Recoverable Potassium Fluxes Variations following Adsorption of T₄ Phage and their Ghosts on Escherichia coli B

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SUMMARY

Adsorption of T₄ phages or their ghosts to Escherichia coli B resulted in the following events. (a) Addition of either phage or ghost caused rapid enhancement of potassium efflux and a corresponding decrease in the intracellular concentration of potassium. (b) Within the first 2 min after infection, the intracellular concentration of potassium started to recover in phage, but not in ghost-infected cells. The recovery, which corresponded presumably to some repair process, was completed 4 to 5 min after infections. (c) The K⁺ influx was enhanced slightly, and reduced 10-fold, in the phage and in the ghost-infected cells, respectively. The repair process in the phage-infected cells was inhibited by pre-treatment of the bacteria with chloramphenicol, or by lowering the temperature from 37° to 20°C. Formalinized phages affect the bacteria in the same manner as the ghosts. Damage which resulted from ghost adsorption, and was characterized by K⁺ transport and infective centres experiments, could be repaired to some degree by infective phage adsorption. The effects due to ghost adsorption could be partially reduced by resuspending the treated bacteria in a medium which contained a concentrate of the non-dialysable metabolites that leaked out of the ghost-treated cells.

INTRODUCTION

T₄ bacteriophages and their ghosts inhibit synthesis of macromolecules and induce various changes in the membrane properties of their host bacteria (French & Siminovitch, 1955; Herriott & Barlow, 1957a, b; Silver, Levine & Spielman, 1968; Duckworth, 1970a, b). The damage to the bacteria caused by T₄ phage differed from that induced by the phage ghost.

Duckworth stated that the phage and ghost induce different initial damage (Winkler & Duckworth, 1971; Duckworth & Winkler, 1972). However, others (Tolmach, 1957; Luria & Darnell, 1967; Duckworth, 1970a) suggested that the initial stage of the damage was similar in both cases, but that later the infective phage activated repair mechanisms in the host, while the ghost did not.

In order to trace the time sequence which would correlate phage and ghost damage in Escherichia coli, in this work we studied the potassium fluxes, during phage and ghost adsorption.

Leakage of metabolites from cells treated with phage or ghost has already been described (Puck & Lee, 1954, 1955; Sechaud, Kellenberger & Streisinger, 1967; Duckworth, 1970a).

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The leakage induced by ghosts was more extensive than that induced by phage. Most of the leakage was observed in the first 3 min after infection. More refined results were obtained by Silver et al. (1968) who studied K⁺ fluxes during infection by either phage or u.v. inactivated phage.

In the present work, a different experimental arrangement enabled us to analyse the transitional state during infection. Rapid recovery of the damage due to phage adsorption was observed. No recovery was detected during ghost infection.

In order to localize the damage in bacteria we assayed cells by conditions under which the leaked metabolites and ions would re-enter the bacteria.

METHODS

Organisms and media. Escherichia coli, strain B was grown at 37 °C in two types of media:

(1) M9 with low potassium: 8.5 mM-NaCl; 2 mM-KCl; 14.4 mM-NH₄Cl; 1 mM-MgSO₄; 10⁻² mM-FeCl₃; 0.4 % glucose; 0.25 % casamino acids; 70 mM-sodium phosphate buffer at pH 7.4.

(2) Tryptone broth: Bacto tryptone 2 %; 170 mM-NaCl.

Logarithmic phase Escherichia coli B, about 5 × 10⁸ cells/ml were infected with T₄ phage or its ghosts after the addition of 100 µg/ml tryptophane to facilitate adsorption.

Bacteriophage. High titre 1 to 2 × 10¹³ purified stocks of T₄ phage were prepared by growing the phage in Escherichia coli B in tryptone broth (m.o.i. was about 2). Purification and concentration of the phage was achieved by differential sedimentation and incubation with enzymes (DNase 100 µg/ml, RNAse 50 µg/ml).

Phage titration, as well as other techniques, were performed according to Adams (1959). Phage ghosts were prepared by a modified method of Duckworth (1970b).

K⁺ influx and intracellular concentration. 0.5 to 1 min after infection with phage or ghost (m.o.i. 3 to 6) 50 µl of 42K was added to each 1 ml of Escherichia coli (6 to 8 × 10⁸ cells/ml) suspension.

Influx of K⁺ was determined according to Giberman & Rosenberg (1970).

42K (aqueous solution of 10 mM-KCl; 500 µCi/ml, 140 mM-NaCl at pH 7.2) was obtained from the Atomic Energy Commission, Israel.

