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Amino Acid and Sugar Transport in *Escherichia coli* (ColIb) During Abortive Infection by Bacteriophage T5

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T5 bacteriophage cannot replicate in *Escherichia coli* containing the colicinogenic factor ColIb. We show that active transport of proline and glutamine begins to decline at about 10 min after infection, the same time at which macromolecular synthesis stops during abortive infection. Uptake of α-methylglucoside is stimulated, however, and this change is evident even by 5 min after infection. These changes in membrane function do not occur during infections that are productive because of mutations on the plasmid or phage. The results suggest that the abortive infection is caused by membrane depolarization.

Bacteriophage replication can be prevented by at least four recognized mechanisms: resistance (1, 15, 41), superinfection exclusion (2, 3, 14), restriction (29), and abortive infection (see, for example, reference 13). Resistant bacteria lack functional phage receptors, and adsorption is thereby inhibited. In superinfection exclusion, however, attachment to receptors is apparently normal. Instead, a primary infecting phage in some way prevents normal entry of DNA from a subsequently adsorbed virus (2, 3). When phage genetic material does manage to penetrate the cell envelope, specific restriction endonucleases, if present, digest unmodified, foreign DNA. In abortive infection, on the other hand, phage adsorption and DNA entry are normal, and the viral genome is not broken down. The normal infectious process is initiated, but the presence of a lysogenized phage or a plasmid in some way dictates that the normal events shall cease prior to the production of progeny phage. Among the best-known examples of abortive infections are T3 or T7 infections of F (fertility factor)-containing *Escherichia coli* (7, 8, 12, 13, 32), T-even *r*II mutant infections of λ lysogens (9, 16, 19), T-even or T5 infections of P2 lysogens (17), and BF23 or T5 infections of colicin Ib (ColIb)-containing *E. coli* (10, 28, 31, 33, 34, 38-40).

Abortive T5 infections of ColIb$^+$ *E. coli*, as do the other types of abortive infection, begin as if a productive infection were to occur (20, 33). Class I (pre-early) proteins are synthesized, and the host cell is killed as usual. At a time 6 to 10 min after infection, however, transcription and translation cease, before or just after class II (early) gene expression begins. Furthermore, no phage DNA replication occurs, although the infecting DNA remains intact.

Host range (h$^-$) phage mutants, able to grow on colicinogenic bacteria, have been isolated (4, 31, 39). Likewise, colicinogenic strains that are permissive hosts for T5 wild-type phage have been found. These bacteria allow T5 replication because of chromosomal mutations (23) or ColIb plasmid mutations (28; Richard Moyer, personal communication). Thus, the interaction of at least three proteins (one specified by the host, one specified by the phage, and one specified by the plasmid) is necessary for abortive infection. The nature of the interaction producing the inhibition of phage multiplication is, however, unknown.

Previous work in this laboratory (10) indicated that cell membrane function is defective during abortive T5 infection. ATP, assayed enzymatically, and ^{32}P-labeled material were found extracellularly in abnormally high amounts. Although no significant drop in intracellular ATP level was detected, the T5-infected colicinogenic hosts lost much of their ability to actively transport thiomethyl-β-D-galactoside. This suggested that functional membrane defects might be responsible for the cessation of macromolecular synthesis previously observed (33). However, since membrane malfunction and cessation of the phage infectious process occurred at approximately the same time, definite conclusions regarding the role of the membrane in contributing to the abortive infection (whether to cause the arrest or only represent a side effect of the process) could not be made.

In the present paper, we describe further studies of membrane function during abortive T5 infection, including the involvement of both phage and plasmid *h* genes in producing the observed changes. The principle aim was to de-

termine whether a defective membrane could be the primary cause of the abortive infection.

MATERIALS AND METHODS

Organisms. Our bacterial strains are characterized in Table 1. Richard Moyer supplied us with the three isogenic strains used. RM 42 contains no colicinogenic factor; RM 43 bears wild-type ColIb; RM 39 has a mutant ColIb plasmid that renders the host permissive for T5 (Moyer, personal communication). We extracted DNA from these three strains and confirmed the presence of plasmid DNA in RM 43 and RM 39. This was accomplished by demonstrating form I DNA on a CsCl-ethidium bromide gradient, using the method of Clewell and Helinski (11) (data not shown). Further, we showed that supernatants of 3-day broth cultures of RM 43 and RM 39, but not of RM 42, contain a substance inhibitory for growth of *E. coli* B and *E. coli* C indicator strains (Table 1).

Wild-type phage T5 was obtained from Rolf Benzinger, and D. J. McCorquodale provided us with T5h12$^-$. We confirmed, by determining the time course of host DNA breakdown, that this mutant adsorbs and initiates infection at the same rate as T5 wild type. Relative plating efficiencies of the phage stocks on our bacterial strains are also shown in Table 1.

Media and growth of bacteria. Basic growth medium is defined as M9 phosphate-buffered balanced salt solution (21), supplemented with glucose (0.5%), yeast extract (0.05%), $CaCl_2$ (5×10^{-4} M), and thymine (50 μg/ml). Other additives were present as indicated. Bacteria were grown from a 5% inoculum of an overnight culture. Growth was followed by monitoring turbidity on a Klett-Summerson colorimeter (660-nm filter), which had been previously calibrated to numbers of bacterial colony formers.

