Studies in Biochemical Adaptation. The 'Mass Action' Theory of Enzyme Adaptation

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The occurrence of enzyme adaptation, known since the beginning of the century, has aroused increasing interest in the last fifteen years. Earlier 'explanations' were purely teleological until Yudkin (1938) put forward his 'mass-action' theory.

According to this theory the adaptive enzyme is formed from a postulated precursor with which it is in equilibrium. Combination of the enzyme with any substance—its substrate or a drug—upsets the equilibrium and more enzyme is produced from the precursor until balance is restored. The theory has been criticized by Spiegelman (1946) and Spiegelman & Reiner (1947) on the grounds, for instance, that it is not possible to predict either an exponential rate of adaptive enzyme formation, or competition between enzyme-forming systems. While these predictions are not directly deducible from the original theory they are certainly not in contradiction with it (see below).

It is beyond the scope of this paper to deal in detail with these criticisms or with alternative theories that have been proposed (see, for example, Monod, 1947; Northrop, 1949). However, the theories of Hinshelwood (1946) and of Spiegelman (1946) require brief mention.

Hinshelwood assumes a series of enzymes linked so that the end product of one reaction becomes the substrate for the next. Each enzyme catalyses its own formation according to the scheme

enzyme + substrate → more enzyme + products.

This implies that the enzymes of the cell, and therefore the protoplasm generally, increase exponentially. Adaptation can, however, occur in organisms which are not growing in this way—in animals (see Weinland, 1905–6; Abderhalden, 1937; Davies & Yudkin, 1951), or in washed suspensions of bacteria —and it is difficult to see how the theory would apply in these instances.

Spiegelman's theory (1946) postulates three self-duplicating units—the gene, the plasmagene and the plasmagene-substrate-enzyme complex—each producing partial or complete replicas of itself. Although the theory accounts for many of the facts it is somewhat cumbersome, and, like Hinshelwood's, does not seem applicable to enzyme adaptation in animals or in non-growing bacteria.

It would be of advantage to have a theory which would apply to animals as well as bacteria, which would explain the relevant facts simply and which would lead to qualitative and quantitative predictions by which it could be tested. It seemed that these requirements could be largely fulfilled by extending Yudkin's original 'mass action' concept to cover a wider range of biochemical reactions.

'*Mass action*' *enzyme model*

We shall examine the consequences of the general assumption that chemical equilibria exist between the various protein components of the cell, postulating also that: (*a*) proteins are synthesized from elementary substances; (*b*) the end products of an enzyme reaction may be used for the building of cell material in general and enzymes in particular.

Since no account has been taken of the important part played by genes in the control of enzymes, this model will clearly give an over-simplified picture.

The model constructed on the above premises is shown in Fig. 1. *B* is a pool of elementary building blocks from which proteins and enzyme precursors are synthesized. The term 'building blocks' will be held to include sources of carbon and nitrogen as well as any vitamins, inorganic ions, etc., that may be needed. The amount of material in the pool may be only a small proportion of the total cell material, but, owing to the equilibria existing, there will always be some free building material. The various cell proteins (Pr_1, Pr_2, etc.) and enzyme precursors (P_1, P_2, etc.) are synthesized from, and are in equilibrium with, the materials in *B*. The enzymes E_1 and E_2 are held to be formed directly from their specific precursors by a unimolecular reaction, and are shown as reacting with their substrates in the usual way. Part of the product of an enzyme reaction is contributed to *B* as building material while the rest is catabolized. The proportion of the product used for each of these two purposes will vary according to the nature of the product and the physiological conditions. Thus the proportion 'fed back' to *B* will be large or small depending on whether the organism is or is not growing.

