular solution in which we are interested. This can be written in the form

$$a_0 = \beta_{00} + \Sigma\beta_{0i}\, e^{-\lambda_i t}, \tag{12'}$$

$$a_j = \beta_{j0} + \Sigma\beta_{ji}\, e^{-\lambda_i t},$$

where we have adjoined a second subscript to each β to designate the particular λ with which it is associated. The initial conditions give us

$$\beta_{00} + \Sigma\beta_{0i} = 1, \tag{13'}$$

$$\beta_{j0} + \Sigma\beta_{ji} = 0.$$

Also, for each i, the β_{ji} must satisfy the homogeneous equations of determinant $\Delta(\lambda_i)$. These equations are seen to be equivalent to

$$\beta_{oo} = \beta_{jo} = x_o, \tag{14'}$$

$$\beta_{oi} = (\alpha_j - \lambda_i)\,\beta_{ji}/\alpha_j = x_i,$$

where the x_o and the x_i are hereby defined. These will turn out to be the coefficients in $a_o(t)$, which is the only part of the solution required. To evaluate the x's, we express the β's in terms of them by (14') and substitute into (13'). After trivial algebraic simplification the result is

$$x_o + \Sigma x_i = 1, \tag{15'}$$

$$x_o/\alpha_j + \sum_i x_i/(\alpha_j - \lambda_i) = 0.$$

These equations in the x's can be solved formally by an artifice as follows: Consider the fractions $\varphi(\lambda)/\Delta(\lambda)$ whose numerator is a polynomial of degree n and whose denominator is a polynomial of degree n + 1. The zeros of the denominator are, as we know, $\lambda_o = 0$ and the λ_i. Hence the fraction can be resolved into partial fractions in the form

$$\varphi(\lambda)/\Delta(\lambda) \equiv C_o/\lambda + \Sigma C_i/(\lambda - \lambda_i), \tag{16'}$$

where the C's are constant. By virtue of equation (8') this can also be written

$$[\lambda g(\lambda)]^{-1} \equiv C_o/\lambda + \Sigma C_i/(\lambda - \lambda_i). \tag{17'}$$

These are algebraic identities and must be satisfied for any value λ may take on. In particular if we set $\lambda = \alpha_j$, then since $\varphi(\alpha_i) = 0$, we have

$$0 = C_o/\alpha_j + \Sigma C_i/(\alpha_j - \lambda_i).$$

These equations for j = 1, 2, ..., n, are the same as the last n of the equations (15') with the C's replacing the x's. Also (17') can be written

$$[g(\lambda)]^{-1} \equiv C_o + \lambda\Sigma C_i/(\lambda - \lambda_i),$$

and as $\lambda \longrightarrow \infty$ we have the limit

$$1 = C_o + \Sigma C_i$$

which is the same as the first equation (15') with the C's replacing the x's.

We may summarize as follows: *The specific activity of the outer compartment will be given at time* t *by*

$$a_o(t) = x_o + \Sigma x_i \, e^{-\lambda_i t}, \tag{18'}$$

where the λ_i *satisfy the equation* $g(\lambda) = 0$ *and the* x's *are defined by the algebraic identity*

$$[\lambda g(\lambda)]^{-1} \equiv x_o/\lambda + \Sigma x_i/(\lambda - \lambda_i). \tag{19'}$$

The function $g(\lambda)$ *is defined by* (10').

We have carried on our discussion as though the relative exchange rates α_i and A_i were known in advance. Ordinarily this will not be so, but we shall have, instead, an empirical function $a_o(t)$, or values of it obtained by measuring the specific activity at various times t. We may then seek to fit these empirical points by a sum of exponentials of the form (18'), thereby obtaining estimates of the x's and the λ's. By means of these we can construct the sum (19'), deriving thereby the function $g(\lambda)$ (after collecting terms and inverting). When $g(\lambda)$ is separated into partial fractions according to (10'), we recover the A's and the α's.

Two or more identical compartments. - We remarked above that it is no restriction to suppose the α's all distinct. Suppose, on the contrary, we had, for some i and j,

$$\alpha_i = \alpha_j = \alpha.$$

If we set

$$b = a_i - a_j,$$

then the two equations in a_i and a_j give

$$db/dt = -\alpha b,$$

$$b = b_o \, e^{-\alpha t}.$$

But since $b(o) = 0$, $b_o = 0$ and hence $b(t) \equiv 0$. Consequently $a_i(t) = a_j(t)$ and

so far as $a_0(t)$ is concerned the system behaves as though only one were present but with an A corresponding whose value is $A_i + A_j$.

