HISTORY OF PHAGE RESEARCH AND PHAGE THERAPY

William C. Summers

Bacteriophages are widely distributed and exhibit dramatic manifestations both in liquid cultures and on solid media, so it is surprising that they were not recognized for almost 40 years after the beginning of bacteriology in the 1880s. Looking back, however, there are several reports in the bacteriological literature that suggest the presence of bacteriophages, but these papers did not present fruitful avenues for further research. For example, Hankin reported in 1896 that the waters of the Jumna and Ganges Rivers in India could kill many kinds of bacteria, especially the cholera vibrio (34). He showed that this antiseptic activity was filterable and sensitive to heating to a boiling temperature and concluded that the bacteriocidal property was caused by a volatile chemical substance. In 1901, Emmerich and Löw described a substance in autolyzed cultures that caused the lysis of diverse cultures, could cure experimental infections, and provided prophylactic immunity to subsequent inoculations (29). There is also a substantial amount of literature on bacterial autolysis, reviewed by Otto and Munter in 1923 (60), that includes work by Gamaleya, Malfitano, Kruse, and Pansini. While some of these observations suggest the action of bacteriophages, others are compatible with bacteriocin effects, and some may be attributed to lytic enzyme production. These early experiments were limited to observations of liquid cultures because in this early period of bacteriology, a culture was conceptualized as an organism in itself rather than in terms of the population dynamics of individual cells. This did not change until the 1920s, when a significant reconceptualization focused on the bacterial cell as the organism rather than on entire cultures (80). The first clear and dramatic experiments on bacteriophages used bacterial cultures spread on solid medium and were based on the observation of localized bacteriolysis, i.e., plaques.

A paper written by Frederick W. Twort and published in 1915 is usually considered the beginning of modern phage research. In this work, he reported the rather odd phenomenon of "glassy transformation," and it was 8 years later, after the pioneering work by Félix d'Herelle, that Twort's report was recognized as dealing

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with bacteriophages. Twort, a student of the famous British bacteriologist William Bulloch, worked at the Brown Institution, a London veterinary hospital (86). He was attempting to grow vaccinia virus on agar media in the absence of living cells when he noted that many colonies of contaminating micrococci grew up and that some of these colonies appeared mucoid, watery, or glassy. This glassy transformation could be induced in other colonies by inoculation of the fresh colony with material from the watery colony. Twort found that this transformation could be propagated indefinitely, and when he examined the glassy colonies under a microscope, he noted that the bacteria had degenerated into small granules that stained red with Giemsa stain. Twort's interpretation of glassy transformation was tentative and less than clear, but he concluded that ". . . it [the agent of glassy transformation] might almost be considered as an acute infectious disease of micrococci" (87).

For different reasons, and certainly independently, in 1917 Félix d'Herelle described a "microbe" that was antagonistic to bacteria, lysed bacteria in liquid cultures, and killed bacteria in discrete patches, which he termed plaques, on the surface of agar spread with a film of bacteria (16). d'Herelle thought of these invisible microbes as "ultraviruses" that invaded bacteria and multiplied at their expense, and so he called them bacteriophages. Working at the Pasteur Institute in Paris under wartime conditions, d'Herelle investigated an outbreak of bacillary dysentery in a group of French soldiers (84). He used this opportunity to pursue his interest in the question of why enteric bacteria were sometimes pathogenic and sometimes not. He based his approach on earlier studies of the etiology of hog cholera, which was thought to be caused by a filterable virus but exacerbated by the presence of a bacterium (*Salmonella enterica* serovar Choleraesuis) (83). d'Herelle filtered the cultures of dysentery samples in the hope that some filterable viruses might be found that could alter the growth and pathogenicity of bacteria from dysentery patients. To his surprise, he observed lysis of the bacteria in liquid cultures and the formation of clear spots (which he later called *taches vierges*, or virgin spots) in the confluent bacterial growth that covered his agar slants. Inasmuch as he was looking for an invisible microbe, his conception of the bacteriophage as a parasite of bacteria was certainly logical. His investigations were guided by this hypothesis, and he observed that the bacteriophage multiplied indefinitely, that it needed living cells to multiply, and that cell lysis seemed required for the multiplication process. He conceived of the bacteriophage as particulate, i.e., an organized microbe, and he astutely realized that the plaque count provided a way to enumerate these invisible microbes. He was able to show by the use of liquid cultures that the phage multiplied in steps, which he interpreted as demonstrating cycles of infection, multiplication, release, and reinfection.

d'Herelle's research took two important directions, focusing on (i) the therapeutic use of the phage in infectious diseases and (ii) the biological nature of the bacteriophage itself. From his first studies of clinical samples from dysentery patients, he observed that phage titers rose just as recovery was taking place. He reasoned that phages might represent natural agents of resistance to infectious diseases, and from this hypothesis he went on to advocate phages as therapeutic agents in the preantibiotic era.

