136. *On the Chemical Kinetics of Autosynthetic Systems.*

By Sir Cyril N. Hinshelwood.

A general treatment is given of the chemical kinetics of certain types of co-ordinated reactions. It is shown that where several components are synthesised by the use of mutually supplied intermediates there is a steady state in which each separate component increases according to the simple exponential law of autosynthesis. Before the steady state is reached there may be lag phases, or even periodic variations in rate. The conditions under which the kinetic equations can be applied to material subject to cell division are considered. Changes of proportions accompanying transfer to new media or to media containing inhibitors are shown to be possible, and the rate and relative permanence of these adaptive changes are discussed.

The relation of the equations derived to the phenomena shown by living cells is indicated, and a hypothesis about the mode in which new enzymes may arise in response to new substrates is outlined.

Masses of living matter reduplicate themselves by processes involving an elaborate co-ordination of enzyme reactions. Quite apart from their biological significance such systems present problems which are novel and important from the point of view of chemical kinetics generally. Various kinetic propositions about autosynthetic mechanisms have been formulated from time to time in connexion with experimental studies of bacterial growth made in this laboratory, but they have been published in various places and sometimes in biological journals. The object of this paper is partly to collect and systematise some of these propositions, partly to elaborate and extend them, and partly to indicate the chemical interest of the biological facts without any obscuring of the issues by bacteriological technicalities.

Equations of Autosynthesis.—In constant surroundings living matter such as that contained in a unicellular organism reproduces all its parts according to the equation

$$\mathrm{d}X/\mathrm{d}t = kX \text{ or } X = X_0\mathrm{e}^{kt} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

This occurs by the interplay of enzyme reactions. Yet enzymes in isolation do not increase autocatalytically, so the origin of equation (1) is of interest.

Suppose we have two enzymes each of which increases its substance by the addition of something derived from the working of the other. Then we shall have

$$\mathrm{d}X/\mathrm{d}t = \alpha Y \text{ and } \mathrm{d}Y/\mathrm{d}t = \beta X \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The solutions of these equations are

$$X = P_1\mathrm{e}^{kt} + Q_1\mathrm{e}^{-kt} \text{ and } Y = P_2\mathrm{e}^{kt} + Q_2\mathrm{e}^{-kt} \quad . \quad . \quad . \quad . \quad (3)$$

where $P_1 + Q_1 = X_0$ and $P_2 + Q_2 = Y_0$, X_0 and Y_0 being the amounts of X and Y at time zero.

It follows that

$$X = \tfrac{1}{2}\left(X_0 + \frac{\alpha}{k}Y_0\right)\mathrm{e}^{kt} + \tfrac{1}{2}\left(X_0 - \frac{\alpha}{k}Y_0\right)\mathrm{e}^{-kt} \quad . \quad . \quad . \quad . \quad (4a)$$

$$Y = \tfrac{1}{2}\left(Y_0 + \frac{k}{\alpha}X_0\right)\mathrm{e}^{kt} + \tfrac{1}{2}\left(Y_0 - \frac{k}{\alpha}X_0\right)\mathrm{e}^{-kt} \quad . \quad . \quad . \quad . \quad (4b)$$

where $\alpha\beta = k^2$.

When t is sufficiently great the terms in e^{-kt} vanish and the ratio X/Y assumes the constant value $\left(X_0 + \frac{\alpha}{k}Y_0\right)\Big/\left(Y_0 + \frac{k}{\alpha}X_0\right) = \alpha/k$. If now a portion of the system in which this constant ratio is established is separated and becomes the starting point of

a new autosynthetic system (as when bacteria are subcultured), $X_0/Y_0 = \alpha/k$. In these circumstances (4*a*) and (4*b*) reduce simply to

$$X = X_0 e^{kt} \text{ and } Y = Y_0 e^{kt}$$

and each separate component increases with time as though it were formed by the simple autocatalytic law (1).

It is of interest to note that this result holds generally when the interplay is between more than two components. For the case of three we have

$$dX/dt = \alpha Y, \quad dY/dt = \beta Z, \quad dZ/dt = \gamma X \qquad . \; . \; . \; . \; (5)$$

Differentiation shows that $d^3X/dt^3 = \alpha\beta\gamma X = k^3X$, with similar expressions in Y and Z. The solutions are of the form

$$X = P_1 e^{kt} + Q_1 e^{\mu_1 kt} + R_1 e^{\mu_2 k}$$

where $P_1 + Q_1 + R_1 = X_0$ and 1, μ_1, and μ_2 are the three cube roots of unity, so that

$$\mu_1 = -\tfrac{1}{2} + i\frac{\sqrt{3}}{2} \text{ and } \mu_2 = -\tfrac{1}{2} - i\frac{\sqrt{3}}{2}$$

