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Stimulation of Derepressed Enzyme Synthesis in Bacteria by Growth on Sublethal Concentrations of Chloramphenicol

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Culturing of *Salmonella typhimurium* or *Escherichia coli* cells in the presence of low concentrations (≤ 1 μg/ml) of chloramphenicol (CAP) permitted exponential growth, but at doubling times up to twice those of controls. When such cultures were subsequently starved for uracil or arginine, derepression of aspartate transcarbamylase (ATCase) or ornithine transcarbamylase, respectively, was enhanced three- to 10-fold as compared to cultures not exposed to CAP. Enhancement of β-galactosidase synthesis by prior exposure to CAP was also observed in uracil-starved *E. coli* cultures. Stimulation of enzyme synthesis appeared to be a specific effect of CAP; low levels of erythromycin, puromycin, sparsomycin, tetracycline, and rifampin did not show such effects. Derepression of ATCase synthesis in exponentially growing cells in the presence of CAP did not result in stimulation of enzyme synthesis by CAP. A prior history of growth of a culture in the presence of CAP was shown to be necessary for enhancement of enzyme synthesis by CAP; furthermore, continued presence of CAP in the medium during starvation was not necessary for enhanced enzyme synthesis and inhibited it in some instances. Enhanced enzyme synthesis in starving, CAP-treated cultures could be blocked by rifampin, which suggested that CAP treatment allows prolonged or more extensive messenger ribonucleic acid synthesis.

It has been known for some time that growth of bacteria in the presence of low concentrations of the antibiotic chloramphenicol (CAP) causes effects on enzyme synthesis that cannot be explained as simply resulting from general inhibition of protein synthesis. Sypherd et al. (30, 32) and Paigen (25) have shown that the synthesis of β-galactosidase and other inducible enzymes was inhibited to a far greater extent than total protein synthesis when *Escherichia coli* cells were induced while growing in the presence of low concentrations (0.5 to 1.0 μg/ml) of CAP. Synthesis of several "constitutive" enzymes was not preferentially inhibited. Low concentrations of other antibiotic inhibitors of protein synthesis, but not other growth inhibitors, also preferentially inhibited synthesis of β-galactosidase. Further studies by Sypherd and DeMoss (29) eliminated the possibility that the effects of CAP were a manifestation of catabolite repression as had been suggested by others (21, 25). Preferential inhibition of β-galactosidase or tryptophan synthetase synthesis by CAP was shown to require functional regulatory genes (i^+ or $trpR^+$, respectively) (29). Furthermore, preferential inhibition of β-galactosidase synthesis could be shown to result from a variety of conditions that increase the rate of ribonucleic acid (RNA) synthesis (31). Sypherd and Strauss (31) have suggested that the accelerated RNA synthesis that takes place in the presence of low concentrations of CAP directly or indirectly enhances repression of β-galactosidase. Most other studies of the metabolic effects of CAP have used much higher concentrations of the antibiotic (≥ 50 μg/ml), which completely inhibit protein synthesis and cell growth.

In the course of a study of derepression of enzymes involved in pyrimidine biosynthesis (34), the observation was made that low concentrations (≤ 1 μg/ml) of CAP stimulated the derepression of aspartate transcarbamylase in *Salmonella typhimurium* strain *pyrA81* (J. Olszowy, unpublished observation). This result was unexpected, both from a consideration of the action of CAP as an inhibitor of protein synthesis and from the effects of CAP on induced enzyme synthesis described above. The results reported here describe the nature of this apparently paradoxical effect of CAP on enzyme synthesis. An accompanying paper (12) describes the effects of low concentrations of CAP on nucleotide pools and attempts to relate these effects to enzyme synthesis.

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MATERIALS AND METHODS

Bacterial strains. The strains of *S. typhimurium* and *E. coli* used in this study are listed in Table 1.

Antibiotics. CAP was purchased from Sigma. Puromycin and tetracycline were purchased from Nutritional Biochemicals. Sparsomycin was obtained from the National Cancer Institute, National Institutes of Health. Rifampin was the gift of H. Heymann, CIBA Pharmaceuticals, and erythromycin was provided by K. L. Rinehart, University of Illinois.