d'Herelle believed that phages were responsible for much of the recovery from infectious diseases. Because he observed increasing titers of phage during the course of recovery from dysentery and typhoid, he concluded that the gradual adaptation of lytic phages to specific pathogens and their subsequent multiplication and lysis of the pathogen were the mechanism of recovery. He termed phages "exogenous agents of immunity." This ecological concept of phages and disease supported his effort to employ phages as therapeutic and prophylactic agents for a wide variety of infectious diseases.

d'Herelle's belief that phages are filterable viruses of bacteria was not widely accepted during the first two decades of phage research, however. Until the early 1940s, the leading authorities in microbiology thought that the lytic

phenomena associated with bacteriophages resulted from an autocatalytic activation of induced endogenous lytic enzymes. d'Herelle's second research program examined the biological nature of bacteriophages, partly to respond to his critics and partly to assert his claim of priority for the discovery of phages in a long-running dispute with Twort's supporters. All of his evidence suggested that phages are organized infectious agents that are obligate intracellular parasites. He also discovered that the host range specificities of phages as well as their antigenic properties were characteristic of given "races" of phages. In retrospect, at least, it appeared from the earliest research that phages might be fruitful organisms for genetic study.

d'Herelle's view of phages as agents of immunity was a direct challenge to the prevailing dogma held by many bacteriologists, including Jules Bordet, who had just received a Nobel Prize in 1919 for his work demonstrating complement-mediated bacteriolyis. Bordet and his colleagues in Belgium immediately started to work on phages and their possible role in Bordet's bacteriolytic phenomenon. Bordet's experimental system was quite different from d'Herelle's bacteriologic approach. He searched for phages in peritoneal extracts of immunized mice and tested these extracts on bacteria. Typically, Bordet injected bacteria (and sometimes phage) into the host animal, and he emphasized the role of the host-bacterium interaction in producing phage. He isolated bacteria from infected animals, and these bacteria often retained the property of giving rise to phage upon laboratory culture or upon treatment with peritoneal extracts. He termed such bacteria lysogenic. Bordet believed that the phages were produced from the bacterium itself upon induction by some perturbation in the metabolic state of the bacterium (such as an antibody treatment). He also favored the concept of phages as lytic enzymes rather than particulate ultramicrobes.

Bordet was a Nobel Prize winner and the director of the Pasteur Institute in Brussels, while d'Herelle, on the other hand, was an obscure, unpaid volunteer researcher at the Pasteur Institute in Paris, a situation that strongly influenced the general perception by the wider scientific community of the nature of phages. Bordet's attack on d'Herelle's views about phages did not rest on scientific evidence alone. It also involved a priority dispute (24, 84, 88). In 1923, Twort's paper on glassy transformation was discovered by Bordet, and he immediately challenged d'Herelle's priority in the discovery of bacteriophages. Twort, probably because of his wartime responsibilities, did not pursue his work on glassy transformation, and had it not been for Bordet's use of his 1915 paper to challenge d'Herelle, Twort's work might have been completely ignored. d'Herelle responded to Bordet by asserting that Twort's phenomenon of glassy transformation was fundamentally different from the bacteriophage phenomenon. He performed a series of experiments to characterize the biological nature of phages, which he might not have undertaken without this challenge by Bordet. This controversy raged on for about 10 years, with Twort occasionally participating as the reluctant surrogate for Bordet in what became well known as the "Twort-d'Herelle controversy." In retrospect, the arguments were often petty and involved insignificant differences in the heat and pH resistance between d'Herelle's phages and Twort's agent of glassy transformation. The dénouement of this controversy finally came in 1932 with a scientific duel of sorts: d'Herelle and André Gratia, a protegé of Bordet, agreed to an independent comparison of Twort's material and d'Herelle's material conducted by highly respected independent scientists representing the two opposing camps. Paul-Christian Flu, director of the Institute of Tropical Medicine at Leiden (representing d'Herelle), and E. Renaux, professor of microbiology at Liege (representing Gratia), conducted the comparisons and finally concluded that the Twort and d'Herelle phenomena were identical (31).

The study of the biological nature of phages was, however, overshadowed by the therapeutic possibilities of phages for treating infectious diseases. Basic research on phages gave way to research on their commercial and medical possibilities. When d'Herelle first observed that phage

titers increased in stool samples from dysentery patients, the possible use of bacteriophages for treating infectious diseases became apparent. The first reported therapeutic use of a phage was the treatment of cutaneous boils (furuncles) by Bruynoghe and Masin from Louvain, who injected a staphylococcal phage preparation into the local region of the infection (4). They reported a reduction in swelling and pain as well as some reduction in fever.

