ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1972, p. 449-455

Vol. 2, No. 6
Printed in U.S.A.

Catabolite Repression in Inhibition of β-Galactosidase Synthesis by *Escherichia coli* in the Presence of Agents Producing Translation Errors

M. ARTMAN, S. WERTHAMER, AND PHYLLIS GELB
Section of Microbiology, Methodist Hospital of Brooklyn, Brooklyn, New York 11215

Received for publication 4 August 1972

Studies were made of the synthesis of β-galactosidase by *Escherichia coli* in the presence of 5-fluorouracil, streptomycin, and subinhibitory concentrations of chloramphenicol. The preferential inhibition of β-galactosidase synthesis observed in the presence of the above drugs was found to be caused by catabolite repression.

The best known and most extensively studied agents producing translation errors are 5-fluorouracil and aminoglycoside antibiotics (11, 14). Recently, other antibiotics that interfere reversibly with various aspects of ribosomal function (chloramphenicol, erythromycin, tetracycline, and spectinomycin) were shown to influence, at subinhibitory concentrations, the frequency of misreading (13).

5-Fluorouracil produces translation errors due to substitution of messenger ribonucleic acid (mRNA). Aminoglycoside antibiotics and chloramphenicol induce errors in mRNA reading by affecting the conformation of ribosomes, which influences codon-anticodon pairing. The notion that translation errors occur in vivo is supported by circumstantial evidence: phenotypic suppression of certain nonsense mutations, together with the production of enzymatically inactive, immunologically cross-reacting proteins (11).

The phenotypic reversals of certain rII mutants of phage T4 and of an amber alkaline phosphatase mutant of *Escherichia coli* by 5-fluorouracil were reported by Champe and Benzer (4) and Rosen, Rothman, and Weigert (18), respectively. Phenotypic repair by streptomycin and chloramphenicol of defective genomes in *E. coli* was reported by Gorini and Kataja (6-8) and by Kirschmann and Davis (13), respectively.

The production of faulty proteins (β-galactosidase) in the presence of 5-fluorouracil and aminoglycoside antibiotics was reported by Bussard et al. (3), Nakada and Magasanik (15), and Bissell (1). Horowitz and Kohlmeyer (9) showed that the extent to which β-galactosidase synthesis was inhibited by 5-fluorouracil depended on the carbon source in the medium, and assumed that catabolite repression may play a role in the inhibition of β-galactosidase. Chloramphenicol-promoted inhibition of β-galactosidase was assumed to be due to inhibition of lac mRNA synthesis and to inhibition of some later steps in protein synthesis (19).

In this report, we present evidence to show that the inhibition of β-galactosidase in *E. coli* by streptomycin, chloramphenicol, and 5-fluorouracil is not due to translation errors but is caused by catabolite repression.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. *E. coli* strain B was grown in the synthetic medium of Davis and Mingioli (5) supplemented with 0.25% glucose or 0.4% glycerol as the carbon source. All cultures were grown at 37 C with vigorous agitation and were in the exponential phase at a density of 5×10^8 cells/ml when used for the experiments. Growth was determined in a photoelectric nephelometer calibrated with viable counts. Viability was determined by spreading 0.1 ml of the appropriate diluted bacterial cultures on the surface of agar plates.

Induction of β-galactosidase. The kinetics of β-galactosidase formation was measured at 37 C. The enzyme was induced by addition of the gratuitous inducer isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 10^{-3} or 5×10^{-3} M to cultures shaken in a water bath.

Removal of inducers and inhibitors. Inducers and inhibitors were removed by collecting the bacteria on grade DA membrane filters with a pore size of 0.65 μm (Millipore Corp., Bedford, Mass.). The cells were washed on the filters with prewarmed medium lacking carbon source (taking precautions that the bacteria were not sucked dry) and then resuspended by shaking the filters in fresh warmed medium of the desired composition. The entire procedure takes less than 1 min.

