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papers in
MICROBIAL GENETICS
bacteria and bacterial viruses

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PREFACE

THE FIRST purpose of this book is to supplement a lecture course on microbial genetics with original readings in a form most readily available to the student. At most universities, the papers included in this collection will be represented by single copies of the original journals. Thus, it is usually difficult for any number of students to read the papers at all, and impossible for anyone (including the instructor) to read and perhaps annotate them at leisure. This volume is not intended to include all the reading on this subject which should be required for a course. I hope, however, that it will make available most of the papers requiring the most general or the most intensive study.

The scope of the collection must obviously have some limits in size and cost. These have led to the restriction to bacteria and bacterial viruses, although consideration of fungi, protozoa, algae, and viruses is essential to a well-rounded course, even to one organized, as at Wisconsin, primarily for students majoring in bacteriology. Within this field, there are a great many important papers which have necessarily been omitted. No apologies need be offered for a selection which must be largely arbitrary. The papers selected were those which seemed to illustrate best certain concepts and methodologies for the benefit of a course in microbial genetics. In some choices, weight had to be given to other circumstances such as the length and availability of alternative papers. It must be emphasized that these selections do not reflect historical priority or scientific worth as such. A bibliographic list is included to help remedy the unavoidable deficiencies of the collection.

This book could not have been produced without the magnanimous cooperation of the publishers and copyright holders of the original papers, to whom specific acknowledgment is made elsewhere. Special thanks are due to the authors who have been very helpful, especially in providing scarce reprints for use as copy. I must also record an obligation to my colleagues who did their best to advise me on the proper scope and contents of this collection.

J. L.

Madison, April 1, 1951

INTRODUCTION

Mutation and adaptation.—The problem of organic adaptation in bacteria has been resolved into two points of view which are closely analogous to "neo-Darwinian" and "Lamarckian" concepts of organic evolution, respectively. The first holds that adaptive changes occur spontaneously, as sporadic mutations, not in any specific relationship to environmental conditions, and that natural selection functions to fix the best adapted genotypes. The Lamarckian viewpoint suggests that the adaptive mutation is itself directed by the environment, and that natural selection plays a subsidiary role. Many of the papers reprinted show experiments which support the spontaneous mutation hypothesis (1, 3, 5). One (6) deals with the end result of bacterial adaptation to chloromycetin and also argues for spontaneous mutations with accumulative effect by separating the components by recombination. There is no representative here of the alternative point of view which is defended strongly by Hinshelwood and his associates (28, 67, 70). In considering this problem it is essential to keep in mind physiological adaptations which are nonheritable, but whose interplay with heritable changes may trap the unwary (97, 120, 45-Lwoff). General discussions of this problem are included in most of the review articles (32, 54, 81, 82, 87, 121, 129).

Mutations for resistance to bacteriophage had been considered some years before Luria and Delbrück's paper (1), notably by Burnet (34). This author drew the same conclusions concerning the spontaneity of the resistance mutations, mainly through his ability to pick out resistant mutants by their colonial morphology without the use of phage as a selective agent. Much of the current work on *E. coli* phages (43, 44, 21) involves a set of 7 virus strains described as T1 through T7. Mutation rates for resistance to these phages have been recorded, together with useful information on cross-resistance patterns (48).

The physiological study of mutant characters is known as phenogenetics, in contrast to formal or cryptogenetics which deals directly with the mechanism of hereditary transmission. One would reasonably expect that a gene mutation would require a period of time to work its effects on the phenotype or outward behavior of the organism. This lag in bacterial mutation effects (phenotypic or phenomic lag) was first noticed directly with phage-resistance mutations

induced by radiations (45–Demerec). Later, Newcombe postulated a similar lag for spontaneous mutations to account for discrepancies in the estimation of mutation rates by different methods, according to whether they counted mutant individuals or mutant clones (4). In both of these instances, however, the interpretation is complicated by the cytological structure of the bacterial cell (15) which speaks for the concurrence of several nuclei (and therefore of several replicate gene sets) in each cell. Recessive mutations such as phage or drug resistance would require for their phenotypic expression that mutant and nonmutant nuclei be segregated, so that there would also be a segregation lag in the genesis of the mutant phenotype (103, 14, 47). The description of phenomic lag in the development of nutritionally sufficient "back-mutations" induced by ultra-violet light (UV) (7) avoids this difficulty, for these mutations are presumably dominant, and would not require segregation for their expression. A final caution which must be entered is that we cannot be entirely certain that the mutation-producing event within the cell is coincidental with the experimental irradiation (mutation delay hypothesis) (26, 49).