Phage growth. Phage stocks were prepared in one of two ways: by the confluent lysis technique of Adams (1), or by recovering phage from liquid medium by polyethylene glycol precipitation (42). No differences in results were obtained with the different types of phage preparation.

Phage infection. Bacterial hosts were grown, as outlined above, to a concentration of approximately 6×10^8 cells/ml. The bacteria were then centrifuged at 4°C for 10 min at 10,000 rpm in an SS-34 rotor on an RC-5 Sorvall centrifuge and resuspended in 1/10 volume of fresh growth medium. Phage were added at a multiplicity of infection of 10, and, after 1 min, the bacteria-virus mixture was rediluted in prewarmed growth medium to 6×10^8 cells/ml. The time of redilution was called time zero.

Macromolecular synthesis. RNA synthesis was monitored by measuring incorporation of [^{3}H]uridine into trichloroacetic acid-insoluble material at different times after infection. Bacteria were grown in basic growth medium with 50 μg of cold uridine per ml. Bacteria were centrifuged, concentrated, infected, and rediluted as outlined above. At time zero, [^{3}H]uridine (1 μCi/ml, 5 μCi/μmol) was added, and, at indicated intervals thereafter, 0.9-ml samples were removed and added to 0.1 ml of cold trichloroacetic acid (5% final concentration). Of this mixture, 0.4 ml was filtered through Whatman glass fiber filters (GF/F) and washed with 15 ml of cold 5% trichloroacetic acid. The filters were subsequently dried in an oven at 60°C for 1 h and counted in a toluene-based scintillation fluid using a Beckman LS200 counter. The results represent total (cumulative) counts per minute incorporated into RNA in approximately 2.2×10^8 cells.

Protein synthesis was measured in the same way, except that cold tyrosine (25 μg/ml) was added to the basic growth medium, and [^{3}H]tyrosine (1 μCi/ml, 7.2 μCi/μmol) was added at time zero. Experiments to measure incorporation of glucose into macromolecules were also performed as described above. These experiments were performed with [^{14}C]glucose (0.5 μCi/ml, 0.1 μCi/μmol).

Proline and glutamine uptake. In these experiments, the amount of amino acid taken up by the cell in a 30-, 60-, or 90-s pulse was measured at various times after infection. Bacteria were grown in basic growth medium, spun, concentrated, infected, and rediluted as above. At the indicated times, 2-ml samples were removed and added to 2 μl of chloramphenicol (100 μg/ml in 95% ethanol). One minute later, after protein synthesis had completely stopped (unpublished observations), 0.9 ml of the suspension was added to 0.1 ml of the labeled amino acid solution. In the proline assay, 1 μCi of [^{3}H]proline (25 μCi/μmol) was present, while in the glutamine experiments, 0.5 μCi of [^{14}C]glutamine (5 μCi/μmol) was present. At indicated intervals after infected bacteria were added to the labeled amino acid, 0.4-ml samples were removed, filtered on Whatman glass fiber filters (GF/F), and washed with 8 ml of M9. A positive control was done with uninfected cells; a negative control was done with cells treated for 15 min with NaN_3 (1% final concentration) before the addition of chloramphenicol. The results are presented as percentage of uptake in infected cells relative to that in uninfected controls.

αMG uptake. α-Methylglucoside (αMG) uptake experiments were done with bacteria grown and infected in basic growth medium, as outlined above. At times indicated, 1 ml was removed from infected cul-

TABLE 1. *Characterization of bacteria and phage*

E. coli strain	Genotype	Colicin production[a]	Relative plating efficiency	
			T5 wild type	T5h12
RM 42	W3110 (*thy*$^-$ ColI^{R_b})	−	1	1
RM 43	W3110 (*thy*$^-$ ColI^R ColIb P9)	+	10^{-7}	0.5
RM 39	W3110 (*thy*$^-$ ColI^R ColIb h^{-c})	+	1	3

[a] Measured by killing of *E. coli* B and C.
[b] ColI^R indicates resistance to the external action of colicin Ib.
[c] ColIb h$^-$ indicates the mutant ColIb P9 factor, which allows T5 growth.

tures, spun in an Eppendorf model 3200 centrifuge for approximately 30 s, resuspended in an equal volume of M9, and spun again. After the second spin, the bacteria were resuspended in basic growth medium, modified by having only 18 μg of glucose per ml. Of this suspension, 0.9 ml was added to 0.1 ml of labeled [^{14}C]αMG solution (1 μCi/ml, 184 μCi/μmol). At indicated intervals, 0.4-ml aliquots were removed and filtered through Whatman glass fiber filters (GF/F) and washed with 8 ml of M9. The filters were dried in a 60°C oven for 1 h and counted. A positive control was done with uninfected cells; a negative control was included, using cells treated for 15 min with 0.07 M NaF and NaN_3 (1% final concentration), before the assay. The results are presented as percentage of the control value.

