Determination of Kinetic Parameters in a Two-Pool System by Administration of One or More Tracers*

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Methods for the estimation of rates of production and of metabolism of a compound in blood from isotopic data obtained after the intravenous injection of a tracer of the compound have been examined mathematically. This analysis has been carried out assuming that the kinetics of the disappearance of the tracer can be rationalized by a two-pool model. When the two pools represent two peripherally interconvertible compounds, the rate constants of their interconversion and of removal of the compounds from the pools can be estimated by injecting tracers of both compounds and following the temporal change of the concentrations of the isotopes in only one of the pools. The special case in which one of the tracers can be labeled with an isotope which is eliminated during the conversion to the second compound has also been considered. In this case, the administration of such a tracer together with the other two permits a more accurate determination of the rate constants. Furthermore, it has been shown that equivalent information can be obtained either when the tracers are administered as a single injection or when they are infused at a constant rate.

When the concentration of an intravenously administered tracer of a compound is measured in samples of plasma, quantitative information concerning parameters of distribution and of metabolism of the compound can be obtained. In this paper, mathematical expressions which provide means by which such parameters may be estimated from experimental data are presented. Two methods of administration of the tracers are considered: In one the tracers are introduced by means of a rapid injection, and in the other the tracers are infused at a constant rate.

The production rate (*PR*) of a compound in *blood* has been defined (Gurpide *et al.*, 1963) and shown to be equivalent to the rate at which the compound enters into circulation for the first time. The production rate can be estimated from the area under the curve resulting from a plot of the *specific activity* of the compound in plasma versus time (Gurpide *et al.*, 1963; Tait, 1963). When a tracer dose of this same compound is infused intravenously at a constant rate, the production rate of the compound in *blood* is given by P/a_{eq}, where P is the infusion rate and a_{eq} is the specific activity of the compound "at equilibrium," that is, when the specific activity remains experimentally constant. The volume of plasma containing a weight of the compound numerically equal to its production rate is equal to that volume of plasma which is cleared of the compound per unit of time, and has been designated as the rate of metabolic clearance (*MCR*) of the compound in plasma (Berson and Yallow, 1957; Tait *et al.*, 1961; Tait, 1963). From this definition it follows that the *MCR* equals the production rate divided by the concentration of the compound in plasma. Therefore the *MCR* may be estimated from the area under the curve corresponding to the plot of the *concentration* of the tracer in plasma against time after injection. The *MCR* also equals the ratio of the rate of infusion and the concentration of the tracer in plasma "at equilibrium."

The calculation of the *PR* or the *MCR* of a compound in plasma does not require the formulation of models which supposedly represent the physiological situation. In other words, no consideration of other spaces of distribution of the compound or its reversible transformation to other products need be given. However, when rates or rate constants of metabolism are to be estimated, the existence of such different spaces and of related compounds must be taken into account.

If a pool is defined as a compound in a space, a system of compounds distributed in one or more spaces can be schematically represented by models of properly connected pools. It is on the basis of such models that particular kinetic parameters are calculated using data obtained from tracer experiments. In this paper the two-pool model, illustrated in Figure 1, is considered. In each of the cases discussed below a different experimental design is shown, together with the description of the information which can be obtained from the experimental data. The derivations of the expressions used to make these calculations are also given.[1]

The requisite experimental data are the concentrations of the isotope in samples from each pool. The parameters which are derivable from these data include volumes, metabolic clearance rates, and rate constants of metabolism. If, by other experimental means, the concentration of the unlabeled compounds in the pools can be measured, then the total amount of the compounds in the pools, production rates, and rates of metabolism can also be obtained. Thus, if the *specific activities* of the compounds rather than isotopic *concentrations* are measured, all the above-mentioned parameters can be evaluated.

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[1] The solutions in equations (4) and (5) take a different form if $\alpha = \beta$. In this case, equations (7) and (8) become $2\alpha = -(k_{AA} + k_{BB})$ and $\alpha^2 = k_{AA}k_{BB} - k_{AB}k_{BA}$. Thus, $(k_{AA} + k_{BB})^2 = 4\alpha^2 = 4(k_{AA}k_{BB} - k_{AB}k_{BA})$. Then $(k_{AA} - k_{BB})^2 = -4k_{AB}k_{BA}$. Since both $k_{AB} \geq 0$ and $k_{BA} \geq 0$, it follows that $k_{AA} = k_{BB}$ and $k_{AB}k_{BA} = 0$. Then α and β are equal only when the two-pool model possesses special characteristics, i.e., when the rate constants of total removal from each of the pools are equal and transfer between the two pools does not occur in both directions. This special case will not be considered further.

