# VIRUS GROWTH AND VARIATION

NINTH SYMPOSIUM OF THE
SOCIETY FOR GENERAL MICROBIOLOGY
HELD AT THE
SENATE HOUSE, UNIVERSITY OF LONDON
APRIL 1959

CAMBRIDGE
*Published for the Society for General Microbiology*
AT THE UNIVERSITY PRESS
1959

# CONTENTS

# VIRUSES: A SURVEY OF SOME CURRENT PROBLEMS

S. E. LURIA

*Department of Bacteriology, University of Illinois, Urbana*

An outline of current perspectives in a field evolving as rapidly as virology could easily become an extensive survey of our science in all its complexity and multidirectional developments. To limit the size and scope of this introductory paper, I shall discuss only a few outstanding recent advances, mainly in the bacteriophage field, which have led to some unifying concepts in virology. In the light of these concepts I shall try to indicate the directions in which progress may be expected and the areas where more concentrated efforts are desirable. The immediate test of the ideas that I am presenting will be whether they provide a useful framework for the other papers in this symposium and whether they can at least stand confrontation with the facts presented in those papers. To use the jargon of our trade, my speculations, if defective, can be prevented from establishing a persistent defective infection of our minds by interference from the almost immediate challenge of healthy, effective data.

Of the two aspects of virology, a branch of cellular biology and a branch of pathology and medicine, the major theoretical advances since 1952, the date of our Society's last symposium on viruses, have occurred in the former. This reflects the general progress in the genetic, biochemical, and cytochemical analysis of cellular organization; in fact, virology has made a distinguished contribution to this progress. Major recent advances in applied virology—such as the effective vaccination against poliomyelitis and the controlled epidemiology of myxomatosis—do not represent an introduction of new concepts, but rather an exploitation of advances at the fundamental level. It seems likely that future practical advances, as in chemotherapy, may be based directly on advances in the study of viruses at the level of cellular phenomena.

## VIRUSES AND NUCLEIC ACIDS

The first and major advance has been in the establishment of the primacy of nucleic acids in viral infection. The basic landmarks were, for phage, the injection experiments of Hershey & Chase (1952); for

tobacco mosaic virus, the reconstitution experiments of Fraenkel-Conrat (1956) and the RNA infectivity tests by Gierer & Schramm (1956); for animal viruses, the extraction of infective, RNase-sensitive materials from several viruses (Colter, Bird, Moyer & Brown, 1957).

The identification of phage DNA with the major and possibly the only essential initiator of phage infection; the discovery of the structure of DNA (Watson & Crick, 1953); the advances in bacterial genetics, especially the unravelling of the mating process (Wollman, Jacob & Hayes, 1956) and of the role of DNA in this process (as well as in transformation phenomena; see Hotchkiss, 1955); and the analysis of lysogeny and of its chromosomal determinants or *prophages* (Jacob & Wollman, 1957; Bertani, 1958) have led to a unified view of bacterial genetics having DNA as its protagonist. DNA has emerged as the essential genetic constituent of the bacterial chromosome, of the prophage, of the vegetative form of the phage, and of the infectious phage particle. The concept of phage infection as cellular parasitism at the genetic level (Luria, 1950) has assumed new significance beyond the original one of a competition between two incompatible sets of genetic elements; we now consider phages as segments of bacterial DNA which possess a specific viral function, that is, a genetically determined potentiality for active release and transfer to other cells.

The contribution of phage research to basic genetics has been outstanding. Benzer's (1957) analysis of the fine genetic structure of phage by means of recombination has not only produced clarification of genetic concepts by defining operationally the units of mutation, of recombination, and of genetic function (*muton*, *recon*, and *cistron*), but has also shown the way to the interpretation of genetic specificity in terms of DNA chemistry. The report that specific mutational sites in phage are selectively affected by thymidine analogues (Benzer & Freese, 1958) is a promising beginning.

Along these lines, major advances may be predicted in two directions. First, we expect rapid progress in the correlated studies of DNA biosynthesis and of genetic replication. While DNA provides the basic template for phage production, and while multiplication of phage DNA is the essential outcome of vegetative reproduction of phage, this reproduction entails more than an increase in phage DNA (Hershey, 1957; Stent, 1958). The relative roles of DNA, RNA, and proteins in phage DNA replication, as well as the roles of the recently discovered polynucleotide-synthesizing enzymes (Ochoa & Heppel, 1957; Kornberg, 1957), will be a major area of biochemical virology, tying it to the wider field of nucleic acid biosynthesis.

