# THE ISOLATION OF A STAPHYLOCOCCAL PHAGE VARIANT SUSCEPTIBLE TO AN UNUSUAL HOST CONTROL*

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PLATES 11 AND 12

## INTRODUCTION

The experiments described in this paper are concerned with a staphylococcal phage variant whose particles are altered by propagation on certain bacterial host cells. In the authors' opinion, the proof of a host control over the properties of a virus rests upon the ability to demonstrate modifications of its particles in the first progeny released from singly infected "controller" host cells. These alterations must be detectable by physicochemical methods (changes in morphology or chemical composition) or biological tests (alterations in host range, adsorption rates, latent periods, burst size, plaque appearance, etc.). They must be dependent upon the host of production, in contrast to spontaneous mutations, which, by definition, may occur independently of the host. In addition, they must be independent of any substances produced in culture filtrates of the host cell.

The authors have found evidence indicating that a phage variant, derived from a stock staphylococcal phage $P_1$, is susceptible to a host control with respect to one of its major biological activities—its host range; that this susceptibility is heritable by every particle in the phage strain; and that the observed changes are not caused by extracellular substances.

In a previous publication (6), a report was presented of the isolation, production, and host-control susceptibility of phage 14. Recent publications by Luria and Human (5) and Bertani and Weigle (1) established the occurrence of a similar host control over viral variation in *E. coli* and *Shigella* phage–host cell systems, thus indicating that the phenomenon is general in character and is not peculiar to only the one phage. In view of the far reaching and self-evident implications of this observation details of the experimental progress with the staphylococcal system are presented.

## Materials and Methods

### The Phage $P_1$-Staphylococcus aureus System

Since its isolation in 1928, the phage $P_1$, also designated K, has been propagated on a *Staphylococcus aureus* strain $K_1$. It is a highly lytic phage, producing sterile broth lysates over a great range of initial phage-bacterium (P/B) infection ratios. Resistant bacteria have been isolated with difficulty from its host, and these revert rapidly to phage sensitivity. It has an extensive host range, lysing a number of staphylococcal strains, including at least 22 of 28 phage typing bacteria.

On the parent host the phage forms small, clear plaques, ranging from 0.5 to 2 mm. in diameter. On several occasions attempts have been made to isolate mutant types from plaques of varying sizes and turbidity, and from those developing on transiently resistant cultures, with uniformly negative results. These failures might be indicative of high reversion rates in this phage-host cell system.

Successful isolation of phage variants resulted from studies of phage production on an alternate host, strain *S. aureus* WF 145. This culture supports excellent phage formation in broth media, while in agar the plaque size is smaller than is typical of the same phage on the parent $K_1$ cells. The strain has been used for staphylococcal typing, is susceptible to a phage 51, and is lysogenic. Rountree has shown that it carries as many as five phages, of which none is active on strain $K_1$ nor is related serologically to phage $P_1$ (7).

In these laboratories, reciprocal cross-tests for lysogenicity between strains $K_1$ and WF 145 have been negative on all occasions, whether tested by the spotting technic or by cross-plating culture filtrates with host cells.

### The Two Host Assay Technic

Throughout these studies, activity was determined by titration of phage aliquots on two assay hosts, strains $K_1$ and WF 145. Changes in the assay ratio, R 145/$K_1$, under standard conditions reflected alterations in phage activity, and in the case of one variant, demonstrated a host control over certain of its properties. Suspensions for plaque count were made from 18 hour tryptose-phosphate slants, adjusted to optimal concentrations for plaque formation (3).

The term assay ratio, or R 145/$K_1$, expresses the ratio of plaque counts of aliquots assayed on host 145 and host $K_1$. In effect it is similar to the term "E.O.P.," or "efficiency of plating," as used by Hershey and Davidson (2) and others, to indicate the plating efficiency of two phages on the same host, or of the same phage on two hosts or under two different environmental conditions. In this report, the assay ratio 145/$K_1$ may refer to aliquots of free phage, of infected cells, or of mixtures of free phage and infected cells, assayed on the two hosts, under standard conditions.

#### EXPERIMENTAL

### The Isolation of Variants from Staphylococcus Phage $P_1$

Because preliminary attempts to isolate plaque type mutants of $P_1$ from growth on host $K_1$ had failed, an alternate host was selected; *viz.*, *S. aureus* WF 145. In order to determine its plating efficiency, the stock phage was as-

sayed on both hosts. Quite surprisingly, the titer for host 145 proved to be almost threefold higher than that for host $K_1$, despite the fact that a series of studies had been made to develop optimal conditions for plaque formation on the parent strain, $K_1$. All available phage lots were then assayed in a similar manner, as shown in Table I. It became apparent that a small but significant difference existed between the titration values so that the ratio 145/$K_1$ always exceeded 1.

This suggested the presence of phage particles within the stock which heretofore had been undetected. Two possible explanations were considered: (a) either the two host strains differed in their sensitivity to a homogeneous phage,

### TABLE I

*Titration of Different Phage $P_1$ Preparations on Two Hosts*

| Phage lot | Test No. | Titre/ml. by plaque count on host | | Assay ratio 145/$K_1$ |
|---|---|---|---|---|
| | | $K_1$ | WF 145 | |
| A | 1 | $4.4 \times 10^8$ | $1.3 \times 10^9$ | 3.0 |
| | 2 | $1.7 \times 10^9$ | $2.2 \times 10^9$ | 1.3 |
| B | 1 | $6.8 \times 10^9$ | $8.9 \times 10^9$ | 1.3 |
| C | 1 | $1.6 \times 10^8$ | $1.9 \times 10^8$ | 1.2 |
| D | 1 | $1.9 \times 10^{10}$ | $2.4 \times 10^{10}$ | 1.3 |
| | 2 | $1.9 \times 10^{10}$ | $2.5 \times 10^{10}$ | 1.3 |
| E | 1 | $2.8 \times 10^9$ | $4.0 \times 10^9$ | 1.4 |
| F | 1 | $1.8 \times 10^{10}$ | $2.6 \times 10^{10}$ | 1.4 |

or (b) the phage was non-homogeneous and contained a certain proportion of particles with differing abilities to form plaques on the two hosts. A third possibility involving the condition of lysogenicity could be eliminated by negative tests for any carried phage reactive for either host.

I

*Isolation of Phage Variants from the Staphylococcal Phage $P_1$*

A comparative study of phage $P_1$ formation on the two hosts, $K_1$ and WF 145, indicated that the rates of adsorption (Text-fig. 1), latent periods, rates of phage formation, and total phage yield (Text-fig. 2) were very similar, so that the originally observed assay ratio of the stock $P_1$ lysates did not appear to be related to the kinetics of phage production on either host. When comparisons were made of the assay ratios of free phage $P_1$ and infected cells, it
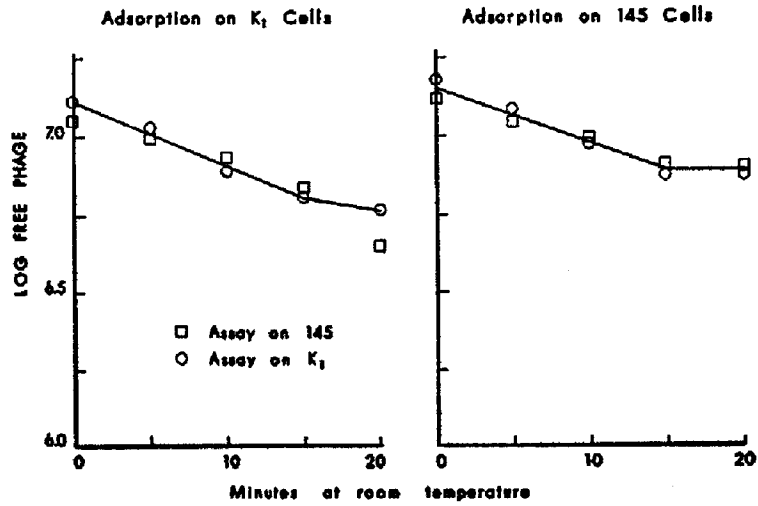
TEXT-FIG. 1. Adsorption of $P_1$ on strains $K_1$ and 145.
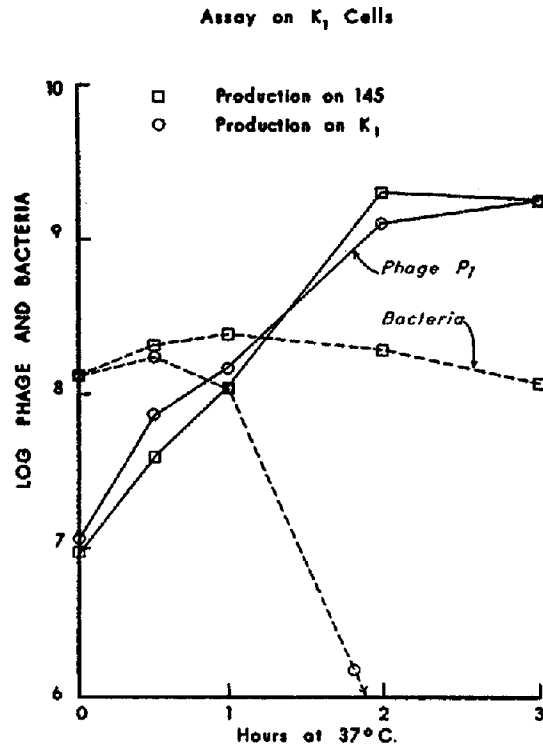


TEXT-FIG. 2. $P_1$ production on $K_1$ and 145 cells in trypticase-soy broth.

was found that whereas the free phage assayed in ratios greater than 1, the infected cells produced ratios closely approaching unity. This indicated that certain particles which adsorbed on host $K_1$ were either incapable of forming mature phage or the cells released insufficient phage to initiate plaque formation on either indicator strain.

## 1. Alterations of Ratio 145/$K_1$ during $P_1$ Production on Host 145.—

Stock $P_1$ was added to log phase 145 cells in trypticase-soy broth, and samples were removed at intervals for assay, to determine the changes in titration ratio. The initial infection was P/B = 1/200. The titration ratio 145/$K_1$ increased from 1.3 at 0 hour to 3.5, 3 hours later, as indicated in Table II and shown graphically in Text-fig. 3.

