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Altered Hexose Transport and Salt Sensitivity in Cyclic Adenosine 3',5'-Monophosphate-Deficient *Escherichia coli*[1]

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A cyclic adenosine 3',5'-monophosphate (cAMP)-deficient mutant strain of *Escherichia coli* K-12 was studied to determine the effect this cyclic nucleotide has on the overall growth and metabolism of this organism. Deficient cells were found to be more susceptible to growth inhibition by salts than were their cAMP-sufficient counterparts. The deficient cells transported α-methylglucoside by passive diffusion, whereas the parental cells or mutant cells grown in the presence of exogenous cAMP were able to take up α-methylglucoside by the normal active transport process. When viewed together with earlier studies conducted on cAMP-deficient cells, these findings support the view that cAMP plays a key role in regulating the construction and operation of the *E. coli* membrane system.

Although a number of cyclic 3',5'-adenosine monophosphate (cAMP)-requiring mutants of *Escherichia coli* have been isolated in recent years (5, 9, 13, 17), the overall physiological and morphological consequences of a cAMP deficiency in this organism have not been fully evaluated. It is known that such mutant cells are devoid of flagella (17) and are unable to grow on substrates whose dissimilation requires the production of catabolite-repressible enzymes because these particular enzymes are not synthesized to any appreciable extent in the absence of cAMP (7). It is also known that these mutants are able to grow on substrates such as glucose or fructose in the absence of cAMP, but that their rate of growth on these substrates is considerably lower than the rate observed with either cAMP-supplemented mutant cells or the corresponding parental strains (9). This latter observation raises the possibility that cAMP is involved in influencing other metabolic functions in *E. coli* besides those involved in catabolite repression.

The data presented in this report show that cAMP-deficient cells are sensitive to salt inhibition and have an impaired hexose transport system. These findings coupled with earlier reports have led to the conclusion that cAMP plays a key role in regulating the construction and operation of a number of membrane functions in *E. coli*. Also described in this report are some of the general characteristics associated with a cAMP deficiency in *E. coli*.

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MATERIALS AND METHODS

Bacterial strains. The parental, wild-type strain used in this study was *E. coli* K-12 (701) obtained from R. J. White (15). The cAMP-deficient mutant, designated *E. coli* strain C-57, was isolated in our laboratory from the K-12 parent by P. Goldenbaum, using the procedure described by Perlman and Pastan (9). The mutant was unable to produce detectable levels of cAMP as determined by using the Schwarz/Mann (Schwarz/Mann, Orangeburg, N.Y.) cAMP radioimmunoassay kit, and as determined by D. Namm (Burroughs-Wellcome Co., Research Triangle, N.C.) by the phosphorylation assay for cAMP according to Wastila et al. (14). cAMP production by these cells was also determined by using the cAMP assay kit produced by the Amersham/Searle Corp. (Arlington Heights, Ill.). Cultures for these assays were grown on glucose and assayed for 30 to 70 min after entry into stationary phase (10). The methods used for these assays and for measurement of adenyl cyclase (EC 4.6.1.1) and cAMP phosphodiesterase (EC 3.1.4.17) in these cells is described elsewhere (W. S. Dallas, and W. J. Dobrogosz, manuscript in preparation). Essentially no cAMP was found in cells or culture filtrates of strain C-57. High levels of cAMP were detected in cell filtrates of K-12 cultures, and these cells had 3.2 μM levels of intracellular cAMP. The specific activities (picomoles of cAMP formed per minute per milligram of protein) of adenyl cyclase were 21.2 and 0.13 for K-12 and C-57 cells, respectively. The specific activities for cAMP phosphodiesterase (picomoles of 5'-AMP formed per minute per milligram of protein) were 7.4 and 11.1 for K-12 and C-57 cells, respectively. On these bases we were able to conclude that K-12 had the genotype *cya*$^+$ and C-57 had the genotype *cya*$^-$. Another *cya*$^-$ culture, *E. coli* 5336, was obtained from R. Perlman.

In contrast to the K-12 parent, the C-57 strain loses viability when stored at 4 C in either nutrient

broth or in paraffin-sealed stab cultures maintained in semisolid nutrient agar medium. We have found that stocks of this mutant can be successfully stored by using either of the following procedures. (i) A thick mat of cells is spread on plates of the lactose-galactose tetrazolium selection medium used by Perlman and Pastan (9), grown overnight, and stored at 4 C. These plates are prepared fresh monthly. (ii) One-milliliter aliquots of a fresh nutrient broth culture of C-57 are added to 0.67 ml of a sterile mixture containing 1 part glycerol and 1 part mineral salts basal medium [grams per liter: K_2HPO_4, 7.0; KH_2PO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.10; $(NH_4)_2SO_4$, 1.0; pH 7.2]. After thorough mixing the cultures are frozen and can be maintained for long periods (>6 months) under these conditions.

Before use in any experiment, each C-57 stock culture was subjected to the series of growth experiments shown in Fig. 1 to insure that it had maintained its original mutant characteristics. Using the basal medium described in Fig. 1, the mutant grew on glucose with a mass doubling time of approximately 210 min in the absence of cAMP and a doubling time of approximately 70 min in the presence of cAMP after a 90- to 150-min adjustment period. In the absence of cAMP the mutant exhibited no growth on glycerol or lactose and only a minimal growth on casein hydrolysate or on a combination of lactose or glycerol and casein hydrolysate. Normal, wild-type growth on these substrates resulted when the medium was supplemented with 1.25 mM cAMP.