β-Galactosidase assay. A series of small tubes, each containing 100 μg of chloramphenicol in 0.5 ml of dis-

tilled water and a drop of toluene were placed in an ice bath, where they were maintained until the entire series had been taken. At the time of sampling, 0.5 m of bacterial culture was added to each tube, shaken vigorously, and returned to the ice bath. When all samples had been taken, the tubes were incubated at 37 C for 30 min and shaken at intervals to evaporate the toluene. The assay was carried out according to Pardee et al. (16) except that incubations were done at 37 C. One unit of enzyme is defined as producing 1 nmole of *o*-nitrophenol/min at 37 C, *p*H 7.0.

Incorporation of radioactive precursors into protein and RNA. Exponentially growing cultures at a density of 5×10^8 cells per ml were treated with 5-fluorouracil, chloramphenicol, or streptomycin and exposed to the following radioactive compounds: uracil (*6-³H*), 15 μCi/ml (specific activity, 3 μCi/0.08 μmole); adenine (*2-³H*), 15 μCi/ml (specific activity, 3 μCi/0.08 μmole); 5-fluorouracil (*6-³H*), 15 μCi/ml (specific activity, 3 μCi/0.08 μmole); *2-¹⁴C*-leucine, 0.4 μCi/ml (specific activity, 1 μCi/0.5 μmole). At zero time and after various time intervals, 1-ml samples were added to equal volumes of ice-cold trichloroacetic acid (10%, w/v). Cells with incorporated radioactive leucine were heated at 90C for 20 min, cooled, left at 4 C for 30 min, filtered through cellulose nitrate filters (Millipore Corp.), dried, and counted. For samples with radioactive precursors of nucleic acids, the heating step was omitted.

Scintillation counting. All samples were counted in a Picker Anstron scintillation counter. The scintillation fluid contained 0.4% 2,5-diphenyloxazole and 0.1% 1,4-bis-(phenyloxazol-2-yl)benzene in toluene.

Materials. The radioactive compounds were purchased from Schwarz/Mann, Div. of Becton, Dickinson & Co., Orangeburg, N.Y. Cyclic adenosine monophosphate (AMP), IPTG, *o*-nitrophenyl-β-D-galactopyranoside (ONPG), chloramphenicol, 5-fluorouracil, and dihydrostreptomycin sulfate were obtained from Calbiochem, Los Angeles, Calif. All chemicals were of analytical grade.

RESULTS

Effect of streptomycin on β-galactosidase synthesis. The amount of streptomycin required for inhibition of *E. coli* B growing in synthetic medium supplemented with glucose or glycerol was 2.5 μg/ml of medium. The length of time during which the cells exposed to streptomycin remained viable varied depending on the drug concentration. With 5, 10, and 100 μg of streptomycin/ml of medium, the cells remained viable for 60, 15, and 5 min, respectively, before killing effect became apparent. After the addition of streptomycin, the cells continued to synthesize protein for some time (Fig. 1A). In the presence of 10 and 20 μg of streptomycin/ml of medium, the rate of protein synthesis during the initial 10 min of incubation with the drug was almost the same as that of exponentially growing cells and then gradually decreased. Cells exposed to 50 and 100 μg of streptomycin/ml continued to synthesize protein for approximately 10 to 15 min at a steadily decreasing rate. The net synthesis of protein at the end of 10 min of exposure, just prior to the cessation of protein synthesis, was 70 to 75% that of the control cultures.

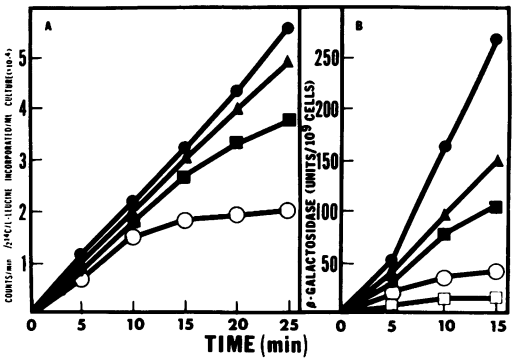

FIG. 1. *Effect of streptomycin on protein and β-galactosidase synthesis. A culture of E. coli B was grown exponentially in synthetic medium with glycerol as carbon source to a density of* 5×10^8 *cells/ml and split into five portions. Each portion was incubated with streptomycin, 2-¹⁴C-leucine, 0.4 μCi/ml (specific activity 1 μCi/0.5 μmole), and IPTG,* 5×10^{-3} M. *At zero time and after various time intervals, samples were taken for the determination of acid-precipitable radioactivity (A), and for β-galactosidase synthesis (B). No drug, ●; streptomycin, 10 μg/ml, ▲; streptomycin, 20 μg/ml, ■; streptomycin, 50 μg/ml, ○; streptomycin, 100 μg/ml, □. (Note: ○ in A represents incorporation of radioactive leucine in the presence of 50 and 100 μg of streptomycin/ml.*