TABLE II

Phage $P_1$ Production on Host WF 145 in Broth

| Time | Titre/ml. of sample assayed on strain | | Ratio 145/$K_1$ | Bacterial count/ml. (Klett readings) | |
|---|---|---|---|---|---|
| | $K_1$ | 145 | | Infected cells | Uninfected cells |
| hrs. | | | | | |
| 0 | $2.8 \times 10^6$ | $3.9 \times 10^5$ | 1.3 | $1.0 \times 10^8$ | $1.0 \times 10^8$ |
| 0.5 | $2.9 \times 10^6$ | $4.4 \times 10^6$ | 1.5 | $1.1 \times 10^8$ | $1.2 \times 10^8$ |
| 1.0 | $1.5 \times 10^7$ | $1.6 \times 10^7$ | 1.0 | $2.6 \times 10^8$ | $2.8 \times 10^8$ |
| 1.5 | $1.0 \times 10^8$ | $1.2 \times 10^8$ | 1.2 | $5.0 \times 10^8$ | $5.1 \times 10^8$ |
| 2.0 | $3.3 \times 10^6$ | $4.0 \times 10^8$ | 1.2 | $8.5 \times 10^8$ | $9.2 \times 10^8$ |
| 2.5 | $1.7 \times 10^9$ | $2.3 \times 10^9$ | 1.3 | $1.1 \times 10^9$ | $1.4 \times 10^9$ |
| 3.0 | $2.6 \times 10^8$ | $9.3 \times 10^8$ | 3.5 | $1.2 \times 10^9$ | $1.8 \times 10^9$ |
| 3.5 | $1.2 \times 10^9$ | $3.4 \times 10^9$ | 2.8 | $1.3 \times 10^9$ | $2.3 \times 10^9$ |
| 4.0 | $0.8 \times 10^9$ | $1.4 \times 10^9$ | 1.8 | $1.2 \times 10^9$ | $2.5 \times 10^9$ |

To test the possibility that the change in ratio reflected a selection of particle types, a series of isolations was made from plaques developing on strain 145, plated with samples which had been removed at 3.0 and 3.5 hours. Plaque contents were suspended in saline, and aliquots were plated with both host cells. The titres were recorded and a second passage was made from plaques appearing on host 145. The third showed no essential changes in the titration ratios of plaque contents. Counts for the first two passages are shown in Table III.

Of thirty-two isolates, Nos. 15 and 16 formed small plaques on host $K_1$; one, No. 14, maintained a high ratio 145/$K_1$ throughout three transfers of its growth on host 145; and two, Nos. 5 and 10, appeared to be typical of the stock phage in that they assayed in ratios approaching 1. Peculiarly, some of the isolates showing high ratios 145/$K_1$ on first test reverted to titration ratios approaching 1 during the second passage on host 145. The reason for this has

not yet been investigated. Strains 5, 14, 15, and 16 were selected for further investigation.

*2. Multiplication of Phage Variants on Host 145 in Medium III Broth.—*

Plaque contents from the third passage in host 145 were suspended in broth and filtered through Seitz filter pads. Each race was then propagated in broth
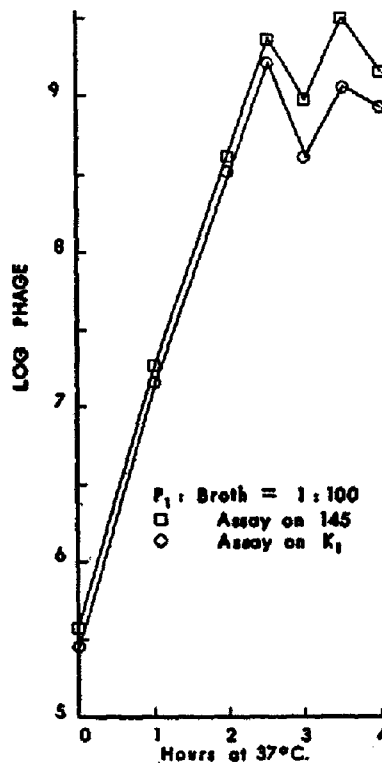


Text-Fig. 3. $P_1$ production on host 145 in trypticase-soy broth.

on host 145 through three successive passages. Phage obtained from the third broth lot was then added to log phase 145 cells in medium III in an initial ratio of P/B = 1/10 at 37°C. Samples were removed at intervals and were titrated on both hosts. The production of each strain was compared to stock $P_1$ formation on host 145 under identical conditions.

As is evident from Text-fig. 4, the addition of phage strains to aliquot suspensions of log phase 145 cells, in similar infection ratios, resulted in the formation of phage at rates characteristic for each strain and differing from the stock phage. Inspection of the curves for strains 15 and 16 indicated that they

## TABLE III

*Titration Ratios of $P_1$ Virus in Individual Plaques Formed on Strain WF 145*

| Isolate No. | Passage 1*<br>Aliquots of plaque contents of $P_1$<br>on WF 145 plated on strain | | | Passage 2<br>Aliquots of plaque contents of $P_1$ on WF 145<br>plated on strain | | |
|---|---|---|---|---|---|---|
| | $K_1$ | WF 145 | R145/$K_1$ | $K_1$ | WF 145 | R145/$K_1$ |
| 1 | 56 | 155 | 2.8 | 323 | 341 | 1.0 |
| 2 | 180 | 255 | 1.4 | 245 | 240 | 1.0 |
| 3 | 117 | 169 | 1.4 | 1000 | 1000 | 1.0 |
| 4 | 321 | 330 | 1.0 | 263 | 227 | 0.8 |
| 5 | 254 | 502 | 2.0 | 339 | 353 | 1.0 |
| 6 | 201 | 229 | 1.1 | 321 | 167 | 0.7 |
| 7 | 83 | 83 | 1.0 | 1000 | Complete | — |
| 8 | 1000 | 1000 | 1.0 | Complete | Complete | — |
| 9 | 350 | 499 | 1.4 | 301 | 321 | 1.0 |
| 10 | 132 | 169 | 1.3 | 148 | 161 | 0.9 |
| 11 | 260 | 228 | 0.9 | 68 | 58 | 0.9 |
| 12 | 271 | 233 | 0.8 | 63 | 48 | 0.8 |
| 13 | 12 | 44 | 3.7 | 76 | 54 | 0.7 |
| 14 | 20 | 263 | 13.0 | 105 | 498 | 4.7 |
| 15 | 48‡ | 166 | 3.5 | 743‡ | 744 | 1.0 |
| 16 | 15‡ | 45 | 3.0 | 43‡ | 47 | 1.0 |
| 17 | 527 | 552 | 1.0 | 573 | 346 | 0.6 |
| 18 | 15 | 66 | 4.4 | 600 | 600 | 1.0 |
| 19 | 59 | 16 | 0.3 | 274 | 173 | 0.6 |
| 20 | 126 | 67 | 0.5 | 150 | 135 | 0.9 |
| 21 | 1000 | 1000 | 1.0 | 53 | — | — |
| 22 | 260 | 386 | 1.5 | 0 | 12 | >12 |
| 23 | 358 | 378 | 1.1 | 0 | 195 | >195 |
| 24 | 34 | 163 | 4.8 | 465 | 187 | 0.4 |
| 25 | 138 | 182 | 1.3 | 450 | 237 | 0.5 |
| 26 | 430 | 113 | 0.3 | 52 | 14 | 0.3 |
| 27 | 31 | 300 | 10.0 | 380 | 154 | 0.4 |
| 28 | 20 | 201 | 10.0 | 320 | 216 | 0.6 |
| 29 | 114 | 94 | 0.8 | 190 | 121 | 0.6 |
| 30 | 180 | 189 | 1.0 | 103 | 104 | 1.0 |
| 31 | 48 | 200 | 4.0 | 1000 | 1000 | 1.0 |
| 32 | 0 | 0 | — | 111 | 165 | 1.4 |

\* Strain WF 145 was infected with stock $P_1$ phage. At intervals, samples were removed and aliquots were assayed on both hosts. For passage 1, contents of plaques developing on host 145, from samples removed 3.0 and 3.5 hours after infection, were analyzed.

‡ Small plaques.

were duplicate isolations. This was substantiated further by the observation that both formed small plaques on host $K_1$. Phage 5 resembled stock phage most closely, although the fact that the assay ratio approached 1 with strain 5, whereas it diverged slightly with stock $P_1$, may indicate significant differences.
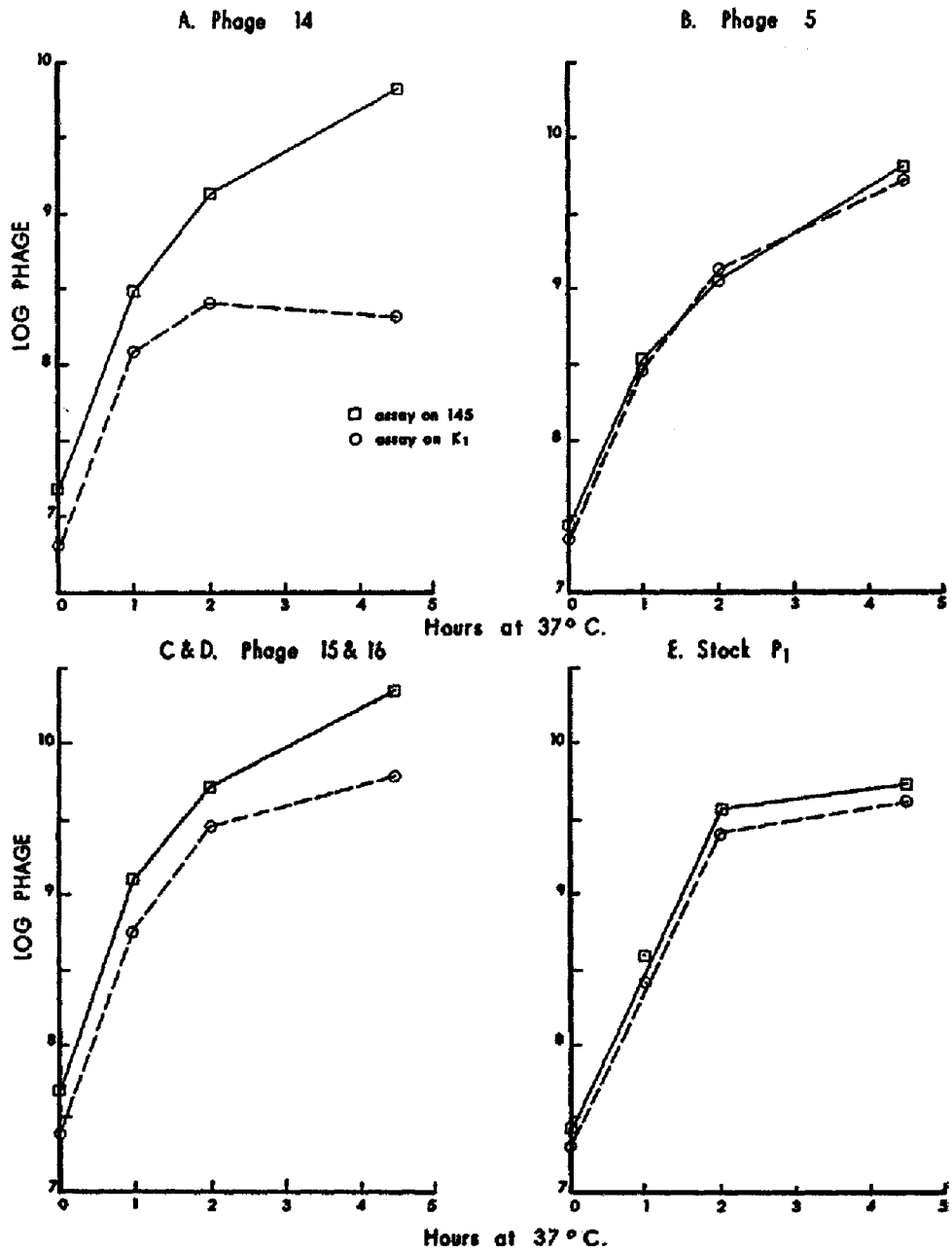
Text-Fig. 4. Production of phage variants on WF 145 in medium III broth.

