Autosynthesis.

FARADAY LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY AT THE ROYAL INSTITUTION, LONDON, ON MARCH 26TH, 1952.

By SIR CYRIL HINSHELWOOD.

SCIENCE, we believe, possesses an underlying unity. Faraday certainly seems to have been guided in his work by such a faith, and never ceased to search for connexions between apparently disparate natural forces, light and magnetism, magnetism and electricity, electricity and chemical affinity, and the insight with which he found them is part of the world's history.

I believe he would have wanted to find one between the intricate and beautiful kinetic mechanisms of chemistry and the mysterious self-reproductive power of living matter. He was indeed no materialist, but with his natural philosophic insight he would have seen that ultimate problems are unaffected by a successful translation of purely biological descriptions into the scientifically more fundamental language of physics and chemistry. In the translation the complex grammar of both languages must be borne in mind.

Genes and autosynthetic units

Biologists have long postulated fundamental self-reproducing units which they call genes, and they have for some purposes established what is rather like an atomic theory of these entities.

Organisms have been supposed to contain them, in a first approximation at least, fixed and unalterable, reproducing themselves, and transmissible by heredity. When cells of different types unite, the characters inherent in them are added and mixed. When cells divide there is a segregation, or division, of the character between the new-formed progeny. Many striking phenomena of heredity have been interpreted in terms of the resulting play of probabilities as the genes change their association like the coloured marbles in a mathematical exercise. The famous laws of Mendel revealed (in appropriately chosen examples) simple numerical ratios which could be interpreted in just this way.

As living cells fuse or divide, then, properties can be transmitted, gained, or lost, as though they resided in material entities possessed by some cells and not by others and subject to the laws of chance as discrete particles would be. For the biochemical properties of cells the principle of " one gene one enzyme " has been suggested.[1]

This whole conception, it is true, has its difficulties. To account for the ways in which even the simpler chemical functions of the cells of micro-organisms change in cultivation or on propagation, very complex sets of muliple genes have to be postulated, and they have to be assumed subject to the influence of what are called " modifiers " and " suppressors " to a degree which largely removes the simple numerical relations from which their existence was originally inferred.

Yet the power of living things to reproduce their kind, and the combinatory possibilities which are, in spite of all, quite manifest in their unions and divisions, have led most insistently to the idea of the self-replicating units of structure, and to the question of what special virtue resides in these strange entities.

There are, of course, in ordinary chemistry suggestive, though imperfect, parallels. In the growth of crystals, existing patterns guide the deposition of new material in ways which are remarkably precise, and new large crystals readily cleave to form numerous similar copies of an original small model. When attractive forces exist between units a copying process will generally give a structure of lower potential energy when the copy is exact, and this principle must surely play its part in autosynthesis. But this is not enough. The analogy of moulds and templates and the corresponding casts, and of positive and negative replicas, has often been invoked. It carries the same principle a stage further, but still seems to provide only a possible element in the solution of the problem, rather than the solution itself.

In the polycondensation reactions so much studied by chemists in recent years there is a continuous growth of substance, often not altogether unlike living matter in molecular structure, and there is even replication in so far as the well-known kinetic mechanism of the

[1] Cf. Monod, Biochemical Society Symposia, No. 4, 1951, p. 51.

" chain transfer " process cuts up what is formed into separate units. But this too can be but one element in the solution of the problem.

The obtaining of certain viruses in the form of crystals certainly illustrates the relevance of these various analogies, but it illustrates also their incompleteness, for viruses cannot reproduce themselves in isolation, and need to invade other living cells. Their own nucleoprotein substance increases not at the expense of the infected cell, but of medium constituents,[2] so that it is the machinery of the cell which they can use, not its material upon which they necessarily prey.

This in a general way is really the key to the whole problem. Just as the virus is not a self-duplicating unit in its own right, so there is probably no other single substance or unit which possesses this property. Viruses have been likened to genes and this parallel in turn has been challenged. But they may well have this in common that neither is the elusive autosynthetic unit, which indeed may well have no more independent existence than the philosopher's stone or the elixir of life.

The substances of which living cells are built are complex and thermodynamically unstable. They are not formed spontaneously under ordinary conditions, and a laboratory synthesis would demand a long series of very elaborately arranged experiments with numerous intermediates first separated and then brought together again in appropriate combinations. Great reductions in entropy have to be compensated.

Such compensation in itself is easily possible if the reactions of negative entropy change are linked with others of positive change, such as oxidations, and this linking commonly occurs by the intervention of common active intermediates in the coupled system.