The uptake of the radioisotope by cells was obtained from the measurements of the radioactivity of the bacterial pellets at different time intervals. The cells were separated from the growth medium by differential flotation (Ballentine & Burford, 1960; Giberman & Rosenberg, 1970). Separation was carried out in polyethylene tubes of about 0.4 ml. Each tube contained 0.05 ml of a separating fluid (the density of which was 1.024 g/ml) to which 0.1 ml of bacterial suspension was added. The tubes were centrifuged for 2.5 min at 10⁴ g in a Beckmann Microfuge centrifuge. The radioactivity was measured in a Packard Gamma Scintillation Counter.

In other experiments, Escherichia coli cells were loaded with 42K until isotopic equilibrium was obtained. Phages or ghosts were then added and the potassium content of the cells was determined at different time intervals, following the infection.

Infective centre formation was measured by incubating a bacterial suspension, treated with phages or ghosts. In various experiments, phages and ghosts were added either simultaneously, or separately, or one preceding the other by varying intervals of time. After 8 min of incubation, bacteria were diluted 1:10 in phosphate buffer containing antiserum against T₄ phages (this reduced the unadsorbed phages or ghosts by 6 log units). After further dilution, the suspension was plated with excess host bacteria, and infective centres were recorded as plaques appearing on the plates after 18 h of incubation.
Preparation of antisera. Antibodies to T₄ phage were prepared in rabbits injected subcutaneously with 1 ml each of 1.5 × 10¹² phage particles twice a week for 3 weeks. Following an intermission of 3 weeks, a booster injection with the same phage suspension was given, and after an additional 2 weeks the serum was collected. The neutralization index (K) (Adams, 1959) of the serum was 420.

Preparation of formalin-treated phage. A suspension of 2 × 10¹⁰ phage/ml was diluted 1:10 in 0.05 % (v/v) of formalin (35 % HCOH) in phosphate buffer and incubated for 1 h at 37 °C. The suspension was then dialysed overnight at 4 °C against phosphate buffer to free it from formaldehyde.

Reconstitution experiment. Escherichia coli was grown in tryptone media to a density of 5 × 10⁹/ml, centrifuged and concentrated to about 5 × 10⁹ cells/ml in the same media. Ghosts were added to the bacteria at m.o.i. of about 5, incubated for 10 min at 10 °C (in order to complete adsorption but to prevent leakage). The bacteria were then centrifuged at 10000 g for 10 min, the pellet resuspended in water (in order to prevent high levels of salts and other media constituents in the subsequent freeze-drying) and incubated for 15 min at 37 °C. The supernatant fluid was collected and freeze-dried. The powder was resuspended in water at 1/500 of original vol. and the material was once more centrifuged at 50000 g. The resulting supernatant solution was the leaked metabolites (LM). A similar medium was prepared from bacteria which were not treated with ghosts. For reconstitution experiments Escherichia coli B was grown to a density of 5 × 10⁸, centrifuged, concentrated twofold in the same media. Following the addition of LM, infective centre formation, killing of bacteria and β-galactosidase activity were determined.

β-galactosidase synthesis. The synthesis of β-galactosidase (EC 3.2.1.23) was estimated from the rate of hydrolysis of o-nitrophenyl-β-D-galactoside (ONPG) after induction in bacteria infected with phage or with ghost, as well as induction with isopropyl-thio-β-D-galactoside (IPTG). The assay was done as described by Duckworth & Bessman (1965).

Sonic treatment of phage. 2 × 10¹¹ T₄ phage/ml was sonicated for 30 s, 2 to 12 Hz in MSE Ultrasonic Disintegrator (Measuring and Scientific Equipment, London). The survival of the phage was 1 % and 0.01 % after 5 and 10 Hz, respectively.

RESULTS

Influx and efflux variation of potassium in phage and ghost treated bacteria

Following infection by ghosts, the influx of [⁴²K] in Escherichia coli decreased 4- and 10-fold, at 37 °C and 20 °C, respectively (Fig. 1, 2). Within 2 min after infection by ghosts 90 % of the intracellular potassium leaked out, at both 37 and 20 °C (Fig. 3, 4). Both influx and intracellular concentration of potassium decreased in ghost-infected cells. The rate of potassium leakage was considerably higher than that of influx in infected cells. It follows that the efflux of potassium has increased, by an order of magnitude, during infection.

While infection of Escherichia coli by phages at 37 °C (Fig. 1) enhanced K-influx by 30 to 50 %, no enhancement of K⁺ influx was observed at 20 °C (Fig. 2).