It is important to note that the model implies a balanced metabolic system. For example, the pro-

teins Pr_1 and Pr_2 will be in a fixed ratio to one another and to all the other proteins and enzymes of the cell. The relative concentrations of the cell components will depend on the nature of the equilibria, and an alteration in the concentration of any one substance will affect the concentrations of substances in equilibrium with B. In addition, no qualitative distinction is made between 'adaptive' and 'constitutive' enzymes. Instead it is assumed that for a 'constitutive' enzyme the over-all equilibrium between B and the enzyme lies in the direction of the latter. For an 'adaptive' enzyme the opposite holds.

concentration, i.e. the enzyme is autocatalytic and its rate of increase is given by

$$\frac{de_1}{dt} = k'e_1. \quad (1)$$

If s_1 is not large enough to be regarded as constant

$$\frac{de_1}{dt} = ke_1s_1. \quad (1a)$$

It may be noted in passing that these are among the fundamental equations of Hinshelwood's kinetic studies (see above).

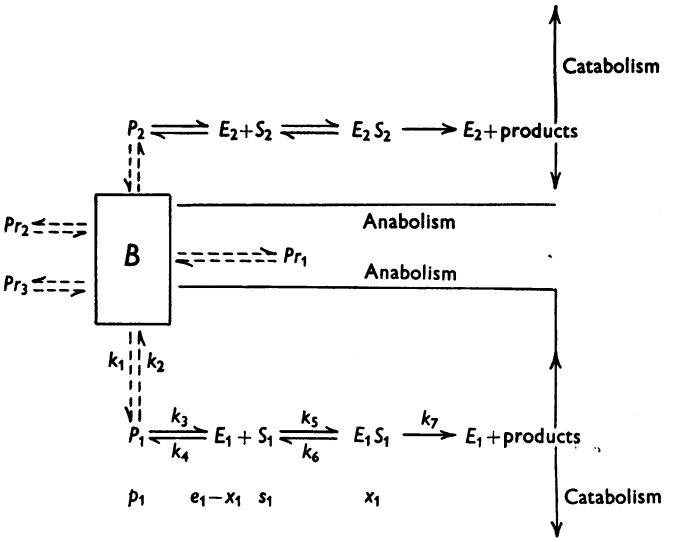

Fig. 1. Model of enzyme adaptation by 'mass action'. B, pool of building blocks; Pr_1, Pr_2, Pr_3, proteins; P_1 and P_2, enzyme precursors; E_1 and E_2, enzymes; S_1 and S_2, substrates; p_1, e_1, s_1 and x_1 are concentrations; k_1, k_2, etc. are velocity constants. Broken arrows indicate that a number of reactions may be involved.

Growth of organisms in terms of the 'mass action' model

In the growth of micro-organisms the medium becomes partly transformed into protoplasm. If S_1 (Fig. 1) is the sole source of carbon and energy, then a large part of the reaction product is ultimately contributed to the pool of 'building blocks'; either the product itself, or substances derived from it after further interaction, are 'fed back' to B. In either case, the rate at which material passes to B is proportional to the rate at which S_1 is broken down, and this in turn depends on the product of concentrations of E_1 and S_1. Now in a fresh inoculum, S_1 will be relatively so large that its concentration may be regarded as constant and the growth of B then depends directly on the concentration of E_1. From Fig. 1 it can be seen that an increase in B leads to an increase in P_1 and hence in E_1. Therefore the rate of growth of E_1 is proportional to its own instantaneous

Integration of Equation (1) indicates that the growth of E_1 will be given by an exponential equation. Furthermore, since B is available not only for the synthesis of E_1 but of protoplasm generally, it follows that the growth of the culture as a whole will be represented by a similar equation.

In animals the picture is more complicated. The growth phase may be considered as similar to that of the micro-organism in that a large proportion of the products of enzyme reactions passes to B. As the animal matures the proportion becomes smaller and smaller, and in the adult it is sufficient only to maintain the body structure.

Enzyme adaptation caused by the substrate

According to the 'mass action' theory, combination of the enzyme with its substrate disturbs the equilibrium with the precursor and more enzyme is synthesized. The process will be considered in two

situations: (*a*) Adaptation in a non-growing system, e.g. in an animal or in a washed bacterial suspension. In other words, it is assumed that there is no 'feed back' of products to B. (*b*) Adaptation accompanied by a 'feed back' of materials to B.