Another source of compensation is illustrated in crystal growth itself. Here an ordered structure is produced from a disordered group of molecules and so entropy decreases. But energy is released and must be conducted away to raise the temperature and entropy of something else, and thus the balance is preserved. For the ordered structure to arise, however, a model must be there to copy, or else the initiation of the process may be long, or in complicated cases, almost indefinitely delayed. Copying is far easier than invention.

In the reproduction of a cell there is indeed copying, but degradation of energy also occurs, and highly complex series of reactions combine, as it were symphonically, to give among the total products certain substances of very low entropy. It is the interplay of all these processes which must make autosynthesis possible, not the replication of individual genes as such.

The guiding of synthesis by existing structures is indeed necessary, and these existing structures are in general so complex as to be nearly incapable of spontaneous appearance. Living matter is thus largely the slave of heredity.

But the two aspects of the gene, that, namely, which is responsible for the fundamental character of a cell on the one hand, and the self-reproducing unit on the other, must be regarded separately. The types may be permanent but the elaborate and subtle interplay between them is a matter of organization and balance, and these are things which unlike the basic entities themselves are labile.

The interplay presents kinetic problems of great interest, and the biological analogies which the results indicate, and which will be briefly mentioned, are highly suggestive. They are certainly of significance though in just what ways is perhaps hardly yet agreed. In the meantime we may at least say that we are studying a new type of chemical reaction system.

Mutually dependent reaction systems

Some fundamental principles are illustrated by a very simple system of kinetic equations. Suppose there are two substances each of which is itself being formed at a rate determined by the catalytic action of the other so that

$$dX/dt = \alpha Y \text{ and } dY/dt = \beta X$$

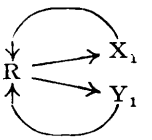
Neither X nor Y in isolation could increase in an autocatalytic manner, yet these two equations show that after a period of rather complex adjustment the amounts are given by

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

X_0 and Y_0 being the values at time $t = 0$. The steady state reached by the system after a long time is described then by the equations $dX/dt = kX$ and $dY/dt = kY$, each constituent being apparently self-duplicating. The result is similar when any number of constituents are formed in such a way that the increase of each depends upon the catalytic action of the others.

[2] Cf. Andrewes, *Proc. Roy. Soc.*, 1952, *B*, **139**, 313.

If we have the set of equations $dX_1/dt = \alpha X_2$, $dX_2/dt = \beta X_3$, $dX_3/dt = \gamma X_4$, $dX_4/dt = \delta X_5$ . . . . it can be shown that in the steady state which is finally reached $dX_1/dt = kX_1$ $dX_2/dt = kX_2$, and so on. Nor does this apply only to a single linear sequence. Suppose we have a system coupled in the following way



where R forms X_1 or Y_1 from different substrates in a complex system, X_1 and Y_1 being, as it were, modifications of similar enzymes, but each guiding the further formation of R. We should then have the equations $dX_1/dt = \alpha R$, $dY_1/dt = aR$, $dR/dt = \beta X_1 + bY_1$.

The solutions, even with this very simple cycle, look rather complicated : they are

$$R = \tfrac{1}{2}\left\{R_0 + \frac{\beta(X_1)_0 + b(Y_1)_0}{k}\right\}e^{kt} + \tfrac{1}{2}\left\{R_0 - \frac{\beta(X_1)_0 + b(Y_1)_0}{k}\right\}e^{-kt}$$

$$X_1 + \frac{\alpha b(Y_1)_0 - ab(X_1)_0}{k^2} =$$

$$\tfrac{1}{2}\left\{(X_1)_0 + \frac{\alpha b(Y_1)_0 - ab(X_1)_0}{k^2} + \frac{\alpha R_0}{k}\right\}e^{kt} + \tfrac{1}{2}\left\{(X_1)_0 + \frac{\alpha b(Y_1)_0 - ab(X_1)_0}{k^2} - \frac{\alpha R_0}{k}\right\}e^{-kt}$$

$$Y_1 = \frac{a}{\alpha}\{X_1 - (X_1)_0\} + (Y_1)_0$$

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

As soon as t is great enough, however, the following ratios are established since the terms in e^{-kt} vanish,

$$X_1/R = \alpha/k = \alpha/(\alpha\beta + ab)^{\frac{1}{2}}$$

and $$Y_1/R = a/k$$

If now a small sample of matter in which these proportions are established is transferred to a fresh environment of similar character $(X_1)_0$, $(Y_1)_0$ and R_0 are in these ratios, and, from $t = 0$, the above equations reduce to the form

$$R = \tfrac{1}{2}\left\{R_0 + \frac{\alpha\beta R_0 + abR_0}{k^2}\right\}e^{kt} + \tfrac{1}{2}\left\{R_0 - \frac{\alpha\beta R_0 + abR_0}{k^2}\right\}e^{-kt}$$
$$= R_0 e^{kt}$$

and similarly $X_1 = (X_1)_0 e^{kt}$ and $Y_1 = (Y_1)_0 e^{kt}$.

