ADAPTATION IN MICRO-ORGANISMS

THIRD SYMPOSIUM OF THE
SOCIETY FOR GENERAL MICROBIOLOGY
HELD AT THE
ROYAL INSTITUTION, LONDON
APRIL 1953

CAMBRIDGE
Published for the Society for General Microbiology
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EDITORS' PREFACE

The organization of this Symposium was along lines essentially similar to those described for the previous one on *The Nature of Virus Multiplication*. The Committee of the Society for General Microbiology invited a number of authorities to submit contributions, under the general title of 'Adaptation in Micro-organisms'. These contributions, which form the main text of this book, were then printed and circulated in galley proof to all members of the Society some two weeks before the meeting at the Royal Institution, London, on 14 and 15 April 1953. At that meeting the papers were taken as read, and the time devoted to discussion, question and argument. No verbatim record of the proceedings was taken, but each contributor to the discussion was asked to send in his comments or questions. These were then sent to the principal contributors for their replies or counter-comments. From the material so obtained the Editors have attempted to construct a somewhat idealized account of the discussion.

Inevitably many of the points raised in discussion failed to reach the Editors; and some of those that did have had to be omitted, since they depended in their turn upon missing questions or comments. This means that much of the spontaneity of the discussion is lost. In compensation, however, questions that went unanswered on the day nevertheless appear here with neat and concise replies, often including references to the literature. The Editors hope that, whatever the shortcomings of the reported discussions, they will refresh the memories of those present at the meeting, and provide an indication of the range, scope and depth of the deliberations for those unable to be present.

The Society wishes to record its thanks to the following, whose generous assistance made possible the organization of the Symposium: the CIBA Foundation, Glaxo Laboratories, Guinness and Co., Imperial Chemical Industries, the Rockefeller Foundation, and the Wellcome Research Foundation.

Department of Biochemistry
University of Cambridge
28 *May* 1953

R. DAVIES
E. F. GALE

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ADAPTATION, EVOLUTIONARY AND PHYSIOLOGICAL: OR DARWINISM AMONG THE MICRO-ORGANISMS

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The task of providing an introduction to this symposium, which I accepted somewhat lightheartedly, turned out to be extremely difficult. 'Adaptation' is an amoeboid notion. In the attempt to give it some definite form, I was eventually driven back to first principles, and decided to try the use of an evolutionary approach; but in a microbiological context this raised a great many questions to which I have been unable to find satisfactory answers. Indeed, I fear that my well-meant effort at clarification and containment has been engulfed by its ostensible object, and I must therefore ask for your indulgence towards this discursive and somewhat interrogatory essay.

BIOLOGICAL PROPERTIES OF MICRO-ORGANISMS

At the risk of being trite, I shall start by summarizing the special biological features of micro-organisms which seem relevant to the problem of microbial adaptation. The first point to be considered is the genetic one. Sexuality has a very spotty distribution through the microbial world, and the sexual process often shows a low frequency in species which can undergo sexual reproduction. These facts imply that genetic recombination of the orthodox sort must play a relatively restricted role in the evolution of microbial genotypes; most micro-organisms would appear to have closed genetic systems. This last statement requires some modification, and may require more as our knowledge of microbial genetics is extended. The mycelial fungi, for example, possess in heterokaryosis a unique mechanism for maintaining an open genetic system without the necessity of sexual fusion. A fungal mycelium can be regarded as consisting essentially of a multinucleate cytoplasmic mass motile in a system of tubes (the mycelial wall); cytoplasmic fusion between two contiguous mycelia can take place, followed by nuclear migration, with the consequence that two genomes come to occupy a common cytoplasm. In bacteria, too, there is evidence for the existence

of non-sexual mechanisms of gene transfer. The classical example is, of course, the transformation of pneumococcal types (Ephrussi-Taylor, 1951). Hotchkiss (1951) has recently extended our knowledge of the range of characters so transferred in pneumococci, by demonstrating the existence of transforming substances for penicillin resistance. In the light of recent work (Lwoff, 1952) the induction of lysogenicity can also be regarded as a non-sexual mechanism of gene transfer; the infected bacterium acquires a foreign set of genes—namely, those of the entering virus—which are so well integrated into the host cell that they cause only rarely the formation of new virus. If the ability to make virus sporadically and then undergo lysis were the only property conferred on the bacterium by this transaction, its evolutionary significance (at least from the standpoint of the bacterium) might well be questioned. However, the remarkable discovery (Freeman, 1951; Freeman & Morse, 1952) that non-toxinogenic strains of *Corynebacterium diphtheriae* become toxin-producers after lysogenicity has been established in them suggests the possibility of the acquisition of additional properties in this way, and the equally remarkable observations of Zinder & Lederberg (1952) on the mode of transfer of single characters between *Salmonella* types point in the same direction. We must therefore, I think, be prepared to accept the possibility that micro-organisms have developed a variety of special non-sexual mechanisms which maintain an open genetic system, although the nature, extent and significance of these mechanisms cannot be properly evaluated at the present time.