(*a*) *Adaptation in the absence of 'feed back'.* Let E_1 (Fig. 1) be the enzyme under consideration. The rate of formation of E_1 from P_1 is given by

$$\frac{de_1}{dt} = k_3 p_1 - k_4(e_1 - x_1). \qquad (2)$$

If enough substrate is present to saturate the enzyme

$$e_1 = x_1 \quad \text{(very nearly)},$$

and (2) becomes

$$\frac{de_1}{dt} = k_3 p_1. \qquad (2a)$$

A number of possibilities arise, some of which may now be considered.

(1) *Large concentration of the precursor.* If p_1 is large in comparison with the amount of enzyme finally formed, it may be regarded as almost constant, that is

$$\frac{de_1}{dt} = \text{constant},$$

and E_1 will appear at a linear rate until the equilibrium is restored (Fig. 2*A*).

(2) *Small concentration of the precursor.* If the initial amount of P_1 is small relative to the final amount of enzyme it will, after addition of the substrate, fall almost to zero. The rate of enzyme synthesis thereafter will depend on the rate of formation of P_1 from B. Although the amount of material in B may be small, any lowering of its concentration will be corrected by degradation of the proteins which are in equilibrium with B. If the amount of E_1 is small in relation to these proteins, B may be regarded as a reservoir whose contents are continuously adjusted to a fixed level. The rate of production of E_1 from this constant reservoir will be constant and the graph obtained will again be linear.

On the other hand, if the replenishment of B is restricted, i.e. if a building block essential for the synthesis of E_1 is limited in quantity and is becoming exhausted, there will be a continuously falling rate of enzyme formation (Fig. 2*B*). This type of curve is not likely to occur frequently for, if the building blocks needed for E_1 are constituents of normal proteins, it might be difficult to obtain enough restriction in building material to produce a noticeably falling rate of adaptation.

(*b*) *Adaptation accompanied by 'feed back'.* With bacteria, the products of the action of E_1 upon S_1 may not be entirely catabolized, especially when some alternative source of energy is present. If the 'feed back' of these products is sufficient to affect the pool of building blocks, the situation becomes similar to that discussed under the heading of bacterial growth—E_1 will increase autocatalytically and the curve obtained will be of the form shown in Fig. 2*C*. If other necessary factors are present there may be concurrent growth and cell division.

In experiments on adaptation of washed yeast cells to galactose it was found that the rate of adaptation was either linear or exponential (Type 2*A* or 2*C*). Whenever the rate was linear there was no increase either in the total count or the dry weight of the cells. An exponential rate of adaptation was usually, but not invariably, accompanied by a significant increase in one or both (Mandelstam & Yudkin, 1952*a*).

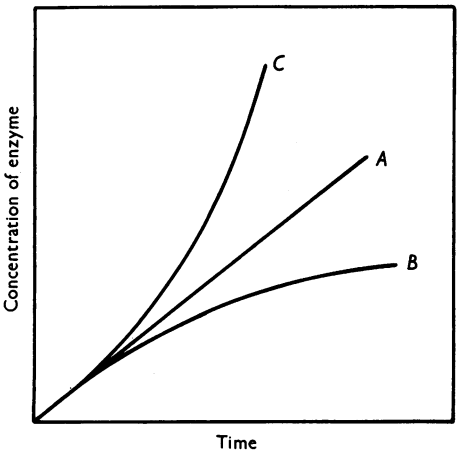

Fig. 2. Theoretical possibilities for rate of adaptation. (*A*), linear rate when final amount of enzyme formed is relatively small; (*B*), falling rate when final amount of enzyme formed is relatively large; (*C*) increasing rate when there is a continuous increase in the amount of building material.

Concentration of substrate and rate of adaptation

In the presence of substrate, enzyme formation will continue until equilibrium between free enzyme and precursor is restored; that is, it will cease when the requisite number of free enzyme molecules is present. For any particular degree of saturation, the number of molecules of free enzyme will be proportional to the total amount of enzyme present. Thus, when the degree of saturation is high and the amount of enzyme small, there will be so few free enzyme molecules that the rate of enzyme formation is nearly maximal and, at this stage, further increase in substrate concentration would not noticeably affect it. As the amount of enzyme increases, the number of free enzyme molecules increases even if the degree of saturation is unaltered. The rate of formation then begins to fall and

eventually no more enzyme is formed. Thus, if a series of concentrations of substrate is used, all of which virtually saturate the enzyme, the initial rates of enzyme formation will be identical, but the lower concentrations will allow equilibrium to be attained earlier. Thus, the final amounts of enzyme formed will depend on the substrate concentration. These predictions were confirmed in experiments on galactozymase in yeast (Mandelstam & Yudkin, 1952*a*).