Mutant characters.—Something is known of the phenogenetics of phage resistance. The extraction of sensitive and resistant cultures results in preparations differing in the ability to neutralize the phage "in vitro." This argues strongly for the concept that phage sensitivity is correlated with the presence of a specific receptor substance (44, 61). Another metabolic correlate of phage resistance is less explicable. In certain specific combinations of bacterium and phage, the resistance mutation simultaneously leads to new nutritional requirements, (often for tryptophane or proline). Unfortunately, this correlation has not been found in strains which can be studied by recombination, so that it is not certain whether a simple genetic change causes the two effects, (23, 139, 45–Luria).

Phage resistant mutations play such a large part in genetic study because of the ease with which they can be selected out of large populations, simply by adding suspensions of the virus under appropriate conditions. This facility is shared by antibiotics and other antibacterials as selective agents. The advantage that the "drug" is not another biological system whose variability must be watched is counterbalanced by the fact that resistance to most chemicals is a quantitative rather than an all-or-none phenomenon, and that a great many genes may be involved. The probable reason for this is that antibiotics interfere with key metabolic steps that are linked to a wide diversity of other reactions, so that any one of a number of biochemical changes may influence the growth response to an inhibitor. The initial reaction between phages and cells is, on the other hand, directly associated with the presence of a receptor substance with a high order of specificity.

Current interest in drug-resistance mutations is largely motivated by their importance in limiting the effectiveness of chemotherapy (95), but they have provided interesting material for more theoretical genetic studies as well. In

most cases, resistance may be developed in a series of individually small steps, speaking for the cumulative action of a number of gene mutations as postulated above. The evidence for this comes primarily from kinetic studies of increasing resistance under selection (5, 107, 135). It has been confirmed by direct crossing experiments involving chloromycetin resistant mutants of *E. coli* (6). Progeny of crosses between a sensitive and a fully-adapted (many-step) resistant parent showed a segregation of many intermediate grades of resistance, representing the reassortment of the sensitive and resistant genes in a variety of combinations.

Streptomycin-resistance shows some unique features in contrast to the agents just summarized. In many species, a mutation conferring full resistance occurs at a rate, about 10^{-10} per fission, which overshadows the smaller step mutations characteristic of resistance to other agents (5, 28, 100, 135). This low rate is perhaps the smallest mutation rate to be accurately measured in any organism. A more striking oddity is the mutation which over-adapts the cell to streptomycin so that the resistant mutant is dependent upon streptomycin for growth (95, 100). The function of streptomycin is possibly to regulate an over-expanded enzyme system, analogous to the precedent of a sulfonamide-dependent mutant of Neurospora (141).

Amino acids and vitamins are not commonly thought of as antibiotics but it has long been known that they sometimes interfere with, rather than promote, bacterial growth (78, 111, 117–Snell, 140). The very ubiquity of these compounds imparts a special interest to them as possible natural regulators. Of equal interest is the correlation between sensitivity to amino acids and virulence in Salmonella and Brucella (12, 109) which may reflect a hitherto unsuspected general principle.

It is likely that a biochemical basis will be found for the effects of all types of mutations (29), but microorganisms have been especially prolific in the production of mutants with overt effects on metabolism. For this reason, microbial and biochemical genetics are intimately associated and often confused. Gene mutations affecting anabolic processes are usually detected as nutritional or auxotrophic mutants, whose growth depends upon an external supply of the missing metabolite. Auxotrophs have been used for the exploration of many biosynthetic pathways, which are remarkably similar in bacteria, molds, and mammals. Davis' paper (7) outlines this methodology, which is based on the pioneer investigations of Tatum, Beadle, and other workers on the production and characterization of auxotrophic mutants in fungi (30, 69, 117–Tatum) and in bacteria (63, 126, 127, 112). Mutations leading to catabolic defects have been especially useful in bacterial work, both as genetic markers and in the analysis of fermentation pathways (53).