The most striking curves were found with strain 14. Its assay ratio rose from 2.5 to 29 during its production on host 145. More specifically, soon after infection, the increase of particles assayable on $K_1$ paralleled those active for host 145. Then, at the time of rapid lysis, the phage exhibited a radical change in assay ratio: the titre of particles producing plaques on host 145 rose, while those assaying on host $K_1$ remained almost constant. (It is to be noted that samples removed prior to mass lysis represented mixtures of infected cells and free phage, and that those removed after lysis represented mainly free phage.)

Virus from a total of sixteen plaques which had developed on host 145 from samples removed at 4.5 hours (lysis completed), was suspended in saline, and aliquots were assayed on both hosts; all sixteen exhibited assay ratios $145/K_1$ exceeding 5, the average ratio being 12, and the range 5 to 46. A plaque re-isolated from WF 145 cells, containing phage which assayed in the ratio 46/1, plated in a ratio 13, so that it appeared that the variation observed was not important, but that the maintenance of the high titration ratio $145/K_1$ by free phage released from host 145 was a characteristic of strain 14.

### 3. Purification of Phage Strains by Repeated Single Plaque Isolation.—

The phage strains 5, 14, and 15 were then subjected to ten successive plaque passages on strain 145. Phage propagated on this strain is referred to as P5(145), P14(145), or P15(145). At the same time a similar series of passages was made with host $K_1$, designated P5($K_1$), etc. On the first, second, third, and tenth passages, plaque contents of each phage were plated on both hosts, and the resultant ratios were recorded, as shown in Table IV.

From the results several significant observations were made:

(a) Phage 14, propagated on host 145, maintained a high titration ratio through ten passages. Phage 15, behaved similarly, although the ratio $145/K_1$ of its plaque contents was not quite as high as with phage P14(145).

(b) Phage 14, when passaged on host $K_1$, exhibited a ratio $145/K_1$ approaching 1. This occurred on the first passage of P14(145) on host $K_1$, yielding P14-($K_1$) particles, and the ratio was increased immediately upon the return of the phage to host 145. When phage 15 was produced on host $K_1$, it, too, assayed in a ratio $145/K_1$ approaching 1, the plaque size on that host remaining small throughout all passages.

From such observations it was apparent that the activity of the phage variants P14 and P15 was dependent upon the host of propagation, so that the lysates P14($K_1$) and P15($K_1$) possessed properties different from those of P14(145) and P15(145).

(c) Phage 5 assayed in a ratio $145/K_1$ approaching 1, regardless of the host used for its propagation.

Fig. 1 shows the appearance of the plaques produced by each strain after the tenth passage on the two hosts, in agar media. It demonstrates that the

plaques of all three strains, produced on host 145, were indistinguishable, regardless of the previous passage history; whereas those of strain 15 on host $K_1$ were always smaller than those of the other two, regardless of the passage history. (It should be stated, however, that plaque appearance may be modified by altering the plating conditions, so that it may become possible to create an environment which will render each strain distinguishable by its plaque type.)

Of the three variants, phage 14 was selected for more detailed study. As will be demonstrated in the following section, this phage was found to be susceptible

TABLE IV

Serial Passage by Plaque Isolation of Phages 5, 14, and 15

| Phage variant | Passage No. | Transfer on host $K_1$ Titre/ml. plaque suspension assayed on cells | | | Transfer on host WF 145 Titre/ml. plaque suspension assayed on cells | | |
|---|---|---|---|---|---|---|---|
| | | $K_1$ | 145 | $\frac{R145/}{K_1}$ | $K_1$ | 145 | $\frac{R145/}{K_1}$ |
| 5 | 1 | $1.3 \times 10^5$ | $1.3 \times 10^5$ | 1.0 | $1.3 \times 10^4$ | $1.5 \times 10^4$ | 1.1 |
| | 2 | $2.8 \times 10^4$ | $2.9 \times 10^4$ | 1.0 | $6.7 \times 10^5$ | $1.3 \times 10^5$ | 1.9 |
| | 3 | $2.8 \times 10^6$ | $3.9 \times 10^6$ | 1.4 | $4.4 \times 10^4$ | $4.7 \times 10^4$ | 1.1 |
| | 10 | $2.1 \times 10^6$ | $3.4 \times 10^6$ | 1.6 | $1.1 \times 10^6$ | $1.4 \times 10^6$ | 1.3 |
| 14 | 1 | $2.4 \times 10^4$ | $1.9 \times 10^4$ | 0.8 | $2.4 \times 10^3$ | $3.2 \times 10^4$ | 13.3 |
| | 2 | $8.9 \times 10^4$ | $1.4 \times 10^5$ | 1.6 | $2.4 \times 10^2$ | $8.5 \times 10^3$ | 35.0 |
| | 3 | $1.2 \times 10^5$ | $1.4 \times 10^5$ | 1.0 | $2.3 \times 10^4$ | $8.0 \times 10^5$ | 34.0 |
| | 10 | $1.2 \times 10^6$ | $1.4 \times 10^6$ | 1.2 | $7.0 \times 10^4$ | $3.2 \times 10^6$ | 45.0 |
| 15 | 1 | $6.3 \times 10^3$ | $5.3 \times 10^3$ | 0.8 | $2.4 \times 10^4$ | $1.2 \times 10^5$ | 4.9 |
| | 2 | $4.8 \times 10^4$ | $4.8 \times 10^4$ | 1.0 | $2.4 \times 10^3$ | $1.4 \times 10^4$ | 6.0 |
| | 3 | $4.8 \times 10^4$ | $6.2 \times 10^4$ | 1.3 | $4.7 \times 10^3$ | $1.1 \times 10^4$ | 2.3 |
| | 10 | $6.8 \times 10^5$ | $6.7 \times 10^5$ | 1.0 | $1.3 \times 10^5$ | $8.8 \times 10^5$ | 6.6 |

to an unusual host control; i.e., its ability to infect and produce plaques on host $K_1$ was shown to be related directly to the host upon which it was previously propagated.

The fact that this property (susceptibility to host control) was peculiar to this phage strain (and perhaps to strain 15 also) but not to strain 5, suggests that it may arise by mutation within the wild type stock $P_1$ virus. As such, its occurrence during multiplication on one host might be considered a "masked mutation," in that its positive demonstration would necessitate passage through a controller host, followed by retitration on both systems.

The two host assay technic provides a useful tool for the detection of host-controlled variants. With phage $P_1$, the procedure was repeated successfully with a second phage lot, from which a strain similar to phage 15 was isolated.

Although the assumption was made that growth of the stock phage on the alternate host caused a selection of variants, and although variants were isolated, the results do not constitute positive proof for a mutational origin, and certainly do not provide conclusive evidence for their presence in $P1(K_1)$ lysates prior to phage production on host 145. It is hoped that experiments now in progress will resolve this question.

## II

### STUDIES WITH PHAGE 14

#### Characteristics of the Host Control Phenomenon

Upon first observation it might seem that transfer of P14 through ten consecutive plaque isolations had effected a separation of two distinct mutants, or strains, one which could form plaques equally well on both hosts, and one which exhibited a lowered ability to reproduce on host $K_1$. This interpretation, however, was made less plausible by the finding that even after ten passages, transfer of the phage from one host to the other resulted in an immediate reversal of the assay ratio. Thus, the properties of the phage were dependent upon the host of production. Lysates of host 145, regardless of the source of the infecting P14 virus, contained phage which behaved as a mixture. It became essential to determine more precisely the stage of the infection cycle in which the alteration occurred in order to take up the problem of the nature of the apparent host control. Three distinct possibilities were considered to explain the change of specificity with change of host.

1. The lysates represent a mixture of phages present in varying proportions dependent on the host. The mixture is composed of: (a) two or more genetically distinguishable phage strains, i.e. mutants arising during multiplication of a single particle on either host, separable by suitable isolation procedures, and, barring further mutation, capable of maintaining their characteristic properties, or (b) one phage strain containing particles genetically indistinguishable but phenotypically different as the result of some unusual control by the host.

2. The lysates contain a homogeneous phage but this is altered and reacts differently upon infection of either host, perhaps due (a) to the presence of different proportions of phage-inactivating substances, or (b) to different proportions of susceptible cells in the host strains used for assay.

3. One or the other host releases some non-phage material which serves as a "cofactor" for plaque formation.

It is obvious that the preliminary observations, derived from the characteristic changes in assay ratio $145/K_1$ of phage produced on the two hosts in broth or agar, reflected the sum total of all factors involved in the several infection cycles leading to mass lysis in liquid or solid media. The experimental details reported below present evidence to indicate that, whatever the mecha-

nism, the alteration is effected during the first infection cycle in host 145. A certain amount of preliminary data is presented which tend to support the theory that the host maintains a control over the phenotypic properties of P14 and to contraindicate the possibilities of mutational changes (1 a) or extra-cellular cofactors or inactivators (2 a and 3). A certain amount of evidence indicated that the control involves only a proportion of the particles rather than all of the particles, as suggested in point 2 b.

### 1. The Course of Phage Production in Broth.—

Log phase cells of each host were infected with P14($K_1$) and with P14(145) virus in medium III broth. The mixtures were incubated at 37°C. on a shaker; at intervals samples were removed and aliquots were assayed on the two hosts. The resultant growth curves were compared with those produced by stock $P_1$ infection of the two hosts. As is evident from Text-figs. 5 and 6, the rate of phage 14 formation on host 145 was independent of the host used previously to passage the virus. The assay curves were characterized by an initial parallel production period followed by a wide divergence, in which particles assayable on host 145 increased and those active for host $K_1$ remained almost constant.
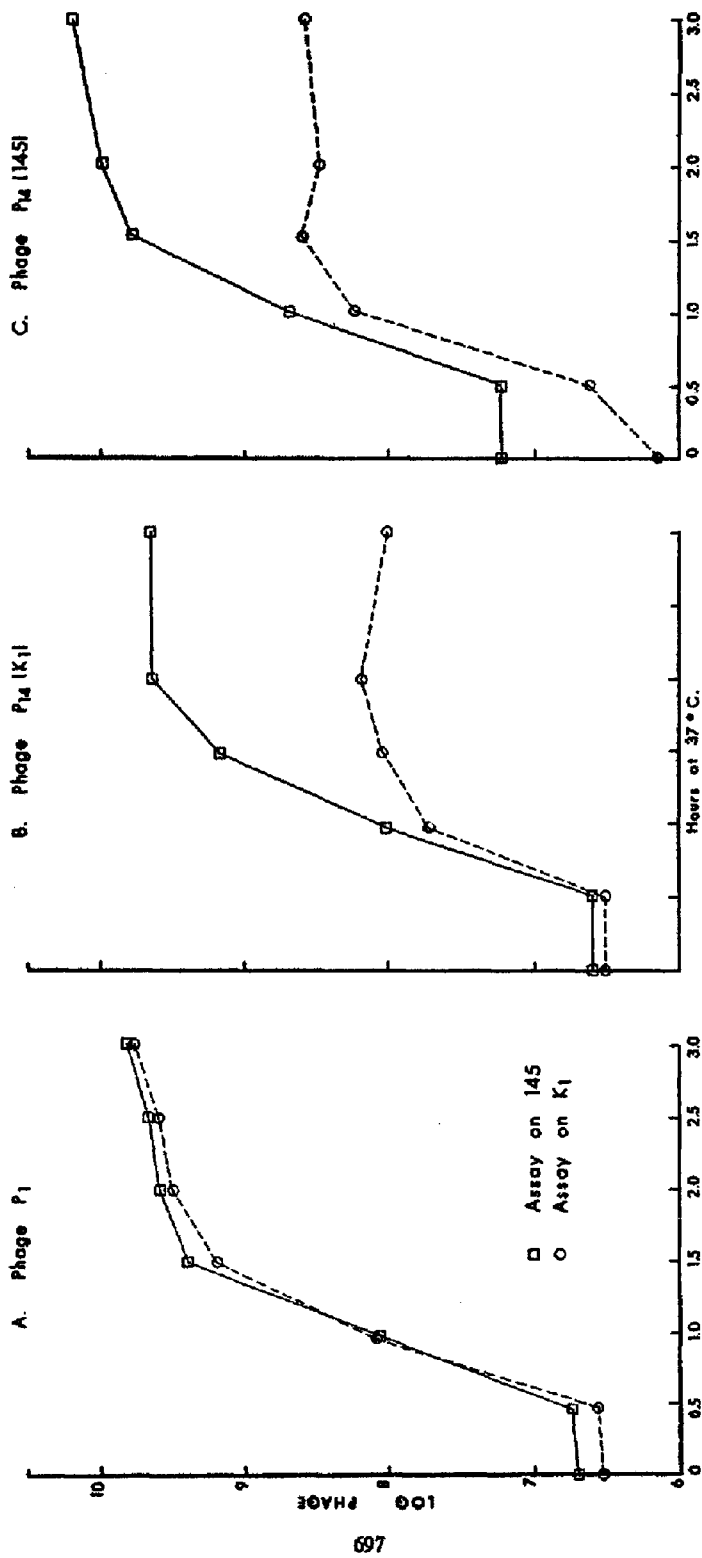
The P14 production on host $K_1$ was similar to that of stock phage $P_1$—with this exception—P14(145) added to $K_1$, showed a progressive initial decrease in titre for host 145. The explanation for this observation becomes apparent from studies of its adsorption and plaque formation, in which it was found that a large proportion of P14(145) particles adsorbed onto but did not multiply on host $K_1$.

