

## Transport in Bacteriophage P22-Infected *Salmonella typhimurium*

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There was rapid efflux of L-leucine, L-phenylalanine, and  $\alpha$ -methyl-D-glucoside after infection of *Salmonella typhimurium* with the clear plaque mutant C<sub>1</sub> of phage P22. The efflux was similar to that observed with cyanide or arsenate treatment except that there was partial recovery in the case of phage infection and almost complete recovery under the condition of lysogeny. There was no efflux after infection with the temperature-sensitive mutant ts16C<sub>1</sub> at nonpermissive temperature. Superinfection of superinfection exclusion-negative lysogen (*sie* A<sup>-</sup> *sie* B<sup>-</sup>) with C<sub>1</sub> led to efflux, whereas the efflux was much less on superinfection of *sie* A<sup>+</sup> *sie* B<sup>+</sup> lysogen. These results indicate that an effective injection process is enough to cause depression in the cellular transport processes.

The transient depression in macromolecular synthesis in *Salmonella typhimurium* after infection with bacteriophage P22 (3) was found to be due to temporary inhibition of the transport of precursors of macromolecules across the membrane of the host (15). The rate of macromolecular synthesis drops down temporarily due to the need for precursor molecules, which have to be transported in from the medium.

It is well known that there is usually efflux of solute molecules from the pool of host bacterium immediately after infection (2, 13, 14, 17, 21). This is thought to be due to leakage as a result of physical damage to the membrane of the host which is, however, repaired subsequently, allowing no further leakage of materials during phage multiplication. The "sealing" is most probably a host-gene-mediated process (17, 21). The question may be raised as to whether the inhibition of transport in *S. typhimurium* after P22 infection represents mere "leakage" of materials from the pool and the recovery of transport indicates the sealing. Therefore transport across the membrane of noninfected and infected *S. typhimurium* was studied. It is well known that there are numerous transport systems responsible for the active transport of various solutes, the energy requirements of which also vary widely (8, 12). In the present studies an analogue of glucose,  $\alpha$ -methyl-D-glucoside, and two amino acids, L-phenylalanine and L-leucine, were chosen.

### MATERIALS AND METHODS

**Chemicals.** L-[<sup>14</sup>C]leucine (66 Ci/mol), L-[<sup>14</sup>C]phenylalanine (99 Ci/mol), and  $\alpha$ -methyl-D-[<sup>14</sup>C]glucoside (4.5 Ci/mol) were obtained from the Bhabha Atomic Research Centre, Trombay, India. All other chemicals were commercial preparations of high purity. Nitrocellulose membrane filters (0.45  $\mu$ m) were obtained from Matheson-Higgins Co. and Schleicher and Schuell Co.

**Bacterial and phage strains.** *S. typhimurium* LT2 (strain 18) and its two lysogens, superinfection exclusion positive (*sie* A<sup>+</sup> *sie* B<sup>+</sup>) and superinfection exclusion negative (*sie* A<sup>-</sup> *sie* B<sup>-</sup>), were obtained from M. Levine (University of Michigan, Ann Arbor, Mich.). The superinfecting phage does not multiply, in either of the two types of lysogen (*sie* A<sup>+</sup> *sie* B<sup>+</sup> and *sie* A<sup>-</sup> *sie* B<sup>-</sup>). But when the prophage is induced to multiply, the superinfecting phage also multiplies in *sie* A<sup>-</sup> *sie* B<sup>-</sup> lysogen, whereas in *sie* A<sup>+</sup> *sie* B<sup>+</sup> lysogen only the prophage multiplies and the superinfecting phage is excluded.

Wild-type phage P22 (C<sup>+</sup>), its clear plaque-forming mutant C<sub>1</sub>, and temperature-sensitive mutant ts16 (previously known as ts25) were also kindly supplied by M. Levine. Wild-type phage (C<sup>+</sup>) lysogenizes the host. Percentage of lysogenization depends on the multiplicity of infection. Mutation in the C region results in failure to lysogenize the host and leads to phage development and subsequent lysis of the host. Mutant ts16 fails to carry out any phage function at nonpermissive temperatures, although it attaches to the host and releases its DNA, at least in part. Gene 16 protein (p16) is a virion protein which acts early but is synthesized quite late during the development of the phage (M. Levine, personal communication).

