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The research undertaken in this field can be reviewed under the following headings:

1. Formal genetics and cytogenetics of Escherichia coli.
2. Genetic control of fermentation enzymes in E. coli.
3. Lysogenic bacterial viruses.
4. Recombination in Salmonella.

1. Formal genetics and cytogenetics of Escherichia coli.

Efforts to unravel the complex life cycle of E. coli strain K-12 were continued during 1950, but with no great clarification of the underlying mechanisms to date. The main question remaining unsolved is the basis for the elimination of genetic material from the zygote which results, for example, in diploid cells which are deficient for part of one gene set (including genes controlling maltose and galactose fermentation, and streptomycin-resistance.) A number of complex hypotheses were tested critically, but none of them were supported by the experimental results. This problem, though difficult, is an important one, since on it hinges the question of how close is the similarity between the genetic organization of bacteria and of other plants and animals whose breeding systems have been successfully exploited for both scientific and technical purposes. The evidence, overall, shows a very close similarity, in particular that the bacterial genes are organized in chromosomes, but there are certain peculiarities that cannot yet be understood in terms of simple chromosome behavior, like that of the more familiar materials of experimental breeding.

Another approach we have been following is a direct cytological examination of haploid and diploid clones of E. coli. These can be distinguished almost absolutely on the basis of their nuclear structure, as illustrated in the accompanying photographs, Figures 1 and 2. However, a study of the nuclear constitution of haploid and diploid bacteria in all growth phases must be completed before too definite conclusions may be drawn. In certain growth phases, haploid bacteria may somewhat resemble what we have regarded as the typical diploid cytological picture, raising the possibility that the morphological differences are based primarily only on the larger size and more open structure of the diploid nuclei, which gives the appearance of a greater number of resolvable granules. The cytological pictures so far do not permit an identification of chromosomes or anything like a count of the chromatic units, so that this level of correlation

of genetic and cytological study remains to be exploited. There are indications that the nuclear bodies may be visualizable in living cells by phase microscopy, and it is hoped that this may help to fill in the picture of the behavior of the nuclear bodies during cell division, genetic segregation, and so on.

In connection with these studies we have also been studying the mode of action of various bactericidal agents, such as ultra-violet light, mustard gas, formaldehyde, and others. These agents have profound effects on the genetic behavior of diploid cells, correlated with disturbances in the nuclear picture. Figure 3 shows a small clone of cells, near recovered from ultra-violet exposure. The large, filamentous cell is typical of sublethal responses to some bactericides; the small cells have probably split off from the large cell at an earlier time, and are multiplying more rapidly.

One of the applications of diploid clones is to the problem of dominance. For a number of reasons, it has been important to determine whether drug-resistance is a recessive or dominant trait. Unfortunately, genes controlling resistance to streptomycin in E. coli are often eliminated during meiosis, so that heterozygotes are difficult to secure; however, one or two exceptional diploids have been isolated which are heterozygous for streptomycin-resistance, and the latter is found to be recessive: i.e., a cell carrying one factor for susceptibility and one for resistance is phenotypically susceptible to this drug. Resistance to bacteriophages has also been found to be recessive, so that it is likely that most mutations for resistance to antibacterial agents will be recessive to the wild-type, dominant, sensitive form. This has some bearing on the mechanisms of development of drug-resistance in the course of chemotherapy, but makes it the more important that we understand the genetic organization of the bacteria we wish to eradicate.

A recent finding which may make it possible to rationalize the genetic behavior of E. coli K-12 is the isolation of other strains which can be crossed

with this strain. Until now, the crossability of E. coli was nearly unique for a single strain, K-12, attempts to use other strains having given inconclusive or methodologically useless results. However, by courtesy of the State Public Health Laboratory, we have received a considerable number of fresh isolates of this bacterium from human sources, and almost 10% of these strains have been found to cross with K-12, and probably with each other, so that the phenomenon of recombination is no longer a unique one. There are indications that some of these strains do not show the aberrant genetic behavior reported for K-12. In addition, the natural differences between the various intercrossable strains provide abundant material for studying the basis of serological and cultural characteristics which are used in the classification of these bacteria as isolated from their natural habitats.

2. Genetic control of fermentation enzymes in E. coli.

The primary fermentation enzymes (in particular the oligosaccharases) of bacteria are usually recognized as "adaptive", that is, they are produced by the bacteria only after contact for some time with the substrate (e.g., lactose). A previous report mentioned that the distinction between "adaptive" and non-adaptive or constitutive enzymes was rather blurred, because small amounts of lactase can be detected, by special techniques, within unadapted cells. It has now been found that "adaptivity" is under genetic control, for in addition to previously described mutations which prevent adaptation, and limit enzyme formation, a new mutation has been found whose effect is to cause the cells to produce lactase abudantly, regardless of previous exposure to lactose or other substrates. This further illustrates the concept that the enzymes are produced as a result of a complex cell machinery in which the genes play a master-controlling rather than a direct role. (The alternative hypothesis, that each gene plays a direct role in the production of a single enzyme has had wide currency as the so-called "one gene-one enzyme theory".)

Another finding provides the first evidence for a possible "position effect" of bacterial genes, and shows how complex these may be. Two lactase-negative mutations had been studied here previously, and thought to be "allelic", i.e., affecting the same gene, for when crossed with each other, these mutants had not given rise to non-mutant offspring. Tests on a larger scale showed, however, that these two mutations were not identical, because non-mutant offspring occurred in a ratio of about 1: 3000. The mutations may therefore be thought of as affecting two genes extremely close together or adjacent. Studies of heterozygous diploids have now shown that a cell which carries one non-mutant gene on one chromosome, and the non-mutant form of the adjacent gene on the other chromosome does not ferment lactose, whereas these genes in the normal position, together on the same chromosome, show normal activity. Several examples of this kind are known in Drosophila; we may speculate that the two genes must lie adjacent because they interact through a non-diffusible primary gene product. Although this material may be more suitable in some respects than Drosophila, it is rather doubtful that any immediate progress on identification of the hypothetical gene products will follow, owing to the insuperable technical difficulties. Further studies in the purification and kinetics of lactase are being pursued in collaboration with Professor H. A. Lardy and his students at the Enzyme Institute.

3. Lysogenic bacterial viruses.

(Since this section must be cleared by Chemical Corps before release, no detailed information will be given here prior to publication.) This investigation deals with the transmission of a latent virus in crosses of carrier, resistant, and susceptible strains. Methods of "disinfection" or removal of the virus from viable, infected bacteria are also under study.

4. Recombination in Salmonella.

For some time, we have attempted to find a system of genetic recombination in a pathogenic bacterium, Salmonella typhimurium, comparable to that we have

been using in E. coli. For nearly three years, concerted efforts in this direction were unsuccessful, although about thirty different strains of S. typhimurium and related species had been laboriously and exhaustively tested in various combinations. We did not have even the lesser satisfaction of a definitely negative result, for certain combinations gave new types which strongly suggested a recombination process, but which for incidental reasons were technically unsuitable for further study. However, a pair of strains has now been found which give quite clearcut evidence of genetic recombination in Salmonella typhimurium, opening up the possibility of an analysis in this species comparable to that in E. coli K-12. The special interest in Salmonella is the opportunity afforded to study genetic aspects of pathogenicity, for which E. coli is unsuitable. However, a detailed analysis of the formal genetics of this material will be necessary before any serious work on the infectious disease aspects can hope to be successful. (Note: Because of the recency of these results, please consult Professor Lederberg before publication.)