Within the first min after phage infection, the observed decrease in the content of potassium at 37 and 20 °C (Fig. 3, 4) was similar to that described previously for ghost infection. One min later, however, a rapid recovery of the bacterial content of potassium occurred at 37 °C (Fig. 3). At 20 °C the recovery was considerably slower (Fig. 4).

Isotopic equilibration had been reached, in the cells, at about 30 min after the addition of ⁴²K. The continuing rise in the kinetics of the ⁴²K uptake function, following that equilibration, was due to the growth of the culture. When the ⁴²K uptake function is corrected
Fig. 1. Kinetics of $^{42}$K uptake by phage- and ghost-infected *Escherichia coli* B at 37 °C. One min before the addition of $^{42}$K (zero time) phage or ghost were added at m.o.i. of 5. ○—○, non-infected controls; ▲—▲, bacteria infected by phage; ●—●, bacteria infected by ghosts; ■—■, bacteria infected by phage and ghost: 1.5 min and 0.5 min before the addition of $^{42}$K, respectively (each at m.o.i. of 5).

Fig. 2. Kinetics of $^{42}$K uptake by phage- and ghost-infected *Escherichia coli* B at 20 °C. ○—○, uninfected control; ▲—▲, bacteria infected with phage; ●—●, bacteria infected with ghost; □—□, bacteria infected with phage 3 min after addition of 100 µg of CAM; ■—■, bacteria infected with formalinized phage. CAM (100 µg) or formalin ($5 \times 10^{-4}$ % v/v) did not affect the influx in uninfected *E. coli*. m.o.i. was 4.5 in each case.
for that growth, the expected plateau is reached. The times in the figures correspond to the beginning of sedimentation after the addition of $^{42}$K to the suspensions. Within 1 min of sedimentation, about 80% of the cells reached the pellet. The uncertainty in the time scale corresponding to the successive separations of cells by sedimentation was about 1 min.

**Potassium fluxes-cumulative effects on bacteria treated by ghosts and phages**

In the previous paragraph we demonstrated that the initial leak produced by infective phage is rapidly repaired, while in ghost-treated cells it is not. One may thus assume that the infecting phage provides the infected cells with a function which the ghosts lack. Consequently one may expect that simultaneous infection by phage and ghost should lead to at least partial repair of the initial damage to the bacterial wall or membrane, resulting from ghost infection. The experiment depicted in Fig. 5 indicates indeed that after addition of a mixture of bacteriophages and ghosts to bacteria loaded with $^{42}$K, there was an expected increase in efflux of potassium, but after about 3 min of adsorption, a slow repair process began, both in respect to efflux and influx values (Fig. 1, 5).

One could also expect that treatment of bacteria with phage before adding the ghosts, at a time when the repair process instituted by the infecting phage is well under way, should offset the damage due to the ghosts. This was indeed the case (Fig. 5). When the order was reversed, i.e. bacteria were first treated with ghost, and 1.5 min later with phage, the repair obtained was found to be smaller than that obtained for ghost–phage mixture and larger than ghosts alone (Fig. 5).
Effect of chloramphenicol on potassium fluxes in phage-infected bacteria

In order to assess the role of protein synthesis during cell repair, bacteria were treated with 100 μg/ml of chloramphenicol (CAM) 1 to 3 min before adsorption of phage, in order to block the protein synthesis.

Using 42K-loaded cells at 37 °C, treated with CAM, we found that (a) the initial damage by phage was similar to that produced in untreated bacteria and (b) that the repair is partially inhibited (Fig. 3). When a similar experiment was performed at 20 °C, the CAM effect was more pronounced than at 37 °C (Fig. 4). There was twofold decrease in potassium influx at 20 °C in bacteria infected by phage with CAM (Fig. 2). Inhibition of growth by CAM did not affect the influx of K+ ions, when phages were absent. On the other hand, following adsorption of phages, the repair was found to be coupled in some way to a process which was essential for growth.

Effect of formalinized phage on potassium fluxes

Phages treated with formalin, like ghosts, adsorbed to bacteria but could not inject their DNA into the cell. The DNA in formalinized phages remain intact and is still endowed with various biological activities (Hershey & Chase, 1952, table 1). In order to assess the importance of phage DNA in the repair process, we examined the effect of 'formalinized' phages in K+ fluxes assay.

