LYSOGENY[1]

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I. FOREWORD

"La seule réalité objective, ce sont les rapports des choses d'ou résulte l'harmonie universelle".—Henri Poincare, *La valeur de la Science.*

Lysogeny occupies a privileged position at the cross roads of normal and pathological heredity, of genes and of viruses. The study of lysogenic bacteria has modified our conceptions concerning the origin, nature, biology and physiology of bacteriophages and posed the problem of cellular immunity. Although our knowledge in the field has increased rapidly, the subject has never been discussed in its whole since the discovery of lysogeny in 1925.

This review aims at giving an idea of: the historical development of knowledge concerning lysogeny; the actual state of the question; the essential data and theoretical conceptions; the orientation of present research; and directing attention to possible analogies between the viral diseases of bacteria on the one hand, and of animals and plants on the other. Although intended essentially for students just beginning their research, I hope that the more mature reader will not be disturbed by this treatment. It is assumed that the reader of this review already has a good knowledge of bacteriophage and its mode of action. Although not exhaustive, this review is sufficiently extensive that a few guide-posts to its organization may be helpful. Each chapter of this review deals with one of the multiple aspects of lysogeny. Owing to the complex interrelations of the phenomena involved, we begin with a somewhat general, although necessarily dogmatic presentation, of the subject. This introductory material together with an accompanying glossary should facilitate the comprehension of the various chapters including the specific terminology of lysogeny (See appendix I).

For many young scientists the future is more important than the past and the history of science begins tomorrow. Nowadays, in the field of lysogeny, many facts and theoretical views are gloriously discovered which were known a long time ago. It has seemed, therefore, desirable to credit early workers for their achievements and also to spare unnecessary efforts directed to later rediscoveries. Moreover, it is interesting to know how phenomena were discovered, how the problems were born, attacked and solved, and how and why our ideas have evolved. The danger of parachuting young enthusiastic scientists into a flower bed of selected data and fully bloomed conceptions should not be underestimated. For these reasons, one section has been devoted to the historical aspects of lysogeny.

Discussing lethal biosyntheses in 1951 in Rome, at the first international colloquium of microbial chemistry (73), I pointed out that bacteria may produce various types of particles: normal particles which are nonlethal and noninfectious, transforming principles which are nonlethal and infectious, bacteriophages which are lethal and infectious. A logical place was available for particles the biosyntheses of which would be lethal and which would not be infectious. A few months later, Jacob, Siminovitch and Wollman (56) demonstrated that the biosynthesis of bacteriocins was a lethal process. Owing to the relationships between bacteriocins and phages on one hand and bacteriocinogenic and lysogenic bacteria on the other, the problem of bacteriocinogeny and of lethal biosyntheses will be briefly considered in section VII.

In addition to the summary of views, conclusions and comments dealing with the present status of lysogeny in Sections XIII, XIV and XV, certain observations have been supplied in the Appendixes. These include a glossary of terms, a chronological table of development, and some asides, peripheral but relevant, whose inclusion in the main body of the text might have interrupted the flow of the argument. Finally, a selected bibliography has been furnished, the references with few exceptions being restricted to papers dealing with lysogeny and to recent papers (1952–1953). Many references concerned with bacteriophage and viruses in general can be found in the monographs and treatises cited in references (1) to (7). In the text these seven references are also used for specific articles and reviews.

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The United States are now, *par excellence*, the holy land of bacteriophage. This is for the great part owing to the successful efforts of M. Delbrück, of S. Luria, and their groups. Like many other workers, I have profited greatly by the methodology, discoveries and conceptions of the American school as well as by personal contacts with their members.

II. GENERAL VIEW OF LYSOGENY

"May possibly be some certain species of animalcula or wonderfully minute living creatures that by their peculiar shape or disagreeable parts . . . cause all the disorders mentioned . . ."—Benjamin Marten, *A new theory of consumption*, London, 1720.

Lysogeny is the hereditary power to produce bacteriophage. *A lysogenic bacterium is a bacterium possessing and transmitting the power to produce bacteriophage.* Each bacterium of a lysogenic strain gives rise to a lysogenic clone (figure 1).

The majority of the individuals of a lysogenic population do not produce bacteriophages. Phages are produced only by a small percentage of bacteria and are liberated by bacterial lysis. The expression of lysogeny, phage production, being lethal, lysogeny can only be perpetuated as a potential character. A given lysogenic bacterium may produce one or several types of phages. When a nonlysogenic bacterium is infected and lysogenized with a given phage, the lysogenic strain produces the original phage. Thus, lysogenic bacteria perpetuate a specific structure (figure 1). But when a lysogenic bacterium is disrupted, no infectious particles are released. Lysogeny is perpetuated in the form of a noninfectious structure. This specific noninfectious structure, endowed with genetic continuity, is called prophgae. *Prophage is the form in which lysogenic bacteria*

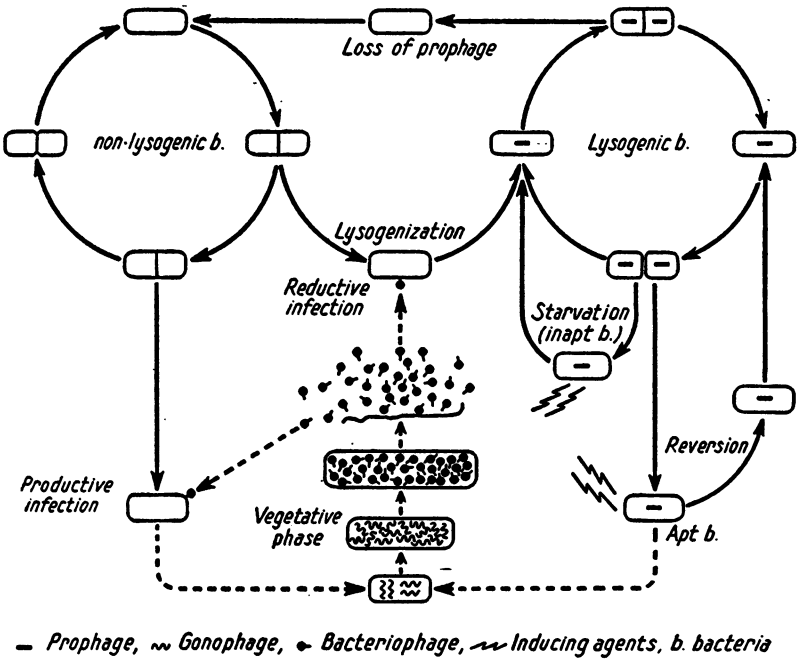


FIG. 1. General view of lysogeny

perpetuate the power to produce phage. Its multiplication is correlated with bacterial reproduction. It seems to be located at a specific site of a bacterial chromosome and to behave in crosses as a bacterial gene.

Any phage entering a bacterium loses its infectious power. Never is a phage multiplied as such by binary fission. If infection is to be *productive*, if phage is to be produced, the infecting phage enters the *vegetative phase* of the life cycle. During the vegetative phase the genetical material of phage is multiplied in the form of *gonophage*, and the specific phage proteins are synthesized together with nucleic acid. The vegetative phase culminates in the organization of *infectious* or *mature* phage particles. When the infection is going to be reductive, when the bacterium is going to be *lysogenized*, the genetic material of the infecting phage or germ is "*reduced*" into prophage.

In some lysogenic systems the spontaneous rate of phage production cannot be modified. In other systems production of phage can be induced at will by the action of some physical and chemical agents. Inducing agents do not unmask a masked phage particle. They convert the prophage into gonophage, and the vegetative phase of the cycle is thus started.

Lysogenic bacteria are *immune* towards the homologous phage which is adsorbed, penetrates into the bacterium, but is not replicated. Although the genetic material of the infecting phage may survive for a few bacterial generations, behaving as an inert cytoplasmic particle, it does not develop. In an immune bacterium a reaction leading towards the vegetative state is blocked. Some lysogenic bacteria may be cured of their lysogeny. The nonlysogenic strains which result have lost their original immunity which is regained by lysogenization. In a lysogenic bacterium, related infecting phages cannot generally be reduced into prophage. This is *incompatibility*. It appears as if the reduction into prophage may take place only at a specific and unique site of the bacterium, which, when occupied by a prophage, is unable to accommodate the new, infecting, related phages. The prophage of a lysogenic bacterium may be visualized as a portion of genetic material containing the phage genes. Prophage blocks phage development and is responsible for bacterial immunity.

In a lysogenic bacterium immunity is a corollary of the presence of prophage. The development of bacteriophage can take place only if immunity is lost, that is to say, when the prophage ceases to be in the prophage state, ceases to be a prophage.

The power to lysogenize is the property of *temperate* phages, as opposed to *virulent* ones. This power to lysogenize may be decreased by mutation. It may also be lost; temperate phages may produce virulent mutants. Some virulent phages have the property to induce the development of a related prophage. Temperate and virulent phages may be crossed. The character "virulence" behaves in crosses like other genetic characters of the phage.

III. HISTORICAL

"Whilst I was asleep a ewe began to browse the crown of ivy that adorned my head. And as she was eating she was saying: Zarathustra is a man of learning no more".—F. Nietsche, *Thus spake Zarathustra*.

Before discussing the early history of lysogeny, a few remarks regarding definitions may be useful. In the early days bacteriophage had been referred to as vitrous material, dissolving substance, lysine, lytic agent, lytic principle, lysogenic principle, fatal principle, or simply "the principle". Lysogenic means etymologically "generating lysis." In 1921, a lysogenic strain was simply a strain containing bacteriophage, a strain in which bacteriophage was produced, and therefore able to induce the lysis of other strains.

It is known today that phage producing strains are of two types:

(1). *Carrier strains*, also called mixed, infected, or pseudolysogenic strains. These are simply mixtures of bacteria and of bacteriophages which are in a more or less stable equilibrium. The majority of bacteria are resistant; however, some sensitive variants appear which, when infected by the extrinsic phage, allow phage multiplication. From now on this type of strain will be referred to exclu-

sively as *carrier strain*. Pseudolysogenic is dropped to avoid confusion with lysogenic.

(2). *Lysogenic strains*. These are strains in which every bacterium is lysogenic, that is to say, perpetuates hereditarily the power to produce phage. From now on, lysogeny will be utilized only with this restricted and very precise meaning. For the conveniency of the history, the expression *phage producing strains* will be kept to designate various strains which were studied before the distinction had been established between carrier and lysogenic strains.

Discovery of Lysogeny

Following the classical studies of Twort and of d'Herelle, Bordet and Ciuca in 1921 recognized that bacterial strains derived from bacterial suspensions often produced bacteriophages when isolated without special caution. They were *lysogenic*. On the contrary colonies obtained from individual bacteria had no lysogenic power. The same year Gildemeister (38) announced that filtrates of feces were lysogenic for some bacteria, that is to say, contained bacteriophages. Thus, the existence of phage producing strains was known as early as 1921, but how was their dual nature recognized?

To follow the historical development of lysogeny, it should be remembered that theories regarding the nature of phage dominated the field between 1920–29 and even later. Twort had considered many hypotheses as regards the nature of bacteriophage (97). It could be an ultramicroscopic virus; an enzyme with the power of growth; a stage in the life history of the micrococcus; an enzyme produced by the micrococcus itself and leading to its own destruction and to the production of more enzyme. In any event, Twort favored the view that the "material" was produced by the coccus and pointed out the connection with the problem of cancer of this "apparent spontaneous production of a self destroying material, which, when started, increases in quantity". For d'Herelle, bacteriophage is a parasite, a virus which penetrated into the sensitive bacteria, develops, and lyses its host (41). This is a correct picture of the relation between a sensitive bacterium and a virulent phage but does not apply at all to lysogeny. Gildemeister, Bail, Otto, Munter, and Bordet took a quite different position: bacteriophage was produced by the bacteria. This theory which was supposed to account for the properties of virulent phages was, from the start, in disharmony with facts. It nevertheless prepared the discovery of lysogeny in accustoming workers to the idea that bacteria could produce bacteriophages without infection.

As stated before, Bordet and Ciuca in 1921 experimentally had obtained phage producing strains; the same year, d'Herelle (41) also observed phage producing cultures and referred to them as "symbiotic". Otto and Munter (85) likewise possessed a phage producing strain. From the fact that a bacterial culture could produce phage, they concluded that phage was not a virus but a harmful, pathogenic enzyme produced by the bacteria. In this very same year, 1921, and later in 1922, Bail (8, 9) who also studied phage producing strains proposed the "splitter theory", according to which phages were pieces of exploded bacteria able to reproduce bacteria. Bail also expressed the opinion that phage was produced

by bacteria. This conclusion was criticized by d'Herelle who stated repeatedly that phage producing cultures were phage contaminated bacterial cultures which were easy to purify, that is to say, carrier strains.

In 1922, Lisbonne and Carrère (68) announced that they obtained a "shigaphagic principle" by bringing into contact certain cultures of *Escherichia coli*, or a *Proteus* X19, with shiga bacilli. Supposedly, the phage was produced as a result of bacterial antagonism, but it was recognized later by Bordet (15) and by Lisbonne and Carrère (69) that phage existed in the original *coli* strain before any contact with *Shigella*. D'Herelle apparently had dealt only with carrier strains and did not conceive of the possible existence of something different. He insisted on the fact that cultures of *E. coli*, when isolated from pathological specimens, were often mixed cultures of bacteria and bacteriophage and concluded without hesitation that Lisbonne and Carrère's strain was of this type (42).

Gildemeister, like Otto and Münter, and Bail were still convinced that bacteria could produce bacteriophage. In 1924, Gildemeister and Herzberg (38) tried to prove that the strain *E. coli* 88 would maintain its "lysogenic power" in the absence of free phage. Their experiments are impressive although not entirely convincing, and it is really difficult to decide whether Gildemeister and Herzberg themselves provided the proof that the strain 88 was lysogenic. What is important is: (a) that Gildemeister and Herzberg conceived of bacteria able to produce phage and keep this property in the absence of extrinsic phage; (b) that the strain 88 was really lysogenic as was demonstrated in 1925 by Bail (10). He confirmed that it contained phage and made isolations from single colonies. Some clones were phage producing; others, not. The phage producing clones were reisolated 6 times serially; each clone was found to produce phages. And Bail concluded, "Die Bakteriophaglieferung muss eine Eigenschaft jeder Bakterien sein".

Bail's paper was published at the end of August, 1925; a few days later, in the beginning of September, Bordet's publication appeared (16). Bordet had studied very carefully the strain of Lisbonne and Carrère and had clearly shown that clones derived from single bacteria produced bacteriophages, whereas this did not happen with clones of a phage contaminated culture (carrier strain). McKinley, in Bordet's laboratory, prepared a serum against the phage of *E. coli* Lisbonne and observed that lysogeny was maintained when the bacteria were grown in the presence of antiphage serum (80). The serum, it was concluded, neutralized the bacteriophages without abolishing the power to produce them. This type of experiment later became a standard technique allowing discrimination between carrier strains from which the phage is eliminated by antiserum and lysogenic strains which remain lysogenic in the presence of serum. And Bordet drew a sharp distinction between *active lysogenic power*, which corresponds to lysogeny and *passive lysogenic power* which corresponds to carrier strains.

Bail, as had Bordet, also discovered that he could obtain lysogenic strains by infecting appropriate bacterial strains with adequate phages. This is lysogenization. As will be discussed later, they also made important observations regarding the discrimination between *strong* and *weak* principles, the future virulent and temperate phages. Thus in 1915, Twort had conceived of bacteria producing

bacteriophages; by 1924, Gildemeister and Herzberg clearly conceived of lysogeny as a property of bacteria which could be maintained in the absence of free phage and produced almost convincing experiments. Finally, in 1925, Bail and Bordet, independently, established the existence of *strains in which each bacterium gives rise to a phage producing clone, this property being maintained in the absence of free bacteriophage.* Now, lysogeny had been discovered, but it was far from being understood.

Development of the Concepts of Lysogeny

Lysogeny and heredity. The faculty to produce bacteriophage, wrote Bordet in 1925, is inscribed in the heredity of the bacterium (16); it is "inserted" in the normal physiology of the bacterium wrote Bordet and Renaux in 1928 (18). The adequacy of this formula in the light of our present knowledge and conceptions is striking. To understand what it meant to the authors, however, it is necessary to analyze their views on heredity and bacteriophage. For Bordet and Renaux, 1928, what is called heredity is only the "continuation of a purely individual physiology". Heredity is only a "regulation which prolongs itself in the course of cell division". Bacteriophagy is a hereditary malnutrition, an autolysis, a transmissible exaggeration of the autolytic phenomenon which already manifests itself in normal bacteria (Bordet, 16). The principle, bacteriophage, contributes to the transmission of certain properties, but it is not the carrier of these properties. Bacteriophages are not "materialized hereditary properties". They act selectively in favoring such microbial types which either possess these properties or the potentialities to acquire them when influenced by the principle. And in his Croonian Lecture (17), Bordet wrote in 1931: "The invisible virus of d'Herelle does not exist. The intense lytic action, to which the name of bacteriophage is given, represents the pathological exaggeration of a function belonging to the physiology of the bacteria".

Bordet did not realize that lysis is not the important fact in bacteriophagy, that bacteria could lyse for various reasons, and that the remarkable phenomenon was the reproduction of a specific particle which multiplied inside the bacterium and bred true to type. Apparently, neither bacterial heredity nor lysogenic power was conceived as associated with specific particles endowed with genetic continuity. Everything was physiological heredity. Lysis of a bacterium by a virulent phage was the exaggeration of a normal, hereditary, physiological property; the lysogenic power was a part of the normal physiology. Having thus conceived of bacteriophage as the result of an exaggerated normal process, Bordet was ready to admit that bacteria could live and multiply while producing bacteriophage. He was, therefore, not surprised to find bacteria endowed with lysogenic power, but apparently, the importance of the discovery was not realized. And perhaps lysogeny was discovered precisely because of these somewhat strange conceptions of bacteriophage and of heredity.

The noninfectious phase. Bail had admitted that in a lysogenic strain each bacterium produces phage and wondered why, in his lysogenic strain, the ratio phage/bacterium was so small. Bordet considered that lysogenic bacteria were

producing "principles" without harm to themselves, were secreting bacteriophages. What actually happens? How is phage perpetuated, how is it produced, how is it liberated? This problem was attacked in 1929 by Burnet and McKie (25). The Australian workers were studying a "permanently lysogenic" strain of *Salmonella enteritidis* Gaertner. The permanence of the lysogenic character led them to assume the presence of bacteriophage or its *anlage* in every bacterium. As lysogenized bacteria produce phages identical to the original phage, the *anlage* was specific.

What is the anlage? Burnet and McKie disrupted lysogenic bacteria with an unrelated extrinsic phage. No infectious particles corresponding to the phage produced by the lysogenic strain were liberated. When a great number of lysogenic bacteria were treated with distilled water, some phages were liberated, and it was found that approximately 0.1 per cent of the bacteria did contain phage. Thus Burnet and McKie reached the following conclusion: Lysogenic bacteria contain in their hereditary constitution a unit potentially capable of "liberating" phage. The lytic principle, or its anlage, is a normal constituent of the lysogenic bacterium; the bacteriophage unit is physiologically coordinated in the hereditary constitution of the bacterium. Phage is "liberated" only if the anlage is activated; "the essential of the process seems to take place entirely within the bacterium, the condition for activation being probably analogous to those concerned in such organic processes as monomolecular reaction or radioactive disintegrations".

Burnet and McKie thus discovered that lysogenic bacteria do not contain infectious particles. They "liberate" phage only under the influence of an activation. Burnet and McKie spoke of the activation *or* liberation of the anlage. But activation and liberation may correspond to two completely different types of processes: unmasking of a preexisting latent virus or development of a phage from something which is not a phage. The idea of an unmasking seems to have prevailed in Burnet's mind for, in his 1934 review on bacteriophage (23), he wrote: "One is almost forced to postulate that each lysogenic bacterium carries in intimate symbiosis one or more phage particles which multiply by binary fission *pari passu* with the bacterium". Later, in 1936, Burnet and Lush investigating a lysogenic coccus observed that when the bacteria were disrupted with the help of a nonrelated phage, no release of the phage corresponding to lysogeny occurred. And although the Wollman's gene hypothesis of bacteriophage was briefly considered in the discussion of lysogenization, the problem of the anlage was not discussed further by the leader of the Australian school. But it was taken over again in Paris, in the Institut Pasteur, by Eugène and Elisabeth Wollman who recognized the existence of an alternation *infectious*→*not infectious*→ . . in the life cycle of bacteriophage.

Before discussing their experiments, a few words should be said on lysogeny in *Bacillus megaterium*, an organism which has played an important role in lysogeny. Lysogeny in *B. megaterium* was discovered by den Dooren de Jong (32). From a sporogenic (lysogenic) strain, the Dutch worker obtained a nonsporogenic strain, the *mutilate*, which turned out to be nonlysogenic and sensitive to a phage produced by the original strain. The same observation was made on *Bacillus*

undulatus. Den Dooren de Jong also discovered that spores of both bacilli heated for 5 to 20 min at 100 C gave rise to lysogenic clones. As phage was known to be killed at this temperature, he concluded that phage could not have been present as such in the spores. The hypothesis of a latent infection was therefore excluded, and the conclusion put forward that *phage was produced by the bacteria*. Later on Vedder (98) showed that dehydrated phages were more heat resistant than phages in aqueous media. And as the bacterial spore was supposed to be more or less dehydrated, the intrasporal bacteriophage could have been in a dehydrated stage, which could have been responsible for the survival of a symbiotic phage responsible for lysogeny. Thus den Dooren de Jong's conclusions have sometimes been considered as devoid of experimental evidence, but we know today that the Dutch worker had made the correct interpretation.

In 1925, Eugène Wollman put forward the hypothesis that some genes could perhaps have a certain stability and be transmitted from cell to cell by the external medium. Discussing in 1928 the transmission of bacterial properties through the external medium which he referred to as *paraheredity*, Wollman (102) expressed the prophetic view that such transmissions could be due to genes; thus, the nature of the future transforming principles was foreseen. Wollman also compared phages to lethal genes and concluded that one has to distinguish *true viruses*, that is, parasitic germs, foreign to their host, and *elements of cellular origin* able to be transmitted either hereditarily or through the medium. These views were severely criticized by Bordet and Renaux (18) who remarked that if bacteriophages were really "materialized hereditary properties", it was difficult to understand how they could be responsible for lytic processes.