With viruses other than phages, an essential step should be to correlate the notion of infectious nucleic acid with a description of the early stages in cellular infection. Specifically, the phenomena of eclipse of infectivity, whose reality is now generally accepted, should be interpreted, as with phage, in terms of the cytochemical events in virus penetration and replication.

Second, we expect a rapid advance in the chemical study of virus replication itself. The cytochemical and immunological analysis of the accumulation of viral materials, correlated with biochemical studies, can tell us not only whether nucleic acid, alone or as a nucleoprotein element, plays the central role in the replication of all viruses, but also in which fractions of the cells the various components of viruses are produced, accumulated, and assembled.

Third, we should witness major progress in the analysis of the functional specificity of RNA and, coupled with a clarification of the molecular configuration of RNA, a full-fledged development of 'RNA genetics'. The role of RNA viruses in this new branch of biology will be even more central than that played by phage in the genetics of DNA systems. Purification and chemical analysis of many more animal viruses, and identification of their nucleic acid components, are needed to provide a basis for a correlated genetic and biochemical analysis of viral growth, as well as to define the chemistry of the essential genetic units.

As yet, there is a single example of a unique nucleic acid constituent of viruses, the hydroxymethyldeoxycytidylic acid of the T-even coliphages (Wyatt & Cohen, 1952). Yet, this finding—in itself almost a monstrosity from the standpoint of biochemical evolution—indicates the desirability of a careful search for similar anomalies among other viruses.

## VIRUSES AND CELLULAR FUNCTIONS

Virus infection brings into a cell a new genetic element. In the virus-infected cell there occur a number of changes in structure and function. We ask, therefore, which of these changes are by-products of viral replication, which changes reflect the action of viral genes irrespective of replication, and which changes are non-genetic effects of the process of infection itself—for example, of a disruption of the cellular surface, or of the biosynthesis of new cellular enzymes in response to viral materials acting as inducers.

Work with phage provides examples of all these types. Thus, on the one hand, the cellular lysis that accompanies phage release depends on phage-specific enzymes formed only after replication of phage DNA

(Jacob & Fuerst, 1958). On the other hand, early death of a bacterium, probably by surface disruption, may follow even the attachment of an empty phage coat, a clearly non-genetic action (Herriott & Barlow, 1957). Initiation of phage replication requires the active function of a number of phage genes *before replication*, the 'essential cistrons', revealed by radiobiological and other studies (see Bertani, 1958). Among the essential cistrons must be those that control the biosynthesis of new enzymes specifically required for synthesis of viral nucleic acid. The cytosine-hydroxymethylating enzyme, which appears in T2-infected bacteria by an apparently *de novo* biosynthesis (Flaks & Cohen, 1957), is a convenient example, although proof of its phage-controlled specificity is still awaited.

Let us consider two groups of bacterial products produced under phage control: the proteins of the phage particles and certain somatic antigens in *Salmonella*, which are specifically determined by some phages (Iseki & Sakai, 1953), an example of the so-called 'conversion' phenomena by phage. In both cases, the biosynthesis and the intrinsic specificity of these cellular products are determined by phage genes (Streisinger, 1956; Uetake, 1958). Yet one group of products, the somatic antigens, whose presence and function are apparently irrelevant to phage growth and maturation, is formed whenever the controlling phage is present in the cell in any form—vegetative phage, prophage, or even non-multiplying phage element (Uetake, Luria & Burrous, 1958). The other group of products, the phage-coat proteins, whose synthesis and assembly into mature phage particles are essential for the transfer of phage elements from cell to cell, that is, for the viral function, is produced only after vegetative multiplication of the phage genetic elements.

Thus, we see that phage genes control cellular functions necessary for the process of viral transfer as well as cellular functions totally irrelevant to this process. All portions of the bacterial genome are potentially transferable from cell to cell, as shown by transformation experiments with purified DNA. What distinguishes the phage elements is that they possess, among their many genetic functions, those needed to determine the formation of a specific phage coat. In other words, phages are elements of bacterial DNA, genetically and selectively adapted for transfer from cell to cell.

In an admittedly broad extrapolation, supported by current ideas on the biosynthesis of tobacco mosaic virus (Jeener, 1956) and of myxoviruses (Burnet, 1956), I have proposed to define viruses as 'elements of cellular genetic material that can determine, in the cells where they multiply, the biosynthesis of a specific apparatus for their own transfer to

other cells' (Luria, 1958). This definition centres our attention on the two essential aspects of viruses; as controllers of genetic functions in the cells, and as specialized agents of infective exchanges of genetic materials among cells.