Fig. 4. Kinetics of 42K at uptake by phage- and ghost-infected Escherichia coli at 20 °C. O—O, uninfected control; ▲—▲, infected with phage; •—•, infected with ghost; △—△, infected with formalinized phage; □—□, infected with phage 3 min after addition of 100 μg CAM. m.o.i. was 5.


**K⁺ flux in E. coli infected by phage and ghost**

Fig. 5. Kinetics of K⁺ uptake by mixed phage-ghost-infected *Escherichia coli* B at 37 °C. O—O, uninfected control; △—△, infected with phage following 1.5 min with ghost; ■—■, infected with ghost following 1.5 min with phage; □—□, infected simultaneously with phage and ghost; ●—●, infected with ghost alone. Phage and ghost suspension were added at m.o.i. 4.5.

**Table 1. Effects of formalinized phage on Escherichia coli B**

<table>
<thead>
<tr>
<th>Formalinized phage inactivated by formalin (v/v %)</th>
<th>0.1</th>
<th>0.5</th>
<th>Ghost</th>
<th>Phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active phage [%]</td>
<td>5 × 10⁻³ ± 10⁻³</td>
<td>2.4 × 10⁻⁴ ± 10⁻⁴</td>
<td>0.5 ± 0.12</td>
<td>100 ± 25</td>
</tr>
<tr>
<td>Colony forming bacteria after infection [%]</td>
<td>4 ± 1</td>
<td>17 ± 4</td>
<td>5 ± 1.2</td>
<td>0.6 ± 0.15</td>
</tr>
<tr>
<td>Inhibition of induction of β-galactosidase [%]</td>
<td>1.3 ± 0.3</td>
<td>4 ± 1</td>
<td>1 ± 0.25</td>
<td>0.1 ± 0.02</td>
</tr>
</tbody>
</table>

* Multiplicity of infection (m.o.i.) = 5.

The results shown in Fig. 2 and 4 indicate that formalinized phages affected influx and efflux of potassium in the same manner as the ghosts. Hence, phage with denatured protein and intact, non-injectable DNA was as effective in making the bacterial cell wall leaky as the ghosts. Secondly, it seemed clear that the entry of phage DNA into the bacterial cell was essential for initiating the repair caused by adsorption of phage.
Effect of sonicated phage on $K^+$ fluxes

The finding that the mere adsorption of phage or phage coat was sufficient for the damage led us to an experiment in which we tried to determine the role of coat subunits in this process. The phage suspension was, therefore, sonicated, so as to decrease its viability to about 0.1%, and the sonicated suspension was assayed on bacteria in all systems tested: $K^+$ fluxes, killing, inhibition of induction of $\beta$-galactosidase. The sonicated suspension did not affect any of these, as was found by Setlow (1957) for X-ray irradiated phage, and by Duckworth (1970a) for fractionated ghosts.

Determination of ghost-induced damage by infective centre technique

We have shown that phage could repair (at least partially) the damage to $K^+$ transport in *Escherichia coli* caused by ghosts. The extent of the repair depended on the relative time when phage and ghost were added. Another experiment that can be used to demonstrate such repair effects involved the measurement of infective centres. Bacteria to which phage and ghost were added simultaneously at 37°C were capable of producing phage (infective centres) to the extent of 12%. When the phage, however, was added 4 min after the ghost only 0.9% of the bacteria produced infective centres (Fig. 6). These results indicate that the damage caused by the ghosts was not fully expressed when phage was simultaneously present at the time of infection. When the ghosts were added 6 min after phage 90% of the bacteria were capable of producing phages.

Assuming that the difference in the production of infective centres stems from repair mechanism initiated by the infective phage, it was predicted that at 20°C the repair would be less efficient. Indeed, bacteria treated with a mixture of phage and ghost at 20°C produced only 60% of infective centres (Fig. 6).

Since it was found that also chloramphenicol prevented the repair mechanism, as determined by the change in $K$ fluxes, we also measured the effect of chloramphenicol on production of infective centres. Arrest of protein synthesis in bacteria treated for 3 min with chloramphenicol caused a drop of infective centres in bacteria treated with a mixture of phage and phage ghosts. In cases where ghosts were added to CAM-treated bacteria after the infective phage, there was no repair of capacity to make phage (Fig. 6).

Reconstitution experiments

Following the adsorption of phage or ghost, considerable leakage of various metabolites and ions occurred (Puck & Lee, 1954, 1955; Silver *et al.* 1968; Duckworth, 1970b; Fig. 1, 4).

