COLD SPRING HARBOR SYMPOSIA
ON QUANTITATIVE BIOLOGY

VOLUME XVIII

VIRUSES

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FOREWORD

The first paper on the subject of viruses to be included in the Cold Spring Harbor Symposia was given at the sixth Symposium, in 1938, by W. M. Stanley and H. S. Loring. Since that time, papers on this topic have been presented at several other sessions, where the main problems under consideration related to genetic mechanisms. This year's Symposium was the first to be devoted entirely to discussion of the fundamental aspects of virus research.

The preliminary plans for this meeting were in progress for several years. The Cold Spring Harbor laboratories have been actively interested in research with viruses ever since 1941, when the T series of phages was used in studies of mutation at the Department of Genetics of the Carnegie Institution of Washington. This interest was considerably stimulated when, in the summer of 1945, Max Delbrück organized a course for research workers, on quantitative methods used in research with bacterial viruses. This course has been given at the Biological Laboratory every summer since then, first by Delbrück, then by Mark H. Adams, and in 1952 by A. H. Doermann while Adams was in Europe. Further stimulation was provided by the presence of phage workers at the Laboratory during the summers, by several phage conferences held here on Delbrück's initiative, and by the addition of A. D. Hershey to the research staff of the Department of Genetics.

Thus the ground was well prepared for a session on viruses. The meeting was originally scheduled for the summer of 1952, but was postponed for a year on account of the phage symposium at Abbaye Royaumont.

This Symposium on Viruses was organized by Max Delbrück, in consultation with Frank L. Horsfall, Jr., and H. M. Weaver. Its primary purpose was to bring together research workers in this rapidly developing field for discussion of basic problems, and to provide an opportunity for those specializing in bacterial, animal, and plant viruses to correlate their findings.

The established procedure of our Symposia has been to schedule two or three invited papers per day and to allow ample time for informal and spontaneous discussion. The scheme was somewhat modified this year. In addition to the twenty authors of scheduled papers, a number of other participants were invited to give prepared discussions, concerned primarily with their own research. These discussions are published here as separate articles.

The last three Symposium volumes were printed by the photo-offset process. This method was used in the hope that publication might be speeded up and also that the cost of production—and thus the price of the books—might be decreased. The first and most important of these anticipations was not realized, however; and since, in addition, the quality of the printing was considered less satisfactory than in previous volumes, we have returned, in this volume, to the typesetting method. We have continued our efforts to complete the publication of the proceedings as soon as possible after the Symposium meeting. As part of this effort, the printer's proofs of all articles in this volume were handled in our editorial office, and no proofs were sent to the authors. In addition to Dr. Katherine Brehme Warren, who served as editor, Dr. Mark H. Adams checked over the proofs.

The Symposium was held from June 5th to June 11th, 1953, and was attended by two hundred and seventy-two scientists. The new lecture hall of the Cold Spring Harbor laboratories was used for the meetings.

The National Foundation for Infantile Paralysis paid the expenses of invited participants, as well as the expenses incurred in organizing the meeting; and I wish to acknowledge this support with grateful appreciation. I wish also to express appreciation for the continued support of the Carnegie Corporation of New York, whose grant for this year has been used to promote the early publication of this volume.

M. DEMEREC

LIST OF PREVIOUS VOLUMES

Volume I (1933) Surface Phenomena, 239 pp.
Volume II (1934) Aspects of Growth, 284 pp.
Volume III (1935) Photochemical Reactions, 359 pp.
Volume IV (1936) Excitation Phenomena, 376 pp.
Volume V (1937) Internal Secretions, 433 pp.
Volume VI (1938) Protein Chemistry, 395 pp.
Volume VII (1939) Biological Oxidations, 463 pp.
Volume VIII (1940) Permeability and the Nature of Cell Membranes, 285 pp.
Volume IX (1941) Genes and Chromosomes: Structure and Organization, 315 pp.
Volume X (1942) The Relation of Hormones to Development, 167 pp.
Volume XI (1946) Heredity and Variation in Microorganisms, 314 pp.
Volume XII (1947) Nucleic Acids and Nucleoproteins, 279 pp.
Volume XIII (1948) Biological Applications of Tracer Elements, 222 pp.
Volume XIV (1949) Amino Acids and Proteins, 217 pp.
Volume XV (1950) Origin and Evolution of Man, 425 pp.
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INTRODUCTORY REMARKS ABOUT THE PROGRAM

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The programs of some previous virus meetings followed the viruses through a life cycle, from the free state through infection to multiplication and release. The present program is also guided by this life cycle idea. One new feature is the recognition that the infecting virus undergoes an essential change before it multiplies. The multiplying form is here called the *vegetative phase*, in analogy to the use of the word "vegetative" in the bacteriology of sporulating bacteria. A second new feature is the bifurcation of the life cycle which leads to the branch called *provirus*.

In formulating the program, we thus start out with the doctrine of the trinity of virus: *infective* (or mature) *virus*, *vegetative virus*, and *provirus*. This doctrine of the trinity is the outgrowth of the work on bacterial viruses of the last decade, and, more specifically, of last year's international phage meeting at the Abbaye Royaumont. It is not the purpose of these introductory remarks to defend the scientific truth of this doctrine. The collective evidence of the papers presented at this meeting will serve to sustain it or to modify it. The worst that may happen to a heretic is that he may be asked to take a hand in formulating the program for a future virus meeting. We merely hope that the doctrine will serve to bring us at once to the most interesting problems of current research, and that it helps to bridge the gaps between the classes of viruses and between the methods of research.