Mutations *to* auxotrophy do not lend themselves to quantitative estimation of rates, despite more or less efficient selective methods for their isolation (88). On the other hand, mutations *from* auxotrophy to the nutritionally wild type or *prototrophic* state are suitable for selective counting methods, but due care

must be taken to minimize residual growth of auxotrophic inocula at the expense of growth factors carried over with the cells or resident as impurities in the test medium (114, 116).

Serological variation is of key importance in medical bacteriology, but its genetic study is barely under way. The work of Kauffmann and of Edwards and Bruner on Salmonella illustrates the provocative information now at hand (8, 9, 10, 71). A compendium of the serological variation of bacteria generally would be inappropriate here, but the subject has been treated exhaustively in excellent reviews and books (66, 36, 54, 32).

Given two alternative forms of a gene—say *a* and *A*, each of which mutates into the other at a definite rate—it is easy to show that an equilibrium will eventually be established such that the ratio of *a* : *A* will be equal to the ratio of the mutation rates *to* the respective conditions (42). Most mutation rates are so low (of the order of one per million or billion cell divisions) that mutational equilibria would take too long for human observation, even if the necessary constancy of the environment were possible. Mutations involving the antigenic structure of the flagella of Salmonella have, however, been found to have unusually high rates (accounting for the readiness with which they have been found), and Stocker (124) has described mutational equilibrium as approached from inocula of either form.

Induced mutations.—Since Muller's announcement in 1928 that X-rays would induce mutations in fruitflies, an extensive segment of genetic research has concerned the discovery of mutagenic agents and the conditions of their effect. Higher organisms like Drosophila and maize are indispensable in the finer analysis of the cytological basis of induced genetic alterations, but microorganisms are very useful tools in the screening of new agents for mutagenic activity, and in the study of the gross quantitative aspects of such activity. The same types of mutants already mentioned as best enumerated on a selective basis are particularly useful here. Dose-response data have been published for mutations induced by X-rays (113, 45) and by UV (49, 104) but their interpretation, especially for UV, is far from simple.

It has been thought that radiations induced mutations by direct photochemical processes, i.e., that the gene itself might be activated by the absorption of a quantum of UV, or by collision with a secondary electron following an X-ray quantum absorption, (60, 80). Some revision of this concept is now necessary on the basis of recent research. The effectiveness of X-rays is potentiated by the presence of oxygen, and there may be a tenfold difference between the doses required for a given effect in oxygen as against an inert atmosphere (47). This argues for a radiochemical intermediate, possibly some free radical (peroxide?) which depends upon oxygen for its production under the influence of X-radiation.

Owing to the powerful penetrability of X-radiation, it has been used in Drosophila studies more extensively than UV, which penetrates through living material so poorly that there are serious experimental difficulties in its ap-

plication in the genetics of plants and animals. The small size of bacterial cells ideally suits them for experiments with this agent, and UV is probably the most convenient and widely used mutagenic treatment in microbial experiments. The analysis of UV-effects has been stimulated by the discovery of photoreactivation (72, 55). A number of workers have found that a treatment of various types of cells (actinomycete spores, bacteria, bacteriophage, mold spores, Arbacia eggs) with visible light partially cancels both the lethal and the mutation-inducing effects of a previous dose of UV (56, 73, 104, 105, 134). This might suggest that a light-sensitive substance is produced by UV, but the possible nature of this substance and its locus within the cell are obscure. Bacteriophage inactivated with UV is photoreactivated by visible light only following its absorption into sensitive cells. The effect of UV in inhibiting enzyme synthesis is also subject to photoreactivation (125) but no systems simpler than intact cells have been shown to give such an effect.

Chemical mutagens.—A byproduct of research on chemical warfare agents during World War II was the realization of the possibility of mutagenic activity of chemicals. The nitrogen and sulfur mustards (β-chloro-alkylamines and sulfides) have been studied especially extensively, and found to be potent mutagens for all organisms studied. In general, their effects are similar to those of X-rays and UV, but there are differences in details (26). The similarity of effects is made the more pronounced by the fact that mutants that are relatively resistant to "mustard" can be selected in *E. coli*, strain B, and these mutants also show augmented resistance to UV and X-ray. (33, 138).