The Wollman's gene conception was well in advance of its time and, until 1950–1951, remained unaccepted or ignored with one exception. In 1936, Burnet and Lush (24), discussing the origin of the induced resistance towards a phage brought by the infection with another phage, noted that the resistance, the changed character, is transmitted indefinitely to the descendants and remarked that the change could come either from an altered genetic constitution of the bacterium or be directly induced by the associated phage at each division. The distinction would disappear, however, according to Wollman's hypothesis that regarded phage as a gene reintroduced into the genetic make up of the organism.

In 1934, the Wollmans, convinced of the validity of their gene theory of the phage (103), started their work on lysogeny. They first rediscovered the absence of infectious particles in lysogenic bacteria. Lysozyme lyses *B. megaterium* but does not alter the phage; no infectious particles are liberated when lysogenic bacteria are lysed with lysozyme (104). The same conclusion was reached independently and almost simultaneously by Gratia (38a) who did not comment on the phenomenon.

According to Wollman's theory, each lysogenic bacterium should produce one phage at each division, a conclusion that was not confirmed by the later experiments. The gene theory had nevertheless been fruitful in allowing the discovery of the noninfectious phase in the life cycle of bacteriophage. The Wollmans had observed that bacteriophages adsorbed on sensitive bacilli, whether *B. megate-*

rium or *B. subtilis*, were not detectable after disruption of bacteria by lysozyme, despite the fact that the infected bacilli would produce phage later. Phage exists, they concluded, in two different forms or phases: the mature, infectious particle and the latent, noninfectious form (105). Although neither quantitative nor chronological data are to be found in this paper, the Wollmans certainly saw the disappearance of the infectious power after infection and understood its significance. Some years later Doermann (31b) rediscovered the phenomenon and provided precise and elegant experiments that proved the correctness of Wollman's observations and interpretation.

The comparison of phage multiplication in lysogenic and nonlysogenic bacteria led the Wollmans to conclude that in both phage may be present in a *cryptophagic* form. Between the penetration of a bacteriophage and the appearance of bacteriophage after lysis is an inactive phase; phage particles are not the direct descendants of preexistent phages. Bacteriophages in lysogenic bacteria are produced from a structure which is not a phage particle. Bacteriophage is not present as such in lysogenic bacteria but is maintained through heredity. In infection as in lysogeny, there is an alternation of an infectious and a noninfectious phase in the life cycle of bacteriophage (105). The Wollmans realized that the solution of the problems concerning lysogeny would come from experiments with single bacteria and secured a micromanipulator. But their work was tragically interrupted in 1943 when Eugène and Elisabeth Wollman were arrested in the Pasteur Institute, deported to Germany, and disappeared in one of the *Vernichtungslagern*.

The secretion hypothesis. Already in 1929, Burnet and McKie had provided evidence that only a small proportion of bacteria in the lysogenic *Salmonella* contained phage (25). Later, in 1936, Burnet and Lush (24) concluded that, probably, liberation of phage is always associated with the destruction of the bacterium from which it is derived. These two papers seem to have been over looked. The problem of the mode of liberation of phage in lysogenic population was taken up by Northrop in 1938 (83).

Starting from the idea that some viruses and phages are proteins, Northrop decided to study the kinetics of phage production in growing populations of bacteria. The increase in phage or virus concentration in the presence of living cells was considered as formally analogous to the formation of enzymes by cells or their formation from precursors, as well as to the growth of bacterial cultures. From measurement of the growth of a lysogenic *B. megaterium*, the increase of gelatinase and the production of phage, he found that initially all three increased logarithmically and concluded that phage is produced during bacterial growth and not during lysis.

But Northrop's conclusions are open to a serious criticism. If in a lysogenic population, each bacterium produces and secretes one phage at each division or if one bacterium out of 100 produces 100 phages, the end result would be identical: the ratio phage/bacterium would be one, the over-all kinetics would be the same. The study of large populations cannot yield the solution to this problem; to know what things really happen, one has to study a single lysogenic bacterium.

Lysogeny at the unitary scale. Lwoff and Gutmann made such a study (74).

A single washed lysogenic *B. megaterium* was isolated in a microdrop, and aliquots of the medium tested at intervals. As they found that lysogenic bacteria could grow and divide without liberating phage, phage liberation is not a necessary corollary of bacterial growth and multiplication. When a single isolated bacterium was allowed to divide 19 times, each isolated daughter bacterium gave rise to a lysogenic clone. As no free phage appeared during the experiment, lysogeny was maintained in the absence of free, extrinsic phage. McKinley's fundamental conclusion drawn from the maintenance of lysogeny in the presence of antiphage serum was confirmed and established on firm ground.

When microclones of lysogenic *B. megaterium* growing in microdrops are carefully and constantly watched for a reasonable length of time, the lysis of some bacteria may be observed. A bacterium is there, and suddenly it disappears. When this happens, phage is found in the droplet, around 100 phages per lysed bacterium (28, 74). Thus, in agreement with Burnet and McKie's fundamental observation, only a small fraction of the lysogenic bacteria in a lysogenic population produce bacteriophages. Moreover, phages are liberated by bacterial lysis. The production of bacteriophage is a lethal process: lysogenic bacteria only survive if they do not produce phage.

Definition of lysogeny. When nonlysogenic bacteria are lysogenized, the new lysogenic strains, as discovered in 1925 by Bail (10) and by Bordet (16), produce the original phage. From this type of experiment, Burnet and McKie had rightly concluded that something specific is perpetuated in lysogenic bacteria, a noninfectious "anlage" which, later on, Burnet considered as an intracellular phage multiplying by bipartition, while the Wollmans interpreted it as a different phase of the life cycle. For this specific noninfectious particle endowed with genetic continuity, Lwoff and Gutmann proposed the name probacteriophage or *prophage*. Prophage was defined: the form in which lysogenic bacteria perpetuate the power to produce bacteriophage.

Since the expression of lysogeny is lethal, prophage must be considered as a potential lethal factor. Lysogeny can only be the hereditary perpetuation of the *power* to produce bacteriophage. The lethal character of the expression of lysogeny being disclosed, as well as the noninfectious nature of the specific prophage, it is possible to conceive of a lysogenic bacterium as a bacterium which possesses and transmits the power to produce bacteriophage, a bacterium which possesses the genetic capacity to produce phage.

This definition contains the essence of lysogeny. It should be considered as a collective achievement, as the outcome of the efforts of numerous workers, and as a synthesis of our knowledge concerning not only lysogeny but also bacteriophage, biological specificity and heredity (see Appendix II).

IV. PROPHAGE

"Certainement, c'est dans la parfaite abnégation que l'individualisme triomphe, et le renoncement à soi est le sommet de l'affirmation".—André Gide, *Journal.*

Lysogenic bacteria perpetuate hereditarily the power to produce bacteriophage. An artificial lysogenic strain produces phages identical to the original type. To

account for this fundamental fact, we shall admit with Burnet and McKie (25) that lysogenic bacteria perpetuate a specific anlage which is now called prophage. What is the prophage? The hypothesis that lysogenic bacteria perpetuate a nonpathogenic bacteriophage particle has been accepted often in the past. Many facts speak against this view. That prophage is neither a masked phage particle nor a phage in the vegetative phase of its life cycle will become evident from the study of immunity. But we have to consider here a more static aspect of lysogeny.

Disruption of lysogeic bacteria does not liberate infectious particles (25). This means either that lysogenic bacteria do not contain bacteriophages or that the infective properties of the bacteriophages are masked. Could induction correspond to an unmasking and lysogeny correspond to the duplication of a masked phage particle? Bacteriophage particles *as such* certainly do not undergo binary fission. When phage particles are produced, the bacterium is always bound to undergo lysis. Moreover, the phenomena taking place in a bacterium during phage development are essentially similar whether the development starts from a prophage or from an infecting temperate phage.

The conclusion seems reasonable that lysogenic bacteria do not, can not, perpetuate a latent or masked phage particle (Appendix III. 4); the production of phage from prophage thus appears, not as an unmasking, but as a development (79). It seems reasonable to suppose that in an induced lysogenic bacterium, as in an infected bacterium, phage develops from a similar, not necessarily identical, sort of material. What can this material be? We have no example of a protein endowed with genetic continuity, whereas it is known that specific nucleic acid may be the basis of genetic continuity: (a) transforming principles of bacteria are known to be desoxyribonucleic acids since the work of Avery and his associates (7a); (b) bacteriophage T2 may be reproduced from its nucleic acid which seems to function as the sole agent of its genetic continuity (45). Chromosomes are sometimes visualized as composed of macromolecules of nucleic acids lined on histone fibers, and the idea emerges that the nucleic acid could be responsible for the specificity of genes. In harmony with these hypothetical views, prophage could be tentatively considered as a macromolecule of nucleic acid.

Nucleic acids being devoid of antigenic properties, lysogenic bacteria should, if prophage is only a nucleic acid, be devoid of phage antigens. Efforts have been made by F. Lanni to detect phage antigens in the lysogenic *Shigella dysenteriae* (P2) (personal communication). Sonic vibrated preparations of bacteria were tested for their ability to decrease the neutralizing activity of an antiphage P2 serum. No antigen was discerned under conditions such that a serological yield equivalent to about one phage particle per 20 bacteria could reasonably have been measured. It thus appears that the lysogenic bacteria (P2) do not contain the serological equivalent of the serum-blocking of the tail protein of the mature phage P2. And it must be concluded therefore that this specific protein is synthesized only when the lysogenic bacterium produces bacteriophage. The theoretical importance of Lanni's experiments is obvious. They will take their full

significance when his conclusions will be extended to complement-fixing antigens and when their generality would have been ascertained.

Prophage could be cytoplasmic or nuclear. If cytoplasmic, it could be either free in the endoplasm, like some other cytoplasmic particles, or bound to a specific bacterial plasmagene endowed with genetic continuity. Lysogeny, except for a few exceptions which will be discussed later, is regularly transmitted to daughter bacteria. If prophage were an independent (not self sufficient) cytoplasmic body, it would be necessary to assume the presence of a number of particles to account for the regular inheritance of lysogeny.

The number of prophages can be determined only indirectly, theoretically by studying their killing curves. Unfortunately, irradiation is of little use as it not only kills the prophage but also suppresses the capacity of bacteria to produce phage. One method of estimation consists in infecting induced lysogenic bacteria with a related phage and studying the progeny as has been done with *E. coli* Lisbonne (14) and with *Pseudomonas pyocyanea* (*Pseudomonas aeruginosa*) (58). With the latter, two *compatible* phages, 8^+ and 8u, *i.e.*, able to develop simultaneously, are used. They are also of equal "strength": if bacteria are infected with one particle of each, both phages appear in the progeny in equal number. The ratio of phages in the progeny varies with the ratio of infecting particles. Now one may lysogenize *P. pyocyanea* with phage 8^+ and then follow the development of lysogenic 8^+ phages in the induced lysogenic (8^+) bacteria infected with varying multiplicities of phage 8u. It is found that the induced lysogenic bacteria (8^+) produce phages 8^+ and 8u in equal number only when infected with about 3 phages 8u. If it is assumed that the relation of the two phages is the same in a lysogenic induced and infected bacterium and in a doubly infected one, it can be concluded that the lysogenic bacterium possesses 3 prophages. This is the average number of nuclei in an exponentially growing culture. It could well be that there is only one prophage per bacterial nucleus. This conclusion will be reinforced by the study of incompatibility.

Therefore, each lysogenic bacterium possesses a small number of prophages which reproduce in harmony with bacterial multiplication. This does not mean that prophage is nuclear; it could be associated with some structures, *e.g.*, present in the polar bodies, and its reproduction coordinated with the duplication of these structures which is itself coordinated with bacterial multiplication.

Probably we would have only hypotheses as to the location and number of prophages if the Lederbergs had not, in 1951, discovered lysogeny in a bacterial strain able to undergo recombination. E. M. Lederberg (65) made the first observation of transmission of lysogeny during bacterial crosses. Then the behavior of the character "lysogeny" was extensively studied by the Lederbergs and by Elie Wollman who independently reached the same general conclusion. The following results have been obtained: (*a*) crosses between two lysogenics yield only lysogenics; (b) crosses between nonlysogenics yield only nonlysogenics; (c) crosses between lysogenics and nonlysogenics yield lysogenics and nonlysogenics. When the character galactose IV is used as a bacterial marker, one finds that (a) all lysogenics have the character galactose IV of the lysogenic parent;

(b) all the nonlysogenics have the character galactose IV of the nonlysogenic parent (66, 107). E. and J. Lederberg have also crossed a lysogenic homozygous diploid, lysogeny + and galactose +, with a nonlysogenic homozygote lysogeny − galactose −. Diploid heterozygous bacteria have been obtained which are lysogenic + and galactose +. These diploids produce spontaneously haploids which are almost exclusively one of the parental types: lysogeny + galactose + or lysogeny − galactose −.

Unfortunately, the nature of the sexual process in *E. coli* K12 is still obscure: one does not know if a fusion takes place or if one parent furnishes to its mate only a limited part of its genetical material. The interpretation is complicated by the existence of a polarity. Some strains behave in recombination as donors (F+); some others, as receptors (F−) (40). Both parents contribute unequally to the genetic constitution of the recombinants which possess essentially the character of the receptor strain. For example, when the receptor is lysogenic and the donor nonlysogenic, 94 per cent of the recombinants are lysogenic. Therefore, data concerning linkage can be considered useful only if one studies the character coming from the donor parent F+. Available data show that although lysogeny λ and the galactose IV character are found together in recombinants, definite proof that both characters may be transmitted from a donor to a receptor has not yet been given. The existence of a true genetic linkage between lysogeny λ and a bacterial gene can be considered only as probable. In agreement with Elie Wollman, we may conclude that the linkage with a specific bacterial gene suggests that the character lysogeny is not independent, thus favoring the hypothesis that lysogeny has a nuclear determination.

Is the genetic difference in the crosses just mentioned identical with prophage? Such a difference could tentatively be ascribed to a bacterial gene allowing the maintenance of a cytoplasmic particle of the "kappa" model, and the prophage would then be the cytoplasmic particle itself. However, the data (to be discussed later) concerning lysogenization, incompatibility, immunity, and the number of prophages are against this hypothesis but are in agreement with the conclusion that the gene-like unit involved in these crosses is the prophage itself. Prophage, like the gene, is a specific structure endowed with genetic continuity. It is located at a specific chromosomal site; it is replicated in coordination with bacterial reproduction; it behaves in sexuality like a chromosomal locus. What could be the reason for the strict localization of prophage? Since during the synaptic pairing, homologous parts of loci of chromosomes are specifically paired, the germ of the infecting phage may be specifically attached to a structurally related part of the bacterial chromosome. The presence of this allelic structure would be a condition for lysogenization. Its uniqueness would account for some features of lysogenic bacteria to be discussed later, and especially for incompatibility. This conclusion is strengthened by Appleyard's observations concerning double lysogenic bacteria carrying two related prophages. *E. coli* K12 perpetuating two triply marked prophages λ have been obtained, which eventually will give segregant clones some of them producing only one type of phage. Some of them possess markers of both parents diversely combined. It really appears as though

related prophages were able to undergo recombination. Such an exchange of genes suggests that they are adjacent or perhaps allelic (see section *Incompatibility*).

Thus, we may visualize a lysogenic bacterium as possessing at a given site of a chromosome a specific gene-like structure, the multiplication of which is coordinated with genic, chromosomal and bacterial reproduction. This structure, the prophage, is the material basis of the genetic continuity of the homologous potential phage. Lysogeny may be tentatively interpreted as the hereditary perpetuation of the genom of the phage integrated with the genom of the bacterium. (See Appendix III. 4 for discussion for some questions of terminology on this subject.)

V. INDUCTION

"Our only hope therefore lies in a true induction".—Francis Bacon, *Magna Instauratio*, 1620.

The proportion of lysogenic bacteria which produces phages spontaneously is variable. Shall we consider this situation as a statistician, draw mortality curves, calculate the probability for a given bacterium to produce bacteriophage in the interval of two divisions, and be satisfied with a formula expressing the state of health of the bacterial population in terms of greek symbols? Or, acting as a biologist, a biochemist, or as the sorcerer's apprentice, shall we attempt to intervene in the course of events which transform an innocuous particle into a virus? The reader knows already that the question would not be raised if an answer could not be provided. This answer is induction.

The observation of a number of bacterial microclones of *B. megaterium* developing in microdrops had shown that in some clones, a high proportion of up to 10/10 bacteria lysed and liberated phages, whereas in others only one bacteria in 100 to 200 produced phage. This strongly suggested that phage production was controlled by external factors (74). A study of the kinetics of phage production during the evolution of bacterial cultures revealed that the curves of phage production and of bacterial growth were not always parallel. Sudden, considerable phage increases occurring at certain definite periods of the development of the culture gave rise to the conviction that phage production was controlled by some changes of the medium induced by the bacterial metabolism. After a large number of experiments, it was finally discovered that irradiation with UV light was followed by phage formation in almost the entire bacterial population (79); forty-five minutes after irradiation, the bacteria lysed and liberated bacteriophages. Evidently, UV light induces the development of prophage into bacteriophage. The synthesis of a particle possessing the characteristic traits of a virus from a particle possessing the behavior of a gene can therefore be induced and studied under defined conditions, as will be discussed in this section.

Genetic Factors Controlling Inducibility

Soon after induction was discovered in *B. megaterium*, several lysogenic systems investigated were found to be inducible: *E. coli* K12 (λ) (99), *Micrococcus*

(*Staphylococcus*) *aureus*, and *P. pyocyanea* (50). Since it appeared that in each bacterial species some systems are inducible whereas others are not, the question immediately arose: is inducibility controlled by the properties of the phage or of the bacterium?

Two phages of *B. megaterium* were studied and it turned out: (a) that when a bacterium is lysogenized with a phage coming from an inducible strain, the new strain is inducible: (b) that when lysogenization is performed with a phage from a noninducible strain, the new strain is noninducible; (c) that when a noninducible strain is lysogenized with a phage from an inducible strain, the double lysogenic, after irradiation, produces only the phage coming from the inducible strain (49). Similar observations have been made by F. Jacob on various strains of *P. pyocyanea* (54). Thus, inducibility and noninducibility are not controlled by the bacterial strain but appeared to be properties of phages.

Inducibility, like many properties of phages, may change by mutation. "Noninducible" phages have been isolated among the phages produced by an inducible strain of *B. megaterium* (Lwoff, 1).

But it would be unwise to conclude that inducibility is always and exclusively controlled by the genetic constitution of the bacteriophage. When a number of individual *E. coli* are lysogenized with phage λ, the proportion of inducible bacteria in each clone may vary from 0 to 100 per cent (Elie Wollman, unpublished). Obviously inducibility may be a property either of the prophage or of the lysogenic system.

It may also happen, as will be seen later, that lysogenization by an inducible phage gives an inducible system, but that induction is followed by an abortive development of the phage. Thus, inducibility of a phage is generally controlled by its genetic constitution, but it may be controlled by the properties of the system prophage bacterium. It should be noted that the spontaneous production of inducible phages is much higher than the spontaneous production of noninducible ones. For example, in *P. pyocyanea* 13 (8) (1), the probability to produce the inducible phage 8 is 10^{-3}; it is 10^{-5} for the noninducible phage 1 (54). The degree of stability of various prophages obviously varies to a considerable extent.

Inducing Agents

The induction of the development of prophage was first observed after an irradiation with UV light of a wavelength around 2537 A (79). X-rays of average wavelength 0.9 A, which correspond approximately to photons of 14 ekv, are also inducers (64) as are *gamma* rays (average 600 ekv) of radioactive cobalt (H. Marcovitch, personal communication). For the same dose of X and γ rays as expressed in roentgens, the inducing effect on *E. coli* K12 (λ) is quantitatively the same (H. Marcovitch). Apparently, other types of radiations have not been studied.

Several chemicals including such reducing compounds as thiomalic acid, reduced glutathione and ascorbic acid (78) have an inducing effect on *B. megaterium* 899-1, whereas strains of *P. pyocyanea* and *E. coli* K12 (λ), inducible with UV, were refractory to these. It was found that reducing substances would induce

only in certain types of media, "active media", which could be made inactive by various treatments and especially by treatment with 8-hydroxyquinoline (oxine); oxine treated, *i.e.*, inactivated, media were reactivated only by copper (72). Finally, it was discovered that the inducing effect of reducing substances in the presence of copper containing media was due to the hydrogen peroxide produced (75). The apparent qualitative difference between *B. megaterium* and *P.*

TABLE 1

Mutagenic, carcinogenic and inducing agents (Lwoff, 1)

AGENT	MUTAGENIC	CARCINOGENIC	INDUCTION OF:		
			Prophage development		Synthesis of colicin
			B. megaterium *P. pyocyanea*	*E. coli*	*E. coli*
UV rays	+	+	+	+	+
X rays	+	+	+	−	−
Methyl bis-(β-chloroethyl)-amine	+	+	+	+	+
Dimethyl-β-chloroethylamine	0	0	0	−	−
H_2O_2 in inorganic media	0	−	0	−	−
H_2O_2 in organic media	+	−	+	0	+
Tertio-butyl peroxide	+	+	+	−	+
2:4:6-*tris*-ethylene-imino-1:3:5-triazine	+	+	+	−	−
Butadiene-1:3-diepoxide	+	+	+	−	−
Ethylurethan	+	+	0	−	−
1:4-bis-sulfonyloxymethyl-butane *	−	+	0	−	−
9:10-dimethyl-1:2-benzanthacene, 2-acetamino-fluorene, methylcholanthrene, β-naphthylamine, benzidine	+	+	0	−	−

* Substances with a low solubility in water; the negative results may not be significant.
\+ Mutagenic, carcinogenic or inducing activity.
0 Devoid of mutagenic, carcinogenic or inducing activity.
− Not examined.

pyocyanea is only quantitative: a greater concentration of hydrogen peroxide is necessary to induce *P. pyocyanea* than *B. megaterium*. No inducing effect of H_2O_2 has been observed on *E. coli* K12 (λ) which is probably "buffered" against peroxides.