A second genetic peculiarity of micro-organisms is the general, although not universal, predominance of the haplophase in their life cycles.* Apart from the special case of the heterokaryotic fungi, haploidy renders micro-organisms highly exposed to the chill winds of selection. Mutant genes cannot be masked by dominance, so that the accumulation of a store of alleles which can be drawn on to cope with environmental changes is impossible; most micro-organisms have a poor genetic buffering capacity. In asexual organisms, however, haploidy does have the compensation that it permits a far more rapid adjustment to environmental changes than is possible to diploid forms.

Microbial populations also have peculiarities, measured against the norm of plant or animal populations, which bear on the problem of adaptation. First, the total number of individuals in many microbial

* This remains inferential for groups where sexuality is very rare or unknown. In bacteria, for example, predominance of the haplophase has been shown conclusively for only one species, *Escherichia coli* (Lederberg, 1949). Nevertheless, the similarity of mutation rates in other bacterial species to the rates observed in *E. coli* suggests the generality of the haploid state.

species* is enormous by comparison. To take a trivial example, there are probably more representatives of the bacterial species *Streptococcus faecalis* in the intestinal tract of any one mammal than the total number of representatives of the mammalian species in question. This abundance reflects the extremely short generation time of micro-organisms. A second peculiarity of microbial populations is their very marked capacity for dormancy. This is obvious enough in spore-forming species, but also characterizes non-spore-formers. Many bacteria can remain viable but non-proliferating in the vegetative condition for a period that is extremely long when measured against their generation time.

NATURE OF THE MICROBIAL ENVIRONMENT

To conclude this rapid survey, we must consider the peculiarities of microbial ecology. The microbial environment is both wider and narrower than that of the plant or animal. It is wider in the sense that there are usually no *geographical* limitations to the distribution of a species, except in the case of parasites whose distribution is governed by that of their host organism. As far as free-living micro-organisms are concerned, a very broad ecological classification into terrestrial, marine and fresh-water forms is generally possible, but localization on a macroscale can go no further.† The reason for this well-nigh universal distribution is that microbial environments are micro-environments, hundreds or even thousands of which lie concealed from the gross ecological eye in any gram of soil. A single cellulose fibre provides a specialized environment with its own characteristic microflora, yet may occupy a volume of not more than a cubic millimetre. Such environments are omnipresent, and do not differ significantly whatever their geographical location. For the study of micro-ecology, there exists a special method, the enrichment culture method, which was largely developed and very widely applied by Beijerinck (1922). The investigator fixes the environment, by preparing a culture medium of defined chemical composition, which can be maintained after inoculation under any desired set of physical conditions. The medium is inoculated with a mixed microbial population—usually that contained in a small amount of soil—and the

* The purist will no doubt object to the word 'species' being applied to such organisms as bacteria. Unfortunately, there is no other convenient designation; and this one will not cause confusion, if we define it, in the context of asexual organisms, as consisting of a bundle of clones with many common properties.

† This statement may not be wholly valid for fungi. For example, Emerson (1941) has shown that the free-living phycomycete *Allomyces*, a soil inhabitant, occurs only in tropical and warmer temperate regions (approximately latitude 40° N. to 40° S.); within this band, distribution is world-wide.