The system

yields still more complex relations for the non-steady state. The formation of X_2 may on occasion lag behind that of Y_2, or *vice versa*, and extraordinary fluctuations in the relative amounts of the components may occur. These phenomena of the non-steady state, as we shall see, may be of great importance. But eventually the simple exponential law of increase is followed by everything.

Thus neither the length of the chain of mutually dependent processes, nor a bifurcation in the sequence, affects the establishment of what appears as an autosynthesis. It may therefore reasonably be concluded that a large variety of quite complexly interlocking patterns of reactions will be subject to the same principle.

It sems not unlikely, then, that there are after all no individual units endowed with mysterious powers, but that the mystery, indeed the miracle, lies in the initial conjunction of partial systems which minister to one another's needs in the appropriate way. What sorts of system would perform such functions? Catalytic degradations and syntheses are of course performed by enzymes which are largely protein. Cellular nuclei, which are the seat of special activity, contain nucleic acids; viruses themselves are largely nucleoprotein in nature.

The nearest thing to a system autosynthetic in its own right—though it is of course not really so—is a virus, and the nucleoprotein character of viruses has often suggested the great importance of protein–nucleic acid interactions. There is indeed experimental evidence for such

a relation in the patterns of mutually dependent reactions which have just been referred to. Malmgren and Heden [3] found that the nucleic acid content of bacteria at various stages of their growth cycle was correlated with the rate of growth at that stage. The amount of ribose nucleic acid in *Bact. lactis aerogenes* cultured in a wide range of different conditions has been found, moreover, nearly proportional to the rate at which the cells have grown.[4] This last relation follows from the equations $d[\text{Protein}]/dt = \alpha[\text{Nucleic acid}]$ and $d[\text{Nucleic acid}]/dt = \beta[\text{Protein}]$, with α a more or less universal constant. This result supports the idea which is already suggested by Astbury's structural considerations that in the synthesis of protein the nucleic acid guides the order and determines the rate of accretion of the amino-acids, and that conversely the protein is, as it were, the heterogeneous catalyst by which the nucleotide units are built together. Characteristic relations of " polypeptide chains and nucleotide columns " have existed from the beginning, as Astbury says " along a line of molecular heredity which may not be broken except at the cost of starting at the beginning again."

Kinetic principles of cell growth

If, then, the autosynthetic function is not inherent in any one cell component, but emerges from the harmonious co-operation of many, it is obvious not only that the growth of the cell and the reproduction of its matter are very intimately linked with the working of the enzymes, but that the reproduction of these enzymes is intimately linked with their own working, since it is this which in turn enables others to work and provide the synthetic material for them. As we have seen, the complex equations of the interlocking reactions are in many respects equivalent to another set $dX/dt = kX$, $dY/dt = kY$, and so on, so that we have a rational basis for a formulation which in effect runs :

$$\text{enzyme} + \text{metabolite 1} = \text{more enzyme} + \text{metabolite 2}$$

This I shall call the *first kinetic principle*.

It is a simple mathematical consequence of this that on continued growth the proportions of the various kinds of material present in a system will settle down to stable values. If, moreover, conditions of growth change in such a way as to alter the velocity constants, these proportions will change also. This we might call the *second kinetic principle*.

We must next bear in mind that living matter is organized in cells, and that these from time to time divide so as to maintain a roughly constant size. The detailed mechanism of cell division need not be discussed. It must, however, come about as a result of the area/volume changes accompanying increase in size. These lead to changed internal concentration of metabolites and products. What is important for the present purpose is the simple and natural hypothesis that at the moment of division some important cell component or set of components plays the rôle of a key substance, and that the process is initiated as soon as the amount of this substance reaches a more or less constant critical limit. This will be the *third kinetic principle*.