(a) Assays of Free Phage during P14 Production on Strain 145.—Titrations of total phage in samples removed early in the course of production on strain 145 assayed in a ratio 145/$K_1$ approaching 1. The total phage represented mixtures of infected cells and free phage. The ratio of the free phage was then determined by filtration of aliquots of samples removed at 0.5, 1.0, and 1.5 hours after infection. They assayed in ratios 145/$K_1$ = 46, 37, and 100 respectively, indicating that the free phage produced in the early stages was similar to that formed in the last bursts, and that no inhibitor accumulated in the culture filtrate to destroy infectivity for host $K_1$.
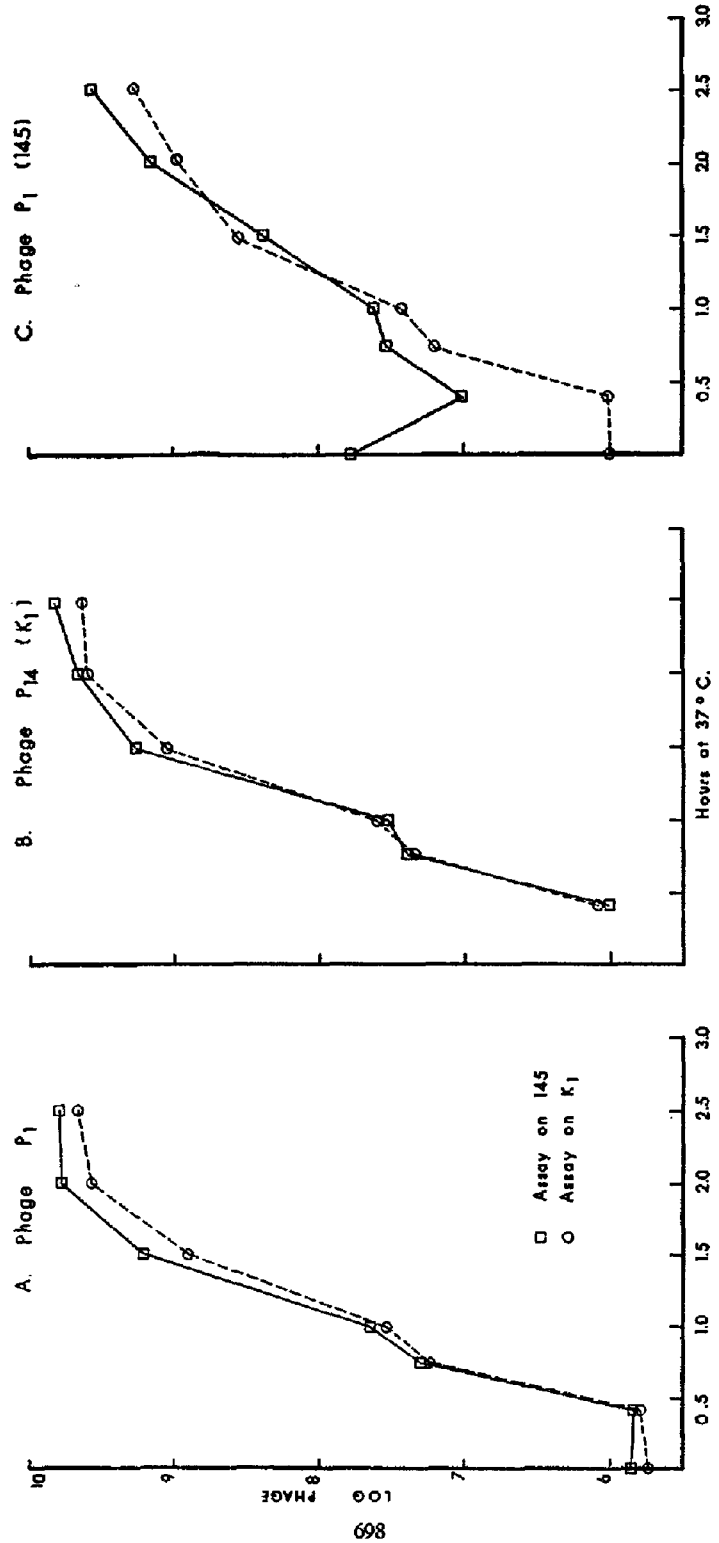
### 2. Adsorption of P14($K_1$) and P14(145) Phage and the Plaque-Forming Ability of Infected Host Cells.—

Resting cells were mixed with P14($K_1$), P14(145), and stock $P_1$ in a ratio P/B = 1/10, at an initial cell count of 1.8 × 10⁸/ml. The mixtures were shaken at 37°C. for 20 minutes, chilled, and filtered through super-cel (4). Cell-free controls were treated similarly. Samples were then assayed on both hosts.

In a second experiment, the mixture was centrifuged in the cold after the 20 minute adsorption period. The infected cells were resuspended to volume

TEXT-FIG. 5. Production of phages $P_1$ and $P_{14}$ on strain WF 145.

A. Phage $P_1$

B. Phage $P_{14}$ ($K_1$)

C. Phage $P_1$ (145)

☐ Assay on 145
○ Assay on $K_1$

LOG PHAGE

Hours at 37° C.

TEXT-FIG. 6. Production of phages $P_1$ and $P_{14}$ on strain $K_1$.

and assayed on the two hosts in order to estimate the number of infected cells capable of forming plaques on either host.

Inspection of the data tabulated in Table V revealed that the amount of adsorption onto either host in 20 minutes was independent of the strain used previously to passage the virus, and was similar to the amount of stock $P_1$ adsorbed. Furthermore, the assay ratios $145/K_1$ of the residual free phage

TABLE V

*Assay Ratios of Free Phage and Washed Infected Cells of Strains $K_1$ and $WF$ 145*

| Phage | Assay host | Total phage (filtered lysate) | Adsorbed on $K_1$ cells* | | | Adsorbed on WF 145 cells* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Infected cells/ml. (b) | Residual free phage (a) | | Infected cells/ml. | Residual free phage | |
| | | | | Per ml. | Per cent adsorbed | | Per ml. | Per cent adsorbed |
| 14($K_1$) | $K_1$ | $1.0 \times 10^8$ | $5.6 \times 10^7$ | $1.1 \times 10^8$ | 98.9 | $4.4 \times 10^7$ | $1.6 \times 10^8$ | 98.4 |
| | 145 | $1.4 \times 10^8$ | $5.8 \times 10^7$ | $1.3 \times 10^8$ | 99.1 | $5.6 \times 10^7$ | $2.5 \times 10^8$ | 98.2 |
| | Ratio | | | | | | | |
| | $145/K_1$ | 1.4 | 1.0 | 1.2 | | 1.2 | 1.5 | |
| 14(145) | $K_1$ | $2.0 \times 10^8$ | $1.3 \times 10^8$ | $1.1 \times 10^4$ | 99.4 | $3.6 \times 10^7$ | $2.0 \times 10^4$ | 99.0 |
| | 145 | $6.8 \times 10^7$ | $1.5 \times 10^8$ | $4.7 \times 10^5$ | 99.3 | $5.6 \times 10^7$ | $6.8 \times 10^5$ | 99.0 |
| | Ratio | | | | | | | |
| | $145/K_1$ | 34 | 1.1 | 42 | | 1.6 | 32 | |
| Stock $P_1(K_1)$ | $K_1$ | $6.9 \times 10^7$ | $6.2 \times 10^7$ | $5.2 \times 10^5$ | 99.2 | $6.6 \times 10^7$ | $5.1 \times 10^5$ | 99.2 |
| | 145 | $9.4 \times 10^7$ | $6.0 \times 10^7$ | $6.2 \times 10^5$ | 99.3 | $7.2 \times 10^7$ | $6.5 \times 10^5$ | 99.3 |
| | Ratio | | | | | | | |
| | $145/K_1$ | 1.4 | 1.0 | 1.2 | | 1.1 | 1.3 | |

* Adsorption mixture $P/B = \dfrac{1 \times 10^8}{2 \times 10^8} = 1/2$. Shaken 20 minutes at 37°C. Chilled. (a) Filtered through super-cel to collect free phage. (b) Centrifuged in cold to obtain infected cells, resuspended to volume. Since some cells are lost, the comparison between assays of total free phage and infected cells is only an approximation.

remained unchanged, indicating that there was no preferential adsorption of particles from any one P14 lysate.

Examination of the assay ratios of the infected cells indicated that all combinations assayed in ratios approaching 1. The titre of P14(145) phage, however, which had been adsorbed onto host $K_1$ and then had been plated on host 145, was reduced about 40-fold; *i.e.*, to the titre of the free phage P14(145) assayed on host $K_1$. That is to say, when the free phage P14(145) produced $1 \times 10^8$/ml. plaques on host $K_1$ and $4 \times 10^9$ plaques on host 145, an equivalent volume, adsorbed first on host $K_1$ and then plated immediately on host 145 produced only $1 \times 10^8$/ml. plaques. If the same amount had been adsorbed onto host 145, and the infected cells had then been plated on both hosts, the

titre would have been $4 \times 10^9$/ml. Thus, most of $K_1$ cells, infected with P14-(145) released no phage capable of forming plaques on either assay host, but all the 145 cells, infected with the same phage, released at least one particle per cell capable of forming plaques on both hosts.