Streptomycin inhibited the synthesis of β-galactosidase to a greater extent than the synthesis of total protein (Fig. 1B). With 10, 20, 50, and 100 μg of streptomycin/ml of medium, there was, respectively, 40, 50, 80, and 90% inhibition of β-galactosidase formation during the initial 10 min after addition of the drug.

The streptomycin-induced preferential inhibition of the synthesis of β-galactosidase has been attributed to misreading of the lac mRNA (7). According to this hypothesis, streptomycin should affect the synthesis of β-galactosidase when present during the phase of lac mRNA translation.

The separation of the process of β-galactosidase synthesis into the phase of enzyme induction and the phase of enzyme production allows experiments designed to answer the question whether streptomycin affects the synthesis of β-galactosidase on the level of transcription or translation. The results of such experiments are illustrated in Fig. 2 and 3. In these experiments, the inducer was removed by filtration at a time before the enzyme had been formed but after the transcription of the

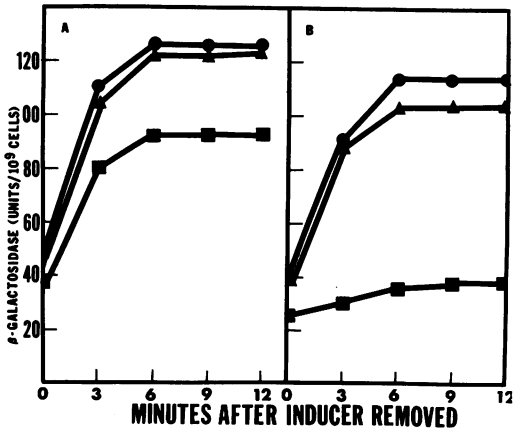

FIG. 2. *Induction of β-galactosidase in the presence of streptomycin. Exponentially growing cells at a density of 5 × 10⁸ cells/ml were induced with IPTG for 3 min, rapidly collected on filters (47 mm in diameter, 0.65 μm pore size), and washed with several 5-ml portions of prewarmed basal salts medium. The bacteria were then resuspended in growth medium without inducer and allowed to synthesize β-galactosidase for 12 min. No drug present during the phase of enzyme induction, ● (A and B); streptomycin present during the phase of enzyme induction, ■, 10 μg/ml (A), 100 μg/ml (B). Streptomycin present during the phase of enzyme production, ▲, 10 μg/ml (A), 100 μg/ml (B).*

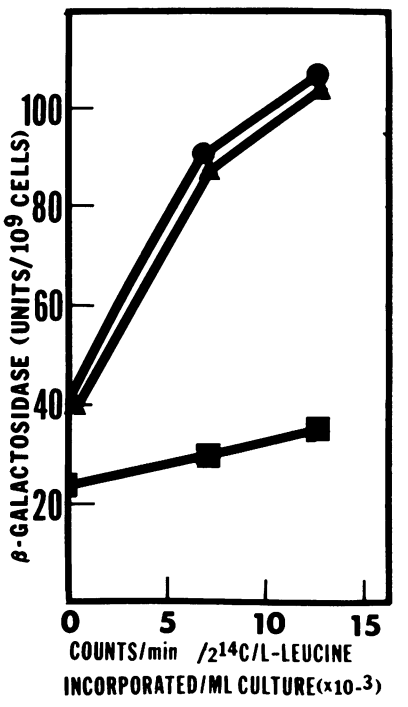

FIG. 3. *Differential rate of β-galactosidase synthesis by cells exposed to 100 μg of streptomycin/ml. Control cells, ●; streptomycin present during the phase of enzyme induction, ■; streptomycin present during the phase of enzyme production, ▲.*