An outbreak of avian typhosis in France during the summer of 1919 provided d'Herelle with the opportunity to perform extensive tests of the use of phages as a prophylaxis against natural infections of chickens by "*Bacillus gallinarum*." He described these studies in his first monograph on phages, *Le Bactériophage: Son Rôle dans l'Immunité*, published in 1921 (17). Even by current standards, this early research on the use of phages to control an epidemic of avian typhosis appears reasonable. Some pens of chickens were treated with phage prior to deliberate inoculation with "*B. gallinarum*," while others were left untreated; several groups of chickens, some phage treated and some not, were exposed under natural conditions to infected animals. Phages were administered by the oral route in order to obviate the possibility that bacterial debris in the phage lysate might act as an immunogen. As d'Herelle reported, phage treatment offered a high degree of protection. Moving from the laboratory approach to actual field trials in rural areas of France that were undergoing epidemics, d'Herelle inoculated (either by the oral route or by injection) many flocks in several widely separated regions. The overall results suggested that phage-treated flocks suffered many fewer deaths and had shorter epidemics and that recurrent rounds of the infection were prevented. These results were confirmed in Holland by a Dutch investigator. The absence of a double-blind design was probably the main deficiency in these early phage experiments; however, note that this level of rigor was very uncommon at the time. d'Herelle was a meticulous experimentalist whose studies were invariably conducted according to the best scientific standards of his day.

d'Herelle also evaluated phage therapy against bovine hemorrhagic septicemia (barbone) in field trials in Indochina. For this disease, too, it appeared that the parenteral inoculation of phage specific for the causative bacterium protected water buffaloes against subsequent experimental inoculation with what is now called *Pasteurella multocida*, which usually causes a highly fatal infection (20).

Having demonstrated the therapeutic effectiveness of phages for both gastrointestinal disease (avian typhosis) and septicemic disease (barbone) in animals under natural conditions, d'Herelle extended his investigations to human infectious diseases. The conduct of human trials in the 1920s, from both scientific and ethical viewpoints, seems crude and inadequate by current standards, but d'Herelle's approach was typical. He tested the safety of his phage preparations by self-administration; he reported both the ingestion and subcutaneous injection of phage lysates tested on himself as well as his family members (21). He also injected his coworkers with phage. This testing was considered sufficient to evaluate the safety of this material: "After being assured that no harmful effects attended the ingestion of the Shiga-bacteriophage, this treatment was applied for therapeutic purposes to patients afflicted with [culture-confirmed] bacillary dysentery" (22).

The work that attracted the most attention for phage therapy was d'Herelle's report of the successful treatment of four cases of bubonic plague with an antiplague phage. d'Herelle was stationed at the League of Nations Quarantine Station in Alexandria, Egypt, and he observed four patients on a ship passing through the Suez canal, all of whom had laboratory-diagnosed bubonic plague. d'Herelle treated all four of these patients by the direct injection of antiplague phage into buboes (the infected inguinal and axillary lymph nodes). In what was considered a remarkable result, all four patients recovered rapidly. This result was soon reported in the widely read French medical periodical *La Presse Médicale* (18). Because of this report, d'Herelle was invited by the India Office of the British government to work on phage therapy of plague at the Haffkine Institute in Bombay.

A short visit to India in 1926 resulted in the later establishment of "The Bacteriophage Inquiry" to study the application of phage therapy in India, especially for cholera epidemics, which occurred regularly in association with religious festivals and pilgrimages (23, 82).

Cholera, rather than plague, provided an ideal test case for phage therapy: the infection is initially confined to the gastrointestinal tract, rapid killing of the bacteria reduces the burden of the pathogenic toxin, the mode of transmission and epidemiological characteristics of the disease were well known, and effective vaccines were not available. The initial reports from India in the 1920s and 1930s (23, 82) consistently reported that the severity and duration of cholera symptoms and the overall mortality from the disease were reduced for patients given cholera-specific phage by mouth. Phage therapy for cholera seemed to be established as helpful for the treatment of patients with the disease; phage prophylaxis for cholera, however, was less clear-cut.

Enthusiasm for phage therapy has waxed and waned since its first use in the 1920s. The initial excitement was followed by critical skepticism and later abandonment. There has been, however, renewed interest and reappraisal in the past several years. Changing attitudes toward phage therapy reflect both scientific and cultural influences. Many, but certainly not all, early phage therapy trials appeared be successful, and phage preparations were marketed by major pharmaceutical firms (e.g., Parke-Davis and Lilly in the United States). In the late 1930s, The Council on Pharmacy and Chemistry, established in 1905 by the American Medical Association to set standards for drugs and lead the battle against quack remedies, undertook an evaluation of phage therapy. The council (25) concluded with an ambiguous assessment of the literature on phage therapy, acknowledging that there were both positive and negative reports in the literature. The poor understanding of the biological nature of phages was a major concern expressed in the council report. Furthermore, the report noted that the lack of standards for the purity and potency of phages made it impossible to compare most of the published studies.