What contribution can we expect from an awareness of the genetic effects of viruses on cellular properties?

First, at the biochemical level, viruses like phage and TMV should provide the best materials for correlating genetic structure with genetic function; that is, for deciphering the code that translates DNA or RNA structure into the molecular structure of proteins.

Second, if present assumptions are valid, it should be possible to interpret the morphogenesis of all viral particles, like that of phage particles, as a triple process: replication of viral genomes, synthesis of specific accessory constituents, and assembly into mature particles. Advances in this direction have already been made with a number of viruses (Sanders, 1957).

Third, we may expect progress in interpreting cellular alterations as due to specific functions of the viruses. Some cellular damage may be bound to specific steps in the growth cycle of a virus, in the same way as bacterial lysis is bound to phage maturation; and these steps, once visualized, may become amenable to directed chemical or nutritional control. Other cellular alterations, such as the release of cells from normal growth-restraining influences observed in virus-induced tumours, may become understandable in terms of specific effects of viral genes, whose function may be as irrelevant to the replication and maturation of the virus as the control of somatic antigens is apparently irrelevant to the viral function of *Salmonella* phages. That is, we may be able to interpret some of the effects of viruses on their hosts as infective heredity rather than as parasitism.

Such a programme of interpretation of viral action as infective heredity has now become feasible for animal viruses because of the major recent advances in mammalian cell microbiology along genetic, nutritional and biochemical lines (Eagle, 1955; Puck, Cieciura & Fisher, 1957; Siminovitch, Graham, Leslie & Nevill, 1957). It is apparent that in the study of the genetics and cytochemistry of mammalian cells the animal viruses will play the same role that phage has played in the analogous study of bacteria.

The development of methods for microbiological handling of plant cells is long overdue. For some plant viruses a useful substitute may well be looked for by developing modern tissue culture methods for the cells of their arthropod vectors (Grace, 1958).

## VIRUSES AND CELLULAR CONSTITUENTS

The genetic material of phage, in its prophage form, is part of the bacterial chromosome. As prophage, it is materially and functionally integrated with the genetic system of the bacterial cell, even though the material integration is probably less solid than that among other chromosomal elements, as shown by loss or substitution of prophage *in toto* (Bertani, 1958). The details of the integration process and the precise nature of the bonds between prophage and bacterial chromosome are still unclear. Matings between lysogenic bacteria provide one line of attack (Jacob & Wollman, 1957). Another, more exciting one, is provided by certain transduction phenomena in which groups of bacterial genes become stably incorporated into prophages, hence into phage particles, with which they can be transferred to other cells (Morse, Lederberg & Lederberg, 1956; Arber, Kellenberger & Weigle, 1957). In this 'specialized transduction' we see how non-viral chromosomal elements can acquire viral properties, that is, become adapted to infective transfer by recombination with viral elements. In turn, the latter may have acquired their viral function by mutations; as prophages, they may return to pair with their homologous counterparts in the bacterial chromosome. If, even as prophages, they appear to be less completely integrated within the chromosome, this may simply reflect an incompleteness of the residual homology between prophage and the attachment region. Conversion phenomena can easily be interpreted within this framework (Luria, Fraser, Adams & Burrous, 1958).

In bacteria there are several genetic determinants or 'episomes' (Jacob & Wollman, 1958) which appear to have a dual multiplication pattern, as vegetatively reproducing elements on the one hand, and as integrated chromosomal elements on the other. The phage elements should be considered as episomes endowed with the ability to determine a transfer apparatus.

With viruses other than phage, this approach to an experimental analysis of the relationship and interactions between the viruses and the genetic elements of their host cells has barely begun. The study of persistent experimental infections of animal cells in tissue cultures (Puck & Cieciura, 1958; Ackermann, 1958) has revealed a variety of situations, none of which can as yet be interpreted in terms of 'proviruses', that is, of persistent viral genomes harmoniously integrated within the cellular genome. The most promising material is that of tumour viruses, where virus persistence is the primary cause of the abnormal growth pattern. Clearly, progress in the knowledge of the genetic status of proviruses

(if they exist) in animal or plant cells will depend on the expected progress in the study of the genetics of these cells.