Media and growth conditions. All cultures were grown with vigorous aeration by rotary shaking at 37 C. In most of the initial studies the basal medium had the following composition (grams per liter): K_2HPO_4, 28.0; KH_2PO_4, 8.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; pH 7.2. The level of potassium phosphate, 0.22 M in this case, was later varied from 0.022 to 0.4 M as described. Other salts were also added to this medium as described in the text. Growth substrates were sterilized separately and usually added at a final concentration of 0.02 M. Vitamin-free, acid-hydrolyzed casein (Nutritional Biochemicals Inc., Cleveland, Ohio), when used, was added at a 0.50 to 1.0% level.

α-Methylglucoside uptake. Cultures were harvested by centrifugation and washed twice with 10 ml of cold 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer containing 0.02 M potassium phosphate (pH 7.2). The cells were diluted to a final concentration of 0.080 mg of cell dry weight per ml in prewarmed (37 C) HEPES-phosphate buffer containing chloramphenicol (20 μg/ml) for exactly 10 min before the addition of methyl-α-D-gluco-[U-^{14}C]pyranoside (α-MG). The reaction mixture was shaken, and after exactly 30 s a 1.0-ml aliquot was transferred to a Gelman filter (25 mm, 0.45 μm) in a membrane filter apparatus (Millipore Corp., Bedford, Mass.) and washed immediately with three 5-ml aliquots of the HEPES-phosphate buffer, also at 37 C. The filters were dried and placed in mini-vials, and the associated

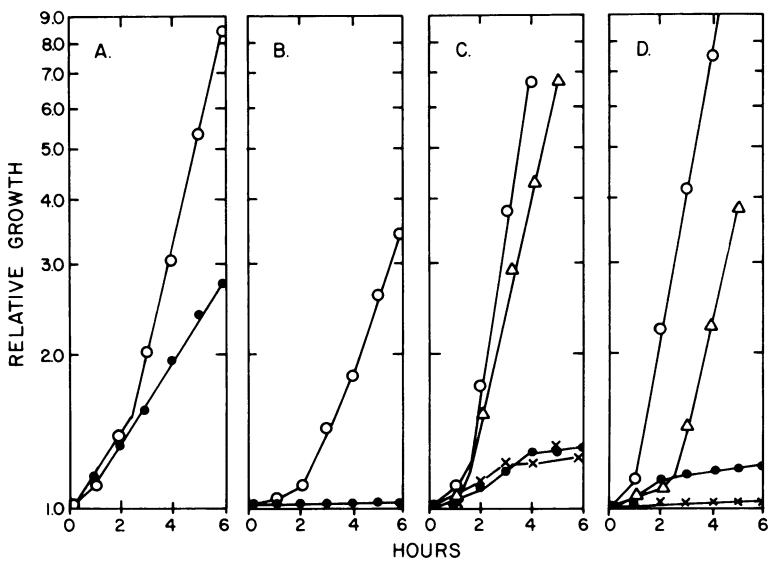

FIG. 1. *Growth characteristics of the cAMP-requiring mutant E. coli C-57. A culture of strain C-57 was grown overnight in the minimal medium containing 0.22 M potassium phosphate and 0.02 M glucose. These cells were harvested, resuspended in sterile 0.05 M sodium phosphate buffer (pH 7.5), and inoculated into the high-salt (0.22 M potassium phosphate) basal medium containing: (A) 0.02 M glucose without (●) and with (○) cAMP; (B) 0.04 M glycerol without (●) and with (○) cAMP; (C) 0.04 M glycerol plus 0.5% casein hydrolysate (CH) without (●) and with (○) cAMP, and 0.5% CH alone without (×) and with (△) cAMP; (D) 0.02 M lactose without (×) and with (△) cAMP, and 0.02 M lactose plus 0.5% CH without (●) and with (○) cAMP. The final cAMP concentration was 1.25 mM in each case, and the inoculum cell mass was in the range of 20 to 50 μg of cell dry weight/ml.*

radioactivity was counted after the addition of 6.0 ml of 0.4% 2,5-diphenyloxazole-0.1% 1,4-bis-(5-phenyloxazolyl)benzene in toluene. The counts were used to calculate the nanomoles of α-MG taken up per milligram of cell dry weight during the initial 30-s period. No significant amounts of [^{14}C]α-MG were detected in heat-killed cells or found non-specifically adsorbed to the membrane filters.

Extraction and identification of the products of α-MG transport. The various strains were grown on glucose in the 0.056 M potassium phosphate-mineral salts medium with and without cAMP. The cells were harvested by centrifugation, washed, and then incubated in the α-MG uptake assay medium as described above with the following modifications: (i) the cell concentration was 500 μg of cell dry weight/ml; (ii) the α-MG concentration was 76 μM; (iii) α-MG uptake was stopped after 10 min instead of 30 s; (iv) large (47 mm) filters were used; and (v) the cells on these filters were washed seven times with 5.0 ml of the HEPES-phosphate buffer at 37 C.

The cells thus treated were extracted by placing the filters in cold 0.3 N $HClO_4$ and agitated for 15 min. The suspension was then adjusted to pH 7.5 with KOH and the precipitated $KClO_4$ was removed by centrifugation. The supernatant fluid was then chromatographed on columns (0.5 by 8.0 cm) of AG2-x8 (100 to 200 mesh, Cl^- form) (Bio-Rad Laboratories, Richmond, Calif.). The radioactive materials were eluted from these columns with either a 0 to 0.15 N HCl linear gradient or by batch elution with 3.5 column volumes of water followed by the same volume of 0.15 N HCl. Identical results were obtained with both elution procedures. Fractions collected were counted by using the Triton-toluene scintillation containing (per liter): 666 ml of toluene, 333 ml of Triton X-100, 0.15 g of 1,4-bis-(5-phenyloxazolyl)benzene, and 5.0 g of 2,5-diphenyloxazole.