Unfortunately, World War II and the discovery of antibiotics effectively diverted efforts away from further study and development of phage therapy in the United States. In Europe, however, phage therapy was continued in a distinctly military context; the Soviet Union's war against the Finns produced many battle casualties, and phage therapy was extensively used to treat these war-wounded individuals. The German military also promoted phage therapy: standard German war medic kits captured in North Africa from Rommel's forces contained vials of phages ready for injection or oral administration (W. C. Summers, unpublished observations).

As a consequence of the widespread success and availability of antibiotics, phage therapy trials in the United States and most of Western Europe ceased after World War II, but they continued in the Soviet Union and some other Eastern European countries. The institute founded by Georgyi Eliava and d'Herelle in Tbilisi was one of the main centers for such work (64).

In the late 1960s, the World Health Organization set up an international trial of phage therapy for cholera in Dacca, Pakistan. It employed widely accepted international standards and was conducted with the support and review of the National Institutes of Health. The use of high doses of anticholera phage (calculated to give a multiplicity of infection [MOI] of 100 to 200 phage per vibrio) tested the idea that phages might be able to kill bacteria in vivo even if they were not able to complete many cycles of replication and amplification (55). Bacteriophage therapy for patients in hospitals was compared to tetracycline treatment and to fluid replacement alone as a control. The significant finding of this study was that very-high-dose phage therapy was comparable to tetracycline treatment in terms of reducing the excretion of vibrios in the stool; this reduction, however, did not lead to overall clinical improvement, that is, a shorter duration of diarrhea or a more rapid recovery. After this initial study, a larger study (53) was undertaken with randomization of patients, placebo controls, and comparisons of oral phage, orally and intramuscularly injected

phage, and tetracycline. This more adequately designed study, however, was conducted with much lower phage doses (MOI of about 0.05 to 0.1 phage per vibrio). No significant effects of phage treatment were discerned in this low-MOI study. Problems which complicated this study of phage therapy for cholera were the diversity of serotypes of vibrios, the varying susceptibilities of these bacteria to phage, and the rapid transit of ingested phage through the gastrointestinal tracts of cholera patients, a fact which may have precluded second rounds of phage infection, which are essential for low-MOI therapy.

Williams Smith and his colleagues in the United Kingdom performed a well-publicized series of studies on phage treatment for *Escherichia coli* diarrhea in calves in the 1980s (94–97). They focused on a known pathogen of calves, *E. coli* O18:K1:H7 ColV$^+$, and cleverly exploited phage receptor biology by selecting phages that require the presence of the K1 antigen for infection. If rapid in vivo mutation to phage resistance was a major cause of failure of phage therapy and if the mechanism of such phage resistance was a loss of phage receptors, then even if the pathogenic bacteria mutated to have resistance to the anti-K1 phages, the loss of the K1 antigen would greatly reduce bacterial virulence. Single-dose phage treatments were more effective than multiple doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim plus sulfafurazole. As expected, phage-resistant bacteria which were isolated in vivo were found to lack the K1 antigen.

Recent phage therapy research on model systems with adequate experimental designs have been encouraging. Barrow et al. (1) confirmed and extended the results of Williams Smith by using anti-K1 phage therapy for experimental *E. coli* infections of both calves and chickens. Soothill (77) used a mouse model, and Park et al. (61) and Nakai et al. (59) explored the use of phages to treat or control specific fish pathogens and concluded that phages were effective. Conversely, Greer and Dilts (32) employed phage to reduce the bacterial contamination of beef and found that while viable bacterial counts were significantly reduced, the overall rates of meat spoilage were not. They noted, however, that phage-resistant organisms were a significant cause of spoilage, suggesting that this study probably should not be overinterpreted. A phage therapy trial for rabbit diarrhea induced by enteropathogenic *E. coli* O103 showed a long-term persistence of phage in the spleen, but no significant effect on the prevention of disease (65).

From both a practical and a theoretical point of view, the use of phages to control fish diseases and other infections in aqueous environments seems particularly promising. The ecology of these bacteria and their phages is similar to laboratory culture conditions, and the host organisms, i.e., fish, mollusks, or crustaceans, live in aqueous media in which the therapeutic phage can have continuous and intimate physiological access to the pathogens.