### 3. Survival of Host Cells Infected with P14(145).—

Resting cells of each host were infected with P14(145) in a ratio $P/B = 5/1$. After adsorption for 20 minutes at 37°C., the mixtures were diluted and plated for viable count. The per cent reduction was estimated from the counts of total uninfected cell controls and the number of survivors.

TABLE VI

Survival of $K_1$ and WF 145 Cells Infected with P14(145)

| Bacterial strain* infected | Phage added (3 × 10⁸/ml.) | Bacteria/ml. after 20 min. at 37°C. | Survivors |
|---|---|---|---|
| | | | *per cent* |
| $K_1$ | None | $6.10 \times 10^7$ | 100 |
| $K_1$ | $P_1(K_1)$ | $1.18 \times 10^6$ | 1.9 |
| $K_1$ | P14 $(K_1)$ | $1.22 \times 10^6$ | 2.0 |
| $K_1$ | P14(145) | $8.10 \times 10^6$ | 13.3 |
| WF 145 | None | $6.80 \times 10^7$ | 100 |
| WF 145 | $P_1(K_1)$ | $1.03 \times 10^6$ | 1.51 |
| WF 145 | P14($K_1$) | $1.24 \times 10^6$ | 1.82 |
| WF 145 | P14(145) | $0.91 \times 10^6$ | 1.34 |

* Resting cells of each strain were mixed with phage lots in initial ratios $P/B =$ approximately 5. After incubation at 37°C. for 20 minutes, the mixtures were chilled and diluted for plate count on tryptose-phosphate agar. The adsorbing medium was tryptose-phosphate broth.

The cell count was reduced 86 per cent for $K_1$ and 98 per cent for host 145, as shown in Table VI. A total of $3.5 \times 10^8$ particles/ml. had been added to $6 \times 10^7$ cells/ml. Of this virus, only $3 \times 10^6$/ml. particles were capable of producing plaques on host $K_1$. If none but the plaque-forming particles were responsible for the drop in viable count, a reduction of $3 \times 10^6/6 \times 10^7$ would have occurred, or 5 per cent. Since the total reduction was in the order of 86 per cent, a high percentage of particles must have been adsorbed onto and must have killed the cells, although they were incapable of forming plaques.

### 4. Lysis of Phage-Infected Cells.—

Log phase cells of strains 145 and $K_1$ were mixed with P14(145) in a ratio $P/B = 6/1$. A second set was infected with P1($K_1$). Following adsorption, the cells were centrifuged and resuspended in broth to the original turbidity. Titrations of the supernate showed that 90 per cent of the phage had been ad-

sorbed on strain 145 and 87 per cent on strain $K_1$, an amount equivalent to $5 \times 10^8$/ml. phage for $1 \times 10^8$/ml. cells. Similar results were obtained for $P_1$ stock. The infected cells were then incubated with shaking at 37°C. and turbidity readings were made.

As may be seen from Table VII, no significant lysis of $K_1$ cells "infected" with P14(145) occurred within 2 hours. It will be remembered that only 1/40 of such infected cells were plaque-formers. Detection of their lysis would have been impossible since this involved only $2.5 \times 10^8$/ml. cells in a total of $1 \times 10^8$/ml. With infection of 145 cells, lysis occurred within 40 minutes.

### 5. Latent Periods and Average Burst Sizes.—

Phage was added to both resting and log phase cells. Following suitable adsorption periods, the suspensions were diluted, and titrations were made on both hosts before and until lysis was completed. Free phage was estimated by

TABLE VII

*Turbidity Changes in Infected Cell Suspensions of Strains $K_1$ and WF 145*

| Strain infected | Phage added (ratio P/B = 5) | Bacteria/ml. $\times 10^7$ after minutes at 37°C. (Klett) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 10 | 40 | 70 | 120 |
| WF 145 | P14(145) | 9 | 9 | 5 | 5 | 3 |
| $K_1$ | P14(145) | 7 | 7 | 7 | 7 | 7 |
| WF 145 | Stock $P_1(K_1)$ | 8 | 8 | 2 | 2 | 2 |
| $K_1$ | Stock $P_1(K_1)$ | 8 | 8 | 2 | 1 | 1 |

filtration through super-cel, and the value subtracted from total phage to obtain the number of infected cells. In some experiments, the free phage was removed by centrifugation, and the infected cells were resuspended and diluted in fresh broth. Tables VIII and IX summarize the results of such studies.

Resting cells of both hosts, infected with P14(145), required longer latent periods than log phase cells. With resting cells of strain WF 145, no increase in titre for host $K_1$ could be detected in 165 minutes, so that the latent period could be reported only for assays made on host 145.

With log phase cells, the rise of particles assayable on host $K_1$ appeared to parallel the rise for those assayable on host 145. The latent periods of cells infected with P14($K_1$) were similar to the latent periods of cells infected with P14(145), regardless of the host used for assay.

From the data on burst size determinations, it may be concluded that resting cells formed less phage than log phase cells. Resting cells on strain WF 145, which had been infected with P14(145), released only 1 particle per cell capable of forming plaques on host $K_1$, but at least 20 particles per cell able to produce plaques on host 145. When the same experiment was performed on log phase

cells, 2 to 3 particles per cell formed plaques on host $K_1$ of a total of 70 to 90 capable of producing plaques on host 145. When P14($K_1$) was adsorbed on strain WF 145, similar yields were obtained. Infected log phase cells produced about 75 particles per infected cell, of which only 2 to 3 could form plaques on

TABLE VIII

*Minimum Latent Periods of Phages $P_1$ and $P14$ in Hosts $K_1$ and WF 145*

| Phage | Adsorbed on bacterial host | Initial P/B | First increase observed after minutes at 37°C. assayed on | |
|-------|---------------------------|-------------|----------------|--------|
| | | | $K_1$ | WF 145 |
| 14(145) | Resting $K_1$ | 3/1 | 45–50 | 45–50 |
| 14($K_1$) | Resting $K_1$ | 3/1 | 45–50 | 45–50 |
| $P_1$($K_1$) | Resting $K_1$ | 3/1 | 45–50 | 45–50 |
| 14(145) | Log phase $K_1$ | 3/1 | 30–35 | 30–35 |
| 14($K_1$) | Log phase $K_1$ | 3/1 | 30–35 | 30–35 |
| $P_1$($K_1$) | Log phase $K_1$ | 3/1 | 30–35 | 30–35 |
| 14(145) | Resting 145 | 3/1 | No rise in 120 min. | 45–50 |
| 14($K_1$) | Resting 145 | 3/1 | No rise in 120 min. | 45–50 |
| $P_1$($K_1$) | Resting 145 | 3/1 | 45–50 | 45–50 |
| 14(145) | Log phase 145 | 3/1 | 25–30 | 25–30 |
| 14($K_1$) | Log phase 145 | 3/1 | 25–30 | 25–30 |
| $P_1$($K_1$) | Log phase 145 | 3/1 | 25–30 | 25–30 |

TABLE IX

*Average Burst Size for Cells Infected with $P14(K_1)$, $P14(145)$, and Stock $P_1(K_1)$*

| Strain infected | Phage 14($K_1$) Average burst/cell calculated from assay on | | Phage 14(145) Average burst/cell calculated from assay on | | Phage $P_1$ ($K_1$) Average burst/cell calculated from assay on | |
|-----------------|------|-----|------|-----|------|-----|
| | $K_1$ | 145 | $K_1$ | 145 | $K_1$ | 145 |
| Resting $K_1$ | — | — | 23 | 18 | — | — |
| Resting 145 | — | — | 1 | 24 | — | — |
| Log phase $K_1$ | 100 | 100 | 58 | 70 | 80 | 85 |
| Log phase 145 | 2.3 | 56 | 2.3 | 75 | 58 | 62 |

host $K_1$. Such results indicated that the particles formed on WF 145 cells were independent of the strain used previously to passage the phage.

After infections of log phase $K_1$ cells by P14(145) the average yield of particles per infected cell was 58 and 70, for hosts $K_1$ and WF 145 respectively. Following infections by P14($K_1$), the average burst was 100 for both hosts. Therefore phage released from infected host $K_1$ cells, regardless of the prior host, assayed in a ratio 145/$K_1$ approaching 1.

*6. The Relation of the Physiological Condition of Host Cells to the Plaque-Forming Activity of Phage 14.—*

The ability of particles derived from host 145 to infect host $K_1$ successfully was found to be related to the physiological state of these cells, a greater number of particles forming plaques on log phase host $K_1$ than on resting cells. The titres of the stock phage $P_1$ and $P14(K_1)$ remained unchanged under the same conditions.

When resting strain $K_1$ was incubated in medium III broth at $37°C.$, a twofold increase in susceptibility to $P14(145)$ occurred within 0.5 hour, before any detectable cellular multiplication. It rose to a peak during the logarithmic phase of growth and then dropped gradually. On one occasion, a 30-fold increase in plaque-forming ability was recorded, which was not followed by the usual decrease with cell age; this has never been repeated.

From the experiments on P14 multiplication, the following is indicated: Regardless of the host cell which has served as the source of the virus, P14 is adsorbed on both hosts. On strain 145, following a latent period of about 25 to 30 minutes (log cells), virus is released with an average total yield in the order of 60 particles per infected cell. All the particles form plaques on host 145. All the particles adsorb on host $K_1$, but only 2 to 3 of the total produce plaques on this host. The remainder of the "infected" cells are apparently destroyed without lysis and yield no phage active for either host. The number of infections that result in successful plaque formation is dependent upon the physiological condition of the $K_1$ cells. The host control phenomenon is therefore concerned with an alteration of the plaque-forming activity of the phage with respect to host $K_1$. This change is effected during multiplication in host 145 cells. Those particles which succeed in forming plaques on host $K_1$ produce phage which remains susceptible to the host control, since the next cycle on host WF 145 reproduces the high titration ratio $145/K_1$.

While the above data narrow the stage at which P14 is altered to the first infection cycle in host 145, they still provide no information as to the mechanism involved. The rapidity of the change, together with observations indicating that all particles remain susceptible to alteration, suggests that host 145 controls the formation of some "phenotypic" property of this particular virus. The identification of a specific constituent requisite for virus formation in host $K_1$, but non-essential for infection of host 145, would, of course, provide conclusive evidence for such a theory. It is necessary, however, to consider alternative possibilities before attempting extensive investigation to establish the validity of such a theory.

*Attempted Separation of Genetically Distinct Phages from $P_{14}$ Lysates*

Following the assumption that the changes in assay ratio $145/K_1$ following production of $P_{14}$ on the two hosts reflected a presence of two (or more) viruses

formed by mutation, or by some unusual recombination between an infecting particle and some internally activated prophage, attempts have been made to isolate distinct components by inactivating or reducing the concentration of one through serum or heat treatment.