The effect of restoring the metabolites to phage- and ghost-infected bacteria was tested in two systems: infective centre production and killing efficiency of bacteria by ghosts. When bacteria were treated simultaneously with ghost + phage in normal medium, 10% ± 2.5 of the cells still produced infective centres. When the same experiment was performed in the presence of leaked metabolites (LM), a threefold increase (30% ± 7.5; Fig. 7) was recorded. In the presence of LM, addition of phage, 4 min after the ghosts, resulted in an infective centre count which was 12 times higher than in similar controls in the absence of LM. We have also tested in a similar experiment the survival of bacteria subject to attack by ghosts at a m.o.i. of 5. The survival (colony-forming ability) of bacteria treated with ghosts drops to 3.5% ± 1. However, when the bacteria were incubated in the presence of LM, the surviving fraction increased to 18% ± 5. It is important to note that the survival
*K* + flux in *E. coli* infected by phage and ghost

Fig. 6. Effect of ghost on infective centre formation. *Escherichia coli* B was grown in tryptone broth to $5 \times 10^8$ cells/ml, at zero time phage and ghost were simultaneously added. The right part of the figure (to be read from left to right) shows the change in number of infective centres when phage added at zero time was followed by ghosts at various time intervals. At the left part (to be read from right to left) the ghosts were added at zero time followed by phage at various time intervals. The total incubation period was 8 min. Subsequently samples were diluted into anti-serum (Methods). The number of infective centres which produced phage was taken as 100 %, ○—○, cells infected at 37 °C; △—△, cells infected at 20 °C; ○—○, cells infected at 4 °C; ■—■, cells infected at 37 °C 3 min after addition of CAM (100 μg). m.o.i. was 5 2 in each case.

of bacteria treated with infective phage was the same in presence or in absence of LM 0.3 % (Table 2).

Two kinds of control experiments demonstrate that the LM effect is due to specific phenomena. (a) The LM effect could result from competition of receptors on the cells and in the LM for the ghosts. This was not the case since the number of infective centres obtained
Fig. 7. Effects of leaked metabolites (LM) and ghosts on infective centres formation. *Escherichia coli* was grown in tryptone broth to $5 \times 10^8$ cells/ml and concentrated twofold. To every tube equal amounts of one of the following reagents were added: (a) leaked metabolites (LM); (b) dialysed (DLM) or (c) the same concentrated medium from lyophilized untreated bacteria. The m.o.i. of phage or ghost was 52.

Survival of bacteria following infection with phage alone was 0.6%, whereas after addition of ghost in the case of the control it was 3% and 10 and 9% in bacteria supplemented with LM, DLM, respectively. For additional details see Fig. 6.

in bacteria treated with phage alone at high and low multiplicities was the same in the presence or absence of LM (Table 2). (b) The LM effect was not an artificial one since a parallel control without ghosts had no effect on bacteria (Fig. 7).

Dialysed LM gave the same recovery, as described in Fig. 7.
Table 2. Effects of leaked metabolites (LM) on biological activities of phage and ghost

<table>
<thead>
<tr>
<th></th>
<th>Ghost</th>
<th>Phage</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LM</td>
<td>DLM*</td>
</tr>
<tr>
<td>Colony formation after</td>
<td>18±0.45</td>
<td>16±0.4</td>
</tr>
<tr>
<td>infection by the agent</td>
<td>(m.o.i. = 5) (%)</td>
<td></td>
</tr>
<tr>
<td>Infective centres</td>
<td>0.69±0.11†</td>
<td>0.4±0.1†</td>
</tr>
<tr>
<td>(m.o.i. = 5) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infective centres</td>
<td>0.25±0.08†</td>
<td>0.2±0.05†</td>
</tr>
<tr>
<td>(m.o.i. = 1) (%)</td>
<td></td>
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</tbody>
</table>

* DLM = Dialysed leaked metabolites.
† These numbers correspond to the active phages which remain in the ghost preparation.

DISCUSSION

Leakage of metabolites labelled by 32P and 35S from phage-infected cells has been described by Puck & Lee (1954, 1955). This leakage occurred during the first 3 to 5 min after infection. From this and other indirect evidence they concluded that the recession of leakage was due to a 'sealing' effect, though they did not have any direct proof for this. The cessation of leakage was also interpreted as the depletion of the pool (Duckworth, 1970a).

In this work we found direct evidence for the existence of a repair mechanism induced by an infective phage (Fig. 3). We have shown that adsorption of infective phage to bacteria caused a rapid leakage of potassium within the first min. This leak terminated, and the potassium content of the cells was restored to the original level, within the next 3 min.

