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APPENDIX

Determination of the Kinetic Constants of Two Consecutive First-Order Reactions

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The efflux of [^{14}C]valine from the chopped brain tissue was first considered as a sequence of two consecutive irreversible first-order processes such that any label lost from the intracellular compartment, A , would have to enter the extracellular compartment, B , before entering the bulk phase, C . This can be represented thus:

with kinetic constants k_1 and k_2 respectively. Therefore:

$$dA/dt = -k_1A \quad (1)$$

$$dB/dt = k_1A - k_2B \quad (2)$$

$$dC/dt = k_2B \quad (3)$$

Eqn. (1) is simply solved:

$$A = A_0 e^{-k_1 t} \quad (4)$$

and eqn. (2) becomes:

$$dB/dt = k_1 A_0 e^{-k_1 t} - k_2 B \quad (5)$$

The solution to this equation was first obtained by Esson (1866):

$$B = B_0 e^{-k_2 t} + \left(\frac{A_0}{1 - k_2/k_1} \right) (e^{-k_2 t} - e^{-k_1 t}) \quad (6)$$

[Note: the changes taking place in A and B may be represented in a relative manner as their 'reduced concentrations' A/A_0 and B/B_0 , which, from eqns. (4) and (6), are:

$$A/A_0 = e^{-k_1 t}$$

$$B/B_0 = e^{-k_2 t} + \left(\frac{A_0/B_0}{1 - k_2/k_1} \right) (e^{-k_2 t} - e^{-k_1 t})]$$

Since at any time:

$$A + B + C = A_0 + B_0 + C_0$$

combining eqns. (3), (4) and (6) gives:

$$C = C_0 + A_0(1 - e^{-k_1 t}) + B_0 \left[(1 - e^{-k_2 t}) - \left(\frac{A_0/B_0}{1 - k_2/k_1} \right) (e^{-k_2 t} - e^{-k_1 t}) \right] \quad (7)$$

(see Benson, 1960). Since $C_0 = 0$ and $A_0 + B_0 = 1$, then:

$$C = \left[A_0 - A_0 e^{-k_1 t} + B_0 - B_0 e^{-k_2 t} - \left(\frac{A_0 k_1}{k_1 - k_2} \right) e^{-k_2 t} + \left(\frac{A_0 k_1}{k_1 - k_2} \right) e^{-k_1 t} \right] \quad (8)$$

$$= \left[1 + \left(\frac{A_0 k_2}{k_1 - k_2} \right) e^{-k_1 t} - \left(\frac{k_1 - B_0 k_2}{k_1 - k_2} \right) e^{-k_2 t} \right] \quad (9)$$

Let $\phi = C - (A_0 + B_0) = C - 1$, $\theta = k_1 t$ and $\rho = k_1/k_2$, then eqn. (9) reduces to:

$$\phi(\rho - 1) + (\rho - B_0)e^{-\theta/\rho} - A_0 e^{-\theta} = 0 \quad (10)$$

This is a transcendental equation that may be solved graphically for θ given any particular value of ρ (Stephenson, 1961, p. 347).

If θ is taken as an approximate value then the corrected value, θ_1 , is given by:

$$\theta = \theta_1 + h.$$

Using Newton's method of successive approximations

$$h = -\frac{f(\theta)}{f'(\theta)}$$

where $f'(\theta)$ is the differential coefficient of $f(\theta)$. Thus from eqn. (10):

$$h = \frac{\phi(\rho - 1) + (\rho - B_0)e^{-\theta/\rho} - A_0 e^{-\theta}}{(1 - B_0/\rho)e^{-\theta/\rho} - A_0 e^{-\theta}} \quad (11)$$

and repeated application of this method to an approximate value of θ gives θ accurately. Adopting the approach of Swain (1944) a table of values of θ that fitted eqn. (10) for a series of values of ρ , when the reaction had proceeded to 15, 35, 50 and 70%, was calculated. The solution for eqn. (10) and then for eqn. (11) was done by a computer programmed to carry out ten Newtonian approximations in the determination of any value of θ .

