WHAT IS HEREDITY?

By Dr. A. C. R. DEAN and Sir CYRIL HINSHELWOOD, O.M., F.R.S.

Physical Chemistry Laboratory, University of Oxford

(1) Introduction

LUCRETIUS likened heredity to the handing on of the torch of life by runners. The present image is that of the genetic code. This is less poetically fanciful in that it refers to the permutations and combinations of known chemical units in perfectly definite macromolecules; but it is still metaphorical in so far as it suggests by analogy rather than defines how the chemical sequences determine the properties of progeny.

The picture of endless replicas of a code being printed off in some copying process gives a rather rigid view of heredity, which in the absence of sexual recombinations can only change as a result of 'mistakes' in copying. When, however, more detailed thought is given to the translation of the code, which involves the functioning of entire cells, heredity turns out to be a somewhat more complex idea than appears at first, even with unicellular organisms which multiply by binary fission. Since germ cells in general multiply in this way, the extra complexity must be present in sexually reproducing organisms in addition to all that associated with sexual recombination itself.

We have already discussed the way in which the general organization of the cell reactions can determine some characteristic biological behaviour of micro-organisms[1,2]. This kind of approach is sometimes said to imply a Lamarckian view of heredity. In what follows we examine the way in which cellular organization may persist during successive generations. The conclusion is that, in this connexion at least, the Lamarckian debate has little significance and that heredity may cover a wide range of biological phenomena.

(2) Morphology of Living Matter

One of the most wonderful things about animate Nature is the complex variety of its morphology. This somehow is conditioned by the chemistry of living matter, and the information which prescribes it lies latent in the so-called genetic codes, which themselves are in effect simply chemical formulæ.

To understand in detail the translation of genetic information into structure is a formidable task, but the basic idea is simple. The initial units of information are separated in space, strung out, for example, along a chain of deoxyribonucleic acid (DNA). They are the seat, severally, of chemical reactions of some sort, the substances formed diffusing away to react presently with one another or with extraneous materials. Thus the concentrations of chemical substances form spatial patterns and vary from place to place. Essential synthetic reactions will often occur at rates determined by some functions of these concentrations. Such functions may also determine processes like precipitation or coagulation. The required values of the relevant functions will only be reached at special places. With a spatial pattern of precipitation we have the beginnings of structure. The well-known example in the inorganic world of rhythmic precipitation to give bands of insoluble material separated by clear medium (Liesegang rings) is easily explained in terms of counter-diffusion and the values of ionic concentration products. Again, diffusion counter-currents from leaves and root must clearly play a part in determining plant morphology.

The intermediates involved in biochemical reactions *in vivo* will generally be labile substances which readily suffer alternative fates if the proper partner is not available at the right moment. The timing depends on the distance this partner has had to travel from its own point of origin, and this again emphasizes the importance of spacing, right back to the location of the genetic information.

We have no intention of entering into the fascinating field of morphology in general. What we wish to emphasize for the further development of the present argument is how much any living system must depend on the availability of the right substance in the right place at the right time.

(3) Internal Map of the Cell

Although in a more subtle way than a complex multicellular organism, a single cell such as a bacterium must possess a sort of internal structure of its own. Different chemical processes are initiated at different points of the code-bearing DNA for one thing. For another, the ingress of nutrients is conditioned by the often highly specific permeability of the surface layers. Enzymes are also located in the surface where they must be arranged to form a map or chart which will guide the release of substances into the interior. The interior itself is not a physically homogeneous mass. It possesses a constellation of particulate matter and forms, as it were, a semi-fluid three-dimensional mosaic.

The conception of this internal structure has been present in the literature for some time. For example, Sir Rudolph Peters[3] speaks of "some tenuous network by the action of which the cell's enzymic activities are co-ordinated (the term cytoskeleton was given to this by J. Needham; it is a good description provided that it does not convey the idea of the rigidity of a bone)", and L. Monné[4] speaks of the concatenation of parts of the cell by cytoplasmic fibrils. F. Dickens[5] speaks of the emphasis which he says has been rightly placed "on the cellular compartments for ATP, inorganic phosphate, DPN, TPN, enzymes, substrates and so on", while C. De Duve[6] writes, "One of the simplest consequences of structural organization is the fact that given groups of enzymes are associated together and separated from others". N. M. Sissakian[7] writes, "In this connexion, heterogeneity of the protoplasm has become the prerequisite for the localization of the biochemical properties within the cell. It is owing to heterogeneity coupled with selectivity of the individual cellular constituents that the biochemical functions have become associated with definite cell structures. In this sense one may speak of the absolute meaning of adjustment of biochemical functions to cellular structures, for wherever life becomes manifest we find heterogeneity and some kind of spatial distribution of biochemical processes and functions which results in the disconnectedness of some processes and in a close contact between others". P. Weiss[8] has some pertinent comments on this theme. For example, he says, "Cytoplasm behaves as a viscous liquid. It can be thoroughly mixed up by stirring or centrifugation, and yet its countless chemical workshops will continue to operate in good order, which implies proper spatial segregation". . . . "Manifest cell organization results from the response of organized elements to fields of organized (that is, non-random)

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physical and chemical conditions, here tentatively identified with conditions prevailing along interfaces". He also speaks of the combination of molecules "to yield composite structures of rather uniform dimensions, proportions and architecture", and says[9], "Any description of cell constitution and cell behaviour couched solely and directly in molecular terms misses the basic fact of the existence of intermediate supramolecular entities of unit character". He also remarks on the fact "that the heterogeneous mixture of components combined in the complex system operates within a framework of order, the stability of which contrasts sharply with the potential randomness of the individual component events if these were not subject to some over-all control"[9].