RESULTS

Growth on various substrates. The ability of *E. coli* strain C-57 and its wild-type parent to grow on a variety of substrates in the presence and absence of exogenously added cAMP was analyzed (Fig. 2 and 3). Whereas the parent strain was able to grow on all of the 20 substrates tested, the C-57 cells were able to grow in the absence of cAMP only on those substrates normally transported via the phosphoenolpyruvate (PEP) phosphotransferase (PT) system described and characterized by Roseman (11). An exception to this rule was the slow rate of growth seen with gluconate (Fig. 2) and pyruvate (not shown). The C-57 mutant was unable to grow on those substrates that are not transported by the PT system (Fig. 3) with the exceptions that (i) some slight growth was detected (mass doubling time >24 h) when casein hydrolysate was included in the medium, and (ii) growth on glucose-6-phosphate occurred in the absence of cAMP if the salt concentration in the medium was low. A preliminary account of the effect of cAMP and salts on the development of the hexosephosphate transport system in these cells was presented earlier (J. W. Ezzell and W. J. Dobrogosz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, P244, p. 176).

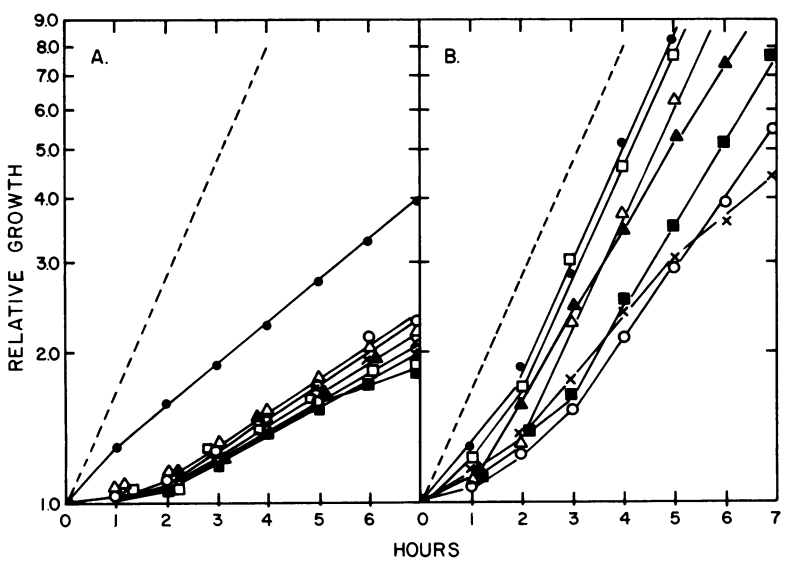

FIG. 2. *Growth of E. coli strain C-57 on PT substrates and gluconate. The basal media and inoculum were as described in Fig. 1. The growth substrates were glucose (●), fructose (○), mannose (×), N-acetylglucosamine (△), sorbitol (▲), mannitol (□), and gluconate (■). (A) No cAMP; (B) 1.25 mM cAMP added to the culture media. The dashed lines indicate the relative growth of the parental K-12 strain on 0.02 M glucose under the same conditions.*

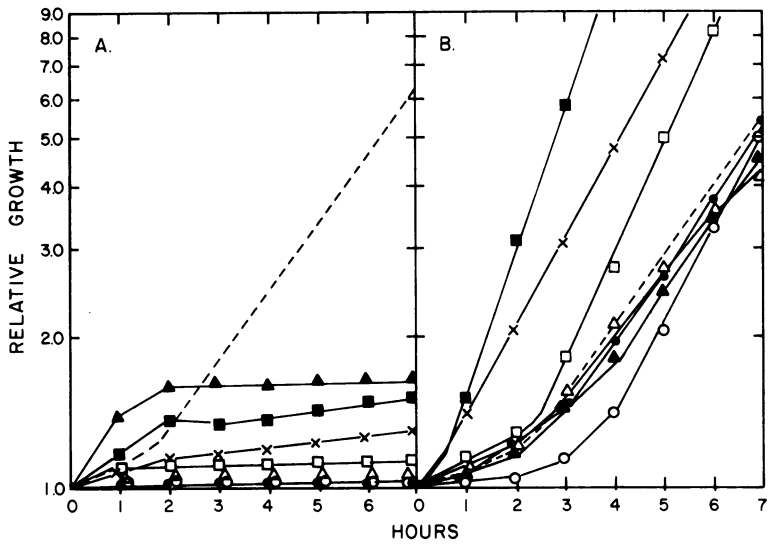

FIG. 3. *Growth of E. coli strain C-57 on non-PT substrates. Conditions were the same as described in Fig. 2 except that the following substrates were used: 0.04 M glycerol (●), 0.02 M lactose (○), 0.02 M glucose-6-phosphate (□), 0.02 M xylose (△), 1.0% casein hydrolysate (CH) (×), 0.04 M glycerol plus 1.0% casein hydrolysate (■), and 0.02 M maltose (▲). (A) No cAMP; (B) 1.25 mM cAMP added to the culture media. The dashed lines indicate the relative growth of the parental K-12 strain on 0.04 M glycerol under the same conditions.*

For reasons dating back to an earlier use for a highly buffered culture medium (2), the potassium phosphate level in our minimal medium was approximately four times higher than the level generally used. In this high-salt medium the C-57 cells grown on glucose have a mass doubling time of approximately 210 min compared with a 70-min doubling time for the K-12 strain grown under the same conditions. To determine whether the salt environment had any specific effect on the mutant cells, the experiments summarized in Fig. 4 and 5 were performed. C-57 and K-12 cells were grown on glucose with and without cAMP in media containing varied levels of potassium phosphate (Fig. 4) or a constant low level of potassium phosphate with varied levels of NaCl (Fig. 5). The initial pH of each culture was 7.2 and the final pH was never less than 6.9.