Media and culture methods. The minimal salts medium of Berkowitz et al. (4) with 0.5% glucose was used in most of these studies. For some experiments, the medium of Irr and Gallant (15) was used with 0.3 mM potassium phosphate and 0.5% glucose. Cell growth and enzyme synthesis were virtually identical in the two media. All media were supplemented at 100 μg/ml with the growth requirements of the strain under study, except as stated in context.

Derepression experiments were performed by growing the appropriate strain overnight at 37 C in supplemented minimal medium. The cells were harvested by centrifugation, washed once with 0.85% saline solution, and inoculated (2%, vol/vol) into minimal medium containing growth-limiting amounts of the appropriate nutrient. The bacteria were grown at 37 C with shaking. Growth was followed turbidometrically on a Klett-Summerson colorimeter with a no. 66 filter. Samples were harvested by centrifugation and frozen until assayed. For some derepression experiments exponentially growing cells were transferred into starvation medium. The cells were grown in supplemented medium at 37 C after a 2% (vol/vol) inoculation from a culture grown overnight in the same medium. After the culture reached 70 Klett units, it was harvested by centrifugation, washed once with 0.85% saline solution, and resuspended in fresh, warm (37 C) medium lacking a necessary metabolite. Samples taken at intervals during incubation in the starvation medium were harvested by centrifugation and frozen.

Enzyme assays. Sonic extracts were prepared by suspending the harvested cells in 1/20 to 1/10 the original sample volume in 50 mM potassium phosphate, pH 7.4 (or 50 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4, if extracts were to be assayed for OTCase [ornithine transcarbamylase]) and exposing them to 15-s bursts on a Sonifier model W185 sonic oscillator. The samples were kept between 0 and 10 C during sonic treatment.

Aspartate transcarbamylase (ATCase, carbamoylphosphate: L-aspartate carbamoyltransferase, EC 2.1.3.2) was assayed by the method of Yates and Pardee (36) using the color test of Prescott and Jones (27) to quantitate carbamyl aspartate. Enzyme activity is expressed as micromoles of carbamyl aspartate formed per hour.

OTCase (Carbamoylphosphate: L-ornithine carbamoyltransferase, EC 2.1.3.3) was assayed by the method of Jones (16) using the color test of Prescott and Jones to quantitate citrulline formation. Activity is expressed as micromoles of citrulline formed per hour.

β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) was assayed by following the hydrolysis of *o*-nitrophenyl-β-D-galactoside at 405 nm on a Beckman DB spectrophotometer thermostatted at 30 C. The reaction mixture contained, in a final volume of 1 ml: 50 mM potassium phosphate buffer (pH 7.1), 10 mM $MgCl_2$, 0.5 mM *o*-nitrophenyl-β-D-galactoside and 20 to 200 μl of cell extract. Enzyme activity is expressed as micromoles of *o*-nitrophenol formed per minute.

Protein was determined by the method of Lowry et al. (18) with crystalline bovine serum albumin as a standard.

RESULTS

Effects of low concentrations of CAP on ATCase derepression. To study the effects of low concentrations of CAP on derepression of ATCase in *S. typhimurium* strain *pyrA81*, cultures were grown with limiting uracil (5 μg/ml) and concentrations of CAP from 0 to 2.5 μg/ml. Growth at all but the highest drug concentration was exponential until the uracil was exhausted, at which time growth stopped. ATCase activity determined in cultures harvested 1 h after cessation of growth is presented in Table 2. After uracil depletion ATCase activity increased considerably over repressed levels. Cells grown in the presence of CAP (≤1 μg/ml)

TABLE 1. *Bacterial strains used in this study*

Organism	Phenotype	Source
Salmonella typhimurium		
LT-2	Wild type	B. N. Ames
LT-2, *pyrA81*	Arg^-, Pyr^- (35)	J. L. Ingraham
LT-2 HD58	Pyr regulatory mutant (constitutive) (23)	G. A. O'Donovan
Escherichia coli		
15T⁻	Arg^-, Ura^-, Thy^-, Met^-, Pro^-, Trp^-	S. Kaplan
CP78	Rel^+, Arg^-, Leu^-, $Threo^-$, His^-, B_1^-, (11)	M. Cashel
CP79	Rel^-, Arg^-, Leu^-, $Threo^-$, His^-, B_1^-, (11)	M. Cashel

showed much greater derepression of ATCase during uracil starvation. Higher concentrations of CAP inhibited ATCase derepression.