resulting predominant microflora is determined. Thanks to the work of Beijerinck and others, a considerable fund of information about microbial ecology has been accumulated in this way, all of which tends to show that the environmental limits fixed on a microscale for organisms with a world-wide geographical distribution are really very narrow. The case of the *Azotobacter* group provides as good an illustration as any. An enrichment medium containing any one of a variety of simple organic compounds, together with the necessary mineral nutrients, but devoid of combined nitrogen, gives rise when inoculated with soil and incubated under aerobic conditions to development of the *Azotobacter* group as the exclusive primary microflora. Isolated in pure culture, *Azotobacter* strains prove perfectly capable of using combined nitrogen; in fact, they grow faster with a combined nitrogen source, and the fixation of atmospheric nitrogen is temporarily suppressed. From this it might be concluded that the isolation of *Azotobacter* strains from nature could be achieved more easily by adding nitrate or ammonia to the enrichment medium. Experiment disproves the assumption; in such a medium, a soil inoculum usually yields a primary population of *Pseudomonas fluorescens*, and even by careful microscopic search the characteristic and easily recognizable cells of *Azotobacter* can never be detected. These experiments show that in nature *Azotobacter* can flourish only in an environment free of combined nitrogen. The ability to fix nitrogen is an essential group character, strongly favoured by biological selection; its essentiality is not apparent under pure culture conditions, because all biological selective pressures have been removed. One general inference can be drawn from these and similar studies. Since the conditions of natural (as opposed to pure culture) development for most microbial groups are so narrowly defined, probability decrees that a micro-organism and its special micro-environment are not always in contact. To put the matter another way, micro-environments are *discontinuous in time*. Once this is realized, the general properties of a microbial population appear singularly well fitted to existence in a micro-environment. Large populations and short generation times guarantee that when a particular micro-environment occurs, it will be occupied and exploited with the greatest possible expedition. Great powers of dormancy guarantee survival of the organism after disappearance of the micro-environment, provided that the micro-environment recurs before the limit of dormancy is reached.

GENERAL DEFINITION OF ADAPTATION

Let us now inquire specifically what is meant by 'adaptation'. In its broadest usage, it describes the totality of the various processes of change which confer on an organism fitness to its environment. There is a simple pragmatic test of fitness, namely, ability to leave offspring, which demonstrates that the organism possesses a constellation of characters having survival value in one of the many possible environments existent in nature, and hence provides *prima facie* evidence for adaptation to that environment. In a sense, 'adaptation' is a biological axiom. Some biologists have denied that reproductivity as such is evidence of adaptation, basing this contention on the spread of so-called 'non-adaptive' characters through certain populations. I cannot see the validity of this argument, since the designation of a character as 'non-adaptive' presupposes a complete understanding of the selective forces at work, an understanding which I would concede willingly only to God. It is perhaps wiser to admit that natural selection may have its reasons which the reason does not know. Furthermore, adaptedness is a property of the whole organism, which involves the contributions and interplay of many genes, so that a particular phenomic property which we pick out and study as a 'character' may well constitute an incidental by-product of gene action which *also* confers other less easily observed, but essential, properties on the organism. A possible microbiological example is provided by certain adenineless mutants of *Neurospora* (Mitchell & Houlahan, 1946), which also differ from the wild type by virtue of accumulating a purple pigment. The pigment is presumed to derive from an unstable intermediate in the biosynthesis of adenine which accumulates behind the block in this particular class of adenineless mutants. It is entirely conceivable that under certain conditions this adenineless state would confer a selective advantage, with a concomitant selection for the 'purple pigment character'. Someone unaware of the biochemical basis of purple pigmentation might be very tempted to describe its spread through the population as 'non-adaptive'.

ADAPTIVE RIDDLES

The subtlety of natural selection is well illustrated by the growth-factor requirements of photosynthetic bacteria belonging to the family Athiorhodaceae. The ecology, morphology, physiology and taxonomy of this group have been studied with great thoroughness and insight by van Niel (1944), who eventually recognized six very well-defined species, belonging to two genera. One aspect of their physiology which he did not examine

was the requirement for vitamins. A subsequent analysis of this question by Hutner (1946, 1950), who used van Niel's strains, showed that each species has a unique set of requirements, the intraspecific variation being remarkably small. Thus, *Rhodospirillum rubrum* usually requires only biotin, while *Rhodopseudomonas spheroides* requires biotin, nicotinic acid and thiamine. Indeed, the correlations are so close that one would make relatively few mistakes by assigning strains to species *solely* on the basis of their vitamin requirements. Since we know that a vitamin requirement can be brought about by a single-gene mutation, there was certainly no *a priori* reason for expecting that in a group where dependency on an exogenous vitamin supply has become the rule, the pattern of dependency should be so rigidly fixed for each species, particularly since the general ecology of the group, governed by its unique photosynthetic properties, is both narrow and uniform. One is forced to conclude that the special constellation of vitamin requirements which characterizes each species must possess great adaptive value, either direct or indirect, although the reason for this remains wholly obscure. The existence of a similar situation in the genus *Bacillus* has been shown by Knight & Proom (1950). Such findings, incidentally, provide much-needed comfort for the poor microbial taxonomist, who has been subjected to a good deal of lofty abuse in recent years by microbial geneticists (e.g. Luria, 1947) for the weight which he places in his taxonomic schemes on highly mutable single-gene characters. Characters of this sort may often be perfectly good taxonomic ones, if they possess adaptive value in a natural environment and are consequently maintained by selection.