We must think then of a bacterial cell as having its own internal map. Disturbances of this map will tend to be resisted, partly because the anchoring of various constituents, including some enzymes, to the cell-wall will impart a certain degree of mechanical stability, and partly because displacement or serious change in volume of the various elements of the internal three-dimensional mosaic will also create pressures or tensions which will call, so far as possible, for relief.

(4) The Network Theorem

Besides this conformity to some kind of spatial map, the cell has an organization of a different kind manifested in what is often called the reaction pattern. As we have shown in previous discussions[1,2,10], nucleic acids, proteins, polysaccharides and other essential cell constituents are all mutually dependent and built up in long series of reactions which, in the nature of things, form networks, liberally supplied with alternative branches. These branches are closed because none of the essential components can be formed without the participation of others (for example, proteins coded by nucleic acids and nucleic acids dependent on the protein-containing enzymes for their synthesis). Reflexion also shows that they must be highly branched as may be illustrated with an example. All enzymes the proteins of which contain a given amino-acid residue (Class A) are dependent on the much smaller number of enzymes (Class B) which produce this amino-acid. Here many things depend on fewer. The enzymes of Class B all contain numerous amino-acid residues and so each one of them in its turn is dependent on a large number of others. In the first case a larger number depend on fewer and in the second a smaller number depend on more. Thus we have both diverging and converging relations, so that the total network of mutual dependences must contain numerous branches.

What we shall refer to as the network theorem shows that the proportions of the various cell components tend to settle down in any given environment to those values consistent with an optimum rate of growth. This theorem predicts the widespread occurrence of adaptive processes and of phenomena bearing a strong resemblance to what is often called enzyme repression.

The theorem is best formulated mathematically, but the general idea is perhaps worth stating in qualitative terms. If reproducing matter is transferred to a new environment in which more demand is made on certain enzyme functions, then, unless all the components are present in such proportions that they can multiply in step, some essential parts will be lagging behind, and this delay constitutes a bottleneck. Only when this has been removed, as it will be when the steady state has been eventually reached, will optimum growth be achieved. The principle can be stated in another way which has some descriptive utility. Any pattern of co-ordinated reproduction of a multi-component system will be selectively replaced in course of time by one which establishes and maintains itself faster than any alternative. As we have pointed out before, reaction patterns are as much subject to selection as abnormal mutant cells[1,10–13].

A reaction network is essentially an organization in time. Individual connexions are expressed by equations of dependence such as:

$$dX_1/dt = \alpha_1 X_2,\ dX_j/dT = \alpha_j X_{j+1}$$

and so on. The term network is, of course, used in an abstract sense to indicate closed systems of mutual dependence. It has no direct geometrical significance. Nevertheless, the values of the constants $\alpha_1, \alpha_2 \ldots \alpha_j \ldots$ are themselves functions of the cell geometry, structural map or cytoskeleton in so far as this determines the lengths of diffusion paths, the supply of materials from outside and the internal transfer.

As a result of this circumstance we have to envisage the interplay of two separate tendencies, the consequences of which are quite profound. On one hand, in a given environment, the various masses of material making up the cell will eventually settle down to form a three-dimensional map in which all tensions and pressures are reduced to a minimum and a maximum stability is achieved. On the other hand, that reaction pattern will be established which gives the maximum growth rate, and corresponding to this there will be a definite set of ratios for the cell components[2,10,13]. For an absolute optimum condition the three-dimensional map, or in P. Weiss's phrase[9] the spatial segregation, must be such as to allow the optimum reaction network with minimum internal stresses. This demands a nicely adjusted disposition of material so as to give strain-free space-filling conditions as well as diffusion paths permitting the best possible values of the α terms.

If the cell is transferred to a new environment, for example, to new sources of nutrient or to a medium containing a toxic agent, the first response will be the establishment of a new reaction pattern in accordance with the network theorem. Although the cell is to a large extent a fluid and elastic system, there will be a limit to possible adjustment since new pressures and tensions will be called forth by the change. So far as it can go, however, the response will be rapid, and will in fact be nearly complete as soon as the cell has multiplied enough times for newly formed material to outweigh the original. This marks stage I of the adaptive process. In accordance with the network theorem it should be readily reversible.