Case 1 describes an experimental situation in which a tracer is injected into one of the pools and samples of both pools are analyzed. The mathematical basis for such an analysis can be found in the literature (Ayers *et al.*, 1962); however, this analysis is repeated here because it offers the opportunity of introducing equations which will be used again in the treatment of the other cases. Furthermore, it shows how much information can be obtained from such single-tracer experiments. For example, if only one pool is sampled and if the tracer is injected into that pool (A), only the volume (V_A), the metabolic clearance rate (MCR_A) of that pool, $k_A + k_{AB}$ and $k_B + k_{BA}$ (see Fig. 1) may be estimated.

Case 1a describes a special situation in which all material leaving pool B is transferred to pool A. The mathematical analysis which applies to this case has been presented by Sapirstein *et al.* (1955) in connection with their studies on the volumes of distribution and clearances of creatinine. Tait *et al.* (1961) have presented a similar analysis in connection with their studies on the distribution and metabolism of aldosterone. When such a model is considered, k_A, k_{AB}, k_{BA}, V_A, and MCR_A can be calculated by following the decline of the concentration of the isotope in pool A with respect to time after the injection of a tracer into that pool A. Furthermore, if all the material entering pool B comes from pool A, V_B can also be estimated from the same experimental data.

Case 2 describes a situation in which two tracers bearing different isotopes are injected simultaneously one into each pool. The concentrations of the isotopes in samples from only one of the pools suffice to permit the calculation of the rate constants of transfer with the other pool in addition to those parameters obtained in case 1. Thus, the analysis of case 2 shows that the administration of two tracers provides considerably more information than the use of only one under the same experimental conditions of sampling and isolation procedures.

Case 3 illustrates how the injection into one of the pools of a third isotopically labeled compound, having the property that the isotope is irreversibly removed during the process of transfer to the other pool, serves to reduce the amount of experimental data required to obtain the same information made available by the experiment described under case 2. Thus it is necessary only to determine the concentrations of the three isotopes in samples of only one of the pools at times when the plots of the logarithm of these concentrations against time are straight lines. This feature may be of considerable practical importance since the experimental data can then be submitted to a least-squares analysis and more accurate results are then to be expected.

Case 4 differs from case 3 only in that the tracers are administered at a constant rate rather than as a single injection.

The accompanying paper (Sandberg *et al.*, 1964) shows the application of the experimental design described under case 3 to the study of the distribution and metabolism of dehydroisoandrosterone sulfate.

Models, Symbols, and Definitions

The open system of pools illustrated by the model in Figure 1 is considered to be in the steady state and the rates at which all processes occur are assumed to be constant. Thus the total amount of material in pool A (M_A) remains constant. Consequently, the sum of all the rates of entry into the pool (S_A from outside the system plus r_{BA} from pool B) must equal the sum of all

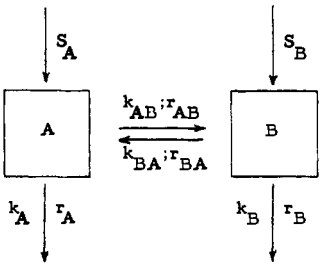

FIG. 1.—Two-pool (A and B) model. Rates and rate constants are denoted by the r and the k values, respectively. S_A and S_B are the rates of entry of material into the pools from outside the system.

the rates of removal from the pool (r_A directly out of the system plus r_{AB} to pool B). A similar material balance holds for pool B. In accord with the symbolism used in a previous paper (Gurpide *et al.*, 1963), the total rate of removal from pool A will be denoted by $-r_{AA}$ and from pool B by $-r_{BB}$. Then,

$$-r_{AA} = r_A + r_{AB} = S_A + r_{BA}$$
$$-r_{BB} = r_B + r_{BA} = S_B + r_{AB}$$

The corresponding rate constants are obtained by dividing each of the rates of removal of pool A by M_A and the rates of removal of B by M_B:

$$-k_{AA} = k_A + k_{AB}$$
$$-k_{BB} = k_B + k_{BA} \qquad (1)$$

The tracers are assumed to have negligible weight; the isotope in a tracer is considered to be stably bound and transferred from one pool to the other, unless otherwise indicated. Furthermore, the fates of labeled and unlabeled molecules are assumed to be identical. To simplify the notation, it will be assumed that the tracer introduced into pool A is labeled with ^3H and that introduced into pool B with ^{14}C. Mixing of labeled and unlabeled molecules within a pool is assumed to be instantaneous.

After the introduction of the tracer as a single dose ($R_A{}^3H$ or $R_B{}^{14}C$) the total amount of an isotope in a pool at a particular time will be designated by the letter y accompanied by a superscript indicating the isotope and a subscript indicating the pool. Thus, the following symbols will be used: $y_A{}^{14}C$, $y_A{}^3H$, $y_B{}^{14}C$, and $y_B{}^3H$.