(a) *By Serum Inactivation.*—Antiserum for purified phage $P_1$ was prepared. Phage 14(145)–antiserum mixtures were incubated at 37°C. and the course of virus inactivation was determined by titration of survivors on both hosts. This was compared with the inactivation of stock $P_1$ (Table X). Essentially similar curves were obtained, indicating that not only was phage 14 closely

TABLE X

*Inactivation of Stock $P_1$ and $P14(145)$ by Antiserum to Stock $P_1$*

| Phage | Time exposed to antiserum | Phage survivors/ml. assayed on | | | Survivors determined from assays on strain | |
|---|---|---|---|---|---|---|
| | | $K_1$ | 145 | R145/K$_1$ | $K_1$ | 145 |
| | *min.* | | | | *per cent* | *per cent* |
| P14(145) | 0 | $1.6 \times 10^6$ | $6.0 \times 10^7$ | 38 | 100.0 | 100.0 |
| | 10 | $5.2 \times 10^5$ | $1.9 \times 10^7$ | 37 | 32.5 | 30.3 |
| | 20 | $1.6 \times 10^5$ | $9.4 \times 10^6$ | 59 | 10.0 | 10.5 |
| | 30 | $4.1 \times 10^4$ | $2.9 \times 10^6$ | 70 | 2.5 | 4.8 |
| | 45 | $9.6 \times 10^3$ | $1.0 \times 10^6$ | 107 | 0.5 | 1.7 |
| | 60 | $2.6 \times 10^3$ | $2.7 \times 10^5$ | 104 | 0.2 | 0.5 |
| $P_1(K_1)$ | 0 | $1.3 \times 10^7$ | $1.6 \times 10^7$ | 1.2 | 100.0 | 100.0 |
| | 10 | $6.8 \times 10^6$ | $5.3 \times 10^6$ | 0.8 | 53.8 | 33.4 |
| | 20 | $9.5 \times 10^5$ | $1.7 \times 10^6$ | 1.8 | 7.3 | 10.6 |
| | 30 | $3.3 \times 10^5$ | $1.0 \times 10^6$ | 3.1 | 2.5 | 6.3 |
| | 45 | $5.6 \times 10^4$ | $1.9 \times 10^5$ | 3.3 | 0.4 | 1.1 |
| | 60 | $1.2 \times 10^4$ | $3.2 \times 10^4$ | 2.7 | 0.1 | 0.2 |

3 ml. phage mixed with 3 ml. antiserum diluted 1/500. Incubation at 37°C. Antiserum dilution was such that concentration present in assay mixtures had no effect on plaque development.

related to phage $P_1$, but also that it did not contain any serologically unrelated component, thus suggesting that no internally lysogenic phage was involved in the behavior of the 145 lysates (none of the 5 carried phages is serologically related to $P_1$).

As a further test, 32 plaques from samples plated after 45 minutes' exposure to antiserum were suspended in saline, and their contents were assayed on the two hosts. All 32 formed plaques on both hosts. The average titration ratio $145/K_1$ of the plaque suspensions was 47, with a range from 25 to 76. When such plaques were repicked, the assay ratio of their contents did not increase. Thus, the last survivors of serum treatment behaved as the untreated phage and the experiment failed to detect genetically distinct phages. Their existence

cannot be ruled out, however, since mutants derived from any given strain have never been shown to lose their antigenic relation to the parent source. Therefore, the serum treatment could not distinguish them unless they happened to exhibit an altered affinity for the antiserum employed.

(b) *By Heat Inactivation.*—Text-figs. 7 and 8 show the course of inactivation of P14(K₁) and P14(145) at 59 and 60°C. Inspection of the graphs reveals that
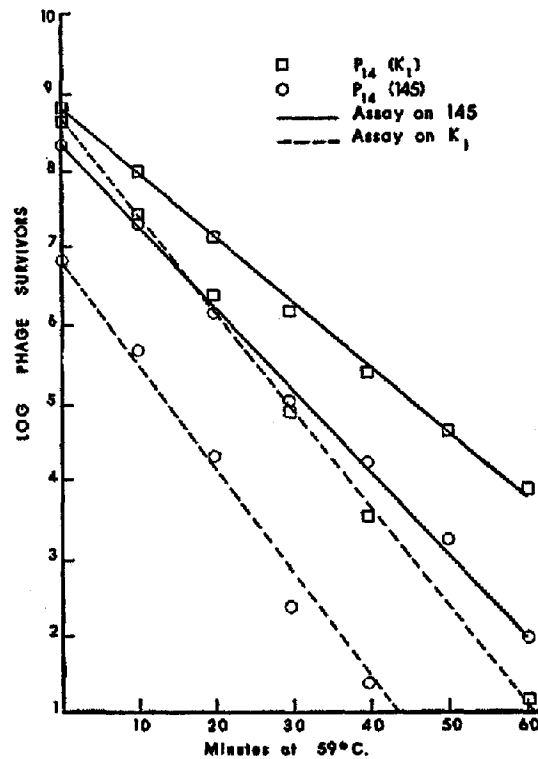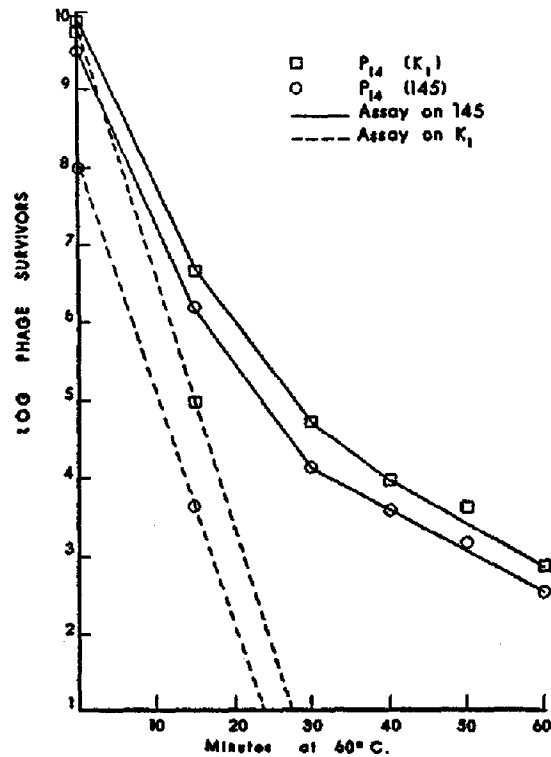


TEXT-FIG. 7. Inactivation of P₁₄(145) and P₁₄(K₁) at 59°C.

regardless of the host used to propagate the virus, the loss of activity for $K_1$ cells proceeds more rapidly than that for 145 cells. It is possible, thereby, to prepare phage which has lost all ability to multiply on host $K_1$, making it appear that there remained a component which might thereafter multiply only on host 145. Plaque isolations were made from the survivors which could still multiply on host 145. Of 68 isolations, all were capable of forming phage on both hosts. The assay ratio 145/$K_1$ of the virus in such suspensions was similar to that of the unheated phage 14(145), as is indicated in Table XI. Thus, particles which had lost all ability to produce plaques on host $K_1$ regained

this property when they were plated on host 145, and again there was formed a preparation behaving as a mixture from which no genetically distinct phage strain could be separated.

There can be no doubt from such an experiment that a particle with ability to multiply only on one host gives rise to a proportion of particles with an increased host range (for $K_1$). The likelihood that such behavior represented two or more genetically distinct phage strains with differing host specificities was



TEXT-FIG. 8. Inactivation of $P_{14}(145)$ and $P_{14}(K_1)$ at 60°C.

reduced by the failures in separation by the ordinary methods available for purifying mixtures of phages. Yet, some sort of mixture exists. Assuming only one phage strain is involved, it follows that host 145 must manufacture two phenotypically distinct particles from P14 phage, 90 per cent of which are lytic failures with respect to host $K_1$. Paradoxically, the same proportion of "defective" particles, adsorbed first on host 145 and then plated with host $K_1$, produce plaques. Thus, host 145 cells behave the same; regardless of whether the infecting phage particle was or was not capable of initiating plaque formation on host $K_1$, the progeny released from such cells act as a mixture.

The above explanation can be acceptable only when at least two other possible factors have been overruled: (1) the production of non-phage extracellular inactivators by one or the other host, and (2) the release of one or more cofactors by infected cells which might be essential for phage multiplication in the alternate host.

### Tests for Inhibitory Substances and Cofactors for Virus Formation

(a) *Bursts into Filtrates of Broth Cultures.*—To test the possibility that the changes in virus behavior following production on each host were related to

TABLE XI

*Assay Ratios of Heat-Inactivated Phage Survivors on Host 145*

| Isolate No. | Phage 14($K_1$) Ratios 145/$K_1$ of virus from plaques on host 145, representing residual phage surviving heat at | | | Phage 14(145) Ratios 145/$K_1$ of virus from plaques on host 145, representing residual phage surviving heat at | | |
|---|---|---|---|---|---|---|
| | 59°C. | 60°C. | | 59°C. | 60°C. | |
| | 60 min. | 30 min. | 60 min. | 40 min. | 15 min. | 60 min. |
| 1 | 46 | 35 | 15 | 21 | 17 | 12 |
| 2 | 22 | 11 | 40 | 11 | 10 | 15 |
| 3 | 30 | 10 | 27 | 23 | 10 | 6 |
| 4 | 30 | 88 | 21 | 24 | 7 | 8 |
| 5 | 41 | 25 | 39 | 42 | 23 | 15 |
| 6 | 18 | 20 | 40 | 25 | 13 | 28 |
| 7 | 19 | 30 | 20 | 34 | 22 | 4 |
| 8 | 28 | 26 | 34 | 69 | 17 | 37 |
| 9 | 29 | 8 | 64 | 33 | 16 | 10 |
| 10 | 19 | 11 | 45 | 30 | 10 | 26 |
| 11 | — | 31 | 13 | — | 21 | 24 |
| 12 | — | 19 | — | — | 24 | 27 |
| Average value | 28 | 28 | 33 | 31 | 18 | 19 |
| Unheated broth control | 1.0 | 1.1 | 1.1 | 30 | 32 | 32 |

differences in release of some cofactor essential for virus formation, infected WF 145 cells were diluted with filtrates of uninfected cells and with plain broth. The bursts were allowed to take place and the phage was assayed on both hosts. No effect on the burst size was observed. Neither was any effect found when P14–infected WF 145 cells were allowed to burst in filtrates of host $K_1$, the phage released and assayable on host $K_1$ being 1.7 and 1.9 per infected cell for control and filtrate-containing samples respectively, and that assayable on WF 145 being 59 and 62 per infected cell.