It is not without direct experimental support. Various observers [5] showed that in certain animal cells the amount of deoxyribose nucleic acid was nearly constant. Caldwell and Hinshelwood [6] found that cells of *Bact. lactis aerogenes* grown under a wide variety of conditions, which gave variable growth rates and widely variable total nitrogen contents, had nevertheless nearly standard contents of deoxyribose nucleic acid. There is no need to suggest a definite identification of deoxyribose nucleic acid with the division-controlling substance or system, but the principle that the amounts of one or more key constituents may be taken as invariants seems to be quite well founded.

From these three kinetic principles there follows a *fourth*, verifiable by mathematical calculations with simple models, namely, that in the kind of systems which have been described those proportions of constituents will finally be established which lead to a maximum rate of growth in the actual environment.

This indeed is something like a process of natural selection. If in a population of cells some individuals could reproduce themselves faster than others then they would outgrow and supplant them. But in a similar way a quantitative scheme of reactions, even one not already established in any cell, will gradually replace other quantitative schemes which offer smaller resultant reaction rates.

[3] *Acta Path. Microbiol. Scand.*, 1947, **24**, 448.
[4] Caldwell and Hinshelwood, *J.*, 1950, 3156.
[5] Boivin, Vendrely, and Vendrely, *Compt. rend.*, 1948, **226**, 1061; Mirsky and Ris, *Nature*, 1949, **163**, 666.
[6] *J.*, 1950, 1415.

Not only would the proportion of enzymic and other material in a cell change in this sense, but what may be termed the " reaction pattern " of the cell as a whole could be superseded by another and more effective one. Cells possess vast numbers of enzymic functions, and many orders and combinations of simple unit processes can be combined to give the final syntheses. If these processes are used in one order and at a given rate, there will be definite ratios of the various material components set up; if in another order and at another rate, different ratios. In the end the available reactions will be used according to that pattern which gives an optimum rate, and the cell composition will correspond.

Simple illustration of an automatic adjustment in a cell containing effectively autosynthetic systems

Suppose an enzyme system I produces a diffusible substance which is used by a second enzyme system II. Let the total amounts of I and II at any time be X_1 and X_2 respectively, contained in n cells. Let the concentration of the intermediate be c. In any cell this will very rapidly reach a steady value determined by the equation

$$dc/dt = A(X_1/n) - B(X_2/n)c - Cc = 0$$

where A, B, and C are constants. The first term of this expression represents formation by I, the second consumption by II, and the third loss by any other means. If II is the component upon the formation of which cell division must wait, then $n = \beta X_2$ where β is a constant. Thus $c = \alpha X_1/X_2$, where α is another constant.

The rates of increase of I and II are given by

$$dX_1/dt = kX_1$$
$$dX_2/dt = k_2X_2c = k_2\alpha X_1$$

Let the ratio $X_1/X_2 = v$

$$\frac{dv}{dt} = \frac{X_2\frac{dX_1}{dt} - X_1\frac{dX_2}{dt}}{X_2^2} = k\frac{X_1}{X_2} - k_2\alpha\frac{X_1^2}{X_2^2}$$
$$= v(k - k_2\alpha v)$$

If $k_2\alpha v < k$ when $t = 0$, dv/dt is positive. Thus v increases with time. Presently, however, v reaches such a value that $dv/dt = 0$, whereupon no further change occurs, since there is no increase in v to alter the value of the differential coefficient. The ratio of X_1/X_2 is now stable and $k_2\alpha v = k$.

Suppose now some agency disturbs the concentration c and so interferes with cell growth. (It could be a toxic substance or a foreign enzyme which destroyed the diffusible metabolite, but this need not be specified.) Since $dn/dt = \beta dX_2/dt$, and dX_2/dt depends upon c, the growth rate is lowered. α assumes the smaller value α'. dv/dt, which was zero, now becomes positive. v now increases until $k - k_2\alpha' v$ is once more zero, whereupon there is a new steady state. $(1/n)dn/dt$, which had fallen below k, has now risen to equal k once more.

This single example illustrates the principle of automatic adjustment.

Biological analogies of systems showing automatic adjustments

So far the theme has been that autosynthesis involves a dove-tailing of mutual supply systems, and that these should possess the property of self-adjustment to show maximum growth rate, whereupon they reach a steady state, in an environment which contributes the proper raw materials. The biological analogies of this picture are obvious. Unicellular organisms show continued logarithmic growth ($X = X_0e^{kt}$) in suitable constant environments. In new environments long periods of adjustment or lag phases may be necessary before the steady state is established. In this state the simple law of autocatalytic growth is closely followed, the amount of material increasing in geometrical progression with time. On transfer from one environment to another they show adaptive responses, initially slow or nearly negligible rates of growth gradually rising to an optimum. The equations which have already been shown predict just such complicated lag phases.