We start out then with a discussion of the *vegetative phase*. For many years it had been supposed that the state in which a virus multiplies would be different from its extracellular state. I do not think, however, that many people considered the possibility that a virus in its multiplying state might be non-infective. Very strong evidence that it is indeed non-infective was obtained by Doermann for some of the phages by the study of intracellular growth curves. A convincing reason for the non-infectiveness was discovered last year by Hershey and Chase who showed that the protein coat of a phage does not even enter the bacterial cell. We thus have a qualitative picture of the transition from the infective to the vegetative phase. It should be clearly borne in mind that a virus particle in the vegetative state escapes every direct method of bio-assay. Nevertheless, indirect evidence about the mode of multiplication can be obtained by the study of genetic recombination, and from bio-chemical studies. Particularly in the latter respect we hope to learn much at this meeting. Last year it seemed as if we were running into a paradoxical situation. From the Hershey and Chase experiments one was led to infer that virus DNA synthesis precedes virus protein synthesis. In conflict with this, there were several lines of biochemical evidence suggesting that there exist immature forms of virus containing protein and no DNA. This issue will be discussed in various connections at this meeting.

The term provirus was coined by Lwoff and Guttman three years ago to describe the state of the phage in lysogenic bacteria. The definition is this: a cell contains provirus if it and all of its descendants have the *potentiality* to produce infective virus. Potentiality means: under proper conditions, the cell will produce infective virus. What are proper conditions? In some cases we know what they are and we then say that these conditions *induce* the transition from provirus to vegetative virus and to infective virus. In other cases we do not know the proper conditions, but nature seems to provide them in a random fashion, apparently, in a very small proportion of the cells. It is clear that provirus is a very shadowy character. It is defined as a condition of the bacterium which confers on it a potentiality. In many cases this potentiality is easily and clearly recognized, but there is no way of ever being certain that a given strain does *not* possess such a potentiality. Clearly, we want to get better acquainted with this shadowy character. Above all, we want to know where he resides in the cell, and how many of them there are within any one cell. The second day of this symposium is devoted entirely to these questions.

There are obvious similarities between a provirus and a gene. In non-sexual reproduction, a gene, too, can only be defined as a condition conferring upon its carrier a potentiality. Our more detailed knowledge of genes stems entirely from its behavior in sexual reproduction. It is therefore of the greatest interest to try to advance our understanding of provirus by studying its behavior in experiments on genetic recombination of cells carrying provirus. Here, of course, we run into the embarrassing situation that the genetics of bacteria is still in a troubled state, and we will have to devote considerable time to an attempt to grasp the present status of this problem.

These remarks should be sufficient to clarify the main outline of the program. There are a number of other papers whose title may not seem to bear any immediate relation to this outline, but anybody at all familiar with the situation will, I believe, find no difficulty in assessing their relation to the other problems.

Special mention should be made of a last minute addition to the program, or rather, to the list of

participants. The discovery of a structure for DNA proposed by Watson and Crick a few months ago, and the obvious suggestions arising from this structure concerning replication seemed of such relevance to many of the questions to be discussed at this meeting that we thought it worth while to circulate copies of three letters to Nature concerning this structure among the participants before the meeting, and to ask Dr. Watson to be present at the meeting.

Perhaps some may feel that the program is biased too heavily towards the bacterial virus side, and that the plant viruses, especially, have been cut out almost completely. Both of these biases may be more apparent than real. At the end of the week more words may have been spoken about the animal and the plant viruses than about the phages. The phages loom so large on the printed program because phage has served to elaborate the doctrine around which the program is built. Plant viruses, even though they have led the field for twenty years in all matters of physico-chemical characterization of the particles in the infective state, do not lend strength to this scheme of things. They fail to do so not by providing conflicting evidence but because of a characteristic shortcoming of the bio-assay techniques now in use for plant viruses. These techniques are not inferior to animal virus assays in their accuracy, but in their efficiency: very large numbers of virus particles are needed to give a minimal biological effect. For this reason most of the experiments needed to test the doctrine are not feasible. In addition, and in consequence of this shortcoming, the plant virus field has been animated for twenty years by an extraordinary amount of controversy. Since most of this controversy would be unintelligible to the outsider, it was felt that no useful purpose would be served by having it aired once more before a larger audience. Let our friends establish a party line among themselves, so we know what to compare with what.

With the animal viruses the situation is infinitely more favorable. There is available a wealth of information on non-infective virus forms both of the vegetative and of the provirus phase. Even genetic recombination has been successfully demonstrated in flu virus. In addition, quantitative and efficient bio-assay methods have been developed and successfully employed to obtain simple growth curves, to study interference and even the phenomena of multiplicity reactivation of ultraviolet treated flu virus. In addition, the extraordinary efforts spent on polio studies have made this group of viruses well known and has led to the development of convenient methods for growing and handling them. These efforts of purely medical research may well turn out to be a boon to pure biology. The paper on the last day of our program is devoted to a technique for the bio-assay of animal viruses which puts them on par with the phages, and wonder of wonders, polio virus appears to be a choice virus for this technique. I think it may safely be predicted that the next few years will see a fierce race between flu and polio for the distinction of being called the "Drosophila of Animal Viruses."

