Papers on

BACTERIAL VIRUSES

Selected by

GUNTHER S. STENT

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GUNTHER S. STENT
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FOREWORD

IN 1951, the collection *Papers in Microbial Genetics; Bacteria and Bacterial Viruses* appeared under the editorship of Joshua Lederberg. That collection has been an indispensable aid to us and many others in the teaching of microbial genetics. It has enabled every student to read a cross-section of the original literature and thus become directly acquainted with the basic experimental data that often outlive the conclusions based upon them.

The Lederberg collection, however, appeared just at the eve of great discoveries which, since that time, have enormously advanced our understanding of the structure and function of the hereditary substance of bacteria and their viruses. The transduction of bacterial genes by bacteriophages, the polarity of bacterial conjugation, the role of DNA as the germinal substance of bacterial viruses, the structure of DNA itself -- all of these were discovered within the two-year period following the publication of Lederberg's collection. New techniques have also greatly changed the course and nature of research in microbial genetics; in particular, the use of isotopic tracers in bacterial virus research and the development of recombination tests of high resolving power for the analysis of genetic fine structure have brought microbial genetics to the molecular level.

Hence it seemed urgent that a new, more up to date, collection should now be made available; we have decided to assume this task, and hope that the present book and its companion volume, *Papers on Bacterial Genetics*, will answer the needs of at least the immediate future. The extent of the changes that have taken place is indicated by the fact that of the more than fifty papers we have selected for the present two volumes, only five have been retained from the twenty papers of Lederberg's collection.

In addition to the reprinted papers, each volume contains an introductory text with its own bibliography, in order to compensate for the necessarily incomplete and arbitrary nature of the selections. The selections themselves were made for the sole purpose of providing students with a cross-section of the literature, at a reasonable cost; in the words of Professor Lederberg "no apologies need be offered for a selection which must be largely arbitrary."

In conclusion, we would like to express our deep appreciation to the copyright owners and the authors who permitted us to reprint these papers, as well as to those who have supplied us with rare reprints from which some of the copy has been made.

GUNTHER S. STENT
EDWARD A. ADELBERG
Berkeley, California, 1960

INTRODUCTION

BACTERIAL VIRUSES were discovered in 1915, when F. W. Twort (149) isolated a filtrable virus which produces a "glassy transformation" of micrococcal colonies during their growth on an agar surface. Twort's paper remained relatively unnoticed, until two years later F. d'Hérelle (64) published his own observations on a filtrable agent, the "bacteriophage," capable of serially transmissible lysis of growing cultures of enteric bacilli. D'Hérelle's announcement caused an immediate sensation among medical bacteriologists, since the bactericidal properties of the bacteriophage offered promise of a generalized prophylaxis and therapy of bacterial diseases. Within two or three years of the publication of his first paper, d'Hérelle had carried out many incisive experiments which allowed him to recognize the essential aspects of these bacterial viruses.

Twort and d'Hérelle did not remain the only bacteriophage workers for very long, and the study of bacterial viruses rapidly became so popular that most of the leading bacteriologists of the decade following the first World War tried their hand at it. This soon led to a number of violent controversies concerning the nature and mode of action of bacteriophages. Some of these controversies were aired at a discussion on the bacteriophage organized by the British Medical Association at its Glasgow meeting of 1922. The presentations which d'Hérelle, Twort, Bordet, and Gratia prepared for this meeting form the first paper of this collection. In his discussion, d'Hérelle demonstrates the self-reproducing, or viral, character of the bacteriophage, a view for which, as is evident from the remarks of Bordet and of Gratia, he had been under attack. Gratia's discussion, however, rectifies two errors of d'Hérelle: the claim that the phenomena discovered by Twort and by d'Hérelle are fundamentally different and the assertion that all bacteriophages represent the same antigen. In 1926, d'Hérelle (65) thus summarized his earlier findings on the multiplication of bacterial viruses: "The first act of bacteriophagy consists in the approach of the bacteriophage corpuscle toward the bacteria, then in the fixation of the corpuscle to the latter . . . The bacteriophage corpuscle penetrates into the interior of the bacterial cell. When, as a result of its faculty of multiplication, the bacteriophage corpuscle which has penetrated into the bacterium forms a colony of a number of elements, the bacterium ruptures suddenly, liberating into the medium young corpuscles which are then ready to continue the action."