These observations show that some culture media contain enough copper to produce a physiologically significant amount of hydrogen peroxide when supplemented with reducing substances. As reducing substances are produced as a result of bacterial metabolism, hydrogen peroxide may be formed as a result of aging. The increased "spontaneous" phage production which is sometimes observed

thus may be caused by the interaction of the product of bacterial metabolism with the constituents of the medium leading to the production of inducing substances. The mutagenic effect of H_2O_2 in organic media is well known since the discovery by Wyss and his co-workers (107a). This mutagenic effect is not observed in synthetic media, whereas mixtures of hydrogen peroxides and various amino acids, formaldehyde or acetone are mutagenic. Organic peroxides are mutagenic according to Dickey, Cleland and Lotz (31a). Tertio-butyl peroxide also induces the development of prophage in *B. megaterium* and *P. pyocyanea*. These bacteria are induced also by butadiene-1:3-diepoxide as well as by ethyleneimine (75). Nitrogen mustard is also an inducer on *P. pyocanea* (53) and on *Salmonella thompson* (101) as is mustard gas (101). Finally, phage formation is induced in some lysogenic strains of *S. thompson*, by sulfathiazole, the effect of which is suppressed by *para*-aminobenzoic acid (101); an extensive study of this should throw some light on the intimate mechanism of induction.

The main results are summarized in table 1. It appears that inducing agents (those which induce the development of prophage into phage) are mutagenic in bacteria as well as in *Neurospora* and *Drosophila* and produce chromosome breakage in plants. Since the pioneer work of Auerbach much work has been done in this direction which has been summarized recently by K. A. Jensen *et al.* (59). Thanks to the work of E. Boyland and E. S. Horning and of W. E. Heston recently summarized by Boyland (22), it is known also that those agents which are mutagenic and inducers are also carcinogenic.

It will be noted (table 1) that some liposoluble carcinogens have given negative results when tested as inducers, but in the absence of any data concerning their penetration in bacteria, these negative results may not be significant. The only water soluble mutagenic and carcinogenic substance which is devoid of inducing effect is urethan. Up to now only a very small number of substances have been examined for the inducing activity. Many more need to be tested before a general conclusion can be reached, but if it turns out that water soluble carcinogens are inducers, then inducible lysogenic bacteria might become a good test for carcinogenic, and perhaps anticarcinogenic, activity.

Phage Development After Infection and After Induction

In *B. megaterium*, the appearance of the first intrabacterial mature phages—as revealed by lysozyme—and spontaneous lysis take place at the same time, whether the nonlysogenic strain has been infected, or the lysogenic strain has been induced with UV light (respectively at 32–35 and 42–45 min) (79). In other systems as *P. pyocyanea*, the latent period is longer after induction than after infection. The latent period varies with the nature of the inducing agent: X rays (J. O. Tobin, personal communication) or UV rays (51). If infection has been preceded by irradiation with UV light, the latent period is also increased (12).

It is therefore possible that the delayed latent period is due to the effects of UV light. As a matter of fact, the latent period is increased (see figure 2) and the burst size decreased when the dose of UV is increased (99). The same phenomenon has been found in *B. megaterium* 1: at 27 C, the length of the latent period is

increased by a factor of 3 for a 16-fold increase of the UV dose (94). As will be seen later, with UV induced pseudomonads a period exists during which photorestoration can take place. The length of this period is of the same order of magnitude as the increase of the latent period. It could be that the "photorestorable phase" does not correspond to a conversion of prophage into gonophage, but to the sequence of events culminating in this conversion which marks the beginning of the vegetative phase (figure 3).

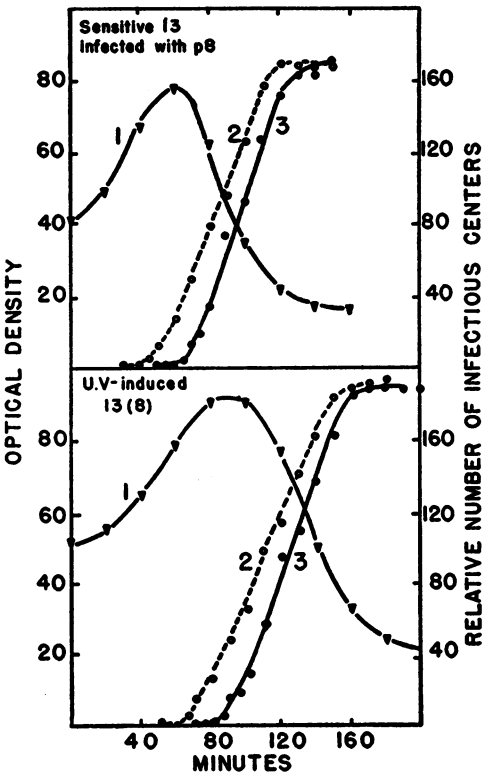


FIG. 2. Development of phage after induction and infection in *Pseudomonas pyocyanea*. *Upper part:* development after infection. *Lower part:* development after induction with UV light. From F. Jacob and Elie Wollman (58).

Mode of Action of Inducing Agents

The effects of radiations and of radiomimetic agents on cells and on substances such as enzymes, collagenous fibers, nucleic acids have been the subject of many studies which have been extensively and clearly reviewed by Ross (88). According to Butler, Press and James (26), nitrogen mustard breaks hydrogen bonds between free amino group and hydroxyl group of two adjacent purine and pyrimidine bases of the nucleic acid, these bridges being responsible for the rigidity of the particle. Whatever the case may be, nucleic acid undergoes analogous modifications under the influence of X rays, hydroxyl radicals produced chemically, and radiometric substances such as bifunctional halogeno-alkoylamines. Also certain mutagens may produce chromosomic breakage. As long as we are ignorant of the structural basis of gene specificity, the mechanism of mutation will remain

obscure, as well as the mode of action of mutagens. But a gene mutation is a rare event. The low probability may be explained if a given mutation corresponds to a specific architectural change such as change in the sequence of an orderly chain of nucleotides.

Induction of phage development or induction of bacteriocin synthesis (see section VII) is quantitatively different from the induction of a mutation. Almost *all* the bacteria submitted to the action of the inducing agent under favorable conditions perform a new specific biosynthesis. It is therefore unlikely that the

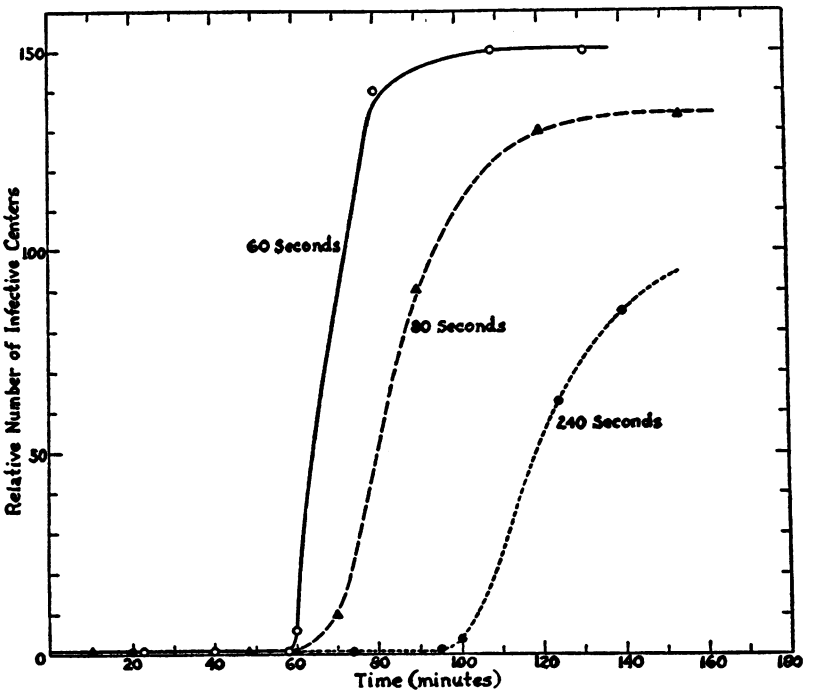


FIG. 3. Influence of the dose of UV light (as inducer) on the latent period of *Escherichia coli* K12 (λ). From J. Weigle and M. Delbrück (99).

event which is the result of induction is of the same nature as gene mutations. The hypothesis has been advanced (73) that inducing agents act by causing a rupture of a weak bond between prophage and the bacterial chromosome; but this breakage is certainly not a direct effect of the irradiation. The question will be discussed in the next section.

Physiological Factors Controlling Inducibility

Aptitude. When the original lysogenic inducible *B. megaterium* 899 (1) was studied, it was observed that the proportion of bacteria which lysed after an irradiation with a given dose of UV light varied; a dose which would induce almost all bacteria grown in yeast extract would not induce bacteria grown in a synthetic medium. The notion of *aptitude* thus emerged (79). Aptitude may be defined as

the quantitative specific response of a lysogenic system to an inducing agent. By specific response, we mean the development of prophage into phage.

One of the most efficient ways to decrease aptitude is starvation. The effect of glucose starvation on the aptitude of *P. pyocyanea* has been extensively studied by Jacob (51).

The aptitude of *P. pyocyanea* is the same whether grown in broth or in a synthetic medium (ammonium salt as nitrogen source, glucose as energy and carbon

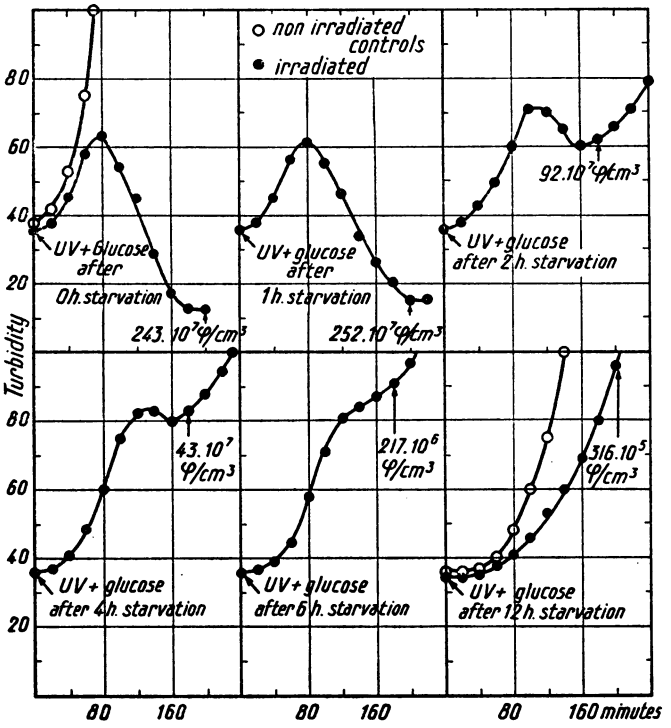


FIG. 4. Decrease of aptitude by starvation. The lysogenic *Pseudomonas pyocyanea* during its exponential growth, or after various periods of starvation, has been irradiated with the same dose of UV light. The bacterial lysis and phage production decrease with the duration of starvation. From F. Jacob (51).

source). If bacteria grown in a synthetic medium are submitted to a starvation in carbon source, the aptitude decreases with the length of starvation and is restored by addition of glucose. If the glucose starvation has lasted 12 hours at 37 C, exponential growth does not follow immediately the addition of glucose. A lag and acceleration period, or a pre-exponential phase, of 40 minutes is observed. With bacteria starved for 12 hours addition of glucose restores aptitude to its original value at a time corresponding to the resumption of exponential growth. Further analysis of the aptitude has led to the conclusion that starvation decreases the number of bacteria induced by a given dose of UV and decreases the sensitivity to a given dose of UV of those bacteria which remain inducible. These

two effects probably represent different expressions of the same phenomenon (see figure 4).

This effect of starvation has been also observed in *E. coli* (λ) by Borek (19). If methionineless, threonineless or leucineless mutants are irradiated after a 3 hour amino acid starvation, no lysis occurs. A 3 hour glucose starvation or nitrogen starvation also produces a decrease of aptitude. Thus, the aptitude of the three lysogenic bacteria studied up to now decreases by starvation whether the missing substance be the energy source, the nitrogen source, or a specific amino

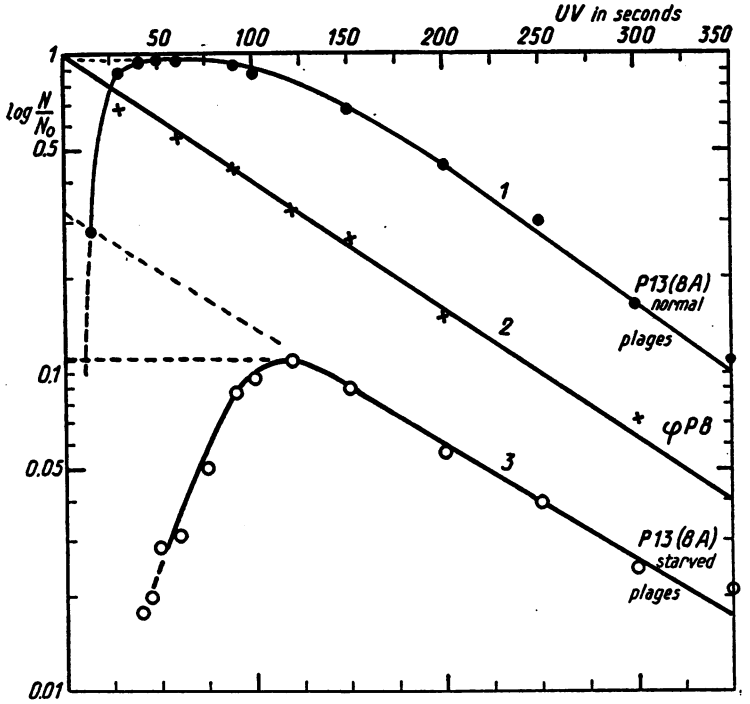


FIG. 5. Decrease of aptitude by starvation. Normal and starved bacteria are irradiated with various doses of UV. For a given dose the proportion of infectious centers (corresponding to induced bacteria) is lower in starved than in normal bacteria. From F. Jacob (51).

acid which the bacterium is unable to synthesize. The only effect of a prolonged starvation on phage development is an increase of the latent period. Phage liberation after addition of glucose to *P. pyocyanea* previously carbon starved for 12 hours takes place after some 120 minutes instead of 80. This is true for induced starved bacteria as well as for infected starved bacteria. The burst size is slightly lower in starved than in "normal" bacteria.

The decreased aptitude brought by starvation has a corollary; when dealing with lysogenic inducible bacteria, the proportion of survivors after a given irradiation is higher in starved bacteria than in normal bacteria. For example, with *P. pyocyanea*, the proportion of survivors for a given dose will be 4×10^{-2} for starved bacteria and 4×10^{-3} for normal bacteria (51). The difference in UV

sensitivity between a starved and nonstarved nonlysogenic bacteria is very small. It is therefore essential, in radiobiological studies, to realize that the death of an irradiated bacterium may be the consequence of an induced lethal development, and that all factors which affect aptitude, or as will be seen later, which affect phage development may modify the "lethal" effect of UV (figure 5).

Remarks on some effects of starvation. If bacteria are irradiated and then starved, neither growth nor lysis is observed. If the missing "growth substance" is added after 2 or 3 hours, residual bacterial growth takes place and lysis occurs. The period between addition of the missing substances and lysis is a little shorter than the normal latent period. Starvation has not suppressed the inducing effect but has stopped the development of the prophage. This conclusion is true for *P. pyocyanea* starved of carbon source (51) and for *E. coli* K12 starved of methionine (19).

Factors Controlling the Bacterial Response After Induction

Physiological factors. The data reviewed in the preceding paragraphs reveal that the fate of an inducible bacterium after irradiation is controlled by: (a) the physiological state of the bacterium; (b) the UV dose. It also depends on the way the bacteria are handled after the UV induction. Consider, for example, a population of *E. coli* K12 irradiated and plated on agar to measure the percentage of surviving bacteria and of phage producing bacteria. The minimum of survival and the maximum of induction are observed when the bacteria are plated at the 50th minute, *i.e.*, soon before the end of the latent period. If bacteria are plated earlier, more colonies and fewer infectious centers are found (99); the same holds for *P. pyocyanea* (51). Whereas 90 per cent of the bacteria plated at 50 min after irradiation will produce phage, only 20 to 30 per cent of the bacteria do so when plated immediately after irradiation. This means that bacteria which would have produced phage if left in broth do not and hence survive if plated early enough after irradiation. Therefore, some of the phenomena which follow immediately the inductive shock are reversible during the early period.

An analogous observation has been made with *B. megaterium*. A culture previously grown in a synthetic medium will grow after a given irradiation if diluted in the same synthetic medium; if an aliquot is suspended in diluted broth, it will produce phage and lyse. A culture grown in yeast extract and irradiated will produce phage and lyse if left in yeast extract, whereas it will not produce phage if suspended in broth after irradiation (72).

Thus, the reactions of the bacteria after irradiation may be modified in one sense or another by the quality of the medium. An irradiated bacterium will or will not produce phage according to its environment after the shock. Essential ions may intervene in these phenomena; *e.g.*, *B. megaterium* in a manganese deficient medium will not produce phage after irradiation, but if supplemented with manganese after the irradiation, bacteriophage is produced. If a culture is grown in the presence of a concentration of manganese just sufficient to allow phage development, phage is produced after irradiation. This induced development is blocked if the medium is supplemented with cobalt or zinc, and the inhibitory effect of Co^{++} and Zn^{++} is released by Mn^{++} (48).

We judge that the development of phage has been induced from the fact that the bacterium will produce phage. If we note that an induced bacterium is prevented from producing phage by a change of the medium, we might say that the effect of induction has been suppressed. But do we have the right to conclude that "phage development" had started and has stopped? Certainly not; not any more than we have to conclude that addition of manganese to an irradiated manganese-deficient bacterium induces the development of prophage.

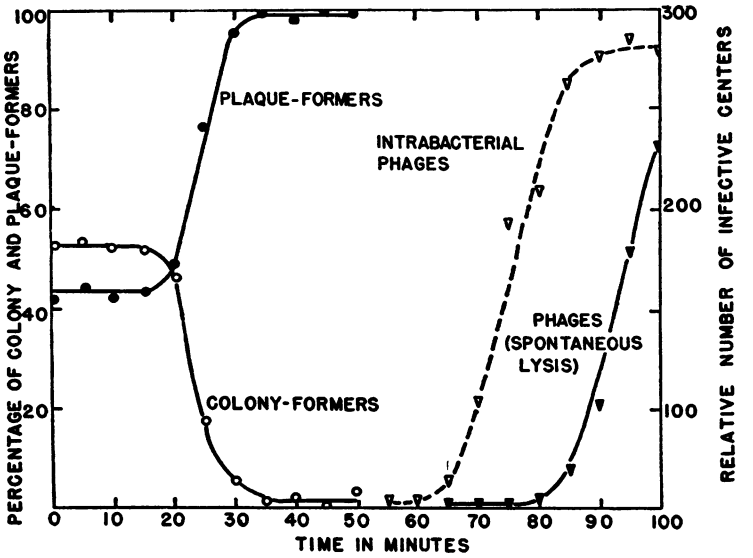


FIG. 6. Photorestoration of UV induced *P. pyocyanea*. A suspension of *P. pyocyanea* 13(8) was irradiated with an optimal dose of UV and then shaken in nutrient medium at 37 C in the dark. At various times during the latent period, samples were removed and exposed to the same dose of reactivating light. After further incubation in the dark, bacteria were plated as infective centers and colony-formers. The percentage of plaque-formers and colony-formers are plotted as a function of the time of restoration with visible light after induction. Phage production is represented as a measure of the latent period. From F. Jacob and Elie Wollman (58).

Photorestoration. The inducing effect of UV is reversed by visible light in a manner similar to that found with other effects of UV. If *P. pyocyanea* is submitted to an irradiation which induces more than 90 per cent of the bacteria and is then, at various intervals, submitted to visible light, a large fraction of the bacteria are photorestored (figure 6). If visible light is applied immediately, 80 per cent of the bacteria do not give infectious centers and a large proportion of these develop colonies. All the surviving clones are lysogenic. Reversion may be obtained until the 25th minute, the latent period being 85 minutes under experimental conditions (58).

Replacement of a prophage by another related one has exceptionally been observed in lysogenic bacteria (see section XII). The possible substitution of prophage during the photorestorable period following an irradiation with UV light

has been examined by Jacob. *P. pyocyanea* 13 (8) was induced, infected with phage 8u (a mutant of 8), and photorestored. As all the clones were lysogenic 8 just like the original strain 8, the infecting phage 8u had not replaced prophage 8 during this precritical period. If induction is a displacement of the prophage from its chromosomal "base", this experiment indicates that during the reversible phase following the irradiation, the prophage is not displaced; otherwise, a prophage substitution could probably occur.

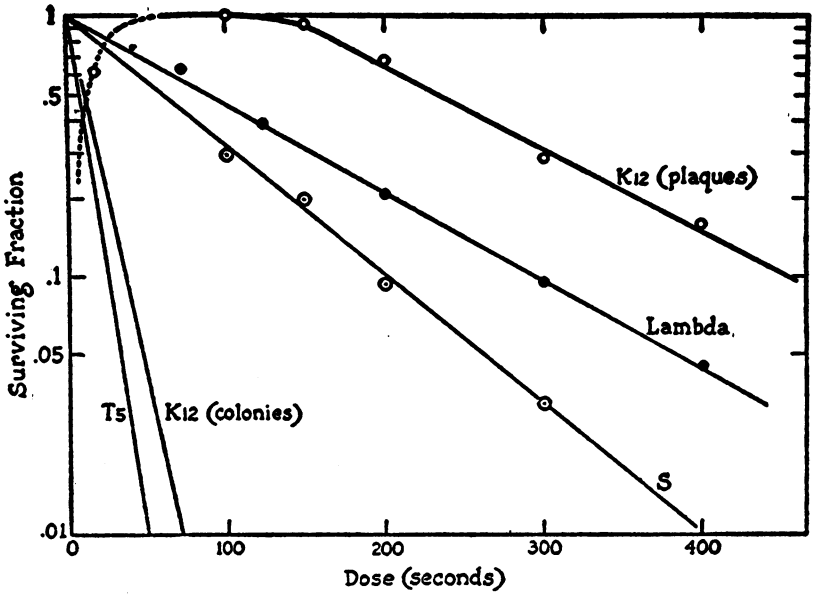


FIG. 7. Induction of *Escherichia coli* K12 (λ) as a function of the dose of ultraviolet light. From J. Weigle and M. Delbrück (99).