To define the effect of CAP on ATCase derepression more clearly, ATCase activity was followed throughout growth (and uracil starvation) of strain *pyrA81* in the presence or absence of CAP. Figure 1 shows growth and ATCase activities of cultures grown in minimal medium with limiting uracil (5.5 μg/ml) and no antibiotic (culture A) or 0.8 μg of CAP/ml (culture B). The loss of turbidity observed after growth stopped is not due to cell lysis since the protein concentration of the cell extracts did not decrease. The ATCase activity of both cultures began to increase at the time that growth stopped. In culture A, ATCase activity reached a maximum value within 30 min. Other experiments (not shown) demonstrated that the ATCase activity remained at this maximum for at least 2.5 h of uracil starvation. In contrast, the ATCase activity of culture B (+CAP) increased for at least 2 h after growth stopped, reaching more than three times the activity of culture A. Other experiments indicated that ATCase activity was near its maximum 2 h after growth stopped.

TABLE 2. *Effects of CAP on ATCase derepression in S. typhimurium strain pyrA81*

Uracil (μg/ml)	CAP (μg/ml)	Doubling time (min)	ATCase sp act (U/mg of protein)[a]	Fold derepression[b]
50[c]	0.0	51	1.2 (±0.6)	
5	0.0	51	21.6 (±2.5)	18
5	0.5	76	154.8 (±15.4)	128
5	0.8	104	81.0 (±8.4)	67
5	1.0	108	80.0 (±6.4)	67
5	2.5	258	9.6 (±0.9)	6

[a] Nine assays of cell extract. Values in parentheses show standard deviation.

[b] Relative to fully repressed culture growing on 50 μg of uracil/mg.

[c] Harvested in mid-log phase.

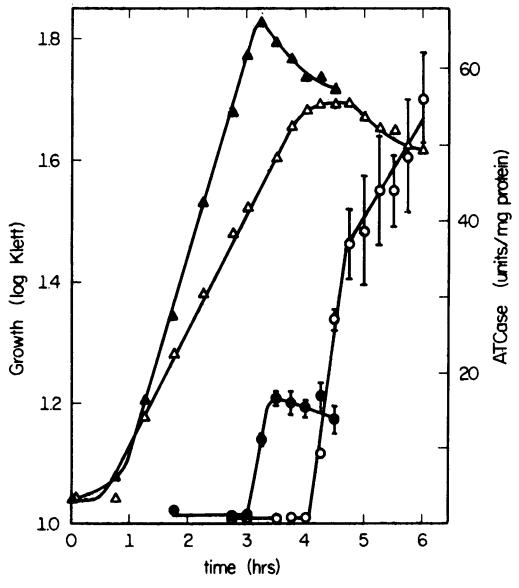

FIG. 1. *Derepression of ATCase in untreated and CAP-treated strain pyrA81. Cultures were grown with 5.5 μg of uracil/ml. Turbidity: culture A (▲), no CAP; culture B (△), 0.8 μg of CAP/ml. ATCase activity: culture A (●), culture B (○). Error bars represent ±1 standard deviation, determined from five assays.*

Effects of CAP on OTCase derepression. To test the generality of the CAP effect on enzyme derepression, and to establish whether or not the effect was limited to conditions of uracil starvation, derepression of OTCase, an enzyme of arginine biosynthesis, was studied by starving strain *pyrA81* for arginine. In the culture without CAP OTCase activity increased immediately upon arginine depletion, reaching maximal activity in less than 20 min after growth stopped. Derepression of OTCase in the culture containing 0.8 μg of CAP/ml continued for at least 2 h after arginine depletion, reaching levels 10 times higher than in the untreated control. This result clearly establishes that the stimulation of enzyme derepression seen upon exposure of cultures to low concentrations of CAP is not limited to enzymes of one biosynthetic pathway or to one means of starvation.

Similar experiments with *E. coli* strain 15T⁻ showed that CAP enhanced derepression of both ATCase and OTCase. These results further substantiate the generality of the CAP effect on enzyme derepression in bacteria.