THE VEGETATIVE STATE IN THE LIFE CYCLE OF BACTERIOPHAGE: EVIDENCE FOR ITS OCCURRENCE AND ITS GENETIC CHARACTERIZATION

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Introduction

It can be stated unequivocally that replication of the genetic material of bacteriophage is accomplished when the viral particle is in a noninfectious condition within the bacterial host cell. This state of the phage has been named the "vegetative state," which implies nothing more than that the virus is in a noninfectious and replicating condition. The purpose of this paper is to summarize evidence indicating the existence of the vegetative state, and to describe, insofar as possible, the genetic phenomena occurring during this state.

Materials and Methods

The material and techniques used in the investigations to be reviewed have been described in many places (for reviews see Delbrück *et al.*, 1950; Adams, 1950). Here it will suffice to discuss what is operationally meant by the term "cross" when used in connection with bacteriophage, and to list briefly the main categories of genetic mutants which have proved useful. Making a cross implies infecting bacteria with phage particles of two genetically different types. It is accomplished by adding a mixture of the phage types in such amount that each of the 10^7 to 10^9 bacteria of the culture will, on the average, be infected with several of each type. The progeny of the cross is usually determined from a sample of the mean yield of a large number of these bacteria. It is possible, although much more laborious, to obtain yields from individual bacteria separately by the so-called single burst technique (Delbrück, 1945) which has been used in some experiments (Hershey and Rotman, 1949). But unless otherwise specified, the experiments mentioned here are from the pooled average of many cells.

Until recently (Bresch, 1953) genetic material in phages other than the closely related T2, T4, and T6 (T-even) group has been too limited to permit extensive investigation, and for this reason all of the experiments referred to in this paper will deal with the T-even series. Generalization to other phages must await more reports on dissimilar groups.

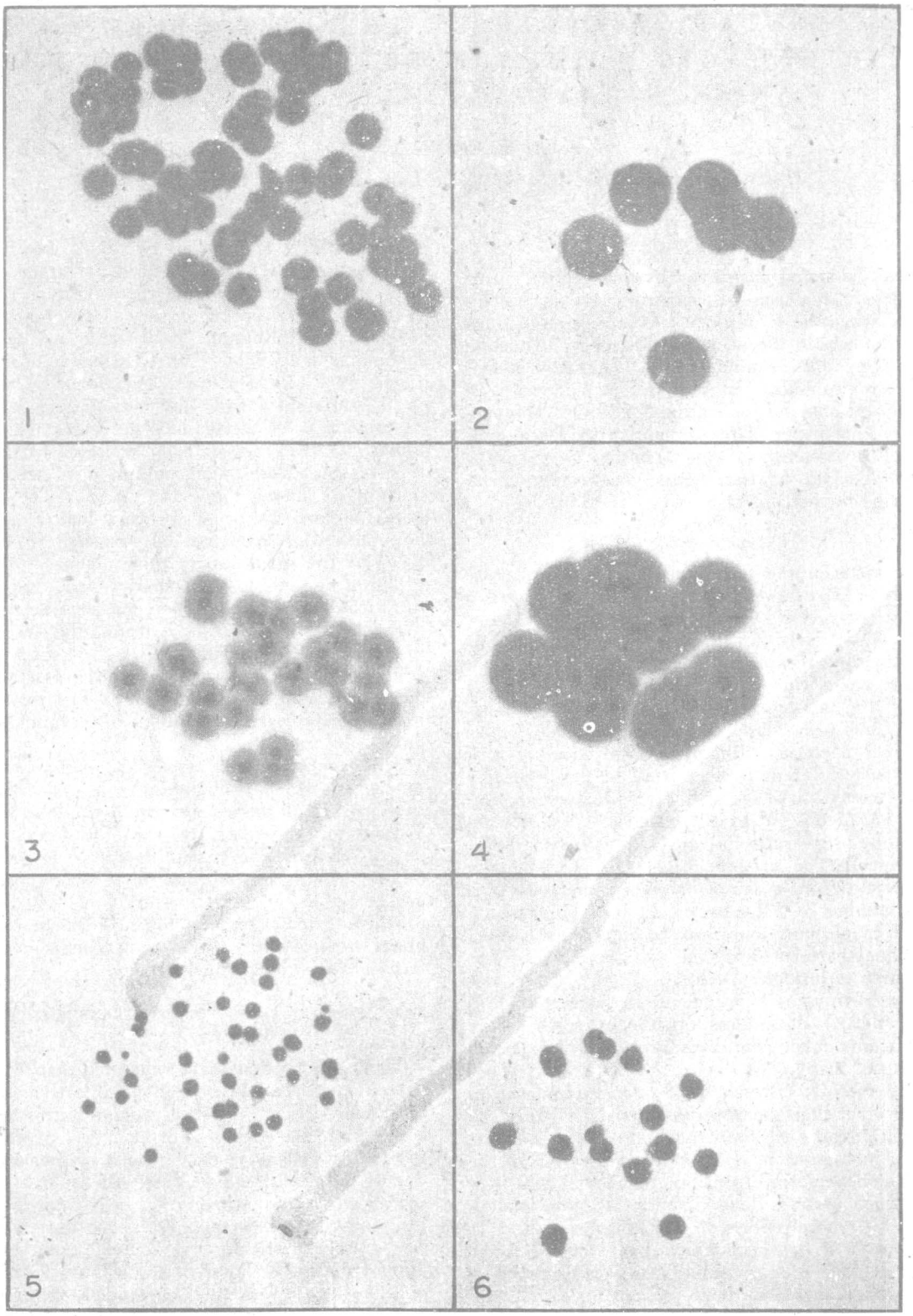
The genetic markers which have proved to be useful in the T-even series fall into four types which are distinguishable by their plaques on agar plates seeded with the appropriate bacteria. The *r* (rapid lysis) mutants are characterized by larger plaques with sharper margins than the wild type plaques. Mutations to *r* occur at many loci both in T2 (Hershey and Rotman, 1948) and in T4 (Doermann and Hill, 1953). The *h* (extended host range, Luria, 1945) mutants have been found at two loci in T2 (Hershey and Davidson, 1951) and are characterized by their ability to attack the indicator strain (B/2) specifically resistant to the wild type phage. Host range mutants have been found only in T2 among the T-even series. The *m* (minute) mutants also occur at several loci (Doermann, unpublished). They are distinguished from other types by the small size of their plaques. The last group of mutants to be extensively studied is of the *tu* (turbid halo) type. The characteristic property of *tu* plaques is the presence around the clear center of a more or less turbid ring which is absent in the wild type. Like the *r* mutants, they occur at many loci (Doermann and Hill, 1953). Figure 1 shows plaques of typical combinations of the mutant types described.