TYPES OF ENVIRONMENTAL VARIATION AND ADAPTIVE RESPONSE

Any environment undergoes two principal types of variation: systematic and fluctuating. Systematic variations are the unidirectional, usually gradual, long-range changes characteristic of geochemical evolution. Fluctuating variations are the short-term variations about a mean, which occur in any environment; they can be further subdivided into cyclic events (seasonal changes of climate, tidal change) and purely random events. The survival of a species requires adaptation to both kinds of environmental change. I shall designate adaptation to systematic changes as *evolutionary adaptation* and adaptation to fluctuating changes as *physiological adaptation.* Each kind may involve what one could term evolutionary and physiological components, but I have been unable to think of better qualifying phrases. Evolutionary adaptation

constitutes the moulding of the genotype of an organism through evolutionary history by selection to fit the mean conditions of its environment; although the results can be seen in an individual organism, the mechanism and pattern can only be comprehended in a historical sequence. Physiological adaptation is less easily defined. If it represents a response to a cyclic event which has a periodicity much longer than the life span of an individual organism, it *may* involve the alternate selection of different genotypes, and thus have a purely genetic, even though evolutionarily static, basis. In practice, it may be difficult to draw the line between very long cyclic fluctuating variations and systematic variations. Random fluctuating variations, on the other hand, occur constantly over periods that are short in comparison to the life span of the individual organism, and in such cases adaptation always involves direct phenomic accommodation to the change. It is with this kind of physiological adaptation that I shall be concerned in the following pages. Underlying such phenomic accommodations there are also genetic factors, since selection must operate on the genome just as effectively in producing physiological adaptiveness—the potential ability to respond rapidly to random environmental fluctuations—as it does in producing evolutionary adaptedness.

SYSTEMATIC VARIATIONS IN A MICRO-ENVIRONMENT

The notion of a systematic change is obvious when one thinks in terms of a macro-environment, but requires clarification in the special context of a micro-environment, which is discontinuous in time. In thinking about the mean conditions of a micro-environment we have to ignore its discontinuities, and consider only its recurrent properties. In the case of the cellulose fibre which was mentioned earlier, the salient mean condition of the micro-environment is the physicochemical structure of the fibre; as long as plants continue to synthesize cellulose with the same properties, this mean will remain unchanged. It is conceivable, however, that the chain length of plant cellulose molecules is increasing through evolutionary time; if this were so, the micro-environment would be undergoing a systematic change. We can infer certain past systematic changes in micro-environments, and even set their time in geological history with rough accuracy. For example, lactose is not known to occur in nature except as a product of mammalian metabolism, and it seems very probable that this disaccharide first appeared during the course of vertebrate evolution, an event which must have led to evolutionary adaptation for its hydrolysis in the microbial world. At the present time, the technological activities of man are producing environ-

mental changes of an unprecedented rapidity which—granting the continuance and spread of industrialization—can be regarded as systematic. Indeed, there is one exceedingly clear-cut example of evolutionary adaptation in bacteria, brought about by human activity in less than a decade: the development and spread in natural populations of genetically determined resistance to such chemotherapeutic agents as sulphonamides and penicillin.

EXPERIMENTAL EVOLUTIONARY ADAPTATION

The biologist who attempts an experimental study of evolutionary adaptation in corn or *Drosophila* is frustrated by the fact that systematic changes in macro-environments occur over very long time spans and are difficult to recognize, let alone control. In these respects, the position of the micro-biologist is infinitely more favourable. Several factors contribute to make micro-organisms ideal biological material for the experimental study of evolution. Their short generation time permits the construction of evolutionary experiments which last only a few days or weeks. Secondly, pure culture methods isolate microbial clones from all selective forces that result from interaction with other biological systems—thus reducing enormously the number of inherent variables. Finally, the physicochemical environment can be specified with a high degree of precision, and designed so as to introduce very subtle selective forces. As a consequence, microbiologists are in a position to perform the operation which Winogradsky (1949) has aptly termed 'forcing the species'; i.e. to induce by systematic variation of the micro-environment a unidirectional series of evolutionary adaptive changes. Winogradsky was concerned with the taxonomic implications of this procedure, since he had realized, long before the advent of microbial genetics permitted a statement of the concept in formal terms, that the microbiologist might exert *unconscious* selection on his pure cultures by his choice of culture conditions, so producing biological artefacts with little resemblance to the naturally occurring forms from which they had been historically derived. This eventually led him to make preliminary observations for taxonomic purposes on still impure enrichment cultures, a perfectly rational procedure to which most bacteriologists, blinkered by the pure-culture routine, have accorded the pained silence which is the customary response to the senile aberrations of Grand Old Men.