**Mineral base medium.** The composition of mineral base medium is as follows: KH<sub>2</sub>PO<sub>4</sub>, 3 g; K<sub>2</sub>HPO<sub>4</sub>, 7 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g per liter of water (7).

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**Basal medium.** The composition of basal medium is as follows:  $\text{KH}_2\text{PO}_4$ , 3 g;  $\text{K}_2\text{HPO}_4$ , 7 g;  $(\text{NH}_4)_2\text{SO}_4$ , 1 g; trisodium citrate  $\cdot 3\text{H}_2\text{O}$ , 0.5 g;  $\text{MgSO}_4$ , 0.1 g per liter of water (5).

**Measurement of transport of  $\alpha$ -methyl- $^{14}\text{C}$ glucoside and L- $^{14}\text{C}$ phenylalanine (or L- $^{14}\text{C}$ leucine).**  $\alpha$ -Methyl- $^{14}\text{C}$ glucoside transport was studied by the method of Hoffee et al. (7), and the method of Ames (1) was followed for measuring the transport of amino acids. Both the methods were slightly modified. *S. typhimurium* was grown in basal medium (5) at 37 C up to the cell density of  $2.5 \times 10^8$ /ml in the presence of 0.2% glycerol as carbon source. The cells were harvested, washed once with mineral base medium (7), and again suspended up to original density in the same medium without any energy source. For measurement of transport of  $\alpha$ -methyl- $^{14}\text{C}$ glucoside the cells were aerated at 37 C for 7 min and then treated with chloramphenicol (final concentration, 50  $\mu\text{g}/\text{ml}$ ) for 3 min.  $\alpha$ -Methyl- $^{14}\text{C}$ glucoside (0.1  $\mu\text{mol}$ ,  $5 \times 10^4$  counts/min) was added per ml of chloramphenicol-treated cell suspension. At desired times, 1-ml samples were filtered through nitrocellulose membrane filters (0.45  $\mu\text{m}$ ) followed by immediate washing of each with 1 ml of ice-cold mineral base medium. The whole process took about 4 to 5 s. The filters were fixed on the planchets, dried, and counted in a windowless gas flow counter (Bhabha Atomic Research Centre, Trombay, Bombay). The counts were corrected for a zero minute incubation blank, which was treated in the same way.

For the measurement of amino acid transport, the cells were harvested and washed as described above and then starved for carbon and nitrogen at 25 C for 90 min. Subsequently they were equilibrated at 37 C for 10 min and treated with chloramphenicol (final concentration, 50  $\mu\text{g}/\text{ml}$ ) for 10 min. L- $^{14}\text{C}$ leucine (10 nmol,  $3 \times 10^4$  counts/min) or L- $^{14}\text{C}$ phenylalanine (12 nmol,  $3 \times 10^4$  counts/min) was added per milliliter of cell suspension. Samples (1 ml) were filtered at desired times through membrane filters followed by immediate washing of each with 5 ml of mineral base medium equilibrated at the temperature at which the experiment was carried out. The whole process took about 5 to 6 s. Under the above conditions, a negligible amount of radioactivity was found to be present in the trichloroacetic acid-insoluble fraction. As in the earlier case, the counts were corrected for zero minute blank reaction.

## RESULTS

**Kinetics of transport of  $\alpha$ -methyl-D- $^{14}\text{C}$ glucoside, L- $^{14}\text{C}$ leucine, and L- $^{14}\text{C}$ phenylalanine into noninfected and  $\text{C}_1$ -infected *S. typhimurium*.** The clear plaque-forming  $\text{C}_1$  mutant of phage P22 was used for infection of *S. typhimurium*. Kinetics of transport of the different substrates seem to be different even in the noninfected host. For example, the uptake of  $\alpha$ -methylglucoside attains equilibrium in about 10 min (Fig. 1A),

