

## Effects of Colicins E1 and K on Transport Systems

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The effect of colicins E1 and K on active transport of  $\beta$ -D-galactosides and of  $\alpha$ -methyl-D-glucoside ( $\alpha$ MG) by *Escherichia coli* was studied. These colicins strongly inhibited the accumulation of thio-methyl-galactoside (TMG) by bacteria and caused rapid exit of previously accumulated TMG. The inhibition effect was limited to the accumulation phase of galactoside transport; the rate of hydrolysis of *o*-nitrophenyl galactoside, which is dependent on transport of the substrate by the lactose-permease system, was only slightly affected. The accumulation of  $\alpha$ MG was highly resistant to inhibition by these colicins under conditions which caused complete suppression of TMG accumulation. These effects of the colicins on transport resemble qualitatively those of sodium azide. The findings were interpreted by assuming that colicins E1 and K inhibit the energy-dependent steps in the accumulation of TMG but do not affect facilitated diffusion of galactosides mediated by the specific transport mechanism. The continued accumulation of  $\alpha$ MG was attributed to the fact that this compound is stored by *E. coli* cells as a phosphorylated compound by a phosphoenolpyruvate-dependent transport system rather than by an adenosine triphosphate-linked accumulation mechanism.

Colicins E1 and K are typical of a group of colicins whose action on sensitive bacteria involves a block of biosynthetic processes (8, 16). This block can be reversed by treatment with trypsin, which probably acts by digesting colicin molecules attached to the bacterial surface. Thus, these colicins appear to exert their inhibitory action from the cellular surface, presumably by a reversible effect on the cytoplasmic membrane.

Levinthal and Levinthal (*unpublished data*, cited in 14) studied the action of colicin E1 (Y20) on *Escherichia coli* K-12 strain C600 and concluded that the primary effect responsible for the biosynthetic blocks was an alteration of energy metabolism. Upon adsorption of the colicin, the biosyntheses of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins were arrested simultaneously rather than sequentially, messenger RNA (mRNA) decay was slight, as in the case of *Bacillus subtilis* suddenly deprived of an energy source (13), and adenosine triphosphate (ATP) levels dropped rapidly, although not to zero. Bacteria growing under strictly anaerobic conditions continued to synthesize macromolecular compounds when treated with colicin E1, but biosynthesis stopped immediately upon the introduction of air. The finding that air sensitized the colicin-treated bacteria suggested a specific

effect on aerobic metabolism, perhaps on oxidative phosphorylation, whose enzymatic machinery in bacteria is probably located in the cytoplasmic membrane (*see* 15).

Luria (14) found that colicins E1(ML) and K(K235) inhibited the accumulation of substrates by several specific "permease" systems (2) and interpreted this inhibition in terms of the effects on energy metabolism observed by Levinthal and Levinthal. Similar effects on substrate accumulation were reported by Nomura and Maeda (18).

It has been pointed out, especially by Nomura (16, 17), that studies on colicins may offer an effective approach to the investigation of the functional organization of the envelope of bacteria. The findings presented in this paper revealed a selective inhibition by colicins E1 and K of specific steps in substrate accumulation. These findings, interpreted in terms of current hypotheses of permease function (7, 9, 22), led to further studies on the effects of the colicins on aerobic catabolism of *E. coli*, the results of which are presented in a separate article (6). A third article (5) summarizes results of a comparison between colicin action and abortive phage infection.

### MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this work are listed in Table 1. Cells were usually grown in flasks with vigorous shaking. Growth was followed by

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TABLE 1. *Bacterial strains*

Luria stock collection no.	Strain	Relevant genotype <sup>a</sup>	Source
L-A279a	<i>E. coli</i> K-12 3000	Hfr Hayes <i>thi lac I<sup>+</sup> lac Z<sup>+</sup> lac Y<sup>+</sup></i>	J. Monod
L-A153	3300	Hfr Hayes <i>thi lac I<sup>-</sup> lac Z<sup>+</sup> lac Y<sup>+</sup></i>	J. Monod
L-104	C600	F <sup>-</sup> <i>thi thr leu lac I<sup>+</sup> lac Z<sup>+</sup> lac Y<sup>-</sup></i>	
L-217	200P	F <sup>-</sup> <i>thi thr leu lac I<sup>+</sup> lac Z<sup>+</sup> lac Y<sup>+</sup> str-r</i>	J. Monod
L-A218	200R	F <sup>-</sup> <i>thi lac I<sup>+</sup> lac Z<sup>+</sup> lac Y<sup>-</sup> str-r</i>	J. Monod
L-A633	Y20(E1)	F <sup>-</sup> <i>thi thr leu</i> (Col E1-K30)	F. Levinthal
L-28	Other bacteria <i>E. coli</i> K 235	Prototroph (Col K)	P. Frédéricq

<sup>a</sup> Symbols: *lac* = lactose; *leu* = leucine; *str-r* = streptomycin-resistant; *thi* = thiamine; *thr* = threonine.

measuring the optical density (OD) at 500 nm in a Zeiss spectrophotometer; 1 OD unit corresponds to about  $5 \times 10^8$  cells/ml for *E. coli* K-12 derivatives.

**Media.** Medium 63, containing  $10^{-1}$  M potassium phosphate (pH 7.0), 0.2% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and  $10^{-3}$  M MgSO<sub>4</sub>, was the basic minimal medium. Minimal agar contained medium 63 and 1.5% agar. Minimal medium was supplemented with 1 μg of thiamine per ml for most K-12 strains and with L-amino acids (20 μg/ml) as required. Carbon sources were used at a concentration of 0.4% unless otherwise noted.

Medium 121 (4) contained 0.12 M tris(hydroxymethyl)aminomethane (Tris; Sigma 121), 0.08 M NaCl, 0.02 M KCl,  $10^{-3}$  M MgCl<sub>2</sub>,  $2 \times 10^{-4}$  M CaCl<sub>2</sub>,  $2 \times 10^{-4}$  M FeCl<sub>3</sub>,  $2.5 \times 10^{-3}$  M Na<sub>2</sub>SO<sub>4</sub>, and  $10^{-3}$  to  $8 \times 10^{-3}$  M KH<sub>2</sub>PO<sub>4</sub>; the pH was adjusted to 7.5 with HCl.

**Preparation and assay of colicins.** Colicin E1 was prepared from strain Y20 (col E1). Cultures were grown to  $2 \times 10^8$  cells/ml in medium 121 (supplemented with thiamine, threonine, leucine, and glucose), chilled, and irradiated with ultraviolet light (2 min at 50 cm from a 15-w GE Germicidal lamp, in thin liquid layers). The irradiated cultures were shaken for 5 hr and then chilled. The cells were collected, washed twice in TM buffer ( $10^{-3}$  M Tris,  $10^{-4}$  M Mg<sup>++</sup>, pH 7.4), suspended in 30 ml of TM buffer per liter of cells, and frozen. The next day the cells were broken in a French press and centrifuged for 30 min at  $120,000 \times g$  in a Spinco model L ultracentrifuge. The supernatant fluid was dialyzed for periods of 1 hr each against three 1-liter volumes of TM buffer and was frozen in small portions. Each sample was thawed when needed and was not refrozen more than a few times to prevent loss of titer. Such colicin E1 preparations contained up to  $8 \times 10^{13}$  killing units/ml.

Colicin K was prepared from strain K235 grown to an OD of 0.4 in medium 63 plus 0.2% glucose. The cultures were concentrated 15-fold, irradiated for 1 min, diluted to the original volume, grown for 3 hr, collected by centrifugation, and concentrated 50-fold in TM buffer. The cells were broken by sonic treatment at 10 kc for 75 sec in an MSE ultrasonic disintegrator. The suspension was centrifuged for 15 min at  $12,000 \times g$ , and the supernatant fluid was frozen. Titters of about  $10^{13}$  killing units/ml were obtained.