The taxonomist's misfortune is the evolutionist's opportunity. But it should always be kept in mind that test-tube studies on the evolutionary adaptation of pure cultures have no *necessary* bearing on the

actual evolutionary history, past or future, of the microbial species concerned. The single, and very great, merit of such work is to allow an insight into the mechanisms by which evolutionary adaptation in micro-organisms occurs. There are certain complications about such work, of which the foremost is the difficulty of distinguishing between genetic and physiological mechanisms of adaptation when one is forced to study populations rather than individuals, as the microbiologist usually is. The situation is usually further complicated by the fact that both kinds of adaptive mechanisms may operate, either as separate or as consecutive events, to bring about a single, experimentally observable change of character. To illustrate these complications, let us consider a particularly well analysed case: the adaptations of *Escherichia coli* concerning lactose utilization.

LACTOSE UTILIZATION BY *ESCHERICHIA COLI*

The acquisition of the ability to hydrolyse lactose by so-called *mutabile* strains of *E. coli* has been known for half a century, and in fact constitutes the classical example of a biochemical variation in bacteria. The first really rigorous proof of its primarily mutational basis has just been produced by Ryan (1952), and the following account will be based on his observations. A 'normal' wild-type *E. coli* strain is genetically lac +, but since the enzyme which hydrolyses lactose (β-galactosidase) is an inducible one, the cells will contain only traces of the enzyme when grown in an environment devoid of lactose; a high rate of enzyme synthesis is dependent on the presence of lactose or some other specific inducer in the environment. Consequently, *mutabile* strains of *E. coli*, which are generally lac −, are phenotypically identical with 'normal' strains of *E. coli* when grown in an environment devoid of specific inducer. What differentiates them is their behaviour when lactose enters the environment. Every cell, practically speaking, in a 'normal' *E. coli* population adapts physiologically to the presence of lactose by starting to synthesize β-galactosidase at a high rate. In contrast, a small population of a *mutabile* strain—say 10^4 cells—shows no physiological response to lactose. As this population grows, the occurrence of a mutation from the prevalent lac − condition to lac + becomes increasingly probable. When it occurs, the mutated cell is not *ipso facto* endowed with a full complement of β-galactosidase; it has acquired by mutation only the *potential* ability to synthesize this enzyme if a suitable exogenous inducer is present. The next step is therefore a physiological adaptation of the mutated cell, induced by the lactose in the environment. Once this has occurred selection starts to operate, since physiologically

adapted lac+ cells have considerable selective advantage over lac− cells in the presence of lactose, and will soon overgrow the original population.

Cohen-Bazire & Jolit (1952) have recently shown in a very ingenious way that the evolutionary adaptation of *E. coli* with respect to lactose utilization can be pushed one step further through adroit selective pressure. If a genetically lac+ population is subjected to rapid alternate cultivation in media containing glucose and lactose, respectively, as the sole carbon source, a population which is capable of synthesizing β-galactosidase in the absence of an external inducer emerges, consisting of so-called 'constitutive' mutants. Under the prescribed conditions, the 'constitutive' mutant has an obvious selective advantage, since it will be able to initiate growth without lag when transferred from glucose to lactose, whereas the parental 'inducible' lac+ type loses its content of β-galactosidase during passage through glucose, and therefore shows a brief delay in growth when transferred to lactose. This series of experiments provides a singularly lucid example of artificial evolutionary adaptation which also involves physiological adaptive events. The final evolutionary step probably has no significance in the natural evolutionary history of *E. coli*, since 'constitutive' mutants have never been isolated from nature.

THE ADAPTIVE PROBLEMS OF DRUG RESISTANCE

I should now like to examine another group of adaptations in which the coexistence of evolutionary and physiological adaptive mechanisms is possible, although not yet unambiguously established. These are adaptations to withstand toxic agents, particularly chemotherapeutic substances. When the phenomenon of drug resistance in bacteria was first encountered, many claims were made for physiological adaptation as the causal mechanism. At that time microbial genetics was still in its foetal stages, and the possibility of mutation and selection as a cause of the observed populational changes was not taken into proper account. Once the mutational acquisition of resistance had been discovered by Luria & Delbrueck (1943) in the special case of virus resistance by *E. coli*, analogous methods could be used to show that the genetic component also played an important part in drug resistance (Luria, 1946; Oakberg & Luria, 1947; Demerec, 1948), and the mutational basis of this class of adaptations became more or less an article of faith among genetically inclined microbiologists. At present, there are probably few people who would still deny the importance of mutation in the phenomenon of drug resistance; but it is *also* possible to believe