In this area two specific problems deserve careful attention. First, we need to investigate the nature of the cytogenetic changes that follow, respectively, infection with DNA and RNA viruses. We need to find out whether viral DNA enters into close contact with host-cell DNA or whether it sets up independent and competing centres of DNA synthesis. For example, any DNA entering the cellular cytoplasm might cause cellular damage because of the absence in the cytoplasm of mechanisms for controlling and restraining DNA replication. Conversely, viral RNA or ribonucleoproteins may be prone to replicate first in the cell nucleus, possibly causing specific changes also in the chromosomal DNA. The tools to answer such questions are rapidly being forged by chemical cytology (Brachet, 1957).

Second, we must find out how restricted is the transferability of viral materials. In bacteria all chromosomal elements are transferable from cell to cell as DNA; it is conceivable that in animal and plant cells also some elements endowed with genetic specificity, whether embodied in DNA or in RNA, may be intrinsically transferable, that is, may be capable of functioning if introduced from without into appropriate living cells, even though they do not possess an adaptive mechanism for such transfer. We should search carefully for such transferability of cellular constituents and for what roles it may play, for example, in developmental and regulatory processes in animals and plants. If transferability were a widespread property of cellular constituents, we should beware of considering as viruses all transferable elements encountered in attempts to detect agents of diseases, for example, in cancer research. We should also ask ourselves how much of a genetic change may be required to make a transferable cellular constituent into a virus, that is, to endow it with the ability to direct the biosynthesis of its own vehicle.

## VIRUS DISEASES AND INFECTIVE HEREDITY

As we emphasize the genetic aspects of viruses, the study of virus diseases assumes new dimensions. We begin to consider, not only cellular damage accompanying virus multiplication, but also abnormal cell functions reflecting the presence of the viral genome and its function within the framework of the cellular genome. In this sense, virus diseases may be considered as genetic diseases, more akin to metabolic diseases than to bacterial or protozoan infections. Thus, for example, the much debated dichotomy between the somatic mutation theory and the virus theory of

cancer loses much of its significance. Clearly, similar results may follow either mutational or infective changes in cellular heredity.

Several other implications may be pointed out. Cellular alterations due to viral gene action can provide new approaches to chemotherapy. It may be possible to control viral infections by acting specifically on the new functions of virus-infected cells rather than directly on viral spread or multiplication. In fact, virus-induced tumours, like other tumours, may be controlled by treatments directed to the growth habits of the cells, not to the viral process.

Some viral phenomena, especially the age dependence of the incidence and course of some virus infections, as observed in lymphocytic choriomeningitis (Hotchin, 1958) and in many virus-induced tumours (see Gross, 1958) may be mediated by alterations in cellular antigens. These may in turn give rise to abnormal immunological responses, such as immune tolerance or hypersensitivity, depending on the age at which infection occurred (Burnet, 1955).

There are other conceivable mechanisms by which functional changes of cells due to a virus may produce general body alterations. For example, infection of an endocrine organ might, without cell destruction, but by alteration of cellular functions, give rise to metabolic and morphogenetic abnormalities.

## VIROLOGY AND MODERN BIOLOGY

The most fascinating aspect of virology to me is the role that virus research is playing in the current revolution in biology. As in the transformation of classical physics into modern physics, the current flourishing of biology involves the dialectical resolution of conflicting opposites through clarification of basic concepts and of their content. Biochemistry is unifying the molecular and the structural levels of organization. Enzyme action and template action are seen as inseparable aspects of the process of genetic replication at the molecular level. Genetics is bringing about a synthesis of gene replication and gene function as two chemical expressions of the same elements, the coded molecules of nucleic acids, and is dissolving the opposition between heredity and development.

In molecular biology, life becomes the self-propagating pattern of functional organization of selected molecular species into harmonious wholes. By analogy, the cell plays the role of the atom; cellular biology, that of atomic physics. Within the living world natural selection, like the exclusion principles of atomic physics, restricts and stabilizes the states which a cell or organism can successfully assume. Within the cell the basic system of coded macromolecules recalls the atomic nucleus; indeed,

it is gathered into a nucleus, whose organization preserves the stable associations of valuable molecular patterns, in the same way as in the atomic nucleus special forces bind together the stable groups of elementary particles.

What role do viruses play? Can we not claim for them, in this analogy, the role of the particulate beams that the physicists use to probe into the organization of atoms and nuclei? And proceeding a step further, may we not feel that in the viruses, in their merging with the cellular genomes and their re-emerging from them, we observe the units and processes which, in the course of evolution, have created the successful genetic patterns that underlie all living cells?