Active programs are under way in several laboratories to screen compounds for mutagenic activity, with bacteria prominent among the test organisms. The methodology of such a program, and some of the precautions needed to justify a positive conclusion, are illustrated in Witkin's paper (11). There is a wealth of further literature on this subject, and it is likely to remain an active field. There is so far no rational basis which can be used to predict the activity of new compounds, and substances as diverse as formaldehyde, acriflavine, urethane, caffeine, hydrogen peroxide, and manganous ion are credibly reported as active in one or another system (26, 49, 58, 65, 47). In general, reagents with a high reactivity for labile organic H groups, e.g., formaldehyde, organic peroxides, acyl halides and sulfates, ethylene oxide, acetic anhydride, and diazomethane are probably mutagenic (86) but this does not account for the mutagenicity of such chemically inactive compounds as caffeine or urethane.

Much more careful work is needed in this field, but so far there is no convincing evidence for any appreciable specificity in the mutagenic capacities of any of these chemicals or physical treatments. Each of them induces a wide diversity of mutations (as far as this has been investigated), and in general, the results of, e.g., X-ray treatment, would not be readily distinguishable from that of formaldehyde. On the other hand, it is possible that different gene forms may differ in the frequency with which they will respond to different

mutagenic treatments; but if there is any specificity, it has so far been of a second order. In this respect, induced resemble spontaneous mutations, whence some genes may mutate more frequently than others, but not in such a way that the environment can be said to direct the mutation of a specific gene preferentially to the exclusion of others.

Spontaneous mutation.—We may return at this point to the mechanism of "spontaneous mutation," keeping in mind that the study of experimentally controlled variables on mutation erases the distinction between spontaneous and induced. So long as the concept connoted by these terms is kept clearly in mind as one distinct from that of *directed* mutation, there need be no confusion.

Evidence bearing on the relationship between growth and mutation is especially paradoxical, for mutations to phage resistance, for instance, apparently do not accumulate in a resting culture (1). On the other hand, cultures whose growth is regulated in a special steady-state, controlled-flow culture vessel ("chemostat") mutate at nearly constant rates per unit time, whether the cells are proliferating slowly or rapidly (106). One interpretation is that spontaneous mutations are due not to intramolecular accidents or reproductive errors, but rather to the action of intracellular chemical mutagens formed by metabolic processes. In this connection it is worth noting that formaldehyde and hydrogen peroxide are both fairly common metabolic intermediates, and that two other mutagens, caffeine and allyl isothiocyanate (mustard oil), are also natural products. Information on the effect of temperature changes on mutation rate would be especially valuable if it could be dissected from effects on growth or metabolism. Under conditions of steady growth a temperature increment of 10° accelerated mutations in *E. coli* by a factor of about 2, and a similar increment is reported in other systems (85).

Bacterial populations.—The necessity for thinking of bacterial cultures always in terms of populations, which may have genotypically diverse components, can scarcely be over-emphasized. The process reviewed to this point, mutation, is the fundamental source of genetic variation, but in view of the smallness of spontaneous mutation rates, it is obvious that the occasional change of a cell from one genetic condition to another can make little impression upon the composition of bacterial populations. The forces that determine which genetic types will predominate in bacterial cultures are the subject of population dynamics. In diploid sexual organisms, population genetics is greatly complicated by recombination and by the concealment of genetic variation in the heterozygous condition, so that the most drastic culling may have to be carried out for a great many generations to have a marked effect on the relative frequency of different gene forms. Selection in bacteria is, as a rule, more straightforward, as shown, for example, by the quantitative isolation of phage-resistant mutants by a single application of the virus. The physiological interactions of bacteria in dense cultures lead to less trivial problems in population dynamics. Such interaction may involve an obvious competition for nutrients,

or the production of auto- or trans-inhibitory metabolites. An especially clearcut example of the latter in an economically important organism is reprinted here (12). The intricate interrelationships of the production of and sensitivity to species-specific antibiotics ("colicins") have been thoroughly analyzed for *E. coli* by Fredericq (59). One of the most exhaustive analyses of selection dynamics in bacteria will serve best to illustrate the complexity of populational interactions in which several distinct effects may be superimposed (115). A number of populational interactions have been described with a less complete analysis (138, 136, 85).