Killing titers were determined by incubating growing cells of a sensitive bacterial strain with different dilutions of colicin preparation for 5 or 10 min at 37 C. Each sample was then assayed for viable bacteria. The number of killing units was calculated from the multiplicity *m* of killing units obtained from the equation  $B/B_0 = e^{-m}$ , where  $B/B_0$  is the survival ratio.

**Galactosidase assay.** β-D-Galactosidase was induced by addition of  $5 \times 10^{-4}$  M isopropyl-thio-β-D-galactoside (IPTG) to cells growing in medium 63. Unless stated otherwise, the carbon source was 0.4% glycerol. Galactosidase was estimated from the rate of hydrolysis of *o*-nitrophenyl-β-D-galactoside (ONPG) by toluene-treated extracts of growing cells according to the method of Revel et al. (19). When hydrolysis by *in vivo* hydrolysis by intact cells, extracts were prepared in medium 63.

**Galactoside permease assays.** β-D-Galactoside permease was assayed by three methods. The first method measured the accumulation of <sup>14</sup>C-methyl-thio-β-D-galactoside (TMG). <sup>14</sup>C-TMG was added to growing cells which had been incubated with 100 μg of chloramphenicol per ml and treated as needed for each experiment. Samples (1 or 0.5 ml) were removed at intervals, added to 8 ml of chilled medium 63 in a precooled filter funnel, and washed with three 4-ml portions of chilled medium within about 20 sec. The filter was dried and counted. Background was measured by the addition of <sup>14</sup>C-TMG to cells and medium precooled in the filter apparatus, followed by immediate filtration.

Galactoside permease was also tested by measuring the rate of hydrolysis of ONPG by intact cells (10). Cells were treated with colicin or buffer in the presence of chloramphenicol, and 0.5 ml of cells was added to 0.5 ml of growth medium in a cuvette in a Zeiss spectrophotometer. ONPG ( $3 \times 10^{-3}$  M in 0.25 M sodium phosphate buffer, pH 7.0) was added, and the production of *o*-nitrophenol (ONP) was measured at 30- or 60-sec intervals by readings of the OD at 420 nm.

Hydrolysis of the fluorogenic substrate fluorescein-di-(β-galactopyranoside) (FDG) was also employed (20). Cells were diluted in medium 63 to an OD of 0.03,

and  $4 \times 10^{-4}$  M FDG was added. Colicin E1 or K was added directly to the tube containing cells and substrate, and readings in a Turner fluorometer were continued for 20 min at 37 C.

IPTG, ONPG, and TMG were products of Mann Research Laboratories, Inc., New York, N.Y.,  $^{14}\text{C}$ -TMG was from New England Nuclear Corp., Boston, Mass., FDG was a gift of Boris Rotman, and chloramphenicol was provided by Parke, Davis & Co., Detroit, Mich.

**$\alpha$ -Methylglucoside accumulation.**  $\alpha$ -Methyl-D- $^{14}\text{C}$ -glucoside ( $\alpha\text{MG}$ ) accumulation was measured in glucose-grown cells which had been washed, resuspended in medium 63 containing 50  $\mu\text{g}$  of chloramphenicol per ml without an added carbon source, and kept at 4 C until used.  $^{14}\text{C}$ - $\alpha\text{MG}$  was added at a final concentration of  $2 \times 10^{-4}$  M (0.02  $\mu\text{c}/\text{ml}$ ), and samples were collected on filters, washed, dried, and counted as described for TMG. Nonradioactive  $\alpha\text{MG}$  was from Mann Research Laboratories, and  $^{14}\text{C}$ - $\alpha\text{MG}$  was from Calbiochem, Los Angeles, Calif. In some experiments (see Table 2), TMG accumulation was also measured under the same conditions used for measurement of  $\alpha\text{MG}$  accumulation.

