

# Comparison of the Action of Colicins E1 and K on *Escherichia coli* with the Effects of Abortive Infection by Virulent Bacteriophages

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Abortive infection of certain strains of *Escherichia coli* or *Shigella dysenteriae* with phages of the T-even group or with phage T5 resembles the action of colicin E1 or K on sensitive bacteria, especially in the effects on biosynthetic processes. Tests on transport systems and on adenosine triphosphate levels suggest, however, that different mechanisms are involved in the two cases. Abortive infection appears to cause damage to the permeability barrier of the cell, whereas the colicins interfere more directly with the energy metabolism of the bacteria.

Abortive infection by certain phages is known to occur under a variety of conditions (1). A special case is that of phages that do not grow on certain lysogenic derivatives of otherwise permissive hosts. The T-even and T5 coliphages give abortive infection in *Shigella dysenteriae* lysogenic for phage P2 (2, 8), as well as in some strains of *Escherichia coli* (6, 10a). In comparing the effects of phage infection with those of some colicins, Luria (9) observed that abortive infection with T-even phages resembled in many respects the action of colicin E1 or K (10). This was particularly evident in *E. coli* Co270, in which the T-even phages, as well as T5, regularly give abortive infection (6).

The present paper compares the effects of abortive infection of *E. coli* Co270 and *S. dysenteriae* Sh(P2) with the recently reported effects of colicins E1 and K on cellular functions (4, 5). The results indicate that, although both sets of effects are probably due to membrane-related alterations, the mechanisms are probably quite different, abortive infection causing drastic alterations of membrane permeability not observed after colicin action.

## MATERIALS AND METHODS

**Bacteria.** *E. coli* Co270 was obtained from P. Frédéricq; *S. dysenteriae* Sh and its derivative Sh(P2) were obtained from G. Bertani (8). *E. coli* B was used for phage assays. Lactose-positive derivatives of Sh and Sh(P2) were produced by transfer of *F-lac* episome from an appropriate strain of *E. coli* (*F lac*).

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**Bacteriophages.** *E. coli* bacteriophages T2, T4, T5, and T6 and antiphage sera were used according to standard phage methods (1). The indicator strains were *E. coli* B and *S. dysenteriae* Sh.

**Media.** In addition to the media used previously (4), medium 121-Sh was used for growing *S. dysenteriae* Sh strains; medium 121 base (see 4) was supplemented with phosphate (50 µg/ml), 10 L-amino acids (arginine, aspartic acid, cysteine, glutamic acid, histidine, methionine, phenylalanine, proline, and serine, each at 20 µg/ml), and five vitamins (thiamine, biotin, *p*-aminobenzoic acid, nicotinamide, each at 1 µg/ml, and riboflavine, 0.1 µg/ml).

**Methods.** The procedures for measuring permease functions, incorporation of labeled substrates, O<sub>2</sub> consumption, and adenosine triphosphate (ATP) levels have been described (4, 5). T2-induced deoxycytidylate hydroxymethylase was assayed as described by Wiberg and Buchanan (13). All experiments were carried out at 37 C.

## RESULTS

**Abortive infection of *E. coli* Co270 and *S. dysenteriae* Sh(P2).** *E. coli* Co270 was reported by Frédéricq (6) to give abortive infection with phage T6. This organism is insensitive to phage P2; its culture fluids produced a few tiny plaques when plated on P2-sensitive hosts, but no lysate could be obtained. It is sensitive to colicins E1 and K.

*S. dysenteriae* Sh gives normal productive infection with T-even or T5 phage, whereas Sh(P2) gives abortive infection. This pair of strains, in addition to Co270, was used so that the effects of abortive versus productive infection could be compared with those of colicin action. No plaques are formed by the restricted phages

on Co270 or Sh(P2); the fraction of T2-infected cells that yields phage is about  $10^{-4}$  (8). The host-killing efficiency per phage particle is one for all phage-host combinations.

