JOURNAL OF BACTERIOLOGY, Mar. 1976, p. 1024-1031

Vol. 125, No. 3
Printed in U.S.A.

Changes in Active Transport, Intracellular Adenosine 5'-Triphosphate Levels, Macromolecular Syntheses, and Glycolysis in an Energy-Uncoupled Mutant of *Escherichia coli*[1]

MICHAEL A. LIEBERMAN AND JEN-SHIANG HONG*

Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

Received for publication 28 October 1975

The temperature-sensitive *Escherichia coli* mutant *ecf*^{ts} *metC** (Lieberman and Hong, 1974), previously shown to be defective in the coupling of metabolic energy to active transport, is also altered in a wide variety of cellular activities at the nonpermissive temperature. These alterations include a lowering of intracellular adenosine 5'-triphosphate levels, an alteration of glucose metabolism such that large quantities of pyruvate and dihydroxyacetone phosphate are excreted into the medium, excretion of accumulated potassium ions, and a cessation of deoxyribonucleic acid, ribonucleic acid, and phospholipid synthesis. Since these effects closely mimic the action of colicins E1 and K on *E. coli* cells, the possibility that the *ecf* gene product is the primary biochemical target for these colicins is discussed.

The bacterial cell membrane plays an important role in a wide variety of cellular functions. In addition to being a selective permeation barrier and the site for oxidative phosphorylation, it has been shown to be involved in deoxyribonucleic acid (DNA) synthesis (16), phospholipid synthesis (9), motility, and chemotaxis (1).

It is therefore conceivable that mutations or agents affecting a specific function of the membrane may also affect other membrane-associated functions, either directly or indirectly. For instance, a mutant of *Escherichia coli* has been reported that is defective in phospholipid synthesis as well as DNA, ribonucleic acid (RNA) and protein synthesis (14), but is normal in active transport. A recent report suggests that this mutant may also harbor a defective adenylate kinase (15). Similar effects are also observed upon attack of colicins K and E1 on sensitive *E. coli* cells. These bacteriocidal proteins inhibit active transport, oxidative phosphorylation, and DNA, RNA, and protein syntheses in addition to other cellular alterations (for review see reference 20).

We have previously reported a temperature-sensitive mutant of *E. coli* (17) that is defective in active transport and presented evidence (M. A. Lieberman and J.-S. Hong, submitted for publication) showing that the defect of the mutant lies in its inability to efficiently energize the membrane. We report here on the effect of this mutation in relation to a number of cellular activities, including DNA, RNA, and phospholipid synthesis, glucose metabolism, and potassium ion retention.

[1] Publication no. 1059 from the Department of Biochemistry, Brandeis University, Waltham, Mass. 02154.

MATERIALS AND METHODS

Media and organisms. Strains used in this work were derivatives of *E. coli* K-12 and are listed in Table 1.

Media used were nutrient broth and minimal salts medium E (23) or medium 56-LP (7) (0.3 mM phosphate), supplemented with 0.5% carbon source and 40 μM thiamine. Methionine was used at 0.4 mM.

Protein determinations. Protein was determined by the method of Lowry et al. (19), using bovine serum albumin as the standard.

Materials. All labeled compounds were obtained from New England Nuclear Corp.

Preparation of colicin E1. The colicin E1-producing strain, Y20, was obtained from S. Luria. Colicin E1 was prepared according to Fields and Luria (13).

Potassium efflux experiments. Cells were grown in the minimal media of Davis and Mingioli (10) except that Na^+ replaced K^+ in all cases. For growth, KCl was added to a final concentration of 1 mM, glucose to 0.5%, thiamine to 40 μM, and, for JSH210, methionine to 0.4 mM. Radioactive potassium (^{42}KCl, 0.13 mCi/mg of K^+) was added to 25 μCi/ml, and the cells were grown at 25 C for at least three generations. The cells were then prepared as described by Feingold (11); cells were spun down, washed once in minimal media lacking K^+, and resuspended in the same media to a cell density of 1.05×10^9 cells/ml. Tubes containing samples (100 μl) of this cell suspension were then incubated for