This group of characteristics, embracing only four phenotypically distinguishable properties, has been used in all the genetic studies made so far with T2 and T4, and illustrates the need for continued search for genetically useful markers. Even though this material has been quite limited, an appreciable number of experimental observations with it have to some extent defined current ideas concerning phage replication. These experiments form the basis of the subsequent discussion.

Experiments Indicating the Occurrence of a Vegetative State

Many observations have suggested that the phage reproductive cycle involves a noninfectious state of the virus particle. An early suggestion of this fact comes from the results of experiments in which infected host cells were disrupted at various intervals during the latent period. These experiments showed that, regardless of whether the cells were disrupted by sonic vibration or by a lytic procedure, no infectious phage particles were found during the first half of the intracellular cycle. Figure 2 shows an experiment in which the cyanide-lysis procedure was used (Doermann, 1952). Before 21 minutes (at 30° C.), the infected bacteria yielded fewer than one infectious particle per cell, even though an

[1] Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.



average of 17 particles had been adsorbed to each. Genetic evidence indicates that several of the 17, at least, must have cooperated in the production of the progeny in each cell. That the infecting particles disappear, has since been confirmed by a third method which disrupts the cells by rapid decompression (Levinthal and Fisher, 1952). The fact that the infecting particles become noninfectious seems less surprising, perhaps, since the phage particles, soon after entering the host cell, change in both their ultraviolet (Luria and Latarjet, 1947) and their X-ray (Latarjet, 1948) sensitivities. The

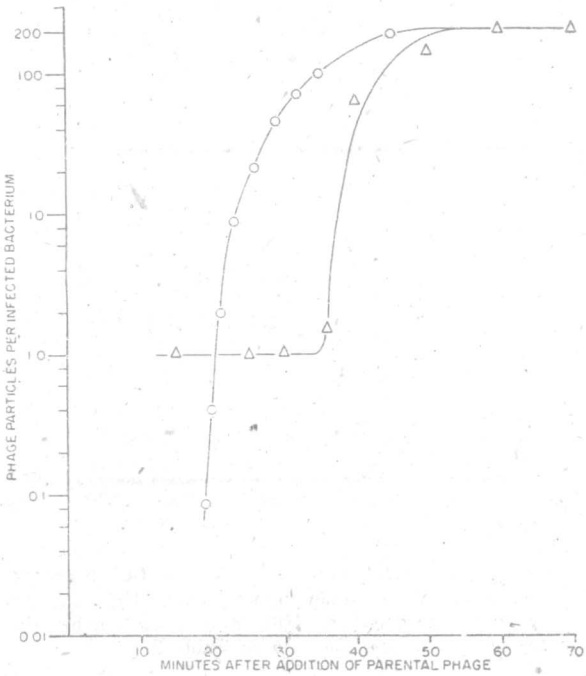


FIGURE 2. The intracellular bacteriophage population during the latent period as determined by the cyanide lysis procedure. Circles are points from samples in which lysis has been induced prematurely by the T6-cyanide treatment. They estimate the intracellular population. Triangles are points from the simultaneously performed one-step growth experiment, in which lysis was permitted to occur normally.

radiation experiments forecast some basic alteration of the virus soon after infection of the host cell.

The disruption experiments suggested the occurrence of a noninfectious intracellular state. It was still necessary, however, to distinguish this hypothesis from an alternative possibility. The latter supposes that the infecting particles may form some temporarily irreversible attachment to the host cell and that, near the middle of the latent period, they again become free within the cell and then proceed to multiply. The hypothesis of a temporarily irreversible attachment was ruled out by experiments on the kinetics of genetic recombination. From Hershey and Rotman's earlier experiments with T2 (1949), it was already known that a cross involving the markers r_{13} and h yielded 2 to 3 per cent recombinants. The cross of $r_{13}h^+ \times r^+h$ was made and aliquots from the culture of infected cells were lysed prematurely by the cyanide-lysis procedure. On the irreversible attachment hypothesis, one would expect the earliest obtainable phage crop to contain no genetic recombinants, since it would simply represent the parental particles which had just been liberated. Table 1 shows that recombinants were already present among the earliest infectious particles found within the cells. This result has been confirmed on a much larger scale with other crosses, so that it is now clear that even at a time when only one bacterium in 50, on the average, contains infectious phage, recombinants are already present. Thus it became necessary to accept the conclusion that genetic recombination, at least, has occurred between the time of infection and the time of appearance of the first phage, and therefore, during some noninfectious state.