In productive infection of strain Sh, protein and nucleic acid synthesis continues during phage development. In contrast, infection of Co270 or Sh(P2) with T2, T4, T5, or T6 results in a rapid cessation of incorporation of labeled uracil, or thymidine or amino acids, into acid-insoluble form. Typical examples are shown in Fig. 1 and 2. Colicins E1 and K block incorporation in strain Co270 as they do in other sensitive *E. coli* strains, but their action on Sh strains is somewhat slower, and incorporation continues for at least 10 min after addition of colicin (Fig. 3). Pulse-labeling tests with  $^{14}\text{C}$ -uracil in abortively infected cells showed no significant incorporation after 5 min. Addition of chloramphenicol did not alter the effects of abortive T2 phage infection on uracil incorporation in *Shigella* strains.

Production of a phage directed enzyme, deoxycytidine monophosphate (dCMP)-hydroxymethylase, was measured after infection of Sh or Sh(P2) with T2. Enzyme appeared in both

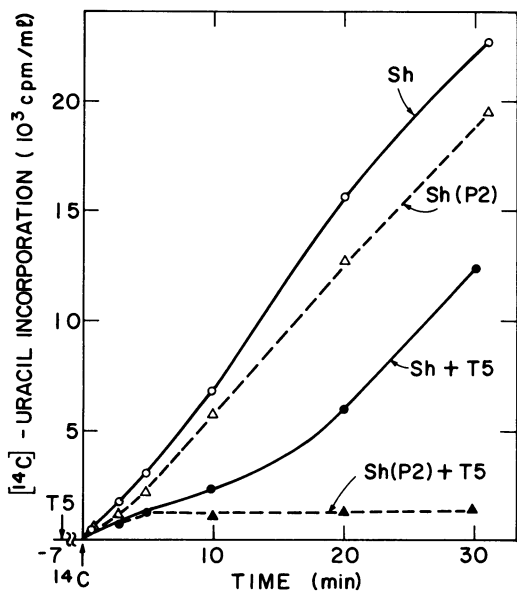


FIG. 1. Effect of phage T5 on incorporation of uracil by Sh and Sh(P2). Bacteria grown in medium 121-Sh were concentrated by centrifugation to  $3 \times 10^9$  cells/ml in the same medium with  $10^{-3}$  M  $\text{CaCl}_2$  and were infected with phage T5. After 7 min, the suspension was diluted 1:40 in medium with  $^{14}\text{C}$ -uracil. Survival at the time of dilution was 1% for Sh and 2% for Sh(P2) cells.

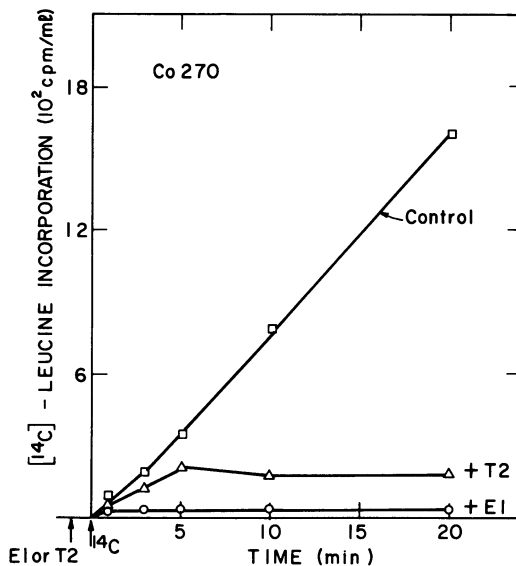


FIG. 2. Effect of colicin E1 or phage T2 on leucine incorporation by *E. coli* Co270. Phage or colicin or buffer was added 1 min before  $^{14}\text{C}$ -leucine to samples of a growing culture in medium 63-glucose. Survival: T2-infected cells, 0.2%; colicin E1, 0.1%.