Quantitative Effect of Inducing Agents

UV radiations. The proportion of bacteria which produce bacteriophage after an irradiation is a function of the UV dose. After a rapid increase the curve flattens, and a plateau is reached. As the dose increases, the fraction of induced bacteria increases; then the number of phage producing bacteria decreases (99) (see figure 7). A dose that induces more than 99 per cent of K12 (λ) kills only 50 per cent of the nonlysogenic detector strain K12 S. At this dose, or lower ones, most of the irradiated bacteria die because phage development has been induced. Similar types of curves have been found in *P. pyocyanea* 13 (8) by Jacob (51). As will be seen later, the decrease of the number of phage producing bacteria with higher doses of UV may be due to the destruction of the capacity of the bacteria to produce bacteriophage.

Chemical inducers. The same type of quantitative response is observed with chemical inducers as with physical inducers as shown by Jacob (53) in a study

of the effect of nitrogen mustard on *P. pyocyanea* (see figure 8). For a given time of action, the inducing effect of nitrogen mustard is a function of its concentration; for a given concentration, the effect is a function of time. The latent period is somewhat shortened and the average yield smaller than after induction with UV light. The survival curves which start exponentially then bend down, this change in the slope of the curve probably being due to the hydrolysis of nitrogen mustard.

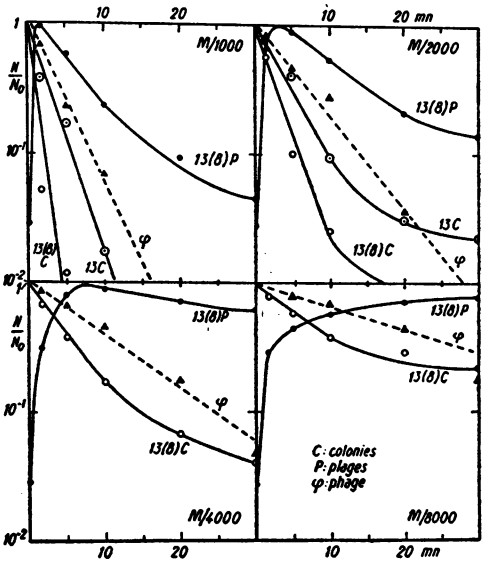


FIG. 8. Induction of phage production in *Pseudomonas pyocyanea* 13(8) by nitrogen mustard as a function of time of exposure to various concentrations. In abscissa, time of exposure to various molecular concentrations of nitrogen mustard. In ordinate, the ratio: infectious centers/original number of bacteria (curve P), surviving bacteria/original bacteria (curve C), surviving free phage 8/original free phage 8 (curve φ). From F. Jacob (53).

Site of Action of Inducing Agents

Relation between spontaneous and induced development of prophage. The study of a double lysogenic *P. pyocyanea* 13 (8) (4) carrying two inducible prophages has provided important data concerning the nature and succession of events in induction (54). Prophages 4 and 8 are both inducible and may develop together in a bacterium. At small UV doses, which induce a low proportion of bacteria, most bursts liberate only one phage, 4 or 8; high doses induce the development of both prophages. Clearly, the development of each prophage of the strain *P. pyocyanea* (8)(4) may be induced independently of the development of the other. Study of the spontaneous production of phage by *P. pyocyanea* (8)(4) has revealed that one bacterium in 600 produces phage spontaneously. Fifty per cent of the producing bacteria give exclusively phage 4; 25 to 30 per cent exclusively phage 8; 20 to 25 per cent yield phages 4 and 8. A high degree of correlation exists between the development of both prophages. The important thing to note is that in both spontaneous production, as well as in the production induced by small doses of UV, twice as many bacteria produce phage 4 than phage 8. In all inducible strains studied so far, both spontaneous and induced production appear to be strongly

correlated, suggesting that the primary phenomenon in the development of prophage is the same in both cases.[2]

The primary causes of phage production could be due either: (a) to a specific change of the prophage or (b) to a modification of the bacterium. Hypothesis (a), which could correspond to a direct hit in induction, or to mutation of the prophage in spontaneous production, is not in harmony with the experimental data. It would imply an absolute independence in the production of each phage, and the probability for one bacterium to produce simultaneously both phages should be less than 10^{-6}, whereas the experimental values are of the order of 5×10^{-4}. The hypothesis of a mutation of the prophage as a cause of the spontaneous phage production in inducible strains does not seem to account for the facts (54). Hypothesis (b) of bacterial modification implies that certain bacterial properties are modified, *e.g.*, the synthesis of a nucleic acid. The development of the prophage is a secondary event, the probability of which is governed by the metabolic state reached by the bacterium. Whatever be the primary effect, conceivably the secondary event might be heterogeneous. This would happen if the development of prophage depends on a substance produced as the result of irradiation in such small amount that it would be largely a matter of chance whether one or the other prophage would be submitted to its effect.

It is possible that, in a small proportion of bacteria, a perturbation caused either by gene mutation, or by a phenotypical change in the metabolism, would end in the production of an inducing substance such as an organic peroxide. The inducing substance could appear in a small or greater amount and then could induce the development either of one or of the two prophages.

Development of Temperate Phage Studied with UV Light

The vegetative phase of the life cycle of virulent bacteriophages has been extensively studied with the help of the Luria-Latarjet method which consists in analyzing the variation of the resistance of the bacterium-phage complex to UV light (see review by Latarjet, 63). The comparative study of the development of temperate phages after infection and induction has been made by Benzer and Jacob (12). For *E. coli* C and phage λ the phenomena are similar in infected and in induced bacteria. If differences exist, they cannot be detected.

The results are quite different in *P. pyocyanea* 13 infected by phage 8. After infection, the survival curve of the complex as infective center is much lower than the survival curve of the phage and of the "capacity" of the bacteria to reproduce bacteriophage (12). This increased sensitivity is observed during the first third of the latent period at which time the survival curve rejoins the curve of bacterial capacity; note that this is the first example of a drop of the resistance of the complex after infection. Induced *P. pyocyanea* 13 (8) shows quite different behavior. The drop in resistance of the complex is not observed here, indicating that the

[2] The study by Bertani (13) of the noninducible triple lysogenic *E. coli* Lisbonne (p1) (p2) (p3) revealed that a single bacterium releases only one type of prophage. The development of each of the various prophages is, in this case, an independent event.

bacterium-phage complex is not in the same state after infection and after induction.

Concluding Remarks

Inducibility is mainly controlled by the genetic constitution of the prophage. But the effects of a given dose of an inducing agent vary with the physiological state of the bacterium before the irradiation, and the ultimate effect of a given dose acting on a given bacterium in a given state may be modified after the irradiation by various factors. Owing to the interplay of these numerous factors, whose mode of action is not well understood, and owing to the lack of knowledge concerning the nature of early phases of phage development, the situation is far from being simple and clear.

Lysogeny is perpetuated in the form of prophage. By definition, as long as the prophage remains prophage, it is unable to develop into phage. Therefore in order that a lysogenic bacterium may be able to produce phage, the prophage must lose its nature of prophage and be converted into gonophage, the latter being the form of the genetic material of the phage during the vegetative phase. A unitary concept of induction will be proposed later. Provisionally, we will adopt the hypothesis that prophage is a specific structure anchored on a defined chromosomal locus, able to develop and to enter the vegetative phase only if it weighs anchor. This would be the effect of inducing agents; but how this effect is produced is another question.

Inducing agents seem to act in altering the chromosome-prophage equilibrium. This could be achieved by an alteration of the chromosome, an alteration of the prophage, or a modification of the environment of the chromosome-prophage system. The discussed data point against the hypothesis of a direct action of inducing agents on the prophage. The similarity between the spontaneous rate of production and the inducibility favors the conclusion that both phenomena are controlled by the same type of alteration. The effect of inducing agents would be to increase the probability of a change of the bacterial chromosome, the change being responsible for the detachment of the prophage.

The noninducible *E. coli* "Li" has a burst frequency of 1/45,000 per cell per generation (13), this low production rate is reminiscent of the rarity of mutations. But as discussed in the section *Immunity*, the development of certain prophages may be induced by virulent mutants of the homologous phage. This suggests that with inducible phages the primary event is not a specific architectural change of the prophage analogous to those that are supposed to be responsible for mutations. It could be simply that the alteration controlling the detachment of the prophage is of a different nature in inducible and noninducible systems.

It is clear from Jacob's experiments that the development of prophage into phage, the vegetative phase, starts only some minutes after the irradiation. It seems as though inducing agents initiate a series of bacterial alterations culminating in the conversion of prophage into gonophage, which might correspond to the breakage of the link of the prophage with the chromosome.

VI. INDUCED ABORTIVE PHAGE DEVELOPMENT

"Je ne me débarrasse pas d'un certain étonnement que les choses soient comme elles sont, et elles seraient tout à coup différentes, il me semble que cela ne m'étonnerait pas beaucoup davantage".—André Gide, *Journal*.

We are used to thinking of viruses as pathogenic particles. That the multiplication of phage is a lethal process is in itself not surprising, but the notion that the synthesis of a nonvirus material may also be fatal is quite recent. Two abnormal, fatal types of biosyntheses will be considered: abortive development of bacteriophage in this section, biosynthesis of bacteriocins in the next.

B. megaterium (1) cured from prophage 1 may be lysogenized with phage 1. Most of the strains behave like the original one: 10^{-2} to 10^{-3} bacteria produce phage spontaneously. After induction, all lysogenic bacteria produce bacteriophage and lyse. But one of the lysogenic strains, 91(1), behaves differently. In exponentially growing cultures, one phage is found for 1,000 bacteria; if one bacterium liberates 100 phages, this means that only one bacterium out of 10^5 produces phage spontaneously. After irradiation, 91(1) shows the classical picture: residual bacterial growth followed by lysis; but lysis is not accompanied by phage liberation.

The abnormal behavior of *B. megaterium* 91(1) may be due to an abnormality of the bacterium, or of the prophage, or to an abnormal interrelation of bacterium and prophage. The phage produced spontaneously by strain 91(1) is normal, and all the bacteria lysogenized with this phage produce phage after induction. It is therefore possible that the abnormality of the strain 91(1) is bound to an abnormality in the prophage-chromosome link (hypothetical link). Lwoff and Siminovitch (78) concluded that the lysis of 91(1) after irradiation is due to an abortive development of prophage. Two questions immediately arise:

(i) *Is 91(1) producing a phage that we are unable to detect?* Electron microscopy shows that it is not the answer.

(ii) *Why is development abortive?* Estimations of desoxyribonucleic acid (DNA) during the latent period show: (a) that during the first half of this period there is no increase of DNA; (b) that during the second half of the latent period, the synthesis of DNA is meager as compared with the synthesis of normal lysogenic strains during the same period (91). Siminovitch concluded that the abortive development of phage in *B. megaterium* 91(1) arises from defect in DNA synthesis. An obvious hypothesis is that UV induces only the development of the protein part of the phage.

If we neglect the one bacterium in 10^5 which spontaneously produces bacteriophage, 91(1) appears as a strain genetically hypersensitive to UV. The potential lethal factor is prophage, but only one part of its development takes place after induction, and the bacteria are killed without phage being produced. Behavior of 91(1) provides a bridge between lethal actions due to the development of a phage and to the *production* of bacteriocins.

VII. BACTERIOCINOGENY AND LETHAL BIOSYNTHESES

"Ne permettes pas que je juge d'après ce que l'oeil aperçoit au dehors, ni que je forme mes sentiments sur les discours insensés des hommes . . ."—*L'Imitation de Jésus Christ*.

Colicins were discovered by Gratia and extensively studied by Gratia and by Frédéricq (review in Frédéricq, 34). Produced by certain strains of *E. coli*, they

are able to kill sensitive bacteria of the same species. Sensitivity is controlled by a specific receptor, and as shown by Frédéricq, some colicins have the same receptor as some phages. That they are not reproduced in the sensitive bacteria that they kill, should be put in parallel with the fact that they are proteins and not nucleoproteins.

Jacob *et al.* (56) discovered that the colicinogenic bacterium, *E. coli* ML, may be induced to produce colicin with UV light (figure 9). Normally, the colicinogenic bacteria do not contain any detectable amounts of colicin, but after irradiation, the colicin synthesis starts immediately, and colicin accumulates inside the bacterium until it lyses, around the 70th minute. During this latent period, bacteria continue to grow, to synthesize respiratory enzymes, ribo- and desoxyribonucleic acids, are able to synthesize "adaptive" enzyme, and they allow the development of a virulent phage. Induction has been obtained with UV light and with chemi-

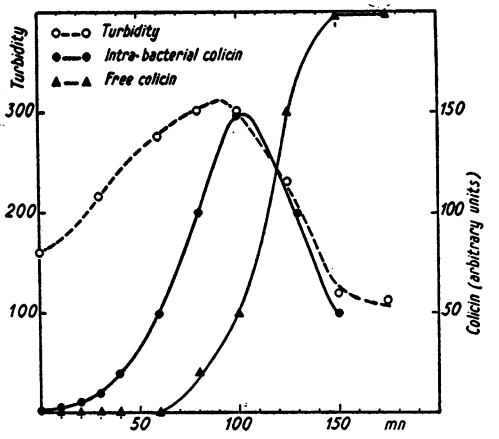


FIG. 9. Colicinogeny. *Escherichia coli* ML has been irradiated with UV light at time 0. The bacteria grow and begin to lyse around the 80th minute. Intrabacterial colicin appears shortly after induction (absence of negative phase). Free colicin appears when the bacteria lyse. From F. Jacob, L. Siminovitch and Elie Wollman (56).

cal inducers (see section on *Induction*). The response to UV of bacteriocinogenic bacteria is controlled by their physiological state just as is the response of lysogenic bacteria. The expression of colicinogeny being lethal just as the expression of lysogeny, colicinogeny can only be perpetuated as a potential property.

The study of the killing of sensitive bacteria has revealed that one particle of colicin is sufficient to kill a bacterium, the adsorption constant, $K = 8 \times 10^{-10}$ cm³/min, being of the same order of magnitude as for "good" phages. A bacterium having adsorbed one particle of colicin immediately ceases to grow. Its respiration remains constant, the induced synthesis of "adaptive" β-galactosidase is no longer possible, and it synthesizes neither RNA nor DNA. Moreover, if colicin is added to bacteria infected with the virulent phage φ_2, less than 7 minutes after infection, no production of phage occurs. The effect of colicin is comparable to that of the protein of the tail of phage T2, responsible for attachment to the receptor and probably for the lethal activity of the "ghost". This tail's protein, as shown by the Lannis (62), is serologically different from the protein coating of the phage. Similarly, a strain of *P. pyocyanea* if irradiated starts synthesizing a bactericidal substance, *pyocin*, which is liberated by lysis and acts on a strain of the same species (F. Jacob, unpublished data). One particle of pyocin is enough

to kill one bacterium. The term bacteriocin has been proposed (57) for the substances possessing the general properties of colicin and pyocin.

Thus, bacteriocinogenic bacteria perpetuate hereditarily the power to produce bacteriocins just as lysogenic bacteria perpetuate hereditarily the power to produce bacteriophages. The analogy of the two situations is striking: (a) both phage and bacteriocin production are perpetuated as potential properties; (b) in both, the lethal biosynthesis allows bacterial growth; (c) both processes may be induced by ultraviolet light and some radiomimetic substances; (d) adsorption is controlled by a specific receptor, sometimes common for phage and bacteriocin; (e) one particle of the substance is enough to kill a sensitive bacterium. The differences are: (a) bacteriophages are nucleoprotein particles whereas bacteriocins are proteins; (b) bacteriophage is generally reproduced in the bacterium which it kills whereas bacteriocins are not reproduced.

The hypothesis can be considered that bacteriocinogenic bacteria are related to lysogenic bacteria in the sense that they possess genetically the ability to form a protein corresponding to the tail's protein of a phage. But bacteriocins being lethal, the hypothetical corresponding phage, because it would kill, could not be temperate (section XIII). Bacteriocinogeny, at least corresponding to the type of bacteriocins we know, therefore cannot be considered as a step in the phylogeny of lysogeny. Thus, the synthesis of bacteriocins is fatal and bacteriocins may kill receptive bacteria. But we may also conceive of a bacterium being killed because its equilibrium is displaced by the synthesis of a substance devoid of pathogenicity for other bacteria. Penicillin kills bacteria only insofar as they grow; the lethal disequilibrium produced by the antibiotic takes place only when syntheses are performed. The hypothesis has been advanced that the lethal effect of some antibiotics could be ascribed to abnormal syntheses (73). Such syntheses of an abnormal material could be responsible for some degenerative processes in plants or animals.

Thus, when considering lysogenic and bacteriocinogenic bacteria and the pathogenic action of temperate and virulent phages and of bacteriocins, we see:

(i) that the development of phage, whether temperate or virulent is always lethal;

(ii) that the abortive development of phage and the synthesis of bacteriocin are lethal;

(iii) that two reasons exist why a bacterium infected by an extreme virulent phage of the T2 type should expect to be killed: because of the impact of the end protein of the tail and because phage develops.

The notion that the mere synthesis of a protein may be lethal has to be kept in mind. And it is important, when studying lethal particles or viruses, to discriminate between the possible effect of their impact on the receptive cell and the effects of their production or reproduction.

VIII. EFFECTS OF DEVELOPMENT OF TEMPERATE PHAGES ON THE BACTERIUM

"Nous n'avons pas cru devoir entreprendre d'expliquer, en tout ni en partie, les Faits singuliers que nous avons rapportés. Il est trop dangereux, en fait d'Histoire Naturelle, d'abandonner l'expérience pour se laisser conduire par l'imagination. On risque de n'arriver, en suivant cette route, qu'à des Hypothèses peu sûres, et qui peuvent devenir nuisibles au progrès de cette Science, si on a le malheur de se prévenir pour elles . . ."—A. Trembley, *Mémoires pour servir à l'Histoire d'un genre de Polype d'eau douce à bras en forme de cornes*, Leiden, 1744.

Since the pioneer work of Seymour Cohen (28a) the effects of infection with virulent phages have been studied extensively. As a result of the penetration of phage T2 in *E. coli*, the following are observed: (a) block of bacterial growth; (b) block of the synthesis of ribonucleic acid; (c) block of the synthesis of respiratory enzymes; (d) inability to synthesize "adaptive" enzymes, a reflection of the inability to synthesize any bacterial constituent; (e) block in the synthesis of bacterial DNA; (f) absence of increase of total DNA during the first part of the latent period, followed by a rapid increase of phage DNA. Herriott's (44a) demonstration that the protein coat of the phage or "ghost" kills the bacteria and apparently blocks all the bacterial synthesis makes it difficult to discriminate in the pathological events following phage infection between the effect of the coat and the effect of phage development proper. Study of lysogenic bacteria allows some clear-cut conclusions regarding this problem.

Residual growth. As soon as induction by UV light was discovered, it was observed that during the latent period, between the inducing shock and lysis, bacterial growth continued; this was called *residual growth* (79), a term which will be justified later. The residual growth, in lysogenic *B. megaterium* as well as in other lysogenic species, corresponds generally to a doubling (occasionally to a three to fourfold increase) of the original material. The bacterial growth rate during the latent period is slower than the normal growth rate and decreases until lysis (79). Residual growth is easy to follow by turbidity measurements. That the increase in turbidity is not a mere swelling is shown by biochemical studies, as during the latent period respiration and RNA increase. The curves of respiration rate and of ribonucleic acid (figure 10) are parallel to the curve of bacterial growth as expressed by turbidity measurements (94). Not only do bacterial syntheses continue, but new syntheses may be started. *P. pyocyanea* grown in lactate media utilizes glucose only after a 40 minute latent period necessary for the synthesis of the specific enzymes involved. The induced synthesis of the system allowing utilization of glucose is possible in induced as well as in noninduced bacteria (50a).

E. coli K12 (λ) grown in the presence of maltose does not contain β-galactosidase. Synthesis of β-galactosidase takes place if lactose is added, even if the lactose is added 50 minutes after the irradiation, *i.e.*, at a period when the first phage particles appear. Once started, the speed of synthesis is constant until lysis. But the later the lactose is added, the lower the rate (93) (see figure 11). It is postulated that induced biosynthesis of enzymes depends on an "organizer" (81, 87); apparently with UV irradiated lysogenic *E. coli* the amount of organizer which can be formed is decreasing while the development of phage is taking place.

The cytological observation of induced *B. megaterium* shows an increase of size during the latent period; a hypertrophy of the bacterial nucleus is also observed (30). During the development of bacteriophage T2 in *E. coli*, the Feulgen positive material of the bacterial nucleus is dispersed in the cytoplasm (69a), but this does not occur during the latent period of induced *B. megaterium*.