Unfortunately, the recent literature on clinical phage therapy is almost entirely anecdotal or describes studies with historical controls. Several large series of clinical trials of bacteriophage for suppurative bacterial infections (8, 38, 57, 70–76, 92, 93) have come from Slopek's group in Wroclaw at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences. Other positive reports have come from Romania (54, 101, 102), France (33, 39, 44, 89), the former country of Czechoslovakia (63), Great Britain (9, 69), and North America (5, 65, 98).

In the first decade of phage research, the biological nature of phages was a neglected topic, but in the 1930s a very few investigators started to examine the chemical composition of bacteriophages in work that was linked to the study of other filterable viruses such as tobacco mosaic virus (TMV) and poliovirus (10). New techniques such as ultracentrifugation, filtration through collodion membranes, and chemical analysis were brought to bear on phages. Both Elford and Andrews in England and d'Herelle and his colleagues in France found that different isolates of phages differed markedly in size (26, 68). These determinations, however, were indirect and without any accepted standardiza-

tion. Until Wendell Stanley was able to obtain poliovirus and TMV in a crystalline form, there had been no criteria for virus purity, and reliable chemical and physical studies of viruses were impossible (10).

The first chemical analyses of these newly purified viruses showed that they were composed of protein, but very soon the presence of phosphorus in these virus preparations suggested a second component, which was subsequently recognized as a nucleic acid. The chemical study of phages was undertaken by a lone scientist, Martin Schlesinger, who worked first in Germany and later in England. In his partially purified phage preparations, he showed the presence of DNA by means of the Feulgen reaction (67). It was the visualization of phage with the newly invented electron microscope that finally settled the smoldering issue of the nature of bacteriophages in 1940 by clearly showing their particulate character and by showing that specific phages have characteristic morphologies (45, 49, 62, 66).

The study of the biology of phage multiplication, viewed as a biological problem, was undertaken in the 1930s and 1940s by three tiny groups of scientists: Frank Macfarlane Burnet in Australia, Eugène and Elizabeth Wollman in Paris, and what came to be called the American Phage Group (6). The acknowledged organizer of this last group was the German émigré physicist Max Delbrück (81). Delbrück was introduced to phages through his meeting with Emory L. Ellis, a postdoctoral researcher at Caltech who was working with phages because he thought their study would contribute to the understanding of the role of viruses in cancer. By 1938, Ellis had developed a research program on the fundamental biology of bacteriophages. His approach to tumor viruses reflected his training as a chemist. Ellis decided that an understanding of the fundamental biology of viruses was a necessary prerequisite to the study of viral carcinogenesis, and so he investigated the process of infection of bacteria by bacteriophages, and in this work he repeated the basic experiments that supported the viral nature of bacteriophages (81). Ellis clearly accepted d'Herelle's view when he was able to replicate d'Herelle's basic stepwise pattern of phage growth.

Although Delbrück is often credited with inventing the so-called one-step growth experiment, as Ellis noted, this result was obtained first by d'Herelle and was crucial evidence for his concept of phage multiplication (19). Ellis recalled: "My first work was to develop the plaque count technique and show the step-wise growth curves showing that the phage multiplied in the bacterium, not in the solution. These step-growth curves really intrigued Delbrück, and I think were responsible for his wanting to join in the work" (27).

Pasadena sewage supplied the first phages isolated by Ellis by the use of d'Herelle's methods. He selected *E. coli* as the host bacterium because it grew well and was available from Carl Lindegren, who was working on microorganisms as one of T. H. Morgan's students at Caltech. Ellis's work on the basic biology of bacteriophages was not unrecognized: it was the subject of a front-page story in the *Los Angeles Times* of 30 April 1938. At this time, Ellis focused on the basic biology of the phage and its role as a model virus. It is clear from the historical record that Delbrück had not yet started his work on phages at the time that Ellis had already established phage work at Caltech (30).

Delbrück had been educated as a theoretical physicist in Germany. He worked on atomic physics with Niels Bohr in Copenhagen and adopted the philosophical outlook of the Copenhagen physicists. In 1932, he entered into a discussion group to consider the nature of the gene and how it might be understood at the physical level. This group included the Russian geneticist Nicolai V. Timofeev-Ressovsky, the German biophysicist Karl G. Zimmer, and the American geneticist Herman J. Muller, who spent 1932 in Berlin working with Timofeev-Ressovsky. Muller believed that understanding the nature of the gene was the critical problem in biology (37). The group discussed how physics might be applied to biology to understand the nature of the gene, and in 1935 Timofeev-Ressovsky, Zimmer, and Delbrück published a famous paper on the nature of gene structure

and mutation (85) aimed at, in the words of Gunther Stent, "a quantum mechanical model of the gene" (79). On the basis of this work, T. H. Morgan, the head of the Biology Division at Caltech, invited Delbrück to visit Caltech as a Rockefeller Foundation fellow. Morgan envisioned Delbrück bringing a new level of theoretical sophistication to his program in *Drosophila* genetics. When he arrived at Caltech, however, Delbrück said that he was discouraged by the details and intricacies of the actual experimental genetics of *Drosophila*, which he found distinctly not to his taste (14).