TABLE 1. OCCURRENCE OF RECOMBINANTS IN CROSS T2Hh $\times$ T2Hr_{13} DURING THE LATENT PERIOD

Expt. No.	Lysis time	Phage per cell	No. of recombinants	Per cent recombinants
1	20	2.3	10	2.0
2	20	6.0	97	2.2
1	25	36	8	1.6
3	27	67	81	2.4
2	29	101	34	2.3
1	30	113	19	3.0
1	Post-burst	288	47	3.0
2	Post-burst	516	131	3.5
3	Post-burst	365	27	2.3

That multiplication must precede or accompany recombination can be deduced from the experiments of Hershey and Rotman (1949), who studied the distribution of recombinants among individual host cells and found the distribution to be random, not clonal. The absence of clones of recombinants in these experiments indicates that recombination could not have preceded multiplication; otherwise an early recombinant would necessarily have grown into a clone. Whether recombination comes about simultaneously with multiplication as a result of cooperative replication by two parental particles is not so easily decided, and will be discussed further

FIGURE 1. Representative combination of genetic markers in T4.

1. Wild type
2. *r*
3. *tu*
4. *rtu*
5. *m*
6. *rm*

in the latter part of this paper.

It appears unequivocal from the results reviewed up to now that the basic processes of replication and recombination must occur at a stage when the phage particles are in a noninfectious or vegetative state. That this conclusion is not an erroneous one may be seen by the agreement with it of many subsequent results. Since these will undoubtedly be detailed in subsequent papers, only two of them will be mentioned here.

The most convincing evidence comes from the experiments of Hershey and Chase (1952) who showed beyond any reasonable doubt that, upon infection of a host cell, the phage particle is separated into two components. One is the protein membrane which is completely dispensable after the phage particle has become adsorbed to the bacterium. It may be removed with no demonstrable effect on the process of replication. The other component, mainly desoxyribonucleic acid (DNA), is injected into the cell at the time of infection and appears to be the part of the phage particle which is responsible for intracellular replication. Presumably, therefore, DNA is one essential component of vegetative phage.

Another series of experiments which agrees with the conclusion that replication occurs while the phage particle is in a vegetative condition is concerned with the transfer of phosphorus from parent to progeny. It has been clearly shown that in both multiple infection (Putnam and Kozloff, 1950; Kozloff, 1952; Watson and Maaløe, 1953) and in single infection (French *et al.*, 1952), only 30 to 50 per cent of the parental phosphorus is found in the progeny particles. Most of the remainder appears in nonsedimentable form in the medium. Thus at the chemical level, the failure to transfer all of the parental material to the progeny indicates the occurrence of some incomplete state between the two.

At this point in the discussion we know that replication of the specificities inside the host cell is going on in a state different and presumably less complex than the whole infectious phage particle. This information creates the problem that we are no longer in a position to enumerate and characterize the replicating particles by simply permitting them to form plaques. What methods are now available to assist in identifying these replicating units? Other speakers in this Symposium will summarize what has been learned by the application of biochemical, immunological, electron microscopical, and other criteria. This paper will start the discussion by reviewing what has been learned about the vegetative phage particle from genetic experiments.

Genetic Structure of the Phage Particle

In order to interpret the experiments on genetic recombination, it will be useful first to come to a decision as to what sort of a structure is involved. The evidence from recombination measurements points to the conclusion that the genetic markers are distributed in linkage groups and, within these groups, in linear sequence. The first recombination experiments with phage by Hershey and Rotman (1948) suggested a linear order in T2H. A genetic map of T4, based mainly on two-factor crosses is shown in Figure 3. It also indicates a

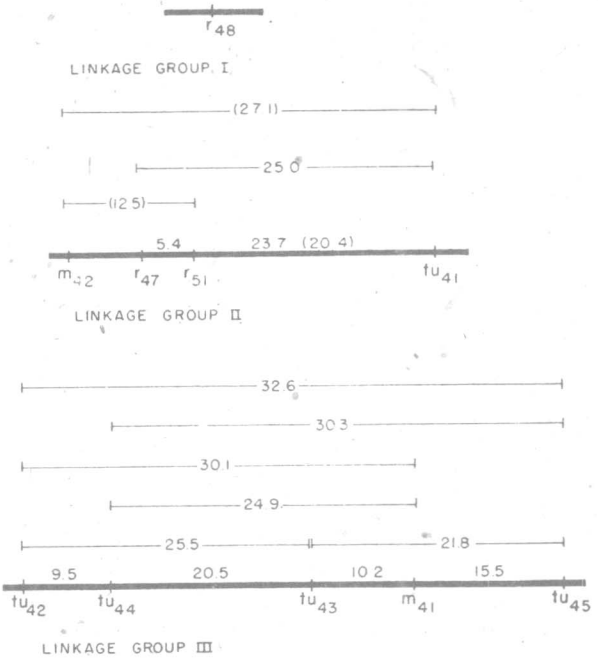


FIGURE 3. Recombination map of phage T4. Numbers indicate recombination values between loci. The data are mainly from Doermann and Hill, 1953. The numbers in parentheses come from the data in Table 2.

linear sequence of the genetic factors. And more recently it has been possible to make crosses in T4 involving three linked markers where each of the eight combinations can be identified. In Table 2, three repetitions of this cross are shown. The results are clearly compatible with the hypothesis of linear order. At least for the T-even phages, a linear arrangement can, therefore, be considered as well established.