The first workers to study in some detail the initial step in bacterial virus

growth, i.e. the fixation, or *adsorption,* of the bacteriophage to its bacterial host cell, were Krueger (91) and Schlesinger (129, 130). In his paper (130), the second of this collection, Schlesinger demonstrates that phage adsorption usually is an irreversible process which follows the kinetics expected from a two-body collision model involving freely diffusing virus particles and bacterial cells. Schlesinger's work, which probably represents the first rigorous application of physicochemical principles to the study of bacterial viruses, was extended by Delbrück (39), who showed that the physiological state of the bacteria affects the rate of adsorption, and by Garen and Puck (59), who demonstrated clearly that, under certain conditions, a *reversible* union between phage and bacterium can take place. Contrary to the predictions of the simple two-body collision model of Schlesinger, however, it turned out that the rate of phage adsorption reaches a maximum at high bacterial concentrations and is strongly temperature-dependent. Hence, it appears that the irreversible fixation of the bacteriophage is a two-step process, involving at least one further, temperature-sensitive, step in addition to the collision of virus and host cell (146). Subsequent electron-optical observations by T. F. Anderson revealed that the organ of adsorption is the phage *tail* (5), in particular, that it is the thin *tail fibers* (155) which are the structures undergoing the stereospecific fixation reaction with the phage receptors on the bacterial surface. (Reviews: 58, 148, 73, 125, 154.)

Convincing support for d'Hérelle's conception that the infecting phage particle multiplies within the bacterium and that its progeny are liberated upon lysis of the host cell was adduced in 1929 by Burnet (31), who showed that 20 to 100 viruses suddenly appear some 20 minutes after a bacterial suspension is infected with a single phage particle. The final demonstration, however, that a burst of progeny of the parent virus is liberated by each infected bacterial cell after a *latent* period was only provided in 1939 in the *one step growth* experiment of Ellis and Delbrück (52), whose paper is presented in this collection. In their publication, Ellis and Delbrück also describe for the first time the *single burst experiment,* which made possible the study of phage growth in individual infected bacteria, rather than in mass culture. The appearance of this paper marks the beginning of modern phage research.

The interpretation of the phage-induced lysis of infected bacterial cultures was one of the violent controversies during the first 20 years of phage research. While d'Hérelle (65) correctly thought that intracellular phage growth leads to lysis of the host cell and liberation of the virus progeny, Bordet and Ciuca (20, 22) maintained that the phage-induced dissolution of bacterial cultures is merely the consequence of a stimulation of lytic enzymes endogenous to the bacteria. Other workers, such as Bronfenbrenner (30), or Krueger and Northrop (92), thought that lysis of the bacteria is only a secondary phenomenon, which may or may not follow the growth of phage, and imagined that the bacteriophage can pass freely in and out of bacterial cells. Delbrück (40)

finally showed, in his second paper of this collection, that these arguments were bedevilled by the circumstance that there exist not one but two completely different processes by which bacteriophages can lyse susceptible bacterial cells. One of these, *lysis-from-without,* represents an immediate dissolution of bacteria, often encountered when the multiplicity of infection is much greater than one phage per bacterium (9, 123, 92, 124). *Loss* of the input phages, rather than their multiplication, ensues from this form of lysis. Only the second of the lytic processes, *lysis-from-within,* is really the form of lysis properly connected with intracellular phage multiplication, and its onset signals the end of the latent period.

The one-step growth experiment demonstrated clearly the nature and kinetics of the process by which bacterial viruses multiply within cultures of susceptible bacteria. It thus brought into focus the question of fundamental biologic interest: what is taking place *inside* the infected cell during the latent period while the parental phage particle replicates itself several hundredfold? In order to study the kinetics of *intracellular* phage multiplication, Doermann (47) broke open phage-infected bacteria at various times during the latent period and assayed the infectivity of the material released by premature lysis. The result of this experiment, published in the paper included in this collection, was that the infectivity associated with the original parental virus is lost at the outset of the reproductive process, since no infective particles whatsoever can be found in any of the bacteria lysed within ten minutes after their infection. After more than ten minutes have elapsed, however, ever-increasing numbers of infective progeny viruses make their intracellular appearance, until the final crop of progeny has been attained which would have been released by spontaneous lysis-from-within at the end of the normal latent period. The stage of intracellular bacterial virus growth during which the infected host cell contains no material capable of infecting another bacterium is the eclipse (107). Subsequent studies showed that the actual multiplication of the infecting virus takes place during the eclipse, i.e. that the phage multiplies in a non-infectious form, the *vegetative phage* (48).