Evaluation of the constants gives

$$X = \tfrac{1}{3}\left(X_0 + \frac{\alpha}{k}Y_0 + \frac{\alpha\beta}{k^2}Z_0\right)e^{kt} + \tfrac{1}{3}\left(X_0 + \mu_2\frac{\alpha}{k}Y_0 + \mu_1\frac{\alpha\beta}{k^2}Z_0\right)e^{\mu_1 k} + \tfrac{1}{3}\left(X_0 + \mu_1\frac{\alpha}{k}Y_0 + \mu_2\frac{\alpha\beta}{k^2}Z_0\right)e^{\mu_2 k} \qquad . \; . \; (6a)$$

$$Y = \tfrac{1}{3}\left(\frac{k}{\alpha}X_0 + Y_0 + \frac{\beta}{k}Z_0\right)e^{kt} + \tfrac{1}{3}\left(\mu_1\frac{k}{\alpha}X_0 + Y_0 + \mu_2\frac{\beta}{k}Z_0\right)e^{\mu_1 kt} + \tfrac{1}{3}\left(\mu_2\frac{k}{\alpha}X_0 + Y_0 + \mu_1\frac{\beta}{k}Z_0\right)e^{\mu_2 k} \qquad . \; . \; (6b)$$

$$Z = \tfrac{1}{3}\left(\frac{k^2}{\alpha\beta}X_0 + \frac{k}{\beta}Y_0 + Z_0\right)e^{kt} + \tfrac{1}{3}\left(\mu_2\frac{k^2}{\alpha\beta}X_0 + \mu_1\frac{k}{\beta}Y_0 + Z_0\right)e^{\mu_1 kt} + \tfrac{1}{3}\left(\mu_1\frac{k^2}{\alpha\beta}X_0 + \mu_2\frac{k}{\beta}Y_0 + Z_0\right)e^{\mu_2 kt} \qquad . \; . \; (6c)$$

From these three equations it is easily verified by substitution that the values when $t = 0$ are X_0, Y_0, and Z_0 respectively, and that the original first-order differential equations are satisfied.

When t becomes great the terms in $e^{\mu_1 kt}$ and $e^{\mu_2 kt}$ vanish. We then have constant ratios of X, Y, and Z. For example in these circumstances

$$X/Y = \left(X_0 + \frac{\alpha}{k}Y_0 + \frac{\alpha\beta}{k^2}Z_0\right)\Big/\left(\frac{k}{\alpha}X_0 + Y_0 + \frac{\beta}{k}Z_0\right) = \alpha/k$$

Also $$X/Z = \alpha\beta/k^2$$

Once again we see that if some of the material is used to start a new system in which X_0, Y_0, and Z_0 are in this proportion, then each of the components continues to follow equation (1), the terms in $e^{\mu_1 kt}$ and $e^{\mu_2 kt}$ now vanishing throughout, since $\mu_1 + \mu_2 = -1$.

For the purpose of what is to follow later it will be convenient to give the solutions for the case of four interdependent components, namely, where

$$dX_1/dt = \alpha X_2, \; dX_2/dt = \beta X_3, \; dX_3/dt = \gamma X_4, \; dX_4/dt = \delta X_1$$

We have $d^4X_1/dt^4 = \alpha\beta\gamma\delta X_1$, where $\alpha\beta\gamma\delta = k^4$. A solution is $X_1 = A_1 e^{\rho kt}$, where ρ is one of the four fourth roots of unity. The general solutions are

$$X_1 = A_1 e^{kt} + B_1 e^{-kt} + C_1 e^{ikt} + D_1 e^{-ikt} \qquad . \; . \; . \; . \; . \; (7)$$

with corresponding expressions for X_2, X_3, and X_4. It is further found by introduction of the values for $t = 0$ that

$$\begin{aligned} A_1 &= \tfrac{1}{4}\{(X_1)_0 + (X_2)_0/a + (X_3)_0/b + (X_4)_0/c\} \\ B_1 &= \tfrac{1}{4}\{(X_1)_0 - (X_2)_0/a + (X_3)_0/b - (X_4)_0/c\} \\ C_1 &= \tfrac{1}{4}\{(X_1)_0 - i(X_2)_0/a - (X_3)_0/b + i(X_4)_0/c\} \\ D_1 &= \tfrac{1}{4}\{(X_1)_0 + i(X_2)_0/a - (X_3)_0/b - i(X_4)_0/c\} \end{aligned}$$

where $a = k/\alpha$, $b = k^2/\alpha\beta$, and $c = k^3/\alpha\beta\gamma$.

Further,

$$A_1 : A_2 : A_3 : A_4 = 1 : a : b : c,\ B_1 : B_2 : B_3 : B_4 = 1 : -a : b : -c,$$
$$C_1 : C_2 : C_3 : C_4 = 1 : ia : -b : -ic,\ D_1 : D_2 : D_3 : D_4 = 1 : -ia : -b : ic$$

Once again we find that when t is large the ratios of X_1, X_2, X_3, and X_4 become independent of time and are, in fact, $1 : a : b : c$. Each component, moreover, now follows a law of the simple form

$$(1/X_n)\mathrm{d}X_n/\mathrm{d}t = k$$

The result is, in fact, general, since if we have a system of r interlinked components we obtain a differential equation of the rth order for each, $\mathrm{d}^rX/\mathrm{d}t^r = kX$. The general solution will be the sum of r terms of the type $Ae^{\rho kt}$, where ρ is the complex rth root of unity. These rth roots form a geometrical series, and only the term in e^{kt} remains of importance when the time is sufficiently great.

The results of this section show, therefore, that non-autosynthetic enzyme systems can combine in such a way that each does in fact in the steady state increase according to the autosynthetic law.

The kinds of equation discussed probably apply with special importance to the interplay of nucleic acids and proteins in cells, each of these constituents appearing to play a decisive part in directing the synthesis of the other (cf. Malmgren and Hedén, *Acta Path. Microbiol. Scand.*, 1947, **24**, 448; Caldwell and Hinshelwood, *J.*, 1950, 3156).

