INTERPRETATION OF TRACER DATA: SIGNIFICANCE OF THE NUMBER OF TERMS IN SPECIFIC ACTIVITY FUNCTIONS

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ABSTRACT It has already been shown that the number of pools in an open system in the steady state cannot be determined from the number of exponential terms in the specific activity function of a pool, even if the data were free from experimental error. However, some information is conveyed by the number of exponential terms. The information is different depending upon whether the data are obtained from the pool into which the tracer is introduced or from another pool. In the latter case, the number of exponential terms is shown to indicate the maximum number of intermediate pools involved in the shortest path of transfer of material from the injected pool to the pool in question. With regard to the former case, this paper is restricted to functions with two exponential terms and shows which systems of n pools ($n \geqq 2$) are consistent with such data. Consequently, biexponential experimental curves can be interpreted in terms of models consisting of an unrestricted number of pools in which each pool is defined in terms of fast mixing. The generalization to cases of functions with more than two exponential terms can be carried out in a similar manner.

INTRODUCTION

The number of terms in the specific activity function of a pool, following the introduction of an isotopically labeled tracer into a system of interconnected pools, can be smaller than the number of pools in the system. Sharney et al. (Sharney, Wasserman, and Gevirtz, 1964) have described n-pool systems which are compatible with specific activity functions consisting of the sum of only two terms. In a previous study[1] we have analyzed n-pool irreducible systems (i.e. all pools exchange material with each other) and have presented relationships among the rate constants of transfer between the pools which are necessary and sufficient for a reduction in the number of terms in *all* pools. It must be concluded, therefore, that the number of pools in the system cannot be determined by counting the terms in the specific activity functions of any number of pools, even if the functions are accurately known and were not affected by imprecision or inadequacy of the experimental data.

[1] Mann, J., and E. Gurpide. 1969. Submitted to *Bull. Math. Biophys.*

A common procedure in interpreting specific activity curves is to consider models consisting of as many pools as terms which are necessary to describe the experimental curve. However, for the reasons given above this approach seems arbitrary, particularly since the number of pools of distribution of a compound in a biological system can be expected to be large. Therefore, conclusions drawn from such simplified systems may be meaningless.

Theorem I, in this paper, gives a set of conditions among the rate constants of transfer between the pools which are *necessary* and sufficient for the specific activity function of the pool initially labeled to be the sum of two terms. The fact that the results were obtained considering n-pool systems and that the conditions are necessary as well as sufficient make this analysis of general applicability. By a similar treatment, results can be obtained for specific activity functions of more than two terms.

The number of terms in the specific activity functions of the pool into which the tracer is injected (pool 1) and of the other pools may be different. Theorem II describes the dependence of the number of terms in the specific activity function of a pool i ($i \neq 1$) upon the number of intermediate pools in the shortest pathway from pool 1 to pool i, regardless of the number of terms in the specific activity function of pool 1.

SYMBOLS AND DEFINITIONS

Throughout this paper, a pool will be defined as an entity determined by a compound (or ion) in a particular space, imposing the condition that the mixing of the compound in that space is instantaneous and homogeneous. Pool 1 will always refer to the pool into which the tracer is initially introduced. The n pools do not necessarily denote n different spaces of distribution of the tracer; they may also include other compounds which are interconverted with the substance under study.

The rate of change in isotope content in pool 1 (y_1 in cpm, corrected for isotopic decay, if necessary) results from the difference between the rate of entry of isotope into the pool and the rate of removal of isotope from the pool, i.e.,

$$\frac{dy_1}{dt} = \sum_{i=2}^{n} k_{i1}y_i - (-k_{11})y_1 = \sum_{i=1}^{n} k_{i1}y_i. \qquad (1)$$

The symbol k_{i1} denotes the rate constant of transfer of material from pool i to pool 1; the symbol $-k_{11}$ denotes the rate constant of total removal of material from pool 1. The minus sign in the latter symbol is introduced for convenience in writing the equations, as is evident from equation 1; it should be noted that $-k_{11}$ is positive and, therefore, k_{11} is negative.