The spatial map in the cell is now, however, not the best possible, and a second stage of adaptation may set in, which will be slower and more difficultly realized. In this there will gradually be established a spatial map allowing the cell components in their new proportions to fit more satisfactorily together. We must, therefore, consider the way in which this packing is determined.

(5) Architectonics of the Cell

Between cell components forces of different orders exist. Primary chemical valencies form the links of long chains, which themselves coil and fold through the action of electrostic forces, hydrogen bonds and van der Waals forces. Regions of nuclear-type material bear a coded arrangement of chemical units. The structure of the surface is specific and in some degree ordered. Thus a kind of polarizing force will operate from the central parts of the cell to the periphery. When a cell is well adapted to its conditions of growth the various packings and foldings will have settled down to some sort of relative minimum of potential energy.

Something very remarkable happens when a cell divides. One field of polarized structure becomes two practically identical ones. We do not know enough for a detailed theory of the division process, but two general statements seem highly probable. In the first place, since two almost identical structures result, it must be a rather orderly process in which the upheaval of the cell substance is kept to a minimum. This impression is strengthened by the almost military evolutions shown in the karyo-

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kinetic figures of certain cells. On the other hand, this important regrouping of the cell parts giving two fields where previously there was one, however orderly, must involve at least some degree of loosening of the whole packing. We may, therefore, take it as probable that the periods of cell division are those when any refolding or reorganization of the packing type has the best chance to occur in conditions where the existing ones have ceased, as a result of environmental change, to be the most stable. But since division is an orderly process this reorganization will probably be quite slow and take place little by little, being completed only during the course of many cell divisions.

From this we would conclude that when a new chemical reaction network has been imposed, the final adjustment of the cell to its optimum spatial map may be a long, slow process.

(6) Rate of Adaptations

Bacterial strains are often maintained by periodic transfer to fresh media. Those used in practice have sometimes an indefinite composition and it is advantageous when quantitative measurements have to be made to use chemically defined ones. Strains of *Aerobacter* and *Escherichia coli* can be grown successfully in a 'minimal medium' containing only a source of carbon, phosphate buffer, magnesium and ammonium sulphates and various trace metals. Glucose is often used as the carbon source, and when cells of these organisms are transferred from a complex medium to a minimal medium containing this sugar it is found that, although growth takes place quite readily within one to two hours, between 10 and 12 daily sub-cultures are necessary before the growth rate climbs to the optimum value.

If cells fully acclimatized to a glucose medium are introduced into one containing a different carbon source, various patterns of behaviour may be observed. For example, the growth rate of *Aerobacter aerogenes* goes on improving gradually during 9 sub-cultures in fumarate medium and during 29 sub-cultures in succinate medium[14]. With acetate as carbon source the following successive phases were observed: (1) slow growth in acetate, optimum rate of growth in glucose; (2) optimum rate of growth in both media; (3) optimum rate of growth in acetate, somewhat impaired growth in glucose; (4) growth rate in both media fluctuating widely but growth in glucose medium never normal. These four phases were observed during 128 twice-daily culturings and stage (2) was not reached until the seventeenth (ref. 15). In a sucrose medium the growth rate of *Aerobacter aerogenes* improved over the first 10 sub-cultures[16], and in a lactose medium a similar pattern was observed in one set of experiments[16], while in another set the adaptation required 20 sub-cultures[17]. A more complex picture is seen in a maltose medium, where the initially slow growth improved gradually during the course of 3 sub-cultures and then remained more or less steady at the new rate for a further 10 before improving again to the final optimum[16].

In the examples given so far the delays preceding growth at the first transfer were small, but when *Bact. coli mutabile* is introduced for the first time into a lactose medium or *Aerobacter aerogenes* into a D-arabinose medium, lags of several days occur. Growth when it eventually takes place is slow and here again the rate improves gradually over many sub-cultures[16,18]. This type of behaviour has also been observed with yeast cells during adaptation to dispense with aneurin or to utilize raffinose as a sole source of carbon[19].

In media containing drugs the slow completion of an initially fairly rapid adaptation is also of common occurrence. For example, when cells of *Aerobacter aerogenes* are introduced for the first time into a medium containing 2 mg/l. of terramycin there is a lag of about 1 day, and then growth takes place. The rate, however, increases gradually over the next 20 or so sub-cultures[20]. At 43 mg/l. of proflavine the initial lag is about 40 h and then growth occurs at about 60 per cent of the rate gradually reached over the next 19 sub-cultures[21]. The gradual approach to the final optimum is sometimes obscured by the method of 'training' organisms to be resistant. A strain 'trained' to a given concentration of drug will always grow in a slightly higher concentration. Thus strains of *Aerobacter aerogenes* which will grow in concentrations of proflavine as high as 3,000 mg/l. can be obtained by stepwise increase of the drug concentration[22]. When highly resistant strains are prepared the drug concentration is often increased in smaller steps than are actually necessary. The time required for completion of adaptation at the highest concentration is naturally shortened if the intermediate stages have been very thoroughly carried out.