Similarly, if the tracers are introduced into the pools by infusion at the constant rates, $P_A{}^3H$ and $P_B{}^{14}C$, the isotope content at a particular time of infusion will be represented by $x_A{}^{14}C$, $x_A{}^3H$, $x_B{}^{14}C$, and $x_B{}^3H$. The constant values that the x's acquire when the state of "equilibrium" with respect to the isotopes is reached after a sufficiently long infusion time will be indicated by the subscript "eq", e. g., $x^{14}C_{A,eq}$.

Case 1. Injection of One Tracer.—Consider that the tracer, labeled with tritium, is injected into pool A. The equations describing the change of the ^3H content in each of the pools with respect to time are:

$$\frac{dy_A{}^3H}{dt} = k_{AA}y_A{}^3H + k_{BA}y_B{}^3H \qquad (2)$$

$$\frac{dy_B{}^3H}{dt} = k_{BB}y_B{}^3H + k_{AB}y_A{}^3H \qquad (3)$$

At the time of the injection all the isotope is in pool A. Hence,

$$y_A{}^3H = R_A{}^3H, \qquad y_B{}^3H = 0$$

when $t = 0$. Then, the solutions of the preceding set of simultaneous differential equations have the following form (see, for instance, Morris and Brown, 1952):

$$y_A{}^3H = C_1e^{-\alpha t} + C_2e^{-\beta t} \qquad (4)$$

$$y_B{}^3H = C_3(e^{-\alpha t} - e^{-\beta t}) \quad (5)$$

where

$$C_1 + C_2 = R_A{}^3H \quad (6)$$

$$\alpha + \beta = -(k_{AA} + k_{BB}) \quad (7)$$

and

$$\alpha\beta = k_{AA}k_{BB} - k_{AB}k_{BA} \quad (8)^1$$

It follows from equations (7) and (8) that α and β are positive. In general the values of α and β are different and we will consider that α has the larger value.

Assume that the only experimental data available are the concentrations (dpm/ml) of the isotope present in a specific compound in the samples. A total volume, V, of the pool determined by a compound in the space from which the sample is taken can then be defined such that V multiplied by the corresponding isotope concentration is equal to the total isotope content in the pool. Therefore, V multiplied by the concentration of the compound in the sample (c) equals the total amount, M, of the compound in the pool. Then, equations (4) and (5) written in terms of isotope concentration are:

$$\frac{y_A{}^3H}{V_A} = \frac{C_1}{V_A}e^{-\alpha t} + \frac{C_2}{V_A}e^{-\beta t} \quad (9)$$

$$\frac{y_B{}^3H}{V_B} = \frac{C_3}{V_B}(e^{-\alpha t} - e^{-\beta t}) \quad (10)$$

The values of α, β, V_A, C_1, and C_2 can be determined by the usual procedures of curve analysis (Sapirstein *et al.*, 1955; Robertson, 1957; Solomon, 1960) from the plot of the logarithm of the concentration of the isotope in samples obtained only from pool A against time. From a similar plot of the concentrations of the isotope in pool B, the value C_3/V_B can be determined. Since these procedures will be referred to subsequently, a detailed discussion of them follows.

(A) CALCULATION OF β and C_2/V_A.—The curve resulting from the plot of the logarithm of the isotope concentration in pool A against time is asymptotic to a straight line (see Fig. 2), since with increasing time the influence of the term containing $e^{-\alpha t}$ in equation (9) upon the value of the function becomes negligible. Elimination of that term from equation (9) and conversion to the logarithmic form gives

$$\ln\frac{y_A{}^3H}{V_A} = -\beta t + \ln\frac{C_2}{V_A}$$

or using decimal logarithms

$$\log\frac{y_A{}^3H}{V_A} = -0.4343\beta t + \log\frac{C_2}{V_A} \quad (11)$$

The slope of the asymptotic part of this curve is then -0.4343β and the value of the intercept resulting from the extrapolation of the asymptote to zero time gives the value of C_2/V_A.

(B) CALCULATION OF α AND C_1/V_A.—Since C_2/V_A and β are now known, the difference $(y_A{}^3H/V_A) - (C_2/V_A)e^{-\beta t}$ which by equation (9) equals $(C_1/V_A)e^{-\alpha t}$ may be plotted against time using a semilogarithmic scale. Then C_1/V_A and α are obtained as in (A).

(C) CALCULATION OF V_A.—The V_A can be calculated from the values of C_1/V_A and C_2/V_A since by equation (6), $C_1/V_A + C_2/V_A = R_A{}^3H/V_A$.

(D) CALCULATION OF C_3/V_B.—The C_3/V_B may be estimated from equation (10) in the same manner as described in (a).