In the absence of cAMP the mutant cells grew at lower rates than any of the control cultures even at the lowest potassium phosphate concentrations tested (Fig. 4). This growth inhibition was completely abolished by cAMP except in the presence of the higher salt concentrations, in which case even growth of the parental cells was inhibited. A similar pattern of salt sensitivity was observed when NaCl was substituted for the potassium phosphate (Fig. 5). In fact, similar patterns were observed when any of a variety of other cation-anion combinations were used including potassium chloride, sodium phosphate, ammonium chloride, or ammonium phosphate. The results presented in Fig. 6 summarize these inhibition patterns in a different manner. Here the relative increase in cell mass 4 h post-inoculation was plotted as a function of the salt level in each growth medium. It can be seen here that whereas the growth of both strains was inhibited when the salt levels exceeded 0.15 to 0.20 M, regardless of the availability of cAMP, the C-57 cells grown without this nucleotide were inhibited by even considerably lower salt concentrations. When cAMP was added, this inhibition was completely reversed. However, since it took 1 to 2 h for this reversal to occur, the total cell mass of the C-57 cells supplemented with cAMP was accordingly lower at the time these measurements were recorded. In each case the C-57 cells grown in the absence of cAMP were more sensitive to these salts than any of their cAMP-sufficient counterparts.

In data not shown here cAMP was unable to reverse the inhibition of growth produced when high levels of nonionic solutes (i.e., sucrose, glycerol, and glucose) were added to an otherwise low-salt medium. Although high levels (>0.8 M) of sucrose, for example, inhibited growth of the C-57 cells on glucose, a similar

inhibition occurred with the K-12 cells, and in neither case was cAMP effective in reversing these inhibitions. These results suggested that the hypersensitivity of the C-57 cells to salts is an ionic effect rather than a general osmotic phenomenon.

Varied cAMP levels. In all of the experiments described thus far, 1.25 mM levels of cAMP were used. The results presented in Fig. 7 show the effect of various cAMP concentrations on growth of C-57 on glucose in a high-salt medium. In the absence of this nucleotide the mass doubling time was 210 min, which decreased to 90 min with as little as 0.25 mM cAMP added. In the range of 0.50 to 2.5 mM cAMP, a minimum generation time of 70 min was observed. In each case, however, there was a 90- to 150-min lag period detected before the stimulating effect of cAMP was observed. Nucleotide levels in the range of 5.0 mM produced a slight inhibitory lag period, whereas levels of 15 mM or higher produced a significant lag and a slight inhibition in the rate of growth as well.

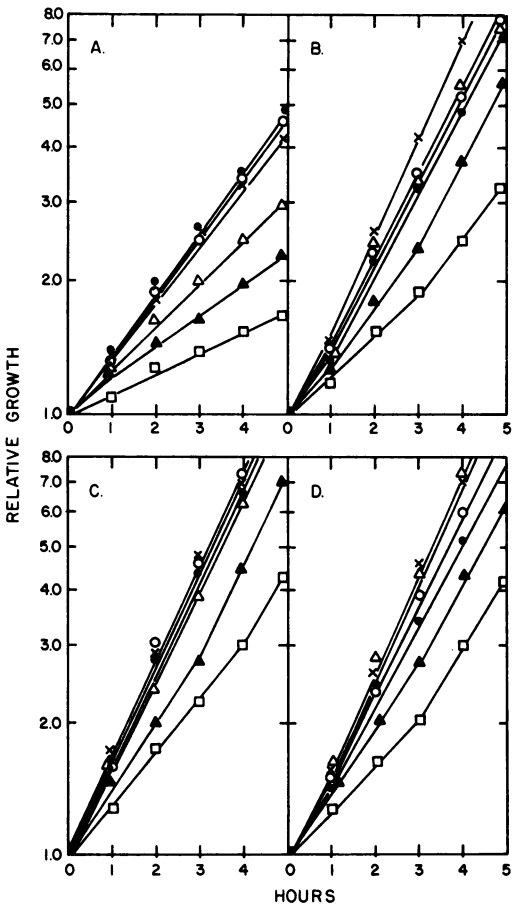

FIG. 4. *Effect of the potassium phosphate concentration of the culture medium and cAMP on growth of strains K-12 and C-57 on glucose. The inocula used in these experiments were prepared as described in Fig. 1. The growth substrate was 0.02 M glucose and the potassium phosphate concentrations (molar) in the media were: 0.022 (●), 0.044 (○), 0.11 (×), 0.22 (△), 0.33 (▲), and 0.42 (□). (A and B) Strain C-57 without (A) and with (B) 1.25 mM cAMP added to the culture media. (C and D) Strain K-12 without (C) and with (D) 1.25 mM cAMP added to the culture media.*