Statistical methods. The means of two sets of data were compared by Student's *t* test, according to the method of Snedecor and Cochran (37). When an equal number of data points was included in each set, *t* values were calculated by the formula

$$t = \frac{(\bar{y}_1 - \bar{y}_2)\sqrt{n}}{\sqrt{S_1^2 + S_2^2}}$$

where y_1 = the mean of data points in set 1, n = the number of data points in each set, and S_1 = the standard deviation of the mean for data collected in set 1. *P* values were derived from a standard table. Application of the *t* test presupposes that the data fit a normal *t* distribution.

RESULTS

Macromolecular synthesis in T5 infection of RM 42, RM 43, and RM 39. It has been previously reported that transcription and translation cease at 6 to 10 min after T5 infection of ColIb$^+$ *E. coli* (33). To determine when these changes occurred in our system, we measured the cumulative incorporation of an RNA and a protein precursor into acid-insoluble macromolecules at various times after infection. Uridine was used as an indicator of RNA synthesis; leucine, tyrosine, or proline was used to monitor protein synthesis. The cells and infecting phage used are desribed in Table 1.

Typical results of experiments measuring incorporation of [^{3}H]uridine can be seen in Fig. 1. RNA synthesis, measured in this way, continued for at least 30 min in productive infections, albeit at a slower rate than in uninfected cells (Fig. 1a). In infection of RM 43 (ColIb), however, uridine incorporation stopped at some time approximately 9 to 12 min after initiation of infection (Fig. 1b). Protein synthesis, as indicated by tyrosine incorporation (Fig. 2), also stopped about 9 min after infection of ColIb-containing cells. Proline incorporation ceased at 9 to 12 min also, whereas leucine incorporation into macromolecules seemed to continue for a slightly longer time in these cells (data not shown). RNA and protein synthesis proceeded for at least 30 min in T5 wild-type infections of RM 39 (ColIb h$^-$) (Fig. 1c and 2c) and in T5h12$^-$ infections of RM 43 (ColIb) (data not shown).

Amino acid uptake in T5-infected RM 42, RM 43, and RM 39. In an attempt to clarify the relationship between the inhibition of RNA and protein synthesis and the membrane changes previously observed (10), and to more specifically define the membrane defects, we looked at uptake of two amino acids. The transport systems of proline and glutamine are fairly well

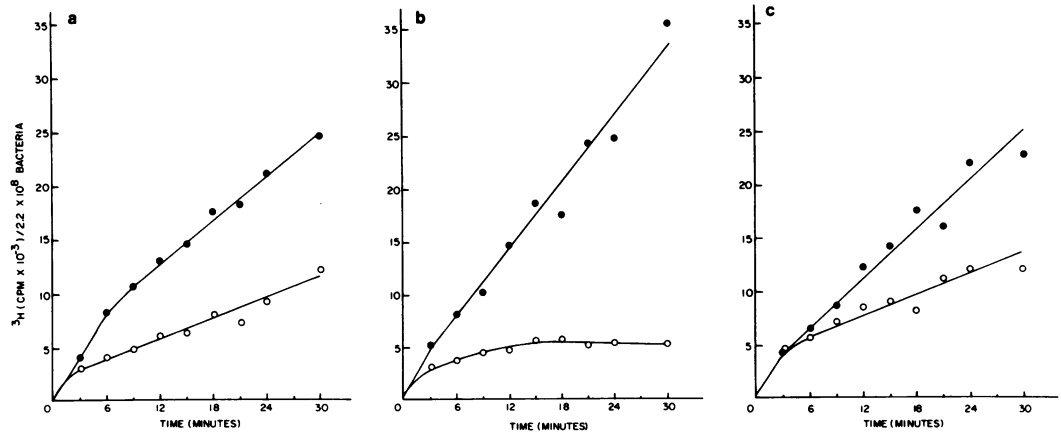

FIG. 1. *RNA synthesis in uninfected and T5-infected E. coli. Cells were grown and infected as detailed in Materials and Methods. [^{3}H]uridine (1 μCi/ml, 5 μCi/μmol) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% trichloroacetic acid. Acid-insoluble material was collected on glass fiber filters, and the filters were washed. The amount of incorporated radioactivity was determined by liquid scintillation counting. (a) RM 42, which contains no plasmid; (b) RM 43 (ColIb); (c) RM 39 (ColIb h$^-$). Symbols: (●) uninfected cells; (○) T5-infected cells.*