TABLE 1. *Bacterial strains used*

Strain	Relevant genotype	Comments
JSH4	Hfr *thi metC* ecf^{ts}*	Described in reference 17
JSH210	F$^-$ *thi strA metC*	
MAL300	F$^-$ *thi strA metC* ecf^{ts}*	From JSH210 by transduction with JSH4 as donor
MAL301	F$^-$ *thi strA metC ecf^{ts}*	A class II revertant (17) of MAL300; phenotypically *Suc*$^+$ at 42 C, transport$^+$ at 42 C; lacks cystathionase activity
MAL303	F$^-$ *thi strA ilv metC*	From JSH210 by diethyl sulfate mutagenesis
MAL304	F$^-$ *thi strA ilv metC* ecf^{ts}*	From MAL303 by transduction with JSH4 as donor
MAL308	F$^-$ *thi strA ilv metC ecf^{ts}*	A class II revertant of MAL304
JSH80	Hfr *thi uncA*	Lacking Ca,Mg-activated ATPase activity
MAL310	F$^-$ *thi strA uncA metC*	From MAL303 by transduction with JSH80 as donor, selecting for Ilv$^+$ and picking Suc$^-$ at 25 C
MAL311	F$^-$ *thi strA uncA metC ecf^{ts}*	From MAL308 by transduction with JSH80 as donor, selecting for Ilv$^+$ and picking Suc$^-$ at 25 C
MAL312	F$^-$ *thi strA uncA metC* ecf^{ts}*	From MAL304 by transduction with JSH80 as donor, selecting for Ilv$^+$ and picking Suc$^-$ at 25 C

various times at 25 or 43 C, diluted with 2 ml of minimal media, and filtered through 0.45-μm membrane filters (Millipore Corp.) that had been presoaked in 0.1 M KCl. The filters were dried and counted in a toluene-based scintillation fluid.

ATP measurements. Cells grown in minimal glycerol medium at 25 C were harvested in mid-log phase, washed once, and resuspended in carbon-free salts medium to 2.8×10^8 cells/ml. No carbon source was added. Samples (1.0 ml) were removed from the reaction flask before and at various times after the flask was placed in a 43 C shaking water bath. Adenosine 5'-triphosphate (ATP) was extracted by placing tubes containing the samples in a boiling water bath for 10 min and then assayed, using the luciferin-luciferase assay described by Feingold (11). A Packard Tri-Carb scintillation counter was used, set for 100% gain, 60 to 65 divisions, with the coincidence switch off. Standard ATP was assayed in carbon-free salts medium, and the assay was linear on a double-log plot over an 8- to 80-pmol range. When ATP measurements were made on growing cells, the standard ATP was assayed in the growth medium.

Enzymatic analyses of metabolites. The enzymatic determinations of various extracellular metabolites were done by the methods of Bergmeyer (3) as modified by Fields and Luria (12). All enzymes were obtained from Boehringer Mannheim except for lactate dehydrogenase, which was from Sigma Chemical Co. Samples were obtained as described below. Cells grown in glucose minimal medium were harvested in mid-exponential phase, washed twice with 70 mM potassium phosphate buffer, pH 7.0, and resuspended in the same medium with the addition of 1.0 mM $MgCl_2$ and 50 μg of chloramphenicol per ml. The final cell density was 7×10^8 cells/ml. Three 5-ml samples were then placed in separate 125-ml Erlenmeyer flasks: to one colicin E1 was added; to a second, nothing was added; and the third was heated at 42 C for 15 min and cooled in ice. The three flasks were then placed at 30 C for 5 min, at which time glucose was added to 1 mM final concentration. After 60 min at 30 C, the samples were centrifuged at 4 C at 30,000 $\times$ *g* for 15 min and the supernatants were assayed as described above. All assays were carried out at room temperature.

Net synthesis of DNA, RNA, and phospholipid. The net synthesis of DNA, RNA, and phospholipids was determined by using $^{32}P_i$ (inorganic phosphate) as a common precursor. Exponentially growing cells in 56-LP medium, supplemented with succinate as the sole carbon source, were labeled with $^{32}P_i$ at 10 μCi/ml for at least three generations before a temperature shift from 26 to 38.5 C. All the phenotypic properties associated with the *ecf^{ts} metC** mutations at 42 C, namely cessation of active transport and inability to grow on nonfermentable substrates, are also observed at 38.5 C. At various times before and after the shift, samples (0.8 ml) were removed and added to 3 ml of chloroform-methanol (1:2), vortexed, and allowed to sit overnight at 4 C. Phospholipids, DNA, and RNA were determined as described by Glaser et al. (14).