The value of the rate constant for total removal from pool A can be obtained from the experimentally determined values of α, β, C_1, and C_2 by comparing equation (2) and the derivative of equation (4) at time zero i.e.,

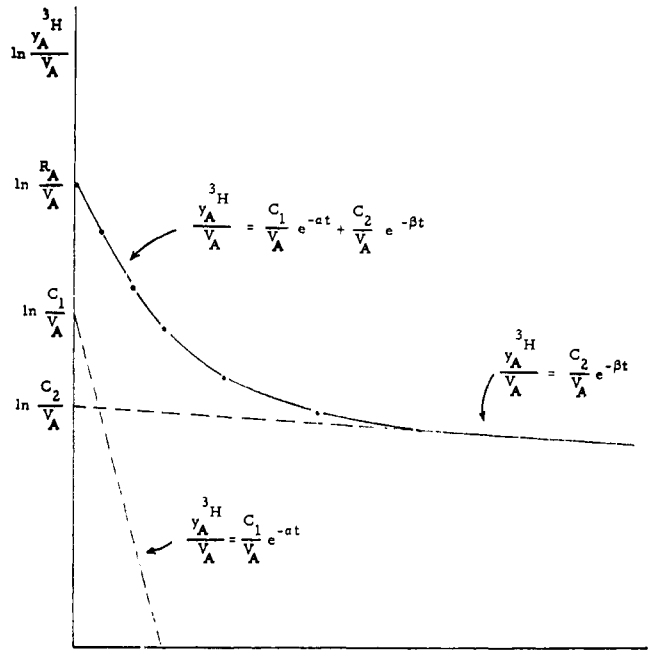

FIG. 2.—Resolution of the time curve of concentration in pool A of the isotope (^3H) introduced into that pool corresponding to the two-pool model shown in Fig. 1. The two components of the original curve are straight lines in a semilogarithmic plot (see text, case 1, for explanations).

$$k_{AA}R_A{}^3H = -\alpha C_1 - \beta C_2 \quad (12)$$

The rate constant of total removal from pool B can also be estimated since, from equation (7),

$$k_{BB} = -(\alpha + \beta + k_{AA}) \quad (13)$$

Equation (4) can be expressed in a more detailed form by substituting the coefficients C_1 and C_2 by the following expressions, obtained from equation (12) using the property shown in equation (6):

$$C_1 = -R_A{}^3H\left(\frac{k_{AA} + \beta}{\alpha - \beta}\right)$$

and

$$C_2 = R_A{}^3H\left(\frac{\alpha + k_{AA}}{\alpha - \beta}\right) \quad (14)$$

Then

$$y_A{}^3H = \frac{R_A{}^3H}{\alpha - \beta}[(\alpha + k_{AA})e^{-\beta t} - (\beta + k_{AA})e^{-\alpha t}] \quad (15)$$

By comparing equation (3) and the derivative of equation (5) at the time of injection ($t = 0$),

$$k_{AB}R_A{}^3H = C_3(\beta - \alpha)$$

or

$$C_3 = -R_A{}^3H\frac{k_{AB}}{\alpha - \beta}$$

Thus equation (5) becomes

$$y_B{}^3H = \frac{R_A{}^3H}{\alpha - \beta}k_{AB}(e^{-\beta t} - e^{-\alpha t}) \quad (16)$$

The value of t which makes the derivative with respect to time of equation (16),

$$\frac{dy_B{}^3H}{dt} = \frac{R_A{}^3H}{\alpha - \beta}k_{AB}(-\beta e^{-\beta t} + \alpha e^{-\alpha t}) \quad (17)$$

equal zero is the same value at which $y_B{}^3H$ is maximum and is given by

$$t_{max} = \frac{1}{\alpha - \beta}\ln\frac{\alpha}{\beta} \quad (18)$$

At the time of maximum the amount of isotope entering pool B equals the amount of isotope leaving the pool, as can be seen by setting equation (3) equal to zero. Then,

$$k_{AB}y_A{}^3H = -k_{BB}y_B{}^3H$$

or

$$r_{AB}a_A{}^3H = -r_{BB}a_B{}^3H$$

where $a_A{}^3H$ and $a_B{}^3H$ are the specific activities of the compound in pools A and B, respectively.

If $S_B = 0$, then $r_{AB} = -r_{BB}$ (i.e., $k_{AB}M_A = -k_{BB}M_B$) and at the time of the maximum $a_A{}^3H = a_B{}^3H$. Furthermore, by subtracting $y_A{}^3H/M_A$ from $y_B{}^3H/M_B$ it follows that

$$a_B{}^3H - a_A{}^3H = \frac{R_A{}^3H}{M_A(\alpha - \beta)}(\beta e^{-\beta t} - \alpha e^{-\alpha t})$$

when $S_B = 0$. Since $y_B{}^3H$ increases with time until the time of the maximum and decreases afterward, it follows from equation (17) that $(\beta e^{-\beta t} - \alpha e^{-\alpha t})$ is negative for $t < t_{max}$ and positive for $t > t_{max}$. Therefore, $a_B{}^3H < a_A{}^3H$ for $t < t_{max}$ and $a_B{}^3H > a_A{}^3H$ for $t > t_{max}$. If $S_B \neq 0$, then $-r_{BB} > r_{AB}$ and at the maximum $a_B{}^3H < a_A{}^3H$.