Behaviour before the Steady State is reached: Lag Phenomena.—The sets of equations (4), (6), and (7) have further properties of great interest in connexion with the properties of living cells. We will first consider the case where t is small so that the steady state is not reached, and where the initial proportions of the components are arbitrary. This would correspond to the transfer of a small amount of material which had reached a steady state in one environment into another where the relevant rate constants were different and where a different steady state would finally be attained.

It will first be convenient to transform the equations of the sets (6) and (7) so as to remove the imaginary quantities, as can be done in the standard way by the introduction of the exponential values for sine and cosine. Equation (6*a*) then becomes

$$X = \tfrac{1}{3}\left(X_0 + \frac{\alpha}{k}Y_0 + \frac{\alpha\beta}{k^2}Z_0\right)e^{kt} + \tfrac{1}{3}e^{-\frac{1}{2}kt}\left\{\left(2X_0 - \frac{\alpha}{k}Y_0 - \frac{\alpha\beta}{k^2}Z_0\right)\cos\frac{\sqrt{3}}{2}kt + \sqrt{3}\left(\frac{\alpha}{k}Y_0 - \frac{\alpha\beta}{k^2}Z_0\right)\sin\frac{\sqrt{3}}{2}kt\right\} \quad . \quad . \quad (8a)$$

and equation (6*b*) becomes

$$Y = \tfrac{1}{3}\left(\frac{k}{\alpha}X_0 + Y_0 + \frac{\beta}{k}Z_0\right)e^{kt} + \tfrac{1}{3}e^{-\frac{1}{2}kt}\left\{\left(2Y_0 - \frac{k}{\alpha}X_0 - \frac{\beta}{k}Z_0\right)\cos\frac{\sqrt{3}}{2}kt + \sqrt{3}\left(\frac{\beta}{k}Z_0 - \frac{k}{\alpha}X_0\right)\sin\frac{\sqrt{3}}{2}kt\right\} \quad . \quad . \quad (8b)$$

with an analogous expression for Z.

The equations of the set (7) are similarly transformed, the value for X_1 being typical of the expressions found, *i.e.*,

$$\begin{aligned} X_1 = {} & \tfrac{1}{4}\{(X_1)_0 + (X_2)_0/a + (X_3)_0/b + (X_4)_0/c\}e^{kt} \\ & + \tfrac{1}{4}\{(X_1)_0 - (X_2)_0/a + (X_3)_0/b - (X_4)_0/c\}e^{-kt} \\ & + \tfrac{1}{4}\{2(X_1)_0 - 2(X_3)_0/b\}\cos kt + \tfrac{1}{4}\{2(X_2)_0/a - 2(X_4)_0/c\}\sin kt \quad . \quad . \quad (9a) \end{aligned}$$

The equations of the sets (8) and (9) show that until the first and major exponential term predominates the trigonometrical terms will give rise to fluctuations in the rates of increase. If, for example, we start with Y_0 and Z_0 zero in (8*a*) or with all the values save that of X_1 zero in (9*a*) then, superposed on the general exponential increase of X (or X_1) there will be a periodic variation. In the actual cases considered this effect is not very important since the amplitudes of the sine and cosine functions are small compared with the rates of increase of the terms in e^{kt}, but the principle is significant. In actual fact when a bacterial culture is prepared in one medium and a small portion of it is transferred to a completely different medium it is often a long time before growth settles down to follow the simple exponential law. In the initial stages there are often very irregular rates of increase in mass with curious bursts of growth and arrests, indicating the non-existence of a harmonious co-ordination of the various reactions involved.

Of more general importance, however, than these small-scale irregularities is the existence of a considerable lag which may precede the establishment of the steady state. This, of course, corresponds to the period of delay which is almost always observed when a bacterial culture is sampled and transferred to new surroundings.

The principle is evident from an inspection of equation (4*a*). If we start with Y_0 equal to zero then

$$X = \tfrac{1}{2}X_0(e^{kt} + e^{-kt})$$

Expansion of the exponential terms leads to the expression

$$X = X_0(1 + \tfrac{1}{2}k^2t^2 + \ldots .) \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

the terms in the first power of t having vanished. Thus the *initial* slope of the curve of X against t is *zero*, and there is a delay in the establishment of the normal rate of increase. The delay increases in an interesting way as the complexity of the interlocking of the various systems increases. With the three-component system we have from (8*a*) when Y_0 and Z_0 are zero

$$X = \tfrac{1}{3}X_0\left(e^{kt} + 2e^{-\frac{1}{2}kt}\cos\frac{\sqrt{3}}{2}kt\right)$$

and when the exponential and cosine terms are expanded in powers of t this gives

$$X = X_0\left(1 + \frac{1}{6}k^3t^3 + \ldots .\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

where not only the first but also the second differential coefficient of X with respect to the time vanishes.

Equation (9*a*) with all the initial values zero save that of X_1 gives

$$X_1 = (X_1)_0\left\{1 + \frac{1}{24}k^4t^4 + \ldots .\right\} \quad . \quad . \quad . \quad . \quad . \quad (12)$$

where the first three differential coefficients vanish at $t = 0$.