If the number of compartments is relatively small, or if the empirical curve $a_0(t)$ can be represented satisfactorily in the form (18') as the sum of a small number of exponentials, the foregoing analysis is sufficient. Even though there may be many compartments, an expression of the form (18') is possible if they fall into a few classes, each class consisting of compartments having the same α. In other circumstances, however, the α's of the separate compartments may have some statistical distribution, which is either known in advance, perhaps from theoretical considerations, or which we wish to infer from the empirical $a_0(t)$. We turn now to a consideration of this circumstance.

Continuous distribution of exchange rates. – We wish to express our relations in terms of integrals that can be generalized to a continuous distribution. Referring, as a guide, to our previous results, we define a step function $A(\alpha)$ as follows:

$$\begin{aligned} A(\alpha) &= 0 && \text{for } \alpha < \alpha_1 \\ &= A_1 && \text{for } \alpha_1 \leq \alpha < \alpha_2, \\ &= A_1 + A_2 && \text{for } \alpha_2 \leq \alpha < \alpha_3, \\ &\cdots\cdots\cdots\cdots \\ &= A_1 + A_2 + \ldots + A_n && \text{for } \alpha_n \leq \alpha. \end{aligned} \tag{20'}$$

We may then define our function $g(\lambda)$ by means of the Stieltjes integral (13) of the function $A(\alpha)$:

$$g(\lambda) = 1 - \int_0^\infty (\lambda - \alpha)^{-1} \, dA(\alpha). \tag{21'}$$

In like fashion we may define a step function $X(u)$:

$$\begin{aligned} X(u) &= 0 && \text{for } u < \lambda_1, \\ &= x_1 && \text{for } \lambda_1 \leq u < \lambda_2, \\ &= x_1 + x_2 && \text{for } \lambda_2 \leq u < \lambda_3, \\ &\cdots\cdots\cdots\cdots \\ &= x_1 + x_2 + \ldots + x_n && \text{for } \lambda_n \leq u. \end{aligned} \tag{22'}$$

Hence we have

$$[\lambda g(\lambda)]^{-1} = x_0/\lambda + \int_0^\infty (\lambda - u)^{-1}\, dX(u), \tag{23'}$$

and

$$a_0(t) = x_0 + \int_0^\infty e^{-ut}\, dX(u). \tag{24'}$$

Now for the finite case the equations (21'), (23') and (24') are equivalent, respectively, to the equations (10'), (19'), and (18'), when $A(\alpha)$ and $X(u)$ are defined by (20') and (22'). But the equations are now in a form to permit of generalization to the infinite case, and we may say that if the exchange rates are distributed in a manner represented by the function $A(\alpha)$, then the time course of the decline in radioactivity $a_0(t)$ is given by (24'), where $X(u)$ satisfies the Stieltjes integral equation (23'). And conversely, if the radioactivity $a_0(t)$ can be represented in the form (24') by means of a suitable function $X(u)$, then the distribution of the exchange rates is represented by the function $A(\alpha)$ satisfying the Stieltjes integral equation (21'), where $g(\lambda)$ is defined by the equation (23'). The finding of a function $A(\alpha)$ necessitates the determination of a suitable analytic representation of a function $X(u)$ that can satisfy (24') with reasonable approximation. From (24') (13) it follows that for $\lambda < 0$

$$\int_0^\infty e^{\lambda t}\, [a_0(t) - x_0]\, dt = -\int_0^\infty (\lambda - u)^{-1}\, dX(u)$$

$$= [x_0 - 1/g(\lambda)]\lambda^{-1}.$$

Consequently for a given $a_0(t)$ we may define $g(\lambda)$ by

$$1/g(\lambda) = x_0 - \lambda \int_0^\infty e^{\lambda t}\, [a_0(t) - x_0]\, dt, \tag{25'}$$

the integration being performed with λ negative. Hence if $a_0(t) - x_0$ is given analytically, $g(\lambda)$ is obtainable from its ordinary Laplace transform. Conversely, if $A(\alpha)$ or $B(\alpha)$ is given analytically, perhaps from theoretical considerations, then $a_0(t)$ is obtained from a Laplace inverse transformation.

In practice, neither the determination of $X(u)$ from (23') given $A(\alpha)$, nor the inverse determination of $A(\alpha)$ from (21') given $X(u)$, is apt to be simple,

and closed expressions, in terms of elementary functions, can be expected in very exceptional cases at best. In thinking of $A(\alpha)$ as a function which represents the distribution of exchange rates, it is natural to think of $X(u)$ as a function which represents the distribution of the "decay constants" λ. This suggests seeking relations between the moments of the two distributions.