(b) *Non-Interaction of P14($K_1$) and P14(145).* Mixtures of phage prepared on each host, both dilute and concentrated, were stored at 4°C. for 8 hours, and then were assayed on both hosts. The values were compared with unmixed

TABLE XII

*Non-Interactions of P14($K_1$) and P14(145) Mixtures after 8 Hours at 4°C.*

*Test of Dilute Mixtures*

| Sample | Treatment prior to assay Mixtures stored 8 hrs. | Plaques/ ml. of average value* | | Per ml. in test mixture assayed on host | |
|---|---|---|---|---|---|
| | | $K_1$ | 145 | $K_1$ | 145 |
| A | 3 ml. $10^{-5.7}$ P14(145) + 3 ml. broth.. | 163 | — | $3.26 \times 10^8$ | — |
| B | 3 ml. $10^{-6}$ P14($K_1$) + 3 ml. broth.... | 206 | 258 | $4.12 \times 10^9$ | $5.16 \times 10^8$ |
| C | 3 ml. $10^{-6}$ P14(145) + 3 ml. broth.... | — | 129 | — | $2.58 \times 10^8$ |
| | Total assayed.................. | 369 | 387 | $7.38 \times 10^8$ | $7.74 \times 10^8$ |
| D | 3 ml. $10^{-6}$ P14($K_1$) + 3 ml. $10^{-6}$ P14-(145)........................... | — | 377 | — | $7.54 \times 10^8$ |
| E | 3 ml. $10^{-5.7}$ P14(145) + 3 ml. $10^{-6}$ P14($K_1$)......................... | 372 | — | $7.44 \times 10^8$ | — |

*Test of Concentrated Mixtures*

| Sample | Treatment prior to assay Mixtures stored 8 hrs. | | Plaques/ ml. of average value‡ | | Per ml. in test mixture assayed on host | |
|---|---|---|---|---|---|---|
| | Contents | Dilution after 8 hrs. | $K_1$ | 145 | $K_1$ | 145 |
| A | 3 ml. P14($K_1$) + 3 ml. broth.. | $10^{-7.0}$ | — | 182 | — | $3.64 \times 10^9$ |
| B | 3 ml. P14(145) + 3 ml. broth. | $10^{-7.0}$ | — | 117 | — | $2.34 \times 10^9$ |
| A + B | Total assayed........... | | — | 299 | — | $5.98 \times 10^9$ |
| C | 3 ml. P14(145) + 3 ml. P14-($K_1$).................... | $10^{-7.0}$ | — | 312 | — | $6.24 \times 10^9$ |
| D | 8 ml. P14(145) + 2 ml. broth. | $10^{-6.7}$ | 239 | — | $0.95 \times 10^9$ | — |
| E | 2 ml. P14($K_1$) + 8 ml. broth.. | $10^{-7}$ | 74 | — | $1.48 \times 10^9$ | — |
| D/50 + E | Total assayed........... | | 79 | — | $1.58 \times 10^9$ | — |
| F | 8 ml. P14(145) + 2 ml. P14-($K_1$)..................... | $10^{-7}$ | 82 | — | $1.64 \times 10^9$ | — |

* Three assays were made of each sample by removing 0.5 ml. aliquots to 4.5 ml. plaque mixture and spreading 0.5 ml. aliquots over each of three slides.

‡ Duplicate dilution series were prepared from each sample. Triplicate aliquots were sampled from each dilution. After mixing with plaque suspension, triplicate slides were prepared from each of the three assay mixtures.

phage controls. Table XII summarizes the findings, showing that the sum titre
of phage incubated singly was equal to the phage in mixtures. Thus, neither
the formed virus nor any postulated extracellular materials present in either
lysate interacted to alter the activity of the virus particles.

An additional experiment to demonstrate possible interaction between the
2 phage lysates was performed as follows: Known volumes of each lysate were
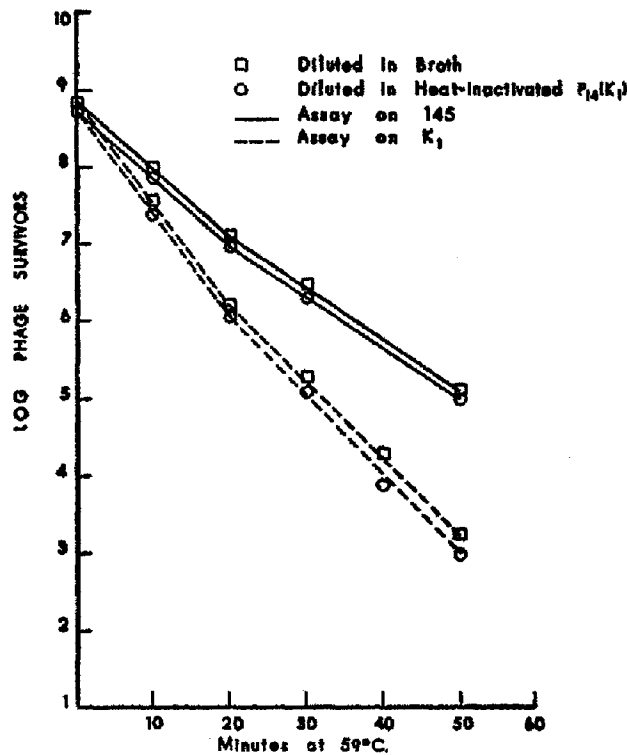


Text-Fig. 9. Effect of heat-inactivated phage on survival of $P_{14}(K_1)$ at 59°C.

centrifuged at high speed to sediment the phage; the supernates were switched
and after 18 hours at 4°C., the preparations were each assayed on the two hosts.
The counts were completely additive; *i.e.*, all virus present in one lysate could
be accounted for after mixing with the centrifuged supernate of the other.
Such results indicated most strikingly that the peculiar behavior of the virus
in the two preparations was closely associated with the virus particles them-
selves. Of course, the data do not exclude the possibility of some heavy mole-
cule being released by strain 145 only during phage infection, which might be
responsible for interfering with plaque formation on strain $K_1$. However, pre-

liminary electron microscope examination, as described below, did not reveal unusual particles peculiar only to one lysate.

(c) *Non-Interaction of Heat-Killed and Active Phage.*—Phage samples were inactivated by heating for 1 hour at 59°C. Active phage P14(K₁) and P14(145) were then diluted 1/10 in heat-inactivated phage, and the course of inactivation at 59°C. was determined. The values were compared with phage controls diluted in broth and heated similarly. Text-figs. 9 and 10 present typical curves
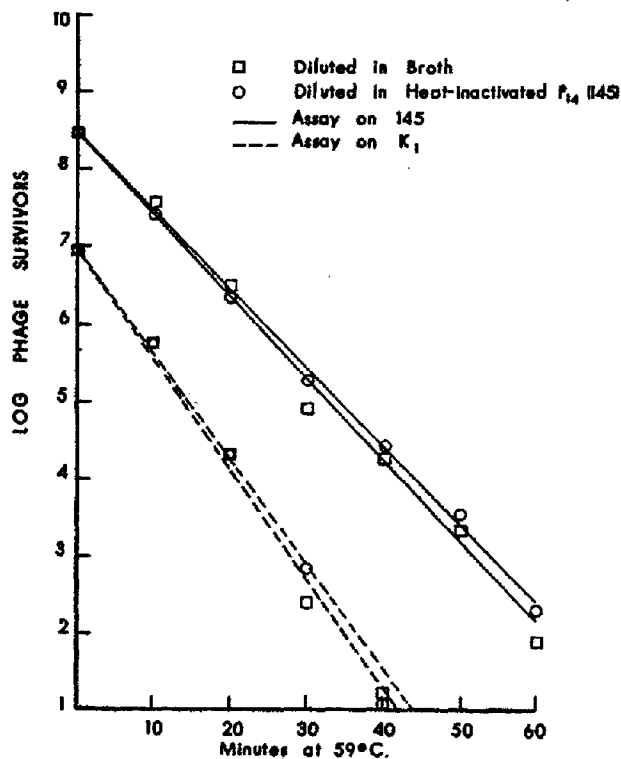


TEXT-FIG. 10. Effect of heat-inactivated phage on survival of P₁₄(145) at 59°C.

of inactivation with respect to activity for both hosts. The presence of heat-inactivated phage did not alter the course of phage destruction indicating that no substances released or formed during heat treatment interacted with P14 phage prepared on either host.

*Morphology of Phage 14*[1]

Filtered lysates of P₁₄(K₁) and P14(145) were subjected to cycles of low and high speed centrifugation at 8,000 and 15,000 R.P.M. in No. 40 rotor of the

[1] Courtesy of Dr. Herbert Gold, Department of Plant Pathology, University of California.

Spinco ultracentrifuge. The partially purified preparations were resuspended in 0.1 ammonium acetate buffer, sprayed on collodion screens, dried, and palladium-shadowed. No morphological differences have been observed under the electron microscope between particles prepared on either host. Unfortunately, most of the preparations obtained to date have revealed particles in various stages of disintegration, so that definite conclusions must await development of more suitable methods of fixing purified phage 14. Figs. 2 and 3 illustrate typical preparations.

## DISCUSSION

### *The Nature of the Host Control over Phage 14*

In considering the data concerning the host control over P14 phage, it would appear that:

1. P14 lysates of strain 145 differ from those of strain $K_1$. The criterion for this distinction lies in the change of assay ratio with change of host. Virus obtained from host 145 behaves as a mixture.

2. It is very unlikely that the change of activity with change of host is related to the presence or absence of extracellular cofactors or phage inhibitors in cell lysates, since (*a*) the two phage preparations did not interact, (*b*) their supernates could be switched with the sedimented phage, (*c*) heat-killed phage did not affect the course of inactivation of active phage, and (*d*) culture filtrates themselves did not alter the burst from either host.

3. The apparent phage mixture does not seem to be composed of genetically distinct phage strains arising by mutation on either host, since attempts at separating and propagating them have been negative. If two strains are involved, the data would require that a single particle of either phage give rise to both at extremely high rates, since the alteration is effected in the first burst of an infected cell.

4. One possible explanation for the observed changes is that the host control involves an alteration of all particles by host 145 in such a way that they behave thereafter as sensitive indicators of the presence of a proportion of resistant cells in host $K_1$. The following observations make this hypothesis very unlikely: (*a*) At least nine different staphylococcal strains exhibit a susceptibility to P14(145) similar to host $K_1$, and have an efficiency of plating, or titration ratio 145/new host $= 145/K_1$. If $K_1$ were composed of a mixture of sensitive and resistant cells with respect to a uniformly altered phage 14(145), then all nine strains would have to contain the *same* proportion of sensitive and resistant cells. This appears unlikely. (*b*) When $K_1$ cells are all singly infected with "altered virus" (at least one particle per cell, regardless of whether a burst results or not), the low percentage, *ca.* 2 to 3 per cent, of plaque formers would indicate that greater than 95 per cent of the cell population was resistant to the altered phage. Such a high proportion of resistant cells would make titration by plaque count virtually impossible. Plaques formed by the phage

14(145) on host $K_1$ show clear centers. If the assay cells contain a large number of resistant cells, one would expect to see turbid plaques, unless one also postulates transient states of resistance. (c) Phage formed by host $K_1$ cells assays equally well on both hosts. This observation, according to the above theory, would require the added explanation that all particles are realtered by $K_1$ cells so that they no longer reflect the mixture of cell types.

5. The authors think it more likely that the host control is concerned with alterations in phenotypic properties of particles belonging to a single and genetically distinct strain. When phage 14 is produced on host 145, a large percentage of the progeny are altered so that they adsorb but cannot form plaques on host $K_1$. Present data make it impossible to determine whether such particles are lacking some factor(s) essential for plaque formation on $K_1$, or whether they are coated with some inhibitor.

The different rates of heat inactivation with respect to assay on each host might reflect (a) the destruction of two different components of a single virus molecule, each essential for virus activity on a given host, (b) the destruction of one component required in different amounts for successful infection of a given host, or (c) unequal rates of inactivation of two distinct phages. Experiments carried out with the stock phage $P_1$ (which is not susceptible to the host control) showed that it, too, exhibited different rates of inactivation with respect to infectivity for the two hosts. A comparison of $P1(K_1)$ and $P14(K_1)$ indicated that their rates of heat inactivation were very similar. Therefore, if two distinct phages are involved, they must be present in similar proportions in the parent $P_1$.