Even before he left Germany for the United States in 1937, however, Delbrück had considered viruses to be appropriate organisms for the study of gene duplication. Before leaving Europe for America in 1937, he wrote some preliminary notes (later appended to his Nobel lecture in 1970) for a talk entitled "The Riddle of Life." He made the point that "we want to look upon the replication of viruses as a particular form of a primitive replication of genes" (13). Others, including Muller, had been interested in bacteriophages for a long time, and as early as 1922 he had suggested that bacteriophages (d'Herelle bodies) were naked genes (58):

> if these d'Herelle bodies were really genes, fundamentally like our chromosome genes, they would give us an utterly new angle from which to attack the gene problem. . . . It would be very rash to call these bodies genes, and yet at present we must confess that there is no distinction known between the genes and them. Hence we cannot categorically deny that perhaps we may be able to grind genes in a mortar and cook them in a beaker after all. Must we geneticists become bacteriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists? Let us hope so.

Delbrück, thinking that a virus, rather than *Drosophila*, might be the "right organism for the job," on his way from Berlin to Pasadena arranged to visit Wendell Stanley at the Rockefeller Institute laboratories in Princeton. He was disappointed to find that TMV infection was too complicated for the simple analyses he envisioned (36). Stanley's colleague at the Princeton Rockefeller laboratories was John Northrop, who was working on bacteriophages, although he was the champion of the autocatalytic theory of phage growth.

At Caltech, Delbrück found a kindred spirit in Emory Ellis. Ellis was a physical chemist who had studied thermodynamics, not genetics or microbiology. He appreciated the formal similarities relating phages, Rous sarcoma virus, and TMV and approached them as "black boxes." Ellis, like Delbrück, had considered other viruses, especially TMV, before turning to phages.

Ellis and Delbrück collaborated for about a year and then published their only joint paper (28). This paper included the "step-curves" that Ellis had developed prior to his collaboration with Delbrück. Delbrück provided a statistical analysis which showed that the infectivity in liquid culture (d'Herelle's terminal dilution experiment) was related to the plaque count on solid media. This analysis was important because some workers used the ability to lyse a culture (or even the time it took to do so) as a measure of phage concentration, while others used the plaque assay. In his fellowship report to the Rockefeller Foundation, Delbrück explained: "The leading idea was the belief that the growth of phage was essentially the same process as the growth of viruses and the reproduction of the gene. Phage was chosen because it seemed to offer the best promise for a deeper understanding of this process through a quantitative experimental approach" (12).

Delbrück focused on the gene, while Ellis wanted to understand the biology of the viral life cycle as a whole. They both recognized that their work on phages was clearly aimed toward Delbrück's goal of understanding the nature and replication of the gene. Delbrück's fellowship report noted that "during the second year Dr. Ellis returned to work on the tumor problem [required by the conditions of his fellowship support] and I carried on alone with the phage-work" (12).

In their famous paper on the nature of the gene, Timofeev-Ressovsky, Zimmer, and Delbrück used an experimental approach known as target theory that was based on methodology derived from atomic physics (40). From the parameters of dose-response curves from radia-

tion inactivation experiments, the target theory provided estimates of the sizes of targets such as genes and molecules within cells. Before the introduction of radioisotopes and modern biochemical methods, radiation biology was a widely used tool.

Physicists interested in biology were drawn to the target theory approach because it was familiar to them from earlier work on atomic structure. Salvador Luria was one such scientist. He was an Italian physician trained in radiology who worked on phages with Geo Rita, an early phage researcher in Rome (48). In 1938, Luria moved to the Radium Institute in Paris, and there he worked on the radiation biology of phages with Fernand Holweck, a well-known French physicist. They used target theory approaches to estimate the size of phages (40). Luria came to the United States in 1940 and at first worked at Columbia University with radiation biologists, but he soon made contact with Delbrück. Together they initiated a long-term relationship that would produce an entire generation of phage researchers in America.

At the same time that Ellis and Delbrück were studying phages in Pasadena and that Luria was working in Paris, Alfred Hershey was studying phage physiology in St. Louis, Mo., at Washington University (78). He was collaborating with Jacques Bronfenbrenner, who had a long interest in the possible metabolic and structural organization of bacteriophages.