(7) Reversibility of Adaptations

In various physical and chemical systems there are great differences in the ease with which an imposed shift of equilibrium is reversed when the original conditions are restored. When a solid is heated to its melting-point there is no delay at all in melting. When, on the other hand, the liquid is cooled to the melting-point or just below, there may be a delay, sometimes almost indefinitely prolonged, before resolidification. The reason in this example for the unsymmetrical relation lies in the fact that the initiation of melting entails a passage from order to disorder while that of crystallization depends on a spontaneous local creation of a small region of order from disorder, which is improbable. Phase changes in general may be associated with highly complex phenomena of hysteresis and delay.

The reversibility of adaptive changes in cells is also a complex business. According to the network theorem, apart from special circumstances considered in section 8, the changed reaction pattern established in a new medium should in its turn be rapidly replaced by the original one when the cells are grown once more in the old medium. The reorientation of the spatial map allowing the slow completion of an adaptive process is more akin to a recrystallization or a phase change than to the establishment of a new reaction network. As we pictured it in section 5, it is usually slow since it depends on a very elaborate repacking of a highly complex system, which we envisage as dependent on chances snatched during the upheavals of successive cell divisions.

According to the view we have adopted the loss of a completed adaptation is not a simple reversal of the process by which it was acquired. There will in effect be a sort of hysteresis cycle which in its simplest form we may represent as follows. Let the reaction network in an original environment be designated by $N(1)$ and the spatial map by $M(1)$. In a new environment there will be established the new network $N(2)$ with initial retention of $M(1)$, which, however, gradually changes into $M(2)$, so that the succession of states is $N(1)M(1)$, $N(2)M(1)$, $N(2)M(2)$. On return to the original environment $N(2)$ will change back as nearly as possible to $N(1)$, so that the first stage of the reversion is through the state $N(1)M(2)$ which, it is important to note, the cells have never been in before, so that reversal does not simply retrace the steps followed in the direct process. New and complex phenomena may, therefore, be expected. As we shall see, they exist in abundance.

At this point we need to make a certain further elaboration of what has been said so far. In the adaptation the cell passes from the state $N(1)M(1)$ through the state $N(2)M(1)$ to $N(2)M(2)$. $N(2)$, which is the best network for the new medium while $M(1)$ persists, may no longer be quite the best for that same medium when $M(2)$ is established, so a further adjustment of the network occurs, and in turn a further adjustment in $M(2)$. These processes can be repeated until we reach the absolute optimum,

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where we have $N'(2)$ and $M'(2)$, N' and M' being various successive stages of adjustment removed from N and M. It is now even more true that on return to the original medium 1 there will not be a simple retracing of the course of adaptation.

In particular, when a succession of mutual adjustments of N and M have occurred, it may happen that some of the intermediate stages of the return from $N'(2)M'(2)$ to $N(1)M(1)$ which have not been traversed before are even less favourable than $N'(2)M'(2)$ itself for growth in the original medium. In this case reversion would not easily occur: or it might go a certain way and then be held up at some intermediate stage. Although the final reverted state, $N(1)M(1)$, would be the optimum in the original medium, this fact will not ensure complete reversion if there is any barrier in the way. A barrier exists whenever an intermediate stage is less favourable than one just before it. This is analogous to the common phenomenon where chemical processes are impeded by the interposition between two states of an intermediate state of higher energy.

Suppose medium 1 is a normal growth medium and that medium 2 has the same composition except for the presence of a drug, the adaptive process in this case being the development of drug resistance. The first stage of the adaptation is the relatively rapid establishment of any reaction network which allows growth at all. According to what we have said, this adaptation would be easily lost. Gradually the more complete adaptation occurs which, as we have seen, may be not nearly so easily lost. The cells, it must be remembered, have not only to grow in spite of the presence of the drug, but they must not lose their capacity for growth in the drug-free medium itself. The state we have referred to as $N'(2)M'(2)$ must, therefore, be consistent with at least fairly good growth in the original medium. If it is fully as good, then there will be no tendency at all to revert to $N(1)M(1)$, and even if it is not quite so good, it may still be better than some of the intermediate conditions which would have to be passed through on the way back. In either case the loss of drug resistance will be slow or partial. There are, moreover, reasons for believing that in some circumstances an established and operative growth mechanism may on occasion actually inhibit the development of one not yet mobilized, even though the second is the more efficient when competing on equal terms with the first (section 8).

Thus we might expect a great variety of types of behaviour ranging from easy and complete loss of adaptations, through slow and partial losses, to very stubborn retention.

Even this is not the whole story. The successive adjustments of reaction network and spatial map must lead not only to minimum interference by drug but optimum use of a medium in which growth is already optimal in the absence of the drug. On occasion these conditions may be best fulfilled by a reaction scheme into which the drug enters directly as a metabolite. Once such a scheme is established, it may, for the reasons mentioned above, be difficult to replace by the original pattern of growth. In such an event we would have the development of an actual dependence on the presence of the drug for optimum growth, that is the phenomenon of drug dependence (cf. ref. 23).