The effect of low concentrations of CAP on OTCase activity was also measured during arginine starvation in the stringent and relaxed pair of *E. coli* strains CP78 (*rel*⁺) and CP79 (*rel*⁻). Cultures were grown on minimal medium supplemented with limiting arginine (12 μg/ml) and all other amino acids in excess (100 μg/ml). In stringent strain CP78, derepression of OTCase synthesis was enhanced twofold by CAP treatment (Table 3). In the relaxed strain, CP79, there was much less synthesis of OTCase upon arginine starvation and CAP had no effect. This result suggests that the effect of CAP may somehow be related to stringent control, since it was seen in *rel*⁺ but not in *rel*⁻ cells. A low concentration of CAP did not overcome the reduced ability of the *rel*⁻ strain to synthesize OTCase upon arginine starvation.

Effects of other antibiotics on ATCase

TABLE 3. *Effects of CAP on OTCase derepression in E. coli strains CP78 and CP79*

Strain	CAP (μg/ml)	Doubling time (min)	OTCase sp act (U/mg of protein)[a]	
			Repressed	Derepressed (2 h)
CP78	0.0	50	2.5 (±0.2)	26.0 (±5.7)
	1.0	65	1.4 (±0.2)	56.4 (±4.6)
CP79	0.0	50	3.4 (±1.0)	10.8 (±1.6)
	1.0	65	0.7 (±0.1)	8.8 (±2.6)

[a] Four assays of cell extract. Parentheses show standard deviation.

derepression. If the effect of CAP on enzyme derepression is due to partial inhibition of protein synthesis or to reduced growth rate, low concentrations of other antibiotics should have the same effect. Several antibiotics were tested for their effect on the derepression of ATCase in uracil-starved *S. typhimurium* strain *pyrA81*. The results of these experiments (Table 4) clearly show that only CAP had a stimulatory effect on ATCase derepression. Neither sparsomycin nor erythromycin, which bind to the ribosome at or near the site of CAP binding (10, 20), caused enhancement of ATCase derepression.

CAP effects on enzyme derepression in growing cells. The results reported above demonstrate the existence of a CAP effect on enzyme derepression only under conditions that preclude growth, since starvation was used to derepress enzyme synthesis. It was desirable to study the effect of CAP on enzyme derepression during exponential growth. Strain *pyrA81* was grown on several concentrations of orotate, which resulted in decreased growth rate and in the derepression of ATCase in this strain (34). A pyrimidine regulatory mutant, strain HD58, which is insensitive to exogenous uracil and synthesizes pyrimidine biosynthetic enzymes constituitively (23), was also tested for enhanced ATCase synthesis in the presence of low concentrations of CAP. The wild-type parent strain LT-2 was tested for the effect of CAP on ATCase synthesis during exponential growth in a medium lacking pyrimidines. Also, strain LT-2 was tested for the effect of CAP on the derepression of ATCase that results from exposure to the pyrimidine analogue 6-azauracil (a competitive inhibitor of orotidylic acid decarboxylase [14]) during exponential growth. These varied conditions resulted in cultures that grew with a wide range of generation times (50 min to 6.5 h) and synthesized greatly differing amounts of ATCase (2 to 400 U/mg of protein). In no case did exposure to CAP (0.5 μg/ml) exert a significant influence on the amount of ATCase activity in the culture. From these results it can be concluded that the CAP effect that we have described for cells starved for a necessary metabolite does not function in exponentially growing cells. Presumably, therefore, the action of CAP is related to alterations in the metabolism of starving or nongrowing cells.

Enzyme derepression upon transfer to starvation conditions. Derepression of ATCase and OTCase was further studied by the technique of transferring exponentially growing cells into starvation media (as described in Materials and Methods). This technique permits one to expose the cells to CAP during growth or during starvation separately and to identify differences related to the time of exposure to the antibiotic. Figure 2a shows the time course of ATCase derepression in strain *pyrA81* after transfer to a medium lacking uracil. Cultures A and B were not treated with antibiotic during exponential growth, whereas cultures C and D were grown in the presence of 0.8 μg of CAP/ml. After transfer to starvation media, cultures B and D were exposed to CAP (0.8 μg/ml), and cultures A and