Experimental Facts about the Recombination Process

In the experimental data indicating linearity there is one apparent difficulty which must be explained; the number of double recombinants is invariably higher by a small amount than is predicted by the hypothesis of linear arrangement with random occurrence of recombinations. In their first paper on genetic recombination Hershey and Rotman (1948) noted a correlation in the occurrence of adjacent recombinations. In other words, the data suggested that a crossover in one region tended

to be accompanied by a crossover in an adjoining region. In order to distinguish between this correlation and others to be discussed later, we shall refer to it as a "negative interference." The original observation of Hershey and Rotman has since been confirmed by Visconti and Delbrück (1953) in three-factor linked crosses in T2; and it has been observed in T4 by Doermann and Hill (1953) in two-factor mapping experiments, as well as in the three-factor data on T4 given in Table 2. Here 2.7 per cent double recombinants (*mtu* and *r*) were expected on the basis of the single recombination values in the same experiments. The percentage found was 3.3.

TABLE 2. PROGENY FROM A THREE-FACTOR LINKED CROSS WITH T4

Category	Class	Genotype	Total plaques	Proportion of total
Parental	A	*m r tu*	3467	0.335
		+ + +	3729	0.361
Recombinant (Region I only)	B	*m* + +	520	0.050
		+ *r tu*	474	0.046
Recombinant (Region II only)	C	*m r* +	853	0.082
		+ + *tu*	965	0.093
Recombinant (both in I and II)	D	*m* + *tu*	162	0.016
		+ *r* +	172	0.017

The three-factor linked cross of $m_{42}r_{51}tu_{41} \times$ wild type was repeated in three experiments. These are the combined data from the mass cultures which showed no significant differences. The probability of recombination in Region I is measured by B + D and in Region II by C + D. The product, (B + D) (C + D), is the probability of a double recombination on the hypothesis of linearity with no interference. The value is

$$(0.096 + 0.033)\ (0.175 + 0.033) = 0.027.$$

Before attempting to explain negative interference, it will be worth while to call attention to several other experimental facts concerned with the recombination process. One was noted by Hershey and Rotman (1948), who found, among the progeny of crosses involving three loci, phage particles containing genetic markers from each of three parents. That triparental recombination is not a rare occurrence, but rather a frequent one, is clear from the subsequent experiments of Hershey and Chase (1951) who showed that the triparental recombinant involving unlinked loci was almost as frequent as would be expected if the population had reached genetic equilibrium.

Results from a second type of experiment suggesting a similar complexity in the phage reproductive cycle are shown in Table 3. The progenies are given for two crosses involving presumably unlinked factors. In both crosses, one parent is greatly in excess of the other. The progenies of both crosses show the same results, namely that the minority parent ($r_{47}tu+$ in cross No. 1) is much less frequent than either recombinant. Had there been no opportunities for multiparental or repetitive mating one would expect that the frequency of either recombinant would, at a maximum, have reached the frequency of the minority parent. Both the occurrence of triparental recombinants and the tendency toward disappearance of the minority parent suggest strongly a recombination process which may be experienced repeatedly by the vegetative phage particles.

A third descriptive fact about the recombination process comes from experiments of a kinetic type. Table 1, showing the data from the cross $r_{13} \times h$, indicated the possibility that, among the first phage particles maturing intracellularly, the frequency of recombinants might be somewhat lower than in the population resulting after normal lysis. This drift in the proportion of recombinants was confirmed by additional experiments with T4. In each of the three crosses shown in Table 4 the proportion of recombinants rose consistently as more phage particles were produced within the cells of the culture.

TABLE 3. PROGENY FROM TWO-FACTOR UNLINKED CROSSES OF T4 WITH UNEQUAL MULTIPLICITY

	$r_{47}tu+ \times r+tu_{43}$			$r_{48}m+ \times r+m_{41}$		
Category	Genotype	Total plaques	Proportion of progeny	Genotype	Total plaques	Proportion of progeny
Majority parent	*r+tu*	3732	0.838	*r+m*	3683	0.852
Recombinant	*r+tu+*	298	0.067	*r+m+*	271	0.063
Recombinant	*r tu*	322	0.072	*r m*	253	0.059
Minority parent	*r tu+*	104	0.023	*r m+*	112	0.026
	Multiplicity of *rtu+* was 2.3; of *r+tu* was 23.0.			Multiplicity of *rm+* was 2.0; of *r+m* was 15.2.		