Case 1a. Particular Situation of Case 1 where $k_B = 0$.—If there is no metabolism via irreversible pathways from pool B, ($k_B = 0$), then from equation (1), $-k_{BB} = k_{bA}$; from equation (7), $\alpha + \beta = -k_{AA} + k_{BA}$ and, from equation (8), $\alpha\beta = k_A k_{BA}$. Therefore from equation (12),

$$k_{AA} = -\frac{\alpha C_1 + \beta C_2}{R_A{}^3H}$$

Then,

$$k_{BA} = \alpha + \beta + k_{AA} = \frac{1}{R_A{}^3H}[\alpha(R_A{}^3H - C_1) + \beta(R_A{}^3H - C_2)] = \frac{\alpha C_2 + \beta C_1}{R_A{}^3H}$$

$$k_A = \frac{\alpha\beta}{k_{BA}} = R_A{}^3H\frac{\alpha\beta}{\alpha C_2 + \beta C_1}$$

and

$$k_{AB} = -k_{AA} - k_A = \frac{C_1C_2(\alpha - \beta)^2}{R_A{}^3H(\alpha C_2 + \beta C_1)}$$

The volume of pool A, V_A, may be calculated as shown in case 1 and MCR_A can be estimated from $MCR_A = k_A V_A$. Furthermore, if $S_B = 0$, the volume of the other pool, V_B, can also be estimated since in that case $r_{AB} = r_{BA}$ and

$$k_{AB}c_A V_A = k_{BA}c_A V_B$$

or

$$V_B = \frac{k_{AB}}{k_{BA}}V_A$$

Case 2. Injection of Two Tracers, One in Each Pool.—The procedure outlined in case 1 yields only incomplete information about the system. Additional experimental data from which all the k values in Figure 1 can be calculated becomes available when a second tracer bearing a different isotope, e.g., ^{14}C, is injected into pool B. Moreover, these data can be obtained from the analysis of samples taken from only one of the pools. In addition to the k values, the volume of the sampled pool can be calculated. Consequently, the experimental demands involved in this design are hardly, if at all, greater than those described under case 1, while the information obtained about the system is notably more complete.

It is again assumed that ^3H is administered into pool A. Then k_{AA}, k_{BB}, V_A, α, and β may be determined from the analysis of the curve resulting from the plot of the logarithm of the concentration of ^3H in pool A against time, as shown in case 1. It will be shown presently that if ^{14}C is introduced into pool B, the $^3H/^{14}C$ ratios in each of the two pools will become experimentally constant and equal. This new datum yields sufficient information to permit the inclusion of k_{AB} and k_{BA} in the above list.

From the symmetry of the model it is easily seen that the ^{14}C content in pool A is a similar function of time as that of ^3H in pool B (equation 16). Thus,

$$y_A{}^{14}C = R_B{}^{14}C\frac{k_{BA}}{\alpha - \beta}(e^{-\beta t} - e^{-\alpha t}) \quad (19)$$

Similarly,

$$y_B{}^{14}C = \frac{R_B{}^{14}C}{\alpha - \beta}[(\alpha + k_{BB})e^{-\beta t} - (\beta + k_{BB})e^{-\alpha t}] \quad (20)$$

Since α is larger than β, then for sufficiently large values of t the term containing $e^{-\alpha t}$ in equations (15) and (19) becomes practically negligible and

$$\frac{y_A{}^3H}{y_A{}^{14}C} = \frac{R_A{}^3H}{R_B{}^{14}C}\cdot\frac{\alpha + k_{AA}}{k_{BA}} \quad (21)^2$$

or

$$k_{BA} = \frac{(^3H/^{14}C)_{injected}}{(^3H/^{14}C)_{constant\ in\ A}}(\alpha + k_{AA})$$

The value of k_{BA} obtained from equation (21) may now be used to evaluate k_{AB}, since from equation (8)

$$k_{AB} = \frac{k_{AA}k_{BB} - \alpha\beta}{k_{BA}} \quad (22)$$

It follows from equations (7) and (8) that

$$\frac{\alpha + k_{AA}}{k_{BA}} = \frac{k_{AB}}{\alpha + k_{BB}}$$

Therefore equation (21) may be written as

$$\frac{y_A{}^3H}{y_A{}^{14}C} = -\frac{R_A{}^3H}{R_B{}^{14}C}\cdot\frac{k_{AB}}{\beta + k_{AA}} \quad (23)^2$$

or

$$k_{AB} = -\frac{(^3H/^{14}C]_{constant\ in\ A}}{(^3H/^{14}C)_{injected}}(\beta + k_{AA})$$

From the values of k_{AB} and k_{BA}, $k_A = -k_{AA} - k_{AB}$ and $k_B = -k_{BB} - k_{BA}$ can be estimated.