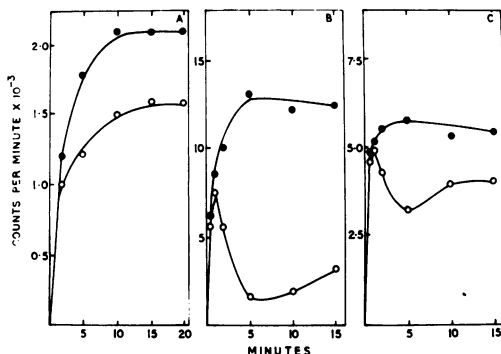


FIG. 1. Kinetics of transport of (A)  $\alpha$ -methyl- $^{14}\text{C}$ glucoside, (B) L- $^{14}\text{C}$ leucine, and (C) L- $^{14}\text{C}$ phenylalanine into noninfected and  $\text{C}_1$ -infected cells of *S. typhimurium*. The cells were treated as described and divided into two batches; one batch was infected at 0 min with  $\text{C}_1$  at a multiplicity of infection of 10, whereas the other batch was kept as a noninfected control. Labeled compounds were added at zero time. In the case of infected cells, the phage was added along with the labeled compound. Subsequent steps were as described. Symbols:  $\bullet$ , noninfected;  $\circ$ , infected.

whereas in the case of the two amino acids the time required to obtain maximum uptake is about 5 min (Fig. 1B and C).  $\text{C}_1$  infection leads to different effects on the transport of  $\alpha$ -methylglucoside and amino acids. In phage-infected *S. typhimurium* the rate of net influx (which is a balance between influx and efflux) of  $\alpha$ -methylglucoside starts to slow down within about 2 to 3 min after infection, and the maximum uptake (attained within about 10 min, as in the case of noninfected cells) is significantly lower than that in the noninfected cells. An entirely different situation is, however, observed in the case of the transport of the amino acids. About 2 to 3 min after the addition of leucine or phenylalanine to the infected cells, there seems to be a net efflux of the amino acids already transported in. In the case of leucine, the rate of net efflux seems to be very fast, and there is a tendency toward recovery after 5 min. With phenylalanine, however, although a similar picture is obtained, the rate of net efflux is somewhat slower than that of leucine, and a significant amount of the transported material remains within the cell. In this case as well there is a tendency toward the recovery of transport. These results clearly indicate that transport of all the three substrates used in this study is affected after phage infection which therefore appears to be a general phenomenon.

**Comparison of the effects of  $\text{C}_1$  infection and cyanide and arsenate treatments on**

**transport.** In these experiments transport was allowed to take place in the noninfected cells, and the phage was added after the attainment of equilibrium. For comparative purposes, cyanide and arsenate treatments were also carried out. Cyanide is known to be a powerful inhibitor of respiration and an agent widely used to prevent the multiplication of phage after infection. It has also been shown to cause efflux of  $^{42}\text{K}$  (9, 17). A concentration of  $2 \times 10^{-3}$  M was chosen for cyanide treatment. Klein and Boyer (10) have shown that arsenate, being an inhibitor of oxidative phosphorylation, does not allow protein synthesis in *Escherichia coli*. This has also been tested in *S. typhimurium*, and it has been found that a concentration of  $10^{-2}$  M completely blocks protein synthesis as well as RNA synthesis.

$C_1$  infection leads to immediate efflux of part (somewhat less than 50%) of transported  $\alpha$ -methyl- $^{14}\text{C}$ glucoside (Fig. 2A). It should, however, be noted that there is a tendency toward

