6. There is evidence that cholinesterase inhibition by insecticidal carbamates may have been significantly underestimated in earlier studies.

The authors are indebted to Dr J. M. Barnes and Dr W. N. Aldridge for a pure sample of *o*-isopropoxyphenyl-*N*-methylcarbamate, blood samples from poisoned rabbits, relevant information and for helpful discussion.

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A Note on the Kinetics of Multi-enzyme Systems

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(*Received* 30 *September* 1963)

The metabolic sequence is a characteristic feature of living systems. Cells are much more complex than collections of enzymes, and there are hundreds of enzymic reactions proceeding simultaneously. Nevertheless, theoretical study of the types of interaction in the simplest systems provides insight that can be gained in no other way. The same features will be present in more 'life-like' situations, but will be less evident. A start must be made with the simplest systems, but there are few studies of the kinetics of multi-enzyme systems (Reiner, 1959; Webb, 1963; Hearon, 1952). The present treatment is confined to simple systems, and stresses the influence of readily reversible reactions. The purpose of this paper is to form a background to thinking about the interplay of enzymic reactions in metabolic sequences, rather than to provide equations that can be directly applied to a given experimental situation.

Linear chains

The simplest linear sequence:

$$A \underset{\text{step 1}}{\overset{E_1}{\rightleftharpoons}} B \underset{\text{step 2}}{\overset{E_2}{\rightleftharpoons}} C$$

will be considered first, and the treatment generalized later. A, B and C are substrates, and

E_1 and E_2 are enzymes, catalysing the first and second steps respectively. The mechanisms may be written:

$$E_1 + A \rightleftharpoons W \rightleftharpoons X \rightleftharpoons E_1 + B$$
$$E_2 + B \rightleftharpoons Y \rightleftharpoons Z \rightleftharpoons E_2 + C$$

where W, X, Y and Z are enzyme–substrate complexes. The reactions are linked, or coupled, because B is both the product of the first step and the starting material for the second step. The treatment considers these reactions as part of an open system; the concentrations of A and C are then taken as constant, and material flows through the system (in the direction A to C) at a rate of flux denoted by v. For the system to be in a steady state, the rate of flux in both steps must be the same, and the linking intermediate B is consumed in the second step as fast as it is formed by the first step. If the concentrations of A, B and C are written as a, b and c, we have for the first step:

$$v = \frac{V_A a/K_A - V_B b/K_B}{1 + a/K_A + b/K_B} \quad (1)$$

and for the second step:

$$v = \frac{V'_B b/K'_B - V_C c/K_C}{1 + b/K'_B + c/K_C} \quad (2)$$

where V_A, K_A and V_B, K_B refer to the maximum rates and Michaelis constants for the forward and reverse reactions in the first step, and V'_B, K'_B and V_C, K_C refer to the maximum rates and Michaelis constants in the forward and reverse reactions of the second step (Peller & Alberty, 1959). We wish now to see how the rate of flux (v) depends on the concentrations (e_1, e_2) of the two enzymes E_1 and E_2. To see this, the concentration of B must be eliminated from equations (1) and (2). This is not straightforward as the equations stand, owing to the terms in b in the denominators. The approximation is therefore made that both b/K_B and b/K'_B are small compared with unity. The concentrations of intermediates are in fact often low compared with their Michaelis constants (Vegotsky & Frieden, 1958). After making this approximation, b may be easily eliminated from equations (1) and (2) to give equation (3):

$$v = \frac{V_A V'_B a/K_A K'_B - V_B V_C c/K_B K_C}{V_B(1 + c/K_C)/K_B + V'_B(1 + a/K_A)/K'_B} \quad (3)$$

The way in which the rate depends on the concentrations of the two enzymes is shown more clearly when equation (3) is rewritten as (4); this utilizes the fact that V_A and V_B are proportional to e_1 and V_B and V_C are proportional to e_2:

$$v = \frac{Q e_1 e_2}{S e_1 + R e_2} \quad (4)$$

where Q, R and S are functions of the rate constants, and of a and c. Even simpler is the reciprocal form:

$$\frac{1}{v} = \frac{1}{Q}\left(\frac{R}{e_1} + \frac{S}{e_2}\right) \quad (5)$$

The reciprocal rate, then, depends on two terms, each of which brings in the concentration of one of the enzymes. Since the dimensions of reciprocal rate are time/concentrations, the two ratios of parameters, R/Q and S/Q, have the dimensions of time. In general, the rate depends on the concentrations of both enzymes. There is no need for there to be a rate-limiting step, but if e_1, for instance, is made large enough the term R/e_1 will obviously be small compared with S/e_2, and the term involving the second enzyme will govern the rate. In other words, from equation (5), either term may dominate the equation, but neither need do so.