Concentration of substrate and extent of adaptation

It has been indicated that the amount of enzyme finally formed depends on the amount of substrate causing the adaptation. Some expressions describing this dependence will now be derived for two experimental conditions in non-growing systems: (*a*) growth of precursor unrestricted, i.e. all the building blocks required for the production of the enzyme are amply available; (*b*) growth of precursor restricted, i.e. only a limited amount of material is available for synthesis of the precursor.

(*a*) *Growth of precursor unrestricted.* Let E be the 'adaptive' enzyme, P its precursor, B the pool of building blocks and S the substrate. We then have

$$\underset{p}{B} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{e-x}{P} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{s}{E+S} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \underset{x}{ES} \xrightarrow{k_7} E+\text{products}$$

with concentrations as shown.

The rate of change of x follows from the law of mass action and is given by

$$\frac{dx}{dt}=k_5 s(e-x)-k_6 x-k_7 x. \tag{3}$$

Initially, that is before addition of S, x will be zero. After S has been added, x will rise rapidly to a small value determined by the amount of E initially present. The further rate of increase of x will depend on the rate of synthesis of fresh enzyme. The process will continue until the system has adjusted itself by producing enough enzyme to restore the enzyme-precursor equilibrium. There will then be, for a short time at any rate, a steady state condition in which x will have reached a maximum and

$$\frac{dx}{dt}=0.$$

Whence $$k_5 s(e-x)-k_6 x-k_7 x=0,$$

or $$x=\frac{es}{s+\dfrac{k_6+k_7}{k_5}}. \tag{4}$$

The term $(k_6+k_7)/k_5$ can be replaced by a single constant which is usually denoted by K_m, the Michaelis constant. Briggs & Haldane (1925) and Haldane (1930) have pointed out that this is not strictly correct unless k_7 is negligible compared with k_6. The approximation is, however, often justified in practice and will be used here for simplicity of notation.

Equation (4) then becomes

$$x=\frac{es}{s+K_m}. \tag{5}$$

Let e_a and x_a be the steady state concentrations when $s=s_a$. Then

$$x=\frac{e_a s_a}{s_a+K_m}. \tag{6}$$

If e_b and x_b are the steady state concentrations for some other value of s $(=s_b)$

$$x_b=\frac{e_b s_b}{s_b+K_m}. \tag{7}$$

The concentration of the free enzyme will be e_a-x_a and e_b-x_b in the two cases respectively. By hypothesis, building blocks are amply available so that the concentration of the precursor is always restored finally to its initial value. If the final concentration of P is constant, the concentration of free enzyme in equilibrium with P must also be constant, and

$$e_a-x_a=e_b-x_b. \tag{8}$$

Substituting in (8) the values for x_a and x_b from (6) and (7)

$$e_a-\frac{e_a s_a}{s_a+K_m}=e_b-\frac{e_b s_b}{s_b+K_m}.$$

Whence

$$e_a\left(1-\frac{s_a}{s_a+K_m}\right)=e_b\left(1-\frac{s_b}{s_b+K_m}\right),$$

and $$e_b=e_a\left(\frac{s_b+K_m}{s_a+K_m}\right). \tag{9}$$

It follows from (9) that, if the enzyme concentration e_a is known for a particular concentration of substrate s_a and K_m is also known, then e_b can be calculated for any other value of S (i.e. s_b). The relationship between the equilibrium amounts of enzyme and substrate is a straight line cutting the vertical axis at a positive value given by

$$e_a K_m/(s_a+K_m).$$

For most 'adaptive' enzymes this value will be nearly zero (see Fig. 3*A*).