It is desirable to write equation 1 in terms of specific activities (σ), which are more susceptible to experimental determination than total isotope content of a pool. Let h_{ij} be defined by the relationship

$$h_{ij}M_j = r_{ij} = k_{ij}M_i \tag{2}$$

where r_{ij} is the rate of direct transfer of material from i to j and M_i denotes the amount of material (moles) in pool i. If both sides of equation 1 are divided by M_1, and if k_{i1} is replaced by $h_{i1}M_1/M_i$, then, since $\sigma_i = y_i/M_i$, it follows that

$$\sigma'_1 = \sum_{i=1}^{n} h_{i1}\sigma_i \tag{3}$$

where σ'_1 denotes the quantity $d\sigma_1/dt$.

Equations similar to equations 1 and 3 can be written for each of the pools in the system. Thus,

$$\sigma'_p = \sum_{i=1}^{n} h_{ip}\sigma_i, \; p = 1, 2 \cdots n. \tag{4}$$

THEOREM I

A necessary and sufficient condition for the specific activity function of pool 1 to have two terms is that there exist a number A such that

$$A \sum_{i=2}^{n} h_{i1}H_{1i}^{(q)} = \sum_{i=2}^{n}\sum_{j=2}^{n} h_{ij}h_{j1}H_{1i}^{(q)} \quad \text{for} \quad 1 \leqq q \leqq n - 1 \tag{5}$$

where n is the number of pools in the system and where

$$H_{1i}^{(q)} = \sum_{p_1=1}^{n} \cdots \sum_{p_{q-1}=1}^{n} h_{1p_1}h_{p_1p_2} \cdots h_{p_{q-1}i} \quad \text{and} \quad H_{1i}^{(1)} = h_{1i}. \tag{6}$$

$H_{1i}^{(q)}$ includes the sum of all the products of the h's involved in each of the possible pathways of transfer from pool 1 to pool i through the intermediacy of $q - 1$ pools.

The proof of this theorem follows from a sequence of lemmas. Let $h_{II\,1}$ equal the sum of the h's corresponding to the transfer of material to pool 1 from all other pools in the system, i.e.,

$$h_{II\,1} = h_{21} + h_{31} + \cdots + h_{n1}. \tag{7}$$

Also, let σ_{II} be a weighted average of the specific activities of all pools other than 1, as seen from pool 1, i. e.,

$$\sigma_{II} = \frac{1}{h_{II\,1}} (h_{21}\sigma_2 + h_{31}\sigma_3 + \cdots + h_{n1}\sigma_n). \tag{8}$$

Lemma 1

A necessary and sufficient condition that σ_{I} have two terms is that there exist constants $h_{\text{I II}}$ and $h_{\text{II II}}$ such that

$$\sigma'_{\text{I}} = h_{11}\sigma_{\text{I}} + h_{\text{II I}}\sigma_{\text{II}}$$
$$\sigma'_{\text{II}} = h_{\text{I II}}\sigma_{\text{I}} + h_{\text{II II}}\sigma_{\text{II}} . \qquad (9)$$

Proof of Sufficiency. If $h_{\text{II I}}$, $h_{\text{I II}}$, and $h_{\text{II II}}$ are constants, then by solving equation 9 by the standard methods (Rainville, 1964) it is seen that σ_{I} (and σ_{II}) has two terms. In fact, if α_1 and α_2 are the negatives of the roots of the equation

$$\begin{vmatrix} (h_{11} - \lambda) & h_{\text{II I}} \\ h_{\text{I II}} & (h_{\text{II II}} - \lambda \end{vmatrix} = 0$$

then

$$\sigma_{\text{I}} = D_{11}e^{-\alpha_1 t} + D_{12}e^{-\alpha_2 t}$$
$$\sigma_{\text{II}} = D_{21}e^{-\alpha_1 t} + D_{22}e^{-\alpha_2 t} \qquad (10)$$

if $\alpha_1 \neq \alpha_2$ and

$$\sigma_{\text{I}} = D_{11}e^{-\alpha t} + D_{12}te^{-\alpha t}$$
$$\sigma_{\text{II}} = D_{21}e^{-\alpha t} + D_{22}te^{-\alpha t} \qquad (11)$$

if $\alpha_1 = \alpha_2 = \alpha$.