The example chosen was one where both enzymic reactions were of the one-substrate (A → B) type. Equation (5) is also obtained from the more common two-substrate type for both reactions. Thus for the reactions:

$$A + Q \rightleftharpoons B + R$$
$$B + X \rightleftharpoons Y + Z$$

the rates will be (Bloomfield, Peller & Alberty, 1962):

$$v = \frac{(V_{AQ}/K_{AQ})aq - (V_{BR}/K_{BR})br}{1 + a/K_A + q/K_Q + b/K_B + r/K_R + aq/K_{AQ} + br/K_{BR} + ab/K_{AB} + qr/K_{QR} + abq/K_{ABQ} + bqr/K_{BQR}}$$

and

$$v = \frac{(V_{BX}/K_{BX})bx - (V_{YZ}/K_{YZ})yz}{1 + b/K'_B + x/K_X + y/K_Y + z/K_Z + bx/K_{BX} + yz/K_{YZ} + by/K_{BY} + xz/K_{XZ} + bxy/K_{BXY} + xyz/K_{XYZ}}$$

With the same approximations as before, the equations for v become linear in b, and b can be eliminated from them. Since V_{AQ} and V_{BR} are proportional to e_1, and V_{BX} and V_{YZ} are proportional to e_2, the solution for v has the same form as equation (4); Q, R and S are here functions of the rate constants, and of a, q, r, x, y and z.

If there are three steps:

$$A \overset{E_1}{\rightleftharpoons} B \overset{E_2}{\rightleftharpoons} C \overset{E_3}{\rightleftharpoons} D$$

in which now both B and C are linking intermediates, the rate equations may be written in the abbreviated forms:

$$v = F - Gb$$
$$v = Hb - Jc$$
$$v = Kc - L$$

In these equations, the same approximation has been made that the concentrations of the linking

intermediates, B and C, are low; F, G, H, J, K and L are functions of the rate constants, and of a and d, and of the concentrations of the enzymes.

Elimination of both b and c gives:

$$v = \frac{FHK - GJL}{HK + GK + GJ}$$

Since F and G are proportional to e_1, and H and J are proportional to e_2, and K and L to e_3:

$$v = \frac{Qe_1e_2e_3}{Re_2e_3 + Se_1e_3 + Te_1e_2}$$

or

$$\frac{1}{v} = \frac{1}{Q}\left(\frac{R}{e_1} + \frac{S}{e_2} + \frac{T}{e_3}\right) \qquad (6)$$

This equation is a simple extension of equation (5), and this formulation clearly holds for any number of steps.

Optimum ratio of concentrations of enzymes

If the total concentration (E) of the enzymes is fixed, we may inquire whether there is a value for the relative concentrations that gives a maximum rate of flux in a linear sequence. We therefore want the conditions for a stationary value of

$$v = \phi(e_1, e_2, e_3)$$

given that $e_1 + e_2 + e_3 = \text{constant}$.

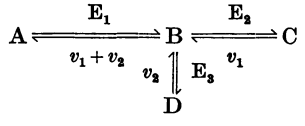
By Lagrange's method of undetermined multipliers (Kynch, 1955), the condition is that:

$$R/e_1^2 = S/e_2^2 = T/e_3^2, \quad \text{or} \quad e_1 : e_2 : e_3 = \sqrt{R} : \sqrt{S} : \sqrt{T} \qquad (7)$$

There is therefore an optimum ratio of the concentrations of the enzymes. This may represent an important factor in the temporal organization in a cell, since it seems reasonable that the relative amounts of different enzymes should be such that the rate is as high as it can be for a given total amount of catalytically active protein.

Branched reactions

The linking intermediates in a metabolic sequence may react further in more than one way (obvious examples of such intermediates are glucose 6-phosphate or pyruvate). The minimum number of steps that must be considered is here three:



The linking intermediate, B, reacts at a rate of v_1 by one route, and at a rate of v_2 by the other; in the steady state it must be formed at a rate of $v_1 + v_2$. With the same approximation as before, the rates of the three reversible enzymic reactions are:

$$\begin{aligned} v_1 + v_2 &= F - Gb \\ v_1 &= Hb - J \\ v_2 &= Kb - L \end{aligned}$$

Elimination of b gives:

$$v_1 = \frac{HF - JK + HL - GJ}{H + K + G}$$

$$v_2 = \frac{JK - HL + FK - GL}{H + K + G}$$

Putting $F = F'e_1$, $G = G'e_1$, $H = H'e_2$, $J = J'e_2$, $K = K'e_3$, $L = L'e_3$, then:

$$\frac{v_1}{v_2} = \frac{e_1e_2(H'F' - G'J') + e_2e_3(H'L' - J'K')}{e_1e_3(F'K' - G'L') - e_2e_3(H'L' - J'K')} \qquad (8)$$

or

$$\frac{v_1}{v_2} = \frac{e_2}{e_3} \cdot \frac{Pe_1 + Qe_3}{Re_1 - Qe_2} \qquad (9)$$