From (10), (11), and (12) we can infer that for the case of n interdependent components the corresponding expression would be

$$X = X_0\{1 + (kt)^n/n! + \ldots .\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

according to which the rate would rise very slowly to its steady value when n is considerable.

The general form (13) can be derived directly as follows. With the n components we shall have differential equations of the form $d^nX/dt^n = k^nX$. The solution of this is of the type

$$X_1 = A_1e^{kt} + B_1e^{rkt} + C_1e^{r^2kt} + \ldots . Pe^{r^{n-1}kt}$$

where $1, r, r^2 \ldots r^{n-1}$ are the nth roots of unity, which are known to form a series in

geometrical progression. The constants A_1 . . . etc., are linear functions of $(X_1)_0$, etc., so that we have, when the initial values of all the components save X_1 are zero

$$X_1 = \frac{(X_1)_0}{n}\{e^{kt} + e^{rkt} + \ldots . + e^{r^{n-1}kt}\}$$

The successive exponential terms can be expanded, and the coefficients of kt found. That of kt is $(1 + r + r^2 + \ldots + r^{n-1}) = (1 - r^n)/(1 - r)$ which is zero since $r^n = 1$. Similarly that of $(kt)^{n-1}$ is found to be zero. That, however, of $(kt)^n$ is

$$(1/n!)(1 + r^n + r^{2n} + \ldots . + r^{(n-1)n})$$

which is

$$\frac{1}{n!}\left\{\frac{1 - (r^n)^n}{1 - r^n}\right\} = \frac{1}{n!}\left\{\frac{1 - p^n}{1 - p}\right\} = \frac{1}{n!}(1 + p + p^2 \ldots . + p^{n-1}) = \frac{n}{n!}$$

since $p = r^n = 1$. Thus

$$X_1 = (X_1)_0\frac{1}{n}\left\{n + \frac{n(kt)^n}{n!} + \ldots .\right\} = (X_1)_0\{1 + (kt)^n/n! + \ldots .\}$$

as in (13).

The general conclusion to be drawn from the above is that the more complex the interdependence of the various processes the longer will be the time required for the establishment of the steady state where the simple exponential law of autocatalytic growth is obeyed. The presumed complexity of the interdependence in living cells readily explains the pronounced tendency for lag phases to declare themselves in the phenomena of bacterial growth. It also appeared from the earlier part of this section that when systems are badly out of adjustment more complicated irregularities such as arrests and accelerations of growth are in principle possible, though the simplified nature of the models here considered hardly does justice to the real examples encountered. A rather more specialised theory on the same general lines as the above and in which very long lags may attend the interdependence of two processes only has been given already (Hinshelwood, " Chemical Kinetics of the Bacterial Cell," Oxford, 1946, p. 82).

Cell Division and Its Introduction into Kinetic Treatments of Growth.—Living matter displays its characteristic properties essentially when organised in cells. When cells have grown beyond a certain size they normally divide. This process is a physicochemical necessity for the maintenance of approximately constant conditions of concentration, even with a standard external medium, since gains and losses from the cell depend upon its surface area while the sum of its synthetic reactions is a function of its volume. It might be thought that the kinetic treatment of cell reactions would demand a detailed hypothesis about the mechanism of cell division, but this is not so, at least for many purposes.

The cell may be imagined to divide when some structure within it exceeds a critical size for stability, or alternatively when the concentration of some active substance within it reaches a critical value. In either case the condition will be that the amount of some cellular component attains a more or less standard value. Such an assumption is strongly suggested by the finding that, for example, the amount of deoxyribose nucleic acid per cell is very nearly constant despite wide variations of growth conditions, but there is no very urgent need to specify the substance or component in question. Suppose, for example, that some concentration, c, within the cell must reach a value c_1 for division to occur. If x is the amount of the enzyme system producing this, then we shall have $dc/dt = ax - bc$, where a and b are constants in a given set of circumstances. This equation expresses the fact that the rate of loss of the active substance, whether by diffusion or by consumption in chemical change, is proportional to c. A steady state will be very rapidly set up in a cell so that $dc/dt = 0$, *i.e.*, so that $c = kx$, and the critical concentration c_1 will correspond to a critical amount x_1 per cell of the enzymic component, just as it would if the condition were an unstable critical size of this component itself.

Without, therefore, making specific assumptions about the precise mechanism of division, we may take cognisance of its occurrence and necessity in the following way. We

represent the *total* amounts, not per cell but in the whole mass of matter present, of the various constituents by X, Y, and so on. The corresponding amounts per cell are x, y, and so on, which are respectively X/n, Y/n . . . , where n is the number of cells. One of the constituents, for example Y, fills the rôle considered above, a critical value y_1 determining the division. Then we shall have y_1 which is proportional to Y/n as a constant, *i.e.*, $Y/n = \beta$.

We now have $(1/n)\mathrm{d}n/\mathrm{d}t = (1/Y)\mathrm{d}Y/\mathrm{d}t$ as an invariant condition. When a steady state prevails $(1/X)\mathrm{d}X/\mathrm{d}t = (1/Y)\mathrm{d}Y/\mathrm{d}t = \ldots$, but when it does not, as in the vitally important periods of adjustment to new environmental conditions, it is only the Y increase which is directly correlated with that of n.

This principle will be illustrated by a discussion of adaptive changes in the proportions of the components of autosynthetic systems in various circumstances.