Schlesinger was also the first to purify a bacterial virus, a feat which he accomplished by high-speed centrifugation of phage lysates (131). Chemical analysis of the purified virus revealed that it consists of approximately equal proportions of protein and deoxyribonucleic acid (DNA) (132). Later studies by Anderson (3, 4) and by Herriott (66) showed that the viral DNA resides within a proteinaceous head membrane, from which it can be released by osmotic shock. Hershey and Chase (75) then demonstrated that the two viral moieties, protein and DNA, have independent functions in the infection process. For, as can be seen in their first paper included in this collection, Hershey and Chase found that practically all of the viral protein remains at the surface of the infected cell, and that it is mainly the viral DNA which enters the bacterium at the outset of intracellular phage growth. The bulk of the phage protein appears to be relieved of any further function in the intra-

cellular reproductive process after the proteinaceous tail has attached the virus particle to the bacterial surface and the DNA has safely entered the interior of the host cell. This historic discovery showed that it must be the viral DNA that is the carrier of the hereditary continuity, i.e. the germinal substance of the extracellular, resting phage. The release of the DNA from its protein envelope at the very moment of infection also accounts for the existence of the eclipse period at the early stages of intracellular virus development. For having been divested of its attachment and injection organs, the DNA of the infecting phage naturally is unable to gain entrance into any further bacterial cells to which it may be presented in the infectivity test.

What happens to the viral DNA after its injection into the host cell? In 1950 Putnam and Kozloff (126) devised an experiment directed toward the question of whether any of the atoms of the parental DNA ultimately reappear among the progeny viruses. In this "transfer experiment," bacteria are infected with phage particles whose DNA is isotopically labeled, and the phage yield issuing from such infected cells assayed for its content of parental isotope. The outcome of Putnam and Kozloff's transfer experiment was that about half of the atoms of the parental DNA were found to be transferred to the progeny. This work was confirmed and extended with improved experimental techniques by Watson and Maaløe (120, 152), one of whose publications is included in this collection. In view of the inference that it is the DNA of the virus which carries the genetic continuity into the host cell, it seemed likely that an understanding of the mechanism of transfer of DNA atoms from parent to offspring might afford valuable insight into the nature of the reproductive process. Further investigations have revealed that the parental DNA complement of a single parental virus is not transferred intact to a single progeny virus, but that the molecular patrimony is dispersed over several offspring phages (76, 143, 101, 145).

Some theories of the nature of phage multiplication envisaged that there are present, within the normal host bacterium, bacteriophage precursors whose metamorphosis into mature bacteriophages is merely triggered by the infecting phage particle (93). This view was finally dispelled in 1948 by an experiment of Cohen (37), designed to determine the origin of the substance of the progeny phages. This work, reported in a paper of this collection, represents the first use of radioisotopes in the study of bacterial viruses. By exposing bacterial cultures to P^{32}, either only prior to or only subsequent to their infection with phage, and analyzing the virus progeny for their relative content of radioisotope, Cohen could show that most of the phage DNA is synthesized from materials still in the growth medium at the moment of infection; hence, the phage particles cannot have been derived from pre-existing bacterial precursors. The complete kinetics of assimilation of phage DNA phosphorus were studied subsequently by modifications of Cohen's original method, by either adding to or withdrawing from the growth medium of bacterial cultures P^{32} at various times before or after their infection. The results of this work led to

the idea that, prior to its incorporation into intact, infective progeny particles, the phage DNA exists in an intrabacterial *phage precursor pool* (144, 71).

Further insight into the process of phage multiplication was gained by the discovery of a variety of "incomplete" phage structures which possess one or another of the properties of the virus without being endowed with the power of self-reproduction, the most complex of all its attributes. Thus, premature lysis of infected bacteria at late stages of the eclipse period liberates newly synthesized proteinaceous material already possessing some of the antigenic properties of the intact bacteriophage (119). The total amount of phage antigen finally liberated upon spontaneous lysis of the cells, furthermore, generally exceeds that incorporated into infective progeny (119, 32, 46). Electron-optical observations of such lysates, furthermore, reveal the presence of structures whose morphology bears some resemblance to the characteristic shape of mature bacterial virus particles (161, 63). Prominent among these structures are the "doughnuts" which Levinthal and Fisher (103) found to appear during the eclipse and then to increase in number at about the same rate as the complete phage particles. Later studies have shown that the doughnuts are, in fact, *empty phage heads,* and that the "maturation" of infective progeny at the end of the eclipse seems to represent the stable union of phage precursor DNA with phage precursor protein into structurally intact virus particles (88, 89).