(8) Special Reasons for Slowness of Reversion

Several mechanisms to explain the retention of an adaptation on return of cells to their original environment have been suggested in the past. They are in no way incompatible with the general considerations of the preceding section, though they are more specific.

The first was applied to drug adaptation. Suppose the drug so affects the working of the cell that the concentration of an intermediate metabolite, which would normally saturate the enzyme processing it for the next stage, is reduced. The adaptation establishes a state of affairs where this concentration returns to something like its original level despite the presence of the drug. If now the adapted cells are grown without drug, the level will rise still higher. This increase would normally force the adaptation into reverse. If, however, the enzyme is saturated, the extra concentration has no effect on the balance of cell processes, and there is thus no reason for the reversion to occur. The adapted state is now metastable[13,15,24,25].

Another possibility is that the reaction pattern established after adaptation is one in which there is high consumption of intermediates needed for the re-establishment of the old reaction pattern. The original mechanism having fallen out of use, it will then have great difficulty in starting up again in the presence of its competitor, even though it is inherently more efficient[10,12]. In such an event we shall see a reversion only after the cells have been so treated as to diminish temporarily the competitive power of that mechanism with the advantage of starting in possession. This argument is easily put into simple mathematical terms[10].

The permutations and combinations of processes in the reaction network are so numerous that in the original medium itself there may be alternative mechanisms for growth, some of which are only a little less efficient than the optimum. It may be one or other of these which becomes established in the course of an adaptation to a new environment. Even in the old environment it now will not be markedly at a disadvantage so its selective replacement by the optimum mechanism can be quite slow.

(9) Reversion

The whole discussion of reversion and stability has led us to expect a wide spectrum of possibilities ranging from rapid reversion to apparently almost complete stability. The experimental evidence on the stability of adaptations to drugs and to new nutrients will now be discussed.

From a survey of many different examples the following general conclusions may be drawn:

(1) After a relatively short period in drug medium, although a quite marked degree of resistance may be reached, this resistance is easily lost by growth in drug-free medium.

(2) Longer periods of growth in drug medium result in a more stable resistance, and there is a direct correlation between the stability of the resistance and the length of time for which the 'training' in the drug medium has been continued. There is considerable variation from drug to drug and even in different experiments with the same drug, but within any given set of experiments the correlation holds.

(3) With the more thoroughly 'trained' strains the resistance is usually only very gradually lost over a large number of generations in drug-free medium. In the majority of examples, however, the end result is a strain practically as sensitive as the original. It is true that in experiments with *Aerobacter* and propamidine isethionate the resistance was not completely lost after 203 sub-cultures in drug-free medium[15], while with the same organism and terramycin, even after only one sub-culture in the drug, the resistance was still more tenaciously held[20]. On growth in the absence of drug it dropped to an 'equilibrium' value intermediate between full resistance and sensitivity and remained in this state without further change for more than 400 generations.

(4) Resistance to very high concentrations of drugs is in general less stable than resistance to lower concentrations.

More highly organized cells such as the yeast *Saccharomyces cerevisiae* and the fungus *Penicillium roqueforti* seem to become resistant to drugs at least as rapidly as bacteria but lose their resistance very much more readily[26-29]. As in the terramycin example just given,

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Table 1. Effect of Growth in Drug-free Medium on the Resistance of *Aerobacter aerogenes* to Drugs

Drug	Concentration (mg/l.)	No. of sub-cultures in drug	No. of subsequent sub-cultures in drug-free medium	Extent of reversion	Ref.
Proflavine	43	*a*. 0·3	1 to 2	considerable	38
		b. 15	8	partial	
		c. 15	20	partial, less than *b*	
	195	*a*. 105	63	none	15
		b. 105	70	partial	
		c. 105	87	almost complete*	
	214	*a*. 203	21	none	15
		b. 203	29	partial	
		c. 203	34	partial, greater than *b*	
		d. 203	75	complete	
Streptomycin	1,000	*a*. 3	15	complete	37
		b. 6	30	partial	
		c. 6	77	almost complete	
		d. 19	82	none	
Thymol	140	*a*. 1	5	almost complete	39
		b. 4	5	partial	
		c. 10	5	less than *b*	
		d. 27	5	less than *c*	
Sulphanilamide	218	3	1	almost complete	40
Chloramphenicol	37	*a*. 79	18	none	15
		b. 79	55	partial	
		c. 79	82	complete	
	69	*a*. 114	11	none	15
		b. 114	22	partial	
		c. 114	46	partial, greater than *b*	
		d. 114	71	complete†	
Propamidine isethionate	267	*a*. 102	24	none	15
		b. 102	43	partial	
		c. 102	203	partial, greater than *b*	
Terramycin	2	*a*. 1	37	partial	20
		b. 1	87	same as *a*	
		c. 20	66	same as *a*	

* Resistance largely lost to 100 and 50 mg/l. also.
† Resistance largely lost to 37 mg/l. also.

however, the resistance of *S. cerevisiae* to 2,4-dinitrophenol dropped readily to an 'equilibrium' state and then remained unchanged for a considerable time[27].