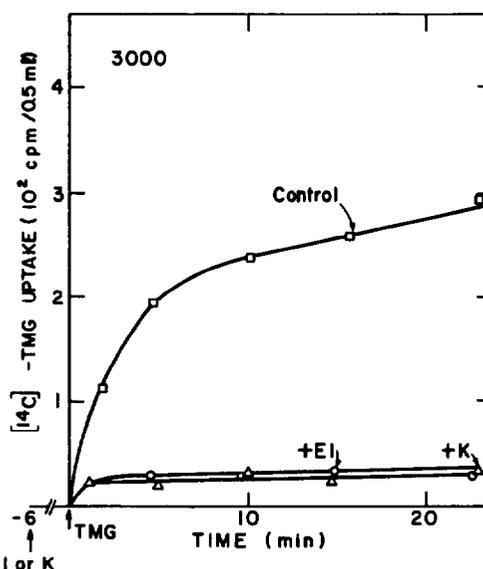
**Measurement of ATP.** ATP was assayed by the method described by Strehler and Totter (21) as modified by Ward and Holt (personal communication). Dried firefly lanterns (FLE-50; Sigma Chemical Co., St. Louis, Mo.) were reconstituted to 10 mg/ml in buffer containing 0.02 M  $\text{MgSO}_4$  and 0.05 M potassium arsenate (pH 7.4). The extract was centrifuged at  $12,000 \times g$  at 4 C for 35 min, and the supernatant fluid was used for the assays.

Samples prepared by adding 0.5 ml of cell suspension (about  $10^8$  cells) to 4.5 ml of water were immediately placed in a boiling-water bath for 10 min. To measure ATP in the medium, the cell suspension was first centrifuged or filtered, and the supernatant fluid or filtrate was diluted and boiled. Boiling did not affect standard ATP solutions, but filtration resulted in about 20% loss of activity.

The assays were done in a Beckman DU spectrophotometer, measuring total light emitted. First, 0.2 ml of enzyme extract was mixed with 0.4 ml of water for 15 sec; then 0.2 ml of the boiled sample was quickly added to the cuvette. Since the amount of emitted light declined exponentially with a half-time of about 30 sec, a single reading was taken exactly 15 sec after addition of the sample. Samples of  $10^{-7}$  M ATP gave readings between 100 and 200 arbitrary units, depending on the enzyme preparation.

## RESULTS

**Uptake of TMG.** Luria (14) and Nomura and Maeda (18) reported that colicins K and E1, but not E2 or E3, inhibit accumulation of various substrates. Figure 1 illustrates the effect of E1 and K on the accumulation of TMG by IPTG-induced cells of *E. coli* strain 3000. Identical results were obtained with strain 3300 (constitutive for the lactose operon) grown either with or without inducer, as well as with several other



E1 or K

FIG. 1. Effect of colicin E1 or K on TMG accumulation in *E. coli* 3000. Cells growing in medium 63 with 0.4% glycerol and  $5 \times 10^{-4}$  M IPTG received 100  $\mu\text{g}$  of chloramphenicol per ml, and colicin E1, colicin K, or buffer. After 6 min,  $^{14}\text{C}$ -TMG was added (1  $\mu\text{c}/\text{ml}$ ,  $2 \times 10^{-3}$  M final concentration). Samples of 0.5 ml were taken at intervals, and the uptake of TMG was measured by filtration. The uptake values are corrected for background (23 counts/min). Survival measured at zero time was 0.02% for colicin E1 and 0.002% for colicin K.

strains of *E. coli*. The inhibition was complete when high multiplicities of colicin were adsorbed, and even with low multiplicities (4% survival) there was at least 70% inhibition.

The effect of colicins on accumulation of TMG could be demonstrated also by adding them to bacteria that had previously accumulated the substrate (see Fig. 4A). Colicin caused a rapid release of TMG from the cells.