β-galactosidase gene had been completed, i.e., 3 min after addition of the inducer (12). When inducer was removed, the enzyme continued to be synthesized for approximately 9 min. The cells were exponentially growing in glycerol-salts medium until the titer of 5 × 10⁸ cells/ml was reached; they were then divided into three portions. Portion I served as control of cells exposed to IPTG for 3 min and subsequently incubated in inducer-free medium. Portion II was exposed to IPTG for 3 min in a medium containing streptomycin (10 or 100 μg/ml) and was subsequently incubated in inducer-free medium in the absence of streptomycin. The cells of the third portion were induced with IPTG in the absence of streptomycin and were subsequently incubated in inducer-free medium containing 10 or 100 μg of streptomycin/ml. The results presented in Fig. 2 reveal that control cells induced with IPTG for 3 min synthesized about 80 units of β-galactosidase after the removal of inducer. Cells induced in the presence of 10 or 100 μg of streptomycin/ml synthesized, after the removal of inducer and streptomycin, 56 and 12 units of β-galactosidase, respectively. The presence of streptomycin, 10 μg/ml, during the phase of enzyme production had no effect on the amount of the enzyme synthesized. With 100 μg of streptomycin/ml, there was a 20 to 25% decrease in the amount of β-galactosidase synthesized, as compared with the control culture. This decrease could be accounted for by the lower rate of protein synthesis in the presence of high streptomycin concentrations, as shown in Fig. 3, which illustrates the differential rate of the synthesis of β-galactosidase in the presence of 100 μg of streptomycin/ml. It can be seen from Fig. 3 that 100 μg of streptomycin/ml inhibited β-galactosidase synthesis by 85% when present during the phase of enzyme induction, but was without any effect on the amount of the enzyme synthesized when present during the phase of enzyme production.

These results show that streptomycin affects β-galactosidase synthesis on the level of transcription. Before considering the mode of action of streptomycin on β-galactosidase synthesis, it should be pointed out that it is highly unlikely that the drug exerts its effect primarily by preventing uptake of the inducer. Since we used the inducer in 10 times the usual inducing concentration, sufficient inducer enters the cell by diffusion to give maximal rate of induction from the start. The inhibitory effect of streptomycin on β-

galactosidase synthesis restricted to the level of transcription may be due to repression of lac mRNA synthesis or to promotion of errors in the course of transcription of the lac operon. The repression of expression of the lac operon is usually caused by a decreased concentration of cyclic AMP (the cause of catabolite and transient repression [17]), and can be overcome by addition of cyclic AMP to the growth medium. The experiments on the effect of cyclic AMP on the synthesis of β-galactosidase in the presence of streptomycin are illustrated in Fig. 4. It can be seen from Fig. 4 that inhibition of β-galactosidase caused by 10 μg of streptomycin/ml amounts to 54% after 20 min of continuous induction, as compared with control cultures, and can be completely overcome by addition of cyclic AMP to the induction medium. The inhibition caused by 20 μg of streptomycin/ml (59% after 10 min of continuous induction) is also largely overcome by cyclic AMP. The effect of cyclic AMP on the synthesis of β-galactosidase in the presence of 50 and 100 μg of streptomycin/ml, however, is not very pronounced. Although cyclic AMP could not overcome inhibition of β-galactosidase synthesis caused by high streptomycin concentrations, the mode of action of the drug seems to be the same at all concentrations studied, affecting the enzyme synthesis at the level of transcription.

It has to be mentioned that inhibition of β-galactosidase caused by relatively low streptomycin concentrations (from 2.5 to 7.5 μg/ml) could be overcome by 10^{-3} M cyclic AMP. With higher concentrations of streptomycin, the beneficial effect of 10^{-3} M cyclic AMP was clearly pronounced; complete relief of inhibition was obtained with 5×10^{-3} or 10^{-2} M cylic AMP. Control experiments have shown that the killing effect of streptomycin in the presence of 10^{-2} M cyclic AMP remained unaltered. It should be mentioned that Broman, Goldenbaum, and Dobrogosz (2) found that the amount of cyclic AMP needed to reverse catabolite repression varies with the bacterial strain employed and the nature of repressor substrate. Seemingly subtle alterations in the composition of the growth medium also have an influence on the responsiveness of cells to exogenous cyclic AMP.