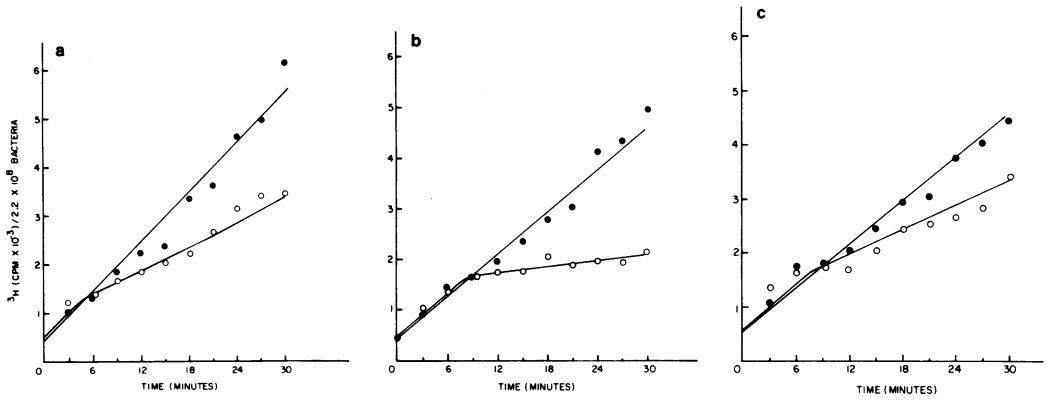

FIG. 2. *Protein synthesis in uninfected and T5-infected E. coli. Cells were grown and infected as described in Materials and Methods. [^{3}H]tyrosine (1 μCi/ml, 7.2 μCi/μmol) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% trichloroacetic acid. Acid-insoluble material was recovered on glass fiber filters, and the amount of incorporated radioactivity was determined. (a) RM 42; (b) RM 43 (ColIb); (c) RM 39 (ColIb h$^-$). Symbols are as in Fig. 1.*

characterized (5, 6), and both systems require an "energized" membrane. Proline requires membrane-bound transport proteins (still present when purified membrane vesicles are made), but does not require a high-energy phosphorylated intermediate. Glutamine, on the other hand, relies upon periplasmic binding proteins and needs ATP to energize its active transport. We wanted to see when, if at all, these uptake systems were inhibited during abortive infection. If they were inhibited, we wanted to know whether they remained functional during infections involving h$^-$ mutants of the phage or plasmid.

In these experiments, cell samples were removed at various times after infection and incubated with chloramphenicol for 1 min (a time sufficient to totally stop protein synthesis [unpublished observation]) before adding the labeled amino acid. The results, therefore, reflect only uptake of the amino acid and are not indicative of incorporation into proteins.

When wild-type T5 infected any of our three bacterial strains, there was a slight drop in proline uptake at 5 min after infection (Fig. 3a, Table 2). By 10 min into the infectious process, however, uptake ability returned to uninfected levels in all cases and continued to increase in both infected RM 42 and infected RM 39 (ColIb h$^-$). In abortively infected RM 43 (ColIb), however, there was a drastic reduction in proline uptake ability between 10 and 15 min. If T5h12$^-$ phage infected RM 43 (ColIb) (Fig. 3b), there was only a slight reduction in uptake ability as compared with that seen during infections of RM 42 or RM 39 (ColIb h$^-$). In comparing results observed during T5h12$^-$ infections of RM 42 and RM 43 (ColIb) by Student's *t* test, only the difference in values at 30 min was significant. This slight loss of uptake ability in colicinogenic hosts was perhaps due to the 50% plating efficiency of the mutant phage on RM 43 (ColIb). When T5 wild-type and T5h12$^-$ infections of RM 43 (ColIb) were compared directly, statistically significant differences were observed at all times after 10 min. In summary, proline uptake ability was drastically reduced between 10 and 15 min in nonpermissive hosts. This inhibition was not observed when gene *h* mutations on either the phage or plasmid allowed productive infection to occur.

Similar results were seen when glutamine uptake was measured (Fig. 4, Table 3). After observing a reduction in uptake ability at 5 min after T5 wild-type infections of any one of the three strains, we saw a gradual recovery in uptake ability during productive infections. When RM 43 (ColIb) was the host, uptake ability fell progressively, and there was a statistically significant difference, as compared with infections of RM 42, by 15 min. Although the 10-min values were not significantly different, in some experiments there was indeed a large disparity. The mean 10-min value of glutamine uptake in infected RM 43 (ColIb) was lower than the value of infected RM 39 (ColIb h$^-$). Infection of RM 43 (ColIb) with T5h12$^-$ or T5 wild type showed that the gene *h* mutation prevented the fall in glutamine uptake. The cell's ability to accumulate glutamine, then, as was the case with proline uptake, was drastically reduced during abortive infection. This dysfunction was prevented by phage or plasmid mutations which also allow productive infection to proceed. There was a tendency for glutamine uptake to decline a bit