Two derivatives of *E. coli* K-12, called 200P and 200R, which are genetically permease-negative (*lacY*<sup>-</sup>), exhibited a slow linear rate of uptake of radioactive TMG, whether grown with or without IPTG as inducer. This uptake was halted by colicins K or E1, but the intracellular TMG was not released from the cells. It is uncertain whether this represents an uptake of TMG by some nonspecific transport system or the uptake of some radioactive impurity present in the commercial  $^{14}\text{C}$ -TMG employed in the present work.

**Effect of colicins on the rate of ONPG and FDG hydrolysis.** The course of hydrolysis of ONPG by intact *E. coli* cells of the *lac* constitutive strain 3300 *lacI*<sup>-</sup> is shown in Fig. 2. Control

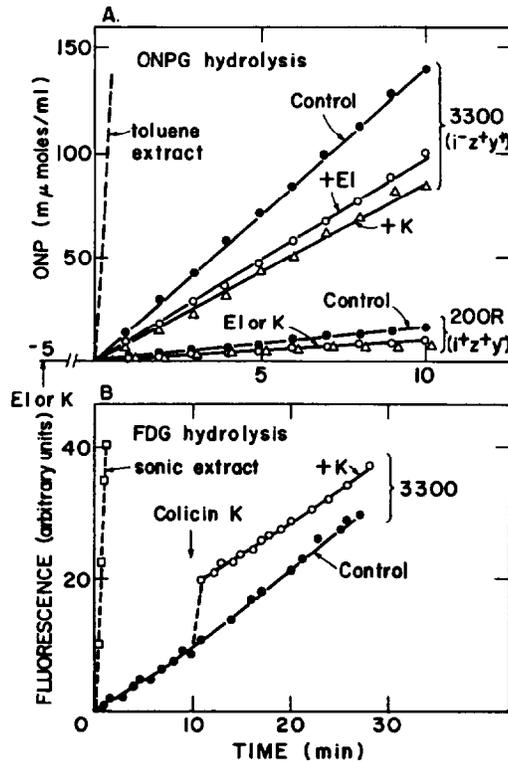


FIG. 2. Effect of colicin E1 or colicin K on ONPG or FDG hydrolysis by *E. coli* K-12. (A) ONPG hydrolysis. Strain 3300 was grown in medium 63 with 0.4% glycerol, chloramphenicol was added at 100  $\mu$ g/ml, colicin E1 or K was added, and after 5 min ONPG hydrolysis and survival were measured. For strain 200R, the same medium plus IPTG was used. Survival was 0.03% or less with either colicin. A sample of the 3300 culture was used to prepare a toluene extract. The ONPG hydrolysis is expressed as nanomoles of ONP produced per milliliter of suspensions normalized to an initial OD at 500 nm of 0.2 to permit comparison between different cell suspensions and with the toluene extract. (B) FDG hydrolysis was measured as described in Materials and Methods with cells of strain 3300 in medium 63-glycerol. Colicin K was added directly to the fluorometer cuvette. The jump upon addition of colicin is due to fluorescent impurities in the colicin K preparation. Survival was 5% with colicin K. A sonic extract of the same culture was used to assay the total  $\beta$ -galactosidase level.

cells hydrolyzed ONPG at a rate 18 times slower than a similar amount of toluene-deoxycholate extract. Colicin E1 or K reduced the rate of hydrolysis by about 30%. Analogous results were obtained with IPTG-induced cells of *E. coli* strain 3000.

Likewise, the rate of hydrolysis of the fluorogenic galactosidase substrate FDG by intact cells

was reduced by about 20% (Fig. 3). The fact that the rates of hydrolysis were reduced rather than increased clearly indicates that these colicins do not simply disrupt the permeability barrier of the cells.