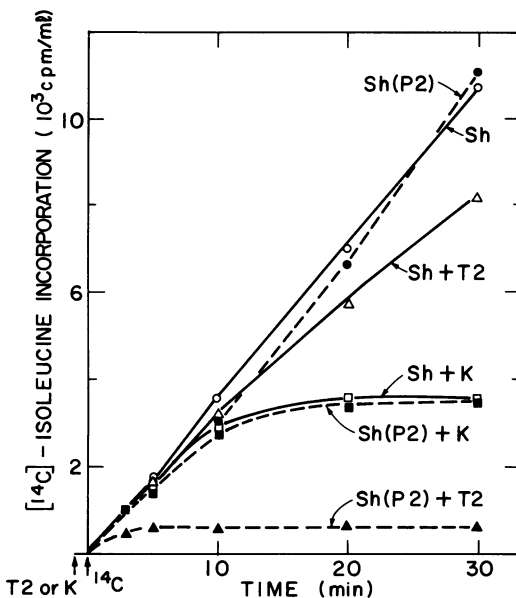


FIG. 3. Effect of colicin K or phage T2 on isoleucine incorporation by Sh and Sh(P2). Phage or colicin or buffer was added to samples of a culture in medium 121-Sh, and 1 min later the cultures were diluted 1:6 in medium with  $^{14}\text{C}$ -isoleucine. Survival 2 min after dilution: Sh + T2, 3.5%; Sh + K, 5%; Sh(P2) + T2, 4%; Sh(P2) + K, 5%.

hosts, but its production in Sh(P2) stopped abruptly by 4 min, and the level reached was about one-tenth that in Sh, where enzyme synthesis continued for 10 min before being shut off.

These experiments indicate that phage-specific syntheses can occur in abortively infected cells, but that the halt in biosynthesis after infection occurs even when phage-specific protein synthesis is prevented.

Oxygen consumption with glucose as substrate was measured with cells grown in glucose media. Infection with phage T2 did not reduce the rate of respiration of Sh cells, but in Sh(P2) the  $O_2$  consumption rate was reduced to about 20%. In *E. coli* strains, colicin E1 does not significantly affect the respiration rate on glucose (5).

**Effects of abortive infection on transport of galactosides and on ATP levels.** The accumulation of  $^{14}C$ -thiomethyl galactoside (TMG) and the rate of hydrolysis of *o*-nitrophenyl- $\beta$ -D-galactoside (ONPG) by normal and phage-infected cells were measured. To use cells of Sh and Sh(P2), which are normally lactose-negative, F-lac carrying derivatives were obtained by transferring the F-lac episome from an *E. coli* strain. The resulting strains Sh(F-lac) and Sh(P2)(F-lac) are stably lactose-positive.

Colicins E1 and K have been shown to block the accumulation of TMG but have only a slight effect on the rate of ONPG hydrolysis by intact cells (4). This result, supported by other evidence, has been interpreted as showing that lactose permease function is affected only at the level of its energy requirement (4).

Accumulation of TMG was stopped by T-even phage infection of Co270 or Sh(P2)(F-lac), but was not stopped in Sh(F-lac), which gives a normal productive infection. In the *Shigella* strains, the levels of TMG accumulation by the lactose-positive strains were more variable than in *E. coli*, but the difference in phage action between Sh and Sh(P2) was unmistakable (Fig. 4). Thus, with respect to TMG accumulation, abortive infection appears to resemble the action of colicins E1 and K.

A very different picture was observed, however, when the rate of ONPG hydrolysis was studied. As shown in Fig. 5, infection with phage T2 increased the rate of ONPG hydrolysis by *E. coli* Co270 by a large factor, whereas colicin E1 decreased it slightly, as it does in other *E. coli* strains (4). Measurement of galactosidase in supernatant fluids of phage-treated cultures showed that there was no significant release of enzyme.