The value of the coefficients in equations 10 and 11 are not necessarily the same.

Proof of Necessity. Since by equation 3, $\sigma'_1 = h_{11}\sigma_1 + h_{21}\sigma_2 + \cdots + h_{n1}\sigma_n$, it follows from the definition of $h_{\text{II I}}$ and σ_{II} in equations 7 and 8 that

$$\sigma'_{\text{I}} = h_{11}\sigma_{\text{I}} + h_{\text{II I}}\sigma_{\text{II}} .$$

Hence, the first equation of 9 is satisfied. In order to show that the second equation of 9 also holds, the proof will now be divided into two cases: when $\alpha_1 \neq \alpha_2$ and when $\alpha_1 = \alpha_2$.

Case 1. If σ_{I} has two terms and $\alpha_1 \neq \alpha_2$, then σ_{I} is given by equation 10 and

$$\sigma'_{\text{I}} = -\alpha_1 D_{11}e^{-\alpha_1 t} - \alpha_2 D_{12}e^{-\alpha_2 t}. \qquad (12)$$

By equation 9, $\sigma_{\text{II}} = (\sigma'_{\text{I}} - h_{11}\sigma_{\text{I}})/h_{\text{II I}}$, and therefore by equations 10 and 12

$$\sigma_{\text{II}} = -\frac{1}{h_{\text{II I}}}[D_{11}(\alpha_1 + h_{11})e^{-\alpha_1 t} + D_{12}(\alpha_2 + h_{11})e^{-\alpha_2 t}]. \qquad (13)$$

It shall now be shown that if the expressions for σ_{I} in equation 10 and σ_{II} in equation 13 are substituted in the second equation of 9, constant values for $h_{I\,II}$ and $h_{II\,II}$ can be obtained. Thus,

$$\frac{1}{h_{II\,I}}[D_{11}\alpha_1(\alpha_1 + h_{11})e^{-\alpha_1 t} + D_{12}\alpha_2(\alpha_2 + h_{11})e^{-\alpha_2 t}] = D_{11}h_{I\,II}\,e^{-\alpha_1 t}$$

$$+ D_{12}h_{I\,II}e^{-\alpha_2 t} - \frac{h_{II\,II}}{h_{II\,I}}[D_{11}(\alpha_1 + h_{11})e^{-\alpha_1 t} + D_{12}(\alpha_2 + h_{11})e^{-\alpha_2 t}]. \quad (14)$$

Equation 14 will be satisfied if

$$D_{11}h_{I\,II} - \frac{D_{11}(\alpha_1 + h_{11})}{h_{II\,I}}h_{II\,II} = \frac{D_{11}\alpha_1(\alpha_1 + h_{11})}{h_{II\,I}}$$

and

$$D_{12}h_{I\,II} - \frac{D_{12}(\alpha_2 + h_{11})}{h_{II\,I}}h_{II\,II} = \frac{D_{12}\alpha_2(\alpha_2 + h_{11})}{h_{II\,I}}. \quad (15)$$

Neither D_{11} nor D_{12} is zero since it is assumed that σ_1 has two terms in equation 10. Constant values for $h_{I\,II}$ and $h_{II\,II}$ may be determined from equation 15 by Cramer's rule if the determinant, Δ, of the coefficients is different from zero. But,

$$\Delta = \begin{vmatrix} D_{11} & -D_{11}\dfrac{(\alpha_1 + h_{11})}{h_{II\,I}} \\ D_{12} & -D_{12}\dfrac{(\alpha_2 + h_{11})}{h_{II\,I}} \end{vmatrix} = \frac{D_{11}D_{12}}{h_{II\,I}}(\alpha_1 - \alpha_2) \neq 0$$

since $\alpha_1 \neq \alpha_2$ and $D_{11} \neq 0$, $D_{12} \neq 0$. Thus, the proof for case 1 is complete.