The 'equilibrium' state is often observed in the adaptation of bacteria to utilize new carbon sources. For example, after *Bact. coli mutabile* had been grown 22 times in lactose medium, two transfers in glucose were sufficient to induce a very slight degree of reversion which remained unchanged for a further 81 culturings, each of which involved about 8 generations[30]. A similar situation is found with *Aerobacter aerogenes* and D-arabinose[31], but with *E. coli* and D-arabinose the stability is less marked and reversion is accompanied by the appearance of types intermediate in character between the 'arabinose-positive' and 'arabinose-negative' forms[31]. If, however, after only a few generations of growth in D-arabinose medium, glucose is added to a culture of *Aerobacter aerogenes*, reversion is rapid and no stably adapted cells can be detected[32]. A strain which had been grown 65 times in acetate medium required 62 culturings in glucose before the 'training' to acetate was lost[15], and in the adaptation of *Aerobacter aerogenes* to utilize glycerol, as measured by growth rate or dehydrogenase activity, the stages (1) adaptation complete but unstable, (2) adaptation complete showing delayed reversion, and (3) adaptation stable with no reversion, were observed as serial sub-culture in glycerol medium was continued[33].

These stages were also seen in the 'detraining' in glucose medium of a strain of *Aerobacter aerogenes* which had been trained to utilize lactose as sole source of carbon[16]. In the adaptation of *S. cerevisiae* to galactose, although delayed reversion was observed after the cells had been grown once in galactose, a further 14 culturings resulted in a stability which withstood 100 culturings in glucose[34].

Sometimes reversion can be hastened if the cells, instead of returning to the original medium, are grown in yet another new one. For example, if *Aerobacter aerogenes* is stably adapted to utilize lactose as a sole source of carbon after having been fully conditioned to glucose, reversion takes place more quickly on growth in maltose than in glucose[16] and in a similar manner the reversion of the adaptations of *Aerobacter aerogenes* to D-arabinose and of *Bact. coli mutabile* to lactose has been hastened by growth in acetate and succinate medium[31,35]. Reversion of proflavine resistance has been brought about by growth in the presence of *m*-cresol[36].

Some illustrations of these general statements are given in Table 1. A 'sub-culture' usually involved about 8 cell generations. Where a fraction is referred to in Table 1 it means that the sample was taken after two or three cell divisions in the course of the first sub-culture. In the experiments with 43 mg/l. of proflavine, 2 mg/l. of terramycin and 218 mg/l. of sulphanilamide, these concentrations were the first to which the cells were exposed. With streptomycin for special reasons[37] cells already given a low grade of resistance by selective growth at 1 mg/l. were introduced into 1,000 mg/l. In all the other examples the cells were 'trained' by serial sub-culture in gradually increasing concentrations of drug until the desired level had been reached.

(10) Can the Code Change?

The dogma that the genetic code cannot be changed is sometimes regarded as sacrosanct, though in a special way which seems still to allow the assumption of spontaneous mutations to account specifically for surprisingly fine shades of biochemical difference. The orthodox assumption is that the changes in base sequence in these mutations must be random, and can be adaptive only in the sense of constituting a possible ground for selective shifts of population.

The point of view which we have been discussing in the foregoing sections is consistent with a very great stability of the genetic material, since it attributes many adaptive phenomena to a change in the cytoplasmic balance in cells with an unchanged code. It could be argued perhaps that, since major transformations like that of one species into another are never observed, this stability is almost absolute, and that those adaptive changes which occur very easily must be of a quite different nature from anything involving an altered code. But in the absence of more detailed understanding of how the code expresses itself in properties of the cell, a quantitative argument of this kind is dangerous*.

Our own hypothesis has not required the assumption of an altered code. Nevertheless the question remains important whether directed changes in it are or are not possible. Mutations are readily assumed by almost all authors. Thus the base sequence, although agreed to be firmly fixed, is not believed to be quite unalterable.

At each step in the polycondensation reaction leading to a nucleic acid there tends to be conformity with a predetermined stereochemical pattern. Departure from this may be unlikely but should not be impossible, since the addition of the 'wrong' base at a certain stage has only the sort of improbability that the formation of a *meta*-benzene derivative has in conditions strongly favouring *ortho-para* substitution. The improbability, in other words, is an affair of activation energy, and an unfavourable activation energy can always be compensated in some measure by increase in the concentration of the appropriate reactants. Suppose a new cytoplasmic organization greatly increases the intracellular concentration of base X. There will be an increased chance that in certain places X will occasionally be incorporated in place of Y even though this means a much less probable reaction.

Are the effects of this abnormality wholly random? A too confident negative answer to this question may not be fully justified. The new code with X in place of Y, once established, tends to persist simply because the altered steric relations now impose this order as the most probable mode of replication in subsequent acts. This

* The stability in question, however, it should be noted, is precisely that of many of the 'markers' used in genetic experiments.