RESULTS

The temperature-sensitive (ts) mutant JSH4 has been shown to be defective in the active transport of amino acids and β-methylthiogalactoside at the nonpermissive temperature (42 C). The defect was shown not to be due to a lack of Ca^{2+}- or Mg^{2+}-activated adenosine triphosphatase (Ca,Mg-ATPase) activity or to an inability to transfer electrons through the respiratory chain (17). Genetic analysis revealed that two mutations, *ecf^{ts}* and *metC**, were necessary for the defect to be observed, and these mutations were located between 55 and 60 min on the *E. coli* chromosome map (17).

We have recently been able to map the mutations by transduction (Lieberman and Hong, submitted for publication); the mutations are found to be closely linked with the *metC* gene at 58 min on the chromosome map. We have also concluded that the physiological defect is most likely due to a reduced efficiency with which

energy is coupled to active transport. We report here the effect of the mutations on a number of cellular functions, using isogenic strains JSH210 *metC*$^+$ *ecf*$^+$), MAL300 (*metC** *ecf*^{ts}), and MAL301 (*metC ecf*^{ts}).

Intracellular ATP levels. Upon attack by colicin K, sensitive *E. coli* cells exhibit a rapid decrease in their intracellular ATP levels (20). Because the primary effect of colicin K appears to be a de-energization of the membrane, this drop in ATP levels was thought to be due to hydrolysis of ATP by the energy-transducing ATPase (Ca,Mg-ATPase), since the membrane could be re-energized via this route. This was recently demonstrated to be the case by Plate et al. (22), using an *uncA* mutant with which the drop in ATP levels did not occur.

Since our mutant appears to be defective in energizing the membrane, we examined intracellular ATP levels at various times after a shift to the nonpermissive temperature (42 C). We show (Lieberman and Hong, submitted for publication) that intracellular ATP levels rise in MAL300 during the first 10 min after a temperature shift. Since a total transport defect was observed after only a 2-min incubation at 42 C, we concluded that the defect could not be due to a loss of ATP. Here we demonstrate that if the ATP levels are followed for a longer time after the temperature shift, the levels are found to drop (Fig. 1). In these experiments, cells were prepared in exactly the same manner as those prepared for transport assays, namely in the absence of any exogenous energy source. Both JSH210 (*metC*) and MAL301 (*metC, ecf*^{ts}) showed an increase in ATP levels during the first 10 min after the temperature shift, and the levels remained fairly steady for the next 45 min. MAL300 also showed an increase in ATP levels during the first 7 min after the temperature shift, presumably due to the increased metabolic rate at the higher temperature, and the level then remained about the same for approximately 5 more min. However, starting at about 10 to 15 min after the temperature shift, the ATP levels decreased steadily for the next 40 min.

We suspected that the drop in intracellular ATP levels might be due to hydrolysis of ATP by the Ca,Mg-ATPase in a situation analogous to that observed with colicin K. To demonstrate that this might be the case, we constructed isogenic *uncA* strains (deficient in the Ca,Mg-ATPase) and measured their ATP levels after a temperature shift. These strains were MAL310 (*metC, uncA*), derived from the wild-type, MAL311 (*metC, ecf*^{ts} *uncA*), derived from MAL301, and MAL312 (*metC**, *ecf*^{ts}, *uncA*), derived from MAL300. ATP levels in both MAL310 and MAL311 rose to about the same level during the first 7 min after the temperature shift, then decreased steadily, and eventually reached a level 60 min after the shift that was only 50% of the preshift level (Fig. 2). In contrast, the ATP level in MAL312 rose more sharply and reached a higher level in the first 7 min after the temperature shift than was observed in MAL310 and MAL311. Starting at 10 min after the temperature shift, however, the ATP level decreased steadily at a rate comparable to that observed with the control strains.

It is of interest to note that under the conditions we used, namely in the absence of any exogenous energy source, the intracellular ATP

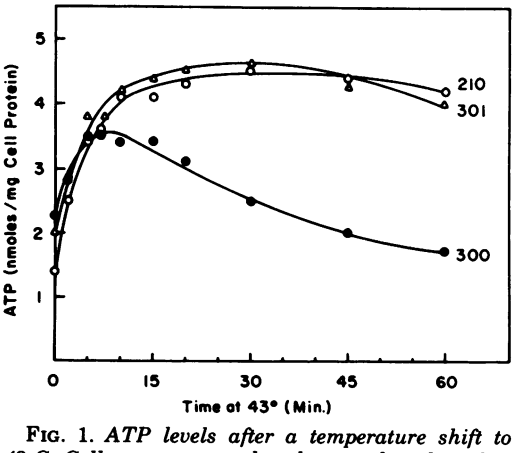

FIG. 1. *ATP levels after a temperature shift to 43 C. Cells were prepared and assayed as described in the text. Symbols: MAL300, ●; MAL301, △; JSH210, ○.*