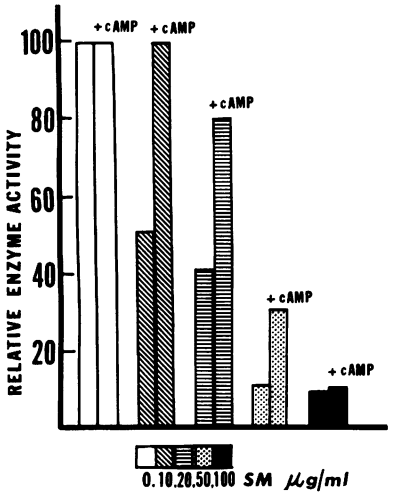

FIG. 4. *Effect of cyclic AMP on the synthesis of β-galactosidase by streptomycin-treated E. coli. Cells growing exponentially with glycerol as carbon source were exposed to streptomycin and induced with IPTG for 5 min in the presence and absence of cyclic AMP. The concentration of cyclic AMP was* 5×10^{-3} *M.*

Effect of chloramphenicol on β-galactosidase synthesis. In the presence of 10 μg of chloramphenicol/ml, bacterial growth and protein synthesis stopped abruptly. With 0.8 μg of chloramphenicol/ml, there was a 50% reduction in the rate of growth and protein synthesis; the latter was determined from the incorporation of leucine-2-^{14}C into trichloroacetic acid-precipitable fraction (Fig. 5A and B). Under these conditions, the inhibition of β-galactosidase synthesis amounted to 80% (Fig. 6). These results are in accord with those of Sypherd and Strauss (19), who showed that chloramphenicol preferentially inhibited the synthesis of certain inducible enzymes in *E. coli*. The results of experiments illustrated in the same figure (Fig. 6) show that addition of cyclic AMP (10^{-3} or 5×10^{-3} M) to the growth medium containing 0.8 μg of chloramphenicol/ml stimulated

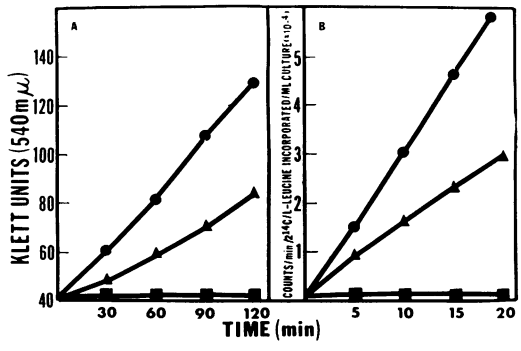

FIG. 5. *Growth and protein synthesis in the presence of chloramphenicol. Exponentially growing E. coli cultures at a density of* 5×10^8 *cells/ml of medium were divided into three portions. All cultures were incubated with leucine-2-*^{14}C*, 0.4 μCi/ml (specific activity, 1 μCi/0.5 μmole). One portion served as control,* ●*; the second portion was incubated with chloramphenicol, 0.8 μg/ml,* ▲*; the third portion was incubated with chloramphenicol, 10 μg/ml,* ■*. Growth (A); protein synthesis (B).*

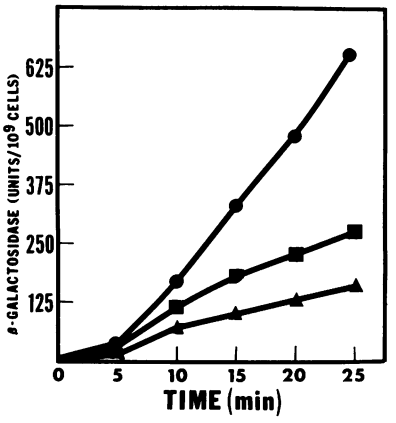

FIG. 6. *Effect of subinhibitory concentrations of chloramphenicol on the synthesis of β-galactosidase. Exponentially growing cells were continuously induced with IPTG. Control culture, ●; chloramphenicol, 0.8 μg/ml, ▲; chloramphenicol, 0.8 μg/ml, + cyclic AMP, ■.*