Delbrück was a natural organizer, and he began to recruit people to work on phage biology. Together with Luria and Hershey, he developed a group of protégés and followers who were indoctrinated mainly with Delbrück's ideas about the important problems of phage research and the legitimate ways to approach them (6). Delbrück believed that by focusing research on a small group of phages, results from different laboratories could be compared. He selected a group of "authorized phages" which were designated the T phages, i.e., T1 to T7 (T for type), and urged phage workers to restrict their studies to this collection of isolates (15).

The most significant of Delbrück's organizational efforts was the establishment of an annual phage course which, starting in the summer of 1945, took place (usually) at the Cold Spring Harbor Laboratories on Long Island, N.Y. While this course was designed to provide laboratory instruction in the techniques of phage research, most importantly it served to recruit and indoctrinate a cadre of students into Delbrück's research program on the "problem of the gene" (92). Both graduate students and senior scholars from major American universities attended the phage course, and after an appropriate education about the mysteries of phages, they returned to their universities as advocates for the "Delbrück school" of phage research. Scientists who had taken the phage course at Cold Spring Harbor formed a loose group of acolytes who kept in close communication through a quasi-periodic newsletter, the "Phage Information Service," distributed by Delbrück. This bulletin provided an alternative to the formal publication of results as well as enforced a sort of orthodoxy of approach that fit Delbrück's ideas of what was acceptable phage research. The annual phage course and the phage meetings often associated with the course became a powerful social force in the early development of molecular biology (6).

While Delbrück's T phages were selected because they caused a virulent lytic response and clear plaques on *E. coli*, phages that exhibited Bordet's lysogenic phenomena had been observed almost from the first days of phage research. The relationship between the phage and host in this lysogenic interaction was unclear, however. In the 1930s, the Wollmans suggested that phages in the lysogenic state seemed to behave as part of the cellular hereditary apparatus (99). In 1950, André Lwoff and Antoinette Gutmann, working in Paris, finally clarified the nature of lysogeny and designated the latent form of the phage by the term "prophage." They monitored phage induction and release from single cells by direct microscopic observations and sampling with a micromanipulator (52). With this clarification, the control of lysogeny became a major focus of phage research in Paris in the 1950s. François Jacob wrote his thesis on lysogeny in *Pseudomonas pyocyanea*, and the study

of lysogeny contributed to the framework for the operon concept of gene regulation proposed in the early 1960s by Jacob and Jacques Monod (56).

In 1951, Esther Lederberg discovered bacteriophage lambda, a lysogenic isolate from a particular strain of *E. coli*. Just as the T phages were the model organisms for lytic phage research, bacteriophage lambda soon became the prototypic lysogenic phage (41). Extensive studies of lambda phage have provided a deep understanding of the physiology of gene expression as well as illuminating the mechanisms of lysogeny.

By the 1960s, it was recognized that phages were but one of several kinds of extrachromosomal genetic structures found in bacteria. The fertility factor F, transmissible drug resistance determinants, and prophages were all examples of what Joshua Lederberg termed "plasmids" (42). The precise structure, both physically and genetically, of these plasmids was not clear: some experiments suggested that the plasmids (the F factor in Hfr strains and some prophages) were attached to the cellular chromosome, while other experiments suggested that they were independent of the host genetic apparatus. In 1962, Alan Campbell proposed a very fruitful model for this reversible association between plasmids and host chromosomes (7). Because the genetic map of phage T4 was circular while the phage DNA appeared to be linear, Campbell reasoned that the intracellular phage might assume a circular form that could, with a single reciprocal recombination event, become linearly integrated into the chromosomal DNA. Later research showed that T4 has a circular genetic map for reasons other than forming a physically circular molecule, but Campbell's model was soon confirmed for phage lambda (which does exist in a circular form) and eventually for many other plasmids. This model of phage lysogeny has provided the conceptual basis for retrovirus integration and excision in animal cells as well.

In the 1930s and 1940s, the stability of the gene was recognized as one of its remarkable features. This stability, together with the random nature and low frequency of genetic change, suggested that mutation might be similar to or governed by a quantized, two-state process. This model appealed to physicists such as Delbrück, Erwin Schroedinger, and Bohr, who thought that a deeper understanding of this paradoxical behavior might reveal new physical laws of nature (30). While no new laws of nature seemed to emerge, deeper insights were indeed provided by strictly formal genetic analyses of phage mutations.