Adaptive Phenomena.—One of the most remarkable properties of bacterial cells is their power of adapting themselves to resist the action of toxic drugs or to utilise new substrates. Without discussion of the biological aspects of these effects it will be shown that autosynthetic systems of the kind described above show these characteristics. The treatment given goes beyond that given in previous papers (Davies, Hinshelwood, and Pryce, *Trans. Faraday Soc.*, 1944, **40**, 412; Hinshelwood and Lewis, *Proc. Roy. Soc.*, 1948, *B*, **135**, 316) in dealing with the questions of the rate of adaptation, the stability of the adapted state, and the mode in which changed substrates induce the adaptive change.

(a) *Resistance to inhibitors.* In the kind of autosynthetic system present in a living cell there is a sequence of enzyme processes each dependent upon material derived from a previous one. Suppose two of the enzymes (or mutually interlocked sets of enzymes) are present in total amounts X and Y. Let the increase in Y be dependent upon the concentration of something derived from the X-set in such a way that we have the equations

$$(1/X)\mathrm{d}X/\mathrm{d}t = k_1 \text{ and } \mathrm{d}Y/\mathrm{d}t = \alpha c Y,$$

where c is the concentration in question. By the principle explained in the last section, c is proportional to the amount x_1 per cell, *i.e.*, to X/n, so that

$$\mathrm{d}Y/\mathrm{d}t = k\alpha XY/n$$

Let Y be the constituent whose amount determines division, so that, by the principle explained above, $Y/n = y_1 = \beta$, a constant. Thus we have

$$X = X_0 e^{k_1 t} \text{ and } \mathrm{d}Y/\mathrm{d}t = k\alpha\beta X$$

whence by integration

$$Y - Y_0 = \frac{k\alpha\beta X_0}{k_1}\{e^{k_1 t} - 1\} = \frac{k\alpha\beta}{k_1}(X - X_0) \quad . \quad . \quad . \quad . \quad (14)$$

X_0 and Y_0 being the amounts at time zero.

When X and Y are large compared with X_0 and Y_0, (14) gives

$$X/Y = x_1/y_1 = k_1/k\alpha\beta = \chi$$

The ratio settles down to the constant value χ, which gives the value of X_0/Y_0, or of x_1/y_1, for a new series of observations made after a small sample of the original material is transferred to a new medium.

Suppose such a transfer is made into a medium containing an inhibitor which in some way reduces the rate of synthesis of the Y system. This means a reduction of k to k' (or an equivalent change in α). Initially the rate of synthesis of the Y system, which determines the increase in the cell number n, is reduced, since $(1/Y_0)\mathrm{d}Y/\mathrm{d}t = k'\alpha x_1$, which is less than the previous value in the ratio k'/k. After continued growth in the new conditions, however, x_1 increases to x_1', since x_1/y_1 changes to χ' which is $k_1/k'\alpha\beta$, and y_1 by hypothesis is invariant. Thus x_1' is finally greater than x_1 in the same ratio as k' is less than k. Thus the final value after continued growth is $(1/Y)\mathrm{d}Y/\mathrm{d}t = k'(k/k')\alpha x_1$,

which is the original value once more. There is thus an adaptive change in the cell proportions which makes the final multiplication rate independent of the inhibitor.

Whenever the stable ratio of X to Y is established, it follows from (14) that $(1/n)\mathrm{d}n/\mathrm{d}t = (1/Y)\mathrm{d}Y/\mathrm{d}t = k_1 = (1/X)\mathrm{d}X/\mathrm{d}t$.

Examples of the complete adaptation of a bacterial culture to resist an inhibitory drug are quite common, and will not be discussed here, the present object being the establishment of the principles by which the kinetic calculations can be made.

(b) *Rate of development of resistance.* We transfer a sample of the material where $X/Y = \chi$ to a new medium where, since k' is less than k, the initial rate of growth is slower, and where the final stable value of X/Y will have to rise to χ' before adaption is complete. X_0/Y_0 will be χ, and *not* χ'. From (14) we have

$$\frac{X}{Y} = \frac{X_0 e^{k_1 t}}{Y_0 + \frac{k'\alpha\beta}{k_1}\{e^{k_1 t} - 1\}}$$

$$= \frac{\chi e^{k_1 t}}{1 + \frac{\chi}{\chi'}\{e^{k_1 t} - 1\}} \quad \text{since } X_0 = \chi Y_0 \quad . \quad . \quad . \quad . \quad . \quad (15)$$

When $t = 0$ this expression is χ, and when t is infinite it is χ'. The ratio approaches its limiting value, however, as soon as $e^{k_1 t}$ becomes substantially greater than unity. This means that a very considerable degree of adaptation occurs quite rapidly. The completion of the process, however, may require a much longer, and in theory an infinite, time.

This result corresponds to what is observed, where the initial stages of adaptive processes are often very rapid, but their completion may demand a great number of subcultures of the organism in the new medium.

(c) *Stability of resistance to inhibitors.* This is a complex problem, and has hitherto occasioned some difficulty in its interpretation.

Stability is judged in the following way. The growing material is adapted to a new medium (containing the inhibitor) : a sample of it is then re-transferred to the original medium where further growth is allowed to take place for a determined time. The material is then tested once more for its growth rate in the medium containing the inhibitor. To varying degrees it displays an apparent memory for its previously induced power to resist the action of the adverse environment. The complete picture of the behaviour is rather a complex one. Sometimes the reversion to the unadapted state is rapid, sometimes very slow. We shall now consider the explanation of these phenomena in terms of the model which has been developed.