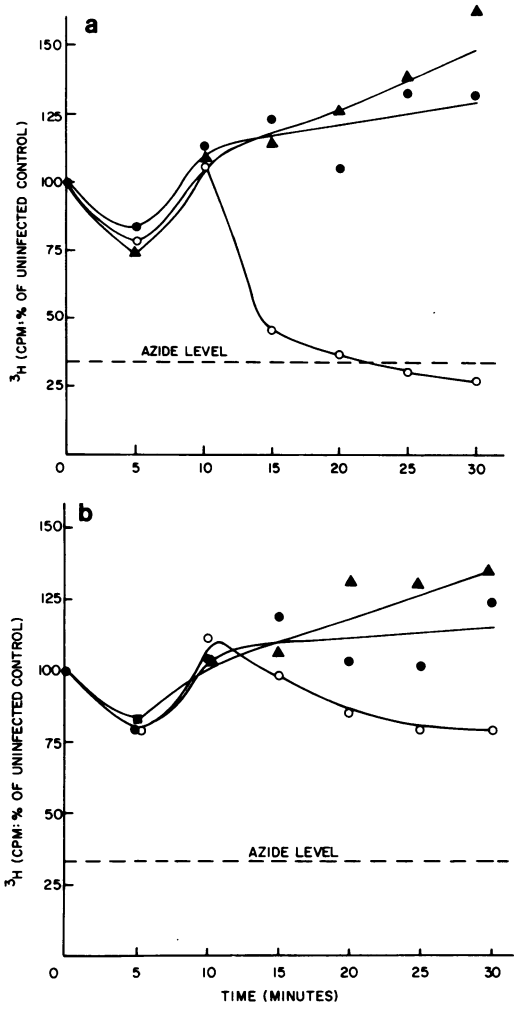

FIG. 3. *Proline uptake by T5-infected permissive and nonpermissive E. coli. Cells were grown and infected as outlined in Materials and Methods. At the indicated times after infection, samples were removed and mixed with [^{3}H]proline (1 μCi/ml, 25 μCi/μmol). Samples were removed at 30 and 60 s thereafter and filtered through glass fiber filters. The amount of radioactive proline accumulated in 60 s by infected cells is expressed as a percentage of the amount taken up by uninfected controls in the same period of time. (a) T5 wild-type infections of E. coli RM 42, RM 43 (ColIb), and RM 39 (ColIb h$^-$); (b) T5h12$^-$ infections of the same three bacterial strains. Symbols: (●) RM 42; (○) RM 43; (▲) RM 39.*

earlier than proline uptake, but the two uptake systems were not compared in the same experiment.

αMG uptake in infected RM 42, RM 43, and RM 39. Since accumulation of either proline or glutamine requires an energized membrane, we decided to measure uptake of a substance that is transported via a group translocation reaction and does not require membrane polarization. αMG is a nonmetabolizable analog of glucose that is taken into the cell by using the phosphotransferase system and energy derived from phosphoenolpyruvate (26). We wanted to see whether this transport system was functional in abortively infected cells.

Phage infection, whether productive or abortive, increased, rather than decreased, αMG accumulation (Table 4). By 5 min after infection, however, the increase was much greater in the abortive infection than it was in the productive infection. As was seen for proline or glutamine uptake, the absence of the normal phage or plasmid gene *h* product eliminated the effect. The augmented accumulation during abortive infection was inhibited by NaF, as is normal for transport systems energized by phosphoenolpyruvate (26), showing that the increased uptake was via the phosphotransferase system and not a new uptake system. After 10 min of infection in a nonpermissive host, the increase in αMG accumulation began to decline, although the total uptake was still much greater than that during productive infection.

Glucose incorporation. The rate of protein synthesis or nucleic acid synthesis measured by incorporation of radiolabeled precursors depends first upon the cells' ability to take up the precursor from the medium. Since the uptake of amino acids into the soluble pools of T5-infected cells was severely depressed during abortive infection, it cannot be said that protein synthesis was necessarily inhibited. Since the glucose transport system appeared to remain functional, as measured by αMG uptake, we looked for the incorporation of label from [^{14}C]glucose into acid-insoluble material (Fig. 5).

Cumulative incorporation of glucose into an insoluble form was inhibited in the abortively infected RM 43 (ColIb) at about 10 min after infection (Fig. 5b). This was not the case during T5 infections of RM 42 or RM 39 (ColIb h$^-$) (Fig. 5a and 5c), nor was it true of T5h12$^-$ infections of RM 43 (ColIb) (Fig. 5b). Since the glucose transport system remained operative for at least 30 min during abortive infection, it appeared that macromolecular synthesis per se was inhibited at about 10 min. This conclusion must still be regarded as tentative, however, since the results could be explained by leakage of intermediary metabolites of glucose. Further investigation of this problem is in progress.

DISCUSSION

It has been known for more than 10 years that T5 is unable to replicate in hosts containing the

TABLE 2. *Proline accumulation by infected RM 42, RM 43, and RM 39 in 60 s*[a]

Condition	RM 42-T5 wt[b]	vs	RM 43-T5 wt (3)[c]		RM 43-T5 wt	vs	RM 39-T5 wt (3)
5 min after phage	83 ± 2		78 ± 7		78 ± 7		74 ± 6
10 min after phage	113 ± 15		106 ± 24		106 ± 24		109 ± 21
15 min after phage	123 ± 27	*[d]	46 ± 7		46 ± 7	*	114 ± 26
20 min after phage	105 ± 19	*	37 ± 6		37 ± 6	*	127 ± 15
25 min after phage	133 ± 32	*	30 ± 7		30 ± 7	*	138 ± 48
30 min after phage	132 ± 51	*	27 ± 5		27 ± 5	*	163 ± 55
NaN_3 + uninfected cells	12 ± 8		14 ± 4		14 ± 4		16 ± 8

Condition	RM 43-T5h⁻	vs	RM 43-T5 wt (3)	RM 42-T5h⁻	vs	RM 43-T5h⁻	vs	RM 39-T5h⁻ (4)
5 min after phage	79 ± 10		78 ± 14	79 ± 16		80 ± 6		83 ± 11
10 min after phage	96 ± 6		82 ± 27	104 ± 26		112 ± 27		103 ± 19
15 min after phage	92 ± 16	*	55 ± 11	119 ± 31		97 ± 5		107 ± 11
20 min after phage	83 ± 12	*	53 ± 7	103 ± 25		85 ± 29	*	132 ± 36
25 min after phage	85 ± 9	*	49 ± 5	102 ± 25		79 ± 12	*	130 ± 26
30 min after phage	83 ± 9	*	46 ± 9	124 ± 26	*	78 ± 12	*	135 ± 30
NaN_3 + uninfected cells	32 ± 2		32 ± 2	32 ± 23		25 ± 12		44 ± 9

[a] ^{3}H-labeled proline accumulation by infected cells was determined as outlined in Materials and Methods. The results represent percentages of uptake, relative to that of uninfected control samples, in 60 s.