The authors suggest the following working hypothesis to explain the observations on host control in this system:

1. The host control involves alterations in phage 14, with the production of at least two phages (phenotypes). The number 14 refers to their genetic composition.

2. The term 14w (wide host range) designates phage which can multiply equally well on both hosts.

3. The term 14n (narrow host range) designates phage which can multiply only on host 145. Any assay on host 145 reflects the activity of both 14n and 14w, whereas assay on host $K_1$ indicates the behavior of only one, 14w. Therefore the assay ratio $145/K_1 = [n + w]/[w]$.

4. Both 14n and 14w adsorb equally well on either host, exhibit similar latent periods and burst sizes, and are closely related serologically to phage $P_1$.

5. Phage 14n adsorbed on host $K_1$ becomes a lytic failure; however, phage 14w is successful and forms mainly more 14w. (Its lysates assay in ratios $145/K_1$ approaching 1.)

6. A single particle of either 14n or 14w infecting a 145 cell may give rise to both 14n and 14w. This becomes evident from the observation that while

free phage 14(145) assays in a high ratio 145/$K_1$, infected 145 cells, themselves, assay to titre in a ratio approaching 1.

7. Heat destroys activity for $K_1$ cells at a much more rapid rate than for 145 cells. It is possible to obtain, thereby, phage with activity only for 145. Such phage is comparable to, but not necessarily identical with 14n. Reinfection of host 145 with such phage yields both 14n and 14w.

The curve of heat inactivation with respect to host $K_1$, reflects destruction of 14w; that with respect to host 145, measures total [14n + 14w]. The curves suggest that the destruction is partial; *i.e.*, a particle unable to multiply on $K_1$ may still do so on host 145.

8. Phage 14n differs from 14w at least in that it lacks a factor essential for $K_1$ activity. This factor is relatively heat-labile and is a phenotypic property of P14. However, the experiments do not distinguish between the possibility that the presence or absence of the host factor is the only point of difference or whether it is but one in a series of differences between two distinct phages.

Until recently there has been no exception to the data indicating that the progeny formed during infection of a host cell by a single particle of a particular strain of virus are identical in size, shape, chemical composition, and biological activity with the infecting particle. Once established in a host, the virus' properties have been considered to be beyond the control of the host; *i.e.*, particles formed in one host are identical in every way with those produced in any other, barring mutation. Heritable changes in viral property have been shown to possess the attributes of a mutation mechanism. Such changes may occur on any susceptible host and can be detected by the use of appropriate conditions for selection of mutant particles.

The recombination phenomenon provides one exception to the rule of identical progeny. In this case, multiple infection of a single cell by two or more closely related viral mutants may result in an exchange of genetic characteristics. The recombinants are, in general, genetically stable; *i.e.*, they may be isolated and passed through sensitive indicator hosts without alterations in their properties.

The work with phage 14 described in this paper has indicated that a second exception to the rule may occur, in which one host may control the formation of certain materials essential for phage production in a second system. A large proportion of particles formed in host WF 145 appear to lack some factor necessary for successful infection of host $K_1$. Under these circumstances, a mixture of phage activity ensues from infection by a single virus particle. The mixture does not seem to consist of genetically distinct phage strains, but more likely appears to be composed of "phenotypically" altered particles, all of which are genetically identical.

It may be that this phenomenon is closely related to the changes described by Rountree (7), in which passage of certain staphylococcal phages through

indicator strains altered their infectivity so that the previously resistant lyso-genic parents behaved as sensitive hosts.

There is no doubt that mutation and selection phenomena provide the explanation for many observed changes in viral activity. The present experiments point to the host cell as an additional mechanism for controlling viral variation. The susceptibility of one of the phage $P_1$ variants to a host control, in contrast to the stability of the stock strain in general, and to a second variant, phage 5, in particular, indicates that an unusual viral mutant may be involved in the host-control effect: a viral mutant which possesses a heritable instability or susceptibility to host control. In the case of phage 14, the susceptibility to change was possessed by all particles of the strain, but the change, itself, was phenotypic in nature and dependent upon the host and experimental environment.

If it be demonstrated subsequently that P14 is actually a mutant strain, derived from the stock $P_1$ during its production on any susceptible host, then it may be possible to establish conclusive evidence for an additional mechanism to explain problems of viral variation. This would not negate what is already known of mutation and selection phenomena, but would add to the gamut of viral mutations a host-controllable variant. Thus, there may be visualized: infection by a single strain followed by mutation to host control susceptibility; alteration by certain host cells to cause the mutant virus to exhibit any one of the following: (1) its retention as a latent infection—perhaps involved in some cancerous growths, in which certain cells provide the controlling host medium, (2) loss of virulence or attenuation, (3) loss of tissue tropism, toxic properties, and (4) their counterparts, all under the influence of the controller host cell.

## SUMMARY

A series of phage $P_1$ variants was isolated from plaques developing on S. aureus WF 145. One in particular, phage 14, was studied in detail because its host range appeared to be dependent on the previous host of production; i.e., it was subject to a host control. When this phage was produced on host $K_1$ its lysate assayed equally well on both 145 and $K_1$ cells. When produced on host 145, however, it assayed manyfold higher on 145 than on host $K_1$. All its particles adsorbed on $K_1$ cells, but only a small percentage were able to produce plaques. No differences could be found in adsorption rates, latent periods, or burst sizes of the phage on the two hosts. No extracellular inactivating substances could be detected which could account for such changes, nor could the results be explained readily on a mutational basis, since distinct phage strains could not be isolated. The change in virus properties was found to occur in the first burst of singly infected host 145 cells, regardless of the previous host or its prior lytic abilities.

Heat inactivation destroyed activity for $K_1$ cells more rapidly than for 145 cells. This was found to be a property of both the stock phage $P_1$ and phage 14. Phage 14 could be heated until there remained particles which could multiply only on strain 145. When the plaques of such survivors were examined they were found to contain phage which could multiply on both hosts in a ratio characteristic of the original unheated preparation.

The data suggest that the observed changes were caused by a host control over the formation of a phage material(s) necessary for successful infection of host $K_1$. Such a substance theoretically could be related to the labile material destroyed by heat and required for plaque formation on host $K_1$.

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EXPLANATION OF PLATES

## PLATE 11

FIG. 1. Phages 5, 14, and 15 plated with strains $K_1$ and 145 after ten plaque passages on each host.

*Plates with Host $K_1$.* A, passaged on host $K_1$; B, passaged on host 145. *Plates with host 145.* C, passaged on host $K_1$; D, passaged on host 145.

FIG. 1

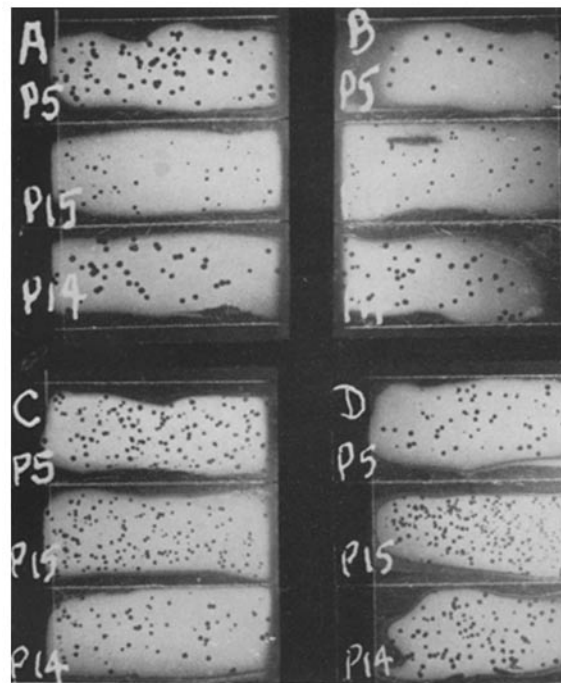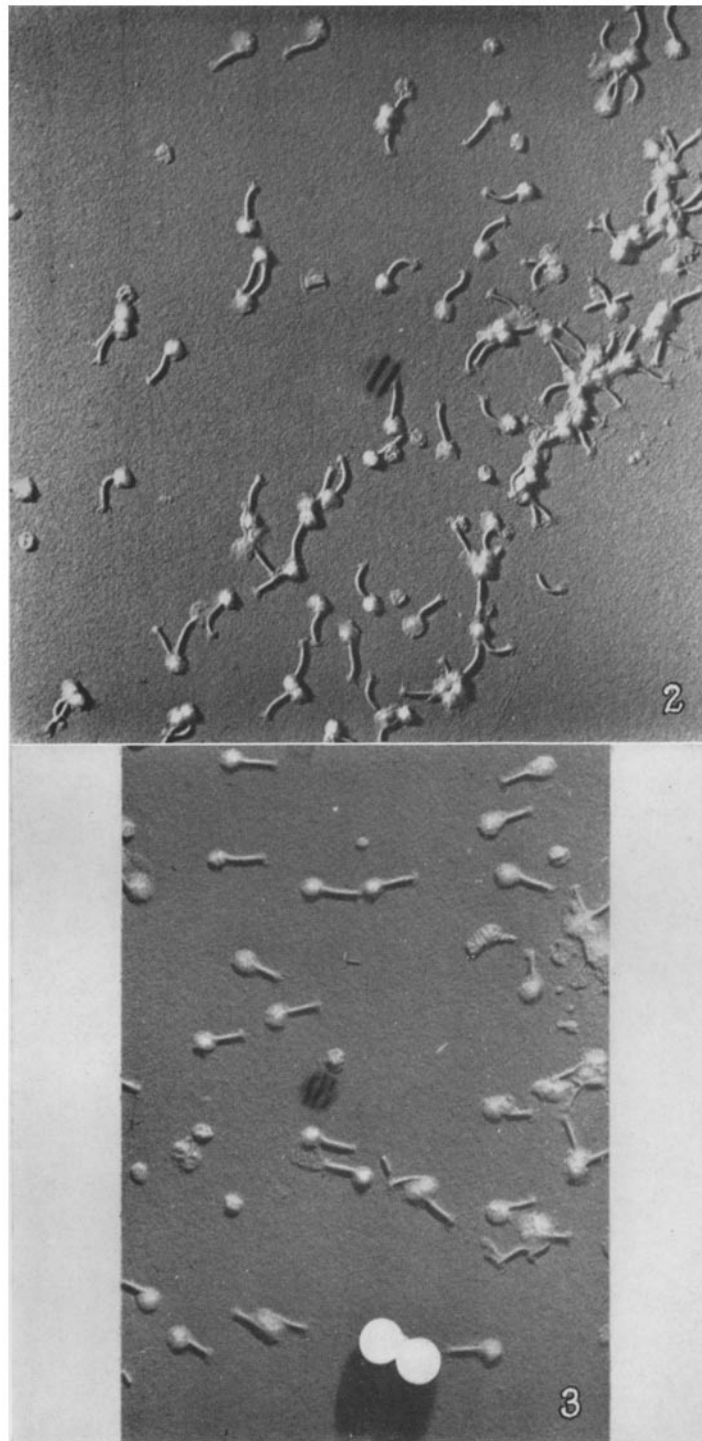Fig. 2. Electron photomicrograph preparation of phage 14(K₁). Approximate magnification, 23,000.

Fig. 3. Electron photomicrograph preparation of phage 14(145). Approximate magnification, 23,000.

(Ralston and Krueger: Staphylococcal phage variant)