The moments of the distribution functions. – For this purpose it is convenient to consider, in place of $A(\alpha)$, the related function $B(\alpha)$ defined by

$$d\,A(\alpha) = \alpha\, d\,B(\alpha), \tag{26'}$$

with, of course,

$$B(0) = 0.$$

This function is the true distribution function for the α's. In place of (21') we write

$$g(\lambda) = 1 - \int_0^\infty \alpha(\lambda - \alpha)^{-1}\, d\,B(\alpha). \tag{27'}$$

We define the moments B_r of the function $B(\alpha)$ by

$$B_r = \int_0^\infty \alpha^r\, d\,B(\alpha) \quad (r = 0, 1, 2, \ldots). \tag{28'}$$

Then $g(\lambda)$ can be expanded formally in powers of λ^{-1}:

$$g(\lambda) = 1 - B_1/\lambda^2 - B_2/\lambda^2 - \ldots - \tag{29'}$$

We note also from (27') and (28')

$$g(0) = 1 + B_0 = x_0^{-1}. \tag{30'}$$

In like manner if we define the moments

$$X_r = \int_0^\infty u^r\, d\,X(u), \tag{31'}$$

then we have the formal expansion of the integral

$$\int_0^\infty (\lambda - u)^{-1}\, d\,X(u) = \lambda^{-1}\,[x_0 + x_1/\lambda + x_2/\lambda_2 + \ldots]. \tag{32'}$$

Hence (23') can be written formally

$$\begin{aligned} 1/g(\lambda) &= x_0 + X_0 + X_1/\lambda + X_2/\lambda^2 + \ldots \\ &= 1 + X_1/\lambda + X_2/\lambda^2 + \ldots, \end{aligned} \tag{33'}$$

since

$$x_0 + X_0 = a_0(0) = 1$$

If we multiply together equations (29') and (33'), we obtain after a minor reduction

$$0 = (X_1 - B_1)/\lambda + (X_2 - B_1 X_1 - B_2)/\lambda^2 + \dots ,$$

and since the right member must vanish identically in λ we obtain the recursion relations

$$\begin{aligned} X_1 &= B_1, \\ X_2 &= B_1 X_1 + B_2, \\ &\dots\dots\dots\dots \\ X_r &= B_1 X_{r-1} + B_2 X_{r-2} + \dots + B_r. \end{aligned} \tag{34'}$$

Often the moments about the mean are more convenient. Let x and b represent the means of the two distributions X and B. Then

$$X_1 = X_0 x, \quad B_1 = B_0 b.$$

Likewise let ν_r and b_r designate the statistical moments about the mean. These are related to the moments X_r and B_r, respectively, by the relations

$$X_r = X_0 \left[\nu_r + \binom{r}{1} x \nu_{r-1} + \dots + \binom{r}{2} x^{r-2} \nu_2 + x^r\right], \tag{35'}$$

and

$$B_r = B_0 \left[b_r + \binom{r}{1} b\, b_{r-1} + \dots + \binom{r}{2} b^{r-2} b_2 + b^r\right], \tag{36'}$$

since $x_1 = b_1 = 0$. These can be substituted into (34') for obtaining a series of relations between the means and the moments about the mean. Recalling that

$$X_0 = 1 - x_0 = B_0/(1 + B_0),$$

we write down only the following:

$$\begin{aligned} b &= x_0 x \\ b_2 &= x_0 \nu_2, \\ b_3 &= x_0 \nu_3 + B_0 b\, b_2. \end{aligned} \tag{37'}$$

Initial behavior of $a_o(t)$. – Equation (24') is equivalent to

$$[a_o(t) - x_o]\, e^{xt} = \int_0^\infty e^{-(u-x)t}\, d\, X(u), \tag{38'}$$

where x is any constant. If we expand the exponential under the integral sign in powers of t and then integrate, the coefficients of the powers of t are the moments of the function X(u) about the point x. In particular if we take x to be the mean of the distribution X(u) as in equation (35') we have

$$[a_o(t) - x_o]\, e^{xt} = X_o\, [1 + \frac{1}{2}\nu_2\, t^2 - \frac{1}{6}\nu_3\, t^3 + \ldots],$$

where the ν's are the ordinary statistical moments about the mean. Hence

$$[a_o(t) - x_o] / X_o = e^{-xt}\, [1 + \frac{1}{2}\nu_2\, t^2 - \frac{1}{6}\nu_3\, t^3 + \ldots],$$

so that on a semilog plot the initial slope is entirely determined by the mean x of the X distribution. If we write

$$\log\, [a_o(t) - x_o] = \log X_o - xt + c(t), \tag{39'}$$

then

$$e^{c(t)} = 1 + \frac{1}{2}\nu_2\, t^2 - \frac{1}{6}\nu_3\, t^3 + \ldots$$

and we find by successive differentiations that

$$\begin{aligned} c(0) &= c'(0) = 0, \\ c''(0) &= \nu_2, \\ c'''(0) &= -\nu_3, \\ c^{iv}(0) &= \nu_4 - 3\nu_2^2. \end{aligned} \tag{40'}$$