For each percentage reaction a plot of ρ (i.e. k_1/k_2) against $\log \theta$ (i.e. $\log k_1 t$) was made on semilog paper. Two such plots for two different percentage reactions correspond to two simultaneous equations for k_1/k_2 in terms of k_1 . Hence with 15 and 35% reaction, for example:

$$\log [\theta]_{15\%} - \log [\theta]_{35\%} = \log [k_1]_{15\%} t_{15\%} - \log [k_1]_{35\%} t_{35\%}$$

then if $[k_1]_{15\%} = [k_1]_{35\%}$ the displacement on the abscissa for the two plots is $\log(t_{15\%}/t_{35\%})$ ($t_{15\%}$ represents the time taken for 15% of the reaction and $t_{35\%}$ that taken for 35% of the reaction). Superimposition of the one graph on the second graph with the abscissa scales displaced by $\log(t_{15\%}/t_{35\%})$ gives a point of intersection that solves eqn. (10) for k_1/k_2 and $k_1 t$ and hence k_1 and k_2 .

Application of this analysis to the experimentally determined efflux curves showed that when 15, 35

and 50% of the efflux had occurred the values of k_1 and k_2 were nearly constant, but this was not the case when 70% of the efflux had been completed.

In eqn. (10) the values of A_0 and B_0 were experimentally determined from the concentration ratio $[3.78 \pm 0.12 (27)]$ after 15 min of incubation with $[^{14}\text{C}]$ valine and the inulin space of the chopped tissue after the same incubation time $[62.4 \pm 1.9\% (12)]$. On correcting the concentration ratio for the weight of the inulin space, which is not taken into account in the experimentally determined concentration ratio, the 'true' concentration ratio (see the Methods section) becomes 10.05. Thus the amount of $[^{14}\text{C}]$ valine in the intracellular water at zero time, A_0 , was 85.8% of the total labelled valine in the tissue, and the quantity of $[^{14}\text{C}]$ valine in the extracellular water at zero time, B_0 , was 14.2% of the total labelled valine in the tissue.

It was possible to obtain a reasonable fit for the experimental curves taking values for A_0 of 70% and for B_0 of 30%; however, to obtain such values the intracellular water must represent only 6% of the total weight of the tissue.

The following calculation was used to obtain a value for the amount of label (C) appearing in the bulk phase with time when the uptake of label from the extracellular compartment into the intracellular compartment was considered. Such a system is described in general terms by the equation:

for which can be derived:

$$dA/dt = -k_{12}A + k_{21}B \quad (12)$$

$$dB/dt = k_{12}A - (k_{21} + k_{23})B \quad (13)$$

$$dC/dt = k_{23}B \quad (14)$$

If one expresses eqns. (12), (13) and (14) as series and assumes for any intermediate (X) the particular solution,

$$X = M_i e^{-\lambda t} \quad (15)$$

then eqns. (12), (13) and (14) reduce to:

$$-\lambda M_1 + k_{12}M_1 - k_{21}M_2 = 0 \quad (16)$$

$$-\lambda M_2 - k_{12}M_1 + (k_{21} + k_{23})M_2 = 0 \quad (17)$$

$$-\lambda M_3 - k_{23}M_2 = 0 \quad (18)$$

By using the notation of Frost & Pearson (1961) eqns. (16), (17) and (18) may be represented in the general form

$$(K_{ij} - \delta_{ij}\lambda)M_j = 0 \quad (19)$$

(where $K_{ij} = -k_{ji}$, when $i \neq j$ but $K_{ij} = \sum k_{ir}$, when $i = j$; $\delta_{ij} = 1$ when $i = j$, but $\delta_{ij} = 0$, when $i \neq j$).