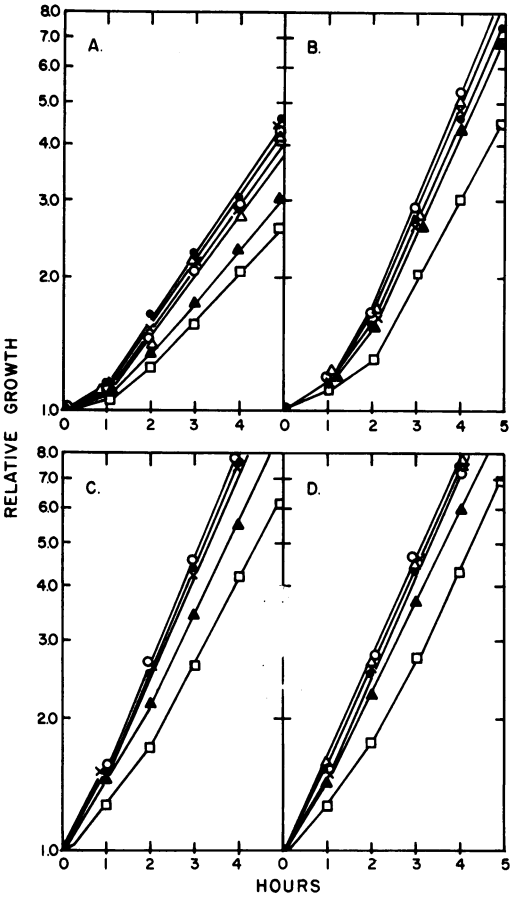

FIG. 5. *Effects of the NaCl and cAMP on growth of strains K-12 and C-57. The inocula used in these experiments were prepared as described in Fig. 1. The growth substrate was 0.02 M glucose, and 0.056 M potassium phosphate was included in each case to buffer the medium. NaCl was added to the media as follows: none (●), 0.028 M (○), 0.055 M (×), 0.11 M (△), 0.22 M (▲), and 0.33 M (□). (A and B) Strain C-57 without (A) and with (B) 1.25 mM cAMP added to the culture media. (C and D) Strain K-12 without (C) and with (D) 1.25 mM cAMP added to the culture media.*

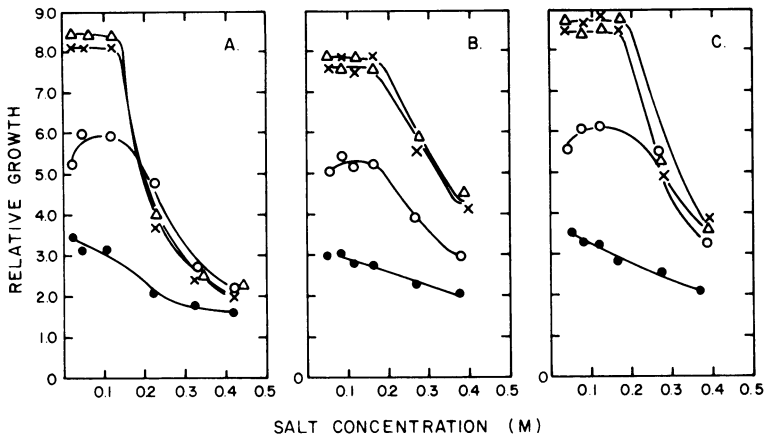

FIG. 6. *Effect of cAMP and salts on growth of the parental and mutant strains. From experiments conducted in a manner identical to those described in Fig. 4 and 5, the relative growth values determined after 4 h of growth were plotted as a function of the molarity of salts added to the various culture media. (A) Sodium phosphate; (B) 0.055 M potassium phosphate plus the indicated concentrations of NaCl; (C) 0.055 M potassium phosphate plus the indicated concentrations of KCl. The pH of the medium in each case was 7.2. Strain C-57 without (●) and with (○) 1.25 mM cAMP. Strain K-12 without (×) and with (△) 1.25 mM cAMP.*

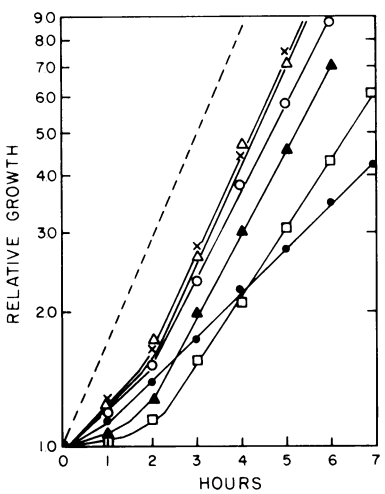

FIG. 7. *Effect of varying the cAMP concentration on the growth of E. coli strain C-57. E. coli C-57 cells were inoculated into the basal medium containing 0.02 M glucose and 0.22 M potassium phosphate. Final cAMP concentrations were: none (●), 0.25 mM (○), 0.50 mM (×), 1.0 mM (△), 5.0 mM (▲), 15.0 mM (□). The dashed line indicates the relative growth of the parental K-12 strain on 0.02 M glucose under the same conditions.*

Hexose transport studies. The cAMP-repairable hypersensitivity of C-57 cells to salts suggested that these cells may have a defective ion transport system and/or a defective hexose transport system. While the ion transport capacity of these cells has not yet been analyzed, their ability to transport glucose was examined, using ^{14}C-labeled α-MG as a nonmetabolizable analogue for this purpose.

Mutant and parent cells were grown with glucose in low- (0.056 M) and high-salt (0.22 M) media in the presence and in the absence of exogenous cAMP. Their ability to take up α-MG was then measured, and the initial velocity (30 s) obtained in each case was plotted as a function of the α-MG concentration in the uptake medium. These results (Fig. 8) showed that the C-57 cells grown in the absence of cAMP in either the high- or low-salt medium had a serious defect in their hexose transport system. In these cells the velocity of initial uptake increased linearly with the external α-MG concentration and was transported by what therefore appeared to be a simple, passive diffusion process. After growth in the presence of cAMP, however, α-MG uptake by these same cells exhibited saturation kinetics, indicating that normal active transport was restored. The only major difference between growth in the low- (Fig. 8A) and the high- (Fig. 8B) salt media in this connection was the fact that a lower V_{max} for α-MG uptake was observed for the cells grown in the high-salt medium than for the cells grown in the low-salt medium.