Case 2. If σ_1 has two terms and $\alpha_1 = \alpha_2 = \alpha$, then σ_1 is given by equation 11 and

$$\sigma'_1 = (-D_{11}\alpha + D_{12})e^{-\alpha t} - D_{12}\alpha te^{-\alpha t}. \quad (16)$$

Again, by equation 9, $\sigma_{II} = (\sigma'_1 - h_{11}\sigma_1)/h_{II\,I}$, so that by equations 11 and 16,

$$\sigma_{II} = -\frac{1}{h_{II\,I}}[D_{11}(\alpha + h_{11}) - D_{12} + D_{12}(\alpha + h_{11})t]e^{-\alpha t}. \quad (17)$$

As before, by substituting equations 11 and 17 in the second equation of 9, it follows that

$$-\frac{1}{h_{II\,I}}[D_{12}(\alpha + h_{11}) - D_{11}\alpha(\alpha + h_{11}) + D_{12}\alpha - D_{12}\alpha(\alpha + h_{11})t]e^{-\alpha t}$$

$$= D_{11}h_{I\,II}\,e^{-\alpha t} + D_{12}h_{I\,II}\,te^{-\alpha t} - \frac{h_{II\,II}}{h_{II\,I}}[D_{11}(\alpha + h_{11})$$

$$- D_{12} + D_{12}(\alpha + h_{11})t]e^{-\alpha t}. \quad (18)$$

Equation 18 will be satisfied if

$$D_{11}h_{1\,\mathrm{II}} - \frac{D_{11}(\alpha + h_{11}) - D_{12}}{h_{\mathrm{II}\,1}} h_{\mathrm{II}\,\mathrm{II}}$$

$$= \frac{1}{h_{\mathrm{II}\,1}} [D_{12}(\alpha + h_{11}) - D_{11}\alpha(\alpha + h_{11}) + D_{12}\alpha] \quad (19)$$

and $$D_{12}h_{1\,\mathrm{II}} - \frac{D_{12}(\alpha + h_{11})}{h_{\mathrm{II}\,1}} h_{\mathrm{II}\,\mathrm{II}} = D_{12}\frac{\alpha(\alpha + h_{11})}{h_{\mathrm{II}\,1}}.$$

Since the determinant of the coefficients in equation 19

$$\begin{vmatrix} D_{11} & -\dfrac{D_{11}(\alpha_1 + h_{11}) - D_{12}}{h_{\mathrm{II}\,1}} \\ D_{12} & -\dfrac{D_{12}(\alpha_1 + h_{11})}{h_{\mathrm{II}\,1}} \end{vmatrix} = -\frac{D_{12}^2}{h_{\mathrm{II}\,1}} \neq 0$$

values for $h_{\mathrm{II}\,1}$ and $h_{\mathrm{II}\,\mathrm{II}}$ can be obtained from equation 19. Therefore, the proof of Lemma 1 is complete.