Population complexities may also arise when more than one mutation occurs, so that the population consists of several categories of genotypes. One example has been cited already in the stepwise development of resistance by the cumulative effect of serial mutations. A second has been described so recently that its implications are still under discussion. In independent experimental work, three groups of investigators noticed a perplexing sequence of cycles in bacterial populations under conditions of continuous or reiterated culture (25, 106, 124). "Marker" mutant cells increased in proportion as mutations accumulated, but instead of increasing indefinitely, their ratio was subject to sporadic downward shifts. The same interpretation was independently formulated for each case: an adaptive mutation increasing the fit of the bacteria to their rather artificial in vitro environment. Owing to the overwhelming preponderance of the cells not carrying the marker, the adaptive mutation will usually occur in an unmarked cell, the descendants of which will then displace the rest of the population, markers and all. After the changeover, marker mutations accumulate again until a possible second changeover takes place to complete another cycle. It is not predicted that this process could alter the ultimate equilibrium of cell types under mutation "pressure," but that it would alter the short-term course of cultures in which marker mutation pressures outweigh selection effects is apparent. Such adaptive mutations undoubtedly serve as models for evolutionary specialization; as the streptomycin-dependent mutation cited earlier shows most strikingly, genetic adaptations are often quite specific for the immediate environment. This type of specialization undoubtedly accounts for the often noted loss of virulence encountered frequently among pathogenic bacteria maintained on artificial culture media.

Interclonal variation: Sexual recombination.—The preceding discussion of bacterial populations has supposed that each bacterial cell is genetically isolated from its partners, i.e., that reproduction is exclusively vegetative or clonal. A large body of evidence is now at hand, however, which shows that this picture is incomplete, and that some account must be taken of interclonal processes. One such process was discovered in 1946 by Tatum and Lederberg (45, 84, 128) and described as genetic recombination, for the experiments are based on the selective isolation of genetic factor combinations from mixtures of different kinds of mutant cells. The conditions under which these genetic exchanges take place, and the patterns in which they result, led these authors

to conclude that recombination in *E. coli,* strain K-12, results from a sexual process (13). This conclusion is based upon evidence which is, to date, entirely negative, indirect, or genetic. It is substantiated, however, by single-cell studies on heterozygous diploid cultures (14, 142), and by a direct demonstration of the nonfiltrability of the agents of recombination (40). A variety of selective techniques can be used for the isolation of recombinants from strain K-12 of *E. coli.* In addition to the selection of prototrophs from mixtures of auxotrophs which is most commonly used, one may select for dually resistant recombinants from mixtures of cells each resistant to one inhibitor (83). A combination of these methods has been profitably employed to detect new strains crossable to K-12; a number were found, some with many properties differentiating them from each other. Developments in the author's laboratory to the date of this writing have been summarized (47–Lederberg).

Recombination in strain K-12 has been applied by a number of other workers for various problems (102). The genetics of resistance to chloromycetin (6) and to streptomycin have been contrasted (46, 101). The very occurrence of factorial segregation shows, of course, that bacterial enzymes are not to be identified with the genes required for their formation (98). In fact, it is controversial whether the patterns of gene-enzyme interrelationships in bacteria support the "one gene-one enzyme" proposed as a generalization from the properties of auxotrophs in Neurospora (69, 47–Lederberg, Horowitz, Bonner).

Transformations.—The history of "type transformation" is often regarded as dating from Griffith's classical experiment on pneumococcal types in 1928 (64), but this work is antedated by a confusing array of studies which date at least as far back as the controversy over the etiology of typhus fever. When the rickettsial etiology of this disease was finally established, many workers suggested that the serological reaction of Proteus OX-19 with rickettsial antibodies (Weil-Felix reaction) was due to a transformation of non-reactive Proteus by rickettsial products, and gave the name "paragglutination" to induced serological variations such as were supposed to be involved here. In support of this hypothesis, (to which no credit is now given) many workers reported that the serological reaction of *E. coli* and other enteric bacteria could be modified by cultivation in filtrates, extracts, or lysates of serologically distinct cultures (reviewed in 81). Griffith's demonstration was, however, the first which proved to be readily reproducible by other workers, and remains a proper starting point for modern discussions of transformation. His paper is unfortunately too lengthy to be appropriate for reprinting here, but his observations, and those of other workers leading to the isolation and characterization of the active principle responsible for the pneumococcus transformation are summarized in the paper reprinted here (16).

