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The nature and action of the gene in bacteria. Lederberg

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1. Formal genetics of Escherichia coli; effects of irradiation.

2. A new mechanism of genetic transmission in Salmonella typhimurium.

3. Genetic basis of drug resistance: replica-plating and indirect selection techniques.

4. Lysogenicity (confidential).

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The analysis of formal genetics of Escherichia coli is being continued. Special emphasis is being given to discrepancies between experimental data and the theory of a linear chromosome as the basis of heredity in this bacterium (as it is in higher forms). Single cell studies on segregating diploids suggest that some discrepancies may not be inherent in the actual genetic ratios, but in differential growth of different types. This work is necessarily slow, and definite conclusions will not be available until it is completed.

A study of the genetic effects of radiations on diploid E. coli has been initiated, with contract support from the Atomic Energy Commission. At this point, the project is very modest. The most significant finding to date is that single cells treated with UV give progenies with a variety of effects: either the cell (probably the nucleus as well) is not a unit target, or the effects of radiations persist as a biological disturbance despite the apparent growth-recovery of the cell. These effcts then later give rise to the variety of changes in different offspring.

The isolation of new strains of E. coli that can be crossed with existing fertile strains was a major subject this year. Earlier estimates were optimistic: of about 1500 strains tested, only 30, or 2% could be crossed. These 30 strains are being studied closely for a) their linkage patterns, b) the possibility of compatibility groups and c) characters of interest for genetic study. So far as can be determined so far, the new strains resemble the original E. coli K-12 in their fundamental biology and life cycle. However, many of them are culturally and serologically distinct. We are now planning studies on the genetic basis of the natural (as opposed to laboratory-created) differences between the strains. The serological differences, in particular, permit of an immunogenetic analysis

For a variety of reasons, bacteria provide excellent experimental material to study the biological bases of gene-controlled antigenic differences such as are the foundation of blood-typing in man, cattle, and other mammals. In addition, although E. coli is "not pathogenic", the genetic basis of serotypes is of intrinsic interest to the student of infectious diseases. The Research Committee provided funds (W.A.R.F.) to initiate this program until definitive support was secured from the Rockefeller Foundation. Dr. P. D. Skaar has undertaken its immediate direction, and has completed preliminary experiments and preparation of antiseral reagents.

Salmonella typhimurium (mouse-typhoid; enteric fever group) has been the subject of genetic study in this laboratory since 1947. During the past year, a reasonably clear picture of its behavior has developed for the first time. The Salmonella bacteria were chosen for this study because they provide good experimental material for studies on virulence, because they are readily cultured in the laboratory and are related to the E. coli familiar to us, and because their serological characters (presumably under genic control) are important in public health bacteriology.

Mr. Norton Zinder undertook this problem in 1948, and is using the experimental data for his Ph. D. dissertation (officially in Medical Microbiology). The plan was to parallel the work with E. coli. Biochemical mutants were made in a large number of strains, and attempts were made to detect crossing by plating mixed cultures on a selective, minimal agar medium. The results obtained were very confusing, as illustrated in previous reports, until it was realized that the recombination mechanism here is quite different from that in E. coli. In E. coli, all the evidence points to a typical sexual process, wherein recombination results from the fusion, and later segregation, of two intact nuclei. In Salmonella, under certain conditions, the cells release fragments of genetic material into the medium. These fragments may be either single "genes" or small aggregates, but much less than an entire nucleus. Other cells may absorb these fragments. The next steps we can only guess at, but an end result is that a "recombinant" cell is sometimes formed in which the absorbed fragment becomes a part of the genetic mechanism of its new host cell. To distinguish this process from fertilization, which is the union of two essentially equal genotypes, we have designated ~~this process~~ it as genetic transduction.

Transduction is an infectious process, if regarded in a certain light, and one may ask whether it does not merely depend on the transmission of a virus "disease" from one cell to another. However, a great many characters have been examined, and every one of them is transducible in the same way. These include many different nutritional requirements, fermentation differences, resistance to streptomycin, and type-diagnostic antigens, the same groups of markers that are inherited as if carried on a chromosome in E. coli. The converse possibility, that genic transduction may throw light on the origin of viruses, should be given close consideration.

From the point of view of adaptive plasticity, transduction is not so efficient as fertilization. In this experiments, only a single factor is transducible to a cell at one time, so that "crosses" of cells differing in many factors result in only a small fraction of all the possible gene combinations.

The Salmonellas have been subjected to very close serological study, and a very large number of antigenic types or "species" are recognized in the diagnostic scheme. These types represent different combinations of somatic antigens (designated by roman numerals) and flagellar antigens (arabic numerals and lower case letters). For example, S. typhimurium is designated as IV,V,XII; i; 1,2,3., while S. typhi is given as IX, XII; d—. Bacteriologists have often speculated on the evolution of the Salmonella group, and the origin of the different antigenic combinations, but owing to the lack of convincing precedents, recombination was not implicated. By transduction, however, a hybrid of S. typhi x typhimurium has been obtained, with the antigenic formula IX, XII; i —. This hybrid has not previously been described as a Salmonella type. If it had been isolated (and it might well be anticipated to occur in a patient suffering from a double infection) from a carrier or patient, it would certainly have been recognized as a new species. On this precedent, we may pre-

dict that many Salmonella types have arisen, and will arise again, from the recombination of factors of previously established forms.