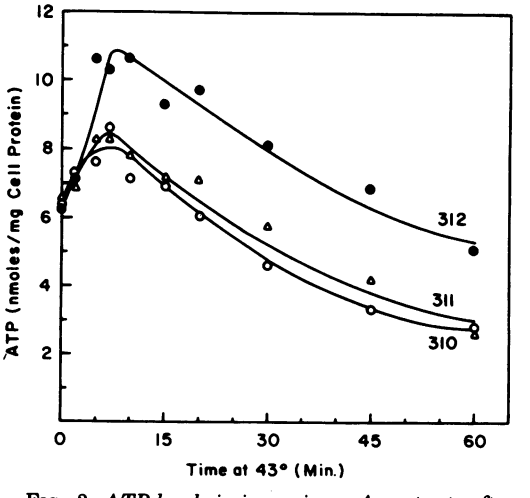

FIG. 2. *ATP levels in isogenic uncA mutants after temperature shift to 43 C. Symbols: MAL310, ○; MAL311, △; MAL312, ●.*

levels in the *uncA* mutants dropped rather rapidly after a shift from 25 to 42 C. This may reflect an intrinsic shortage of energy reserves in these mutants due to their altered physiology. This interpretation is supported by the observation made by Berger (2), that *uncA* mutants were much more easily starved than the wild type.

Because of the high background loss of ATP from the *uncA* strains, it was difficult to observe any loss due to the *metC* ecf*^{ts} mutations (0.2 nmol of ATP/min per mg versus 0.05 nmol of ATP/min per mg). These measurements therefore do not support or refute our contention that the drop in ATP levels is due to ATP hydrolysis by the Ca,Mg-ATPase. However, that is the most likely possibility.

DNA, RNA, and phospholipid synthesis. The effect of the *ecf*^{ts} *metC** mutations upon DNA, RNA, and phospholipid synthesis was examined in MAL300 upon a temperature shift from 25 to 38.5 C with cells growing on succinate as a sole carbon and energy source (Fig. 3). The net synthesis of each component was followed by using $^{32}P_i$ as a common precursor to ensure that there were no transport barriers for specific precursors at the nonpermissive temperature. Cells were grown in the presence of $^{32}P_i$ for at least three generations before the temperature shift. It is evident that within 10 min after the temperature shift, growth and the synthesis of DNA, RNA, and phospholipid had ceased in MAL300. However, the synthesis of these macromolecules and growth in JSH210 continued at an increased rate. It is also clear that there was no net degradation of DNA, RNA, or phospholipid in MAL300 after the temperature shift, since the amount of material produced before the shift remained unchanged.

At present it is not clear whether the effect on macromolecular synthesis is due to a direct effect of the *ecf*^{ts} mutation or a secondary effect due to, perhaps, a drop in intracellular ATP levels or a change in the critical adenylate nucleotide ratios (12).

Colicins K and E1 also inhibit macromolecular synthesis (20), and in the case of colicin K it has been recently shown that the lowering of ATP levels is largely responsible for this inhibition (22). This was demonstrated by using an *uncA* mutation; in the presence of this mutation, the lowering of ATP levels was not observed and the inhibition of macromolecular synthesis by colicin K was essentially lifted.

There have been reports of other mutants of *E. coli* that exhibit a simultaneous loss of DNA, RNA, and phospholipid synthesis (5, 14). One of these mutants (4–6) contained a *ts* adenylate kinase, and upon a shift to the nonpermissive temperature, the intracellular ATP levels in this mutant were found to fall rapidly. This observation led the authors to conclude that either the drop in ATP levels or the change in adenylate nucleotide ratios was responsible for the cessation of macromolecular synthesis.

Another mutant, containing a *ts* glycerol-3-phosphate acyltransferase *(plsA)*, was also shown to be defective in macromolecular synthesis at the nonpermissive temperature (14). A reexamination of this mutant (15), however, revealed that it also possesses a *ts* adenylate kinase, and that ATP levels drop at the nonpermissive temperature as well. These investigators also found that the *ts* mutant reported by Cousin and Belaich (5) also contained a *ts* glycerol-3-phosphate acyltransferase. It therefore appears that the cessation of macromolecular synthesis is closely associated with the general lowering of intracellular ATP levels, as observed in colicin-treated cells, in cells with thermosensitive adenylate kinases, and perhaps in the *metC* ecf*^{ts} mutants.