The limited effect of colicins E1 and K on the hydrolysis of substrates of galactosidases stands in sharp contrast to their drastic action on the accumulation of TMG and of substrates of other permease systems. A plausible explanation is provided by the model of permease action proposed by Koch (10) and by Fox and Kennedy (7),

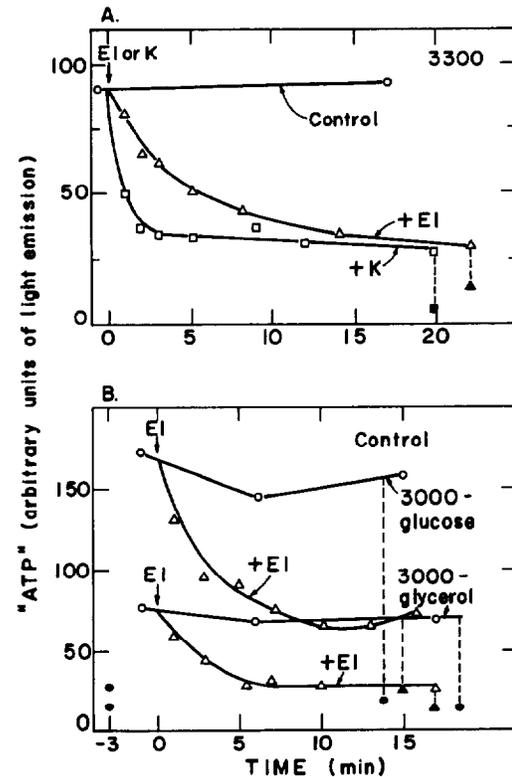


FIG. 3. Effect of colicins E1 and K on ATP levels in 3300 and 3000. (A) Strain 3300 was grown in medium 63-glycerol, and at zero time cells were removed from the control culture and added to colicin E1 or colicin K. ATP was determined at intervals in the total culture (open symbols) and at the end of the experiment in a sample of the filtered medium (closed symbols) by means of the firefly assay. Survival after 4 min: colicin E1, 0.7%; colicin K, 0.07%. (B) Strain 3000 was grown in medium 63 with glucose (upper curves) or with glycerol (lower curves) and was treated with colicin E1. ATP was then measured in the total culture (open symbols) or in the supernatant fluid of centrifuged samples (closed symbols). Survival at 5.5 min: glucose-grown cells + E1, 7%; glycerol-grown cells + E1, 5%.

according to which the galactosidase permease system consists of a facilitated transport component which specifically carries galactosides into the cell in an energy-independent reaction, and of an energy-dependent system which permits accumulation of nonutilized substrates. If colicins E1 and K act by interfering with energy metabolism, they would be expected to prevent accumulation of galactosides by the energy-dependent system, but not to affect the facilitated transport of galactosides and the subsequent hydrolysis of the hydrolyzable ones by galactose-positive cells.

The findings with colicins, interpreted in terms of a selective interference with the energy-dependent component of the permease system, are in good agreement with the findings of Koch (10) and Boniface and Koch (1) on the effects of sodium azide on ONPG hydrolysis (24 to 28% reduction) by cells of *E. coli* ML 308 grown on succinate; *E. coli* K-12 was reported to behave similarly.

The partial reduction of the rates of hydrolysis by colicin-treated cells suggests that in bacteria with a full complement of galactosidase the facilitated transport system is inadequate to maintain saturation of enzyme with substrate and that the energy-dependent accumulation system contributes in some measure to the normal rate of hydrolysis in vivo.

The effect of colicin E1 on the rate of ONPG hydrolysis by *E. coli* strain Co270 and by *Shigella dysenteriae* Sh(F-lac) was also studied (5) and proved to be even less than in *E. coli* K-12 derivatives, suggesting that in these organisms the energy-dependent accumulation mechanism contributes less to saturating the cellular galactosidase. TMG accumulation was fully inhibited by colicin E1 in these bacteria.