Phage precursor protein and phage precursor DNA are not the only materials whose synthesis within the host cell is induced, or presided over, by the DNA of the infecting parental virus. For, at the outset of intracellular phage growth, the formation of some non-precursor proteins must proceed before replication of the viral DNA can begin. One of these "early" proteins was identified by Flaks and Cohen (53, 54) as the enzyme deoxycytidylate hydroxymethylase, essential for the synthesis of the specific components of the viral DNA, 5-hydroxymethylcytosine (160). Studies by Kornberg, Zimmerman, Kornberg, and Josse (90), presented in a paper of this collection, revealed the phage-induced formation of four further enzymes, all of which are demonstrably involved in the synthesis and replication of the viral DNA. It is important to realize, therefore, that the phenotypic expression of the genetic substance of the phage is not confined solely to the construction of materials that find incorporation into the mature, infective progeny virus.

In common with other organisms, bacterial viruses sport occasional hereditary variants, or *mutants,* in the course of their growth (34, 68, 133). These mutants can differ from their parents in a variety of characteristics, such as the type of plaque formed on agar seeded with sensitive indicator bacteria (67), the strains of bacteria which the phage can infect (105), or the physical or chemical properties of the virus particle (2, 43, 26). The mutation of the vegetative phage during its intracellular growth was used by Luria (108) to probe the nature of the self-duplication of the hereditary material of the infecting particle, as shown in a paper of this collection. In his experiment,

Luria examined the individual phage yield of many thousands of phage-infected bacterial cells and scored the single bursts for the presence of progeny viruses possessing a certain plaque-type mutant character. On the basis of the observed clonal frequency distribution of these mutants, Luria was able to infer that the replication of the hereditary material of the phage is geometric, i.e. that it proceeds by a number of successive cycles of self-duplication, since other conceivable reproductive models, e.g. successive replications of the initial parental element or chain replication of the last element produced, would have led to mutant distributions quite different from that actually found.

In 1946, a most important discovery was made independently by Delbrück and Bailey (44) and by Hershey (67), who examined the genetic character of the phage yield issuing from bacterial cells infected with two related parent viruses differing from each other in two mutant factors. It was found that among the progeny of such mixed infection there appear virus offspring carrying one of the mutant factors of one and one of the mutant factors of the other of the two parents, demonstrating that bacterial viruses can undergo *genetic recombination*. The first detailed study of genetic recombination in phage was undertaken by Hershey and Rotman (77), their paper being included in our collection. This work showed that, on the basis of the frequency with which recombinant progeny for various mutated characters appear in such "crosses," it is possible to construct a *genetic map* of the phage on which the mutant loci can be arranged in a linear order. Hershey and Rotman also examined the frequency of complementary recombinant types in the yields of individual mixed infected bacteria and found that the formation of complementary types does not seem to occur in a single event [Bresch (28) was able to establish this conclusion even more convincingly in a later study]. This fact led Hershey and Rotman to entertain the notion that recombination in phage might not be the consequence of a reciprocal exchange of preformed genetic structures, such as chromosomal recombination in higher forms, but that it might be an act incidental to the replication of the genetic material itself. This hypothesis, which came to be called "partial replicas" (69), or "copy choice" (97), now forms one of the basic concepts in the understanding of the molecular basis of self-duplication and genetic recombination. As more data concerning the process of genetic exchange in phage accumulated, it became evident that the theoretical analysis of a phage "cross" is a problem in population genetics. It was seen that within each mixedly infected bacterial cell, growth and recombination of the numerous vegetative phage replicas proceed concurrently. In 1953, Visconti and Delbrück (150) developed, therefore, a theory which succeeded in explaining quantitatively the recombinant frequency observed in different phage crosses under various conditions. This theory assumes that replication and recombination of vegetative phages proceeds in an intrabacterial *pool*, in which phages repeatedly mate pairwise and at random until lysis of the host cell, and from which pool the vegetative phages are withdrawn irreversibly for maturation into infective progeny

phages. A concise statement of this theory can be found in Adams' book (1), and a more generalized formulation is presented in two later analyses of this problem (137, 29).