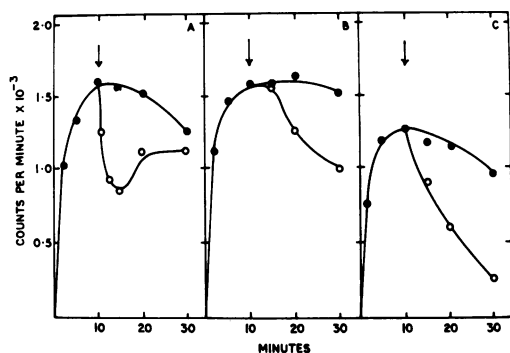


FIG. 2. Comparison of the effects of (A)  $C_1$  infection and treatments with (B) cyanide or (C) arsenate on the transport of  $\alpha$ -methyl- $^{14}\text{C}$ glucoside into cells of *S. typhimurium*. (A) Experimental procedure was the same as described in the legend to Fig. 1 except that noninfected cells were allowed to incorporate  $\alpha$ -methyl- $^{14}\text{C}$ glucoside for 10 min. At 10 min the cells were divided into two batches, and to one batch  $C_1$  was added immediately at multiplicity of infection of 10, whereas the other batch was kept as a noninfected control. At indicated times 1 ml of cell suspension was passed through a membrane filter, and the remaining procedure was the same as in the legend to Fig. 1. (B) The experimental procedure is the same as described for (A) except that cyanide ( $2 \times 10^{-3}$  M) was substituted for  $C_1$ . (C) The cells were grown and washed once with 0.025 M Tris-hydrochloride (pH 7.6) and resuspended in the same buffer to the original cell density. The remaining procedure was the same as described for (A) except that arsenate ( $10^{-2}$  M) was substituted for  $C_1$ . Symbols: ●, noninfected; ○, infected or treated with cyanide (or arsenate).

some loss of the transported material in the noninfected cells as well if the experiment is carried out for longer time. Both cyanide and arsenate treatments lead to similar efflux of  $\alpha$ -methylglucoside (Fig. 2B and C) although the effect is much more prominent in the case of arsenate treatment. An interesting difference, however, is observed between these two treatments and infection with  $C_1$ . There is a tendency toward partial recovery of the transport process about 10 min after phage infection (Fig. 2A), whereas no such recovery is discernible in the case of treatment with either cyanide or arsenate, which was expected since these compounds permanently block the energy metabolism of the cells. A similar situation was observed in the case of transport of the two amino acids, although there were some quantitative differences (data not shown). It appears that phage infection represents a similar type of interference which follows treatment with cyanide and arsenate; the infected cells, however, tend to recover from such an effect. It is quite possible that phage infection blocks the cellular energy-yielding process which later tends to recover. The ability to recover can be tested in an infection system in which the cell can survive the infection, i.e., from infection leading to lysogeny. This will be made clear below.

#### Transport of $\alpha$ -methyl-D- $^{14}\text{C}$ glucoside after infection leading to lysogeny.

The apparent immediate efflux of transported material from phage-infected cells is followed by a tendency to recover the rate of influx (Fig. 2). However, there is not complete recovery of the transport, which may be due to the fact that the conditions employed for the measurement of efflux and influx were not entirely physiological. Further, with the progress of infection the cells become very fragile, and no proper measurement of efflux or influx is possible. Therefore, the efflux and influx were followed in  $C^+$  infection which leads to lysogeny (Fig. 3). There is an almost immediate efflux of about 50% of the intracellular  $\alpha$ -methyl-D-glucoside on infection with  $C^+$ . A balanced state of influx and efflux is maintained for about 10 min, after which the rate of influx increases and approaches that in noninfected cells. Similar results were obtained in cases of leucine and phenylalanine (data not presented), although the recovery was not as prominent.

**Effect of ts16C<sub>1</sub> infection on the transport of L- $^{14}\text{C}$ leucine.** The temperature-sensitive mutant ts16C<sub>1</sub> fails to carry out any phage-controlled function at nonpermissive temperatures, for example, 39°C (Levine, personal communi-

cation). It has already been demonstrated in our laboratory (3) that although there is no transient depression of protein synthesis after infection with this mutant at nonpermissive temperatures, there is some depression in the rate of RNA synthesis under the same condition. Further, it has now been agreed upon that effective injection process requires the function of gene 16. To test whether effective injection of phage DNA is necessary to induce efflux, the following experiment with *ts16* was carried out. The cells were infected with this mutant at nonpermissive temperature, and the efflux of an amino acid across the membrane of the host was followed. The results presented in Fig. 4 clearly point out that there is no efflux (on the other hand, there is somewhat more influx) of L-[ $^{14}$ C]leucine after infection with *ts16C<sub>1</sub>* at 39 C, whereas the control infection with *C<sub>1</sub>* mutant shows remarkable transient net efflux. The results are in agreement with the earlier observation that there was no transient depression in the rate of protein synthesis after infection with this mutant.