It should be noted that, since $e^{-\alpha_1 t}$ and $e^{-\alpha_2 t}$ in equation 14 are linearly independent, the only constant values of $h_{1\,\mathrm{II}}$ and $h_{\mathrm{II}\,\mathrm{II}}$ which satisfy equation 14 are those given by equation 15. Similarly, the only constant values of $h_{1\,\mathrm{II}}$ and $h_{\mathrm{II}\,\mathrm{II}}$ which satisfy equation 18 are those given by equation 19 since $e^{-\alpha t}$ and $te^{-\alpha t}$ are linearly independent. Furthermore, both in cases 1 and 2,

$$h_{1\,\mathrm{II}} = -\frac{(\alpha_1 + h_{11})(\alpha_2 + h_{11})}{h_{\mathrm{II}\,1}} \quad (20)$$

and

$$h_{\mathrm{II}\,\mathrm{II}} = -(\alpha_1 + \alpha_2 + h_{11}). \quad (21)$$

In particular, if $\alpha_1 = \alpha_2 = \alpha$ these last two equations become

$$h_{1\,\mathrm{II}} = -\frac{(\alpha + h_{11})^2}{h_{\mathrm{II}\,1}} \quad (22)$$

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[2] In particular, if the specific activities of all the exchanging peripheral pools are proportional at all times ($\sigma_i = C_{i2}\sigma_2$, $i \neq 1$), the constant

$$A = \frac{\sum_{i=2}^{n}\sum_{j=2}^{n} C_{i2}h_{ij}h_{j1}}{\sum_{i=2}^{n} C_{i2}h_{i1}}$$

will obviously satisfy equation 24. In a previous study[1] it was shown that, in this case, all the σ_i have two exponential terms.

$$h_{\mathrm{II\,II}} = -(2\alpha + h_{11}). \tag{23}$$

The constant $h_{\mathrm{II}\,1}$ is not zero by definition, since otherwise no tracer would re-enter pool 1 and σ_1 would not be given by equation 10. Hence, since $h_{1\,\mathrm{II}}$ cannot be negative, it must equal zero because of equation 22. Then $\alpha + h_{11} = 0$ and, by equation 23, $h_{\mathrm{II\,II}} = -\alpha$.

Lemma 2

A necessary and sufficient condition that there exist constants $h_{1\,\mathrm{II}}$ and $h_{\mathrm{II\,II}}$ so that equation 9 holds is that there exist a constant A so that

$$A \sum_{i=2}^{n} h_{i1}\sigma_i = \sum_{i=2}^{n} \sum_{j=2}^{n} h_{ij}h_{j1}\sigma_i \tag{24}$$

for all values of t.

Proof of Necessity. By differentiating equation 8 with respect to t and replacing each σ_i in the resulting expression by equation 4, it follows that

$$\sigma'_{\mathrm{II}} = \frac{1}{h_{\mathrm{II}\,1}} [(h_{12}h_{21} + \cdots + h_{1n}h_{n1})\sigma_1 + \cdots + h_{n2}h_{21} + \cdots + h_{nn}h_{n1})\sigma_n]. \tag{25}$$

The right hand sides of the second equation in 9 and 25 are, therefore, equal and since at $t = 0$, $\sigma_1 \neq 0$, $\sigma_i = 0$ for $i \neq 1$ and $\sigma_{\mathrm{II}} = 0$, it follows that

$$h_{1\,\mathrm{II}} = \frac{h_{12}h_{21} + \cdots + h_{1n}h_{n1}}{h_{\mathrm{II}\,1}}. \tag{26}$$

Consequently,

$$h_{\mathrm{II\,II}}\sigma_{\mathrm{II}} = \frac{1}{h_{\mathrm{II}\,1}} [(h_{22}h_{21} + \cdots + h_{2n}h_{n1})\sigma_2 + \cdots + (h_{n2}h_{21} + \cdots + h_{nn}h_{n1})\sigma_n].$$

But this is equation 24 with $A = h_{\mathrm{II\,II}}$, since by equation 8 $h_{\mathrm{II}\,1}\sigma_{\mathrm{II}} = \sum_{i=2}^{n} h_{i1}\sigma_i$.