According to equation (15), the re-transfer of adapted material to its original environment would result in a change of χ back to the old value at about the rate of the forward change. Thus, further tests in presence of inhibitor would show that a rapid reversion had taken place. This, in fact, is what is often observed with bacterial cultures which have just become nearly but not very thoroughly adapted to a changed environment, so that to this extent the prediction of the model system corresponds to the reality.

But there is often a state of what may be termed metastable adaptation where the material preserves its newly acquired property through many generations of culture in the original medium removed from the inducing agent, displaying, as it were, memory of its first training. This happens usually only when the initial process of adaptation has been carried on for a long time and reached completion.

(d) *Slowness of reversion.* To account for this we must reconsider a previous assumption, namely, that the rate of functioning of the Y-enzyme in section (a) is directly proportional to the concentration, c, of the active intermediate derived from the enzyme system which supplies it. In general, the rate will be governed by an adsorption isotherm, and should be set proportional, not simply to c, but to $c/(a + c)$, *i.e.*, should follow a Langmuir isotherm, or some similar equation. This expression gives simple proportionality to c when c is small, but becomes independent of it when c is great enough.

Now the two cases of adaptation to the inhibitor and reversion to the original state in its absence differ in one important respect. In the adaptive process c will have been reduced to a low value and gradually returns to a normal value. Thus it is quite reasonable to assume, as has been done so far, that the rate of functioning of the Y-system is directly proportional to c. In the reversion, x_1 and, therefore, c start at a greatly enhanced value for the original medium and should gradually fall. But when c is great, the enzyme system Y may well be nearly saturated with the intermediate, so that the rate of formation of Y does not show any marked increase. In such circumstances reversion may be very slow. As an approximate representation of the adsorption isotherm function $c/(a + c)$ when c is great we may write proportionality to $c^{1/p}$, where p is a number greater than unity. The nearer to saturation, the greater will be the effective value of p.

The rate of functioning of the Y-enzyme system will now be proportional, not to x_1, but to $(x_1)^{1/p}$, and the equations of sections (a) and (c) assume the following forms :

$$\begin{aligned} \mathrm{d}Y/\mathrm{d}t &= k\alpha {x_1}^{1/p} Y = k\alpha X^{1/p} Y/n^{1/p} = k\alpha X^{1/p}(Y/n)^{1/p} Y^{1-1/p} \\ &= k\alpha\beta' X^{1/p} Y^{1-1/p}, \text{ where } \beta' = (Y/n)^{1/p} \\ \mathrm{d}X/\mathrm{d}t &= k_1 X \end{aligned}$$

whence by integration we obtain a modified form of equation (14), namely,

$$\frac{X}{Y} = \frac{(X_0/Y_0)\mathrm{e}^{k_1 t}}{\left\{1 + \dfrac{k\alpha\beta'}{k_1}\left(\dfrac{X_0}{Y_0}\right)^{1/p}(\mathrm{e}^{k_1 t/p} - 1)\right\}^p} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

When t is very great this expression assumes the value $(k_1/k\alpha\beta')^p = \chi_1$.

If the material has undergone an adaptive change, χ_1 will change to a new value χ_1' and the reversion from the latter to the former on re-transfer to the original medium will be governed by equation (16) with $X_0/Y_0 = \chi_1'$, *i.e.*, $(k_1/k'\alpha\beta')^p$. Thus we shall have

$$\frac{X}{Y} = \frac{\chi_1' \mathrm{e}^{k_1 t}}{\left\{1 + \left(\dfrac{\chi_1'}{\chi_1}\right)^{1/p}(\mathrm{e}^{k_1 t/p} - 1)\right\}^p} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (17)$$

For the reversion to be appreciable $\mathrm{e}^{k_1 t/p}$ must now considerably exceed unity, and if p is great enough this may require very large times indeed, for in the limit, where the active intermediate produced by X entirely saturates Y in normal conditions, p approaches infinity and the reversion becomes infinitely slow.

Approaches to this case are actually encountered. Sometimes, adapted bacterial cultures survive hundreds of generations in their original medium without losing the power of rapid growth in a second medium to which they had become acclimatised. But their state is essentially not one of true stability since reversion usually occurs in the long run, or may be induced by special means.

(e) *Dependence of reversion on the thoroughness of the previous adaptation.* The following typical set of facts illustrates the problem to be considered. If certain bacteria have just been adapted to resist a drug, then cultivation in the absence of the drug causes rapid loss of resistance, as shown in further tests with the drug restored to the medium. If, however, the adaptation has been long continued, then the resistance is more nearly permanent. It is not the object of this paper to discuss alternative biological explanations of this behaviour, but to show in what circumstances the kinetic model under investigation would predict it.