[b] wt, Wild type.

[c] Number in parentheses indicates the number of experiments done in each group.

[d] *, Difference between the two samples with confidence level $P < 0.05$.

ColIb factor (34, 38). Very little is known, however, about the mechanism whereby phage development is inhibited. Originally, no class II (early) or class III (late) proteins or RNA were seen on polyacrylamide gels. Because of this, it was hypothesized that a class-specific transcriptional block occurred (33). Later, though, it was found that some class II (early) proteins do actually appear. Additionally, functional RNA polymerase can be recovered from abortively infected cells (40), and no DNA template changes can be found that explain the abortive infection (20, 25). Thus, there appears to be no evidence to implicate a primary transcription or translation dysfunction. The finding that T7-infected F^+ hosts develop membrane permeability lesions (8, 13), and the resulting controversy regarding a proposed specific translational block (7, 12, 32), led us to be suspicious of any postulated specific control mechanism. Also, since membrane defects have been found to play key roles in other abortive systems, such as T-even *r*II mutant infections of λ-lysogenized cells (9, 16, 19) and T-phage infection of P2-lysogenized bacteria (17), we decided to look for changes in membrane function during T5 infections of ColIb$^+$ hosts. Previously (10), we observed that uptake of thiomethyl-β-methyl-D-galactoside, a nonmetabolizable lactose analog taken up by an ATP-dependent active transport mechanism, is inhibited during the abortive infection. In the present study we sought to further define the membrane's functional defects by looking at uptake of three additional substances, proline, glutamine, and αMG, each of which is taken up by a different mechanism.

Glutamine uptake is inhibited during abortive infection and begins to decline by 10 min after infection. Since active transport of glutamine is an ATP-dependent process requiring periplasmic binding proteins, decreased uptake ability during abortive infection could be due to a decrease in available ATP, a loss of periplasmic proteins, or marked permeability changes that allow leakage of the glutamine and prevent accumulation against a concentration gradient. The last of these explanations probably cannot totally explain the data, since at a time when glutamine accumulation is markedly depressed, αMG accumulation is three- to fourfold greater than that in uninfected cells. Regarding the loss of binding proteins, the inner membranes of abortively infected cells do not become sensitive to sodium dodecyl sulfate-induced lysis until much later in infection (10), so it is unlikely that the outer membrane integrity is destroyed to an extent necessary for periplasmic proteins to leak, at least by 10 min after infection. We think the most likely explanation for the inhibition of glutamine uptake is that adequate energy is no longer available. Proline uptake, unlike that of glutamine, does not require ATP or periplasmic binding proteins (5, 6). Energy is derived from the energized membrane, and a high-energy phosphorylated intermediate is unnecessary. Proline accumulation decreases during abortive infection at about the same time, or slightly later than the decline of glutamine uptake. Hence, we

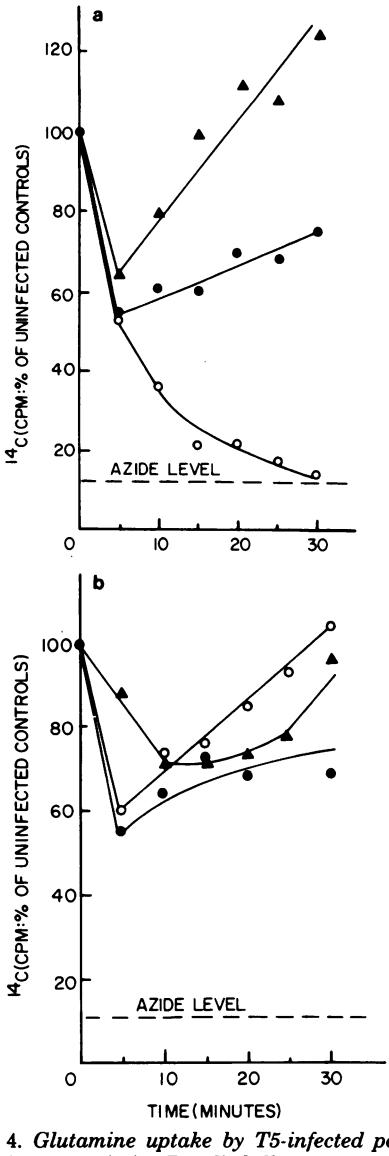

FIG. 4. *Glutamine uptake by T5-infected permissive and nonpermissive E. coli. Cells were grown and infected as detailed in Materials and Methods. At the indicated times, samples were removed and mixed with [^{14}C]glutamine (0.5 μCi/ml, 5 μCi/μmol). At 30, 60, and 90 s thereafter, samples were removed and filtered through glass fiber filters. The results here are for the 90-s pulses, expressed as a percentage of the amount taken up by uninfected controls in the same period of time. (a) T5 wild-type infections of E. coli RM 42, RM 43 (ColIb), and RM 39 (ColIb h$^-$); (b) T5h12$^-$ infections of the same three strains. Symbols are as in Fig. 3.*

feel that the most likely explanation for the inhibition of both proline and glutamine uptake is that membrane energization is inadequate during abortive infection. Further justification of this view is derived from the Mitchell chemiosmotic theory (30).