Silver et al. (1968) also demonstrated leakage of 42K, but did not demonstrate the repair directly. They concluded that following infection the influx of potassium was the same as in the control, uninfected bacteria. In our influx experiments we have observed that the initial rate of influx with phage-treated bacteria was higher than with the controls.

This finding supports our hypothesis as to the nature of the initial step of phage infection affecting potassium efflux: the cells that are depleted of K⁺ within the first 30 s take up K⁺ at a higher rate than uninfected bacteria.

A transient inhibition in the transport properties of bacterial membranes (Salmonella) has also been described by Koteswara Rao, Chakravorty-Burma & Burma (1972).

Silver et al. (1968) demonstrated that Mg²⁺ (25 mM) reduced the leak of K⁺ ions, in the presence of phages. The infection was not affected by that Mg²⁺ concentration. We plan to investigate in the future the effects of Mg²⁺ ions and various metabolic reagents on the K⁺ fluxes, following infection of bacteria with ghosts and u.v. irradiated phages.

In the present work addition of chloramphenicol to bacteria inhibited seriously the repair process. This finding supports the hypothesis that the repair mechanism requires protein synthesis. The repair mechanism and the protein synthesis occurred in the presence of phage DNA inside the infected bacterium, and is missing in ghost-treated or formalinized phage-treated bacteria.

Chloramphenicol did not completely prevent the repair mechanism. This may be due to existence of two repair mechanisms only one of which is CAM sensitive, as postulated by Nomura et al. (1966) and Swift & Wiberg (1971, 1973) in relation to inhibitory effect of phages and ghosts on the action of colicins.

The K⁺ efflux experiments, performed at 37 and 20 °C, support the hypothesis of repair
mechanisms. At 20 °C the rate of protein synthesis in bacteria is drastically reduced, and indeed the repair of the damage is much slower than in 37 °C (Fig. 2, 4). At 37 °C the repair mechanism becomes visible within the first min of phage infection and almost completed 4 to 5 min later. This time sequence corresponds to the time during which early phage proteins are synthesized. In that respect the phage-induced repair is similar to the superinfection exclusion mechanism. Both occur very rapidly and in both there is a drastic change in the properties of the bacterial membrane (Dulbecco, 1952; Anderson & Eigner, 1971). It is thus possible that the change induced by phage infection which manifests itself as a superinfection exclusion is identical with the change we call repair, and is induced by a protein specified by the phage genome. There have been recently two types of mutant of T₄ imm⁻ which have reduced ability to 'immunize' infected bacteria (Childs, 1972; Mufti, 1972; Vallee & Cornet, 1972; Vallee, Cornet & Bernstein, 1972). Such mutants could therefore be used to determine whether the repair mechanism and superinfection exclusion in phage-infected bacteria are due to the same phage-induced protein.

Adsorption of ghosts or formalinized phage to bacterial cells causes two effects: (a) it induces a rapid leakage of K⁺ in the membrane (this leak is the same at 37 and 20 °C); (b) inhibition of the K⁺ active influx. The fact that formalinized phage acted like ghosts (Fig. 2, 4), suggested the importance of the DNA of the phage in the repair mechanism of the membrane.

The data presented in this work point to a common mechanism in the nature of damage to the bacterial cell immediately after adsorption. This hypothesis is supported also by the data of Duckworth (1970b), Puck & Lee (1954, 1955), and Silver et al. (1968) indicating that the initial damage is the same but the 'sealing' mechanism is different. The experimental evidence for the hypothesis of the common nature of damage by ghosts and phages is based on the same rate of efflux of potassium from cells during the first min. When phages and ghosts are added together the same initial damage occurs and then the repair process takes place. The repair occurring during simultaneous infection of bacteria with phage and ghosts is also demonstrable by measuring the capacity for the infective centres. Thus 10% of bacteria treated simultaneously with phage and ghost would produce infective centres, but only 1% of them would do so when phage is added 4 min after ghosts. Our data are supported by the results of Vallee et al. (1972) but are contradicted by that of Duckworth & Winkler (1972).

In order to prove that the main damage of ghost-infected cells is due to the leakage of metabolites, we have increased, during infection, the extracellular concentration of the leaked metabolites (LM) to a level which approached their intracellular concentration. We found that in this case the capacity of the bacteria to form phages and to divide and form colonies had increased. Restoring the original intracellular concentration of potassium by using high-level extracellular K⁺ did not result in a significant recovery of the damaged cells.

REFERENCES

K+ flux in E. coli infected by phage and ghost


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