Desoxyribonucleic acid. At a temperature of 27 C, after a given dose of UV, the first intrabacterial phage of *B. megaterium* appears around the 80th minute, the first bacterial burst occurring around the 95th minute. During the first 30 min-

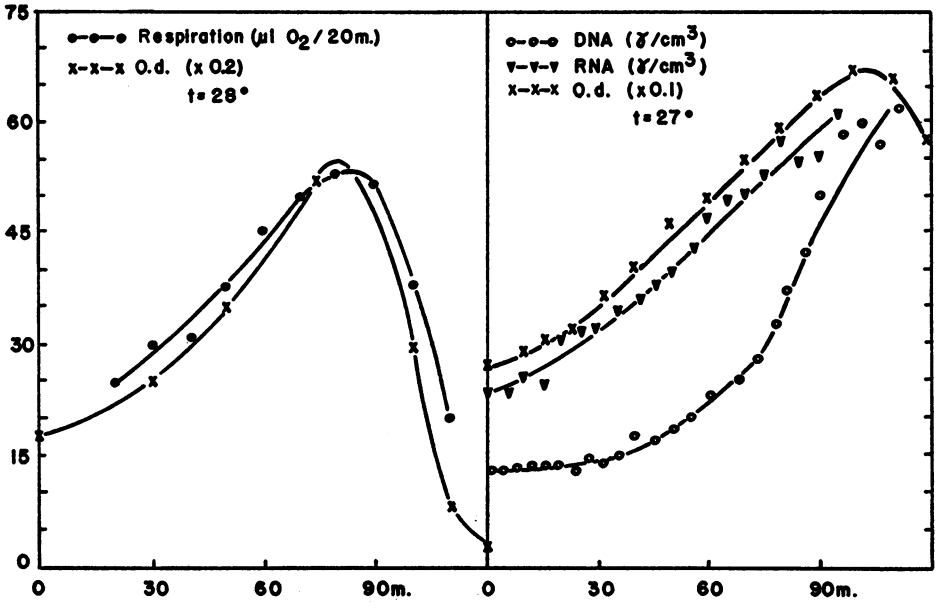


FIG. 10. Residual bacterial growth after UV irradiation. *Bacillus megaterium* has been irradiated at time 0 and the optical density, respiration, desoxy- and ribonucleic acid measured at various intervals. From L. Siminovitch and S. Rapkine (94).

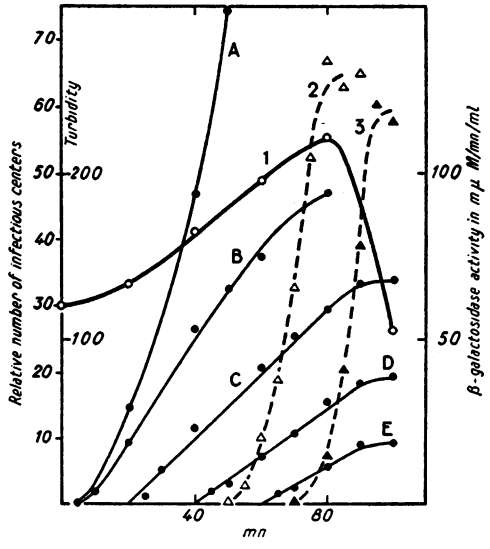


FIG. 11. Synthesis of β-galactosidase during the induced development of bacteriophage in *E. coli* K12 (λ).

1. Growth and lysis of induced bacteria; 2 and 3. phage production: (2) bacteria opened with cyanide; (3) spontaneous liberation of phage. A to E. β-Galactosidase activity. The origin of the curves corresponds to the time at which lactose is added as inducer of the synthesis of β-galactosidase (maltose as carbon source). A. Nonirradiated control; B to E. Lactose added at various times after irradiation. From L. Siminovitch and F. Jacob (93).

utes of the latent period, the amount of total DNA remains constant, then it increases at a rate higher than the normal DNA synthesis of the noninduced bacterium (94) (see figure 10). The length of the period when the DNA remains constant varies with circumstances. At 27 C after heavy irradiation, it is 50 min

(latent period 140 min); at 37 C it is 15 min (latent period 43 min). In irradiated nonlysogenic bacteria, no block in DNA synthesis is observed.

* * * *

The continuation of bacterial syntheses during phage development has been observed after infection by a temperate phage as well as after induction of the development of prophage in *B. megaterium*, *P. pyocyanea*, *E. coli*, and *Salmonella typhi-murium*. The arrest of all observed syntheses during the development of phage T2 in *E. coli* also occurs as the result of the impact of the protein coat or ghost, *i.e.*, in the absence of any phage development. The block of the synthesis of bacterial DNA is therefore difficult to interpret.

In view of the general continuation of syntheses during the development of temperate phages, the block in DNA synthesis observed after induction of *B. megaterium* during the first phase of the latent period takes its full significance. This block was considered as specifically related to the development of phage (94). But it has been recently discovered by L. Simonovitch (personal communication) that after infection of *E. coli* K12 by the phage λ c or of *S. typhi-murium* by the phage Ac the synthesis of desoxyribonucleic acid continues, the curve of DNA being parallel to the curve of bacterial growth during the first phase of the latent period. From these observations, it is concluded that some temperate phage may develop without necessarily blocking the synthesis of bacterial DNA.

Nature of residual growth. Residual bacterial growth can be experimentally suppressed by submitting bacterial population to a low and constant "*régime*" or rate of feeding. The *régime* is defined by the number of molecules of substrate available per unit time (52). This type of feeding is obtained for *P. pyocyanea* by the system lactose plus β-galactosidase which liberates glucose and galactose at a constant speed. Neither lactose nor galactose is utilized by the bacterium which can feed only on the glucose produced by the enzyme at a rate proportional to its concentration. With low concentration of enzyme or low *régimes*, the increase of bacterial substance is linear; this means that bacterial growth takes place at a constantly decreasing rate.

In the presence of an excess of glucose, the normal generation time is 70 min; after induction or infection, residual growth takes place. When the *régime* is fixed at a rate such that the bacterial mass would double in 6 hours in the noninfected control, the respiration is decreased to one-third of the normal, and no residual bacterial growth is observed after induction or infection. But the latent period of the phage is not modified; only the average yield is slightly decreased. For lower *régimes*, the latent period is increased and the average yield decreased a little more (52) (see figure 12). Thus, during the development of a temperate phage, the decrease of the *régime* affects the bacterial growth before affecting the development of phage. It appears as if, below a certain *régime*, the available glucose is utilized only for phage synthesis; only when the *régime* is higher, is a surplus of glucose available for bacterial synthesis. This is not what would be expected if there was the equivalent of an equilibrium between phage and bac-

terial syntheses. Taking advantage of this method, Jacob calculated that a bacterium of strain 13-8 of *P. pyocyanea* needs 10^{10} molecules of glucose to double itself. For the production of around 50 phages p8, 2.6×10^9 molecules are needed, this figure being probably higher than the real need.

It is also possible to limit the bacterial growth rate by means of the nitrogen *régime*, the nitrogen source being provided by the system: urea + urease. Here

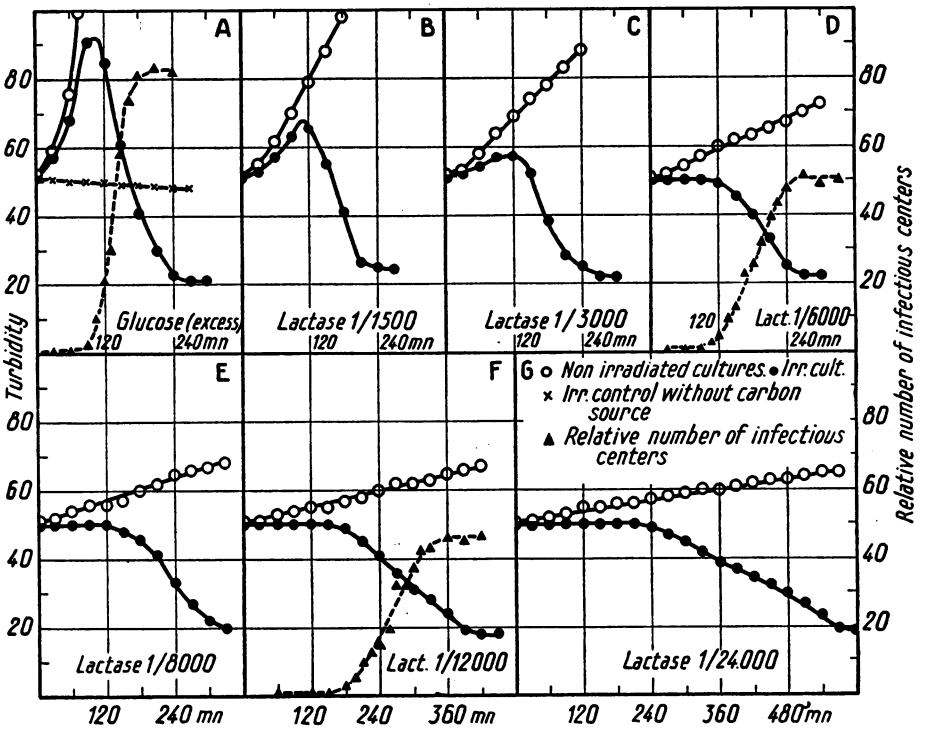


FIG. 12. Suppression of the residual growth of bacteria by a low "régime". The lysogenic *Pseudomonas pyocyanea* is fed by the glucose liberated from lactose by the lactase (β-galactosidase) at various concentrations. Note that the growth curve of the control is linear and that the residual growth is suppressed for low *régimes* corresponding to low enzyme concentrations. From F. Jacob (52).

again, for a low *régime*, the bacterial growth is suppressed, whereas the development of phage continues. Obviously the bacterial growth observed during the latent period takes place at the expense of the materials, nitrogen and carbon source, which are not utilized by the phage. This justifies the expression *residual bacterial growth.*

IX. LYSOGENIZATION

"The body which was outisde the real being, thus came into the real being, and took its place in the living world . . ."—Plotinus, *6th Ennead.*

History

Lysogenization is the action of conferring to a bacterium the hereditary power to produce a given phage. Lysogenization, as lysogeny, was discovered in 1925

by Bail (10) and by Bordet (16) who observed that when nonlysogenic bacteria are mixed with certain phages some of the surviving clones may be lysogenic. This is the first example of a specific hereditary property being conferred to an organism by a specific extrinsic particle. As understood by Bail and by Bordet and clearly demonstrated by Burnet and McKie (25), lysogenization is specific: a lysogenized bacterium produces a phage which is identical with the original infecting particle. Bordet (16) also observed that different bacterial strains reacted differently to various phages. With some phages the survivors were rare and nonlysogenic, whereas with others the survivors were numerous and lysogenic.

The survival of bacteria exposed to *virulent* bacteriophages is not the result of the action of the bacteriophage but represents the selection of preexisting bacterial mutants (4, 25). For the time being, this is known to be true only for virulent phages. Studying in 1936 the lysogenization of a micrococcus, Burnet and Lush (24) concluded that the alternative of selection by the temperate phage of preexistent variants was definitely excluded, a conclusion based on the fact that 10 to 20 per cent of the phages would, under experimental conditions, induce the appearance of a "resistant" lysogenic colony. The lysogenic cocci studied by them normally produced a phage giving turbid plaques, but mutants may appear which give clear ones. The lysogenic cocci "turbid" are immune towards the phage "clear". The Australian workers realized that this system would be useful for the study of lysogenization. Unfortunately, the adsorption in their system was poor, and the nature of resistance, immunity or nonreceptivity, was not established with certainty.

Before examining the various aspects of lysogenization as disclosed by recent research, a few words concerning the reactions of bacteria towards phage infection are necessary.

Bacterial Responses to Temperate Phage

Let us mix temperate phages and a nonlysogenic bacterial population and consider the fate of individual bacteria. A bacterium is either infected (I) or not (II).

I.—The Bacterium Has Been Infected.

Either it survives (A) or not (B).

A. *The bacterium survives*

(i) It gives rise to a lysogenic clone. Infection has been reductive. The infecting phage has been reduced to prophage. The bacterium has been lysogenized.

(ii) It gives rise to a nonlysogenic clone. The infecting phage has disappeared. The infection has been *abortive*. The bacterium behaves as if noninfected (situation II B).

B. *The bacterium is killed.* It may, or may not, produce phage.

(i) It produces phages. The infection has been *productive*.

(ii) It does not produce phages. The infection has been *abortive*.

II.—The Bacterium Has Not Been Infected or Behaves As if Noninfected.

A. *The survivor gives rise to a nonreceptive, nonlysogenic clone.* A preexisting, nonreceptive mutant has apparently been selected.

B. *The survivor gives rise to a clone, the members of which behave as the original population.* Either the bacterium has not been infected or it has been infected and the phage has disappeared (see I Aii).

A bacterial strain may be used as a detector only if under the conditions of phage estimation the majority of phages give rise to a plaque, that is to say, if the majority of bacteria are able to adsorb and to reproduce phage. Therefore, under the conditions of phage estimation, a detector strain is composed by a majority of bacteria giving a productive response, that is to say, behaving as sensitives. As will be seen later, the members of a nonlysogenic population may be involved in a variety of situations in which the fate of the individual bacteria is controlled, not only by their genetical constitution, but also by extrinsic factors. The bacteria of a detector strain, although genotypically sensitives, may be largely phenotypically resistant. They could be called *detector bacteria.*

Genetic Control of Lysogenic Response

It has been known for a long time that temperate phages producing turbid plaques, that is to say, those allowing a high proportion of lysogenic response, may give rise to mutants producing clear plaques, in which a small number of lysogenics are recovered, or sometimes no lysogenics at all. These types of mutants have been found on a phage of *Micrococcus* (24), *B. megaterium* (82), *S. typhi-murium* (21), and *E. coli* K12 (58a and M. Lieb, unpublished). Phage A_1 producing turbid plaques on the indicator strain of *S. typhi-murium* forms mutants Ac producing clear plaques. Whereas the percentage of lysogenic responses to phage A_1 may be as high as 80, it is around 0.1 for phage Ac (77). Similar examples of variation have been found with phages of *P. pyocyanea.* The proportion of bacteria giving a lysogenic response is 10^{-2} with the phage $P8u^+$ and 10^{-4} with the mutant P8u (58).

Thus, the lysogenic response of a bacterium depends on the genetic constitution of the phage. It has been seen that it depends also on the phenotypical properties of the bacterium, and as will be seen, it is also controlled by the bacterial genetical constitution. When in a given medium a given phage is allowed to act on two different bacterial strains, the result may be quite different. One phage of *B. megaterium* produces turbid plaques on two strains but clear plaques on another (H. Ionesco, unpublished). The same type of observations has been made for one phage of *P. pyocyanea* (F. Jacob, unpublished). Here the response of the bacterium to a given phage seems to be controlled by bacterial genetic constitution.

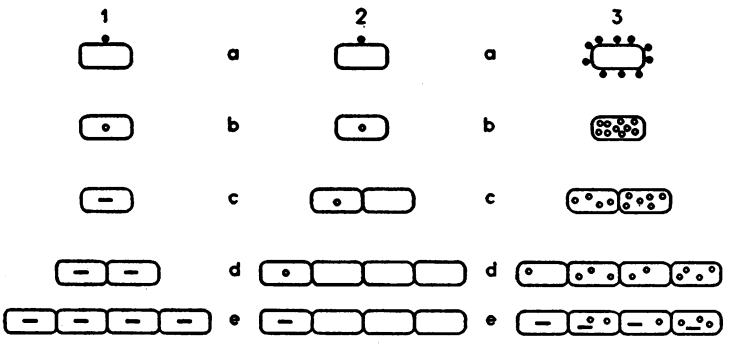
The problem of lysogenization is complicated by the fact that the bacterial response does not depend solely on the genetic constitution of the phage and of the bacterium as such, but on the interrelation or interaction of genetic materials.

Thus, the bacterial response to a temperate phage is under the triple control of the genetic constitution of the phage and of the genotypical and phenotypical constitution of the bacterium. When studying the responses of a given bacterial population to a given phage, one reaches the impression that this response is controlled by environmental factors. But all properties are in the last analysis controlled by the genetic constitution of the bacterium. And it is quite possible

that in some cases, only some rare bacterial mutants are able to give the lysogenic response. Theoretically, lysogenic survivors could sometimes correspond to phage-selected bacterial mutants.

Nongenetic Factors

Independently, Bertani and Lieb have attacked the problem of the nature of the factors controlling the lysogenic response of a bacterium. According to Bertani (14) the fate of *Shigella dysenteriae* infected by the temperate phage P1 depends in particular on the temperature. The proportion of infected bacteria producing phage is 4 to 5 times higher at 37 C than at 25 C; a higher percentage of lysogenization is obtained at 25 C than at 37 C. From an experiment of this



• *Infecting phage*, – *Prophage*, ∘ *Genetic material of the infecting phage in the pre-reduced state.*

FIG. 13. Lysogenization. Scheme in the system *E. coli* K12 and λ

1. Single infection. The reduction into prophage takes place before any bacterial division. All descendants are lysogenic. (*E. coli* K12 and λ at 25 C.)
2. Single infection. The reduction takes place after two divisions. 1 clone/4 is lysogenic (*E. coli* K12 and λ at 37 C.)
3. Multiple infection at 37 C. The reduction takes place after two divisions. All the clones are lysogenic.

type, obviously the productive or reductive response does not depend only on the genetic constitution of the bacterium or of the bacteriophage. This has been established by Lieb (67) to whom we owe an extensive study of the system *E. coli* K12 + phage λ. All types of bacterial responses are found when λ is added to *E. coli* K12. The important point is that these responses are controlled by the previous history of the culture, and especially by its physiological condition.

The proportion of the receptive bacteria varies according to the phase of the development in broth, indicating that the modification of the broth brought about by the microbial metabolism induces changes in the properties of the bacteria. "Old" bacteria adsorb phage much better than "young" ones, old and young referring here to the phase of development of the culture. The power of adsorption of a given bacterium is not modified by the suspension in an "old or young" medium. The adsorbing activity of old bacteria suspended in a young medium is significantly modified only after one or two hours (67). This difference between

young and old bacteria is not absolute. Young bacteria may be infected provided the multiplicity is high, suggesting that poor adsorption is the result of a low efficiency of the collisions between phage and bacteria. The poor adsorbers give a greater percentage of lysogenic response than the good adsorbers. According to Lieb, the decision as to whether the infection of *E. coli* K12 will be productive or reductive is taken either during infection or immediately after and cannot be modified. As will be seen, this does not mean that the phage is reduced into prophage immediately. The proportion of lysogenic descendants of a single infected bacterium varies with the temperature. It is higher at 25 C than at 37 C, this being true for a low multiplicity. For a multiplicity of 10 at 25 C, the infected bacteria give rise to clones containing lysogenics. At 42 C, one-half of the infected bacteria give rise exclusively to nonlysogenic clones (figure 13).

A lysogenic *E. coli* K12 (λ) cannot be cured from its lysogeny at 45 C: the prophage is not affected by high temperatures. The fact that bacteria can be cured from their potential lysogeny just after infection shows that the specific particle is not yet in the state of prophage. The time necessary for the infecting phage to attain the temperature resistance of the prophage is around one hour at 37 C, more than one hour at 25 C.

The following picture of lysogenization derives from Lieb's experiments. The response of *E. coli* to phage λ is determined by its previous life history; the fate of the infecting phage, or of its genetic material, is decided at the time of infection and might depend on the properties of the bacterial receptors or other related bacterial properties. A rapid, so far as we know, irreversible modification is undergone by the infecting phage. Apparently, it is either a reaction on the way to vegetative phase (*i.e.*, to phage development) or a reaction on the way to reduction into prophage. The future prophage will remain protoplasmic for one hour at 37 C; unable to duplicate and temperature sensitive. As the bacterium divides, the number of daughter bacteria possessing at least *one* such particle will depend: (a) on the initial number of particles; (b) on the rate of bacterial division; (c) on the rate of conversion of the particle into prophage. As the rate of the "reduction" is less sensitive to temperature variation than the bacterial multiplication, more lysogenic clones are obtained at 25 C than at 37 C from the descendants of one singly infected bacterium. According to Lieb's interpretation, the final step of the reduction is the attachment to a regularly inherited bacterial structure, the most obvious location being a chromosome. It should be added that the growth rate of nonlysogenic and lysogenic bacteria is the same, whereas the multiplication rate of a bacterium undergoing lysogenization, that is to say between infection and reduction, is markedly decreased.

According to Bertani (personal communication), during the one hour period preceding reduction, *Shigella* is not immune towards the clear mutant of the temperate phages. This phenomenon will be further discussed in the section on *Immunity*. Thus, the future prophage is a cytoplasmic structure, unable to divide, which behaves during bacterial division as an inert particle. Its presence does not confer immunity.

Influence of Multiplicity

Boyd (21) discovered in 1951 that *S. typhi-murium* behaves differently towards a phage A according to the multiplicity of infection. No visible lysis occurs when the ratio phage/bacterium is 10, whereas visible lysis does occur when this ratio is 1. When the detector strain is plated with "excess" phage, 72 per cent of the initial bacteria may be recovered as lysogenic colonies, and a smaller percentage with lower phage concentration. This effect would be, according to Boyd, because the population of phage A contains two types of phages, only some of which are able to lysogenize.

Starting from this interpretation, Boyd has proposed a new conception of lysogeny, according to which the development of prophage into phage is the consequence of a mutation. The mutated prophage develops and then back-mutates. Thus, two types of phages would be produced, only one being temperate. At present, an influence of multiplicity on lysogenization has been observed only with the phage A of *S. typhi-murium* and seems therefore to be a rare property. It is perhaps dangerous to build a general conception of lysogeny on an entirely hypothetical interpretation of this phenomenon, implying an *ad hoc* mutation of the prophage and a reverse *ad hoc* mutation of the genetic material during the vegetative phase.

Actually, the character "multiplicity" may be lost as a result of a phage mutation. As already stated, a mutant of phage A producing clear plaques, Ac, has been isolated (76, 77). Whereas the percentage of lysogenic response reaches 80 per cent with phage A, it is around 0.1 per cent with phage Ac and is the same when the multiplicity varies from 5 to 96. For all practical purposes the mutant Ac is stable, and the lysogenic strain *S. typhi-murium* (Ac) produces only phage Ac. But as will be seen, the response of the bacteria is controlled not only by the genetic constitution of the phage and by multiplicity, but also may be modified by a variety of factors acting either after or before infection.

The percentage of lysogenic response is controlled by the composition of the medium in which the bacteria are growing. Various substances (lactic, pyruvic, fumaric, succinic, L-malic, citric and malonic acids) added to broth decrease the proportion of lysogenic responses. Factors or substances altering the metabolism that produce the same effect include: anaerobiosis, heating at 42 C for 10 minutes after infection, or 2-4-dinitrophenol 6×10^{-4} M, a concentration that allows bacterial growth at reduced rate. Bacterial growth that has ceased as a result of the action of sulfanilamide or of 4-amino-pteroyl glutamic acid also results in a greatly reduced (10 to 20-fold) percentage of lysogenic responses. If bacteria are irradiated with UV light before infection, the percentage of productive response is considerably increased (75).