These adaptive responses include the development of the power to use with maximum efficiency unfamiliar sources of nitrogen or carbon, or to grow with unimpaired speed in the presence of toxic agents which initially are inhibitory. It is an interesting hypothesis that during the lag phases and periods of adaptive response a given reaction pattern is establishing itself, sometimes in competition with another. Here we are presented with systems which are

of the greatest interest not only in biology, where the phenomena shown by adapting cells are of great fascination, but also in chemical kinetics.

Until recently there has been reluctance of many biologists to admit even in principle the possible interpretation of such phenomena in terms of kinetic mechanisms, and they have believed most of the apparent adjustments to depend upon natural selection of spontaneously occurring mutant cells. But more recently there seems to be an increasing willingness to admit the possibility of both mechanisms. And in particular cases there is indeed very strong evidence for the occurrence of just the sort of automatic adjustments which have been described.

Reversibility of adjustments

The whole state of affairs suggested by the foregoing considerations is very different from that where biochemical properties reside in self-replicating genes which are unchangeable save by a catastrophic event of some kind (usually called a mutation).

The results of experiments on automatic adjustments in cells suggest rather an interlinked system of mutually dependent parts, no one of which could grow in isolation. There is, according to this view, no autosynthetic type of structure as such, and the permanence of genes (and the essential dependence of really fundamental cell properties upon them) simply reflects the stability of many chemical compounds and the fact that complex structures of low entropy are in any case extremely unlikely to arise *de novo*. Any characteristic piece of cell texture would thus constitute a gene in the sense that it might determine a property on the one hand, and on the other hand be continuously resynthesised in the co-ordinated interplay of cell processes.

We have thus two major factors: fundamental key structures, and their quantitative kinetic relations to one another. Both are of importance in the building of cells with given qualitative and quantitative properties. Fundamental key structures are likely to be relatively permanent, quantitative relations to be highly variable.

An important question therefore is how far changes in properties shown by cells in new circumstances persist in the progeny when the same circumstances no longer prevail. Not infrequently they do seem to persist. Once a bacterium, for example, has learnt how to use a new sugar for growth, it often behaves as though it remembered how to do this even in the absence of the sugar itself. Inheritable properties of this kind might indeed seem to indicate discontinuous and irreversible structural changes.

But the story is not quite so simple. To begin with, it is quite a question what is meant by heredity in a unicellular organism which increases in size and then splits in two. Changed chemical organization of the so-called parent cell is inevitably preserved to some extent in the two cells formed by binary fission, and the problem of how many generations inherit the new property may be no more than that of how long a new organization imposed on a mass of autosynthetic material requires before it can give place to another.

The establishment in a fresh environment of a new reaction pattern accompanied by different proportions of the cell components should, according to the kinetic propositions already set out, be reversible on return to the original environment. But there are various chemical reasons why the rate of reversion may be slow, or even very slow indeed. One of the most important is the lag which may theoretically be expected to attend the establishment of a new reaction pattern. If growth by an alternative route is proceeding actively during this lag the new pattern may never have a chance to develop.

According to this conception the problem is not so much one of stability of adapted forms as of rate of reversion. And in fact the biological systems to which these analogies may apply do show varying and highly complex time relations in their adjustments.

For example, if bacteria are adapted to a new medium, they may lose their adaptation at a whole series of different rates according to the length of time for which they have, as it is said, been trained. But an eventual return to the original state seems to be the commonest fate (unless in the meantime other factors have intervened), so that the argument from heredity can by itself not abolish belief in the kind of kinetic adjustments to which reference has been made in the foregoing.

Whether or not I am right in what I have said about heredity and about permanence, the fact remains that, on general thermodynamic and kinetic grounds, cells must be systems possessing something at least of the character I have described, and that the equations of such systems predict adjustments as they modulate from one growth regime to another. If the phenomena which look like adaptive adjustments are really something else, then I feel sure that true adaptive responses will still be found in the end to have been going on in secret.

Most of these adjustments are of course limited in range, and so do not in any way weaken belief in a fundamental substratum of substance with which given qualitative characters are necessarily linked. The relation of the adjustable to the permanent elements in cell organisation is indeed one of the major questions, and more will be said of it shortly. But there is first a little more to say about the reaction pattern itself. It is after all even more significant than the key structures in indicating the nature of the kinetic laws by which the cell establishes and maintains its organization.