THE KINETIC NATURE OF THE REPLICATION PROCESS

Genetic information on the replication process itself comes largely from Luria's experiments (1951) in which he examined the distribution of spontaneous mutants in individual bacteria. The

distribution of numbers of phage mutants among the cells should be related to the process of replication of the genetic material, and, if so, several possibilities ought to be distinguishable: (1) Replication may proceed from the pattern laid down by the parent virus particles. If so, and if replicas cannot serve as patterns for further replication, two alternatives exist: (a) The pattern itself might mutate, in which case the mutation would presumably occur with equal probability at any time during the reproduction period. One would then expect mutants to occur in clones, and that mutant clones of various sizes should occur with equal frequency. (b) The production of a mutant might come about by the formation of a faulty replica, and not by the mutation of the pattern itself. One would then expect the mutants to be distributed randomly among the individual bacteria, and with the mutation rate as low as it is, clones of three or larger should be vanishingly rare. (2) If, however, replication proceeds in such a way that replicas themselves may serve as patterns for additional replications, a third result would be expected. An early mutation should produce a large clone, but a late mutation a smaller one. As the number of patterns increases, the chance for a mutation to occur increases similarly, so that the late mutations, producing small clones, should be more frequent than the earlier ones. The distribution of clones with respect to their size should be exponential. The last result is what Luria found, namely an exponential distribution clearly distinguishable from the other possibilities. It appears conclusive that the replication process is characterized by the fact that the replicas themselves may serve as patterns for further replication. Both models mentioned subsequently to this discussion involve replication of this type.

TABLE 4. INTRACELLULAR DRIFT IN THE PROPORTION OF RECOMBINANTS

$r_{47}tu+ \times r+tu_{43}$		$r_{48}m+ \times r+m_{41}$		$tu_{43}tu_{44}+ \times tu_{42}+tu_{41}$	
Phage per cell	Proportion of recombinants	Phage per cell	Proportion of recombinants	Phage per cell	Proportion of recombinants
0.09	0.32	0.05	0.25	0.57	0.061
0.41	0.33	0.34	0.24	0.85	0.062
1.9	0.35	1.1	0.25	2.2	0.057
8.6	0.36	2.3	0.28	9.1	0.061
21	0.39	10	0.28	19	0.080
44	0.38	34	0.30	46	0.079
70	0.40	91	0.33	70	0.088
97	0.40	172	0.36	98	0.098
		323	0.37	127	0.109
Normal lysis		Normal lysis		Normal lysis	
197	0.42	313	0.37	147	0.115
Multiplicities:		Multiplicities:		Multiplicities:	
$rtu+ = 7.1$		$rm+ = 7.8$		$tu_{43}tu_{44}+ = 6.1$	
$r+tu = 10.0$		$r+m = 9.2$		$tu_{42}+tu_{41} = 5.2$	

Samples were taken from the mass cultures at intervals throughout the latent period and induced to lyse by the cyanide lysis procedure. The column, "phage per cell," indicates the average number of particles liberated per cell after such treatment.

THE VISCONTI-DELBRÜCK THEORY

The main groups of results have been put together by Visconti and Delbrück (1953) to form the experimental basis for a model of genetic recombination in phage. The most important features of the model are the following: (1) Upon entering the host cell, the infecting phages become vegetative particles and multiply in an exponential manner to form the vegetative pool. (2) As the size of the vegetative pool increases, the rate of mating becomes appreciable. Mating is assumed to occur between whole vegetative phage particles. (3) Soon after the onset of mating, particles are taken from the vegetative pool and transformed into infectious phage. These infectious particles are assumed not to re-enter the mating pool, nor do they multiply.

The theory has two parameters, namely, the number of repeated mating encounters (m) per progeny particle, and the probability of recombination per mating. (p). By experiments which measured the proportion of a minority parental type containing three unlinked loci, Visconti and Delbrück estimated m for T2 to be about 5 in the progeny found after a normal latent period. The value of p can then be calculated from the recombination value observed after normal lysis on the assumption that each progeny particle resulted from an average of 5 pairwise mating encounters among the vegetative phage particles.

The theory does, in fact, account for the experimental facts just described. The triparental re-

combinants could obviously arise from particles which in one mating become recombinants for two parental types. In subsequent matings they encounter and recombine with particles carrying the marker present exclusively in the third parent itself or in its offspring. The drift in the proportion of recombinants is easily explained by the fact that, when particles of the first progeny crop to mature are examined, an early vegetative population is being considered. In this group the average number of matings per particle is less than in the vegetative population from which the last particles are taken. Thus, in the latter, more recombinants will have been formed.

Perhaps the most important contribution of the theory is that it points up the fact that in phage crosses one is dealing with a population problem which predicts that a negative interference will be encountered. The prediction is based on the assumption that mating opportunities are distributed more or less randomly among the particles. The result of such a distribution can easily be visualized if one imagines the progeny to be divided equally into two classes, one in which no mating encounters have occurred and the other in which the particles have engaged in a single round of mating. Within the latter class, the number of double recombinants may be precisely as expected on the basis of the total recombinations in any given region and the total in an adjacent region. In adding the former class in which no matings and consequently no recombinants have occurred, the recombination value for both regions will be cut in half. The proportion of doubles *expected* should then be reduced to one-fourth. The observed number of doubles, however, will be reduced only by a factor of 2. A qualitatively similar result will be observed if the matings are randomly distributed around any average value.