Transduction is so different from the hereditary patterns familiar to geneticists that it is difficult to reconcile it with the cytological observations that suggest the presence of similar nuclear structures in E. coli and in Salmonella. We will have to learn a great deal more about genetic transduction before we can evaluate its significance for our concepts of the nature of the gene and its relationship to the cell.

The newspapers rdeently have graphically described the continued race that we must anticipate in the development of new antibiotics for the chemotherapy of infections caused by bacteria that have adapted to the old. The medical usefulness of the sulfonamides, penicillin, and streptomycin is already limited, to varying degrees, by the prevalence of pathogens resistant to these agents. The development of drug-resistance can be demonstrated in laboratory cultures of bacteria exposed to a particular antibiotic. The mechanism of this adaptation will not immediately affect the outcome of this race, but is bound to enter into any longterm considerations.

Owing to experimental difficulties, a rather unequal controversy on the mechanism of this adaptation has persisted. Most workers, especially those with genetic interests, have concluded that small numbers of drug-resistant mutants occur spontaneously. The occurrence of these mutants has no relation to the presence of the drug. In its absence, however, they remain undetected and they come to view only when the preponderant sensitive cells are eliminated by the chemotherapeutic agent. This view is precisely analogous to the Darwinian theory of evolution by natural selection of random changes. The alternative opinion has held that the drug-resistant forms do not occur, even in small numbers, in the absence of the drug. The latter must have some direct effect on the mutation. Experiments to settle this question have been difficult to carry out because of the scarcity of the mutants. When only one cell among its billion neighbours is resistant, the only means of detecting it had been to expose the entire population to the drug, and this does not help to decide the controversy. However, a considerable body of indirect evidence has accumulated in favor of the "Darwinian" theory.

In the course of studies unrelated to this problem, a method was devloped for handlädg large numbers of bacterial colönies with a minimum of effort, and this later provided a decisive answer to the problem of drug-resistance.

Much of the laboratory work in microbial genetics (and other aspects of microbiology) involves "picking" each of a great many bacterial colonies growing on an agar medium, and transferring them with a platinum wire needle to several other types of medium to see how they will perform on these. In our laboratory, the number of times this operation is carried out is numbered in the hundreds or thousands per day.

A method was devloped so that an entire plate, containing up to several hundred colonies, could be handled as a unit. A disc of a pile fabric, such as velvet or velveteen was used as a sort of template or stencil. When the fabric is gently pressed onto an agar plate, the colonies become entangled in the hairs of the nap. When the template is then pressed on one ore more fresh plates, a few cells are depesited from the velvet, in positions corresponding to the colonies on the original plate. In effect, it becomes possible to "copy" a pattern of growth on one agar plate to a series of others. With care, the copies are quite faithful (Figures 1 to 3). This technique, which is called replica-plating, has been quite useful in the detection and cäässification of mutants of bacteria and of actinomycetes, also. It should save a great deal of work in similar operations, such as testing groups of bacteria for their reactions to various antibiötics.

It was only after the replica method was well developed that we realized its applicability to the problem of adaptation to antihiötics. Two lines of pertinent evidence arose. If a suspension of bacteria not previously exposed to a drug (streptomycin was used in our experiments) is spread on a plain agar plate, we should expect that a few of the cells placed on the plate are already drug-resistant, if we reason from the "Darwinian" hypothesis. If the plate is

incubated for a few hours, so that the bacteria grow undisturbed, the original resistant cells will have multiplied several times to give a clone or asexually produced family of resistant cells at that place. This was verified by making copies of such a plate to a series of other plates containing the drug. In almost every case, the copies showed resistant cells developing at corresponding places, showing that the original plate must have had the resistant cells in families. The resistant cells must have developed the families on the plain agar before they were exposed to the drug on the copy plates. This is contradictory to the theory of a direct effect of the drug.

An even more convincing result, however, is a procedure that allows us to isolate the resistant cells without ever exposing them directly to the drug. In the previous experiment, families of resistant cells are located on the plain agar at places corresponding to the resistant colonies developing on the copies. When the velvet template is prepared, it does not remove most of the cells on the original plate. The drug-containing copy plates tell us where to look for resistant mutants on the original plate. This plate is too crowded to allow us to tell exactly which cells correspond to the resistant families, but the method narrows the range: instead of looking for over an area of about five square inches (a whole plate), we can locate the clone in an area of about .05 square inches. By using these cells as an inoculum for a fresh plate, we will have concentrated the resistant cells about one-hundred fold, say from a proportion of 1 : 1,000,000 to 1: 10,000. This "enrichment" can be repeated several times, until the resistant cells are well separated on the plate, and can be picked easily.

In all of this, the actual selection line remains on plain agar, and never is exposed to the drug. The sibs that are carried over to make the replica plates

serve to tell us where to look for the resistant mutants. Thus we can practise our selection indirectly, and ean isolate the resistant mutahts without ever exposing them to the drug they are adapted to. This is rather like pedigree-selection of roosters for breeding stock. We can use information on egg-production records of the sib hens of a rooster flock to select the birds with the best genotypes, without ever demanding that the rooster prove his potentialities directly.

This work was done in collaboration with Dr. E. M. Lederberg, and will appear in the January 1952 issuenof the Journal of Bacteriology.