

APPENDIX

Calculation of Reaction Rates in Steady Systems

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Elaboration of problem 1. This problem represents the simplest case and its nature is fully stated in the preceding paper to which reference should be made. In the previous treatment v is calculated from the initial radioactivity (x_0) of a and measurements of the radioactivity x of a at the time t . Alternatively v can be calculated from determinations of the radioactivity y of b . This may serve as a check in some cases. In others it may be a matter of convenience when the isolation and determination of the radioactivity of a offers greater difficulties than the determination of the radioactivity of b .

Three cases may be distinguished.

(1) One measurement of y is used covering the time between t_0 and t :

$$v = \frac{ab}{t(a+b)} \ln \frac{bx_0}{bx_0 - y(a+b)}.$$

(2) Two measurements of y are used (y_1, y_2 , at t_1, t_2). In this case the changes of y occurring between t_1 and t_2 are relevant. This may be advantageous if, owing to a lag period, the starting point of the reaction is not clearly defined or if there is a time lag in the establishment of the steady state:

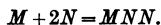
$$v = \frac{ab}{(t_2 - t_1)(a+b)} \ln \frac{bx_0 - y_1(a+b)}{bx_0 - y_2(a+b)}.$$

(3) No value for x_0 is required but three measurements of y are used at t_1, t_2, t_3 , the time intervals between t_1, t_2 and t_3 being equal:

$$v = \frac{ab}{m(a+b)} \ln \frac{y_2 - y_1}{y_3 - y_2},$$

where $m = t_2 - t_1 = t_3 - t_2$. For accurate determinations of v , y should not be near its steady state value.

Problem 2.

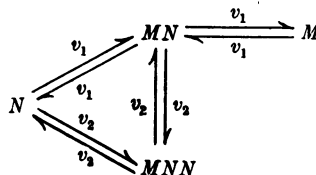


This involves (at least) two separate stages the nature of which may not be known for certain, but three different possibilities are discussed:

- (1) (a) $M + N \rightleftharpoons MN$,
 (b) $MN + N \rightleftharpoons MNN$.
- (2) (a) $2[MN + N \rightleftharpoons MNN]$,
 (b) $M + MNN \rightleftharpoons 2MN$.
- (3) (a) $2[M + N \rightleftharpoons MN]$
 (b) $2MN \rightleftharpoons M + MNN$.

The most likely biological mechanisms in the case of the synthesis of ATP is (2) but (1) cannot be excluded. There is no evidence in support of (3). Only cases (1) and (2) are therefore considered.

Case 1. The reactions taking place in this case are diagrammatically as follows:



It is assumed that a steady state exists, so that the rates of formation and disappearance of each substance are equal.

- Let v_1 = rate at which N exchanges with MN ;
- v_2 = rate at which N exchanges with MNN ;
- a = amount of, x = radioactivity of, N ;
- b = amount of, y = radioactivity of, MN ;
- c = amount of, z = radioactivity of, MNN .

It is assumed that c is small in comparison to a and b and that v_1, v_2 is large in comparison to c/t . Required is $v_1 + v_2$.

It follows from the above assumptions that

$$\frac{dx}{dt} = -\frac{(v_1 + v_2)x}{a} + \frac{v_2 y}{b} + \frac{v_1 z}{c}, \tag{1}$$

$$\frac{dy}{dt} = -\frac{2v_2 y}{b} + \frac{v_2 x}{a} + \frac{v_2 z}{c}, \tag{2}$$

$$\frac{dz}{dt} = -\frac{(v_1 + v_2)z}{c} + \frac{v_1 x}{a} + \frac{v_2 y}{b}, \tag{3}$$

$$x + y + z = x_0. \tag{4}$$

Substitution of (4) in (1) and (3) gives:

$$\left(D + \frac{v_1 + v_2}{a} + \frac{v_2}{b}\right)x + \left(\frac{v_2}{b} - \frac{v_1}{c}\right)z = \frac{v_2 x_0}{b} \quad \left(D = \frac{d}{dt}\right), \tag{5}$$

$$\left(\frac{v_2}{b} - \frac{v_1}{a}\right)x + \left(D + \frac{v_1 + v_2}{c} + \frac{v_2}{b}\right)z = \frac{v_2 x_0}{b}. \tag{6}$$

Elimination of z gives

$$\left\{D^2 + D \left(\frac{v_1 + v_2}{a} + \frac{2v_2}{b} + \frac{v_1 + v_2}{c}\right) + v_2(2v_1 + v_2) \left(\frac{1}{bc} + \frac{1}{ca} + \frac{1}{ab}\right)\right\} x = \frac{v_2(2v_1 + v_2)}{bc} x_0. \tag{7}$$

The initial conditions for x are:

$$x = x_0, \quad Dx = -\frac{(v_1 + v_2)}{a} x_0.$$

The exact solution is therefore of the form

$$x = \frac{ax_0}{a+b+c} + Ae^{-\alpha t} + Be^{-\beta t},$$

where $\frac{(b+c)x_0}{a+b+c} = A + B, \quad \frac{(v_1 + v_2)x_0}{a} = \alpha A + \beta B,$

and α, β are the roots of the quadratic equation

$$\theta^2 - \theta \left(\frac{v_1 + v_2}{a} + \frac{2v_2}{b} + \frac{v_1 + v_2}{c} \right) + v_2(2v_1 + v_2) \left(\frac{1}{bc} + \frac{1}{ca} + \frac{1}{ab} \right) = 0. \tag{8}$$

The result is too complicated for a simple adaptation to experimental data, but if c is small in comparison to a and b , a suitable approximation to x and z may be obtained.