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circumstance itself now creates a certain local demand for X rather than Y. The provision of X rather than Y will in fact give better growth, and, as we have seen, the response to particular needs is the expansion of enzyme systems capable of supplying them. Thus a changed metabolic pattern could render a code-change more likely (that is, favour a mutation), and this change, by creating a new demand, could tend to favour the kind of metabolism which would preserve it.

It is difficult to say how large the effect of this undoubted tendency would be in practice, nor have we, for our present purposes, any urgent need to attribute great significance to it. Nevertheless, it seems likely that the relation of codes and their expression is more complex than is sometimes suggested. In the last resort, moreover, we must remember that there are few (if any) examples of a situation where A has an effect on B while B has absolutely none on A. If a change in cytoplasmic organization can never in principle affect a code at all, a rather special kind of chemistry seems to be indicated, and possibly even the abrogation of Newton's third law.

(11) What is Heredity ?

The argument from 'stable heredity' has been used a good deal to discredit adaptive mechanisms of a physiological kind which assume, rather than a simple shift in the balance of a population, the modification of most of the individuals. Transmission of an 'acquired' character has been frequently ruled to be 'Lamarckian' and impossible.

We have shown, however, that adaptive changes, such as development of drug resistance or improved utilization of new substrates, are in fact not really permanent at all. Perhaps, therefore, they might be ruled not to be stably heritable, and, if they are not, then the Lamarckian objection loses its point. Perhaps, on the other hand, they might be pronounced not to represent acquired characters, since they depend essentially on the enhancement of capacities already present. In that event, too, the objection lapses.

Nevertheless, as we have seen, some of these changes represent quite considerable modifications, a cell capable, for example, of resisting 1,000 mg/l. of chloramphenicol differing in quite an important way from one capable of resisting only 10 mg/l. The quantitative aspect of the adaptation is great enough to represent what for many purposes can be regarded effectively as the appearance of a qualitatively new form, even if the difference is far less than corresponds to a species change. Furthermore, the persistence of the changes, through subsequent generations, although far short of absolute stability, is quite marked.

What happens in many adaptations can be given a straightforward interpretation in physicochemical terms. If then we really wish to regard these adaptations as the development of new characters and if we wish to count the relatively slowness of reversion as equivalent to stability, and if, further, anyone wishes to call the interpretation Lamarckian, then all that can be said is that this designation must lose the depreciatory connotation usually associated with it. There is no real need to bring Lamarck into the discussion at all, but it may just be observed that his name has been bandied about in a rather unscientific way. As Graham Cannon has pointed out[41,42], people have misquoted him, and caricatured his views, forgetting that Darwin himself took them seriously.

The more important question is what status, in terms of heredity, is to be given to the kind of adaptive changes we have been discussing. We have seen, at one extreme, that there is an inherent possibility that a long-imposed change in reaction pattern might conceivably stabilize itself by causing some direct modification of the genetic code. If this sort of thing could occur at all, then there would be no question of the potential evolutionary significance of physiological adaptations of cells.

But, even short of this extreme, quite good explanations of the observed facts about the persistence of adaptations can be given without the assumption of code changes. Modified reaction patterns and spatial maps can be reflected in long-range effects without them. By persisting long enough, moreover, they may expose the cell to still further changes which would not have occurred had reversion from the earlier ones been more rapid. It would seem then that in any event, according to physicochemical principles (which, as B. Commoner[43] has well argued, are never at variance with biological principles provided that both sets are soundly applied), cytoplasmic organization can have important and far-reaching effects on the transmission of cell properties.

Unicellular organisms are in a special position, in that, multiplying by binary fission, they cannot help transmitting at each generation the cytoplasmic organization as well as the genetic code from parent to progeny. Thus in some ways all transmission in them is akin to what is called cultural inheritance in higher forms. If anyone wishes to deny the application of the term heredity to these phenomena he is presumably at liberty to do so. But whatever terms are used there is more to the development of living matter than the stencilling off of replicas, with or without an occasional slip. In complex organisms with sexual reproduction the segregated germ cells are maintained in a nearly constant environment. But somatic changes in the individual will be associated with adaptive changes in some of the many types of differentiated cell which it contains, and these adjustments in their turn can affect the biochemistry of the organism and thereby lead to second order effects even on the remote germ cells. Whether or not these effects can ever assume major importance over long periods of time is an open question; but to deny their possibility on the strength of the usual type of experiment designed to test for Lamarckian phenomena is rather like looking down a low-power microscope and dismissing the atomic theory as a myth.

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X-RAY DIFFRACTION AND THE SYMMETRY OF THE ACTINOMYCIN MOLECULE

By HILDA T. PALMER, REX A. PALMER and RICHARD E. DICKERSON

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena

BACHMANN and Müller[1] have recently reported three crystal forms of actinomycin. From space group symmetry arguments and model building they have expressed a preference for the double pentapeptide ring structure rather than a decapeptide model. Perutz[2] has since pointed out that the conclusions which they have drawn are unjustified, and that the results presented provide no basis for a choice between these two alternative structures. We should like to present information on the molecular symmetry of actinomycin, crystallographic and non-crystallographic, obtained from a systematic investigation of the symmetry of the Patterson vector map. This information places limits on possible model structures and provides a preference, of the most tentative sort, for the decapeptide.