**Effects of superinfection of *sie A<sup>+</sup> sie B<sup>+</sup>* and *sie A<sup>-</sup> sie B<sup>-</sup>* lysogens with *C<sub>1</sub>* mutant.** The presence of the superinfection exclusion

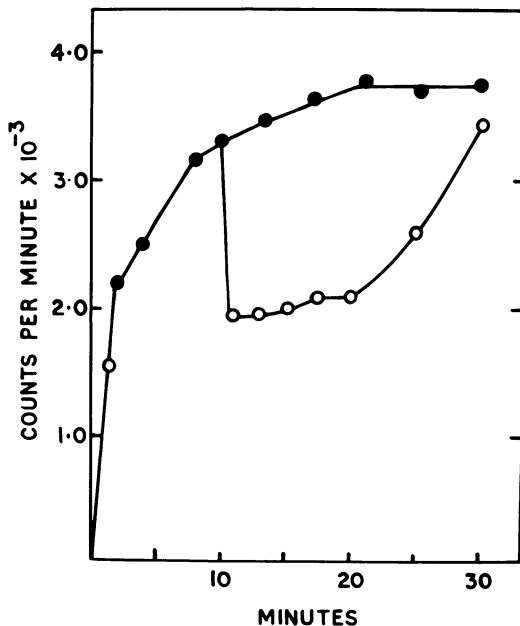


FIG. 3. Transport of  $\alpha$ -methyl-D-[ $^{14}$ C]glucoside after infection leading to lysogeny. Experimental procedure was the same as described for Fig. 2A except that the infection was carried out with wild-type phage (*C<sup>+</sup>*) at a multiplicity of infection of 20 so that the infected cells lysogenize. Symbols: ●, noninfected; ○, infected.

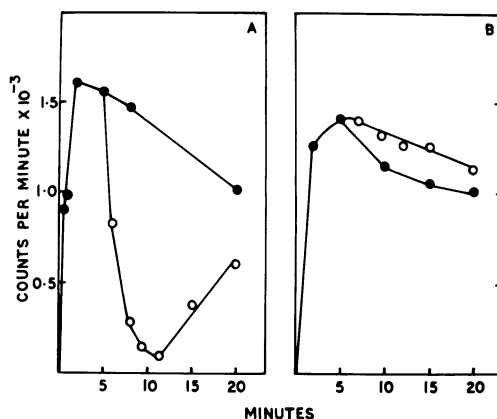


FIG. 4. Effect of *ts16C<sub>1</sub>* infection on transport. The experiment was carried out as described in the legend to Fig. 1 except that the cells were equilibrated at 39 C instead of 37 C and the incorporation of leucine was also carried out at 39 C. Further, the infection was carried out with (A) *C<sub>1</sub><sup>+</sup>* and (B) *ts16C<sub>1</sub>* 5 min after the addition of L-[ $^{14}$ C]leucine. Symbols: ●, noninfected; ○, infected.

gene (*sie* gene) in the prophage of lysogen does not allow the superinfecting lytic phage to multiply, even when the repressor is withdrawn by UV light treatment or chemical agents which allow the induction of integrated phage DNA (16). Susskind et al. (19) presented evidence that there are two systems of exclusion by P22. The A exclusion system excludes phages P22, MG178, or L. The B system, on the other hand, acts only on the heteroimmune phages. The two exclusion systems act independently. There is no transient depression of macromolecular synthesis after infection of *sie<sup>+</sup>* (actually *sie A<sup>+</sup> sie B<sup>+</sup>*) lysogen with *C<sub>1</sub>*, whereas this depression is observed in superinfection of *sie<sup>-</sup>* (*sie A<sup>-</sup> sie B<sup>-</sup>*) lysogen (3) and was found to be due to transient block in the transport of precursors of macromolecules (15). Due to the present observation that there is efflux of transported solutes after phage infection, the effects of superinfection of both *sie A<sup>+</sup> sie B<sup>+</sup>* and *sie A<sup>-</sup> sie B<sup>-</sup>* lysogens with *C<sub>1</sub>* were studied. There was comparatively rapid efflux of transported L-leucine from *sie A<sup>-</sup> sie B<sup>-</sup>* lysogen immediately after infection with *C<sub>1</sub>* (Fig. 5A). The minimum pool size was attained within 5 to 7 min after infection, after which there was very small, although significant, recovery in another 10 min. However, in the *sie A<sup>+</sup> sie B<sup>+</sup>* lysogen, the efflux was very little (Fig. 5B).