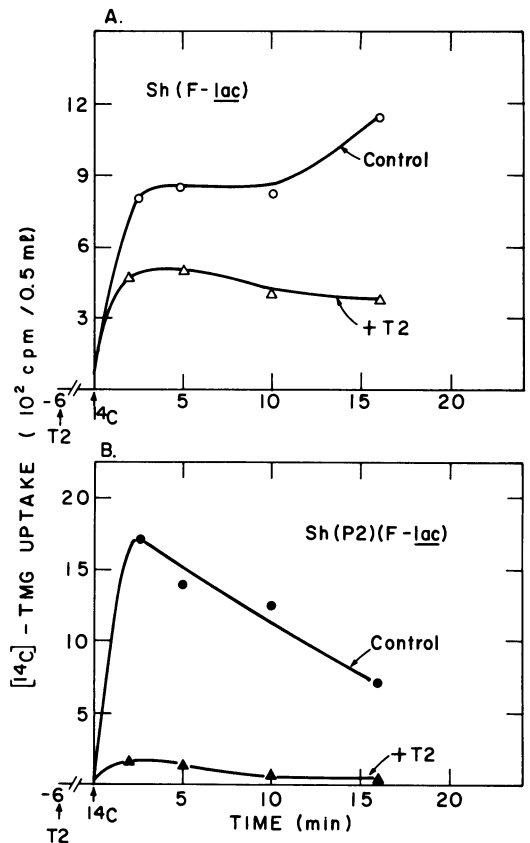


FIG. 4. Effect of phage T2 on TMG accumulation by Sh(F-lac) and Sh(P2)(F-lac). Cells growing in medium 121-Sh with glucose and  $5 \times 10^{-4}$  M isopropyl thio-galactoside IPTG) were harvested, resuspended at  $5 \times 10^8$ /ml in medium without IPTG, and infected with phage T2;  $^{14}C$ -TMG ( $0.9 \mu\text{C}/\text{ml}$ ,  $3 \times 10^{-4}$  M) was added 6 min after infection. (A) Sh(F-lac); survival after T2 infection, 1.7%. (B) Sh(P2)(F-lac); survival, 2.2%.

Likewise, there was a substantial increase in the rate of ONPG hydrolysis by Sh(P2)(F-lac). At high phage multiplicities there was also some increase in hydrolysis rate by T2-infected Sh(F-lac) cells, but no increase was observed in experiments with fewer than five phages per cell. This suggests that *Shigella* cells are more sensitive to lysis from without than are *E. coli* cells.

This, the findings with ONPG hydrolysis, which were confirmed for Co270 by fluorometric experiments with another galactosidase substrate, fluorescein digalactoside (12), revealed a significant difference between the effects of colicins and those of abortive infection on the same bacteria. Abortive infection clearly increased

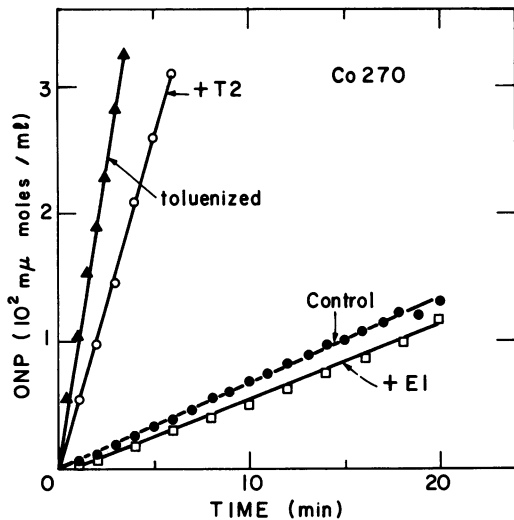


FIG. 5. Hydrolysis of ONPG by cells of *E. coli* Co270. Cells grown in medium 63-glycerol-IPTG were centrifuged and resuspended in medium with chloramphenicol (100  $\mu$ g/ml) and without IPTG. Phage T2 or colicin E1 was added, and after 5 min the samples were chilled, bacterial survival was measured (T2, 2%; E1, 0.3%), and the rate of ONPG hydrolysis was measured with control, phage-infected, colicin-treated, or toluenized cell suspensions. Assay of total  $\beta$ -galactosidase activity in the supernatant fluid of centrifuged samples showed that, compared to toluenization, T2 released about 10% of the enzyme activity and E1 released less than 0.5%.

permeability to substrates of galactosidase, which suggested a nonspecific damage to the permeability barrier of the bacterial cell. Such damage would also explain the reduced rate of respiration, if metabolic intermediates were to flow readily out of the cells.