Actinomycin is an antibiotic of molecular weight about 1,260, made up of ten amino-acid residues and a phenoxazone chromophore. According to Brockmann[3], there are two pentapeptide rings, each closed by a lactone linkage and attached separately to the chromophore (Fig. 1). It has since been suggested that the molecule might as easily be made up of one decapeptide ring like gramicidin *S*, the tyrocidins, and several other similar antibiotics. The ring would then be closed at opposite ends by two lactone linkages and attached to the chromophore at two points (Fig. 2). Chemical evidence on this choice so far is inconclusive.

The particular amino-acid sequence shown in Figs. 1 and 2 is that of actinomycin C_1, also known as B_1, D_{IV} or IV. Other naturally occurring actinomycins differ generally in the substitution of D-alloisoleucine for one or both D-valines and of hydroxyproline or 4-ketoproline for one or both prolines[4]. All amino-acids are in the L-form except for those at the D-valine sites. Actinomycin C_2 has one D-alloisoleucine and C_3 (ref. 1) has two. The material examined here, actinomycin *B*, is a mixture of the component of Figs. 1 and 2 (29 per cent) and two others with one 4-ketoproline (59 per cent) or with one hydroxyproline (10 per cent)[5], with an average molecular weight of 1,266.

Crystal Examination (Table 1)

A three-dimensional structure analysis of actinomycin *B* is now in progress with multiple isomorphous replacement phase analysis using the parent compound, chloroactinomycin and bromoacetylactinomycin. The original sample of actinomycin *B* was obtained through the courtesy of Dr. W. D. Celmer of Chas. Pfizer and Co. Roughly equidimensional parent crystals of 0·2–0·5 mm size can be grown with ease in 1–2 days from the solvent pairs of Table 1. Derivative crystals grow much less readily and are much smaller, but are isomorphous with the parent crystals.

Table 1. ACTINOMYCIN CRYSTAL DATA

Actinomycin:	C_3	C_3	C_3	*B*
Crystal type:	*A*	*B*	*C*	new
Source:	Ref. 1	Ref. 1	Ref. 1	This paper
Crystal class:	Trigonal	Monoclinic	Monoclinic	Monoclinic (triclinic)
Space group:	*R*32	$P2_1$	$P2_1$	*C*2 (C1)
Cell Parameters:	*a*: 18·7 Å	21·4 Å	17·6 Å	26·8 Å
	b: 18·7	27·2	19·9	18·5
	c: 38·4	14·0	23·1	25·7
	β: (90°)	107·9°	97·8°	95·0°
Volume:	11,629 Å^3	7,755 Å^3	8,016 Å^3	12,700 Å^3
Molecules per cell:	6	4	4	8
Molecules per asymmetrical unit:	1/3 (disorder ?)	2	2	2 (4)
Solvent of crystallization:	Methanol + H_2O Ethanol + H_2O Ethyl acetate	Benzene + ligroin	Chloroform + ligroin	Methanol + dibutyl ether Methanol + butanol Ethyl acetate + CS_2
Observed density:	1·19 g/cm^3	1·19	1·22	1·315 ± 0·01
Calculated density:	1·10	1·10	1·06	1·322
Limit of resolution of diffraction pattern:	1·67 Å	2·5 Å	1·1 Å	Around 1 Å

Bracketed quantities in right-hand column refer to the reduced symmetry triclinic cell.

At first sight the appearance of the diffraction pattern and the absence of reflexions with $h+l$ odd suggest the monoclinic space group *C*2. (*Cm* or *C*2/*m* are ruled out by the presence of asymmetric amino-acids in only one form each.) The measured density then requires eight molecules per cell or two per asymmetric unit, with no detectable liquid of crystallization. The unit cell dimensions are unchanged on drying the crystal. A closer examination of some but not all crystals, however, shows intensity differences between reflexions of the type (h, k, l) and $(\bar{h}, k, \bar{l})$, indicating a breaking of the two-fold symmetry and a reduction of the true space group to triclinic *C*1 (or *P*1 with a change of axes). The approximation to a two-fold axis about *b* and to monoclinic symmetry is extremely good out to 3·5 Å resolution, beyond which point it deteriorates markedly. A low-resolution Patterson vector map will be virtually unchanged by the use of full triclinic data, and arguments based on Patterson symmetry will be the same. The work described here has been carried out with the monoclinic data.

The diffraction pattern (Fig. 3) extends out to approximately 1 Å resolution. The decrease of average intensity in three dimensions with sin θ is smooth and shows no sign of the 10 and 5 Å maxima found in several proteins and in gramicidin *B*[6]. A prominent group of four or five strong reflexions is found around $(\bar{2}, 0, 7)$ as can be seen in Fig. 3, and produces layers of vector density in the

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