Recently, the chemiosmotic theory has gained renewed and widespread acceptance for its ability to explain energization of active transport (35). Briefly, the proposal states that the cell stores energy for transport by generating electrical and pH gradients across the membrane (30). Previously, we found that phosphorylated compounds begin to appear in the medium between 10 and 20 min after abortive infection is initiated (10). It is difficult, in terms of Mitchell's theory, to visualize a membrane that is able to maintain electrical and chemical polarization, while at the same time being permeable to phosphorylated compounds. We think it is likely, therefore, that deterioration of both proline and glutamine uptake is due to loss of membrane polarization between 10 and 15 min after abortive infection. Loss of membrane polarization, in turn, might be due either to an inability to couple energy to membrane activation or to a primary permeability defect.

αMG accumulation, unlike proline or glutamine uptake, is markedly enhanced by 5 min after infection of a ColIb-containing host. αMG is taken up by the phosphotransferase system, wherein the sugar is phosphorylated as, or just after (18), it crosses the membrane; an energized (polarized) membrane is not required for its uptake. Since the cell membrane is impermeable to the phosphorylated sugar, it is retained in the cell. The phosphorylation reaction may be followed by an energy-requiring dephosphorylation reaction (22). This converts the αMG-phosphate back into αMG, which can then diffuse out of the cell. Inhibition of this dephosphorylation would have the effect of increasing the amount of αMG retained by the cell. This is apparently the explanation for the increase in αMG uptake caused by colicins K and E1 (24). It is possible, therefore, that the apparent augmentation of αMG uptake ability which we observe reflects an inhibition of the dephosphorylation step. Alternatively, it might be due to a real increase in the rate of sugar uptake. Experiments are in progress to distinguish between these possibilities. What is clear, however, is that the effect cannot be explained by invoking membrane damage. Since the increased uptake of αMG is inhibited by NaF, as is the normal uptake, we feel that a new uptake system is not in operation. After being stimulated very soon after infection of a ColIb$^+$ host, the increase in αMG uptake begins to decline. In order to explain this, we are measuring internal and external αMG-phosphate concentrations at both early and later

TABLE 3. *Glutamine accumulation by infected RM 42, RM 43, and RM 39 in 90 s*[a]

Condition	RM 42-T5 wt[b]	vs	RM 43-T5 wt (4)[c]		RM 43-T5 wt	vs	RM 39-T5 wt (3)
5 min after phage	54 ± 25		50 ± 11		57 ± 3		64 ± 6
10 min after phage	61 ± 33		35 ± 13		37 ± 19	**[a]	80 ± 11
15 min after phage	60 ± 30	*	21 ± 11		21 ± 12	*	100 ± 48
20 min after phage	69 ± 31	*	18 ± 10		25 ± 21	*	112 ± 39
25 min after phage	68 ± 45	*	17 ± 10		14 ± 7	*	108 ± 38
30 min after phage	75 ± 48	*	16 ± 7		12 ± 4	*	125 ± 55
NaN_3 + uninfected cells	17 ± 17		15 ± 3		15 ± 3	*	22 ± 12

Condition	RM 43-T5h⁻	vs	RM 43-T5 wt (4)	RM 42-T5h⁻	vs	RM 43-T5h⁻	vs	RM 39-T5h⁻ (1)
5 min after phage	65 ± 14		56 ± 3	55		60		88
10 min after phage	66 ± 21	*	38 ± 16	64		74		71
15 min after phage	75 ± 16	*	24 ± 12	73		76		73
20 min after phage	71 ± 19	*	25 ± 15	68		85		74
25 min after phage	73 ± 28	*	18 ± 10	77		93		78
30 min after phage	74 ± 28	*	16 ± 8	68		104		96
NaN_3 + uninfected cells	12 ± 5		12 ± 5	2		5		

[a] ^{14}C-labeled glutamine accumulation by infected cells was determined as outlined in Materials and Methods. The results represent percentages of uptake, relative to that of uninfected control samples, in 90 s.
[b-d] See Table 2.