TABLE 4. *Effects of various antibiotics on ATCase derepression*

Drug	Amount (μg/ml)	Doubling time (min)	ATCase sp act (U/mg of protein)[a]	
			Repressed	Derepressed (2 h)
CAP	0.0	45	1.2 (±0.1)	19.2 (±1.6)
	0.5	60	1.3 (±0.1)	43.8 (±12.6)
Erythromycin	0.0	54	1.2 (±0.1)	25.2 (±1.6)
	4.0	69	3.0 (±0.4)	21.0 (±3.1)
	8.0	93	4.2 (±0.4)	13.5 (±1.3)
Puromycin	0.0	60	1.3 (±0.1)	21.0 (±1.3)
	30.0	78	1.4 (±0.1)	12.3 (±2.1)
Rifampin	0.0	45	1.2 (±0.1)	19.2 (±1.6)
	2.0	60	1.3 (±0.1)	21.0 (±1.2)
Sparsomycin	0.0	42	1.1 (±0.1)	23.8 (±1.9)
	1.5	54	1.1 (±0.1)	18.6 (±1.7)
Tetracycline	0.0	42	1.8 (±0.3)	19.5 (±3.3)
	0.04	54	1.4 (±0.3)	11.7 (±2.2)
	0.08	63	1.9 (±0.4)	10.5 (±2.3)

[a] Five assays of cell extract. Parentheses show standard deviation.

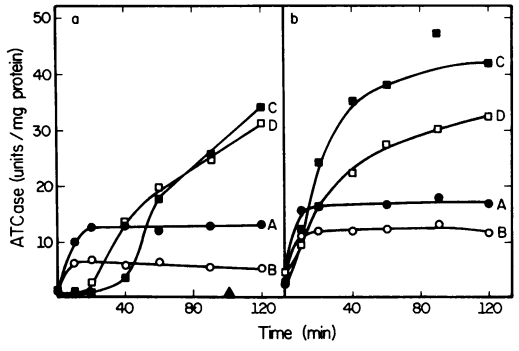

FIG. 2. *ATCase derepression upon transfer to uracil starvation medium. Strain pyrA81 was grown and transferred to uracil starvation medium as described in text. Growth and starvation were in (a) the absence or (b) the presence of supplemental amino acids. CAP, when present, was at 0.8 μg/ml. Symbols: culture A (●) grown minus CAP, starved minus CAP; culture B (○) grown minus CAP, starved with CAP; culture C (■) grown with CAP, starved minus CAP; culture D (□) grown with CAP, starved with CAP. Control not starved for uracil (▲).*

C were not treated with the antibiotic. In both cultures A and B derepression of ATCase synthesis began immediately after transfer into the starvation medium (lacking uracil). Maximal activities were reached within 30 min. The observation that culture B, exposed to CAP only during uracil starvation, did not synthesize ATCase as well as did culture A can be explained by the action of CAP as a protein synthesis inhibitor. Cultures C and D, which were grown in the presence of CAP, lagged for 20 min before derepression of ATCase synthesis began. Once ATCase activity began increasing, it did so for at least 1.5 h and reached much higher activities than in cultures A and B. Continued presence of CAP during starvation (culture D) reduced the lag of ATCase synthesis, but had little effect on the extent of ATCase derepression. The surprising observation is that for CAP to enhance derepression of ATCase upon uracil starvation, it must be present during exponential growth but need not be present during starvation. This result makes it unlikely that the enhanced enzyme synthesis seen in the presence of low concentrations of CAP is a direct result of exposure to the antibiotic. More likely it is an indirect effect, mediated by a change in the metabolism of the cell.

In an analogous experiment, the derepression of OTCase was studied by transfer of exponentially growing cultures of strain *pyrA81* to media lacking arginine. In cultures that were exposed to CAP (0.8 μg/ml) during exponential growth and subsequently starved for arginine for 2 h, OTCase reached a specific activity three to four times that of cultures not exposed to CAP during growth. In this experiment addition of CAP at the time of arginine starvation caused slight enhancement of OTCase synthesis.