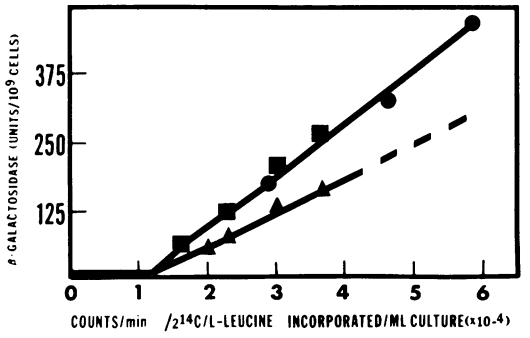

FIG. 7. *Differential rate of β-galactosidase synthesis in chloramphenicol-treated cells in the presence and absence of cyclic AMP. The data are taken from experiments presented in Fig. 5b and 6. No drug, ●; chloramphenicol, 0.8 μg/ml, ▲; chloramphenicol, 0.8 μg/ml, + cyclic AMP, 10^{-3} M, ■.*

twofold the synthesis of β-galactosidase. Since the cells were growing with glycerol as the carbon source, the addition of cyclic AMP to control cultures during the phase of enzyme induction had no effect on the amount of the enzyme produced. The relief by cyclic AMP of chloramphenicol-promoted inhibition of β-galactosidase synthesis is best presented by the differential rate of the synthesis of the enzyme, e.g. the rate of β-galactosidase synthesis relative to the overall rate of protein synthesis. The differential rate of β-galactosidase synthesis by cells exposed to subinhibitory concentrations of chloramphenicol in the presence and absence of cyclic AMP is presented in Fig. 7. The data were taken from experiments presented in Fig. 5B and Fig. 6. The results show that in the presence of cyclic AMP the differential rate of β-galactosidase synthesis by chloramphenicol-treated cells is exactly the same as that observed in normal exponentially growing cultures. In the absence of cyclic AMP, chloramphenicol brought about a 30% decrease in the differential rate of enzyme synthesis.

Effect of 5-fluorouracil on β-galactosidase synthesis. When 5-fluorouracil was added to *E. coli* growing exponentially in synthetic medium with glycerol or glucose as the carbon source, deoxyribonucleic acid synthesis stopped and growth became linear. During the initial 20 min of exposure to 5-fluorouracil, the rate of RNA synthesis per cell slightly diminished and 5-fluorouracil was extensively incorporated into RNA (on a molar basis, 5-flurouracil was incorporated to the extent of 70% as compared to uracil incorporation by exponentially growing cells [10]). Protein synthesis continued at a rate comparable to that observed in exponentially growing cells. The synthesis of β-galactosidase, on the other hand, was severely inhibited, 90 and 75% inhibition after 12 min of exposure to 5-fluorouracil in glucose- and glycerol-containing media, respectively (Table 1). 5-Fluorouracil affected the synthesis of β-galactosidase only when present during the phase of enzyme induction. When added during the phase of enzyme production (after removal of the inducer), 5-flurouracil had no effect on the amount of β-galactosidase formed.

These results are consistent with either (or both) hypothesis regarding the mode of action of 5-fluorouracil (translation errors due to substitution of mRNA or catabolite repression). To distinguish between the two possibilities, we studied the effect of cyclic AMP on the synthesis of β-galactosidase by 5-fluorouracil-treated cells. The results of these experiments, illustrated in Fig. 8, were as follows. Cyclic AMP stimulated twofold the synthesis of β-galactosidase in control cultures growing with glucose as carbon source when added during the phase of enzyme induction. Cyclic AMP had no effect on the amount of enzyme synthesized when added after removal of the inducer. Cyclic AMP had no effect on β-galactosidase synthesis in cultures growing with glycerol as carbon source. Cyclic AMP when added to 5-fluorouracil-treated cultures growing with glucose or glycerol restored the rate of β-galactosidase synthesis to the level characteristic for normal, exponentially growing cells. 5-Flurouracil-promoted inhibition of β-galactosidase synthesis could be overcome by cyclic AMP only when the latter was present during the phase of enzyme induction.