Even the K-12 cells exhibited this lowered V_{max} for α-MG uptake as a result of growth in the high-salt medium. In addition, the K-12 cells showed an unexpected stimulation of this α-MG transport system resulting from growth in the presence of exogenous cAMP. Although the K-12 cells were able to transport α-MG via a process exhibiting saturation kinetics even

when grown without exogenous cAMP, their growth in the presence of this nucleotide resulted in an α-MG transport system having an increased affinity for this substrate. A 1.25 mM cAMP concentration was needed for the development of this low-K_m (transport) system when these cells were grown in the low-salt medium, but a 5.0 mM level was needed for this purpose when they were grown in the high-salt medium.

The effect of cAMP on the development of the low-K_m system in the mutant cells grown in the low-salt medium on glucose is summarized in Fig. 9. These results showed that the α-MG transport system evolved gradually from the defective system which exhibited only passive diffusion of α-MG when grown in the absence of cAMP to a fully competent, low-K_m process when cAMP was present in at least a 5.0 mM concentration. These data plotted according to the double-reciprocal method of Lineweaver-Burk are shown in Fig. 10. cAMP-deficient cells (curve 1) exhibited $x = 0$, $y = 0$ intercepts as would be the case if only passive diffusion were involved in the uptake process. With as little as 0.25 mM cAMP (curve 2), uptake via saturation kinetics became discernible with a K_m calculated at 160 mM. A 0.63 mM level of cAMP yielded an uptake system (curve 3) showing a K_m of 16 μM and a V_{max} of 10 (micromoles of α-MG taken up per milligram of cell dry weight per 30 s). As the external cAMP levels were increased to 6.25 mM (curves 4 through 6), the K_m values decreased to approximately 10 μM (see insert, Fig. 10) and the V_{max} values remained unchanged. Also shown in this figure (see insert) are the K_m values calculated for K-12 cells grown under these same conditions.

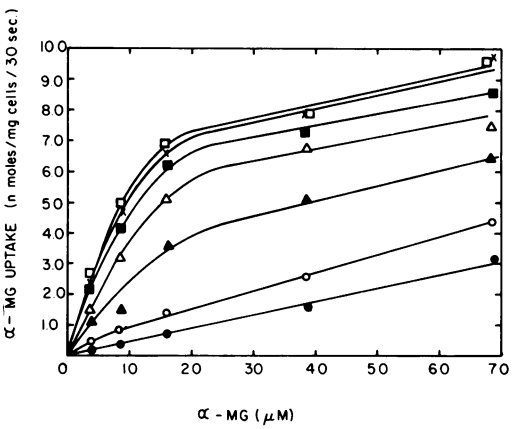

FIG. 9. *Effect of cAMP concentration on the development of the α-MG uptake system in the E. coli C-57 mutant. The experimental system was identical to that described in Fig. 8 except that in this experiment the C-57 cells were grown on glucose (0.02 M) in the low-salt (0.056 M potassium phosphate) medium. These cells were grown in the presence of the following concentrations (millimolar) of cAMP: none (●), 0.25 (○), 0.625 (▲), 1.25 (△), 2.5 (■), 5.0 (□), and 6.25 (×).*

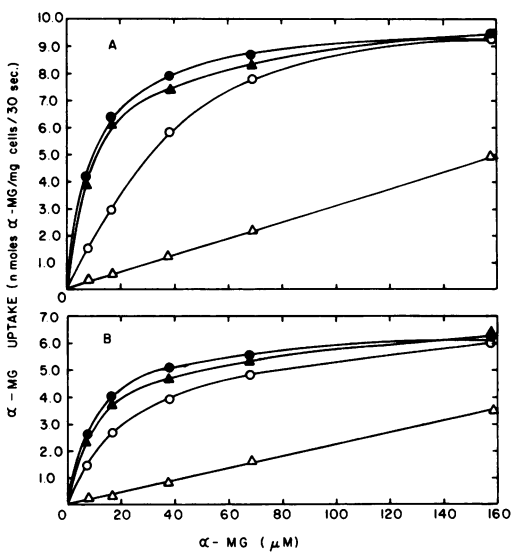

FIG. 8. *Effect of cAMP and salts on transport of α-MG. E. coli K-12 and C-57 cells were grown in the basal medium containing 0.02 M glucose in either the low- (0.056 M) salt medium (A) or the high- (0.22 M) salt medium (B). K-12 cultures without (○) and with (●) 6.25 mM cAMP added. C-57 culture without (△) and with (▲) 6.25 mM cAMP added to the culture medium. α-MG uptake was measured as described in the text as the nanomoles α-MG taken up per milligram of cell dry weight per 30 s. This initial velocity of uptake was plotted as a function of the α-MG concentration present in the uptake reaction system.*

For both the K-12 and C-57 cells the amount of cAMP needed for the full development of the low-K_m system for α-MG uptake increased as the salt level of the medium increased. With K-12 cells, 1.25 mM cAMP was sufficient for this purpose in the 0.056 M salt medium, whereas 5 mM was needed in the 0.22 M salt medium. With the C-57 cells 5 and 6.25 mM levels, respectively, were required.