**Effect of colicins on ATP levels.** Levinthal and Levinthal first observed a rapid decrease of ATP in cells of *E. coli* K-12 strain C600 treated with colicin E1; ATP disappearance, however, was never complete. These findings were confirmed in the present study, of which typical results are shown in Fig. 3. The decrease in ATP was faster when the killing multiplicity of colicin was higher. Note that the residual ATP levels represented intracellular rather than extracellular ATP.

It should be pointed out that the firefly assay for ATP used in this work is not strictly specific. Levinthal and Levinthal, however, demonstrated by electrophoresis that some ATP remained present after colicin E1 treatment, a finding that was also confirmed in the present work.

**Effect of colicins on accumulation of  $\alpha$ -methylglucoside.** The hypothesis that the sharp decrease in accumulation of TMG by colicin-treated cells reflected a reduced ATP level led us to consider

the possible effects of colicins E1 and K on the accumulation of  $\alpha$ MG, a substance whose accumulation is mediated by a phosphoenolpyruvate (PEP)-dependent phosphotransferase system (9, 11, 12). Glucose metabolism by colicin-treated cells continues through normal pathways as far as pyruvate (6). Hence, PEP should be available for the  $\alpha$ MG transferase reaction.

Typical findings, illustrated in Fig. 4, showed

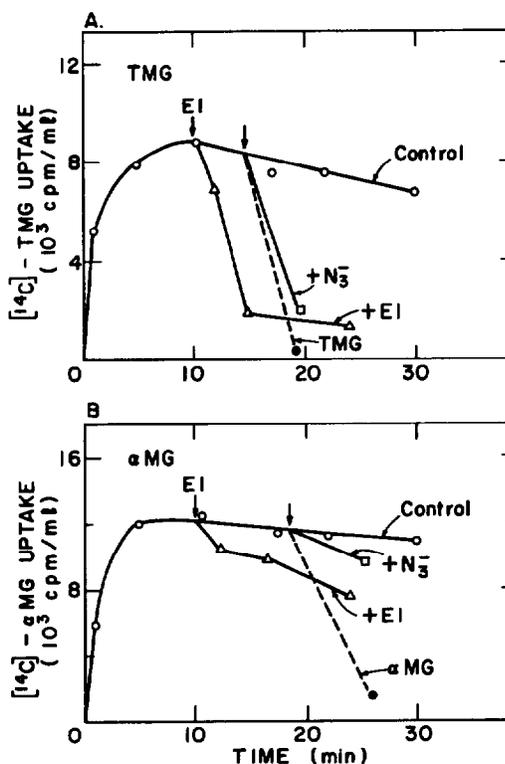


FIG. 4. Effect of colicin E1 on accumulated TMG and  $\alpha$ MG. *E. coli* 3000 was grown in medium 63 with 0.2% glucose and  $10^{-3}$  M IPTG to  $6 \times 10^8$  cells/ml. The cells were chilled, washed in the cold, and resuspended to an OD at 500 nm of 1 in medium 63 with 50  $\mu$ g of chloramphenicol per ml but with no added carbon source. The procedure for parts A and B was the same. Cells were warmed to 37 C for 10 minutes, and the  $^{14}\text{C}$ -labeled compound was added. (Part A: TMG, final concentration 0.5  $\mu$ g/ml,  $10^{-3}$  M. Part B:  $\alpha$ MG, 0.02  $\mu$ g/ml,  $2 \times 10^{-3}$  M). At the indicated times, portions of the control were added to flasks containing colicin E1,  $10^{-3}$  M  $\text{NaN}_3$ , or excess ( $10^{-3}$  M) unlabeled glycoside. TMG or  $\alpha$ MG accumulation was measured in the usual way except that the samples were counted in a scintillation counter. Survival after 3.5 min of colicin treatment was 0.3% in part A and 0.03% in part B. Cell samples without added glycoside were also treated with colicin, and uracil incorporation was measured. In both parts A and B, colicin E1 reduced incorporation by 98%.

that colicin E1 had a much less pronounced effect on the accumulation of  $\alpha$ MG than on that of TMG. The effects of colicin E1 closely resembled those of  $\text{NaN}_3$ . An excess of unlabeled  $\alpha$ MG did chase accumulated  $^{14}\text{C}$ - $\alpha$ MG quite effectively, an indication that intracellular  $\alpha$ MG was in fact available for exchange with the external medium.