Proof of Sufficiency. It must be shown that constants $h_{1\,\mathrm{II}}$ and $h_{\mathrm{II\,II}}$ exist so that equation 9 holds. Let $h_{1\,\mathrm{II}}$ be defined as in equation 26 and let $h_{\mathrm{II\,II}} = A$. Then, if $h_{1\,\mathrm{II}}h_{\mathrm{II}\,1}\sigma_1$ is added to both sides of equation 24, it results

$$h_{1\,\mathrm{II}}h_{\mathrm{II}\,1}\sigma_1 + h_{\mathrm{II\,II}}h_{\mathrm{II}\,1}\sigma_{\mathrm{II}} = h_{1\,\mathrm{II}}h_{\mathrm{II}\,1}\sigma_1 + \sum_{i=2}^{n} \sum_{j=2}^{n} h_{ij}h_{j1}\sigma_i. \tag{27}$$

By equations 26 and 25, the right hand side of 27 equals $h_{\mathrm{II}\,1}\sigma'_{\mathrm{II}}$. Thus, $\sigma'_{\mathrm{II}} = h_{1\,\mathrm{II}}\sigma_1 + h_{\mathrm{II\,II}}\sigma_{\mathrm{II}}$ and the proof of Lemma 2 is complete.

Lemma 3

There will exist a constant, A, so that equation 24 holds if, and only if,

$$A\sum_{i=2}^{n} h_{i1}(\sigma_i^{(q)})_o = \sum_{i=2}^{n}\sum_{j=2}^{n} h_{ij}h_{j1}(\sigma_i^{(q)})_o \tag{28}$$

with the same A, for $q = 1, 2 \cdots (n-1)$.

Proof. Since equation 28 is the q^{th} derivative of equation 24 evaluated at $t = 0$, equation 28 holds whenever equation 24 does. A proof of the converse now follows.

Each σ_i is a linear combination of the same n linearly independent functions; these functions are of the form $e^{-\alpha_i t}$ or $t^k e^{-\alpha_i t}$, where k is a positive integer, depending upon whether some of the α's are repeated and the multiplicity of the repetition. If these functions are called $f_1(t), \cdots, f_n(t)$, then

$$\sigma_i = \sum_{j=1}^{n} D_{ij}f_j(t). \tag{29}$$

If each σ_i in equation 24 is replaced by equation 29, and if like terms are grouped together, then equation 24 may be written as

$$C_1f_1(t) + C_2f_2(t) + \cdots + C_nf_n(t) = 0 \tag{30}$$

where the C's are constants.

Lemma 3 states that if equation 30 holds for the q^{th} derivative at $t = 0$ with $1 \leqq q \leqq n-1$, then it holds for all t. Then, by hypothesis,

$$\begin{aligned} C_1f_1(0) + \cdots + C_nf_n(0) &= 0 \\ C_1f'_1(0) + \cdots + C_nf'_n(0) &= 0 \\ &\vdots \\ C_1f_1^{(n-1)}(0) + \cdots + C_nf_n^{(n-1)}(0) &= 0. \end{aligned} \tag{31}$$

The first equation in 31 follows from the fact that at $t = 0$ each $\sigma_i = 0$ for $i \geqq 2$ so that equation 24 certainly holds for $t = 0$.

The determinant of the coefficients in equation 31 is the Wronskian of the functions $f_1, \cdots f_n$ evaluated at $t = 0$. It does not vanish because the functions are linearly independent (Rainville, 1964). Since the right hand side of each equation in 31 is zero, each $C_i = 0$. Thus equation 30 holds for all t and, therefore, so does equation 24.

Lemma 4

Equations 5 and 28 are equivalent.