## REFERENCES


ACKERMANN, W. W. (1958). Certain factors governing the persistence of poliovirus in tissue culture. In *Symposium on Latency and Masking in Viral and Rickettsial Infections*. Minneapolis: Burgess.

ARBER, W., KELLENBERGER, G. & WEIGLE, J. (1957). La défectuosité du phage lambda transducteur. *Schweiz. Z. allg. Path.* **20**, 659.

BENZER, S. (1957). The elementary units of heredity. In *The Chemical Basis of Heredity*. Edited by W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Univ. Press.

BENZER, S. & FREESE, E. (1958). Induction of specific mutations by 5-bromouracil. *Proc. Nat. Acad. Sci., Wash.* **44**, 112.

BERTANI, G. (1958). Lysogeny. *Advanc. Virus Res.* **5**, 151.

BRACHET, J. (1957). *Biochemical Cytology*. New York: Academic Press.

BURNET, M. (1955). *Principles of Animal Virology*. New York: Academic Press.

BURNET, M. (1956). Structure of influenza virus. *Science*, **123**, 1101.

COLTER, J. S., BIRD, H. H., MOYER, A. W. & BROWN, R. A. (1957). Infectivity of ribonucleic acid isolated from virus-infected tissues. *Virology*, **4**, 522.

EAGLE, H. (1955). The specific amino acid requirements of a mammalian cell (strain L) in tissue culture. *J. biol. Chem.* **214**, 839.

FLAKS, J. & COHEN, S. S. (1957). The enzymic synthesis of 5-hydroxymethyl deoxycytidylic acid. *Biochim. biophys. Acta*, **25**, 667.

FRAENKEL-CONRAT, H. (1956). The role of the nucleic acid in the reconstitution of active tobacco mosaic virus. *J. Amer. chem. Soc.* **78**, 882.

GIERER, A. & SCHRAMM, G. (1956). Die Infektiosität der Nucleinsäure aus Tabakmosaikvirus. *Z. Naturf.* **11***b*, 138.

GRACE, T. D. C. (1958). The prolonged growth and survival of ovarian tissue of the promethea moth (*Callosamia promethea*) *in vitro*. *J. gen. Physiol.* **41**, 1027.

GROSS, L. (1958). Viral etiology of 'spontaneous' mouse leukaemia. *Cancer Res.* **18**, 371.

HERRIOTT, R. M. & BARLOW, J. L. (1957). The protein coats or 'ghosts' of coli phage T2. II. The biological functions. *J. gen. Physiol.* **41**, 307.

HERSHEY, A. D. (1957). Bacteriophages as genetic and biochemical systems. *Advanc. Virus Res.* **4**, 25.

HERSHEY, A. D. & CHASE, M. (1952). Independent functions of viral protein and nucleic acid in growth of bacteriophage. *J. gen. Physiol.* **36**, 39.

HOTCHIN, J. E. (1958). Some aspects of induced latent infection of mice with the virus of lymphocytic choriomeningitis. In *Symposium on Latency and Masking in Viral and Rickettsial Infections*. Minneapolis: Burgess.

HOTCHKISS, R. D. (1955). Bacterial transformation. *J. cell. comp. Physiol.* **45** (Suppl. 2), 1.

ISEKI, S. & SAKAI, T. (1953). Artificial transformation of O antigens in *Salmonella* E group. I, II. *Proc. Japan Acad.* **29**, 121 and 127.

JACOB, F. & FUERST, C. R. (1958). The mechanism of lysis by phage studied with defective lysogenic bacteria. *J. gen. Microbiol.* **18**, 518.

JACOB, F. & WOLLMAN, E. L. (1957). Genetic aspects of lysogeny. In *The Chemical Basis of Heredity*. Edited by W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Univ. Press.

JACOB, F. & WOLLMAN, E. L. (1958). Les épisomes, éléments génétiques ajoutés. *C.R. Acad. Sci., Paris*, **247**, 154.

JEENER, R. (1956). Ribonucleic acids and virus multiplication. *Advanc. Enzymol.* **17**, 477.

KORNBERG, A. (1957). Pathways of enzymatic synthesis of nucleotides and polynucleotides. In *The Chemical Basis of Heredity*. Edited by W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Univ. Press.

LURIA, S. E. (1950). Bacteriophage: an essay on virus reproduction. *Science*, **111**, 507.

LURIA, S. E. (1958). Viruses as infective genetic materials. In *Symposium on Immunity and Virus Infection* (in the Press).