Mandelstam & Yudkin (1952*b*) have carried out experiments on the hepatic arginase of rats in which Equation (9) was applicable. Building blocks were freely available in that the animals were given a full diet, and a steady state was ensured by prolonging the experiment for 22 weeks. At this stage, growth was so slight that the effect of 'feed back' could be disregarded. Under these conditions a linear relationship was found to hold between hepatic arginase and the amount of protein in the diet.

Similar results were also obtained with galactozymase in *Saccharomyces cerevisiae* (Mandelstam & Yudkin, 1952*a*).

(*b*) *Growth of precursor restricted.* By hypothesis only a limited amount of precursor can be synthesized. As a simplifying assumption it will be taken that the total amount of P that can be synthesized is present from the start. We then have

$$P \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + S \underset{k_6}{\overset{k_5}{\rightleftharpoons}} ES \xrightarrow{k_7} E + \text{products}.$$

$$p \qquad e-x \quad s \qquad x$$

The rate of change of p is given by

$$\frac{dp}{dt} = k_4(e-x) - k_3 p.$$

When the system has reached the steady state condition

$$\frac{dp}{dt} = 0,$$

and

$$x = e - p\frac{k_3}{k_4},$$

or

$$x = e - K_e p, \tag{10}$$

where K_e is the equilibrium constant for the reaction between enzyme and precursor. Since p cannot be increased it follows that

$$e + p = \text{constant}.$$

But from (5)

$$x = \frac{es}{s+K_m}.$$

Substituting this value of x in (10) we find that in any steady state

$$K_e p = e - x = e\left(1 - \frac{s}{s+K_m}\right). \tag{11}$$

Whence

$$K_e(e+p) = e\left(1 + K_e - \frac{s}{s+K_m}\right). \tag{12}$$

But $e+p$ is constant, so if s_a and s_b are the substrate concentrations in two steady states and e_a and e_b the corresponding enzyme concentrations,

$$e_a\left(K - \frac{s_a}{s_a+K_m}\right) = e_b\left(K - \frac{s_b}{s_b+K_m}\right), \tag{13}$$

where

$$K = K_e + 1.$$

Rearranging (13)

$$e_b = e_a\left(\frac{K - \dfrac{s_a}{s_a+K_m}}{K - \dfrac{s_b}{s_b+K_m}}\right). \tag{14}$$

This equation provides a means of obtaining the equilibrium constant for the precursor-enzyme reaction. Thus, if two concentrations of enzyme (e_a and e_b) are determined for two concentrations of substrate and if K_m is known, K can be calculated. Now it will be remembered that

$$K_e = K - 1,$$

where K_e is the equilibrium constant for the reaction between enzyme and precursor. For the purposes of calculation it was taken that all the precursor that could be synthesized was present from the start.

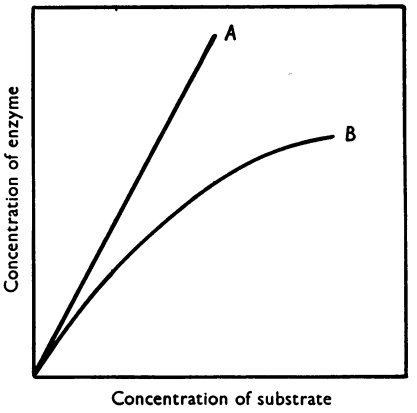

Fig. 3. Theoretical relationship between concentration of substrate and final amount of enzyme formed. (*A*), building material unrestricted; (*B*), building material restricted.

It would probably be more correct to assume that P is not present in full amount initially, but that it is continuously formed from B during the adaptation period. In that case K_e would characterize the overall equilibrium between E and the pool of building blocks, i.e. it would apply to the reaction

$$B \rightleftharpoons E.$$

On this basis, K_e becomes applicable to the problem of competition between enzyme-forming systems (see below).

Table 1. *Relationship between galactose concentration and the amount of galactozymase formed when building blocks are restricted*

(The calculated values were obtained from Equation 14.)