Multiple reaction patterns

The multiplicity of the reaction networks has already been mentioned. They may branch, and alternative sections may operate simultaneously and in competition, in phase with one another, or with one lagging while the other functions. To them correspond a whole series of enzymic organizations of the cell, which represent various possible states of adaptation to various environments.

This conception helps in the understanding of a whole range of remarkable phenomena observed with micro-organisms such as bacteria and yeasts.

Sometimes, to quote one example, a bacterium on transfer from compound A as a carbon source to compound B, attains its optimum growth in B without any impairment of its growth in A. Sometimes on the other hand, growth in A is impaired and the adaptation to the two sources at once seems impossible. What seems to happen is that the cells can grow in B by two alternative reaction patterns, one compatible with that required for growth in A and one not. Sometimes the one of the alternatives lags in its establishment and then the behaviour appears different from what it is when this alternative is mobilized. In the light of kinetic principles such phenomena become generally intelligible.

The great part played by the combination of reaction routes is illustrated by an example taken from the yeasts. These are a little more complex than bacteria and in particular two cells of haploid yeasts may fuse together to give one of more elaborate structure, a diploid. From diploids, haploids may be re-generated in the process of sporulation. Now when two haploids of a certain strain united to give a diploid, its growth rate in the sugar raffinose was found, after a considerable period of adaptation, to be greater than that of either of the original haploids. In other words, the potentially more complex reaction pattern could achieve a greater overall efficiency. But before the adaptation the diploid initially showed a much greater lag in utilizing the sugar than either of the haploids. Although it possessed all the potentialities for more rapid growth, the disturbance of organization on cell union did actual harm, and the more elaborate pattern of the reactions used by the diploid required longer to establish.[7]

Organization and substratum

However, it must not be forgotten that the gene of the biologist is sometimes an entity which passes from parent to offspring according to the play of probability. What is the physico-chemical basis of Mendelian heredity?

May we pause a moment to remember Faraday once more? His laws of electrolysis provided the main support of dualistic chemistry, and yet his discovery of benzene opened a field of investigation where, as strikingly as in any, there has been revealed the subtle ways in which polar and non-polar influences combine to determine the behaviour of chemical substances. His liquefaction of chlorine, and his work on heterogeneous catalysis paved the way for the understanding of the multitudinous modes of interaction of matter and the elaborate hierarchy of chemical forms. It is the complexity of this hierarchy which must now be invoked.

To behave as a Mendelian gene an element of cell structure must be transferable as a single unit in processes such as division. It must therefore be localized and possess a certain degree of mechanical independence. It could therefore be a single unit of macromolecular structure of definite chemical composition, or else an association of units constituting a separable phase in the physical sense.

Not only are chemical molecules themselves of widely varying stability, but their modes of interaction with others of the system in which they exist is also graded over a wide range. They form aggregates which enter into all the complex relations of phase equilibria and of colloid chemical phenomena. Macromolecules have their own laws of regimentation and they form sheets and chains folded in elaborate ways which react in turn on their relations to their neighbours. In macromolecular chemistry the ordinary differences between molecules and

[7] Kilkenny and Hinshelwood, *Proc. Roy. Soc.*, 1952, *B*, **140**, 352.

masses, surface and bulk phases, homogeneous and heterogeneous systems lose much of their definiteness. In such a world mechanical and chemical separability must show wide variations. If some key molecular unit or combination of units is mechanically separable from the rest without too drastic an upset, then it can be gained and lost as a whole when cells unite or divide. We then have the condition that some entity is recognizable as a Mendelian gene.

But even so the complete manifestation of its properties, and still more the provision of material for its synthesis, depends upon its quantitative relation to other structures, and upon the proportions of other constituents in immediate reaction with it, and with other key structures And all these are subject to the subtler changes envisaged in theories of adaptive response.

Yeast cells provide an illuminating example of this thesis. Diploid yeasts sometimes divide to give four haploid spores from which haploid sub-strains can be cultivated. In certain examples the diploid will utilize the sugar galactose for growth, while of the four derived strains two will do the same while two (to all intents and purposes) will not. Evidently two key structures responsible for galactose metabolism have divided themselves bodily between four spores, so that two of these spores are fortunate and two are not. Yet even the two positive spore cultures show the clearest adaptive phenomena, and develop their optimum power of growth in galactose only after long periods of adjustment which there is every reason to believe may involve changes in reaction pattern of the kind already discussed.[8]

Nor is this all; even the negatives, if left with galactose for perhaps ten or twenty times as long as the positives require even in the unadapted state, eventually develop yet another reaction route by which they succeed in growing with utilization of the sugar.