TABLE 1. *Effect of 5-fluorouracil (5-FU) on RNA, protein, and β-galactosidase synthesis*[a]

Cells	Time (Δ min)	Incorporation of radioactive precursors (nmoles/ml)				β-Galactosidase (units/ml)	
		Leucine	Adenine	Uracil	5-FU	In glucose	In glycerol
Control	3	1.42	0.67	0.29	—	15	36
	3	1.42	0.67	0.29	—	30	90
	3	1.45	0.70	0.33	—	60	120
	3	1.46	0.73	0.36	—	70	130
Cells treated with 5-FU	3	1.42	0.55	—	0.170	3	6
	3	1.42	0.55	—	0.170	3	18
	3	1.40	0.56	—	0.168	6	25
	3	1.40	0.55	—	0.168	7	35

[a] Cells were growing in synthetic medium with glycerol as carbon source, unless stated otherwise. For experimental procedures, see Materials and Methods.

DISCUSSION

The main finding of this work is that inhibition of β-galactosidase by fluorouracil, low to moderate concentrations of streptomycin, and subinhibitory concentration of chloramphenicol is not due to errors in translation, but is caused by catabolite repression,

Streptomycin causes misreading in vitro and suppresses certain mutations in vivo, presumably by inducing errors in translation (11). It has been postulated that streptomycin-induced misreading is not confined to the repair of mutated proteins but inevitably leads to the formation of faulty proteins (8). This concept explained the streptomycin-induced inhibition of β-galactosidase (1). This explanation cannot, however, be reconciled with the results of our experiments which show conclusively that streptomycin, at all concentrations studied (10 to 100 μg/ml), affects the synthesis of β-galactosidase during the phase of induction (level of transcription) and has no effect on the formation of the enzyme after removal of the inducer (level of translation). These data, in conjunction with the finding that cyclic AMP can relieve streptomycin-promoted inhibition of β-galactosidase synthesis, implicate catabolite repression as the mechanism by which β-galactosidase synthesis is inhibited in streptomycin-treated cells. The finding that at concentrations which are lethal to the cell streptomycin does not affect enzyme synthesis on the level of translation suggests that streptomycin lethality is not related to streptomycin-induced errors in translation.

The results obtained with cells exposed to subinhibitory concentrations of chloramphenicol were analogous in all respects to those obtained with streptomycin-treated cultures, and therefore led to similar conclusions, namely, that chloramphenicol-promoted inhibition of β-galactosidase synthesis was caused by catabolite repression.

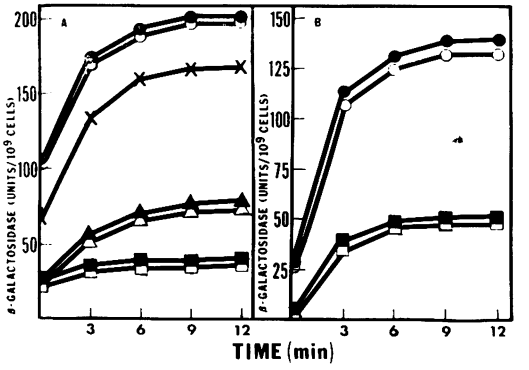

FIG. 8. *Effect of 5-fluorouracil on the synthesis of β-galactosidase by E. coli. For experimental procedures, see Fig. 3. (A) Cells grown with glucose as carbon source. Control culture without cyclic AMP, ▲; control culture, cyclic AMP during the phase of translation, △; control culture, cyclic AMP during the phase of enzyme induction, ●; 5-fluorouracil during the phase of enzyme induction, without cyclic AMP, □; 5-fluorouracil during the phase of enzyme induction, cyclic AMP during the phase of enzyme translation, ■; 5-fluorouracil and cyclic AMP during the phase of enzyme induction, ×; 5-fluorouracil during the phase of enzyme translation, cyclic AMP during the phase of enzyme induction, ○. (B) Cells grown with glycerol as carbon source. Control culture with or without cyclic AMP during the phase of enzyme induction, ●; 5-fluorouracil and cyclic AMP during the phase of enzyme induction, ○; 5-fluorouracil during the phase of enzyme induction, cyclic AMP during the phase of enzyme translation, ■; 5-fluorouracil during the phase of enzyme induction without cyclic AMP, □.*

The complete reversal by cyclic AMP of 5-fluorouracil-promoted inhibition of β-galactosidase synthesis indicates that in this case too the inhibition is caused by catabolite repression.