Galactose concentration (%)	Activity of galactozymase	
	Experimental	Calculated
2	16	17
3	26	24
4	30	30
5	36	35
6	40	39
8	47	47

Returning to Equation (14), it can be seen that if e_a has been determined for one concentration of substrate s_a, then e_b can be calculated for any other value of S (s_b). The relationship between substrate

concentration and extent of adaptation given by this equation is a curve of the type shown in Fig. 3*B*.

The galactozymase formed in washed suspensions of *S. cerevisiae* was found to be related to the galactose concentration in this manner (Mandelstam & Yudkin, 1952*a*).

In Table 1 the theoretical amounts of enzyme calculated from (14) are compared with the experimental values.

Competition between enzyme-forming systems

Qualitative considerations. The formation of an adaptive enzyme may be retarded or reversed by the presence of the 'normal' substrate (Dienert, 1901; Stephenson & Yudkin, 1936). It has also been found that, in certain conditions, one enzyme may be synthesized at the expense of another (Spiegelman & Dunn, 1947). Monod (1947) has shown that if an organism, capable of utilizing two sugars, S_1 and S_2, is inoculated into a medium containing both, the type of growth curve observed depends on the nature of the relevant enzymes E_1 and E_2. If E_1 and E_2 are both 'adaptive' or both 'constitutive' the two sugars are metabolized simultaneously and there is a single growth phase. But if E_1 is 'constitutive' and E_2 'adaptive' there are two successive growth phases. In the first phase S_1 is selectively attacked until it is completely expended. There is then a lag period at the end of which S_2 is used. This effect, which Monod has called diauxie, is found even when the cells have been previously adapted to S_2.

Let us now examine the question of competition between enzyme-forming systems on the basis of the 'mass action' enzyme model. It is necessary to stress again that 'constitutive' and 'adaptive' enzymes are assumed to differ quantitatively only. By reference to Fig. 1 a number of deductions will be seen to follow directly.

(1) The enzymes E_1 and E_2 are in equilibrium with one another through B. If a building block, needed by both enzymes, is restricted and the organism is then forced to produce E_1 adaptively, it can only do so at the expense of E_2 and of the other proteins in equilibrium with B. The extent to which E_2 will be drawn upon, rather than Pr_1, Pr_2, etc., will depend on the equilibria involved. It also follows that this competitive effect would be reduced by providing more of the building block in question.

(2) If both S_1 and S_2 are present the relative amounts of E_1 and E_2 will depend, other things being equal, on the overall equilibria between these enzymes and the pool of building blocks. If E_1 and E_2 are both 'constitutive' or both 'adaptive' their equilibrium constants, and hence their concentrations, will be of the same order, and both substrates will be metabolized simultaneously. On the other hand, if E_1 is 'constitutive' and E_2 is 'adaptive', the former will be at an advantage in the competition for building blocks, and the concentration of E_2 will remain low. Even if the cells have been previously adapted to E_2, the addition of S_1 will establish a drain on B and hence on E_2, which will then become depleted.

(3) It has so far been assumed that S_1 and S_2 are related substances (e.g. sugars). From the model it is clear, however, that any two enzymes may become involved in competition for building material. On this basis it becomes possible to explain the fact that a carbohydrate may inhibit the formation of a proteolytic enzyme (for examples of this see Gale, 1943).

Competition between enzyme-forming systems. Quantitative formulation for non-growing organisms

For purposes of calculation the simplest case only will be taken. It will be assumed that (*a*), only one building block, Q, is the subject of competition between E_1 and E_2; (*b*), the full amount of Q that can be drawn upon is present from the start; (*c*), the reactions involved are unimolecular (or pseudo-unimolecular).

No restricting assumption is necessary as to the 'constitutive' or 'adaptive' nature of either E_1 or E_2, nor are the substrates necessarily related.