The *metC* ecf*^{ts} mutant we have isolated is clearly distinct from the glycerol-3-phosphate acyltransferase and adenylate kinase mutants isolated. First and most important, active transport remains normal in *plsA* strains even after 20 min at the nonpermissive temperature (14). Second, *plsA* maps at 13 min (8) on the *E. coli* chromosome map (both the *ts* glycerol-3-phosphate acyltransferase and the *ts* adenylate kinase phenotypes were transferred together in transduction by P1 phage [15]), and the *ecf*^{ts} is at 58 min. Third, adenylate kinase assays in cell-free extracts of MAL300 showed no temperature sensitivity in the enzyme activity (data not shown).

Excretion of glycolytic intermediates. We have already demonstrated that the effect of the *metC* ecf*^{ts} mutations is very similar to that of the action of colicins E1 and K in regard to cessation of macromolecular synthesis and lowering of intracellular ATP levels. Fields and Luria (12) have demonstrated that the addition of colicins E1 or K to sensitive *E. coli* cells, in the presence of glucose, results in the excretion of a wide variety of glycolytic intermediates. The compounds that were excreted in significant amounts were glucose-6-phosphate, pyruvate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, and 3-phosphoglycerate. Because of the marked similarity between colicin action and the effect of the *ecf*^{ts} *metC** mutations, we investigated glucose metabolism in MAL300.