LURIA, S. E., FRASER, D. K., ADAMS, J. N. & BURROUS, J. W. (1958). Lysogenization, transduction, and genetic recombination in bacteria. *Cold Spr. Harb. Symp. quant. Biol.* **23** (in the Press).

MORSE, M. L., LEDERBERG, E. M. & LEDERBERG, J. (1956). Transduction in *Escherichia coli* K-12. *Genetics*, **41**, 142.

OCHOA, S. & HEPPEL, L. A. (1957). Polynucleotide synthesis. In *The Chemical Basis of Heredity*. Edited by W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Univ. Press.

PUCK, T. T. & CIECIURA, S. J. (1958). Studies on the virus carrier state in mammalian cells. In *Symposium on Latency and Masking in Viral and Rickettsial Infections*. Minneapolis: Burgess.

PUCK, T. T., CIECIURA, S. J. & FISHER, H. W. (1957). Clonal growth *in vitro* of human cells with fibroblastic morphology. *J. exp. Med.* **106**, 145.

SANDERS, F. K. (1957). The multiplication of animal viruses. In *The Nature of Viruses*. Ciba Foundation Symposium. Edited G. E. W. Wolstenholme and E. C. P. Millar. London: Churchill.

SIMINOVITCH, L., GRAHAM, A. F., LESLIE, S. M. & NEVILL, A. (1957). Propagation of L strain mouse cells in suspension. *Exp. Cell Res.* **12**, 299.

STENT, G. (1958). Mating in the reproduction of bacterial viruses. *Advanc. Virus Res.* **5**, 95.

STREISINGER, G. (1956). The genetic control of host range and serological specificity in bacteriophages T2 and T4. *Virology*, **2**, 377.

UETAKE, H. (1958). Mutations affecting the specificity of the somatic antigens determined by *Salmonella* bacteriophage $\epsilon^{15}$ (to be published).

UETAKE, H., LURIA, S. E. & BURROUS, J. W. (1958). Conversion of somatic antigens in *Salmonella* by phage infection leading to lysis or lysogeny. *Virology*, **5**, 68.

WATSON, J. D. & CRICK, F. H. C. (1953). The structure of DNA. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 123.

WOLLMAN, E. L., JACOB, F. & HAYES, W. (1956). Conjugation and genetic recombination in *Escherichia coli* K-12. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 141.

WYATT, G. R. & COHEN, S. S. (1952). A new pyrimidine base from bacteriophage nucleic acids. *Nature, Lond.* **170**, 1072.

# GROWTH OF BACTERIOPHAGE

EDOUARD KELLENBERGER

*Laboratoire de Biophysique, Université de Genève, Switzerland*

## INTRODUCTION

The developmental cycle of bacteriophage is in essence the same as that of most other viruses; in particular, infection is characteristically followed by the so-called eclipse period in which no infective particles can be found inside the cell. For phage it was possible to show that this eclipse period coincides with the replication of phage DNA. The basic experiments of Hershey & Chase (1952), demonstrating that the protein coat of the infecting phage is left outside the cell, have been followed by still further evidence strengthening the hypothesis that DNA is the sole carrier of genetic information.

Two main problems can be studied with the system of bacterial viruses: (1) the replication of the injected DNA in the form of new phage genomes which are later integrated into the progeny; (2) the direction by that DNA of the synthesis of the phage proteins and the assembly of complete phage particles. These problems have the potentiality of contributing to our knowledge of what is nowadays called the chemical basis of heredity. It is desirable to search out the connexions between all the manifestations of phage reproduction and the genetic information contributed by phage DNA. These questions will be treated in other contributions to this symposium.

Before concentrating on vegetative phage and the formation of mature virus particles, we will briefly recall the main results obtained concerning phage structure and the mechanism of infection, as this knowledge is important for understanding the functional differentiation of phage components. We will not always proceed historically, because newer results have often supplanted the original interpretations.

The author apologizes for putting some exaggerated emphasis on recent morphological results: it is, after all, so satisfactory to see now what was formerly deduced by indirect means.

Most of the experiments concerning phage have been made on the even-numbered *Escherichia coli* T phages. The main reason for this is that the DNA of these phages had been found to be of a composition different from that of the host DNA: cytosine is replaced by 5-hydroxymethylcytosine (Wyatt & Cohen, 1952). It is this property which enables

one to follow the metabolism of phage DNA without any confusion with that of bacterial DNA. This fundamental difference is probably reflected in other particular properties; care should therefore be taken in extrapolating results from these T-even phages to other systems.