In the absence of both substrates the situation may be represented as follows:

$$E_2 \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} Q \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_1.$$

After addition of the substrates S_1 and S_2 we have

$$E_1 + S_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_1S_1 \xrightarrow{k_5} E_1 + \text{products}$$

$$e_1 - x_1 \quad s_1 \qquad\qquad x_1$$

$$k_1 \Big\| \; k_2$$

$$q \quad Q$$

$$k_1' \Big\| \; k_2'$$

$$E_2 + S_2 \underset{k_4'}{\overset{k_3'}{\rightleftharpoons}} E_2S_2 \xrightarrow{k_5'} E_2 + \text{products}$$

$$e_2 - x_2 \quad s_2 \qquad\qquad x_2$$

Under certain conditions (see below) a steady state will be reached in which both enzymes will be in equilibrium with Q. At this stage

$$k_1 q = k_2(e_1 - x_1).$$

But $$x_1 = \frac{e_1 s_1}{s_1 + K_{m_1}} \quad \text{and} \quad \frac{k_1}{k_2} = K_e,$$

and so $$q = \frac{e_1 K_{m_1}}{K_{e_1}(s_1 + K_{m_1})}. \qquad (15)$$

A similar equation holds for the second enzyme. Hence

$$\frac{e_1 K_{m_1}}{K_{e_1}(s_1+K_{m_1})} = \frac{e_2 K_{m_2}}{K_{e_2}(s_2+K_{m_2})},$$

or

$$\frac{e_1}{e_2} = \frac{K_{e_1} K_{m_2}(s_1+K_{m_1})}{K_{e_2} K_{m_1}(s_2+K_{m_2})}. \qquad (16)$$

We thus have an expression for the relative concentrations of the two enzymes in terms of their Michaelis and equilibrium constants and the concentrations of the substrates. The equation indicates that if s_2 is kept constant, the ratio e_1/e_2 will vary linearly with s_1.

Equation (16) can, however, be quantitatively applied only in restricted conditions, for it was derived on the assumption that both enzymes reach a steady state condition at the same time. In general, this is likely to occur much more slowly than when only one enzyme is involved. Thus, it is probable that one enzyme, E_1 say, will increase more rapidly and will reach a maximum first. The second enzyme, E_2, will then have to acquire material from a depleted pool of building blocks. Its formation will accordingly be retarded and may be very slow if much E_1 has already been formed. Nevertheless, E_2 will increase, removing further material from the pool, thereby upsetting the equilibrium with E_1 which will then be diminished. Consequently, the ratio e_1/e_2 will vary continuously, but will tend to approach a limiting value defined by Equation (16). The greater the concentration of the substrates, and the greater, therefore, the adaptive response, the greater will be the depletion of building material and the more slowly will the limiting value be approached.

Bearing these considerations in mind, the following deductions can still be made. In an equimolecular mixture of S_1 and S_2, and assuming K_{m_1} and K_{m_2} to be of the same order, it follows that the ratio e_1/e_2 will depend on the ratio K_{e_1}/K_{e_2}. When E_1 is 'adaptive' and E_2 is 'constitutive' e_1/e_2 will be very small because K_{e_1}/K_{e_2} is very small (certainly not greater than 10^{-2}, and in most instances probably far less). It is clear that s_1/s_2 has to be made very large before any measurable adaptation can occur. If S_2 is present in any reasonable quantity, S_1 would have to be raised to a concentration beyond the physiological limits of the organism. In other words S_2, the 'normal' substrate, acts as an efficient inhibitor of adaptation.

Under these conditions, i.e. when one enzyme is 'adaptive' and the other 'constitutive', Equation (16) has no practical value. If, however, E_1 and E_2 are both 'adaptive' (K_{e_1} and K_{e_2} are of the same order) it will be possible to obtain measurable variations in e_1/e_2 by altering the relative concentrations of the two substrates. An increase in s_1/s_2 should cause an increase in e_1/e_2.

These predictions were shown to hold for the maltozymase and galactozymase systems of *S. cerevisiae* (Mandelstam & Yudkin, 1952*a*). Thus, it was found that the observed variations in e_1/e_2 corresponded with those calculated from Equation (16) provided that the concentrations of the sugars were low. Furthermore, an increase in the ratio of galactose to maltose produced a linear increase in the ratio of the enzymes.

SUMMARY

1. A model of enzyme adaptation by 'mass action' is proposed mainly on the assumption that enzymes and other proteins are in equilibrium with one another through a common pool of building material.