We still envisage the two-enzyme systems X and Y of the previous paragraphs. By the equations already given we have

$$\mathrm{d}X/\mathrm{d}t = k_1 X \text{ and } \mathrm{d}Y/\mathrm{d}t = \alpha c Y = k_2 Y$$

For a steady state $k_1 = k_2$: otherwise the ratio of X to Y changes, and at a rate which depends upon the difference between k_1 and k_2. The amount of X per cell, x_1, adjusts itself in the process of adaptation as has been shown. If αc is lowered by any agency then,

as shown, x_1 increases and causes a rise in c which in turn increases k_2 till k_1 and k_2 can once more be equal. Suppose in the normal state the Y-system is nearly but not quite saturated with the intermediate, and suppose that the inhibitor somehow interferes with the supply and reduces c. Adaptation raises c from the lowered value c' to the normal value c, *i.e.*, from well below the value required to saturate the Y-system nearly to this value again. The Y-system responds. Now if the inhibitor is removed, the increased proportion of x_1 raises c to a new value c'', which corresponds more nearly to saturation than did the original value c. Thus k_2 now exceeds k_1, and the ratio of X/Y regresses, *i.e.*, reversion occurs. But the process may be quite slow (see last section) if we are near enough to saturation for the increase in k_2 to be small.

So far we have considered two consecutive sets of enzymes, but cells are much more elaborate organisations. Although we have assumed αc to suffer the main effect of the inhibitor, the relations of all sorts of other cell processes may have been affected in a secondary degree, and in the course of adaptation numerous secondary adjustments are likely. Suppose now that, although the main effect of the inhibitor is on k_2, yet k_1 also suffers some small reduction. The adaptive change which leads to the restoration of k_1 can be very slow, partly because the reduction in any case is not great. In the meantime k_2 can rise, not to k_1, but to this lowered value k_1'. Presently, however, k_1 is restored to its initial value, and may now rise to a still higher value k_1'' on return to the normal conditions. Since k_2 was in any case near to its saturation value, an extremely small increase in k_1'' will be sufficient to balance the increase which has occurred in k_2'', and to convert a moderate rate of reversion into an extremely slow one.

There are other possibilities depending upon the complicated interlocking of cell processes, but they will not be further discussed here.

(f) *Adaptation to new substrates.* Bacterial cells are capable of utilising fairly varied sources of carbon, though the compounds which will support growth are not usually wholly unrelated. Glucose and many other sugars form one of the main groups, and the other consists of the series of breakdown products derived from glucose, namely, glyceraldehyde and its derivatives, pyruvic acid, and various acids of the dicarboxylic and tricarboxylic acid series which are connected in a definite metabolic pattern (Krebs cycle and its variants).

It is a fundamental question whether in the adaptation to utilise a new substrate existing enzymes are used in changed proportions, so that their previous ratios in the cell are quantitatively altered, or whether existing enzymes undergo qualitative alteration. An outline of the theory of the first type of process has already been given (Hinshelwood, *op. cit.*, p. 180; Hinshelwood and Lewis, *loc. cit.*), and the second type has been dealt with in a qualitative way only. It is proposed here to develop a view which is a synthesis of both these alternatives, and which links together all the preceding parts of the present paper.

We have already envisaged the autosynthetic system as consisting of interlocking parts, *e.g.*, a certain type of nucleic acid which guides the formation of a certain type of protein and vice versa. The relevant equations are

$$\mathrm{d}R/\mathrm{d}t = A_1P_1 \text{ and } \mathrm{d}P_1\mathrm{d}t = B_1R \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

where R is the amount of the nucleic acid, and P_1 that of the protein, A_1 and B_1 being constants. (The specific identification of R and P_1 as nucleic acid and protein is of course not essential, but illustrative: what matters is the interlocking system.)

Now let it be supposed that the protein P_1 constitutes the enzyme (enzyme X of the previous paragraphs) which by interaction with a substrate S_1 gives an intermediate (concentration c_1) which in turn is used by the enzyme system Y, where Y, as before, is the critical division-determining component.

As previously shown, in the steady state (18) leads to $R = R_0\mathrm{e}^{k_1t}$, $P_1 = (P_1)_0\mathrm{e}^{k_1t}$ where $k_1^2 = A_1B_1$ and $R/P_1 = (A_1/B_1)^{\frac{1}{2}}$. As in section (a) we have also

$$\mathrm{d}Y/\mathrm{d}t = \alpha c_1Y = k\alpha x_1Y = k\alpha XY/n = k\alpha P_1Y/n = k\alpha\beta P_1$$

Then as in (14) we have

$$Y - Y_0 = (k\alpha\beta/k_1)\{P_1 - (P_1)_0\} \quad . \quad . \quad . \quad . \quad . \quad (19)$$

In the steady state $P_1/Y = k_1/k\alpha\beta$ and $R/Y = A_1/k\alpha\beta$, since $k_1 = (A_1B_1)^{\frac{1}{2}}$. The growth rate constant $(1/n)\mathrm{d}n/\mathrm{d}t = (1/Y)\mathrm{d}Y/\mathrm{d}t = (1/R)\mathrm{d}R/\mathrm{d}t = k_1$.

When growth occurs in the substrate S_2 we must suppose that a different enzyme-protein, P_2, is involved, and that this leads to the formation from the new substrate, in one or more stages, of the intermediate required for Y. We shall now have

$$\mathrm{d}Y/\mathrm{d}t = \alpha c_2 Y = k'\alpha\beta P_2,$$

c_2 and k' being different from c_1 and k, respectively.