The maximum concentration of ^{14}C in pool A is reached at that time when the value of the derivative of equation (19) is zero:

$$\frac{dy_A{}^{14}C}{dt} = R_B{}^{14}C\frac{k_{BA}}{\alpha - \beta}(-\beta e^{-\beta t} + \alpha e^{-\alpha t}) = 0 \quad (24)$$

This equation has the solution given by equation (18). Therefore ^{14}C in pool A and ^3H in pool B reach maximum values at the same time.

If pool B is also sampled, new data are obtained from which V_B can be calculated in the same manner as described for V_A in case 1.

It might be useful to point out that the $^3H/^{14}C$ ratio also becomes constant in pool B and is identical with the experimentally constant ratio of the isotopes in pool A.

[2] The absolute values of k_{AA} and k_{BB} are included in the interval between the value for α and that for β. This conclusion follows from equations (21) and (23) since the ratios of the concentrations of isotopes and α, β, k_{AB}, and k_{BA} have positive values and k_{AA} and k_{BB} are negative. Then $\alpha + k_{AA} > 0$, $\beta + k_{AA} < 0$, and from equation (7), $\alpha + k_{BB} < 0$ and $\beta + k_{BB} < 0$. Therefore, $\beta < |k_{AA}|$, $|k_{BB}| < \alpha$. If one of the rate constants of total removal is known, the limits between which the value of the other lies can be defined more precisely by the condition resulting from equation (8), $k_{AA}k_{BB} > \alpha\beta$.

This is seen by dividing equation (16) by equation (20). At values of t sufficiently large this ratio becomes

$$\frac{y_B{}^3H}{y_B{}^{14}C} = \frac{R_A{}^3H}{R_B{}^{14}C} \cdot \frac{k_{AB}}{\alpha + k_{BB}}$$

which is identical to equation (23) since $\beta + k_{AA} = -(\alpha + k_{BB})$ from equation (7).

Case 3. Injection of Three Tracers, One of Which is "Metabolically Labile."—In what follows proof will be given that the injection of a "metabolically labile" tracer alone into one of the pools provides sufficient information so that the rate constant of total removal and the volume of that pool can be calculated. Moreover, with this information, all four rate constants shown in Figure 1 can be calculated from only the asymptotes of the ^3H and ^{14}C curves described under case 2. By a "metabolically labile" tracer is meant a tracer labeled with an isotope which is irreversibly removed from the compound during the process of reversible transfer to the other pool. As an example, pool A could represent a sulfate ester of a steroid and pool B the unconjugated compound. If a ^{35}S-labeled tracer of pool A is administered, the isotope will be lost during the process of reversible conversion to the corresponding steroidal alcohol. The [^{35}S]sulfate ion, once cleaved from the steroid, is not reused in subsequent reconjugation. Then the kinetics of the disappearance of ^{35}S from the pool in such a system will be the same whether a single- or a two-pool model is considered. If the compound is labeled with another, stably bound isotope, e.g., with tritium, the rate of disappearance of this isotope from pool A will be slower than that of ^{35}S, assuming that the tritium re-enters pool A from pool B.

In an analogous fashion, if a steroid is reversibly converted to the corresponding ketone, a tritium atom attached to the carbon bearing the hydroxyl group will be lost during the process.

Consider that a tracer for pool A containing a ^{35}S label, which is metabolically labile with respect to its transfer to pool B, is administered. Hence

$$\frac{dy_A{}^{35}S}{dt} = k_{AA}y_A{}^{35}S$$

and since $y_A{}^{35}S = R_A{}^{35}S$ when $t = 0$, then

$$y_A{}^{35}S = R_A{}^{35}Se^{k_{AA}t}$$

or

$$\frac{y_A{}^{35}S}{V_A} = \frac{R_A{}^{35}S}{V_A} e^{k_{AA}t} \qquad (25)$$

and

$$\log \frac{y_A{}^{35}S}{V_A} = 0.4343k_{AA}t + \log \frac{R_A{}^{35}S}{V_A} \qquad (26)$$

Then the plot of the logarithm of the concentration of ^{35}S in pool A against time will result in a straight line from whose slope and intercept with the ordinate axis k_{AA} and V_A can be estimated.

In order to calculate rate constants in cases 1 and 2, the study of the "complete" curve of disappearance of ^3H from pool A is necessary; i.e., the concentrations of ^3H in samples of that pool must be measured immediately after injection of the tracers. However, if a metabolically labile tracer is also injected into pool A, examination of the early portions of the disappearance curves is unnecessary. Thus, as described by equation (11), the asymptotic part of the tritium curve in pool A gives the value of β and also C_2 since V_A is known. Since k_{AA} is also known from the ^{35}S data (equation 26), α can be calculated from equation (12) which is rewritten as

$$\alpha = \frac{\beta C_2 + k_{AA}R_A{}^3H}{C_2 - R_A{}^3H} \qquad (27)^3$$

Now that α, β, and k_{AA} are known the other k values are obtained as before using equations (7), (21), and (22) or (23).