Addition of 5-methyl tryptophan may produce a 25-fold decrease of lysogenic response, provided it is added before the 6th minute after infection; it has no effect after the 9–10th minute. Incubation of infected bacteria with 5-methyl tryptophan does not modify the proportion of lysogenic responses provided tryptophan is added before the 6th minute after infection, but tryptophan does

not reverse the 5-methyl tryptophan effect if added after the 10th minute. Thus, under experimental conditions, the fate of an infected bacterium may be modified by 5-methyl tryptophan only between the 6th and the 10th minute. An irreversible process towards gonophage or towards prophage takes place during this critical period. What could this reaction be? Cohen and Anderson (28b) have shown that 5-methyl tryptophan blocks completely the development of phage T2 in *E. coli* and that its action is reversed by tryptophan. The latent period is increased by a period equal to that during which the bacteria have been in contact with 5-methyl tryptophan. This substance blocks completely protein synthesis, while allowing the synthesis of desoxyribo- and of ribonucleic acids (L. Siminovitch, unpublished).

In *S. typhi-murium*, the appearance of intrabacterial infecting phages, as revealed by Doermann's technique with cyanide, starts exactly at the same time (around the 20th minute) and follows the same curve, whether or not 5-methyl tryptophan has been present for 6 minutes after infection. If bacteria have lived for 18 minutes with 5-methyl tryptophan, infecting phage appears 12 to 13 minutes later than in the control. Therefore, in those bacteria which are to produce phage, there is a 6 minute delay between infection and the beginning of protein syntheses. In those bacteria which would become lysogenic, no irreversible reaction takes place during this neutral period. The fate of the system will be decided during the following 3 minute interval when, in the absence of 5-methyl tryptophan, reduction will take place. In the presence of 5-methyl tryptophan 96 per cent of the would-be lysogenics will produce phage. Thus, the irreversible reaction leading to the vegetative phase can proceed in the presence of 5-methyl tryptophan. For the time being the only conclusion that can be drawn from these experiments is that lysogenization is bound to a much more delicate balance than the reaction germ → gonophage.

Thus, the fate of *S. typhi-murium* seems to depend on whether one or the other reaction, germ → gonophage or germ → prophage, will take place first. The conclusion that the fate of the bacterium does not follow immediately the penetration of phage is confirmed by the fact that bacteria infected with the mutant Ac may be rescued, that is to say, may be prevented from producing phage Ac if infected simultaneously by phage A. This is apparently sufficient to allow the reduction of phage A into prophage, conferring immunity towards phage Ac, to be completed before the irreversible step in the development of phage Ac has taken place. The period during which the infected bacterium may be saved is increased by glucose starvation and by chloromycetin (chloramphenicol). Moreover, in phage Ac as in phage A, the first phase of 6 minutes after infection is not affected by 5-methyl tryptophan. Thus, the rate of the irreversible process, infecting phage → gonophage, is decreased in the absence of an energy source.

The interpretation of these facts is difficult. It appears as though the fate of the infecting phage is controlled by the probability of one or another reaction taking place. It could well be that only one reaction is involved, but that the effects are different according to the state of the phage-material in the bacterium at the time of the reaction, *e.g.*, lysogenization would take place only if the germ is in

the vicinity of a given chromosomal locus. It would then be readily understood why the probability of lysogenization increases with multiplicity. But the difficulty, of course, is that multiplicity acts only, so far as we know, in the case of phage A of *S. typhi-murium*.

Whatever the reason may be, the reaction of *S. typhi-murium* to phage A is obviously not controlled solely by the genetic constitution of the phage. The productive or reductive response of a bacterium is determined under given conditions by the multiplicity. And, for a given multiplicity, the response may be modified by various agents or treatments acting or applied before or after infection.

Conclusions

From all these data, which unfortunately concern a very small number of systems, the following conclusions may be drawn. The lysogenic response of a bacterium towards a phage is controlled by the genetic constitution of the bacterium and of the phage, by the interaction of these genetic structures, by the phenotypical traits of the bacterium, by its actual metabolism, and with one organism, at least, by the multiplicity of infection. With one strain of *E. coli* the fate of the infecting phage, namely, whether it will enter the vegetative phase or be reduced, is determined immediately after its penetration, and the reduction into prophage takes place after one hour. With *S. typhi-murium* the fate of the infecting phage is determined between the 6th and the 10th minutes after infection, and immunity seems to be established within the same limits of time. Evidently, a comparative study of a variety of systems is necessary to provide essential data, not only on lysogenization but also on the phases of phage development.

X. INCOMPATIBILITY

"Angriffstellen aller Heilstoffe sind bestimmte Rezeptoren der Zelle."—Paul Ehrlich, *Aus Theorie und praxis der Chemotherapie.*

It has been long known that some lysogenic bacteria may perpetuate a number of different prophages: some staphylococci may produce 5 types of phages (100). Let us consider the reactions of a receptive immune lysogenic bacterium. The homologous phages and some of their mutants can penetrate into the lysogenic bacterium which is not killed, and the genetic material of the infecting phages is able to survive but does not multiply nor develop. Bertani (14) has studied the fate of *S. dysenteriae* after infection with various mutants of the homologous phage. Double lysogenic strains may be obtained, each bacterium when producing phages, yielding *both* phages in the ratio 1/1. Most of the lysogenic bacteria infected with a mutant continue to be lysogenic for the originally carried prophage. But, some have been found to produce the mutant instead of the original phage; the original prophage has been replaced by a mutant.

Thus, a *substitution* of one prophage by a related prophage may take place, whereas, generally, an *addition* of one related prophage to another is more rare. This is *incompatibility* as discovered and defined by Bertani. It appears as though

the reduction into prophage of the germ of an infecting phage is impossible in a lysogenic bacterium carrying an homologous or related prophage.

According to Bertani, the most likely hypothesis is that each lysogenic bacterium or, perhaps, each nucleus of a lysogenic bacterium carries only one prophage which behaves as a bacterial genetic locus or piece of chromosome. As a matter of fact, one simple hypothesis which may account for incompatibility is that reduction can take place only at one specific locus of the bacterial chromosome, different for each phage. If the position is occupied, the reduction cannot take place. In this conception, a different locus of the bacterial chromosome corresponds to each structurally different temperate phage. No difficulty is had in visualizing multiple heterologous lysogeny. Double lysogenic bacteria perpetuating related prophages would, according to Bertani's interpretation, be diploid bacteria, heterozygous for the prophage, or bacteria having duplicated that particular phage-carrying part of the chromosome. Let us recall (see section on *Prophage*) that related prophages may undergo recombination in double lysogenic bacteria, thus behaving like allelic structures. In general, it is difficult to account for incompatibility otherwise than as the result of the occupation of a unique specific functional position.

Both hypotheses lead to the same conclusion: prophage is located on a unique specific bacterial structure which can "accept" or take care of only one prophage. Ehrlich would have called it a prophagoceptor. Apparently, reduction of phage into prophage can occur only if the alleged unique chromosomal spot is not occupied by a related prophage. It is known from the study of synaptic pairing that homologous parts of chromosomes have an elective affinity which is clearly expressed in chromosomal inversion. Lysogenization may have something in common with pairing.

XI. LOSS OF LYSOGENY

". . . whereby hidden diseases are often contracted, the cause of which even the physicians themselves cannot properly understand".—Columella, *ca.* 60 B.C.

References are often found in papers around 1925–1930 to nonlysogenic strains which spontaneously became lysogenic, or to lysogenic strains which spontaneously lost their lysogeny and then spontaneously regained it. It is known today that some temperate phages are exceedingly fragile. They are easily adsorbed on porcelain filters and readily inactivated by monovalent cations in the absence of divalent cations. Moreover, some of them give very tiny plaques on indicator strains and only in certain samples or batches of broth or peptone. The question of the origin of phage will be discussed later, but it should be said immediately that no example is known of a nonlysogenic bacterium becoming spontaneously lysogenic. The nonlysogenic "multilate" of *B. megaterium* obtained in 1932 by den Dooren de Jong, still maintained in many laboratories, has never regained its lysogeny except by infection. It seems highly probable that the losses and gains of lysogeny reported around 1925–1930 were due to cyclical failures to recognize the presence of phage.

Loss of lysogeny was obtained experimentally for the first time by den Dooren

de Jong, by cultivating *B. megaterium* and *B. undulatus* in media containing 10 per cent peptone. Asporogenic strains or "mutilates" were thus selected which were nonlysogenic and had lost the original immunity towards the homologous phage. No necessary correlation exists between lysogeny and sporogeny. From sporogenic lysogenic strains of *B. megaterium* nonlysogenic sporogenic clones are obtained (unpublished). And asporogenic strains, mutilates, of *B. megaterium* may be lysogenized without regaining their sporogenic character.

Clarke (27) has found another way of obtaining nonlysogenic bacteria. If a lysogenic *B. megaterium* is subcultured in a synthetic medium containing citrate, lysogenicity was lost after 61 subcultures. Clarke's experiments are easy to repeat, and I have obtained regularly nonlysogenic strains after 25 to 34 subcultures in synthetic media, whether or not a calcium binding substance (oxalate) was present. This works only for phage 1 and not for prophages 2 and 3 (H. Ionesco, personal communication).

How is lysogenicity lost? Prophage being a specific particle endowed with genetic continuity, the loss of lysogeny means a loss or a permanent alteration of prophage. If prophage were a cytoplasmic particle, loss of prophage could be produced by a relative decrease of the multiplication rate of prophage in certain media. As prophage appears to be linked to a bacterial chromosome, the loss of prophage can be visualized, either as an irreversible mutation of prophage or as a loss of a piece of bacterial chromosome, or as the result of an accidental absence of prophage replication. This may perhaps happen frequently. But in media where phages are numerous and where adsorption is good the nonlysogenic bacteria have a good chance to be reinfected and either lysed or lysogenized. Therefore the probability of selecting nonlysogenic variants is theoretically greater in media where the survival chances of phages are low and where adsorption is bad.

Unpublished experiments of J. Weigle, F. Jacob, and E. Wollman suggest that lysogenic bacteria may be cured from their lysogeny by heavy irradiation with UV light. This seems to be an excellent method of killing the prophage.

The intimate mechanism by which lysogeny is lost, is for the time being not clear. The only certainty that we have is that all lysogenic strains studied up to now have, by losing their lysogeny, also lost their immunity.

XII. EXCLUSION, COMPETITION

"For exclusion is evidently the rejection of simple natures".—F. Bacon, *Magna Instauratio.*

Exclusion of a temperate phage by a virulent phage. When a bacterium infected with phage T1 is superinfected with phage T2, only T1 develops; the preestablished T1 prevents the establishment of T2. *Vice versa*, the preestablished T2 prevents the establishment of T1. This phenomenon, discovered by Delbrück and Luria, has been called *mutual exclusion.* It is observed only between nonrelated phages and not between mutants differing by a small number of mutational steps. According to Lesley, French, Graham and von Rooyen, the first infecting phage T2 modifies the bacterial surface in such a way that the late comers, although adsorbed, are broken down and some of their nucleic acid liberated into

the medium. But this is not always so; phage T2 is able to penetrate into T1, T3, or T7 infected bacteria and to block, even after several minutes, the development of the odd numbered phages.

What happens when virulent phages are allowed to compete with temperate ones? Weigle and Delbrück (99) induced the development of prophage in *E. coli* K12 (λ) and infected the induced bacteria with phage T5. From the analysis of the bursts, it appeared that production or maturation of phage λ is blocked by T5 in 99 per cent of the bacteria, even in those containing already λ particles. Thus, production of phage λ is blocked by phage T5, whatever the period of development λ has reached. Although the mechanism of this inhibition is yet unknown, note that it is neither a mutual exclusion nor a cross-exclusion, but a one way exclusion.

Another closely related type of "exclusion" has been observed between two phages of *E. coli*, the wild type λ+ and the virulent λv which certainly differs from λ+ by more than one mutational step (F. Jacob, Elie Wollman, L. Siminovitch, unpublished). Bacteria infected simultaneously with both phages produce only phage λv. When *E. coli* (λ+) is induced and then infected with λv at various periods after induction, mixed bursts are observed. The later the infection with phage λv, the more phages λ+ are produced. Obviously, λv is able to compete successfully with λ+. If λv is handicapped by an early start of λ+, both phages are produced; the absence of λ+ observed in certain cases is the manifestation of a selective advantage of λv. Both phages being able to multiply and to mature in one bacterium, this phenomenon should be described as a competition rather than an exclusion.

Exclusion between temperate phages. This type of exclusion has been discovered by Jacob (54) with the temperate phages 1 and 8 of *P. pyocyanea*. After mixed infection, a great majority of bacteria produce phage 1, a few produce 8, but a single bacterium never produces both phages. This is a typical mutual- or cross-exclusion. But phage 1 is "stronger" than phage 8: in induced *P. pyocyanea* 13(8), an infection with 1 blocks the development of 8. Bacteria produce phage 8 only if the infection by 1 has taken place 10 to 15 minutes after induction of 8 (F. Jacob, unpublished).

Another phenomenon has been observed in *P. pyocyanea* with two phages which, provided the infection is synchronous, are compatible or congenic, *i.e.*, can develop simultaneously. If a bacterium in which one of the phages has been developing for 35 to 40 minutes is infected with the heterologous phage, no phages are produced, and the bacterium does not lyse (F. Jacob, unpublished). Lacking any data concerning the mechanism by which a phage may arrest completely the formation of another, discussion would be superfluous, but it is important to obtain some information concerning the nature of the mechanism involved.

XIII. IMMUNITY

"Corpora nun agunt nisi fixata".—Paul Ehrlich.

As observed by Bail and by Bordet in 1925 lysogenic bacteria are not lysed by the homologous phage. The existence of a relation between lysogeny and a

modified resistance of the bacterium seems to have been conceived for the first time by Burnet and Lush in 1936. The original nonlysogenic coccus they were studying was receptive, whereas the lysogenic strain was nonreceptive. The Australian workers had noticed that the "modified reaction" appeared less than one hour after infection and wrote (24):

"This changed character is then transmitted indefinitely to its descendant. It is not possible to say whether this surface change results from an altered genetic constitution of the bacterium or is directly induced by the associated phage at each generation. According to Wollman's hypothesis the distinction between the two alternatives would disappear, the phage being regarded as a gene reintroduced into the genetic makeup of the organism".

Lysogeny and nonreceptivity. Although this section deals essentially with the bacterial properties correlated and controlled by lysogeny, the question of nonreceptivity should be discussed and especially the problem of phage production by nonreceptive bacteria. Burnet and Lush (24), to whom we owe the first accurate study of lysogenization, had observed that the lysogenic coccus SF was unable to adsorb the homologous phage. Two remarks are necessary: (a) the "normal", nonlysogenic coccus is a poor adsorber; (b) the Australian workers studied the adsorption on heat killed bacteria. Their conclusion that one hour after infection the previously receptive coccus SF had become nonreceptive as a result of lysogenization may be true. But it is, to now, a unique example and the existence of a correlation between lysogeny and nonreceptivity cannot be admitted before others of this type have been found.

But one of Burnet and Lush's conclusions has been confirmed, *viz*, that nonreceptive lysogenic bacteria are able to produce phage. The rough variants of lysogenic *S. typhi-murium* and of *P. pyocyanea*, unable to adsorb the homologous phage, can produce and liberate phage (A. Lwoff, unpublished; F. Jacob, unpublished).

The conclusion is tempting that phages may be produced in a bacterium devoid of the specific receptor for phage adsorption. But we know today that some nonreceptive bacteria may possess the specific receptor in a masked form (see P. Nicolle, 1953, 1). It is known also that some rough pneumococci possess a small amount of the specific polysaccharide. It is therefore difficult to decide, without a special search, that a nonreceptive bacterium is completely devoid of the specific phage receptor, or of some part of this receptor, and whether or not a given phage can be produced in bacteria which are unable to synthesize the specific phage receptor.

Immunity proper. It has been known for many years that lysogenic bacteria are able to adsorb the homologous phage. In the Wollmans' paper of 1938 (106), this is considered as "classical", but I have been unable to identify the author responsible for the first observation. Nevertheless, all that was known up to 1953 is that free homologous phages disappear as infectious units in the presence of lysogenic bacteria. It was believed that the homologous phage did penetrate the bacterium and that resistance of lysogenic bacteria represented something quite peculiar, which was called immunity. But the reality of the penetration was proven for the first time by Bertani in 1953. Bertani's experiments which were discussed in the previous section on lysogenization show that a related phage

may substitute for the "normal" prophage. A second proof of the penetration has been provided by Jacob (unpublished) with *P. pyocyanea* and by F. Jacob and E. Wollman (unpublished) with *E. coli*. After infection of a lysogenic bacterium with a mutant of the homologous phage, it was found that, as a function of time, the infecting particles are distributed in the daughter bacteria, diluted out and finally lost (figure 14).

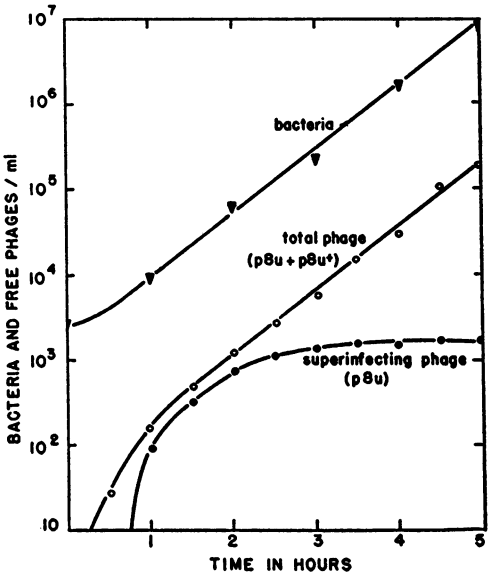


FIG. 14. Immunity. The lysogenic *Pseudomonas pyocyanea* 13 (p8+) has been infected with the mutant phage p8u towards which it is immune (initial multiplicity about 8). The bacterial growth is followed as well as the number of phages corresponding to spontaneous production. During the first period of around 2 hr both phages are produced. Then, the production of the infecting phage p8u decreases and stops. From F. Jacob and E. L. Wollman (58).

It appears as though the genetic material of the infecting phage persists in the protoplasm of the bacteria but is unable to undergo replication. Whereas the reproduction of prophage is correlated with bacterial reproduction, the nonreduced germ of the infecting phages is unable to undergo replication in an immune lysogenic bacterium (58). The proportion of bacteria producing phage spontaneously is not modified by infection with the mutant phage; each phage-producing bacterium yields both phages, but this only for a short time after infection.

Thus, from Bertani's and Jacob's data, it is certain that related phages adsorbed by lysogenic bacteria do penetrate in the bacterium, but that they do not develop. The bacteria are not killed. This survival, different from the survival due to nonreceptivity, has been ascribed to a state of "immunity" (figure 15). Immunity is the property of a lysogenic bacterium allowing it to survive an infection by an homologous or related phage. From the preceding experiments, clearly immunity is not due to a destruction of the infecting phage, which may survive in the bacterial protoplasm for several generations, but to the fact that the infecting phage does not develop. In lysogenic bacteria, both the prophage and the infecting phage are unable to develop. But when the prophage undergoes development, either spontaneously or as the result of an extrinsic induction, the infecting phage develops also: immunity is bound to the persistence of the prophage as such.

Specificity of immunity. Thus, it seems that in a lysogenic bacterium, one reaction is blocked which controls the development of the prophage and of the infecting homologous or related phages. To what extent is immunity specific? One-way immunity has sometimes been described. A phage 1 will give plaques on a lysogenic bacterium B(2) whereas phage 2 will not produce plaques on B(1). The obvious conclusion, that prophage 1 immunizes against phage 2 whereas prophage 2 does not immunize against phage 1, is not justified as shown by the following data.

When *P. pyocyanea* is infected simultaneously with phages 4 and 8, both phages develop: there is no exclusion. Although phage 4 gives plaques on *P. pyocyanea* (8), whereas phage 8 does not give plaques on *P. pyocyanea* (4), this absence of plaque formation is not due to immunity. The study of the fate of individual *P. pyocyanea* (4) bacteria infected with phage 8 shows: (a) that 2 to

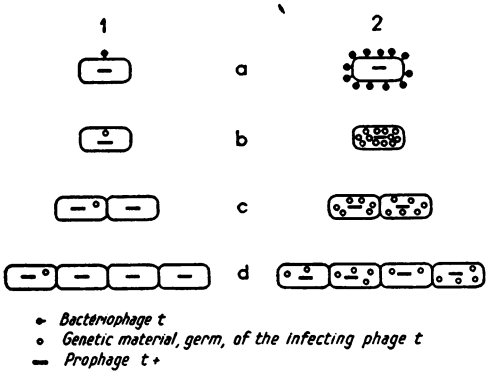


FIG. 15. Immunity. The infecting phage, whatever its form or state may be, persists in the immune bacteria and behaves as an inert particle. In singly infected bacteria, the percentage of bacteria carrying the infecting phage decreases exponentially.

5 per cent of the infected bacteria produce phage 8; (b) that 50 to 60 per cent are killed, whereas 40 per cent survive (F. Jacob, unpublished). What may be said is that in the lysogenic *P. pyocyanea* (4) the reactions towards phage 8 are modified by the presence of the prophage 4. Bacteria are "allergic" but not immune. Since the absence of plaques here is not due to a bacterial immunity, all cases in which one way resistance has been found should be reinvestigated in the light of Jacob's finding.

Until the proof of the existence of one-way immunity has been given, we have to expect that if one prophage *a* immunizes against a phage *b*, prophage *b* should immunize against phage *a*. In other words immunity is specific or at least group specific. Thus, in lysogenic bacteria, so far as we know, the process responsible for immunity controlled by the prophage appears as group specific. It is possible that one of the first steps of the development of infecting phages involves pairing with a homologous section of a bacterial chromosome. The presence of the prophage at this site might easily be conceived as preventing the initial pairing.