It is very desirable that our knowledge be extended to other phages, in particular to the temperate phages, which are able to integrate themselves into the host genome, thus forming lysogenic cells. These bacterial viruses are more closely related to the bacteria than are the T-even coliphages, and show even in their lytic cycle a much greater dependence upon the host cell. In the present paper we will consider some observations related to the growth of a temperate coliphage; but the scope of the review does not allow us to go further. For the general problems of lysogeny, we refer the reader to a recent review by Bertani (1958).

## STRUCTURE OF BACTERIOPHAGE

The even-numbered T phages can be easily disrupted by osmotic shock into two parts (Anderson, 1950): the DNA moiety, comprising about 40 % of the total phage weight, and the protein (Herriott, 1951), representing the phage head membrane, the tail and some other minor components. In other phages which are not sensitive to osmotic shock, it was possible to show at least that the existence of a head membrane is general, since various methods of inactivation result in the production of empty head membranes (Lark & Adams, 1953; Kellenberger & Kellenberger, 1954).

The structural complexity of the T2-protein has been investigated by electron-microscopy. The tail has been found to consist of several substructures (Kellenberger & Arber, 1955; Williams & Fraser, 1956). At the centre is an inner core, which has recently been found to be a hollow tube (Brenner, Streisinger & Horne, 1958). This core is surrounded by a sheath, which normally covers the whole length of the tail. By chemical means—mainly oxidation, or hydrolysis of thiolester bonds—this sheath can be made to contract to half its length, uncovering the distal part of the tail core (Kellenberger & Arber, 1955; Brown & Kozloff, 1957; Kozloff, Lute & Henderson 1957). It is not yet known if some other proteins of a 'cement' nature go into solution at the same time. On the tip of the core are fixed the tail fibres. The latter seem to be important in adsorption (Williams & Fraser, 1956). Very recently Brenner (personal communication) succeeded in separating and purifying by chemical means the three main constituents of the tail, namely core, sheath and fibres. This opens the way to more thorough

investigations of the chemical structure and physiological functions of these components in relation to the genetic control of their production.

All phages studied so far contain DNA of the usual base composition, with the exception of the T-even phages which contain hydroxy-methylcytosine in place of cytosine. No other exceptional base has as yet been observed. The molecular constitution of T2 DNA has been studied in some detail, but without reaching a final conclusion about its significance. It has been found by autoradiography of $^{32}P$-containing phage that the DNA is composed of a large piece, representing about 40 % of the total, and several much smaller pieces (Levinthal & Thomas, 1957). Two different DNA fractions had also been found by chromatography (Brown & Martin, 1955). It is interesting to note that the T-even phages contain glucose associated with the hydroxy-methylcytosine, in amounts which are different for the various strains (Sinsheimer, 1956; Jesaitis, 1956), and that the association shows unusual inheritance (Streisinger & Weigle, 1956).

It is not yet clear how the free acid groups of DNA are neutralized. A recent report, however, gives evidence that this role may be taken by basic polyamines. Indeed, spermidine and putrescine have now been found in phage T2 (Ames, Dubin & Rosenthal, 1958).

## THE INFECTION OF THE CELL

Since our primary subject is phage growth, we will not discuss in detail our knowledge concerning the adsorption and penetration of bacterial viruses. An extensive review of this subject has been made by Tolmach (1957). We may just mention recent work showing that there is a double interaction between the bacterial wall and the tail of the phage, in the sense that on the one hand contact with the wall seems to induce structural changes in the tail of the phage (Kellenberger & Arber, 1955; Kozloff, Lute & Henderson, 1957), and on the other hand that an enzyme on the phage tail breaks down some of the constituents of the cell wall (Brown & Kozloff, 1957; Weidel & Primosigh, 1957).