The genetic interpretations of this transformation are currently a subject of lively discussion. Its description as a specific induced mutation is probably less fruitful than as a transfer of hereditary material from one cell to another (47–Taylor). The main questions which still have to be answered include

a) the further physical and chemical characterization of the agent, b) its genetic complexity (that is, whether it involves single characters, the full hereditary material of the pneumococcus, or something intermediate), and c) its cytological relationships to nuclear or extranuclear structures in these cells. The current approaches to these problems are summarized in a number of papers and reviews, (27, 91, 93, 130, 131).

In addition to the volume of older literature which must be supposed to carry some grain among the chaff, there are a number of more recent reports of transformations in various organisms. These include *E. coli* (31), *Hemophilus influenzae* (22), *Shigella paradysenteriae* (133), *Alkaligenes radiobacter* (39), and staphylococcus (38). Discussion of these and other transformations should take into account complications which might arise from bacterial life cycles more complex than usually regarded.

This presentation has emphasized the contrasting features rather than the similarities of the phenomena described as genetic recombination vs. transformation. Both phenomena tend to the same genetic result: the elaboration of the cells whose hereditary traits are derived from more than one parent. When more is known of the morphological basis of "sexual" genetic recombination on one hand, and of the genetic properties of transformations, on the other, a more profitable synthesis of these contrasting concepts may issue.

The recrudescence of interest in bacterial cytology has been largely independent of, though contemporary with the development of bacterial genetics documented in this book (77). The geneticist notes at least two fields where cytological information is indispensable to him: a) the form and behavior of the bacterial nucleus, and b) the possible existence of complex "life cycles." Reliable information on both these subjects is relatively meagre, but the evidence of at least the existence of nuclei in bacterial cells is relatively convincing. Robinow's paper reprinted here (15) may be taken as one point of departure for the more recent work on this subject (41, 74). With the improvement of techniques, we may look forward to rapid progress in the establishment of the details of the nuclear cycle at cell division, segregation, etc. (99, 118, 92, 122, 47).

The expression "life cycle" has come to carry many connotations in bacteriology which hinder a careful discussion of the often conflicting observations of generations of bacteriologists. At least one type of cycle is indisputable, the formation of highly resistant endospores by many bacteria. Whether endosporogenesis has any genetic significance is controversial. At the least, they may be supposed to represent the stage at which the presence of only one nucleus per cell (in contrast to the two, four, or more, characteristic of most rods) is the most likely (see 54), and on this basis may be particularly useful for certain types of genetic experiments. More than one student of the mode of nuclear segregation into the endospore has proposed a sequence of nuclear fusion and meiotic reduction (i.e., autogamy). However, the cytological figures are not easily interpreted, especially in view of the pitfalls of logical recon-

struction of fixed cells, and in this form autogamy is a blind alley without the possibility of genetic novelty, and therefore not amenable to genetic test, (74).

The genetic significance of another cycle, the "L-forms," is equally obscure. It is expected that this problem will be clarified in the very near future to an extent that makes a detailed discussion of its present status here premature. The reader is referred to provocative discussions published elsewhere (50, 51, 52, 75, 76, 132, 123) These studies reopen the possibility of the existence of diminutive forms of many bacteria which may be capable of passing through ordinary bacterial filters. These forms may also be highly resistant (like the endospores) to heat and antiseptics. Since they also may have special nutritional requirements, they may remain dormant in ordinary bacteriological media which support the normal bacterial form. If these observations are confirmed, the filtrable L-forms might well play a role in some of the transformation experiments cited earlier. However, the transforming agent would consist not of a single genetic factor transferred from one cell to another, but of a diminutive form of one cell which germinates under the influence of another. The analogy between the diminutive forms and the gametes of other plants and animals is obvious on a physical plane; the possibility that they may play a similar function in bacterial biology is a provocative one which will require careful study.