The absence of translation errors in β-galactosidase synthesis in the presence of 5-fluorouracil is

consistent with the data of Rosen et al. (18), who studied the mechanism of phenotypic reversal of mutants by 5-fluorouracil and found this effect to be site-specific, suggesting that 5-fluorouracil mispaired during translation only at the site of amber codons. The mechanism of the site specificity of 5-fluorouracil-induced errors in translation is at present unknown.

Phenotypic reversal by streptomycin of certain mutations would suggest, therefore, that errors in translation induced by aminoglycoside antibiotics may also be restricted to the site of certain mutated codons.

LITERATURE CITED

1. Bissell, D. M. 1965. Formation of an altered enzyme by *Escherichia coli* in the presence of neomycin. J. Mol. Biol. **14**:619–622.
2. Broman, R. L., P. E. Goldenbaum, and W. J. Dobrogosz. 1970. The effect of amino acids on the ability of cyclic AMP to reverse catabolite repression in *Escherichia coli*. Biochem. Biophys. Res. Commun. **39**:401–406.
3. Bussard, A., S. Naono, F. Gros, and J. Monod. 1960. Effets d'un analogue de l'uracile sur les proprietes d'une proteine enzymatique synthetisée en sa presence. C. R. Acad. Sci. **250**:4049–4051.
4. Champe, S. P., and S. Brenner. 1962. Reversal of mutant phenotypes by 5-fluorouracil: an approach to nucleotide sequences in messenger RNA. Proc. Nat. Acad. Sci. U.S.A. **48**:532–546.
5. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
6. Gorini, L., and E. Kataja. 1964. Streptomycin-induced oversuppression in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. **51**:995–1001.
7. Gorini, L., and E. Kataja. 1964. Phenotypic repair by streptomycin of defective genotypes in *E. coli*. Proc. Nat. Acad Sci. U.S.A. **51**:487–493.
8. Gorini, L., and E. Kataja. 1965. Suppression activated by streptomycin and related antibiotics in drug sensitive strains. Biochem. Biophys. Res. Commun. **18**:656–663.
9. Horowitz, J., and V. Kohlmeier. 1967. Formation of β-galactosidase by *Escherichia coli* treated with 5-fluorouracil. Biochim. Biophys. Acta **142**:208–218.
10. Horowitz, J., J. J. Saukkonen, and E. Chargaff. 1960. Effects of fluoropyrimidines on the synthesis of bacterial proteins and nucleic acids. J. Biol. Chem. **235**:3266–3272.
11. Jacoby, G. A., and L. Gorini. 1967. The effect of streptomycin and other aminoglycoside antibiotics on protein synthesis. Antibiotics. **1**:726–747. Springer Publ.
12. Kaempfer, R. O. R., and B. Magasanik. 1967. Mechanism of β-galactosidase induction in *Escherichia coli*. J. Mol. Biol. **27**:453–468.
13. Kirschmann, C., and B. D. Davis. 1969. Phenotypic suppression in *Escherichia coli* by chloramphenicol and other reversible inhibitors of the ribosome. J. Bacteriol. **98**:152–159.
14. Mandel, H. G. 1969. The incorporation of 5-fluorouracil into RNA and its molecular consequences. In Progress in Molecular and Subcellular Biology. **1**:82–135.
15. Nakada, D., and B. Magasanik. 1964. The role of inducer and catabolite repressor in the synthesis of β-galactosidase by *Escherichia coli*. J. Mol. Biol. **8**:105–127.
16. Pardee, A. B., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by E. coli. J. Mol. Biol. **1**:165–178.
17. Perlman, R. L., B. DeCrombrugghe, and I. Pastan. 1969. Cyclic AMP regulates catabolite and transient repression in *E. coli* Nature (London) **223**:810–812.
18. Rosen, B., F. Rothman, and M. Weigert. 1969. Miscoding caused by 5-fluorouracil. J. Mol. Biol. **44**:363–375.
19. Sypherd, P. S., and N. Strauss. 1963. Chloramphenicol-promoted repression of β-galactosidase synthesis in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **49**:400–407.