An important clue to the nature of the elementary recombinational event in phage was uncovered by Hershey and Chase (74), described in their second paper of this collection. They noted that among the progeny of mixed infections about 2% of the particles are *heterozygous*, in that these individuals carry homologous loci, or alleles, from both parents of the cross. The heterozygosity is only partial, however, in that in any one heterozygote virus only a very limited segment of the genome is actually of biparental provenance, most of its loci being homozygous, or derived from only one or the other of the parents. The structure and behavior of these heterozygotes suggested to Hershey and Chase that the formation of heterozygotes and the formation of recombinants might be related processes. The nature of heterozygotes was considered further by Levinthal (100), who demonstrated that such viruses are *recombinant* for genetic loci on opposite sides of the limited region of heterozygosity. Levinthal then inferred that recombinant phages, in fact, arise through the formation of heterozygotes in the course of phage reproduction by the partial replica, or copy-choice, recombination mechanism. This inference found further support from Levinthal's calculation that the observed frequency of heterozygotes is great enough to explain the observed frequency of recombinational events.

Once the viral DNA had been identified as the germinal substance, it became possible to consider in actual chemical terms how the hereditary information is stored in the resting phage and how it is replicated in the vegetative phage. After deoxyribonucleic acid was discovered by Miescher in 1871, some 60 years of chemical study of this substance revealed that its building block is the *nucleotide*, composed of one molecule each of phosphoric acid, deoxyribose, and either adenine, guanine, thymine, or cytosine. More recently, it was established that DNA molecules are, in fact, polymers of very high molecular weight, each molecule containing more than 10^4 nucleotide units joined through phosphate diester bonds linking successive deoxyribose molecules (cf. 36). The actual molecular architecture of DNA was worked out finally by Watson and Crick (151), whose paper is presented here. Watson and Crick showed that the DNA molecule consists of two helically intertwined polynucleotide chains laterally held together by a pair of hydrogen bonds between a complementary pair of purine and pyrimidine residues on opposite chains. The nature of the DNA molecule suggests that the only specific aspect which could distinguish one DNA macromolecule from another is the precise sequence of the four possible purine-pyrimidine base pairs along the complementary nucleotide chains, i.e. that the hereditary information is a message written into the DNA macromolecule in an alphabet containing four letters. This structure also suggested to Watson and Crick a mechanism by which the DNA molecule could replicate itself; for if the two complementary

polynucleotide chains separate and each parental chain acts as the template for the *de novo* synthesis of a complementary daughter chain, a *pair* of DNA molecules would be generated, each half-old, half-new, whose specific purine-pyrimidine base pair sequence is identical to that of the parent molecule. A genetic mutation, from this point of view, would then be a rare copy error in the replication process by which a nucleotide carrying an incorrect base is introduced into the replica nucleotide chain, thus producing a change in the genetic information. Even though a number of modifications of this replication scheme of Watson and Crick were subsequently proposed (45), later experiments have shown that in the replication of bacterial DNA the distribution of the atoms of the parental molecules appears to proceed by the *semi-conservative* route (122), a central feature inherent in the Watson-Crick scheme.

One of the first successful attempts to bridge the gap between chemistry and genetics was made by Benzer (12) in his first paper of this collection. Benzer discovered a method for scoring very rare recombinant viruses appearing in phage crosses between parents bearing extremely closely linked mutant loci. This allowed him to construct a *fine structure map* of a large collection of mutants situated in a very restricted region of the phage genome. In consequence of this work, the concept of the gene, traditionally regarded as the unit of recombination, mutation and function, became clarified. For Benzer showed that these three aspects of the genetic material are operationally separable and hence cannot share a common unit. Translated into molecular terms, the unit of recombination appears to represent one, or a few, nucleotide pairs along the DNA molecule, whereas the unit of mutation can be of variable length, ranging from the alteration of a single nucleotide pair, in case of a *point* mutation, to long-span alterations of the phage genome, covering hundreds or thousands of nucleotide pairs. Finally, the unit of function, or *cistron*, assumed to determine the specific chemical structure of an enzyme protein, or more precisely, of a polypeptide chain, is of the order of 1000 nucleotides in length (13).

