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SOME BIOLOGICAL ASPECTS OF  
BACTERIAL GENETICS

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Bacterial genetics has grown up so rapidly<sup>1, 2, 3</sup> that it may be difficult to say what it is about and what it may have to do with the genetics or biology of higher organisms, and their cells and tissues. Many kinds of problems and organisms have been studied, but there are some general principles that can be fairly inferred. Perhaps the most fundamental cleavage in the thinking of microbiologists has had to do with the impressive adaptive plasticity of bac-

teria. I need only cite the rapidity with which some pathogens have caught up to our chemotherapeutic progress by the development of "drug-resistance"<sup>4</sup>. Some workers, of whom the physical chemist, Sir Cyril Hinshelwood, is perhaps the most articulate spokesman have interpreted such adaptations as a direct chemical reaction of the bacteria to the drug. To the challenge that such adaptations pose a genetic, as well as a physiological problem, a new theory of heredity has been offered by which bacteria are sharply set off from other organisms, for which Lamarckian theories have long since been abandoned<sup>5</sup>.

Largely as a result of the biometric analysis of bacterial resistance to bacteriophage by Luria and Delbruck<sup>6</sup> most biologists have reached a rather different conclusion: that these adaptations result from a Darwinian struggle for survival, more precisely that a few individuals in the large populations tested are marked by random, pre-adaptive resistance. The spontaneous variations or mutations occur sporadically and are inherited by their descendants in the absence of the drug. The mutants hold no significance for the natural history of the population unless and until the drug is encountered, whereupon the mutants will overgrow until some other ecological factor becomes limiting. By this theory, the most significant action of the drug is selective, not inductive.

The selective theory has successfully accounted for every inherited adaptation<sup>7, 8, 9</sup>. (This discussion is not applicable to the numerous instances of enzymatic adaptation that are characterized by a rapid attenuation after removal of the evoking substrate.) Unfortunately, the only means by which the supposedly spontaneous mutants could be detected has been by the introduction of the drug, so that a satisfactory (or at least a simple) experimental disqualification of inductive effects has been very difficult. This difficulty has been circumvented more recently by the development of a technique of indirect selection. As the details are about to appear in print elsewhere<sup>10</sup>, they will be summarized only briefly. The development of resistance to streptomycin in *Bacterium (Escherichia) coli* will serve as an example.

Indirect selection depends in turn on replica plating, which is a means of producing accurate replicas of the bacterial growth pattern on an initial agar plate. This is done simply by transferring the initial pattern to a sheet of velveteen fabric, which can then be used to imprint a series of fresh agar plates. If the imprints are made to streptomycin-agar, the development of resistant colonies will register the locations of resistant cells growing on an original, plain agar plate. That these cells are present in clones prior to exposure to the drug is signified by the recurrence of resistants at superimposable sites in a series of replica plates. These recurrent sites, in turn, reveal the locations of the resistant clones on the original plate. Fortunately, the replica procedure does not damage the source plate, and part of the clone is left in its initial location, thus revealed. In practice, to find any mutants the initial plate must be so crowded with cells that a single stage of indirect selection does not permit the precise

localization of single, pre-adapted, resistant cells. However, the experimenter can locate these cells to the extent that an inoculum taken from the approximate site is considerably enriched in the proportion of resistant (perhaps by a hundred-fold). Then, by a reiteration of indirect selection, the resistant mutants can be isolated in pure culture. In this experiment, the role of the streptomycin cannot have been inductive, for the resistant cells isolated by indirect selection have never been exposed to streptomycin. The selective action of the drug has, however, permitted us to record the locations of resistant clones that could not otherwise be detected. This approach is, in principle and in practice, applicable to many other systems of bacterial adaptation. As already mentioned, the conclusion is not new. The didactic value of the present argument is its principal justification. But the main purpose of this introductory *divertissement* is its illumination of the underlying philosophy of genetic microbiology, with its focus on the individual cell and its social behavior. A recurrent theme at this conference has been the desirability and achievement of successes in the treatment of tissues and tumors as populations of individual cells for physiological, cytological, virological and genetic analysis. The orientation to a microbiological approach is bound to be the more fruitful as methodological as well as analogical concepts are transferred.

The selective isolation of pre-determined types is a fundamental element not only of mutation study, but in the investigation of genetic recombination and bacterial life cycles. The results have been strikingly different in different bacteria. The most extensive experiments have been conducted with *B. coli*<sup>11, 12</sup>.