One root α is of the form $k_1 + o(c)$ and the other root β is of the form $l_1/c + l_2 + o(c)$, where k_1 and l_1 are both positive.

Substitution of these forms in the equations for A and B shows that A is of the form $A_0 + o(c)$ and B is of the form $o(c)$.

The value of x is therefore of the form

$$\frac{ax_0}{a+b} + o(c) + [A_0 + o(c)] e^{[-k_1 + o(c)]t} + o(c) \exp \left\{ - \left[\frac{l_1}{c} + l_2 + o(c) \right] t \right\}.$$

The leading term in the approximation is obtained by letting c tend to $+0$ and this then takes the form

$$x = \frac{ax_0}{a+b} + A_0 e^{-k_1 t}.$$

Finally equation (5) shows that z/c has a form similar to x when c tends to $+0$.

x is found from equation (7) by equating the coefficient of $1/c$ to zero. This gives

$$\left\{ (v_1 + v_2) D + v_2(2v_1 + v_2) \right\} \left(\frac{1}{a} + \frac{1}{b} \right) x = \frac{v_2(2v_1 + v_2) x_0}{b},$$

the solution of which is:

$$x = \frac{ax_0}{a+b} + \frac{bx_0}{a+b} \exp \left[-K \left(\frac{1}{a} + \frac{1}{b} \right) t \right], \tag{9}$$

where

$$K = \frac{v_2(2v_1 + v_2)}{v_1 + v_2}.$$

Substitution of x from (9) into (5) will give

$$z = \frac{cx_0}{a+b} - \frac{cx_0}{a+b} \left(1 - \frac{a+b}{a} L \right) \exp \left[-K \left(\frac{1}{a} + \frac{1}{b} \right) t \right], \tag{10}$$

where

$$L = \frac{v_1}{v_1 + v_2}.$$

It is assumed in (9) that terms $c/a, c/b$ are negligible and in (10) that $c^2/a^2, c^2/b^2$ are negligible.

Also it is assumed in (10) that the term $e^{-L_1(t/c)}$ is negligible. Since $l_1 = v_1 + v_2$, the neglect of this term implies that $(v_1 + v_2)t$ is large in comparison to c . This means that a sufficient time has elapsed from $t=0$ to ensure that the flux into and from c is large compared to c .

Rearranging (9) gives:

$$K = \frac{ab}{t(a+b)} \ln \frac{bx_0}{x(a+b) - ax_0}.$$

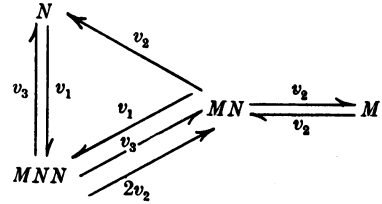
The main result is that (9) is formally identical with the formula derived for the single step case, but the significance of the constant is somewhat different. Thus if a plot of $\ln \frac{bx_0}{x(a+b) - ax_0}$ against $\frac{t(a+b)}{ab}$ gives a straight line the underlying mechanism may be either a one-step or a two-step reaction.

It should be noted that the K is equal to

$$v_1 + v_2 - \frac{v_2^2}{v_1 + v_2}$$

and is therefore a minimum value for the effective speed.

Case 2. The reactions taking place are diagrammatically shown in scheme 2.



Required are $v_2 + v_3$ (which equal v_1). The assumptions are the same as in case 1. The equations are:

$$\frac{dx}{dt} = v_2 \frac{z}{c} - v_1 \frac{x}{a} + v_3 \frac{y}{b}, \tag{1}$$

$$\frac{dz}{dt} = -v_2 \frac{z}{c} - v_1 \frac{z}{c} + (v_2 + 2v_3) \frac{y}{b}, \tag{2}$$

$$x + y + z = x_0. \tag{3}$$

The method used for case 1 is applicable here, since the equations are similar in form.

Substitution from (3) into (1) and (2) gives:

$$\left(D + \frac{v_2 + v_3}{a} + \frac{v_3}{b} \right) x + \left(\frac{v_3}{b} - \frac{v_2}{c} \right) z = \frac{v_3 x_0}{b}, \tag{4}$$

$$\frac{v_3 + 2v_2}{b} x + \left(D + \frac{v_3 + 2v_2}{b} + \frac{v_3 + 2v_2}{c} \right) z = \frac{v_3 + 2v_2}{b} x_0. \tag{5}$$

Taking $z + 0(c)$

$$\left(D + \frac{v_2 + v_3}{a} + \frac{v_3}{b} \right) x - \frac{v_2 z}{c} = \frac{v_3 x_0}{b}, \tag{6}$$

$$\frac{x}{b} + \frac{z}{c} = \frac{x_0}{b}, \tag{7}$$

i.e. $\left(D + \frac{v_2 + v_3}{a} + \frac{v_2 + v_3}{b} \right) x = \frac{v_2 + v_3}{b} x_0.$

The solution

$$x = \frac{ax_0}{a+b} + \frac{bx_0}{a+b} \exp \left[-(v_2 + v_3) \left(\frac{1}{a} + \frac{1}{b} \right) t \right]. \tag{8}$$

Substitution in (7) gives:

$$z = \frac{cx_0}{a+b} - \frac{cx_0}{a+b} \exp \left[-(v_2 + v_3) \left(\frac{1}{a} + \frac{1}{b} \right) t \right]. \tag{9}$$

Rearranging (8) gives:

$$v_2 + v_3 = v_1 = \frac{ab}{t(a+b)} \ln \frac{bx_0}{x(a+b) - ax_0}.$$

This formula is again formally identical with that derived for the single-step mechanism. In the present case the constant signifies the actual rate at which the labile phosphate exchanges.