259. *Induced and Other Variations in Bacterial Cultures. Part I. The Chemical Basis of the Changes.*

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In order to define the theoretical background for the discussion of ensuing experimental studies, various kinds of chemical basis for variations in bacterial characteristics are classified.

There is nothing in the last resort upon which the properties of a bacterial cell can depend except the molecular pattern of its cell substance. This pattern involves (*a*) the structure of individual macromolecules, (*b*) their types of folding, and (*c*) the proportions in which the various sub-patterns are present in the cell as a whole. The existing material is copied with more or less exactness whenever the cell multiplies and divides, the contents being almost equally shared between the two new cells formed.

In principle the essential reproductive parts of the cell may (1) constitute a single whole, more or less equivalent to a uniform phase, or (2) they may separate into mechanically distinguishable units which are virtually separate phases, as appears to happen in cells containing chromosomes. In case (1) the heredity is determined by the direct copying while in (2) it may also be governed largely by redistribution of certain basic structural units when sexual unions are involved.

In both cases the fundamental consideration is the making of replicas of the molecular pattern, but in the second case there is the added variety of recombination or segregation of, as it were, mechanically detachable sub-units of this pattern. The second process is a powerful source of property variations, but can only occur when there are sexual conjugations. With bacteria, such phenomena, although they have been claimed (see review by Lederberg, *Heredity*, 1946, **2**, 145), are at least exceedingly rare and in the vast majority of cases the cells multiply by simple fission without the possibility of recombination phenomena.

The properties of bacterial cells are very much subject to variations. These are sometimes of an adaptive kind, the power to utilise new substrates or to resist inhibitory substances being gradually developed as growth proceeds in suitable media. Other changes in biochemical character are also induced by exposure to ultra-violet light and other radiations or by the action of chemical agencies such as the " nitrogen mustards." Ultra-violet light, in particular, gives rise to mutants which have usually become deficient in certain characters which the cells originally possessed (see, for example, Lea, " Action of Radiations on Living Cells," Cambridge, 1946). Certain biochemical reactions may fail in the mutants, the radiation having evidently destroyed or impaired some part of the molecular structure on which the property depended. These deficient mutants not infrequently recover their lost characteristics when cultivated under conditions which offer the opportunity of exercising the lost or impaired property.

A question of great interest is the chemical basis in general of these various changes, adaptive, mutational, and restitutive. The possible cases of change in general may be classified as follows.

1. Some separable parts of the structure become differently distributed as a result of recombination processes. This with all its subdivisions is essentially the Mendelian hereditary mechanism, which in certain types of phenomenon is of dominant importance. But with bacteria, where on the one hand conjugations are at least very rare (if they occur at all) and on the other hand variability is great, this case does not concern us very much.

2. The structure (including the folding of the macromolecular chains and so on) of a part of the cell may suffer a modification.

3. The *proportions* of different types of structure present in different regions of a cell may suffer *quantitative* modification. (This may be referred to for brevity as a change in enzyme proportions—though " enzyme " is here understood in a rather broad sense.)

Mixed cases of 2 and 3 with 1 would also be possible in suitable organisms.

Both 2 and 3 are subdivided in another way :

A. The change occurs in certain cells or even in one cell of the entire population, the modifications being provoked by agencies having no direct relevance to any adaptive changes involved. Mutations caused by ultra-violet light would come under this heading. They usually deprive a cell of a character which it originally possessed. In such a case they do not have any adaptive value, since the mere loss of a property could hardly enable a cell to compete more effectively with its unchanged neighbours. Changes could also be provoked by unusual thermal activation, encounters with chance impurities, and so on. If they happen to render the cell better suited for growth in some new medium, then in such a medium it can outgrow the unmodified types in such a way as to show what amounts to an adaptive response of the population as a whole. The regain of their lost properties by ultra-violet mutants *could* be due to this.

B. Large numbers or indeed all the cells suffer a direct modification as a result of their growth in changed conditions.

The mechanisms of 2A and 3A include all possible physical and chemical agencies which are capable of affecting molecular structure or indeed colloidal form. Any relation of the agency to the final biological result is caused merely by selection of forms which happen to be favoured by particular media.

The mechanisms of 2B and 3B are of greater interest from the physico-chemical point of view, since we have to show how adaptive responses can arise.

Case 2B might conceivably be analogous to the activation of a heterogeneous catalyst which occurs when it is repeatedly made to catalyse the same reaction. In this process the microcrystalline structure and possibly even the lattice spacings of a solid catalyst are gradually dragged into that configuration which gives an easy adsorption of (and so favours the formation of) the product of reaction. The analogy of enzymes and heterogeneous catalysts is too close for this possibility to be entirely neglected.

Case 3B is the hypothesis which has been explored in detail in this laboratory. Cells utilise very varied substrates to build up the same constituents of their mass. There must be a considerable number of alternative metabolic routes, and these will involve the utilisation of various enzyme systems to different extents. Now it may be shown that, since the effective combinations of enzyme systems reproduce themselves in the course of cell growth, there must be a linking of the functioning and the synthesis of enzymes, and that the enzyme proportions which are synthesised in a given medium will attain that value which corresponds to the optimum rate of growth. This provides for an automatic adaptive response (Hinshelwood and Lewis, *Proc. Roy. Soc.*, 1948, *B*, **135**, 316; Hinshelwood, " Chemical Kinetics of the Bacterial Cell," Oxford, 1946).

What should be specially emphasised (and it is not always realised) is that there is considerable similarity in some respects between 3A and 3B. As regards the total bacterial mass the results are identical. In the one case certain parts of the population increase relatively to the others : in the alternative case certain parts (already in existence) of each cell increase relatively to the rest. This identity in respect of total mass explains why the alternative hypotheses are difficult to choose between experimentally.

It is necessary at this stage to point out that the whole chemical question cannot be ruled out of court by the familiar objection that acquired characters cannot be transmitted, as adaptive changes in fact often are, and that, therefore, these cannot be really adaptive. If the character in the cell had to have been present from all time, then cultures isolated from single cells could not show adaptive phenomena (and they certainly do). If all changes arise from

chance mutations (leaving the recombination phenomenon aside), then the properties dependent on the mutations are just as much acquired as any which arise from changed enzyme proportions caused in turn by such agencies as altered reaction rates. There remains indeed the question whether the modification is independent of, or caused by, the agent towards which its effect later becomes manifest. But this is logically quite unrelated to any considerations about the inheritance of acquired characters. The principle of non-inheritance properly applies to the usual absence of any effect upon germ cells of nutrition, exercise, or mutilation of the entire animal or plant in which they are formed. The reproduction of such organisms is entrusted to special cells to which the acquired characters of the whole body are almost entirely irrelevant. To extend the non-inheritance principle to unicellular organisms which are their own germ cells, and which simply divide into two parts when they multiply is virtually to assert that reproductive mechanisms know how a change is caused in a cell and that they will refuse to copy it if it is induced, but are ready to do so if it is due to chance.

The discussion of the chemical basis of cell changes may therefore be regarded as a purely technical question, not to be prejudged by this general principle.

In the light of the above discussion and classification we propose to examine in detail some of the chemical phenomena which occur when mutations are induced in *Bact. lactis ærogenes* (*Aerobacter ærogenes*). Among other things we shall be concerned with the mechanism of the recovery of lost biochemical characters under defined chemical conditions. We shall also be concerned with the question whether the loss and recovery of properties is gradual and quantitative or abrupt and qualitative, a matter which possesses interest from the standpoint of any kind of theory.

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