While some elegant studies on bacterial mutations in the 1930s by I. M. Lewis did not change many minds, in the 1940s two related experimental approaches gave results that have been considered landmarks in the study of genetic mutation. Both of these employed bacteriophages as experimental tools. From the work of d'Herelle, Delbrück and Luria knew that bacteria often developed heritable resistance to phage lysis. In addition, their backgrounds in atomic physics and their routine use of statistical models for their target theory studies helped them to devise a statistical approach (the afluctuation test) to show that phage-resistant mutants existed in the bacterial population prior to exposure to the lethal effects of a phage (50). This method was indirect and mathematical, as was a related approach devised in 1949 by Howard B. Newcome. In 1952, however, Joshua Lederberg and Esther Lederberg developed a direct and simple method to show that mutations occurred randomly and independently of the selection procedures. They used a velvet cloth as a transfer tool to "print" a very large number of colonies from one plate to another to create replica plates (43). These replica plates could be used to test colonies in large numbers for mutant properties. They used this technique to study phage resistance as well as streptomycin resistance: it was clear that the mutants had appeared randomly before the application of the selective agent (phage or drug).

The elucidation of the formal genetic behavior of bacteriophages was first done by Hershey and Raquel Rotman, who used plaque morphology mutants (large plaques, interpreted as rapid-lysis mutants, or r mutants) to show

that phage crosses could be obtained by simultaneous mixed infections (35). In 1954, Seymour Benzer discovered an odd property of one class of Hershey's r mutants: they did not create any plaques at all (less than one in a hundred million) on bacterial hosts carrying the lambda prophage (K12 lambda) but created large (r-mutant) plaques on the usual bacterial host (strain B). After nearly a year of work on the physiology of this unusual host range phenomenon, Benzer realized that this case of conditional expression of a phage mutation could be used to perform a fine-structure genetic analysis of the T4rII gene (2). Because of the extremely high specificity of the system (the discrimination against the rII mutants in host K12 lambda is >108), very low frequencies of wild-type r^+ recombinants or revertants could be detected, and hence, recombination between very close mutations, calculated to be at about the level of base pairs, could be detected. A detailed genetic analysis of insertion and deletion mutations in the T4rII gene by Francis Crick, Leslie Barnett, Sydney Brenner, and Richard Watts-Tobin also provided strong evidence for the triplet nature of the genetic code (11).

During the course of the study of phage genetic behavior, several investigators discovered a phenomenon that was called host-induced modification. This puzzling phenomenon was found in studies of phage host range mutations and adaptations. Salvador Luria and Mary Human (for T2 phage) (51) and Giuseppe Bertani and Jean Weigle (for lambda phage) (3) found that the host range, as measured by plating efficiency, was sometimes determined by the specific strain of host bacteria in which the phage had most recently replicated. The host bacteria appeared to modify the phage in some way so as to affect its subsequent ability to infect other bacterial strains. Phages with the incorrect modification were restricted in their growth in certain bacterial strains. This strange phenomenon was regarded as a minor peculiarity of phage biology for nearly two decades, but in the late 1960s and early 1970s, both genetic and biochemical investigations of host-induced modification revealed, of course, that it is caused by certain host-specified modifications, usually methylations or glucosylations of the phage DNA, and by sequence-specific nucleases that cleave DNA at sites which lack the cognate modifications. This arcane facet of phage biology has been exploited to provided a major tool, restriction endonucleases, for both fundamental and technological progress in biology in the decades since its elucidation.

In the 1960s, there was a unification of the two main research traditions in genetics, that is, gene transmission and gene expression. The study of phage replication and gene expression provided a strong impetus for this development when it became clear that a full understanding of the workings of genes could be seen as a unified process, captured, for example, in Crick's metaphor of the so-called central dogma of molecular biology. He described the function of genes, both in heredity and in development, in information-theoretic terms when he stated that information flows from DNA to DNA and from DNA to RNA and thence to proteins. Key experimental support for the role of RNA as an intermediary in such an information transfer was given by the finding that in phage-infected cells, the RNA base composition was much more DNA-like than in uninfected cells (90). Thus, it was concluded that phage gene function required the synthesis of RNA molecules that differed from those present in the uninfected cell and that the RNA base composition, and hence its information content, was determined by the DNA of the phage. The discovery of phages with RNA genomes in 1961 (47) and the demonstration that these RNAs function as expected for the postulated genetic message gave strong confirmation of Crick's unifying notion of the central dogma.

Phage research since the 1960s has been greatly advanced by new biochemical approaches originating from the early work of Seymour Cohen, Lloyd Kozloff, and others (6). The availability of radioactive isotopic techniques, improved methods of protein chemistry, and advances in enzymology contributed to the influx of biochemists and the application of

biochemical methods to phage work. This fruitful collaboration between biochemists, geneticists, and microbiologists led to detailed descriptions of the mechanisms of phage replication and transcription and of phage morphogenesis and assembly and to a detailed understanding of phage adsorption and entry phenomena. These mechanistic studies have been paralleled by recent studies of phage ecology which echo some of the earliest interests of phage biologists. Successful investigations of phages and their interactions with host bacteria in natural settings will require both detailed molecular studies and integrated biological research. The chapters in this volume present the current state of work in both directions.

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