In the absence of the above analysis the simple assumption that the relative flow of material along the two pathways was directly proportional to the ratio of the amounts of enzymes might have seemed reasonable. As it is, the ratio in equation (9) depends on the concentrations of all the enzymes; thus the system is affected by reciprocal interactions. This is a property of highly organized systems shown by even such a simplified model as the one being discussed.

There is one important general condition under which the relative flow down the pathways is proportional to the ratio of the amounts of enzymes; i.e., when the two competing reactions are irreversible. Then $J' = L' = 0$, and equation (8) reduces to:

$$\frac{v_1}{v_2} = \frac{e_2}{e_3} \cdot \frac{H'}{K'} \qquad (10)$$

The way therefore in which competing reactions interact is affected by whether they are reversible. Since enzymic reactions differ greatly in whether they are 'practically' reversible, it is useful to have a measure of whether a given reaction is close to equilibrium.

The approach adopted in the derivations given has been to take relatively simple schemes and to see what their predicted behaviour is. The systems have been chosen as the simplest that contain more than one enzyme. Even so, it is necessary to make approximations to get readily comprehensible information about the way in which the rate of flux depends on the concentrations of the enzymes in linear and branched sequences. This approach is complementary to that in which the kinetics of reactions in cells are interpreted by

complex schemes with the aid of computers (Chance, 1960; Chance, Garfinkel, Higgins & Hess, 1960). The theoretical study of simple model multi-enzyme systems will surely play an important role in guiding biochemical thought on the behaviour of living systems.

SUMMARY

1. The variation of the rate of flux with the concentrations of the enzymes in multi-enzyme systems is considered.

2. For linear sequences (when the concentrations of the linking intermediates are relatively low) the reciprocal rate is given by a formula in which each term involves the reciprocal of the concentration of one of the enzymes.

3. Interaction occurs in branched systems, and the relative rate of competing reactions is not proportional to the ratio of the amounts of the enzymes, unless the reactions are irreversible.

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The Effect of Red Light on the Flavonoid Composition of Etiolated Pea Plumules

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(*Received* 21 *May* 1963)

Hillman & Galston (1957) reported that the indolylacetate-oxidase activity of the buds of etiolated pea plants is inhibited by red light (640 mμ) and that the effect is reversed by near-infrared light (730 mμ). In following up this report, Mumford, Smith & Castle (1961) found that pea buds from plants treated with red light possess increased amounts of an indolylacetate-oxidase-inhibiting flavonoid, R_F 0·14 in 20% (w/v) potassium chloride, which yielded kaempferol, glucose and 4-hydroxycinnamic acid on acid hydrolysis. This flavonoid was also present in smaller amounts in extracts of pea buds from plants completely grown in the dark, but the major flavonoid from tissue from plants grown in the dark had R_F 0·28 in 20% potassium chloride. Investigations on the structure of these flavonoids, now reported, show that the compound isolated from plants treated with red light is 3-(4-hydroxycinnamoyltriglucosyl)kaempferol and that from plants grown in the dark is probably 3-(hexaglucosyl)-kaempferol.

EXPERIMENTAL

Flavonoid from pea plumules from plants treated with red light

Acid hydrolysis. The flavonoid, R_F 0·14 in 20% KCl, from pea buds from plants treated with red light was obtained as the dihydrate (mol.wt. 954), as described by Mumford *et al.* (1961). The flavonoid (0·5 mg.; 0·523 μmole) was dissolved in methanol (2 ml.), and the solution heated on a steam bath under reflux with 2N-H_2SO_4 (2 ml.) for 2 hr. The hydrolysate was diluted to 10 ml. with methanol, and the resulting solution assayed quantitatively for kaempferol and glucose by the method of Hörhammer (1956). The results are shown in Table 1.

Alkaline hydrolysis. Flavonoid (10·4 mg.) from pea buds from plants treated with red light was heated with 20% (w/v) NaOH (2 ml.) on a steam bath for 1 hr. The solution was cooled and adjusted to pH 6·6 with dilute HCl. The solution was extracted three times with portions (10 ml.) of ethyl acetate. The ethyl acetate extracts were dried over anhydrous Na_2SO_4, and the ethyl acetate was removed *in vacuo*. The ultraviolet-absorption spectrum of the residue dissolved in methanol (4 ml.) was almost identical with