The development of the α-MG transport system in another cAMP-requiring mutant, *E. coli* strain 5336 (9), was influenced by cAMP in a manner essentially identical to that shown for the C-57 strain (Fig. 11).

α-MG transport products. α-MG is transported in *E. coli* via group translocation using the glucose PT system with α-methylglucose-6-phosphate (α-MG6P), the product of this translocation. Not all of the α-MG is transported into the cells by this route. Winkler (16) has shown,

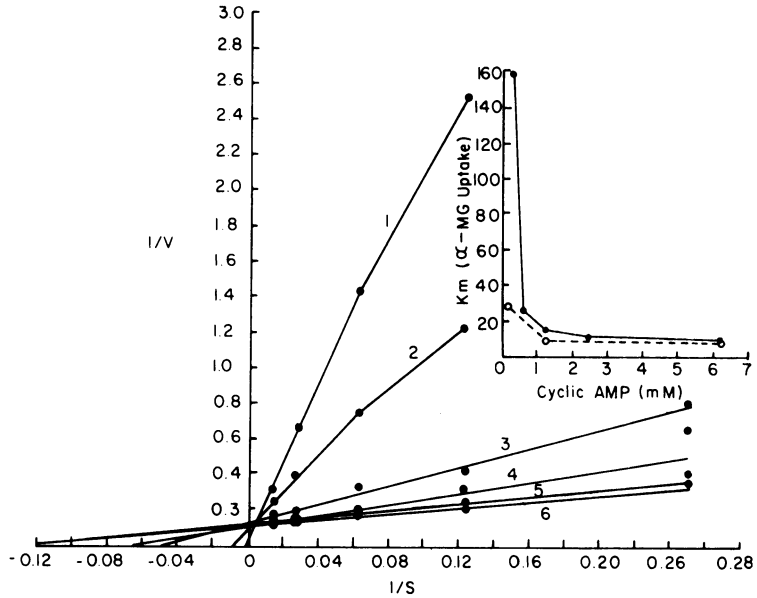

FIG. 10. *Effect of cAMP on the K_m (uptake) of the α-MG transport system. The data presented in this graph are Lineweaver-Burk double-reciprocal plots of the data presented in Fig. 9. Included here are the plots for cells grown in the presence of the following cAMP levels: curve 1, none; curve 2, 0.25 mM; curve 3, 0.625 mM; curve 4, 1.25 mM; curve 5, 2.5 mM; curve 6, 6.25 mM. Insert: A plot of the calculated K_m (micromolar) (uptake) values as a function of the cAMP concentrations in the respective growth media for C-57 (●) and K-12 (○) cells.*

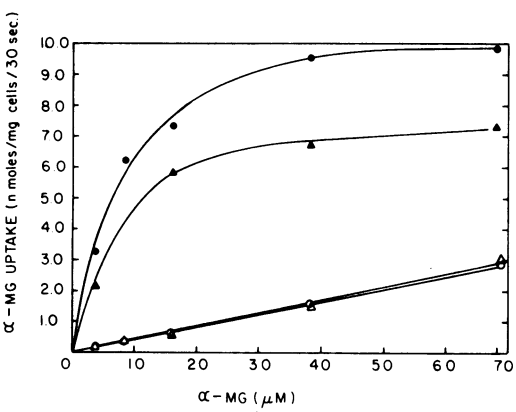

FIG. 11. *Effect of cAMP on the development of the α-MG uptake system in E. coli strain 5336. These experiments were carried out as described in Fig. 8 except that E. coli strain 5336 was used. Cells grown on glucose in the low-salt (0.056 M potassium phosphate) medium without (○) and with (●) 6.25 mM cAMP. Cells grown on glucose in the high-salt (0.22 M potassium phosphate) medium without (△) and with (▲) 6.25 mM cAMP.*

for example, that approximately half of the α-MG taken up by *E. coli* cells can be recovered as the netural sugar and the other half as α-MG6P. The data summarized in Table 1 showed that this was also the case with our K-12 strain and with the C-57 and 5336 mutant strains grown in the presence of exogenous cAMP. When the mutant strains were grown without cAMP only 20 to 30% of the α-MG transported by these cells was in the phosphorylated form.

These findings, viewed together with the kinetic measurements described earlier, indicate that cAMP-deficient cells have a defect(s) in the ability to translocate α-MG via the PT system. Proof that this is the case must await a more detailed examination of the components that make up the PT system. These studies have been initiated and will be reported in the future.

DISCUSSION

E. coli strain C-57 is a cAMP-deficient mutant of *E. coli* K-12 (701) isolated in our laboratory by nitrosoguanidine mutagenesis according to the procedure of Perlman and Pastan (9). This mutant is apparently comparable, at least insofar as preliminary tests have shown, to strain 5336 isolated by these authors. The C-57 strain, however, is more stable and can be grown more conveniently than the 5336 strain by not requiring thiamine, glutamate, or histidine.