Recent experiments of Levinthal and Visconti (1953) are consistent with the Visconti-Delbrück model. By making use of the increased latent period of lysis-inhibited cells (Doermann, 1948) they were able to show that the increase in proportion of recombinants is very closely correlated with the increase in the amount of phage produced, and that the proportion of recombinants does, in fact, progres stoward genetic equilibrium. In addition, they speculatively estimated the size of the vegetative pool, on the basis of the relatively simple assumptions that (1) phage particles are removed from the vegetative pool at random, (2) the size of the vegetative pool remains constant, (3) *each mating gives one duplication.* Granted these assumptions, they were able to calculate that every vegetative particle must mate once every three minutes on the average in order to account for the change in recombinant proportion with time. Since ten particles are produced every minute, it would require a pool size of 30 vegetative particles to give one duplication for every mating. An independent estimate of the size of the vegetative pool would be extremely valuable in testing the validity of the third assumption and in answering the general question whether recombination and replication are associated phenomena.

Residual Heterozygotes of Phage

One class of observations, however, has not yet been accounted for by the Visconti-Delbrück theory, and these appear to be more readily interpretable on a different model. Hershey and Chase (1951), in T2 crosses which involved *r* and *r*+, observed that about 2 per cent of the progeny contained both the *r* locus from one parent and the homologous *r*+ locus from the other. They called these particles residual heterozygotes. From their experiments with the heterozygotes, Hershey and Chase could draw certain generalizations: (1) Among the six genetic loci in T2 which they tested, each showed about 2 per cent heterozygosity. It therefore appears that heterozygosity is more or less uniformly distributed throughout the genetic complex. (2) When two loci are marked, more than 90 per cent of the heterozygotes are heterozygous for only one of them, excepting the case where markers are closely linked. In this situation the majority of the particles may be doubly heterozygous. (3) On the assumption that the number of genetic loci is large, almost all of the phage particles must be heterozygous for some region.

The results with heterozygotes are difficult to interpret within the framework of the Visconti-Delbrück theory. Hershey and Chase (1951) showed that the processes of recombination and heterozygote formation are not independent of each other. The argument is as follows: If the two processes were independent of each other, then in the case of closely linked markers where one finds 2 per cent recombinants, one should find that only 2 per cent of the segregants from heterozygotes are recombinants. In examining these segregants, however, Hershey and Chase found 20 per cent to be recombinants, indicating some connection between the two phenomena.

If the heterozygotes have their origin in the same matings which yield recombinants, and, as the Visconti-Delbrück theory requires, the matings occur between pairs of genetically complete vegetative particles, then the formation of any particle with a duplicate set of loci should be accompanied by the formation of one deficient in the homologous material. Since the over-all frequency of heterozygotes is high, one would expect an appreciable class of genetically deficient particles. There is no evidence for such a class of particles even though one might in some types of experiment expect to find it.

The Partial Replica Hypothesis

The formation of residual heterozygotes is much

more easily visualized on another model which has been called the partial replica hypothesis (Hershey, 1952). At present, this concept is rather poorly defined, since it embraces most of the models which center around the assumption that replication proceeds by formation of genetically specific material on a template. In addition to this feature the basic characteristics of the model are that replicas and perhaps even partial replicas can serve as templates, and that recombination occurs by linking partial replicas from several templates. While the parameters have not been specified with the precision of the Visconti-Delbrück theory, it is immediately apparent that the partial replica hypothesis can, qualitatively at least, account for all of the observations which have been mentioned in this manuscript. Both triparental recombinants and the tendency toward loss of a minority parent could be due to second, third, or higher orders of replication, or to the association of partial replicas from more than two parents. The hypothesis would explain the drift in proportion of recombinants precisely as the Visconti-Delbrück theory does, namely by repetitive recombination encounters. Negative interference could arise from the distribution of these encounters.

On the basis of some new and ingenious experiments, Levinthal has recently been able to develop a specific quantitative model of the partial replica hypothesis. In addition to accounting for the experimental observations on which the Visconti-Delbrück theory is based, it also gives a satisfactory explanation for the formation of residual heterozygotes and its relation to the formation of recombinants. This model will be presented by Dr. Levinthal in this Symposium.

Conclusions

That a noninfectious stage occurs in the reproductive cycle of bacteriophage has been deduced from the accumulation of various kinds of information. The first direct suggestion of this so-called vegetative phage particle came from the failure to recover the infective parental virus from host cells during the first half of the latent period. Genetic evidence has furthermore shown that the population which is first recoverable from the host cells contains a new class of particles which combine genetic properties from several parents. This fact, taken together with the observation that genetic recombinants do not occur in clones in individual bacteria, indicates that recombination must accompany or follow replication.

The genetic description of that entity which is called the vegetative phage particle depends on a small number of observations mainly concerned with the process of recombination. The observations require (1) a linear arrangement of genetic determinants, (2) a replicating mechanism in which replicates may themselves be the parents of subsequent genetic structures, (3) a repetitive series of encounters, any one of which may result in recombination of genetic markers, (4) a distribution of encounters such that the individual progeny particles have not experienced an identical number of encounters, (5) a mechanism to account for progeny particles which are diploid for small segments of the genetic material.

Models to account for these observations fall into two categories. Visconti and Delbrück have proposed a scheme in which the vegetative phage particle consists of a genetically complete unit. The distinctive feature of their scheme is that it maintains that recombination and replication are separate phenomena. Their model accounts quantitatively for all observations except the residual heterozygotes.

The other category consists of those models which visualize recombination as part of the process of replication. This group accounts for the residual heterozygotes as well as the other observations at least in a qualitative sense. It is nevertheless impossible at present to make a certain choice between the two types of concept.

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