The complete gain or loss of a key structure seems, then, to be something of a limiting case. Organizational changes with a kinetic basis are ever-present. What intermediate degrees of change can be envisaged?

Apparently discontinuous changes in cell properties are often called forth by exposure to ultra-violet light or other "mutagenic agents." One can well understand that some key structure may become completely extirpated by that treatment, and indeed in most known cases the change occurring is the loss rather than the gain of a character—a deficiency in the metabolic machinery usually results. The deficiency may be slowly repaired if the cells are cultured for long enough in conditions conducive to adaptation. The question then arises whether a second, spontaneous, mutation reversing the effect of the first has occurred, or whether the key structure, not having been wholly eliminated, is gradually repaired by a quantitative adaptive adjustment.

Since the effect of ultra-violet light varies continuously and quantitatively, and since the rather uneventful process of subculture recreates a lost character while the more potent agency of the ultra-violet light is unable to do so (and causes little save damage), the quantitative interpretation of these phenomena has perhaps something to be said for it.

But there are obvious kinds of qualitative change which should in appropriate circumstances be imposable without an excessively drastic upheaval in the cell economy, and it would be surprising if these did not play their part, the range of chemical forms and textures being so wide and capable of such subtle gradations. Two of the most important kinds are, on the one hand, the sequence and arrangement of peptides in proteins or of nucleotides in nucleic acids, and, on the other hand, the different configurations into which protein chains can fold themselves.

Cohn has found that during the induced formation of β-galactosidase in *E. coli* there is indeed production of a substance with given antigenic properties in place of another with different though related properties.[9] This may of course represent simply the quantitative shift in proportions of two substances both present all the time (though perhaps in minute amount), but it may very well indicate an imposed qualitative modification in the proteins.

If nucleic acid and protein synthesis are indeed mutually guided, it seems not unlikely that a given nucleic acid may guide the formation not of a single protein only but of several related ones. These proteins may then react enzymically each with the appropriate member of a not too diverse series of substrates. If this is so, then during an adaptation to optimum growth in a new medium there may be minor qualitative changes in proteins as well as major quantitative changes in the proportions of the various cell constituents.[10]

[8] Winge and Laustsen, *Compt. rend., Trav. Lab. Carlsberg*, 1937, **22**, 235; Kilkenny and Hinshelwood, *Proc. Roy. Soc.*, 1951, *B*, **139**, 73.
[9] See Monod, Cohen-Bazire, and Cohn, *Biochim. Biophys. Acta*, 1951, **7**, 585.
[10] Cf. *J.*, 1952, 745.

Pauling has discussed the formation of antibodies in terms of protein folding. When a so-called antigen is introduced into blood serum, it induces the formation of an antibody which for a considerable time retains the property of precipitating antigen. According to Pauling, antigens and antibodies have complementary structures in portions of their molecules, and in fact in at least two regions. As a result, the formation of a composite framework, constituting the precipitate, becomes possible. He suggested that the antibody differs from the normal serum only in the way in which certain regions of the globulin polypeptide chains are folded, and he pointed out that there will be many configurations of nearly equal energy, so that many specific antibodies can be induced by different antigens.

The folding of parts of the chain to conform to the shape or force field of another molecule is an example of what is often called " template " action. Some authors have postulated such a relation of mould or matrix to the corresponding casting for the building of genes from their precursors, and indeed have assumed a symmetrical relation for the alternating formation of both. Many variants of such ideas occur, and the Mendelian genes have not been the only entities assumed to be duplicated in this way. Thus Spiegelman at one time envisaged cases where certain enzymes were " capable of self-duplication without the necessity of genic intervention," the sole function of the gene itself being " the initiation of the enzyme synthesis."

It is clear from all this that, even within the framework of essentially constant basal chemical types, not only may quantitative proportions vary but subtler kinds of qualitative modification may occur, sometimes of what we should once have called a colloid chemical nature, and sometimes involving minor shifts in chemical composition. The precise rôle of all the possible kinds of modification is still to be explored and offers a fascinating field of study.

The decline of the cell economy

The theme of the complex interlocked reaction pattern of the cell with its hierarchy of physical and chemical forms in their varying degrees of interdependence has naturally been most in evidence in connexion with synthesis, growth, and adaptive changes. It dominates also the picture of the process by which cells suffer decline and death. The physical chemistry of this is of outstanding interest.