Strain C-57 has already been reported to contain a decreased content of cytochromes b_1 and *o* (1) and a drastically reduced level of membrane-associated respiratory enzymes such as succinic dehydrogenase, D- and L-lactate dehy-

TABLE 1. *Analysis of α-MG transport products*

Strain	5.0 mM cAMP[a]	% α-MG	% α-MG6P
K-12	−	52	48
K-12	+	46	54
C-57	−	80	20
C-57	+	58	42
5336	−	71	29
5336	+	52	48

[a] All cells were grown in the 0.056 M potassium phosphate-mineral salts medium containing 0.02 M glucose with or without 5 mM cAMP as indicated. The cultures were harvested, washed, and resuspended in the α-MG uptake system containing chloramphenicol as described in the text.

drogenases, and reduced nicotinamide adenine dinucleotide oxidase (S. S. Dills, and W. J. Dobrogosz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, P207, p. 179) as determined by crude extract analyses and in membranes isolated and fractionated by the procedures of Schnaitman (12) and Osborne et al. (6). Preliminary studies have indicated that C-57 cells grown in high-salt medium have an abnormal fatty acid pattern (Edwards and Dobrogosz, unpublished observations). These cells are unable to produce flagella (17) and therefore are incapable of chemotactic migrations (3). They grow poorly under anaerobic conditions owing, in part, to an inability to synthesize the anaerobic formic hydrogenlyase system (8). All of these abnormalities are completely eliminated and normal parental functions restored within 2 h after these cells are provided an exogenous source of cAMP.

A number of additional, cAMP-regulated functions in the C-57 strain are described in this report. Except for a slow rate of growth seen on gluconate and pyruvate, the mutant cells are able to grow in the absence of cAMP only on those substrates known to be transported via the PT system, namely, glucose, fructose, mannose, mannitol, sorbitol, and *N*-acetylglucosamine. Even on these substrates, however, the mutant does not grow at rates comparable to those observed with the parental cells, a finding also made with the 5336 strain (9). Although the inability of the C-57 mutant to grow on substrates not transported by the PT system (e.g., glycerol, lactose, L-arabinose, etc.) is now well understood in terms of the role cAMP plays in the catabolite repression phenomenon (7), there is yet no explanation as to why such mutant cells do not grow normally on the PT substrates unless cAMP is available.

The findings presented in this report have begun to shed some light on this matter. It was found that the mutant cells are hypersensitive to their ionic environment. Whereas the K-12 culture and the mutant culture supplemented with cAMP grow normally in media having salt levels as high as 0.18 M, the growth of the mutant in the absence of this nucleotide was inhibited by even the lowest levels of salts tested. These cells lose their sensitivity to the salts approximately 2 h after the addition of cAMP. This effect was found to be nonspecific in that all the various anion-cation combinations tested produced a similar pattern of hypersensitivity. It also appears to be an ionic effect rather than a general osmotic effect because the inhibition of growth produced by adding high levels of nonionic solutes (sucrose, glycerol, and glucose) to the culture media did not result in a similar pattern of cAMP involvement; i.e., when the levels of these latter substances were high enough (generally >1.0 M) to inhibit growth, cAMP was unable to overcome the inhibition. Also, the pattern of growth inhibition by these solutes was similar when the C-57 and K-12 cells were compared with each other.

The reason(s) for the hypersensitivity of the C-57 cells to salts is not known. Direct measurements of ion influx and efflux rates in cAMP-sufficient and -deficient cells must be conducted before any conclusion can be reached in this regard. However, it seems reasonable to predict that cAMP-deficient cells may have a defective ion transport system and consequently are unable to regulate their ionic flux properly. This speculation is consistent with our finding that the C-57 cells have defective substrate transport systems.

cAMP-deficient cells were found to have a defective hexose transport system. Instead of exhibiting normal saturation kinetics, α-MG uptake by these cells occurred by what appeared to be simple passive diffusion, and the α-MG thus transported was recovered primarily as the neutral sugar rather than as the phosphorylated derivative. cAMP-sufficient cells, on the other hand, possessed a normal PT system showing saturation kinetics for α-MG transport and a high internal pool of α-MG6P. On the basis of these findings we have concluded that cAMP is required for the construction of a normal PT system by *E. coli* cells.

The mechanism by which cAMP functions in this regard is another matter. Presently experiments are underway to isolate and compare the individual components of the PT system from both sufficient and deficient cells. Included in these analyses will be an examination of a possible defect in the production of PEP by the deficient cells. An altered PEP-generating system by itself could produce the anomalous α-MG transport kinetics observed in the cAMP-defi-

cient cells. It has been observed, for example, that α-MG is transported into membrane vesicles by passive diffusion when PEP is unavailable for normal PT functions (4).

The development of the PT system in both control and cAMP-deficient cells was also found to be significantly influenced by the concentration of salts present in the growth medium. The V_{max} for α-MG transport was considerably lower in cells that had been grown in a high-salt medium than was the case for cells grown in a relatively normal salt environment. This salt effect will also be included in our more detailed future examination of the PT system in these cells.

However, neither this salt effect nor the need for cAMP can account by itself for the slower growth on glucose observed for the C-57 cells as compared to their K-12 parents (see, e.g., Fig. 4 and 5). This conclusion is based on the fact that glucose can be transported by passive diffusion into the deficient C-57 cells at a rate equivalent to the V_{max} of the normal cells with an external hexose level as low as 0.35 mM. Considering that the glucose concentration in the growth medium was 20 mM, it must be concluded that transport of glucose was not the growth-limiting factor under these conditions.

We are not yet able to determine the specific reason(s) why cAMP-deficient cells grow more slowly on glucose than do their cAMP-sufficient counterparts. We are presently unable to determine whether this is a consequence of the inner membrane's altered transport and respiratory functions (1; Dills and Dobrogosz, Abstr. Ann. Meet. Am. Soc. Microbiol. 1974, P207, p. 179) or altered periplasmic or outer membrane properties (Y. H. Tseng, S. S. Dills, and W. J. Dobrogosz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K121, p. 167), or attributable to still other as yet unidentified functions. Clearly, much remains to be discovered concerning the specific and generalized consequences of cAMP deficiency in bacterial cells.

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