Proof. By changing indexes in equation 4, one gets

$$\sigma'_i = \sum_{p=1}^{n} h_{pi}\sigma_p , \qquad i = 1, 2 \cdots , n \tag{32}$$

and

$$(\sigma'_i)_o = h_{1i}(\sigma_1)_o$$

since, at $t = 0$, all tracer is in pool 1. Differentiation of both sides of equation 32 yields (after a change of indexes)

$$\sigma''_i = \sum_{p_1=1}^{n} h_{p_1 i}\sigma'_{p_1}$$

or by replacing σ'_{p_1} by its value given by equation 32

$$\sigma''_i = \sum_{p_1=1}^{n} \sum_{p=1}^{n} h_{p_1 i} h_{p p_1} \sigma_p .$$

Thus,

$$(\sigma''_i)_o = \sum_{p_1=1}^{n} h_{1p_1} h_{p_1 i}(\sigma_1)_o .$$

By induction it follows that

$$(\sigma_i^{(q)})_o = \sum_{p_1=1}^{n} \cdots \sum_{p_{q-1}=1}^{n} h_{1p_1} h_{p_1 p_2} \cdots h_{p_{q-2} p_{q-1}} h_{p_{q-1} i}(\sigma_1)_o . \tag{33}$$

Consequently,

$$(\sigma_i^{(q)})_o = H_{1i}^{(q)}(\sigma_1)_o , \qquad q = 1, 2 \cdots n - 1. \tag{34}$$

This completes the proof of Lemma 4. The proof of Theorem I results directly from Lemmas 1 through 4.

COMMENTS ON THEOREM I

The constants $h_{\mathrm{II}\,\mathrm{I}}$, $h_{\mathrm{I}\,\mathrm{II}}$ and $h_{\mathrm{II}\,\mathrm{II}}$ in equation 9 (the equations which, according to Lemma 1, hold if σ_1 has two terms) have the same properties as the constants h_{21}, h_{12}, and h_{22} in a simple 2-pool model. That $h_{\mathrm{II}\,\mathrm{II}}$ is negative, as is h_{22}, is seen as follows. Since $h_{\mathrm{I}\,\mathrm{II}}h_{\mathrm{II}\,\mathrm{I}}$ is nonnegative by virtue of equation 26, the factors $(\alpha_1 + h_{11})$ and $(\alpha_2 + h_{11})$ in equation 20 must have opposite signs. If, for instance,

$(\alpha_1 + h_{11})$ is positive, then $(\alpha_2 + \alpha_1 + h_{11})$ is positive since $\alpha_2 > 0$. Therefore, by equation 21, $h_{\text{II II}}$ is negative.

If the conditions of Theorem I are satisfied, all the pools in the system other than pool 1 behave as a single pool as far as pool 1 is concerned. If $h_{\text{II 1}}$, $h_{\text{1 II}}$, $h_{\text{II II}}$ and σ_{II} were replaced by h_{21}, h_{12}, h_{22}, and σ_2, respectively, equation 9 would describe a simple 2-pool system. Hence, even though these $(n - 1)$ pools do not constitute a pool in the sense of implying fast mixing, they behave kinetically as a single pool with respect to pool 1. However, it is possible that neither pool 1 nor any other pool will have two terms in its specific activity function if the tracer is administered into a different pool.

Since, as is indicated above, the differential equations which apply to a 2-pool system are formally identical with 9 the same formulae derived from 2-pool models (Tait et al., 1961; Gurpide et al., 1964) which allow the calculation of h_{11} and h_{22} from experimentally obtained values of D_{11}, D_{12}, α_1, and α_2, can be used to calculate h_{11} and $h_{\text{II II}}$. It should be noted, however, that the physiological meaning assigned to h_{22} could be quite different from the interpretation of the parameter $h_{\text{II II}}$. Furthermore, formulae for the estimation of h_{12} and h_{21} from experimental data, which may be justified by special restrictions in the 2-pool model, cannot be applied to estimate $h_{\text{1 II}}$ and $h_{\text{II 1}}$. An expression for $h_{\text{II II}}$, in terms of the rate constants of transfer among the pools, is obtained from equations 5 and 6 when $q = 1$. Thus, since $A = h_{\text{II II}}$,

$$h_{\text{II II}} = \frac{\sum_{i=2}^{n} \sum_{j=2}^{n} h_{1i} h_{ij} h_{j1}}{\sum_{i=2}^{n} h_{1i} h_{i1}} . \qquad (35)$$

It can be noted that if $n = 2$, $h_{\text{II II}} = h_{22}$.