Assuming $M \neq 0$, this equation has a solution only if

$$(K_{ij} - \delta_{ij}\lambda) = 0 \quad (20)$$

This is the secular determinant (Lowry & John, 1910):

$$\begin{vmatrix} (k_{12} - \lambda) & -k_{21} & 0 \\ -k_{12} & (k_{21} + k_{23} - \lambda) & 0 \\ 0 & -k_{23} & -\lambda \end{vmatrix} = 0 \quad (21)$$

On expansion this gives the polynomial

$$-\lambda^3 + \lambda^2(k_{12} + k_{21} + k_{23}) - k_{12}k_{23} = 0$$

for which there are three solutions (see Frost & Pearson, 1961):

$$\lambda_1 = 0; \lambda_2 = \frac{1}{2}(p+q); \quad \lambda_3 = \frac{1}{2}(p-q) \quad (22)$$

Therefore,

$$(M)^{-1}(X_0) = (Q) \quad (29)$$

Combining eqns. (29) and (26) we obtain:

$$(X) = (M)(E)(M)^{-1}(X_0) \quad (30)$$

(see Lewis & Johnson, 1960), where

$$(X) = \begin{pmatrix} A \\ B \\ C \end{pmatrix}; \quad (X_0) = \begin{pmatrix} A_0 \\ B_0 \\ C_0 \end{pmatrix};$$

$$(M) = \begin{pmatrix} 0 & M_{12} & M_{13} \\ 0 & M_{22} & M_{23} \\ 1 & 1 & 1 \end{pmatrix};$$

$$(E) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & e^{-\lambda_2 t} & 0 \\ 0 & 0 & e^{-\lambda_3 t} \end{pmatrix}$$

and $(Q) = \begin{pmatrix} Q_1^0 \\ Q_2^0 \\ Q_3^0 \end{pmatrix}$. The explicit solutions for eqn. (30)

were obtained and can be written thus:

$$A = \frac{1}{B_{12}B_{23} - B_{13}B_{22}} [A_0(B_{12}B_{23}e^{-\lambda_2 t} - B_{13}B_{22}e^{-\lambda_3 t}) + B_0B_{12}B_{13}(e^{-\lambda_3 t} - e^{-\lambda_2 t})] \quad (31)$$

$$B = \frac{1}{B_{12}B_{23} - B_{13}B_{22}} [A_0B_{22}B_{23}(e^{-\lambda_2 t} - e^{-\lambda_3 t}) + B_0(B_{23}B_{12}e^{-\lambda_3 t} - B_{12}B_{22}e^{-\lambda_2 t})] \quad (32)$$

$$C = \frac{1}{B_{12}B_{23} - B_{13}B_{22}} \{A_0[B_{22}(1 - e^{-\lambda_3 t}) + B_{23}(e^{-\lambda_2 t} - 1)] + B_0[B_{13}(1 - e^{-\lambda_2 t}) + B_{12}(e^{-\lambda_3 t} - 1)]\} \quad (33)$$

[where $p = (k_{12} + k_{21} + k_{23})$ and $q = (p^2 - 4k_{12}k_{23})^{1/2}$].

The M constants can now be solved in the homogeneous eqns. (16), (17) and (18). Thus if $M_{3r} = 1$, then

$$M_{2r} = -\lambda_r/k_{23} \quad (23)$$

$$M_{1r} = -k_{21}\lambda_r/k_{23}(k_{12} - \lambda_r) \quad (24)$$

The general solution for a particular intermediate is obtained as a linear combination of particular solutions:

$$X = \sum_{r=1}^m M_{ir}Q_r^0 e^{-\lambda_r t} \quad (25)$$

Eqn. (25) may be expressed in terms of matrices (see Stephenson, 1961, p. 297)

$$(X) = (M)(E)(Q) \quad (26)$$

which at time $t = 0$ reduces to:

$$(X_0) = (M)(Q) \quad (27)$$

or

$$(M)^{-1}(X_0) = (M)^{-1}(M)(Q) \quad (28)$$

(cf. Lewis & Johnson, 1960). Eqn. (33) was solved (see Fig. 6a) by using the following values from Table 2: $k_{12} = 0.526$; $k_{21} = 6.3$; $k_{23} = 2.4$.

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