Effects of amino acids on enhanced enzyme synthesis. Recovery of growth in cultures after exposure to high levels of CAP (≥50 μg/ml) is known to be faster in an amino acid-supplemented medium than in a minimal medium (22). The addition of amino acids is thought to accelerate conversion of unstable RNA made during exposure to CAP into stable forms (1). Presence of amino acids was shown to accelerate the recovery of growth rate in a culture after removal of low concentrations of CAP. In minimal medium a culture of strain *pyrA81* required 1 h to attain the growth rate of uninhibited cultures after removal of 0.5 μg of CAP/ml, whereas a culture recovering from exposure to CAP (0.5 μg/ml) in a medium supplemented with all 20 amino acids (100 μg/ml each) achieved uninhibited growth rates within 15 min after removal of CAP (data not shown). What effects do amino acids have on the CAP-stimulated enzyme synthesis in nongrowing cells? Figure 2b shows the derepression of ATCase in cultures grown in amino acid-supplemented media (100 μg of each/ml) upon transfer into a uracil starvation medium. The presence of amino acids had no effect on the derepression of ATCase activity in cultures not exposed to CAP during exponential growth. However, the presence of amino acids nearly abolished the lag in the onset of derepression of ATCase in cultures exposed to CAP during exponential growth. The amino acids had little or no effect on the final ATCase activities in any of the cultures. Thus, the effect of CAP on ATCase derepression is not eliminated by the speeded recovery from CAP treatment in the presence of amino acids. Rather, the enhanced enzyme derepression is also speeded by addition of amino acids.

Effect of CAP on inducible enzyme synthesis in nongrowing cells. To test the generality of the effects of CAP on enzyme synthesis further, the induction of β-galactosidase in *E. coli* strain 15T⁻ was studied. It has previously been shown that low concentrations of CAP preferentially inhibit synthesis of β-galactosidase in exponentially growing cells (25, 32), but the effects of CAP on β-galactosidase induction in nongrowing cells were not studied. Cells that had been grown on uracil-supplemented mini-

mal medium with 0.5% glycerol were transferred to starvation media (lacking uracil) containing 10^{-3} M isopropyl-β-D-thiogalactoside. The cultures were exposed to CAP (0.8 μg/ml) during growth and/or starvation as described above. β-Galactosidase synthesis can be induced in the absence of CAP (culture A) under these nongrowing conditions (Fig. 3). Exposure to CAP during exponential growth but not during starvation (culture C) resulted in a twofold enhancement of the synthesis of β-galactosidase. The continued presence of CAP in the growth medium during starvation and induction of β-galactosidase strongly inhibited synthesis of the enzyme (cultures B and D). These results established that prior exposure to CAP brings about enhancement of β-galactosidase synthesis when the enzyme is induced in nongrowing cells. It is interesting to note that this enhanced synthesis is in contrast to the preferential inhibition of β-galactosidase synthesis by the same levels of CAP when the enzyme was induced in growing cells (25, 32).

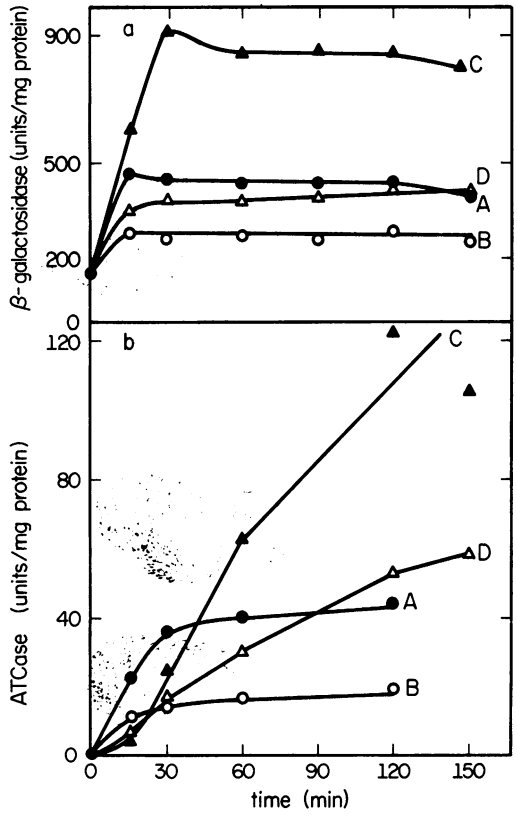

FIG. 3. *Derepression of ATCase and induction of β-galactosidase in E. coli strain 15T⁻. Cultures of strain 15T⁻ were grown and transferred to a uracil starvation medium as described. Isopropyl-β-D-thiogalactoside (10^{-3} M) was added to the starvation medium to induce β-galactosidase. (a) β-Galactosidase specific activity; (b) ATCase specific activity. Symbols: culture A (●) grown minus CAP, starved minus CAP; culture B (○) grown minus CAP, starved with CAP; culture C (▲) grown with CAP, starved minus CAP; culture D (△) grown with CAP, starved with CAP.*

As a control, ATCase was assayed in the same extracts that were assayed for β-galactosidase. Comparison of Fig. 3b and 2a illustrates that derepression of ATCase in *E. coli* strain 15T⁻ shows the same CAP effect as does *S. typhimurium* strain *pyrA81* upon uracil starvation.