All expressions involving h's can be transformed into expressions involving the usual rate constants, k, by applying the relationships shown in equation 2. For instance, equations 5 and 6 are equivalent to

$$A \sum_{i=2}^{n} k_{i1} K_{1i}^{(q)} = \sum_{i=2}^{n} \sum_{j=2}^{n} k_{ij} k_{j1} K_{1i}^{(q)}, \; q = 1, 2 \cdots, (n - 1) \qquad (36)$$

where

$$K_{1i}^{(q)} = \sum_{p_1=1}^{n} \cdots \sum_{p_{q-1}=1}^{n} k_{1p_1} k_{p_1 p_2} \cdots k_{p_{q-1} i}$$

and

$$K_{1i}^{(1)} = k_{1i} .$$

Theorem I makes explicit the properties of the system which determine that the specific activity function of pool 1 have two terms. A similar analysis can be extended to specific activity functions of pool 1 having m terms, $m > 2$. However, a different analysis would be necessary to study the implications of finding m terms in the specific activity function of a pool other than 1. Theorem II shows a relationship between the number of terms (m) in pool i ($i \neq 1$) and the number of intermediate pools in the shortest path of transfer of material from pool 1 to pool i. A "shortest path" of transfer between two pools is a route which involves the minimum number of intermediate pools, regardless of the amounts of material which is transferred by each of the possible paths.

THEOREM II

If the specific activity function σ_i of pool i ($i \neq 1$) has m terms, $m > 1$, there cannot be more than $m - 2$ intermediate pools in the shortest pathway from pool 1 to pool i.

Proof. If s is the number of intermediate pools in the shortest path of transfer from 1 to i, each of equations 33 with $q - 1 < s$ must be zero. This is evident since each term of equation 33 represents a pathway from 1 to i consisting of, at most, $q - 1$ intermediate pools; since such pathways do not exist for $q - 1 < s$, at least one of the h's in each term must be zero. Thus, $(\sigma_i^{(q)})_o = 0$ for $q - 1 < s$.

The theorem is proved by contradiction. Suppose there are more than $m - 2$ intermediate pools in the shortest path from 1 to i, i.e., $s > m - 2$. Therefore, $(\sigma_i^{(q)})_o = 0$, at least for $0 \leqq q - 1 \leqq m - 2$, or equivalently, for $1 \leqq q \leqq m - 1$. Furthermore, since $(\sigma_i)_o = 0$ for $i \neq 1$, it follows that

$$(\sigma_i^{(q)})_o = 0 \quad \text{for} \quad 0 \leqq q \leqq m - 1. \tag{37}$$

By hypothesis σ_i has m terms, i.e.

$$\sigma_i = C_1 f_1 + C_2 f_2 + \cdots + C_m f_m \tag{38}$$

where the f's are functions of t as in the proof of Lemma 3. Equation 38 with the conditions in equation 37 yields

$$\begin{aligned} C_1 f_1(0) + \cdots + C_m f_m(0) &= 0 \\ C_1 f'_1(0) + \cdots + C_m f'_m(0) &= 0 \\ &\vdots \\ C_1 f_1^{(m-1)}(0) + \cdots + C_m f_m^{(m-1)}(0) &= 0. \end{aligned} \tag{39}$$

Again, as in the proof of Lemma 3, the determinant of the coefficients of equation

39 is unequal to zero. Consequently, $C_1 = C_2 = \cdots = C_m = 0$. This contradicts the assumption that σ_i has m nonvanishing terms.

Another enunciation of Theorem II is that if there are $m - 2$ intermediate pools in the shortest pathway from 1 to i, then σ_i must have at least m terms.

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