Table 2 shows a series of measurements made with various combinations of colicins and metabolic inhibitors. Colicin K behaved like colicin E1. The most interesting result was the effective synergism of colicin plus NaF, comparable to the synergism between  $\text{NaN}_3$  and NaF (9). Fluoride is thought to inhibit PEP production by acting on enolase; the incomplete effect of NaF alone suggests that the PEP level may be replenished by some other reaction, probably from pyruvate and ATP (3). This second source of PEP would be eliminated by the effect of colicin or azide on ATP levels.

### DISCUSSION

The evidence suggests that the effects of colicins E1 and K on transport systems, first reported by Luria (14) and Nomura and Maeda (18), are due to effects on energy metabolism. Facilitated diffusion is not blocked nor is the membrane disrupted. Accumulation is strongly inhibited only for certain substances, whose

accumulation presumably involves reactions dependent on high ATP levels.

The low residual accumulation of TMG by cells treated with low multiplicities of colicin might reflect a limited accumulation by systems such as the PEP-dependent phosphotransferase. This may also account in part for the low level of accumulation of TMG by *lacY*<sup>-</sup> bacterial strains.

The partial inhibition of  $\alpha$ MG accumulation by colicins (and by  $\text{NaN}_3$ ) observed in some experiments suggests that the PEP level may be reduced by these inhibitors. As discussed in the following paper (6), this is probably true for colicin E1 acting on glucose-degrading cells, since this colicin causes an excretion of some of the intermediates between glucose and PEP. As already mentioned, the synergistic effect with NaF suggests that colicins, as well as  $\text{NaN}_3$ , may prevent regeneration of PEP from pyruvate and ATP.

In summary, all findings on colicin effects on transport agree with the thesis that colicins E1 and K act by lowering the ATP levels in the cells. An intriguing aspect of this action is that the inhibition of many functions, including TMG accumulation, is more complete than the reduction in ATP levels. Possible interpretations will be discussed in the following article (6).

TABLE 2. Effect of colicins and inhibitors on  $\alpha$ MG or TMG accumulation by *E. coli* 3000<sup>a</sup>

Expt	Treatment	Amt accumulated (counts/min)	
		$\alpha$ MG	TMG
1. Chase	Control	11,500	7,550
	Colicin E1 added at 10 min	9,000	150
2. Pretreatment	Control	9,280	5,600
	Colicin E1, 5 min before substrate	7,160	210
	Colicin K, 5 min before substrate	5,730	420
	$\text{NaN}_3$ ( $10^{-3}$ M), 30 min before substrate	3,520	445
	Background	36	116
3. Pretreatment	Control	2,400	
	Colicin E1, 5 min before substrate	2,100	
	$\text{NaN}_3$ ( $10^{-3}$ M), 5 min before substrate	2,800	
	$\text{NaN}_3$ and E1, 5 min before substrate	2,600	
	KF ( $5 \times 10^{-3}$ M), 5 min before substrate	400	
	KF and E1, 5 min before substrate	<100	
	$\text{NaN}_3$ and KF, 5 min before substrate	<100	

<sup>a</sup> Cells of *E. coli* 3000 were grown and treated as described for Fig. 4. Experiment 1 was a chase experiment like that of Figure 4; the cell-associated radioactivity was tested 10 min after addition of colicin. In experiments 2 and 3, cells pretreated in various ways for 10 or 5 min received  $^{14}\text{C}$ -TMG or  $^{14}\text{C}$ - $\alpha$ MG; samples for assay were taken 10 min after addition of the labeled compounds and were counted in a scintillation counter. Survival after colicin E1 treatment was less than 0.1%.

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