After Benzer had arranged his set of closely linked spontaneous phage mutants into a linear linkage map, it became obvious that there exists a great variability in mutability of different genetic sites within a single functional group, or cistron, since at some loci, or "hot spots," spontaneous mutations recur with much greater frequency than at other, nearby loci. This differential mutability of individual genetic sites very probably reflects the chemical structure of the hereditary molecule corresponding to each locus; e.g. the chance of making a spontaneous copy error at a given site in the course of viral DNA replication might depend on which particular sequence of purine and pyrimidine residues obtains there and which particular base substitution will produce the mutant genotype in question (13). Benzer and Freese (14), therefore, examined also distribution, or *mutational spectrum*, of mutants induced by the action of chemical mutagens, in particular by replacement of thymine by its analog 5-bromouracil in the viral DNA, which replacement, as

Litman and Pardee had found (104), is highly mutagenic in bacterial viruses. Benzer and Freese's investigation, presented in this collection, showed that the set of mutants induced by the action of 5-bromouracil is completely different from the set of spontaneous mutants in the same general region of the viral genome, demonstrating that "the mutagen does not merely enhance the over-all mutation rate, but acts at specific locations in the hereditary structure." Mutational spectra of other chemical mutagens were subsequently established, and it turned out that each of these substances raises the probability of mutation at a restricted number and individually characteristic set of sites (27, 57). Further insight into the chemical nature of the induced mutations was provided by studies that determined the connection between the induction of a mutation at a specific site by a given mutagen and the ability of the same, or of another mutagen, to revert this mutation to the original state. On the basis of these results, Freese proposed that there exist two basic types of point mutation in the viral genetic material: *transversions*, corresponding to the substitution of a purine by a pyrimidine residue, or vice versa, and *transitions*, corresponding to the replacement of one type of pyrimidine by the other or of one type of purine by the other (56).

Not long after the discovery of the bacteriophage it was found that ultraviolet light (UV) kills the virus particle (65), and since then, UV has been the inactivation agent whose effects have been most extensively studied (136, 95, 110, 139). This work has shown that in addition to simply destroying the reproductive power, UV also produces a number of important physiological and genetic effects. The inactivated phages, furthermore, are by no means inert, being still capable of adsorbing to and killing bacteria, and of interfering with the growth of other, unirradiated phages in the same host cell (111). Some of the lethal effects of UV, finally, are reversible under appropriate conditions. An important example of such reversibility is the existence of *photoreactivation*, discovered by Dulbecco (49) in the work presented herein. Dulbecco found that viability is restored to UV-inactivated phages if bacteria infected with such "dead" particles are illuminated with visible light. Dulbecco's quantitative analysis of photoreactivation showed that a fraction of the UV lesions, the photoreactivable sector, is restored by a light-activated enzyme system of the bacterial host cell. Later work by Bowen (23, 24) revealed that photoreactivation consists of two steps: the first step is a dark reaction requiring no light, which generates the substances adsorbing and "activated" by the quanta of visible light for the second, actually reactivating step. Experiments by Lennox, Luria, and Benzer (99) suggested that photoreactivation constitutes a direct reversal rather than a bypass mechanism of the primary ultraviolet damage, a conclusion that now seems certain since Goodgal, Rupert, and Herriot demonstrated the *in vitro* photoreactivation of UV-inactivated transforming DNA by illuminated bacterial extracts (61).

Viability can also be restored to UV-inactivated phages if two or more "dead" particles, each unable to reproduce itself *in solo*, happen to infect

the same bacterial cell. This is the phenomenon of *multiplicity reactivation*, discovered by Luria in 1947 (106) and investigated in some detail by Luria and Dulbecco (112), whose paper is included in this collection. The quantitative results presented here seemed to bear out Luria's proposal that each inactivating UV lesion represents a lethal mutation in one of a certain number of genetic subunits of the phage and that multiplicity reactivation ensues from the genetic exchange of still undamaged units between the two irradiated parent viruses. In order to explain the very high frequency of reactivation, furthermore, it was assumed that phage growth occurs by the independent reproduction of each subunit, followed by reassembly of the units into complete phages. When Dulbecco (50) subsequently continued his multiplicity reactivation studies, he found that the results observed at very high UV doses are no longer compatible with the notion of independently multiplying subunits. In any case, studies on genetic recombination in phage had in the meantime indicated that the genetic material of the phage does not multiply in the form of independent subunits (150). More recently, however, modifications of the original hypothesis of multiplicity reactivation have been proposel by Baricelli (10) and by Harm (62), which still retain that most essential element of Luria's hypothesis that reactivation proceeds by a mechanism of genetic exchange of undamaged parts and which lead to quantitative formulations in satisfactory agreement with the observed data.