Inducing action of a virulent phage. The cases in which immunity fails will help in our analysis. The bacteriophage λ+ of *Escherichia coli* produces spontaneously two types of virulent mutants: λc and λv (58a): the distinctive properties of these two phages are summarized in table 2. The original λ+ is a typical temperate

phage giving turbid plaques on the detector strain K12S; the mutant λc gives clear plaques on K12S but none on the lysogenic K12 (λ+). Resistant bacteria are rare, and all those which were tested proved to be receptive and nonlysogenic. The possibility of λc being a temperate phage with a very low power of lysogenization is not excluded.

The mutant λv behaves quite differently to λc: it produces plaques on K12 (λ+) which is therefore not immune towards it. What is the reason for this absence of immunity?

The experiments of Jacob, Wollman and Siminovitch have shown: (a) that infection of *E. coli* K12 (λ+) with virulent phage λv induces the development of prophage λ+; (b) that the infecting UV inactivated λv may be restored; (c) that recombination between λv and λ+ takes place; (d) that rare phages λv in which the v character has been destroyed by UV are still capable of inductive action. Certainly, as concluded by these workers (58a) a relation exists between

TABLE 2

Properties of the phage λ+ of Escherichi coli K12 and of some of its mutants

PHAGE	IMMUNITY*		POWER TO LYSOGENIZE	INDUCING ACTIVITY	QUALITY OF PHAGE
	K12S	K12(λ)			
λ+	0	+	+	0	temperate
λc	0	+	0?	0	weak virulent (noninducer)
λv	0	0	0?	+	strong virulent (inducer)

* Immunity: 0 the bacterium is not immune; + the bacterium is immune.

induction, restoration, and recombination. The sequence of these phenomena seems, in most cases, to be: (a) induction, (b) restoration, (c) multiplication, (d) recombination. The phage λv does not induce the development of phage in lysogenic *E. coli* C carrying various prophages not related to λ. As phage λv induces only the development of the related prophage, induction here appears to be group specific. Thus, the absence of immunity of *E. coli* K12 (λ+) against the virulent mutant λv could be bound to the fact that λ induces the development of the prophage λ+ (58).

Phage of *S. dysenteriae* produces, according to Bertani, virulent mutants of three types: *weak* virulents towards which the original lysogenic strain is immune; *moderate* virulents which produce a few plaques; and *strong* virulents which produce the same number of plaques on both lysogenic and nonlysogenic strains. It would be important to know if the absence of immunity of the lysogenic bacteria against the strong virulent is here also bound to the induction of the development of prophage.

Conclusions

Under conditions in which the bacteria of a nonlysogenic strain would be lysed after infection, lysogenic bacteria survive infection by the homologous or related phages. It is known thanks to Bertani (14) and Jacob and Elie Wollman

(58) that: (a) the temperate phages adsorbed by lysogenic bacteria studied up to now penetrate into the bacterium, (b) the genetic material persists during a few bacterial generations in the bacterium, where it does not multiply and apparently behaves as an inert protoplasmic particle; (c) the infecting phage develops if the prophage develops, whether spontaneously or after induction.

In an immune bacterium, the development of phage is blocked. Immunity is lost as a result of the spontaneous or induced development of the prophage. It is obviously bound to presence and integrity of the prophage.

It is essential to recall that immunity is specific or group specific. It appears as though in a given lysogenic bacterium a specific process necessary for the development of a specific type of phage cannot take place. That the prophage itself cannot develop is easy to conceive, and it may be that the "active surface" of the genetic material of the prophage is unavailable for an essential reaction. But the development of some related infecting phages is also blocked. Thus, the presence of prophage on the chromosome blocks an essential step of the development of certain types of phages. Unfortunately, nothing is known about the nature of this step which could be the synthesis of a protein or a modification of the nucleic acid.

XIV. SOME COMMENTS AND VIEWS

"For why should I attempt to judge those who are not within the church?"—St. Paul, *First epistle to the Corinthians.*

Temperate and Virulent Phages

Phages have been classified in two categories, temperate and virulent according to the presence of absence of the power to lysogenize. Unfortunately, the definition of the character virulent is purely negative. If, after the action of a temperate phage, most survivors are nonlysogenic, the rare lysogenic survivors, because of their low proportion, may be practically impossible to find. Thus, as a result of the study of a system with a low lysogenization quotient, a temperate phage could be considered as virulent. It would therefore be important to know whether or not the characters *temperate* and *virulent* are correlated with specific properties other than lysogenization.

Table 3 makes immediately obvious that the physiological properties of various virulent phages are quite different. As pointed out, a weak virulent could be a temperate possessing an exceedingly low probability of reduction into prophage. On the contrary, a strong virulent which induces the development of the related prophages seems certainly devoid of the power to lysogenize. But, we must never forget that we are dealing with systems in which the genetic material of the bacterium intervenes, and one may conceive of a phage behaving as a strong virulent in one bacterium and able to be reduced to prophage into another. Nevertheless, when considering the extreme virulent phages of the T2 type, one gains the impression that they are really unable to lysogenize for the simple reason that the "impact" of the tail kills the receptive bacterium. Therefore, when dealing with the power to lysogenize two characters have to be considered: the

TABLE 3

Properties of some temperate and virulent phages and of bacteriocins

	TEMPERATE	VIRULENT			BACTERIOCINS
		Weak	Strong	Extreme	
Power to lysogenize	+	0	0	0	
Power to induce the development of the homologous prophage	0	0	+		
Lethal action of the protein	0	0	0	+	+
Residual bacterial growth after infection*	+	+	+	0	0
Corresponding particles of *E. coli*	λ+	λc	λv	T2	*colicin* ML

* Between phages allowing a doubling of the bacterial substance and phages allowing no measurable growth, intermediary types do probably exist.

TABLE 4

Composition of some virulent and temperate phages

	I T5	II T2	III λv	IV A_1
μg P/10^{11} phages	8	2.5	1.2	0.6
Adenine	29	33.6	21.3	23.4
Thymine	31.7	35.6	28.6	33.3
Guanine	19.1	18.1	22.9	18.8
Cytosine	20.4	0	27.1	24.6
5-Hydroxymethyl cytosine	0	16	0	0

The figures correspond to moles of the bases/100 moles of total base recovered.

I. Virulent T5 from *Escherichia coli*. Phosphorus: J. D. Smith and L. Siminovitch, unpublished. Bases: Wyatt and S. S. Cohen (1).

II. Virulent T2 from *Escherichia coli*. Phosphorus: Hershey. Bases: Wyatt and S. S. Cohen, except 5-hydroxymethyl cytosine from Wyatt, unpublished.

III. Virulent λv from *Escherichia coli*. J. D. Smith and L. Siminovitch, unpublished.

IV. Temperate A_1 from *Salmonella typhi-murium*. J. D. Smith and L. Siminovitch, unpublished.

J. D. Watson and F. H. C. Crick (*Nature*, 1953, p. 737) have proposed a new structural scheme for the salt of desoxyribonucleic acid. Two helical chains are coiled round the same axis. The two chains are held together by purine and pyrimidine bases which are joined in pairs by hydrogen bonds. The structure is such that only the specific purine-pyrimidine pairs adenine-purine and guanine-cytosine can bind. This implies that the ratios of the bases of the two pairs must be equal to one. Figures given here which are not in accordance with the theory should therefore be regarded as tentative.

structure of the genetic material, and the physiological effects of the proteins of the phage.

Considering other possible discriminative characters of virulent and temperate phages, we note that the *morphology* of the temperate phages of *E. coli*, *B.*

megaterium, and *Corynebacterium diphtheriae* is normal. Only the temperate phages of *Bacillus cereus* as described by the Kellenbergers (60) have a strange inflated tail. The *chemical constitution* so far as purine and pyrimidine bases are concerned is for the time being of little help (see table 4). Some temperate phages contain less phosphorus (less nucleic acid) than some virulent ones. This may account for the fact that UV sensitivity of some temperate phages (of *P. pyocyanea*, *B. megaterium* and *S. typhi-murium*) is 5 to 10 times lower than UV sensitivity of phage T2 (figure 14). The study of the *genetics* of the characters *temperate* and *virulent* is quite recent and has been performed only with the λ phages of *E. coli* (58). Crosses involving the virulent and the wild type character yield the parental types and recombinants of the two groups of markers. "Virulence" behaves as other genetic markers of the phage.

Thus, a temperate and a virulent phage may differ by a few, perhaps sometimes one, mutational steps. Therefore their essential difference appears to lie in a structure of the genetic material of the phages, which is not necessarily correlated with detectable morphological or biochemical properties.

Lysogeny and Toxinogeny

D'Herelle noted in 1926 (a footnote on p. 218 of his book, 44) that certain bacterial species, such as toxinogenic strains of *Corynebacterium diphtheriae*, were only known as "cultures mixtes" that is to say, as carrier strains. It could be, added d'Herelle, that the toxic character of the diphtheria bacillus is bound to the presence of bacteriophage which would cause the lysis of bacteria and thus liberate the toxin. In three different instances, d'Herelle had, from toxinogenic strains of *C. diphtheriae*, isolated bacteriophages which were virulent for nontoxinogenic strains. This observation was completely overlooked, and, as a matter of fact, the situation is much more fascinating.

Freeman (35) discovered that a nontoxinogenic strain of *C. diphtheriae* becomes toxinogenic when lysogenized with a certain phage B isolated from a toxinogenic strain. Hewitt (47) confirmed that the property to produce toxin can be conferred to nontoxinogenic strains of *C. diphtheriae*; he also made a quite unexpected observation: some lysogenic strains of *Staphylococcus aureus* produce a phage able to induce the transformation nontoxinogenic → toxinogenic in the diphtheria bacillus.

When sensitive bacteria are lysed by phage B, no toxin is released (26). When the toxinogenic lysogenic strain is irradiated with UV light, phages are produced, but no toxin is liberated (77). The question of the relations between a certain type of prophage and the ability to produce toxin is not yet clear.

Lysogeny and the Origin of Bacteriophage

Bordet and Renaux in 1928 (18) advanced the idea that lysogenic bacteria could be the origin of phage, an idea that has been repeatedly reintroduced since that time. We know that in a lysogenic bacterium, phage develops from prophage which is ontogenetically primitive, and that in the cycle of the virulent T2, phage is produced from its nucleic acid (45). As bacteriophage can reproduce

only inside bacteria, the hypothesis is likely that the genetic material of phage or prophage is not only primitive ontogenetically but also phylogenetically. Prophage could have arisen by mutation of the genetic material of its actual host or of an ancestor of its host. The hypothesis of bacteriophage being the residue of the degradation of a parasite of bacteria is difficult to accept because we do not know of any parasite of bacteria except bacteriophage itself. This favors the theory of the endogenous origin of bacteriophage. If this theory is accepted, the problem of the nature of bacteriophage has to be considered anew.

Twort in 1915 and his followers have considered and discussed many hypotheses concerning the nature of bacteriophage: enzyme, particle or nonparticle, virus or nonvirus, organism or nonorganism, parasite or nonparasite, living or nonliving. Fierce disputes subsequently raged. Apparently, Burnet and McKie in 1929 (25) made the first effort of coordination of such apparently contradictory views. The Australian workers had adopted Bordet's conception of lysogeny, according to which bacteriophage was part of the bacterial heredity and behaved as a harmless particle towards lysogenic bacteria. They noted also that the bacteriophage, in accordance with d'Herelle's views, behaved towards a sensitive bacterium as a "predatory" entity becoming adsorbed on the bacterial surface and multiplied in the bacterium which it destroyed. Burnet and McKie added:

"The difficulty of reconciling these two aspects of bacteriophage phenomena has been responsible for all the current controversy on the intimate nature of phage, whether it is an independent parasite or a pathologically altered constituent of normal bacteria. In our view both these contentions have been completely proved, and the current attitude on both sides of regarding them as irreconcilable alternatives is quite unjustified. According to the particular type of bacterium that is reacting with the phage concerned, it may be useful and convenient to regard the phage as an independent parasite or as a unit liberated from the hereditary constitution of some bacterium, the usage being determined wholly by its functional activity at the time".

It is quite clear that a virulent phage of T2 type *behaves* as a parasite whereas the prophage *behaves* as a gene. The problem as to whether bacteriophage is or is not a parasite or a virus is entirely a matter of definition (see Appendix III, 6, remarks).

XV. VIRAL DISEASES AND IMMUNITY IN PLANTS AND ANIMALS

"Soit pour exemple le virus variolique. . . . Pourquoi reste-t-il ordinairement sans effet dans ceux qui ont déjà eu la petite vérole? Quand on le communique par insertion, il donne la petite vérole à ceux qui ne l'ont pas encore eue. Il faut donc croire que s'il n'agit pas, c'est qu'il ne trouve pas le corps dans une disposition favorable, disposition qui a été détruite dans ceux qui ont eu la petite vérole. Cette même disposition est donc en partie la cause principale de cette maladie: par conséquent l'aptitude à recevoir l'impression des miasmes varioliques, et les divers phénomènes ou effets qu'ils produisent, sont les véritables objets qui méritent l'application du Médecin. Tout le reste n'est qu'accessoire et trop éloigné de sa portée".—Antoine, Théophile et François de Bordeu, *Recherches sur les maladies chroniques*, Paris, 1775.

According to the Bordeu's conceptions of immunity against infectious diseases as illustrated by the example of smallpox, the disposition of the host towards the virus plays a determining part in illness and resistance. A disease is not produced if the state favorable for the virus development has been suppressed by a previous disease. After having discovered the prevention of rabies, Pasteur tried to visualize the mechanism of the induced resistance. "Would it be possible," wrote Pasteur in 1885, "that what constitutes the virus of rabies is formed by two distinct substances, and that nearby the living substance, able to thrive in the

nervous system, another would be present, having the faculty, when present in adequate proportion, to stop the development of the first?" Pasteur added in 1887, in his famous *"Lettre sur la rage"* which appeared at page 1 of number 1 of volume 1 of the *Annales de l'Institut Pasteur*: "a substance that would produce such a state in the nervous system that it would become unable to allow the culture of the microbe". Thus, Pasteur conceived of rabies as produced by the multiplication of a virus in the nervous system and of immunity against rabies as the result of a tissular modification induced by a viral constituent and preventing the reproduction of the virus.

Our conception of immunity in lysogenic bacteria corresponds partly to Pasteur's views: one part of the bacteriophage modifies the bacterium in such a way that the reproduction of bacteriophage is not possible any more. We know that the factor responsible for immunity, the genetic material of the bacteriophage, is perpetuated as prophage together with the genom of the bacterium. Does an analogous type of cellular immunity exist in animals and plants, and if so, is it bound to the genetic material of the virus, to a specific provirus[3], the presence of which at a specific site would prevent the development of homologous or related viruses? The first question is of course whether viruses of plants, insects or animals may or not be perpetuated in the form of a provirus. This problem, far from being solved, has been recently discussed by Shope (4), Koprowsky (5) and Kenneth Smith (95). We may consider the following features:

(a) Analogy of constitution of many viruses and of bacteriophage: many viruses are, like phage, nucleoproteinic particles, with only one type of nucleic acid.

(b) Analogy in the life cycle: many viruses from plants, insects or animals when entering their host cell lose their infectivity (G. Henle, and W. Hoyle, 3). They are not replicated by binary fission in the form of the infective particle.

(c) Existence of abnormal types of development: in many virus infected cells viral antigens are produced although virus particles are not formed, just like in some lysogenic bacteria phage development is abortive.

(d) The development of the polyhedral disease is induced in silkworm by various diets (108) and by injection of hydrogen peroxide (84). But it is not known whether these substances allow the development of a latent, hidden, or masked virus or induce the development of a provirus into virus.

Thus, a sort of "vegetative phase" exists in the life cycle of many viruses comparable to the corresponding phase of phage reproduction. Although no evidence exists for the conclusion that the genetic material of viruses may be perpetuated in the form of a particle possessing the features of prophage, there is no theoretical objection against this view, and it would be of the utmost interest to know whether or not immunity in animals or plants may be correlated with the presence of a provirus-like material.

[3] *Previrus* would be more correct. *Pro* and *phage* are greek; prophage is correctly formed. *Virus* is latin and *provirus* is a greco-latin hybrid. But, as even some purists consider that latin and greek roots have become so acclimatized that they have become part of our languages, we will keep provirus, (a) because it is already in use; (b) because of its balanced phonic analogy with prophage.

We know with certainty that in some plants normal looking cells may contain crystals of virus. The production of a "ribonucleic" virus is thus not necessarily accompanied by the death of the host cell. We know also that a plant cell infected by a given protecting virus may be modified in its ability to reproduce some other viruses. Whether this is due to the presence of a provirus as at a given site is not known. The problem of the resistance of animals towards viruses is a much more complicated one; often antibodies certainly play a role. Whether a cellular immunity, analogous in its essence with the immunity of lysogenic bacteria, exists is unknown. But in one case at least the presence of a virus produces a cellular resistance against another related virus. As this happens in a neoplastic-like condition, a few introductory remarks regarding neoplasms and viruses are necessary.

A neoplastic cell is a cell which is permanently, hereditarily modified in such a way that it is not submitted any more to the factors of coordination. With some such cells, the first and best known being the Rous sarcoma, the neoplastic change is produced by a "virus". The infective Shope's virus of the cotton-tail rabbit, the agent of the benign papilloma, may produce malignant transformation when injected in the blood stream of rabbits in which papillomata have been induced with tar. No infectious particles are present in the neoplastic cells, but antibodies able to neutralize the original Shope's virus appear in the blood of the cancerous rabbits. The malignant cells thus produce a specific antigen (39, 69). In this case, malignancy is not correlated with detectable infectious particles. It is known that Shope's papilloma virus, infectious in the cotton-tail rabbit, generally loses its infectivity in the domestic rabbit, It is sometimes described as a masked virus. So far as we know, this state seems to correspond to an incomplete, noninfectious particle rather than to a provirus.

In a lysogenic bacterium the production of phage is under the control of the constitution of its hereditary material, including prophage, phenotypic factors controlling aptitude and phage development. And finally the expression of the potential properties is eventually controlled by an event, the probability of which can be considerably increased by inducing agents which are carcinogens. This situation recalls the initiation of neoplastic diseases of animals.

The formation of a malignant cell is under the control of the genetic constitution of the host, of phenotypic properties of the cell as controlled by hormonal factors, of extrinsic carcinogenic agents and sometimes of an infective particle. As a result of the conjunction of these factors, a cell may become permanently altered. This hereditary change could be, as postulated by Darlington (29), the result of the formation of a "plasmagene-like" structure. The relation of neoplasia to the genetics of the host has been reviewed by Heston (46), its relation with mutations by Tatum (96) and the general aspects of the neoplastic viruses by Rous (89), Duran-Reynals (33), Shope (4), Kidd (61) and Harris (39). An extensive discussion of the subject would be beyond the scope of this review. For the time being, no evidence may be advanced against the hypothesis that the potential power of a cell to become neoplastic may be perpetuated in the form of a gene-like structure and that carcinogenic agents induce the expressions of the potentiality of this genetic material, which would culminate in the formation of a new particle endowed with genetic continuity.

Thus, the neoplastic potentiality of a cell could be visualized as perpetuated in the form of the genetic material of the neoplastic particle. But, whereas the development of phage in a lysogenic bacterium is lethal, the neoplastic agent is not pathogenic for the neoplastic cell. It is the neoplastic cell which is pathogenic for the organism. This difference being duly taken into account, it is possible that, in potentially malignant cells, the initiation of malignancy, *i.e.*, the initiation of the development of a neoplastic agent, may have something in common with the initiation of phage formation from prophage in a bacterium potentially able to produce bacteriophage.

If an analogy exists between lysogeny and some neoplastic or neoplastic-like diseases, then situations analogous to the immunity of lysogenic bacteria could be found. This seems to be so for the fibroma and myxoma of rabbits; Shope (4) demonstrated that the virus of fibroma protects against infection by the virus of myxoma. The existence of a close relationship between these two viruses is shown by the recombination experiments of Berry and Dedrick; also rabbits which have recovered from a myxoma possess antibodies neutralizing both fibroma and myxoma viruses although the reverse is not true. Moreover, in rabbits injected with the fibroma virus the resistance towards myxoma appears between the 2nd and the 4th day. Since the blood of these fibroma infected rabbits contains antibodies neutralizing only the fibroma virus and devoid of neutralizing activity towards the myxoma virus, Shope excluded the possibility of the protection arising from the presence of neutralizing antibodies. Therefore, the resistance could be the result of a state of cellular immunity essentially analogous to the immunity of lysogenic bacteria towards related phages. But we do not know if the protected cell perpetuates the fibroma virus, an incomplete virus or a provirus.

Sabin and Winsser (90) have recently succeeded in producing resistance against poliomyelitis by feeding monkeys repeatedly with small doses of virus. The acquired immunity was not associated with demonstrable antibodies in the serum. Here again, the question of a possible cellular immunity is raised. For the time being, however, prophage is the only known provirus, and lysogenic bacteria are the only known examples of: (a) organisms perpetuating the genetic material of a virus; (b) cells in which immunity appears to be bound to the integrated genetic material of the virus which, as a result of the integration, prevents the development of homologous and related viruses.[4]

XVI. UNITARY CONCEPT OF LYSOGENY, LYSOGENIZATION, INCOMPATIBILITY, IMMUNITY AND INDUCTION

"I forsee that if even men are roused by my admonitions to betake themselves seriously to experiment and bid farewell to sophistical doctrines, then indeed through the premature hurry of the understanding to leap or fly to universals and principles of things, great dangers may be apprehended . . . against which evil we ought even now to prepare".—F. Bacon, *Magna Instauratio*, 1620.