Case 4. Infusion of the Tracers at a Constant Rate.—The isotope content of a pool during infusion of a tracer at a constant rate is related to the isotope content in the pool after the injection of the tracer as a single dose (Gurpide *et al.*, 1963). This relationship when applied to the ^{35}S concentration in pool A gives

$$\frac{x_A{}^{35}S}{V_A} = \frac{P_A{}^{35}S}{R_A{}^{35}S} \int_0^t \frac{y_A{}^{35}S}{V_A} dt \qquad (28)$$

where $x_A{}^{35}S/V_A$ is the ^{35}S concentration in pool A at time t after the start of the infusion at a rate $P_A{}^{35}S$ and $y_A{}^{35}S/V_A$ is the concentration of ^{35}S in pool A at time t after the administration of the single dose, $R_A{}^{35}S$. By replacing the value of $y_A{}^{35}S/V_A$ in equation (28) with that given in equation (25), the following equation is obtained:

$$\frac{x_A{}^{35}S}{V_A} = \frac{P_A{}^{35}S}{V_A} \int_0^t e^{k_{AA}t}\,dt = \frac{P_A{}^{35}S}{k_{AA}V_A}(e^{k_{AA}t} - 1)$$
$$= \frac{P_A{}^{35}S}{k_{AA}V_A} e^{k_{AA}t} + \frac{x_{A,eq}{}^{35}S}{V_A} \qquad (29)$$

The term $x_{A,eq}{}^{35}S/V_A = P_A{}^{35}S/(-k_{AA})V_A$ and is the constant value of the concentration of ^{35}S in pool A obtained at sufficiently long time of infusion. This constant value is called the concentration of ^{35}S "at equilibrium" in pool A.

From equation (29) it can be seen that the plot of the log of the differences of the concentrations of ^{35}S in the samples and the concentrations at equilibrium against time result in a straight line described by

$$\log\left[\frac{x_{A,eq}{}^{35}S}{V_A} - \frac{x_A{}^{35}S}{V_A}\right] = 0.4343k_{AA}t + \log \frac{P_A{}^{35}S}{(-k_{AA})V_A}$$

Then, from the slope and intercept at the ordinate axis, the values of k_{AA} and V_A can be obtained.

The previous equation shows that, from an experiment involving a single injection of ^{35}S as described under case 3, the same information can be obtained by infusing this tracer at a constant rate.

Assume, as in case 3, that a ^3H-labeled tracer is introduced into pool A. The relationship, similar to equation (28), between administration by single-dose injection and by constant infusion with respect to the ^3H isotope in pool A is given by

$$\frac{x_A{}^3H}{V_A} = \frac{P_A{}^3H}{R_A{}^3H} \int_0^t \frac{y_A{}^3H}{V_A} dt$$

From equation (15),

$$\frac{x_A{}^3H}{V_A} = \frac{P_A{}^3H}{V_A(\alpha - \beta)} \int_0^t [(\alpha + k_{AA})e^{-\beta t} - (\beta + k_{AA})e^{-\alpha t}]dt$$
$$= \frac{P_A{}^3H}{V_A(\alpha - \beta)}\left[\frac{\beta + k_{AA}}{\alpha} e^{-\alpha t} - \frac{\alpha + k_{AA}}{\beta} e^{-\beta t}\right]$$
$$+ \frac{x_{A,eq}{}^3H}{V_A} \qquad (30)^4$$

[3] The denominator in equation (27) may be close to zero and therefore it may be impossible to obtain an accurate value for α. In this situation other experimental data from pool A such as the time at which ^{14}C reaches its maximum concentration (equation 18) or the "complete" ^3H curve may be necessary to determine the value of α.

[4] If the amount of an isotope y in a pool after injection of a dose R of a tracer is given by $y = \sum_{i=1}^{n} A_i e^{-\lambda_i t}$, then the amount of the isotope in the pool during constant infusion at a rate P is

$$x = x_{eq} - \frac{P}{R} \sum_{i=1}^{n} \frac{A_i}{\lambda_i} e^{-\lambda_i t}$$

where x_{eq} is the equilibrium value.

where

$$\frac{x_{A,eq}{}^{3H}}{V_A} = -\frac{P_A{}^{3H}}{V_A(\alpha-\beta)} \times \left[\frac{\beta + k_{AA}}{\alpha} - \frac{\alpha + k_{AA}}{\beta}\right] = -\frac{P_A{}^{3H}k_{BB}}{V_A\alpha\beta} \quad (31)$$

Again, $x_{A,eq}{}^{3H}/V_A$ is the constant value of the concentration of ^3H in pool A obtained at infinite time of infusion (practically reached at a finite time).