Another radiobiological method of inactivation bacterial viruses was discovered by Hershey, Kamen, Kennedy, and Gest (76), who showed that highly P^{32}-labeled bacteriophages lose their infectivity upon decay of radiophosphorus atoms. From the kinetics and efficiency of this inactivation process, it could be inferred that the cause of death is the transmutation of phosphorus into sulfur atoms in the polynucleotide chains of the viral DNA, or the highly energetic nuclear recoil associated with this event. These studies were extended by Stent and Fuerst (141), whose paper appears in this collection. They found that, although the efficiency of killing of one lethal hit per ten P^{32} disintegrations first observed by Hershey and his co-workers also obtains in a variety of different bacteriophage strains, the fraction of disintegrations that are lethal depends on the temperature at which decay is allowed to proceed. A mechanism for the decay inactivation process of the virus was suggested on the basis of these findings. It was proposed that the high proportion of *nonlethal* decays reflects the possibility that the physiological function of the double-stranded DNA molecule is preserved even after radioactive decay has interrupted only *one* of its polynucleotide strands. The *lethal* decays, in contrast, are thought to be those that result by chance in a complete cut of *both* strands of the DNA double helix. The decay of incorporated P^{32} atoms has proven a very useful tool for the study of the structure, physiology, and genetics of bacterial viruses and bacteria. (Review: 142.)

In the hope of measuring the extent to which the infecting parental virus has multiplied within the host cell during the eclipse period before the appear-

ance of any mature progeny, Luria and Latarjet (113) irradiated phage-infected bacteria with UV at various stages of intracellular phage growth. They reasoned that if the UV sensitivity of the vegetative phage is equal to that of the free, extracellular virus, then the result of this irradiation experiment ought to be a family of multiple-hit survival curves from which the instantaneous number of vegetative phages present at the time of irradiation could be inferred. The outcome of Luria and Latarjet's experiment was contrary to their expectation, however; instead of the anticipated multiple-hit survival curves, a family of straight lines of ever decreasing slope was observed, indicative of the fact that the intrinsic UV sensitivity of the vegetative phage is much less than that of the free virus. This is also evident from Benzer's (11) improved experimental design of the Luria-Latarjet technique, presented here as Benzer's third paper. The meaning of the great reduction in UV sensitivity of the vegetative phage has not yet found an entirely satisfactory explanation. On the one hand, as is evident from Benzer's report, some phages do not manifest this effect, so that UV irradiation of bacteria infected with such phages actually gives rise to the family of multiple-hit curves anticipated by Luria and Latarjet. On the other hand, the vegetative phage is also much more resistant to inactivation by decay of incorporated P^{32} atoms than the extracellular P^{32}-labeled virus (138). It seems likely, however, that the reduction in radiosensitivity of the vegetative phage reflects some important aspect of the function and replication of the viral DNA, and some of the possible interpretations have been discussed in several reviews (94, 136, 139, 142). In any case, the method of Luria and Latarjet has found a number of valuable applications in the study of intracellular virus growth, not only with bacteriophages but also with plant and animal viruses (134, 135, 51, 128).

Within a few years of the discovery of the bacteriophage, *lysogenic* bacterial strains were found which appear to "carry" bacteriophages, in the sense that phage particles are always present in the culture fluid of such strains (21, 60). It was soon realized that this association of phage and bacteria cannot be of a casual nature, since it is impossible to permanently free lysogenic strains from the phage they carry by methods which ought to kill or remove the virus particles, such as heating, anti-phage serum neutralization, or single-colony purification (8, 18, 121). The nature and significance of lysogeny then remained a subject of intense controversy for about 30 years, some workers denying the existence of "true" lysogeny and others claiming that lysogeny disproves the whole notion that bacteriophagy involves an infection of bacteria by virus particles. Nevertheless, a few bacteriologists, such as Burnet and McKie (35), and the elder Wollman (156), already envisaged that lysogeny represents an *innate* capacity of bacterial cells for phage production. In order to establish firmly some of the basic but controversial facts of lysogeny, Lwoff began a study of this phenomenon after the Second World War and in 1950 published the paper (116) presented here. In this work Lwoff and Gutmann demonstrate unequivocally that each bacterium of a lyso-

genic strain harbors and maintains a noninfective structure, the probacteriophage or prophage, which endows the cell with the ability to give rise to infective phage without further intervention of exogenous virus particles. The actual synthesis of infective phage, however, proceeds in only a small fraction of the cells of a growing culture of lysogenic bacteria, whose intracellular content of virus particles is liberated by lysis of the phage-producing individual. Lwoff also inferred from these experiments that the *induction* of phage development in a lysogenic cell is under control of external factors, and his subsequent investigations in collaboration with Siminovitch and Kjelgaard (118) showed that treatment with various agents, in particular irradiation with UV light, will indeed induce phage production and ultimately lysis of almost every cell of a culture of lysogenic bacteria. The first preliminary report of this finding (117) is included in this collection. After the publication of Lwoff's papers and reviews (114), the study of lysogeny not only flowered into a distinct branch of bacterial virus research but also became the bridge leading from the genetics of virus to that of host cell. In fact, the recognition of the existence and nature of the *provirus* state engendered entirely new ideas concerning the origin, evolution, and biological function of viruses (79). (Reviews: 114, 17, 85.)