TABLE 4. *αMG accumulation by infected RM 42, RM 43, and RM 39 in 90 s*[a]

Condition	RM 42-T5 wt[b]	vs	RM 43-T5 wt (4)[c]		RM 43-T5 wt	vs	RM 39-T5 wt (3)
5 min after phage	177 ± 40	**[d]	270 ± 41		254 ± 18	*	140 ± 4
10 min after phage	140 ± 12	*	426 ± 72		439 ± 74	*	104 ± 31
15 min after phage	108 ± 19	*	384 ± 70		392 ± 85	*	131 ± 12
20 min after phage	107 ± 20	*	343 ± 26		323 ± 9	*	108 ± 15
25 min after phage	114 ± 18	*	296 ± 52		282 ± 48	*	113 ± 27
30 min after phage	100 ± 28	*	261 ± 33		257 ± 30	*	141 ± 46
NaN_3 and NaF + uninfected cells	25 ± 7		24 ± 14		25 ± 10		18 ± 7

Condition	RM 43-T5h⁻	vs	RM 43-T5 wt (1)	RM 42-T5h⁻	vs	RM 43-T5h⁻	vs	RM 39-T5h⁻ (2)
5 min after phage	202		245	219 ± 6		177 ± 59		137 ± 37
10 min after phage	225		354	137 ± 19		167 ± 17		191 ± 37
15 min after phage	169		313	112 ± 14		211 ± 130		124 ± 15
20 min after phage	164		313	134 ± 24		180 ± 36		108 ± 2
25 min after phage	153		228	118 ± 4		65 ± 13		99 ± 12
30 min after phage	134		260	148 ± 15		157 ± 61		154 ± 20
NaN_3 and NaF + uninfected cells	20		37	18 ± 8		25 ± 6		17 ± 1

[a] ^{14}C-labeled αMG accumulation by infected cells was determined as outlined in Materials and Methods. The results represent percentages of uptake, relative to that of uninfected control samples, in 90 s.
[b-d] See Table 2.

times to determine whether this effect is due to leakage of the phosphorylated sugar. We also plan to look at the concentration of glucose metabolites, both internal and external, to determine whether leakage of these substances could explain why there is no incorporation of glucose-derived carbon into macromolecules.

Amino acid uptake is inhibited and αMG uptake is stimulated during abortive infection, but the observed changes do not occur if the gene *h* mutation, which allows normal phage development, is present on either the phage or plasmid DNA. The presence of membrane alterations, therefore, is correlated with the abortive infection under all conditions examined thus far.

We have, during the course of these studies, been intrigued by the similarities among three apparently unrelated phenomena: (i) the inhibition of bacterial metabolism by colicins E1, K, Ia, and Ib; (ii) the inhibition of bacterial metabolism by phage ghosts; and (iii) the inhibition of phage development during abortive infections of virulent phage in plasmid-containing or lysogenized hosts. A recent paper (36) attributing colicin action to the formation of membrane ion channels fits in very well with our ideas regarding the mechanism of abortive T5 infection. Our belief that these processes are quite similar is certainly not original, as Luria compared their mechanisms as early as 1964 (27). As more data accu-

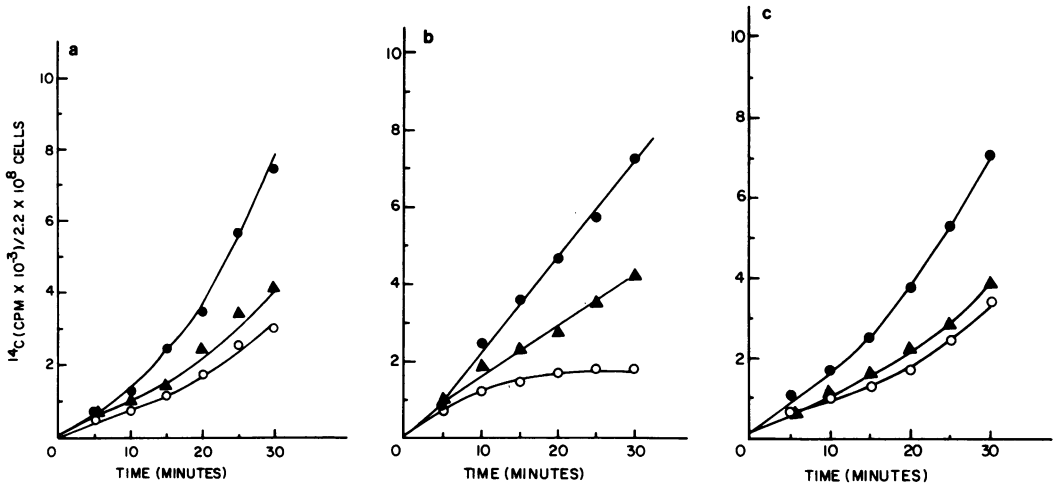

FIG. 5. *Glucose incorporation into trichloroacetic acid-insoluble macromolecules by uninfected and infected E. coli. Cells were grown and infected as detailed in Materials and Methods. [^{14}C]glucose (0.5 μCi/ml, 0.1 μCi/μmol) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% trichloroacetic acid. Acid-insoluble material was collected on glass fiber filters, and the filters were washed. The amount of incorporated radioactivity was determined by liquid scintillation counting. (a) RM 42; (b) RM 43 (ColIb); (c) RM 39 (ColIb h$^-$). Symbols:* (●) *uninfected cells;* (○) *T5-infected cells;* (▲) *T5h12$^-$-infected cells.*

mulate, however, the similarities become more abundant, and the question of why these seemingly dissimilar phenomena occur by a common mechanism becomes even more intriguing.

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