Does CAP alter transcription or translation? Studies of the effects of high levels of CAP (≥100 μg/ml) have indicated effects of CAP on both transcription (2, 33) and translation (7). In the present case, low levels of CAP could enhance derepression in nongrowing cells by permitting more extensive or prolonged messenger (m)RNA synthesis or by increasing the stability of the mRNA. To distinguish between these possibilities, RNA synthesis was inhibited with rifampin at various times during ATCase derepression in cultures of *S. typhimurium* strain *pyrA81* grown on limiting uracil. At intervals throughout growth and starvation, duplicate samples were removed. One was harvested immediately; rifampin (50 μg/ml) was added to the other, and it was incubated for a further 30 min at 37 C before it was harvested. In the culture not exposed to CAP (Fig. 4a), addition of rifampin shortly before growth stopped prevented ATCase derepression entirely (presumably by preventing total depletion of uracil). Addition of rifampin to samples during starvation reduced the derepression only slightly, probably because the derepression was nearly complete before the rifampin was added. In a culture treated with 0.8 μg of CAP/ml (Fig. 4b), addition of rifampin to samples taken during ATCase derepression largely prevented the continuation of the derepression. The small increase in ATCase activity after addition of rifampin could easily represent RNA synthesis which was initiated before the rifampin entered the cells. A similar experiment showed that rifampin also blocks the derepression of OTCase synthesis during arginine starvation in CAP-treated cultures of strain *pyrA81*. These results suggest that, in the presence of CAP, synthesis of ATCase (or OTCase) mRNA continues long after it has stopped in untreated controls. The action of CAP is not mediated by prolonging the lifetime of existing mRNA or increasing the rate at which protein is made from the mRNA,

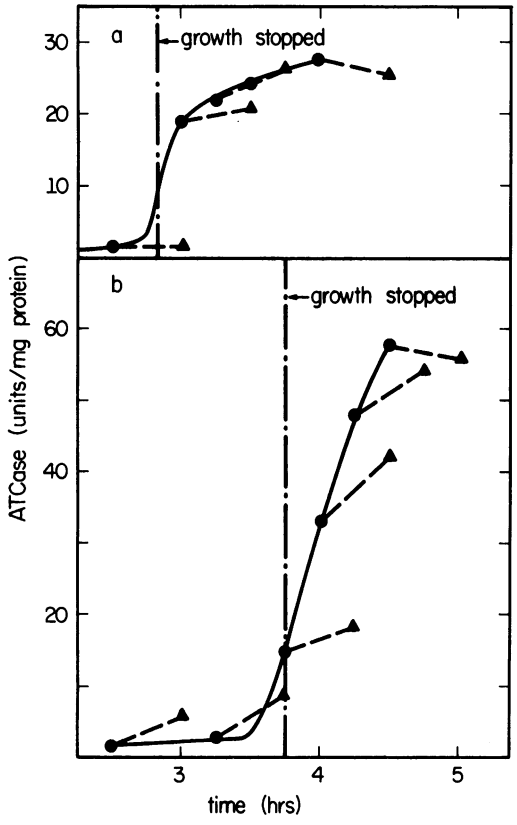

FIG. 4. *Rifampin inhibition of ATCase derepression. Growth of strain pyrA81 was described in text. Duplicate samples were taken throughout ATCase derepression. One was harvested immediately (●). The second was incubated 30 min in the presence of rifampin (50 μg/ml) before being harvested (▲). (a) Cells grown in the absence of CAP. (b) Cells grown in the presence of CAP (0.8 μg/ml).*

because such processes should be insensitive to treatment with rifampin.