We have not yet succeeded in detecting sexual processes in bacteria by direct microscopic observation, chiefly because the frequency with which it occurs is usually too low to encourage such an approach. We may hope that this barrier will soon be breached. Instead, we set up the question in genetic terms. Can we, in mixed bacterial cultures, detect the exchange of genetic factors that is the fundamental meaning of sexuality? To answer this question conclusively, we use different growth-factor-dependent or *auxotrophic* mutants, which are unable to form colonies on a basal medium owing to the lack of the specific growth factors required. If genetic recombination takes place, it should result in *prototrophs*, cells which have reacquired the wild type combination of genetic factors regulating nutrition. Since prototrophs can be isolated selectively by platings on minimal medium, this method is very powerful: a single prototroph is readily detected in the presence of a billion auxotrophs. Somewhat to the surprise of E. L. Tatum and myself, this simple experiment worked the first time it was tried on *B. coli*, strain K-12.

The appearance of prototrophs is the beginning, not the culmination of the analysis. From subsequent experiments, we have convinced ourselves<sup>1</sup> that the prototrophs are not artefacts resulting from spontaneous reverse-mutation of the parent mutants; (2) that the agent of recombination is the intact cell, and

cannot be replaced with smaller units obtained by the extraction, disruption, or sedimentation of bacterial cultures; (3) that all, or almost all, of the characters of the organism are involved in the recombination system; and (4) that the genes or factors controlling these characters are organized in a very definite way, entirely consistent with a linear-linkage chromosome system. The genetic evidence points to a life cycle similar to that of *Neurospora*, with haploid vegetative cells and a transient diploid zygote. Exceptional cultures which persist in a diploid, heterozygous condition provide some of the strongest confirmation of this picture. These cultures can be subjected to rigorous single-cell pedigree isolations, following which the hybrid character of the single cells is confirmed by the segregation of the parental components. In its fundamental organization, therefore, this bacterial species closely resembles such higher forms as yeast, molds, algae and man.

The next question that any naturalist would ask is certainly: "How about other strains and species?" After a time, methods were developed for rapidly screening new strains of *B. coli* for recombination. About two or three percent of some 2,000 strains tested have proved to be fertile; probably many others were not detected. As far as can be seen at present, these new fertile strains, which cross with the original and with each other, are constructed along the same lines as K-12. They do, however, differ in a number of characteristics of which the cellular antigens are perhaps the most promising for further research.

For the question of the genetics of related species, the *Salmonella* group appeared to be a likely choice. *Salmonella* looks enough like *B. coli* that one might expect a similar story. In addition, we could depend on the same general methods for cultivation, obtaining and characterizing mutants, and so on. Technically, *Salmonella typhimurium* has, indeed, behaved very much like *B. coli*. It also has a recombination mechanism, but its details are almost diametrically opposed to those of *B. coli*<sup>12, 13</sup>.

In this species, genetic exchange is mediated by an agent much smaller than the cell, readily passing through bacteria-tight filters, and for this reason non-committally nicknamed "FA"—for filtrable agent. FA is most readily demonstrated in cultures that have been exposed to weakly lytic phages, but since every culture of *S. typhimurium* carries latent phages (lysogenicity) this is not a restrictive requirement. FA is probably associated with granules about 0.1 micron in diameter, easily seen in electron micrographs and barely visible with dark-field microscopy. It has not yet been chemically characterized, but is remarkably resistant to heat, antiseptics, and a number of enzymes of which ribonuclease and desoxyribonuclease are the most significant. The genetic activity of *Salmonella* FA also contrasts with recombination in *B. coli*. In a number of tests, involving altogether thirty or forty characters (mostly biochemical mutants, as in *B. coli*), no instance of associated exchange or linkage has been found. In fact, within the range of our experiments, each exchange has involved no more

than one character per bacterium. For this kind of transmission of hereditary fragments from one cell to another we have applied the term *transduction*. The limited scope of transduction does not prevent the synthesis of new bacterial forms, and we have, for example, obtained serotypic "hybrids" of *S. typhi* with *S. typhimurium*, carrying the somatic antigen of the former with the flagellar characteristic of the latter. Many others of the ever-increasing number of *Salmonella* serotypes can be readily visualized as recombinants of existing forms.

In *Salmonella* transduction, we are dealing with an agent squarely athwart what we now classify as genes and viruses, respectively, and it would be tempting to speculate further in this vein. In some other bacteria, genetic transduction goes under the name of type transformation, for example, in the pneumococcus and the influenza bacterium. The present work has led to the further conclusion that, as far as could be told, the entire heredity of *Salmonella typhimurium* is involved in transduction, if only one fragment at a time. It is difficult to reconcile the experimental facts of transduction, with its fragmentation of the genotype, to any plausible picture of genetic stability which seems to me to require something very similar to an integrated chromosomal system. Perhaps the FA particles correspond to fragmented pieces of chromosomes, as suggested for the pneumococcus by H. J. Muller<sup>14</sup>. Whatever its form, the resolution of this paradox cannot help but challenge our thinking about genes and our insight into their behavior in all organisms.

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