When a large population of bacterial cells is kept in an environment unfavourable to growth the cells gradually die. If the logarithm of the number of survivors is plotted against the time, the curve obtained varies in form, but sometimes approximates to a straight line (as would be shown accurately by the single-stage decay of a radioactive element). The straight-line relation, if absolute, would imply that the probability of death for an individual cell is independent of its previous history. Rather naive interpretations of this conclusion have occasioned much controversy. Death of the cell has been attributed to an accidental encounter between a quantum of radiation or the molecule of a toxic substance, on the one hand, and a highly localized vulnerable centre on the other. The " bullet " theories have been opposed by another group which ascribe death to a progresive deterioration, give to each cell a definite survival time, and rightly emphasize that this time will not be uniform but will show a statistical distribution in the population in accordance with the inherent variability of all biological systems.

The difficulty about such theories is that the statistical distribution which they must assume to account for a logarithmic decline is one which seems inherently improbable and has no independent support: it is, namely, that the largest proportion of the cells possess the minimum resistance rather than one nearer to the average value.

The matter assumes, however, a more natural aspect when looked at in relation to the complex reaction pattern of the cell. In many different ways synthesis and destruction are in continual opposition. Enzymic reactions are reversible. Cell material is labile and breaks down when not continuously built up. Even when a cell is in a state of apparent quiescence, as judged by the criteria of growth and division, it remains the seat of lively metabolic exchanges. Living cells multiply, dead cells suffer lysis, but synthesis and degradation in general are known from the evidence of radioactive tracers to occur far more rapidly than either observable growth or observable decay. The immense network of reaction patterns when no longer working with the complete co-ordination necessary for effective autosynthesis is still capable of constructive functions, and partially disorganized but still not purely destructive activity continues.

When some parts of the cell material break down they yield diffusible compounds which can be incorporated into other parts by the operation of the still effective partial mechanisms. It

can easily be shown that there may result an actual waxing and waning of different enzymic functions. When a number of these undergo quasi-periodic variations bearing no regular phase relation to one another, it becomes, for any individual cell, a matter of chance when the functions happen to pass through, say, their minimum values together. (This, of course, is the essential theoretical basis of the law of radioactive decay.)

We can now form the following picture of cell decline. A certain general decay of all the functions sets in, and until this has reached a certain stage, the chance of death is small. This represents an initial stage of slow progressive decline and corresponds to the period of delay which is sometimes observed to precede the onset of the approximately logarithmic phase. Next comes the phase during which death ensues when various quasi-periodic fluctuations reach unfavourable minima together. The reduction of the living population now follows roughly the exponential decay law. This law may be more or less satisfied to the end, but sometimes an effective degree of adaptation may be achieved. If so, there is a final phase in which a certain proportion of survivors exhibit exceptional longevity. This broad conception of the process covers most of the experimentally observed facts. The general form of the logarithmic survival curve is in fact sigmoid rather than linear except in its central portion.

Conclusion

The physical chemistry of highly co-ordinated reaction systems is in its infancy both theoretically and practically. All I hope to have shown is the cogency and relevance of its problems. There is scope for powerful mathematics in the proper working out of the general conditions for the existence and functioning of such systems, and for every resource of ingenuity and technique in the practical exploration of these problems.

The biological aspects of the question are equally alluring, and, to mention only one, the decision as to which changes occur by modification of individual cells and which by shifts in population balance gives rise to arguments with surprising depths of subtlety. I have not attempted to deal with this here, partly because time is insufficient, but also because I should like the purely chemical aspect to appear in its own right.

It may seem a long way from here to Faraday, but in several respects, as I have indicated in passing, the story is a continuous one, and I do not think he would have deprecated the study of cell function in the light of physico-chemical ideas.

The last words shall be with Faraday himself. Whoever may prove less right in some of the more controversial issues of the moment may perhaps ponder what he says in the Preface to Volume I of *Experimental Researches in Electricity*: "Although I cannot honestly say that I *wish* to be found in error, yet I do fervently hope that the progress of science in the hands of the many zealous present cultivators will be such as, by giving us new and other developments, and laws more and more general in their applications, will make even me think that what is written and illustrated in these experimental researches, belongs to the by-gone parts of science." And to those who doubt the wisdom or expediency of such enquiries, may be quoted the words he used in a letter to Tyndall (June 28th, 1854): "But then our subjects are so glorious, that to work at them rejoices and encourages the feeblest; delights and enchants the strongest."