These experiments (Table 2) were done at

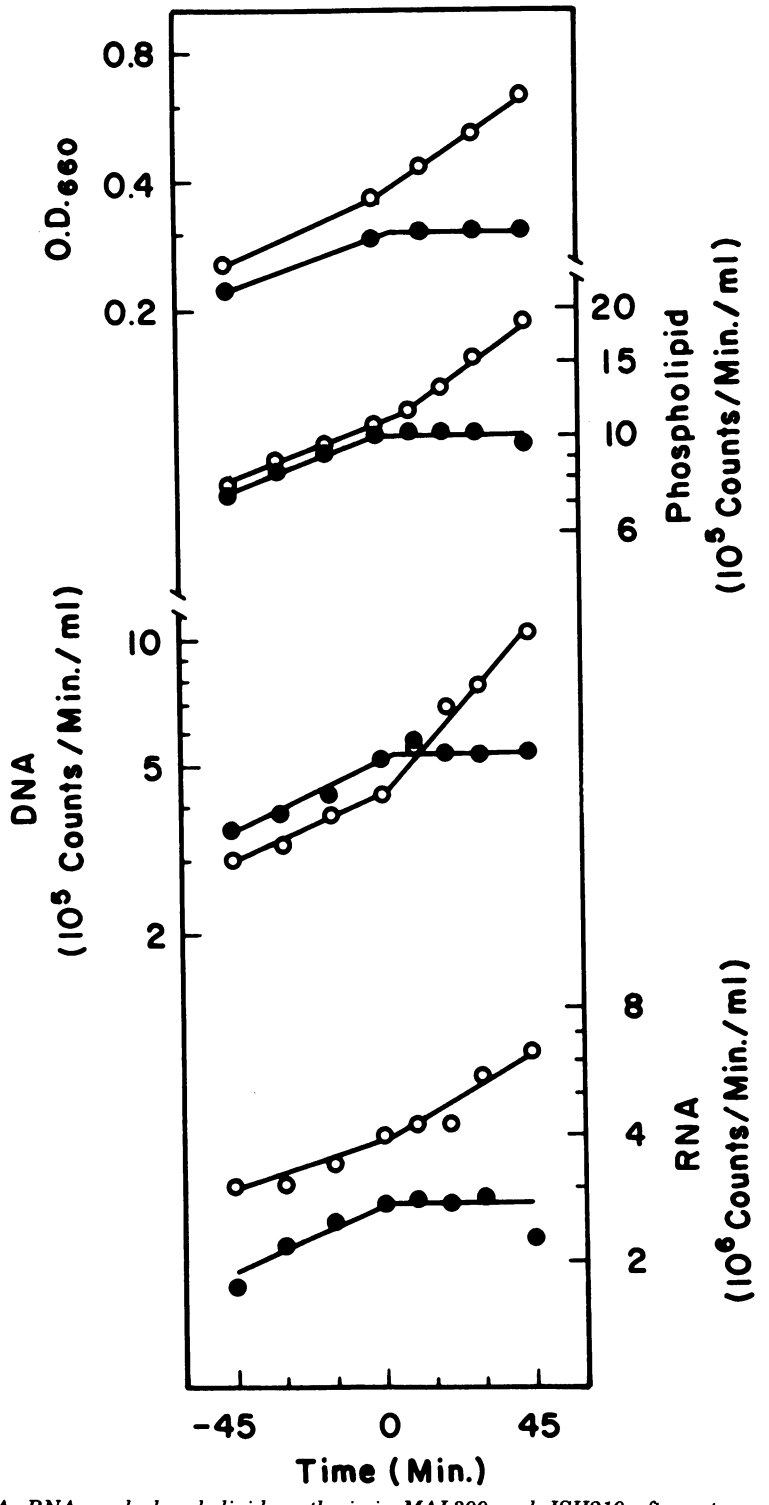

FIG. 3. *DNA, RNA, and phospholipid synthesis in MAL300 and JSH210 after a temperature shift to 38.5 C. Cells were grown and macromolecular synthesis was determined as described in the text. Symbols: JSH210, ○; MAL300, ●.*

TABLE 2. *Excretion of glycolytic intermediates*[a]

Metabolite	JSH210 (wild type) (nmol/ml of supernatant)		MAL300 (mutant) (nmol/ml of supernatant)		
	Nonheated	Heated	Nonheated	Heated	+E1
Dihydroxyacetone phosphate	≤5	≤5	20	73	60
Fructose-1,6-diphosphate	≤5	≤5	≤5	15	16
Fructose-6-phosphate	≤5	≤5	≤5	≤5	11
Glyceraldehyde-3-phosphate	≤5	≤5	≤5	10	≤5
Glucose-6-phosphate	≤5	≤5	≤5	≤5	58
D-Lactate	≤5	≤5	16	13	21
Phosphoenol pyruvate	13	16	13	≤5	10
2-Phosphoglycerate	27	31	30	13	82
3-Phosphoglycerate	11	10	11	≤5	≤5
Pyruvate	15	15	11	120	37

[a] Cells were processed and metabolites were assayed as described in the text. For determination of colicin killing, chloramphenicol was omitted from the assay medium. Survival for colicin E1-treated cells was 0.3%.

30 C, comparing nonheated and heat-inactivated cells as described under Materials and Methods. Colicin E1-treated cells were also examined as a control. Both heated and nonheated wild-type cells (JSH210) excreted a small amount of 2-phosphoglycerate and essentially no significant amount of any of the other compounds assayed. The nonheated mutant cell (MAL300) excreted small quantities of 2-phosphoglycerate and dihydroxyacetone phosphate. After heat treatment, however, large quantities of dihydroxyacetone phosphate and pyruvate were excreted from the mutant. Colicin E1-treated MAL300 (nonheated) cells were found to excrete significant amounts of only pyruvate, 2-phosphoglycerate, dihydroxyacetone phosphate, and glucose-6-phosphate. The amount of colicin-induced excretion observed here was small compared to that found by Fields and Luria (12), but that could be due to strain differences. In fact, we found that the pattern of colicin E1-induced excretion varied from strain to strain (data not shown).

It is clear that both the *ecf* mutation and the action of colicin E1 (or K) lead to excretion of glycolytic metabolites although the excretion is more extensive in both the amount and variety as a result of colicin action than as a result of the *metC* ecf* mutations. The small extent of excretion observed in MAL300 may be due to the fact that the mutant is not totally defective under the conditions used, namely in the presence of glucose. We show (Lieberman and Hong, submitted for publication) that under such conditions re-energization of the membrane could occur, although with a reduced efficiency. It is therefore conceivable that under more severe conditions a more extensive excretion both in amount and variety may be observed.

The mechanisms by which pyruvate and dihydroxyacetone phosphate are excreted from the mutant cells are unknown. The fact that the mutant cells retain normal diffusion barriers for *o*-nitrophenyl-β-D-galactoside (Lieberman and Hong, submitted for publication) and accumulated α-methyl-D-glucoside (17) rules out the possibility that the mutant membrane is grossly altered so as to become freely permeable to charged compounds. Although highly unlikely, it is possible that a selective alteration of the cytoplasmic membrane might occur, allowing these glycolytic intermediates to exit, but not affecting the overall permeability barriers of the cell. Fields and Luria (12) related the loss of glycolytic intermediates by colicin E1 action to a drop in intracellular ATP levels in conjunction with an alteration in the cytoplasmic membrane. This could also be the explanation for the results we obtained with heated MAL300.