When t is sufficiently large, the term $e^{-\alpha t}$ in equation (30) is negligible since α is larger than β. Hence for these values of t

$$\log\left(\frac{x_{A,eq}{}^{3H}}{V_A} - \frac{x_A{}^{3H}}{V_A}\right) = -0.4343\beta t + \log\frac{P_A{}^{3H}}{V_A\beta}\cdot\frac{\alpha + k_{AA}}{\alpha - \beta}$$

The value of β obtained from the slope of the line resulting from the plot of this function may be used in conjunction with the values of V_A and k_{AA} (estimated from the ^{35}S data) to evaluate α from the intercept of this line with the ordinate axis. The value of k_{BB} is given by equation (13).

In a similar manner, when a ^{14}C-labeled tracer is administered into pool B it can be shown that the equilibrium value of the concentration of ^{14}C in pool A is

$$\frac{x_{A,eq}{}^{14C}}{V_A} = \frac{P_B{}^{14C}k_{AB}}{V_A\alpha\beta} \quad (32)$$

which gives the value of k_{AB}. The value of k_{BA} is obtained from equation (8).

From equations (31) and (32) the ratio of the concentrations at equilibrium of ^3H and ^{14}C in pool A may be expressed as

$$\frac{x_{A,eq}{}^{3H}/V_A}{x_{A,eq}{}^{14C}/V_A} = \frac{P_A{}^{3H}}{P_B{}^{14C}}\left(-\frac{k_{BB}}{k_{AB}}\right)$$

Calculation of Production Rates

The production rate of a compound in a pool (PR) has been previously defined and shown to represent the rate of entry of material into the pool excluding the rates of re-entry resulting from reversible transfer to other pools (Gurpide *et al.*, 1963). The production rate is equal to P/a_{eq}, where P is the constant ratio of infusion of a tracer into the pool and a_{eq} is the specific activity at equilibrium of the compound in the pool. Thus for pool A,

$$PR_A = \frac{P_A{}^{3H}}{a_{A,eq}{}^{3H}} = \frac{P_A{}^{3H} \times M_A}{x_{A,eq}{}^{3H}}$$

and, from the relationship between single injection and constant infusion (Gurpide *et al.*, 1963) discussed earlier,

$$PR_A = \frac{P_A{}^{3H}}{\frac{P_A{}^{3H}}{R_A{}^{3H}}\int_0^\infty \frac{y_A{}^{3H}}{M_A}\,dt} = \frac{R_A{}^{3H}}{\int_0^\infty \frac{y_A{}^{3H}}{M_A}\,dt} \quad (33)$$

where

$$\int_0^\infty (y_A{}^{3H}/M_A)\,dt$$

is the area under the curve, after administration of the tracer as a single rapid injection, representing the decline with time of the *specific activity* with respect to ^3H in pool A. These equations are valid for all systems of pools.

In the two-pool model (see equation 15)

$$\int_0^\infty \frac{y_A{}^{3H}}{M_A}\,dt = \frac{R_A{}^{3H}}{M_A(\alpha-\beta)} \times \left(\frac{\alpha + k_{AA}}{\beta} - \frac{\beta + k_{AA}}{\alpha}\right) = -\frac{R_A{}^{3H}k_{BB}}{M_A\alpha\beta}$$

Then,

$$PR_A = \frac{M_A\alpha\beta}{-k_{BB}} = \frac{c_AV_A\alpha\beta}{-k_{BB}} \quad (34)$$

and, from equations (13) and (12),

$$PR_A = \frac{R_A{}^{3H}c_A\alpha\beta}{\frac{\alpha C_2}{V_A} + \frac{\beta C_1}{V_A}} \quad (35)$$

Calculation of Metabolic Clearance Rates

The value of this parameter equals the production rate of the compound in the pool divided by its concentration. Then, from equation (33),

$$MCR_A = \frac{PR_A}{c_A} = \frac{R_A{}^{3H}}{\int_0^\infty \frac{y_A{}^{3H}}{V_A}\,dt} \quad (36)$$

for any system of pools (Tait, 1963). It should be noted that $\int_0^\infty (y_A{}^{3H}/V_A)dt$ is the area under the curve produced by plotting the concentration of the isotope in the pool against the time after administration of the tracer as a single injection.

For the two-pool model, from equations (34) and (35),

$$MCR_A = \frac{V_A\alpha\beta}{-k_{BB}} = \frac{R_A{}^{3H}\alpha\beta}{\frac{\alpha C_2}{V_A} + \frac{\beta C_1}{V_A}}$$

In a still simpler case when the decline of the concentration of the isotope in the pool with time is given by a single exponential, i.e., $y/V = De^{-\lambda t}$, then

$$\int_0^\infty \frac{y}{V}\,dt = \frac{D}{\lambda}$$

and

$$MCR = \frac{R\lambda}{D} \quad (37)$$

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