2. Qualitative and quantitative predictions have been made for the rate of adaptation, the relationship between the concentration of the substrate and the extent of adaptation, and competition between enzyme-forming systems.

3. The conformity of the predictions with experimental results has been shown in experiments described in the two following papers.

4. The extended 'mass action' theory developed in this paper is at this stage presented as no more than a working hypothesis, developed from a limited set of assumptions, to account for the main facts of enzyme adaptation in both animals and micro-organisms. It is not claimed that the conformity of experimental results with the theoretical predictions is a 'proof' of the theory, to the exclusion of other possible explanations.

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Studies in Biochemical Adaptation. The Effect of Variation in Dietary Protein upon the Hepatic Arginase of the Rat

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Much of the recent work on enzyme adaptation has been carried out in micro-organisms. Relatively little has been reported on enzyme adaptation in mammals and further work on this subject is desirable. In particular, a quantitative study would be of interest as a test of the predictions made from the extended mass action theory, which should apply to enzyme adaptation in animals as well as in micro-organisms (Mandelstam, 1952).

The enzyme chosen for study was hepatic arginase in the rat and the effect was investigated of variation in dietary protein.

Previous work in this field is inconclusive. Baldwin (1935) reported that there seemed to be a decrease in arginase in the hepatopancreas of the snail during starvation; Baldwin & Yudkin (1939), however, found such a great variation in the activity of the enzyme in different specimens that it was not possible to draw any definite conclusion concerning the effect of starvation. Seifter, Harkness, Rubin & Muntwyler (1948) reported a decrease in hepatic arginase in rats fed on a protein-free diet for 1–3 weeks. Lightbody & Kleinman (1939) found that in rats fed on diets containing 6, 25, 60 and 75% protein, the amount of enzyme in unit weight of liver was higher with higher amounts of dietary protein. It is possible that the rise in enzyme was due, at least in part, to a general increase in hepatic protein which was not estimated. Folley & Greenbaum (1946) found, with rats, that a diet containing 50% protein resulted in a higher concentration of hepatic arginase than one containing 20% protein though, with the small number of animals studied, the difference was not statistically significant. Kochakian, Bartlett & Moe (1948) estimated hepatic arginase in rats fed on a diet containing either no protein or 80% protein. After 7 days, there was a fall in the arginase in the former, partly due to a general decrease in hepatic protein, and a slight rise in the animals fed on the high protein diet, which the authors do not consider noteworthy.

Hepatic arginase is of interest in relation to the theory of Krebs & Henseleit (1932) that urea is formed in the liver through a series of reactions involving this enzyme. Although this theory has been the subject of some criticism (e.g. Bach, 1939; Trowell, 1942), it is now accepted by the majority of workers. An increase in dietary protein, and so an increase in production of urea, might well, therefore, cause an increase in the arginase involved in the process. The general problem of the use of enzyme adaptation in studies of metabolic pathways is dealt with elsewhere (see, for example, Yudkin, 1952; Davies & Yudkin, 1951).

EXPERIMENTAL

Animals. The rats were bred in this laboratory and were of an albino strain which we have elsewhere designated as *KC*1 (Wiesner & Yudkin, 1951). Preliminary tests showed that male animals have some 15% more hepatic arginase than female animals. In the detailed experiments to be reported, only male animals were used.

Diet. From weaning to the beginning of the experiment, the animals were fed a mixed diet of cubes with additional milk and green vegetables (see Wiesner & Yudkin, 1951). This diet contains approx. 20% protein. The experimental diets containing varying amounts of protein were made according to Table 1. Animals were distributed so that one from each litter was given each of these purified diets. Food and water were given *ad lib.* The animals were weighed twice weekly.

Estimation of arginase. The animals were killed by a blow on the head and the whole liver removed and weighed. Two samples, each of about 20 mg., were accurately weighed on a glass cover-slip. Nitrogen was estimated in these samples by the Kjeldahl method. For the estimation of arginase, about 0·5 g. of liver was accurately weighed and homogenized in a Waring Blendor with 300 ml. distilled water for 90 sec. Into a Warburg cup were placed 1 ml. liver homogenate,