It should be noted that, under the conditions we used, essentially all (99.7%) of the colicin-treated cells were killed, whereas at least 30% of the non-colicin-treated cells remained viable. This may also be a factor in the difference in excretion patterns observed between the effects of the mutation and the action of colicin E1.

Potassium efflux. *E. coli* takes in potassium ions by an active process and can accumulate this ion to a concentration gradient of 10,000 or higher (24). Since the uptake process appears to require an energized membrane state (18), the effect of the *ecf*^{ts} *metC** mutations on the efflux of potassium ion was investigated. Cells of JSH210 and MAL300 were preloaded with ^{42}KCl by growing in the presence of this isotope at 25 C for at least three generations. The washed cell suspensions were then incubated at either 25 or 42 C for varying intervals, and the K^+ retained by the cells was determined. At 25 C both the wild type, JSH210, and the mutant, MAL300, retain about 90% of the accumu-

lated K^+ after 30 min (Fig. 4). At 42 C JSH210 gradually lost the accumulated K^+ such that about 50% was lost after 30 min. In contrast, the mutant MAL300 lost accumulated K^+ much more rapidly than JSH210 and retained only about 15% of the loaded K^+ after 30 min at 42 C.

These results demonstrate that the *ecf* mutant cannot retain intracellular potassium ions at the nonpermissive temperature. We have previously demonstrated efflux of accumulated amino acids upon a temperature shift (17) and attributed that to the inability of the mutant to maintain an energized membrane state. Apparently, under the nonpermissive conditions sufficient energy was not available for the retention of accumulated amino acids against a large concentration gradient, therefore resulting in efflux. This is probably the case for K^+. The efflux of K^+ observed here is also similar to the action of colicin K, which has been shown by many investigators to cause a rapid loss of K^+ from attacked cells (11, 21, 25).

DISCUSSION

The data presented in this paper demonstrate that under nonpermissive conditions the *ecf*, *metC** mutations exert, either directly or indirectly, a simultaneous effect on a number of cellular functions: the ability to couple metabolic energy to active transport is abolished, as has been shown earlier (17); the synthesis of DNA, RNA, and phospholipid ceases soon after a shift to the nonpermissive temperature; the cell is unable to retain K^+ ions; intracellular ATP levels, after an initial rise due to the elevation in temperature, decrease steadily; and glucose metabolism is also altered, resulting in the excretion of large quantities of pyruvate and dihydroxyacetone phosphate into the medium.

To our knowledge this is the first instance in which mutational alterations are known to bring about such multiple effects on cellular activities, affecting both energy metabolism and macromolecular synthesis simultaneously. There is precedence, however, for such effects in the lethal action of colicins K and E1 against sensitive *E. coli* cells.

Because of the striking similarity of effects between the *ecf*, *metC** mutations and the action of colicin K (or E1), we propose that the *ecf* gene product, most probably a protein, is the primary biochemical target of these colicins. Thus, at the nonpermissive temperature, our mutant exhibits cellular alterations as if being attacked by colicins. The primary difference so far observed between colicin action and the effects of the mutation is that whereas colicins bring about cell death, the *ecf*^{ts}, *metC** mutations are not lethal.

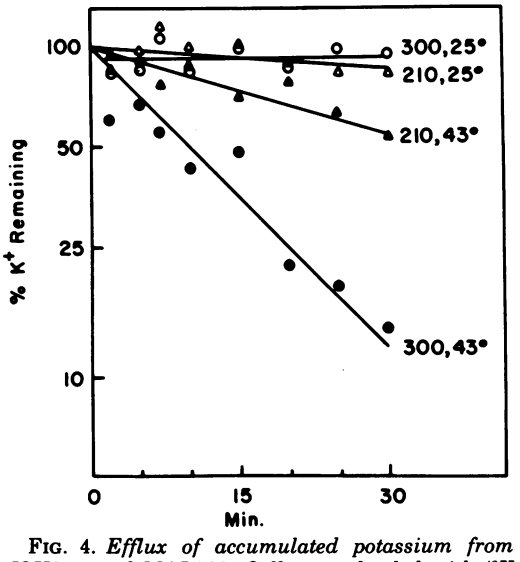

FIG. 4. *Efflux of accumulated potassium from JSH210 and MAL300. Cells were loaded with ^{42}K and efflux was measured as described in the text.*

ACKNOWLEDGMENTS

We thank S. E. Luria for strain Y20 and M. Simon for critical reading of the manuscript.