DISCUSSION

A most remarkable feature of the enhancement of enzyme synthesis by CAP was the fact that a prior history of exposure to the antibiotic was both necessary and sufficient to obtain the effect. The presence of CAP during the starvation (and derepression) phase actually interfered with enzyme synthesis, as would be expected for an inhibitor of protein synthesis. This suggests that CAP must affect some metabolic process during growth in such a manner that it is observed only after growth has ceased due to starvation for a necessary metabolite. These alterations in metabolism then permit enhanced enzyme synthesis during subsequent starvation. The possible nature of the process affected by CAP is considered below and in the accompanying paper (12).

The CAP effect is not the result of the synthesis of altered forms of the enzymes with enhanced activity. The absence of a stimulatory effect of CAP on ATCase synthesis during exponential growth eliminates the possibility that CAP causes synthesis of a more active form of the enzyme. Further, no kinetic differences were found between ATCase activities in extracts of cells which had been derepressed by uracil starvation in the absence or presence of CAP.

The effects of CAP described here are clearly different from the effects of CAP demonstrated by Sypherd et al. (29–32) and Paigen (25). Those authors showed that the same low levels of CAP preferentially inhibited depression of β-galactosidase and OTCase, but only in exponentially growing cultures (29). We have observed enhanced synthesis of the same enzymes, but only in starved cultures. The preferential inhibition by CAP could also be obtained with other antibiotic protein synthesis inhibitors and other nutritional manipulations (30, 32), but enhanced synthesis was specifically induced by CAP. Thus, in spite of intriguing similarities between the two sets of observations, there is no evidence that the two effects are mediated through similar mechanisms.

What effects of CAP could account for enhanced enzyme synthesis during starvation? The results of experiments in which rifampin was added during starvation suggest that CAP acts to allow more extensive or more prolonged mRNA synthesis during starvation. The two starvation conditions used have in common the fact that both limit total RNA synthesis: uracil starvation by depletion of pyrimidine nucleotide precursors and amino acid starvation by stringent control of RNA synthesis. A reasonable hypothesis is that CAP acts in both cases to relieve the limitation of RNA synthesis and hence to permit more extensive synthesis of message for the enzyme whose synthesis is being derepressed by the starvation conditions used. It has been shown previously (17, 19) and in the accompanying paper (12) that low concentrations of CAP stimulate RNA synthesis in growing cells. Low and high concentrations of CAP cause accumulation of "chloramphenicol-RNA", which is believed to consist primarily of immature ribosomal RNA (24). This "chloramphenicol-RNA" can be degraded, at least in the presence of high levels of CAP (13, 22). Thus, in the case of uracil-starved cells, low levels of CAP could cause accumulation of a pool of unstable RNA, which could be degraded during

starvation and provide an additional pool of pyrimidine nucleotides for mRNA synthesis. Tests of this hypothesis are presented in the accompanying paper (12).

In the case of amino acid starvation, it is more difficult to single out the likely effect of CAP. The possibility that growth on CAP allows prolonged enzyme synthesis during amino acid starvation by causing enlarged arginine pools has not been tested experimentally. However, in view of the fact that low concentrations of CAP have been shown to inhibit, rather than stimulate, protein turnover (26, 28), this possibility seems rather unlikely. Precursors to RNA should be abundant during amino acid starvation, although conflicts in the literature exist concerning the effect of the stringent response on nucleotide pools (3, 5, 8, 9). The stringent response has been reported to inhibit pyrimidine uptake (5, 8, 9), so that the uracil auxotroph used in these studies could be starved for pyrimidine nucleotides during amino acid starvation. Finally, it is possible that the stringent response has some direct effect on mRNA synthesis. The failure to observe the CAP effects in starved *rel*$^-$ cells also suggests some relation of the CAP enhancement of enzyme synthesis to stringent control of RNA synthesis. Although high concentrations of CAP have been shown to relieve the stringent response, the concentrations of CAP used in our experiments do not (17). Thus, there is some question whether the effects of CAP in arginine-starved cells can be related to stimulation of mRNA synthesis.

Our rationalizations of the ability of low levels of CAP to enhance enzyme synthesis are thus centered on the possible effects of the antibiotic on nucleotide pools. The accompanying paper undertakes to determine these effects and, as will be seen, severely undermines the hypothesis presented here.

ACKNOWLEDGMENTS

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