The various aspects of lysogeny having been considered, it is now possible to discuss the interrelation of the different features of lysogenic bacteria. We

[4] It is known that the CO_2 sensitivity of drosophila is due to a "genoid" which behaves in the germinal line as a cytoplasmic particle. (See P. l'Heritier, Cold Spring Harbor Symposia Quant. Biol., **16**, 1951, 99–112.) It is transmitted by the male and female gametes in the case of the genoid *alpha*, by the female gamete only in the case of the genoid *omega*. The extracts of sensitive flies contain infectious particles able to confer CO_2 sensitivity to normal flies. After injection, infectious particles are recovered only after a negative latent period of about 24 hours. But certain flies called *rho*, despite the fact that they contain genoids—generally in small number—are insensitive to CO_2, and they do not become sensitive after injection of genoids *alpha*. G. Brun has compared the situation of *rho* flies to lysogeny and has concluded that their genoid could be present in the state of a provirus. But another hypothesis could also be considered, namely that the *rho* situation is due to a defect in the development or maturation of infectious particles.

know that lysogenic bacteria perpetuate a specific particle, the prophage. In order that a bacterium can maintain its lysogeny, replication of prophage has to be correlated with bacterial reproduction. The fate of the prophage must be bound to the fate of an integrated bacterial structure. The number of prophages per bacterium is certainly low, and the experimental data indicate that it is of the same order as the number of bacterial nuclei. In the only case studied, lysogeny behaves as a unit-character associated with a bacterial linkage group; it seems also to be located at a definite locus of a bacterial chromosome. The study of lysogenization has revealed that the presence of one given prophage prevents the reduction of related phages into prophage. All this points towards the conclusion that the nonlysogenic bacterium, or better its nucleus, possesses a unique site on which the genetic material of the infecting temperate phage can become attached. This site could be visualized as an allelic structure of the genetic material of the phage.

In order that a bacterium may become lysogenic, the genetic material of the infecting phage has to be attracted and bound to a specific chromosomal receptor. Prophage thus appears not only as a specific structure but a specific structure localized at a specific site.

The temperate or virulent character of a phage would be the reflection of the structural homologies of the genetic material of the phage and of the chromosomal receptor. A certain degree of correspondence would be necessary in order that attachment can take place.

Lysogenic bacteria survive only if prophage does not develop into phage. In lysogenic bacteria, a reaction must be blocked which is necessary for the development of prophage. As a matter of fact, in lysogenic bacteria the infecting homologous and related phages are also unable to develop. This is immunity.

Immunity is a corollary of lysogeny, or of prophage. A lysogenic bacterium can perpetuate itself only if it is immune. Now, from time to time a lysogenic bacterium expresses its potentiality and produces bacteriophages. Prophage can obviously develop only if immunity ceases to exist, and immunity is bound to the presence of prophage. As a matter of fact, when the vegetative phase is started from prophage, the superinfecting phages, towards which the bacterium was previously immune, also develop. Prophage and immunity cannot be separated. This is the best proof that prophage is functionally different from gonophage, from the genetic material of the phage during the vegetative phase. All this means that the vegetative phase is started, that bacteriophage develops, when prophage having ceased to be in the prophage state, immunity is lost.

In order that the vegetative phase can start, something has to happen which we conceive as a detachment of the genetic material of the phage from its chromosomal locus. This would be the effect, probably secondary, of inducing agents. The inducing effect of some "strong" virulent phages on the related prophages would be due to the detachment of prophage from the bacterial chromosome.

Temperate phages infecting a lysogenic bacterium possessing immunity persist as inert cytoplasmic particles. They are not reproduced and are diluted out at bacterial division. The replication of the genetic material of infecting phages

can not take place in immune lysogenic bacteria. In a lysogenic bacterium, prophage is replicated, but a reaction is blocked which is necessary for the replication of the non-reduced, non-integrated, genetic material of infecting phages.

Thus, in a lysogenic bacterium, prophage does not develop, the related superinfecting phages do not develop and are not replicated. Due to the presence of prophage, a reaction can not take place. The structure prophage can not block a reaction by the sole virtue of its presence. It could block a reaction in modifying the property of some structure, and in order that this should happen, a specific functional key site has to be occupied.

What is the reaction blocked by the prophage which controls immunity? As already stated, it could be that pairing of the genetic material of the phage and of the bacterial chromosome is a necessary step of phage development. Prophage could prevent this pairing.

Another hypothesis may be mentioned. When considering bacteriocinogeny, it appears that the specific reaction occurring as a result of the action of inducing agents is the synthesis of a new protein, of a bacteriocin. The development of phage, the unbalanced multiplication of the genetic material of phage, culminates in the maturation of the infective particle only if the specific proteins of phage are produced. No development is possible in the absence of protein synthesis. The process blocked by prophage in lysogenic bacteria and responsible for immunity could be the synthesis of this specific protein. It could be also the conversion of the genetic material of the phage into gonophage.

The receptor theory of drug action orginated by Ehrlich has culminated in the discovery by Woods and Fildes of the competition of a drug and of an essential metabolite for a cellular structure. The role of specific superficial receptors in the adsorption of bacteriocins and bacteriophages is well known. In lysogenic bacteria, another type of specific structure will have to be considered as a possible attractor, receptor and modifier of the genetic material of the phage. This genetic material, by its attachment to the receptor, would be converted, reduced, into prophage. Prophage itself would in turn modify the properties of the bacterial chromosome. The result of the interaction of the genetic materials of the phage and of the bacterium is the prophage, and the result of prophage is bacterial immunity towards phage. That the effect of genes varies with their position on the chromosome is well known since Sturtevant; that the behavior and accomplishments of a cytoplasmic particle may depend on its geographical localization in its "host" is evidenced by the study of kinetosomes of ciliates (70). A specific structure or particle must not be only considered as such but in its spatial relations with other cellular structures. As a consequence of the study of lysogeny, the "site effect" is introduced in the bacterial or cellular pathology.

The unitary concept of lysogeny, lysogenization, incompatibility, immunity and induction provides a model in which all the properties of lysogenic bacteria are ascribed to the *presence and position* of a specific structure representing the genetic material of the phage, the properties of a lysogenic bacterium being the consequence of the presence of the right particle at the right place. Position is

the fourth dimension of the prophage. Although this unitary concept has the advantage of accounting for the numerous properties of lysogenic bacteria, it is only a hypothesis and not a dogma.

The fact that an organism may, along with its genome, perpetuate hereditarily a structure from which a virus can be produced is in itself remarkable. More remarkable still, this structure, this potential virus, is the very factor responsible for immunity towards the homologous true virus. Attention therefore is called to lysogenic bacteria which, together with lysogeny proper, perpetuate the solution—for them—and the problem—for us—of cellular immunity.

APPENDIX I—GLOSSARY

"C'est en m'occupant de ce travail que j'ai mieux senti que je ne l'avais fait jusqu'alors, l'evidence des principes qui ont été posés par l'abbé de Condillac dans sa logique et dans quelques autres de ses ouvrages. Il y établit que nous ne pensons qu'avec le secours de mots; que les langues sont de véritables méthodes analytiques; que l'algèbre la plus simple, la plus exacte et la mieux adaptée a son objet est a la fois une langue et une méthode analytique; enfin, que l'art de raisonner se réduit a une langue bien faite".—Lavoisier, *Discours préliminaire du traité élémentaire de chimie*, 1789.

An excellent general glossary of bacteriophage is to be found in *Viruses 1950* edited by Delbrück (4). The terminology of lysogeny which is given here, with the exception of a few additions and modifications, corresponds to the glossary of F. Jacob, A. Lwoff, L. Siminovitch and E. Wollman (57).

Abortive infection: Infection followed neither by lysogenization nor by phage production. The infecting material is not reproduced. A bacterium may or may not survive an abortive infection.

Aptitude: Physiological conditions allowing a lysogenic bacterium to react to inducing agents by the development of phage.

Bacteriocin: Protein of the colicin type, the biosynthesis of which is lethal and the adsorption of which is controlled by a specific bacterial receptor.

Bacteriocinogenic bacterium: A bacterium perpetuating hereditarily the power to produce a bacteriocin.

Carrier strain: A mixed population of bacteria and bacteriophage in a more or less stable equilibrium. The bacteria are not lysogenic: they may be separated from the phage by various procedures, such as by plating, or by action of antiphage serum. *Synonym:* pseudo-lysogenic strain.

Detector strain: A nonimmune strain utilized for the detection and estimation of a given phage. The majority of the bacteria of a detector strain allow phage development *under the condition of the test.*

Germ: The part of the bacteriophage which represents the material base of its genetic continuity, probably its nucleic acid.

Gonophage: The genetic material of the phage during the vegetative phase of the life cycle.

Homologous phage or prophage: The phage or prophage corresponding to a given prophage or phage.

Immunity: State preventing phage development conferred by the prophage to a lysogenic bacterium and allowing its survival after infection. A lysogenic bacterium is always immune towards the homologous phage. It may be immune also towards related phages.

Induction of a lysogenic bacterium: Action of provoking the development of bacteriophage. Induction corresponds to a conversion of the prophage into gonophage or to the initiation of the vegetative state.

Infection: See abortive, productive, reductive.

Latent period: The period which extends between infection (or induction) and the bacterial lysis.

Lysogenic bacterium: A bacterium which possesses and transmits, which perpetuates, the power to produce bacteriophage. The nature of the prophage is expressed by the symbol of the phage in parentheses following the name of the bacterium and the identification number of the strain, *e.g.*, *Bacillus megaterium* 899(1). *Synonym:* lysophoric bacterium.

Lysotypy: Identification of the bacterial type by means of bacteriophage.

Production rate: The probability for a given lysogenic bacterium to produce phage in the interval of two divisions.

Productive infection: Infection followed by phage production.

Prophage: (or probacteriophage). The form in which lysogenic bacteria perpetuate the power to produce phage. *Synonyms:* anlage, cryptophage (*pro parte*), symbiotic phage, symbiont, latent phage.

Receptive bacterium: A bacterium able to adsorb phage. Contrary: *nonreceptive.* Nonreceptivity can be due to the genotypic absence of the power to synthesize a receptor, to the phenotypical absence of this receptor, or to the masking of the receptor.

Reduction of bacteriophage: Conversion of the infecting phage into prophage.

Reductive infection: Infection followed by lysogenization.

Resistance: A bacterium resistant to a given phage is a bacterium which is not killed by this phage. This could be due either to nonreceptivity (absence of adsorption), or to a neutralization of the infecting phage, or to a lysogenization. Thus, *resistance* is a general term covering a variety of situations. It should be utilized only when the factor responsible for the bacterial survival has not been identified.

Sensitive bacteria: Receptive bacteria which are killed by the infecting phage and which reproduce it. The phenotypic expression of this genetic property is variable.

Temperate phage: A phage able to be reduced into prophage and to give lysogenic systems. *Synonyms: principe moyen, principe faible,* weak phage, latent phage, symbiotic phage, prolysogenic phage, symbiont.

Vegetative phase: The phase following infection or induction characterized by multiplication of the genetic material of the phage, or gonophage, and the synthesis of the proteins of the phage and culminating in the organization of infective phage particles or maturation.

Virulent phage: A phage unable to give lysogenic systems. *Synonyms: principe fort,* strong phage, lytic phage, prolytic phage, intemperate phage.

APPENDIX II—CHRONOLOGICAL TABLE

"Time which flows forever will reveal all". Aeschylus, *Prometheus bound.*

The chronology of lysogeny is presented here together with the chronology of bacteriophage and ultra-viruses.

50—Cornelius Aulus Celsus. Rabies is caused by a virus.

1882-1887—L. Pasteur. Rabies is caused by an invisible infectious agent or virus. The development of the disease may be prevented.

1891—D. Iwanowski. The agent of tobacco mosaic is filtrable.

1898—M. W. Beijerinck. The agent of tobacco mosaic multiplies only in living cells. It is filtrable and diffuses in agar gels. It can be dried, or precipitated with alcohol from aqueous solutions, without losing its infectivity. It is a *contagium vivum fluidum.*

1915—C. W. Twort. Bacteria may be lysed by an invisible infectious particle.

1917-1922—D. d'Herelle. Bacteriophage is a parasite of bacteria. It penetrates into the bacterium, multiplies, and is liberated by bacterial lysis. Bacteriophage is a particle. Bacteriophage particles may be counted.

1922—H. J. Muller. There are some similarities between bacteriophages and genes.

1923—B. M. Duggar and J. K. Armstrong. The virus of tobacco mosaic may be conceived as a revolted gene.

1925—O. Bail, J. Bordet. *Lysogeny and lysogenization are discovered.*

1928—E. Wollman. *Paraheredity,* bacterial transformations produced by extrinsic agents, could be controlled by a gene-like material. Phage is compared to lethal genes.

1929—F. M. Burnet and M. McKie. *Lysogeny is transmitted in the form of a specific non-infectious anlage. In a nonlysogenic population only a small proportion of bacteria contains bacteriophage.*

1932-1934—den Dooren de Jong. *Heated spores of lysogenic bacilli give rise to lysogenic clones. Lysogenic strains may be cured. The nonlysogenic, cured, strains are sensitive to the phage they produced previously.*

1934-1936—M. Schlesinger. Bacteriophage contains 3.7 per cent phosphorus. It is composed of a protein and of a desoxyribonucleic acid.

1936—E. and E. L. Wollman. Phage undergoes a cycle infective → noninfective. . . . Phage particles are not the *direct* descendants of preexisting phages but are produced from nonphage material.

1936—W. M. Stanley. The virus of tobacco mosaic is crystallized.

1937—F. C. Bawden, N. W. Pirie, J. D. Bernal and I. Fankuchen. The virus of tobacco mosaic contains a protein and a ribonucleic acid.

APPENDIX III—EXPLANATORY NOTES

1. *Remarks on microscopical observations*

"Ah, Alas!"—Aeschylus, *Prometheus bound.*

[The fact that the development of an invisible innocuous particle into another invisible particle may be a lethal process was disclosed as a result of microscopical observations. The attention of young scientists has to be called to the virtue of direct observation of living beings, which, nowadays, is sometimes neglected for the profit of mathematics. Let me recall that Pasteur discovered anaerobiosis in 1861, just in interpreting the movements of some bacteria which he was watching under the microscope. The history of this discovery was revealed by Pasteur only in 1876 (86). Let me recall also that in recent years certain conclusions concerning two different types of cytoplasmic particles have been painfully obtained by statistical analysis of numerous experiments. It appeared later that *one* microscopical examination would have led immediately, and more convincingly, to the same conclusion.

2. *Danger of hypothetical secretions*

"Alas! Alas!"—Aeschylus, *Agamemnon.*

Twenty-five years elapsed between the discovery of lysogeny and its definition. Among the factors responsible for this long delay was the secretion theory which created the suspicion in which lysogeny itself has been held for a time. This theory was probably unconsciously reached by the following reasoning: lysogenic bacteria live and multiply, lysogeny is not lethal; lysogeny being phage production, phage production is not lethal. This view was certainly substantiated by the fact that lysogenic bacteria adsorb homologous phage without being killed; phage is not lethal. Thus, in lysogenic bacteria, prophage is not lethal, perpetuation of prophage is not lethal, phage is not lethal. How should a normally innocuous particle be lethal only when producing another similar innocuous particle? No example of this type of phenomenon was known. Phage must be secreted. Phage is secreted. Phage was secreted.

In early publications the definition of lysogenic bacteria was treated by preterition. But it is obvious that they were generally considered as phage-secreting bacteria, or, at least, as bacteria able to secrete phage. A scientifically minded bacteriophagist interested in lysogeny soon discovered that the secretion theory was devoid of any experimental basis. No evidence of phage secretion being available, he then logically decided that phage secretion was doubtful, that it did not exist. Therefore, phage-secreting bacteria could not exist and lysogeny did not exist. A wrong definition of lysogeny had led to the condemnation, not of the definition, but of lysogeny itself. And whilst lysogenic bacteria were utilized as instruments for the identification, or typing, of bacteria, lysogeny itself had ceased to exist in the thinking of a number of prominent scientists.

Problems do not exist in nature. Nature only knows solutions. The solution, lysogenic bacteria, was enslaved as a typing tool. The ghost of the problem, like those wisps of cloud that a breath of wind dispels, was blown away from the temple of science and a smell of sulfur was left floating in the air. For many years, many eminent scientists have considered lysogeny as a heresy.

3. *Evolution of language*

"Alas! Alas! Alas! Alas!"—Aeschylus, *The Persians*.

The term "virus" has, for 1900 years, designated the principles or agents of transmissible, contagious, infectious diseases. It is still employed with this meaning by some cultured microbiologists. Since their discovery, those agents which are not visible with an ordinary microscope have been successively called filtrable viruses, ultra-viruses and infra-microbes. But as a result of the *principe du moindre effort*, perhaps also because no one was pretentious or modest enough to describe himself as an ultra-virologist or as an infra-microbiologist, the habit has prevailed to refer to invisible viruses simply as *viruses*. As a consequence, the protists or microbes, the visible agents of infectious diseases, have lost their secular right to be called viruses. The evolution of language being irreversible, one can only, with regret, ratify this popular alteration of the sense of the term virus.

4. *Questions of terminology*

"Most certainly alas! always, always!"—Aeschylus, *The Persians*.

Some confusion exists in literature for the description of prophage and of lysogeny. A lysogenic bacterium has been defined as a bacterium infected with a cryptic, symbiotic, latent virus (66a). Etymologically, latent means hidden. May prophage really be considered as a latent phage? An egg is not a latent organism, it is an organism *in posse*, a potential organism. A chromosome is not a latent cell; a gene is not a latent enzyme. *Prophage* is the specific hereditary structure necessary for the production of phage. It is by no means a hidden phage.

Another example shows the results of the use of such terms as *latent*. The "latent virus" of lysogenic bacteria has been described as possessing a "weak lytic power" on sensitive bacteria (66a). If the virus is latent, how can it infect and lyse bacteria, even weakly? By "latent phage" the author has designated the prophage; then, without any warning, latent phage becomes the infectious phage particle.

The same remark is valid for *symbiotic*. Symbiotic phage, in the same paper, at a few lines intervals, designates either the prophage or the temperate bacteriophage particle.

Lysogenic bacteria have been defined also as a "stable association of a bacterium with a bacteriophage". Prophage is not a bacteriophage. The different stages of the life cycle of an organism, the different parts of a cell are not designated by the same term because they belong to the same species. The egg, or the pluteus larva of a sea-urchin, is not referred to as "sea-urchin". The chromosomes of an *Ascaris* are not referred to as *Ascaris*. The gene which controls the synthesis of β-galactosidase is not called β-galactosidase. Whether an organism is a higher animal, a protist or a bacteriophage, the same term should not be utilized to designate a stage of the life cycle, or the genome, and the "adult" or mature organism itself.

5. *Lysogeny and typing*

The fact that adsorption of phage is bound to specific bacterial receptors which correspond to bacterial antigens has been extensively utilized for the identification or typing of various bacteria such as the salmonellae, staphylococci, etc. This subject has been extensively reviewed by Craigie and Felix; Felix, Felix and Callow; Felix and Pitt; and P. Nicolle (see P. Nicolle (1)). The use of temperate phages produced by lysogenic strains has been suggested by Scholtens and by Boyd. And it appears that immunity of lysogenic bacteria intervenes in lysotypy. This problem which carries in itself the substance of a review cannot be discussed here.

6. *Is bacteriophage a virus? What is a virus?*

"L'on ne fait rien de bien si cette rupture d'équilibre n'arrive à se produire, entre le monde réel et la création du cerveau, cellie-ci paraissant, pour un temps, plus réelle que l'autre".—André Gide, *Journal*.

Since Beijerinck the problem of the fundamental nature of viruses has been often discussed. Some microbiologists consider that viruses are small microbes, but as noted by Rivers (7):

"those who work with viruses consider that they differ in certain respects from bacteria and rickettsiae. Perhaps the best short definition of viruses is that they constitute an heterogenous group of infectious agents which are smaller than ordinary bacteria and require susceptible host cells for multiplication and activity".

Viruses have been defined operationally by Luria (4) as "exogenous submicroscopic units capable of multiplication only inside specific living cells". As bacteriophage is always formed inside its host, it could therefore be described as endogenous. When bacteriophages are formed in a lysogenic bacterium that may have perpetuated its prophage for millions of years, may bacteriophage be described as exogenous? If prophage is phylogenetically endogenous, the temperate phage produced by a lysogenic bacterium *must* be described as endogenous. Bawden in his classical treatise (2) has put some emphasis on size and defined viruses as "obligatory parasitic pathogens with at least one dimension of less than 200 mμ." Thus, according to this definition, a spherical particle would or not be a virus according to whether its diameter is 199 or 200 mμ. If dimensions have any meaning at all, it is not by the astrological virtue of a number but because of a correlation between size and some essential properties which are responsible for a fundamental difference between viruses and nonviruses.

We may try to find out if such a natural difference does really exist and consider bacteriophage on one hand, protists and cells on the other:

(a) All cells and protists, including rickettsiae, contain both types, DNA and RNA, of nucleic acid; bacteriophage contains only one type.

(b) Cells and protists are reproduced essentially from the integrated sum of their constituents; bacteriophage is produced or reproduced from its nucleic acid (case of T2) or from prophage (case of lysogenic bacteria).

(c) Cells and protists are able to grow and to undergo binary fission; the bacteriophage particle *as such* is, so far as we know, unable to grow and to undergo fission. Bacteriophage particles are never produced directly by division of a preexisting phage particle but by organization of nonphage material. It appears as though the materials of the phage could not be replicated when in the form of an organized phage particle.

Thus, cells or protists possess both types of nucleic acid, RNA and DNA, are not produced from a nucleic acid, and are able to grow and to undergo binary fission. Bacteriophage possesses only one type of nucleic acid, is possibly reproduced from its nucleic acid and is unable to grow and undergo binary fission. It is quite possible that these three features or attributes are correlated and subordinated. Whatever the case may be, we are entitled to conclude that bacteriophage differs from cells and protists not only by its size but also in its chemical constitution and mode of reproduction.

It is believed that the properties ascribed to bacteriophage, nucleoprotein particles possessing only one type of nucleic acid, unable to grow and to undergo binary fission and reproduced from their nucleic acid, are shared by a number of "small" infectious, obligate parasitic, sometimes pathogenic, particles which are studied by virologists. Perhaps a discrimination between viruses and nonviruses could be attempted on this basis. If this difference between viruses and nonviruses would be found to be justified and generalizable, then the term virus shall acquire, at last, a definite meaning.

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