This work was supported by grant GB40237 from the National Science Foundation, a fellowship from the Medical Foundation, Inc., and Public Health Service research career development award K04-GM-00092 (to J-S.H.) and training grant GM212 (to M.A.L.), both from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Adler, J. 1975.** Chemotaxis in bacteria. Annu. Rev. Biochem. 44:341-356.
2. **Berger, E. A. 1973.** Different mechanisms of energy coupling for the active transport of proline and glutamine in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 70:1514-1518.
3. **Bergmeyer, H. U. (ed.). 1963.** Methods of enzymatic analysis. Academic Press Inc., New York.
4. **Cousin, D. 1967.** Mutants thomosensibles d'*Escherichia coli* K12. II. Etude d'une mutation létale affectant une réaction génératrice d'energie. Ann. Inst. Pasteur Paris 113:309-325.
5. **Cousin, D., and J. P. Belaich. 1966.** Sur une mutation thermosensible d'*Escherichia coli* affectant une fonction énérgetique. C. R. Acad. Sci. 263:886-888.
6. **Cousin, D., and G. Buttin. 1969.** Mutants thermosensibles d'*Escherichia coli* K12. III. Une mutation létale d'*E. coli* affectant l'activité de l'adénylate kinase. Ann. Inst. Pasteur Paris 117:612-630.
7. **Cronan, J. E., Jr., C. H. Birge, and P. R. Vagelos. 1969.** Evidence for two genes specifically involved in unsaturated fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. 100:601-604.
8. **Cronan, J. E., Jr., and G. N. Godson. 1972.** Mutants of *Escherichia coli* with temperature-sensitive lesions in membrane phospholipid synthesis: genetic analysis of glycerol-3-phosphate acyltransferase mutants. Mol.

Gen. Genet. 116:199-210.
9. **Cronan, J. E., Jr., and P. R. Vagelos.** 1972. Metabolism and functions of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta 265:25-60.
10. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine and vitamin B_{12}. J. Bacteriol. 60:17-28.
11. **Feingold, D. S.** 1970. The mechanism of colicin E1 action. J. Membr. Biol. 3:372-386.
12. **Fields, K. L., and S. E. Luria.** 1969. Effects of colicins E1 and K on cellular metabolism. J. Bacteriol. 97:64-77.
13. **Fields, K. L., and S. E. Luria.** 1969. Effects of colicins E1 and K on transport systems. J. Bacteriol. 97:57-63.
14. **Glaser, M., W. H. Bayer, R. M. Bell, and P. R. Vagelos.** 1973. Regulation of macromolecular biosynthesis in a mutant of *Escherichia coli* defective in membrane phospholipid biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 70:385-389.
15. **Glaser, M., W. Nulty, and P. R. Vagelos.** 1975. Role of adenylate kinase in the regulation of macromolecular biosynthesis in a putative mutant of *Escherichia coli* defective in membrane phospholipid synthesis. J. Bacteriol. 123:128-136.
16. **Jacob, F., S. Brenner, and F. Cuzin.** 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. 28:329-348.
17. **Lieberman, M. A., and J.-S. Hong.** 1974. A mutant of *Escherichia coli* defective in the coupling of metabolic energy to active transport. Proc. Natl. Acad. Sci. U.S.A. 71:4395-4399.
18. **Lombardi, F. J., J. P. Reeves, and H. R. Kaback.** 1973. Mechanisms of active transport in isolated bacterial membrane vesicles. XIII. Valinomycin-induced rubidium transport. J. Biol. Chem. 248:3551-3565.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
20. **Luria, S. E.** 1973. Colicins, p. 293-320. *In* L. Lieve (ed.), Bacterial membranes and walls. Marcel Dekker Inc., New York.
21. **Nomura, M., and A. Maeda.** 1965. Mechanism of action of colicins. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. Orig. 196:216-239.
22. **Plate, C. A., J. L. Suit, A. M. Jetten, and S. E. Luria.** 1974. Effects of colicin K on a mutant of *Escherichia coli* deficient in Ca^{2+}, Mg^{2+} activated adenosine triphosphatase. J. Biol. Chem. 249:6138-6143.
23. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli:* partial purification and some properties. J. Biol. Chem. 218:97-106.
24. **Weiden, P. L., W. Epstein, and S. G. Schultz.** 1967. Cation transport in *Escherichia coli*. VII. Potassium requirement for phosphate uptake. J. Gen. Physiol. 50:1641-1661.
25. **Wendt, L.** 1970. Mechanism of colicin action: early events. J. Bacteriol. 104:1236-1241.