Measurements of ATP by the firefly assay method supported the hypothesis of permeability damage. As shown in Fig. 6, abortive infection of Sh(P2) with phage T2 led to a slight rise in ATP levels followed by little or no further change; by 10 min most of the ATP was found to be in the supernatant fluid. Similar results were obtained with *E. coli* Co270. By contrast, productive infection of strain Sh caused the large increase in ATP levels that had been observed by others (Colowick, *personal communication*), almost all the activity being within the cells. As reported previously (4), colicins E1 and K caused a strong reduction in ATP levels but no loss of ATP from the cells.

#### DISCUSSION

Although the present results provide only a very partial description of the events in abortive

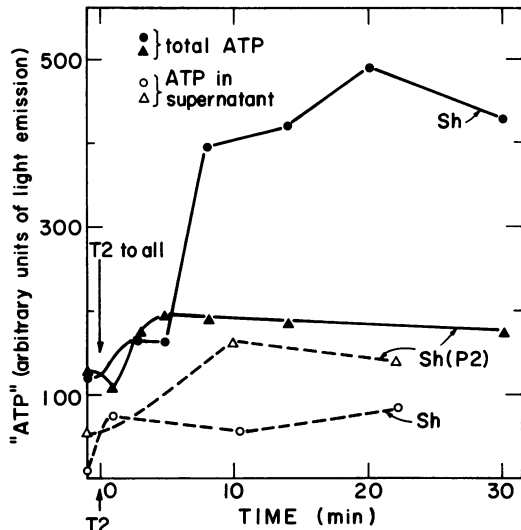


FIG. 6. Effect of phage T2 on ATP levels in Sh(F-lac) and Sh(P2)(F-lac). Cells growing in medium 121-Sh with glycerol and IPTG were infected with phage T2 (survival: Sh(F-lac), 0.8%; Sh(P2)(F-lac), 1.2%). Total ATP or ATP in supernatant fluid of centrifuged samples was measured with crude firefly extract. Assays of  $\beta$ -galactosidase in the supernatant fluid at 28 min after infection showed that less than 1% of the enzyme had been released.

infection by T-even or T5 phage, they indicate that the mechanism of this kind of phage infection differs from the mechanism of action of colicin E1 or K. The common features—arrest of biosynthesis and of other energy-requiring reactions such as accumulation of  $\beta$ -galactosides—probably result from different primary mechanisms in the two cases: interference with ATP generation by the colicins (5) versus damage to the permeability barrier, probably the cytoplasmic membrane, for abortive infection. Clarification of the causes of abortive infection must await a more detailed description of the biochemical events that accompany it.

It seems plausible that in Sh(P2) the presence of the prophage alters the cells in such a way that infection with the phages like T2 or T5 leads to irreparable damage. The role of prophage may be to prevent the action of a repair mechanism normally functional in phage infection. Puck and Lee (11) proposed that infection with T-even phages involved an initial damage to cell permeability, which resulted in a transient leakage of metabolic intermediates and which was subsequently repaired. If such repair failed to occur, abortive infection would result.

The abortive infection observed in *E. coli* Co270 may also reflect the role of a prophage, since this strain, like many other *E. coli* strains,

is probably lysogenic for a P2-related prophage. *E. coli* W, which also gives abortive infection with T-even and T5 phages (10a), has been shown to be lysogenic for a P2-related phage (7) and the prophage is responsible for the restriction (10a). In abortive infection of *E. coli* W, the T2 deoxyribonucleic acid (DNA) is partially broken down, but such DNA breakdown is not observed in T2 infection of Sh(P2) (10a). The genetic background of the bacteria certainly plays a role, since *E. coli* B(P2) does not give abortive T-even infection (8).

The hypersensitivity of some P2 lysogens to T-even infection may be related to the sensitivity to 5-fluorodeoxyuridine caused by P2 prophage in *E. coli* C, a sensitivity also attributed to altered cell permeability (3).

#### ACKNOWLEDGMENTS

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