By expanding around the point t = 0

$$c(t) = \frac{\nu_2 t^2}{2} - \frac{\nu_3 t^3}{6} + \frac{(\nu_4 - 3\nu_2^2)t^4}{24} + \ldots \tag{41'}$$

$e^{c(t)}$ represents the multiplicative correction for deviation of $a_o(t) - x_o$ from exponential behavior, and c(t) the additive correction for its semilog plot.

In terms of the moments of the B function

$$c(t) = \frac{b_2 t^2}{2\, x_o} - [b_3 - x\, b_2\, (1 - x_o)]\, \frac{t^3}{6\, x_o} + \ldots \tag{42'}$$

The function c(t) determines the departure of the semilogarithmic plot from linearity. Initially the deviation is controlled almost entirely by the second moment and is such that the curve is parabolic and convex to the axis of abscissas. As time progresses the higher moments become increasingly effective. These are determined by the more detailed aspects of the distribution such as skew, excess, etc. The convergence of c(t) depends to a considerable extent on the magnitude of the higher moments. Even in the case of a Gaussian distribution these may become quite large. Thus the approximation is usually only good for small t.

The electrical analogy. - Figure 11 shows an electrical analogy to the system in figure 9. The differential equations are

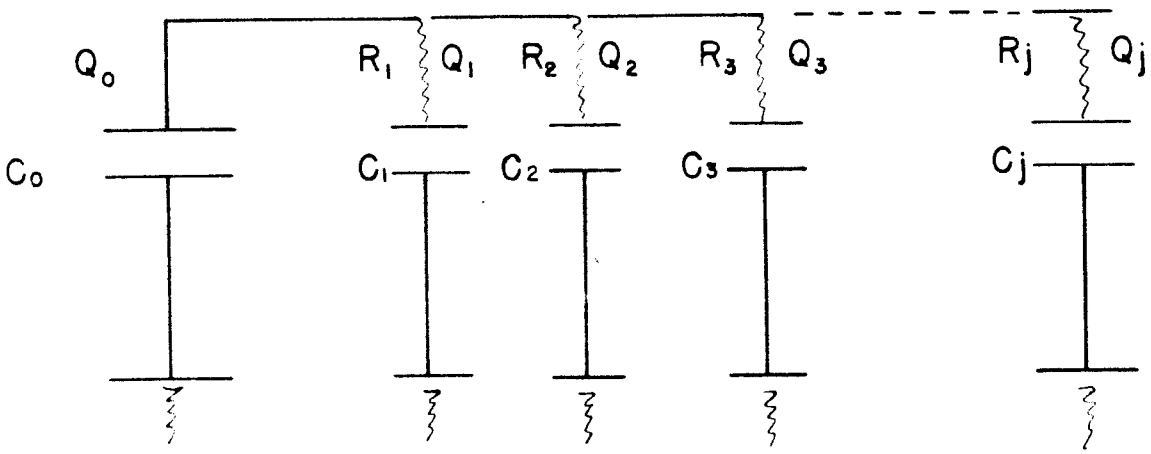
$$d\,Q_o/dt = \Sigma \frac{1}{R_k} \left(\frac{Q_k}{C_k} - \frac{Q_o}{C_o}\right)$$

$$d\,Q_j/dt = \frac{1}{R_j} \left(\frac{Q_o}{C_o} - \frac{Q_j}{C_j}\right)$$

These become identical with equations 2' and 3' if the radioactivity R_j is inserted in place of the charge Q_j on condenser j, the capacitance C_j replacing the S_j and the exchange rate ρ_j replacing the conductance $1/R_j$. Thus an electrical analog computer could be designed to reproduce the behavior of the biological system.

Figure 11

Electrical analogy to the system in Fig. 9. The capacitances C_j represent the amounts S_j, the charges on the condensers Q_j, the radioactivities R_j and the conductances $1/R_j$, the exchange rates ρ_j.


Q0
R1
Q1
R2
Q2
R3
Q3
Rj
Qj
C0
C1
C2
C3
Cj