What is the relationship of the prophage to the remainder of the lysogenic cell? The great stability of the lysogenic character implies that the prophage is transmitted to daughter cells at each bacterial division. This could happen in one of two ways: either the prophage represents numerous *autonomous* structures, replicating in the bacterial cytoplasm in synchrony with the rest of the bacterium and being partitioned at random at each division over the daughter cytoplasms, or the prophage is *integrated* into the nuclear apparatus of the host cell and participates in the specific replication and segregation process which assures, *nolens volens*, that each daughter cell obtains one complete set of parental hereditary factors. The first of these alternatives soon appeared unlikely, when indirect estimates of the number of prophages revealed that each cell seems to carry only one or two prophages per bacterial nucleus (16, 81), and that a given type of prophage appears to saturate a limited number of sites on some bacterial structure (15). Positive indications that the prophage is integrated into the bacterial nucleus became available from experiments in bacterial conjugation, in which non-lysogenic bacteria were crossed with lysogenic bacteria, and a linkage of the lysogeny character with other known genetic factors of the cell inferred from the segregation pattern of the recombinants (96, 158, 7, 55). After the discovery of high-frequency-recombination (Hfr) bacterial strains and of the *oriented* transfer of the bacterial chromosome from donor to recipient cell, Wollman and Jacob (159) could show very clearly in their first paper of this collection that at least one particular prophage has its specific location on the bacterial chromosome; their later work, furthermore, revealed that different prophages have different specific chromosomal sites (83, 84). At the same time, Jacob and Wollman

(82) also discovered the existence of *zygotic induction,* described here in their second paper. For if a chromosome fragment of a donor bacterium bearing a prophage enters a non-lysogenic recipient cell, then the prophage becomes induced, enters the vegetative state, leading to the production of infective progeny and lysis and loss of the bacterial zygote. This phenomenon accounted for the discrepancies that had been observed in earlier attempts to determine the chromosomal location of the prophage by bacterial conjugation experiments, from which different linkage relations could be inferred, depending upon which of the two parents of the cross carried the prophage (160).

A completely independent confirmation of the specific location of the prophage on the bacterial chromosome was provided through transduction experiments by Jacob (78), whose paper is presented here. Transducing virus particles carry a small genetic segment of closely linked loci of a donor bacterium, i.e. the last host cell, into a recipient bacterium, i.e. the next host cell. In this way, recombinant bacteria can arise which have derived a very limited part of their genome from the donor cell (164, 163, 147, 98). Jacob thus showed that a transducing virus can carry the prophage of an entirely unrelated virus strain from a lysogenic donor into a non-lysogenic recipient bacterium, usually in association with the contiguous region of the donor chromosome. The chromosomal region, moreover, turned out to be the same in which the prophage had been already placed by bacterial conjugation studies.

The presence of the prophage not only endows the bacterium with the capacity to produce phage but also confers upon the cell an *immunity* to infection by a homologous phage (8, 18, 121, 157). Such virus particles are usually adsorbed to immune lysogenic bacteria, but the particles neither multiply to give rise to infective progeny nor affect growth and division of the immune cells in any way (16, 15, 81). The immune character is highly specific, in that a bacterium lysogenic for, and hence immune to, infection by one type of phage is not immune to infection by other viruses whose prophage the cell does not happen to carry. It became possible to study the genetic basis of immunity when Wollman and Jacob (84) discovered a number of related phage strains that undergo genetic recombination with one another but that differ in their immune specificity as well as in the locations of their prophages on the host linkage map. The final paper of our collection presents the work of Kaiser and Jacob (87) which established by means of crosses of these related phage strains that there is a definite segment of the viral genome, the C region, which determines the immune specificity of the phage. This same segment also controls the ability of the virus to establish itself as prophage and the locus at which the prophage is situated on the bacterial chromosome. Later experiments by Jacob and Campbell (80) have shown that immunity derives from the presence of a *repressor substance* in the cytoplasm of the lysogenic cell. The specificity of both formation and action of this repressor appears to be determined by the C region of the phage genome.