One of the reasons for including papers on viruses and bacteria in the same volume is the necessity for considering both the host and the "parasite" in any physiological studies of their association. Furthermore, it is becoming apparent that a great many bacterial cultures carry bacteriophages in a symbiotic relationship ("lysogenicity") so that what are taken to be bacteriological investigations have an unavoidable virological component. A second reason is that the phage-bacterium complex may be profitably regarded as a unit for comparison with other cellular systems which carry extranuclear hereditary components (57, 119). Burnet's paper illustrates this very clearly (17).

It might be worth pointing out also the formal analogy between the transfer of a latent virus from a lysogenic to a sensitive bacterium and the transfer of the capsular attributes from smooth to rough bacteria in the pneumococcus transformation. Whether this analogy is more than superficial only time will tell. It is becoming clear, however, that there is a continuous gradation of properties between systems of cytoplasmic heredity such as the chloroplasts of green plants, and the metabolic granules of yeast, and infective associations such as the "killer" factors in Paramecium, rickettsia in the Arthropoda, and viruses in plants and mammals. In many instances at both extremes, "disinfection" or cure is possible by the use of appropriate drugs (streptomycin for chloroplasts, acriflavine for yeast granules) (47, 57).

Burnet's paper also illustrates one of the earliest and clearest cases of mutation in a bacteriophage. In a later study, Luria described host range mutations in phages which restore the virus' ability to attack bacteria which had mutated to resistance to the previous form of the virus (18). This process of compensatory

mutations on the part of bacterium and virus evidently can sometimes be repeated over a great many cycles, and is of some economic importance in the acetone fermentation industry. These mutations in bacteriophage have been used as the basis for further genetic studies which have revealed the occurrence of genetic recombination among viruses (as well as in bacteria) (19, 20). Two lines of attack are exemplified in the papers here. One uses patent markers, such as host range and plaque morphology. For the other, recombination among lethal mutations induced by UV is used to explain multiplicity reactivation, or the cooperation of several particles damaged by UV to initiate phage growth, in contrast to the ineffectiveness of individual damaged particles.

This work has naturally stimulated attempts to show similar phenomena in animal viruses. At present these are represented by reports by Burnet and Lind (37) of probable recombination in the influenza virus, and similar investigations are under way in other laboratories. Plant and animal viruses are likely to be more difficult to study from this point of view, chiefly because of technical difficulties in initiating infections with single virus particles (35, 62, 79).

Genetic study of bacteria and viruses is closely interwoven with the most general problems of their biology; this is not surprising, for the same has happened in other areas of biology. But it is to be hoped that genetics will be regarded not as a unique or isolated part of bacteriological study, but as an element of all teaching and research in microbiology.

THE REPRINTED papers are numbered from 1 to 20; citations to the literature at large are numbered from 21 to 142, alphabetized by authors' names. Choice of citations has been dictated by the same general considerations as were applied to the reprints, and many interesting examples of specific mutant types have necessarily been omitted. Many reviews are available which provide more detailed and balanced accounts of specific subjects; these are listed with an asterisk (*).

The subjects treated in the reprinted papers may be outlined as follows:

I. Bacteria
  - A. Spontaneous mutation
    1. Mechanism of adaptive mutation (1, 3, 5, 6)
    2. Estimation of mutation rates (1, 2, 4)
    3. Categories of mutants
       - a. Resistance to antibacterial agents (1, 2, 3, 4, 5, 6, 11, 12, 17)
       - b. Biochemical mutations (7)
       - c. Serological mutations (8, 9, 10, 12)
    4. Phenotypic lag (4, 7)
  - B. Induced mutations (11)
  - C. Population dynamics (12)
  - D. Interclonal variation
    1. Genetic recombination in *E. coli* (6, 13, 14)
    2. Transfer of symbiotic virus (17)

3. Type transformation in pneumococci (16)

E. Nuclear cytology of bacteria (15)

II. Bacterial viruses

A. Spontaneous mutation (15, 18)

B. Recombination (19, 20)

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