## DISCUSSION

The phenomenon of transient depression of macromolecular synthesis (3) is most probably

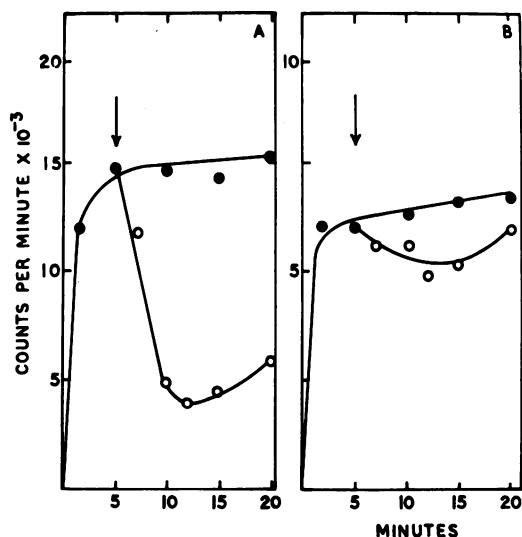


FIG. 5. Effect of superinfection on the efflux of transported L-[<sup>14</sup>C]leucine from lysogens of *S. typhimurium*, sie A<sup>-</sup> sie B<sup>-</sup> lysogen (A) and sie A<sup>+</sup> sie B<sup>+</sup> lysogen (B). The respective lysogens were grown to a cell density of  $2.5 \times 10^8$ /ml in basal medium containing 0.2% glycerol, harvested, and pretreated for the measurement of leucine transport as described. Infection was carried out with C<sub>1</sub> mutant 5 min after the addition of L-[<sup>14</sup>C]leucine. Symbols: ●, non-infected; ○, infected.

directly related to the transport process (16). Transport is a complicated phenomenon and is the result of at least two opposing reactions, influx and efflux. Further, the disturbance of influx is expected to result in net efflux. The efflux after phage infection resembles that in cyanide- and arsenate-treated cells, although in the former there is a tendency toward the recovery of the transport process. It is quite possible that phage infection results in temporary blockage of cellular respiration or other energy-yielding processes which in turn inhibit the transport processes. When the influx is stopped due to phage infection, the efflux naturally becomes prominent. Subsequently the blockage is withdrawn so as to restore the normal respiration and thus the transport.

Since the change in cellular transport process takes place as a result of phage infection even in the presence of chloramphenicol and immunity repressor (superinfection experiments), it is hard to imagine that this is due to phage gene expression. Further, this phenomenon has been observed in all infections except two, infection of normal cells with ts16 and superinfection of sie A<sup>+</sup> sie B<sup>+</sup> lysogen with C<sub>1</sub>. It is agreed that DNA is not effectively injected due to the defect in gene 16 (Levine, personal communication).

Further, Ebel-Tsipis and Botstein (6) suggested that in superinfection of a sie<sup>+</sup> lysogen the superinfection exclusion system acts upon the superinfecting DNA as it is being injected from a phage particle. Recently, Susskind et al. (18) confirmed that the A exclusion system blocks the entry of superinfecting phage DNA. Thus, in both cases the infecting DNA experiences interference while entering into the cellular interior. Our earlier observations on the change in the cellular transport processes (15) as well as the present observations also support the hypothesis that the exclusion system is located in or near the cell membrane. Thus an effective injection process seems to be essential to induce change in cellular transport. Although the phage gene expression seems not to be necessary for this, recent observations in this laboratory (unpublished data) indicate that a preformed phage protein (internal protein) is involved in the recovery of the cellular transport process.

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