One of the most important experiments concerning the infection of a bacterium by a bacteriophage has been made by Hershey & Chase (1952). With $^{35}S$ used as tracer, only the protein of the phage is made radioactive. When bacteria are infected with such phages and some minutes later sheared in a blender, the following results are found: most of the radioactivity remains in the supernatant when the bacteria are centrifuged down; the resuspended bacteria, however, still form infective

centres; observed in the electron microscope (Kellenberger & Arber, 1955) the supernatant is found to contain empty heads alone or associated with half of the tail, so that some very small part of the tail must have been left on the wall or in the bacterium. No sulphur, however, is transmitted to the progeny in measurable amounts (French, 1954). Hence most of the phage protein, composed of the empty head and most of the tail, remains outside the cell after DNA has been injected. Proof of this latter fact is given by $^{32}$P-labelling, which is confined to the DNA; after injection, such phosphorus is found bound to the host cell (Hershey & Chase, 1952), and about half of the isotopic label of the injected DNA is found again in the progeny phage (Watson & Maaløe, 1953; French, Graham, Lesley & van Rooyen, 1952; Putnam & Kozloff, 1950; Kozloff, 1953; Hershey, Garen, Fraser & Hudis-Dixon, 1954). The $^{32}$P of the isotopically marked DNA is not equally distributed among the progeny, but, as shown by decay experiments (Stent & Jerne, 1955; Stent, 1958), remains confined to a very small number of phages.

Since it is basically important to know whether the injected DNA contains the genetic information, or whether there are some components other than DNA which do so, Hershey (1957) made a detailed investigation of the acid-soluble non-DNA fraction of phage which is injected with the DNA. This fraction, which comprises about 3 % of the total carbon of phage, was found to be composed of a polypeptide and two other substances now identified as polyamines (Ames, Dubin & Rosenthal, 1958). The polypeptide is not transmitted from the parental DNA to the offspring and its synthesis in newly infected bacteria is inhibited by chloramphenicol. The other components, however, are transmitted to the offspring and are formed in the presence of chloramphenicol. But since these substances can be supplied to the offspring through the culture medium, Hershey concludes that they do not play an important role in carrying the genetic information.

## THE METABOLISM OF THE PHAGE-INFECTED CELL

Soon after infection with T-even phage the metabolism of the bacterial cell changes markedly: many enzymes are no longer produced, though those already present remain active (Cohen, 1949; Pardee & Williams, 1953), or may even be activated (Kozloff, 1953; Kunkee & Pardee, 1956; Wormser & Pardee, 1957); infected bacteria fail to be induced for adaptive enzymes (Monod & Wollman, 1947); protein synthesis, however, measured as a whole, continues (Cohen, 1948; Hershey, Dixon & Chase, 1953). Antigenically this protein is not related to phage

protein (Watanabe, 1957*a*). It seems important to note, however, that this synthesis seems to be governed by the phage genome: the synthesis of 'non-antigenic protein' is induced by infection in u.v.-irradiated bacteria, in which the synthesis of bacterial protein is largely suppressed (Watanabe, 1957*a*). The synthesis of bacterial DNA is completely stopped upon infection and the existing DNA becomes progressively degraded. Phage-specific DNA, containing hydroxy-methylcytosine, is found about 8 min. after infection (Cohen, 1948; Hershey, Dixon & Chase, 1953; Vidaver & Kozloff, 1957).

The metabolism of RNA is not yet clearly understood. There is no doubt, however, that after infection with T2, new RNA is synthesized as measured by the assimilation of radioisotopes (Volkin & Astrachan, 1956). Some at least of the bacterial RNA is broken down: purines and pyrimidines may even enter the phage-synthesized DNA (Hershey, Garen, Fraser & Hudis-Dixon, 1954).

Some 5 min. before active intracellular phage appears, phage-specific proteins—precipitating with antiphage serum—begin to appear, and increase almost linearly with time. This protein is produced independently of the other proteins synthesized during the first minutes of infection, that is, there is practically no turnover between them (Kiho & Watanabe, 1957; Watanabe, 1957*b*).

If the synthesis of an amino acid is missing from an auxotrophic bacterium, phage growth is inhibited unless the amino acid is supplied (Burton, 1955). Phage does not supply the necessary information for the synthesis of amino acids. Phage T2, however, is able to direct the synthesis of thymine (Barner & Cohen, 1954).

## THE DNA OF VEGETATIVE PHAGE

### *DNA and the genetic pool*

We have already seen that the DNA of phage T2 begins to increase about 8 min. after infection. It then continues to grow almost linearly (Cohen, 1948; Hershey, Dixon & Chase, 1953; Vidaver & Kozloff, 1957). The measurements include all phage DNA, whether integrated into particles or not. We know, however, that the first intracellular phage, and phage-related structures visible in the electron microscope, do not appear before 10 min., at which time about fifty phage equivalents of DNA can already be measured chemically (Hershey, Dixon & Chase, 1953; Hershey, 1956). Using isotope tracers it has been found that this DNA, formed early, is later incorporated into mature phage (Hershey, 1953); its phosphorus needs about 14 min. for transfer from