In the new steady state, R will attain the value given by $R/Y = A_2/k'\alpha\beta$, so that $R_{S_2}/R_{S_1} = A_2k/A_1k'$. If A_2 and A_1 are not very different, *e.g.*, if the two proteins P_1 and P_2 are about equally efficient in guiding the nucleic acid formation, but if k' is much less than k, *i.e.*, if the new substrate is a much less efficient source of the required intermediate, then, just as in the case of the inhibitor action, the response of the cell is an expansion of the basic formative structure R. Until this has occurred the growth rate remains below its optimum k_1'.

For the steady states we have then

$$R_{S_1}/P_1 = (A_1/B_1)^{\frac{1}{2}} \text{ and } R_{S_2}/P_2 = (A_2/B_2)^{\frac{1}{2}}$$

and

$$R_{S_1}/Y_{S_1} = A_1/k\alpha\beta \text{ and } R_{S_2}/Y_{S_2} = A_2/k'\alpha\beta$$

The maximum growth rate constants for the two substrates are $k_1 = (A_1B_1)^{\frac{1}{2}}$ and $k_1' = (A_2B_2)^{\frac{1}{2}}$. The R/P ratios are determined only by A and B and are unaffected by k or k'. On the other hand, the ratio of R and P themselves to Y depends upon k or k' and settles down to a greater value the smaller these constants are. The optimum degree of adaptation is determined therefore by A and B only. If their product is small, no amount of adaptive response will increase the growth rate beyond what they allow. If, on the other hand, their product is large but k' is very small, the initial rate on transfer to the new substrate will be small but can rise to a high value in time. This kind of behaviour is in fact found. *Bact. lactis aerogenes* when first transferred from glucose to D-arabinose shows a very long lag but finally attains a growth rate comparable with but still less than that in glucose.

The lag on transfer from S_1 to S_2 is roughly calculable as follows. In equations (4*a*) and (4*b*), X becomes R, Y becomes P_2, $Y_0 = (P_2)_0 = 0$, $\alpha = A_2$, and $\beta = B_2$; k is k_1'. Then

$$P_2 = (\tfrac{1}{2}k_1'R_0/A_2)(\mathrm{e}^{k_1't} - \mathrm{e}^{-k_1't})$$

so that

$$\begin{aligned}\mathrm{d}Y/\mathrm{d}t = k'\alpha\beta P_2 &= (\tfrac{1}{2}k'k_1'\alpha\beta R_0/A_2)(\mathrm{e}^{k_1't} - \mathrm{e}^{-k_1't}) \\ &= (\tfrac{1}{2}k'k_1'\alpha\beta R_0/A_2)(2k_1't) \text{ approx. for small changes.}\end{aligned}$$

Since $Y = \beta n$, we have for the early stages

$$(1/n)\mathrm{d}n/\mathrm{d}t = k'(k_1')^2(\alpha/A_2)(R_0/n)t \quad . \quad . \quad . \quad . \quad . \quad (20)$$

The definitition of the lag, L, is necessarily somewhat arbitary, but if we take it here as the time at which $(1/n)\mathrm{d}n/\mathrm{d}t$ begins to assume some assigned value Δ, then, since at the start $R/Y = A_1/k\alpha\beta = R_0/\beta n$,

$$\begin{aligned}L &= \Delta(A_2/\alpha)(n/R_0)\{1/k'(k_1')^2\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (21) \\ &= \Delta(1/k_1')^2(k/k')(A_2/A_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)\end{aligned}$$

Equation (22), which makes the lag independent of R_0 in appearance, assumes of course that R_0 has the equilibrium value corresponding to S_1. If k' is small, in particular, the lag is long.

(g) *Stability and reversion of substrate adaptation.* As in the case of drug resistance, the equations so far given predict rapid adaptation and rapid reversion on transfer between two media containing S_1 and S_2 respectively. This is sometimes observed, but often the adaptation is rather stable.

Once again it is necessary to reconsider the tacit assumption that the rate of synthesis of enzyme Y is directly proportional to the concentration c. This assumption leads to

(19). If enzyme Y becomes saturated, the rate cannot rise above a limit. Suppose now we first effect equilibration in substrate S_1 where R/Y reaches a definite value, and then transfer to S_2 where k' is very small, so that R/Y expands greatly. Now re-transfer to S_1. The amounts of R and presently of P_1 per cell will be abnormally great. If there is a linear relation of dY/dt to c, Y will expand again relatively to R and P_1 and the old ratio will be restored. If, however, Y was already saturated in the normal functioning in S_1, the rise in c will produce no response. The values of $(1/R)dR/dt$ and of $(1/Y)dY/dt$ will be k_1, and the second will not rise above it. Hence the abnormal proportions will be preserved, and will give the cells an abiding advantage if at a later stage they are yet again transferred to S_2, since in (21) R_0/n will have, not the value there calculated, but an abnormally great value. This circumstance will not, however, abolish all the lag, since P_2 has in any case to be formed. The lag will, however, be less than it would have been had the previous adaptation not occurred.

That in a common substrate like glucose the enzyme Y may often be working at near saturation is quite possible, since this point would be precisely that at which adaptive processes of other kinds in S_1 would have reached the limit of their effectiveness.

It is not suggested that the mechanisms here discussed in any way exhaust the possibilities for the explanation of the varied phenomena of adaptation to new substrates.

PHYSICAL CHEMISTRY LABORATORY, UNIVERSITY OF OXFORD. [*Received, October 24th*, 1951.]