further broadens the range of related hydrocarbon structures which spread from a common source through the marine environment.

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References and Notes

1. M. Tsujimoto, *J. Ind. Eng. Chem.* **9**, 1098 (1917).
2. Y. Toyama, *Chem. Umschau* **30**, 181 (1923).
3. P. K. Christensen and N. A. Sörensen, *Acta Chem. Scand.* **5**, 751 (1951).
4. M. Blumer and D. W. Thomas, *Science* **147**, 1148 (1965).
5. M. Blumer, M. M. Mullin, D. W. Thomas, *ibid.* **140**, 974 (1963).
6. ———, *Helgoländer Wiss. Meeresber.* **10**, 187 (1964).
7. J. S. Sörensen and N. A. Sörensen, *Acta Chem. Scand.* **3**, 939 (1949).
8. R. A. W. Johnstone and P. M. Quan, *J. Chem. Soc.* **1963**, 5706 (1963).
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Puromycin: Effect on Messenger RNA Synthesis and β-Galactosidase Formation in Escherichia coli 15T⁻

Abstract. *Incubation of* Escherichia coli *15T⁻ in the presence of puromycin inhibits the inducible formation of β-galactosidase to a greater extent than it inhibits protein synthesis. In* E. coli *33.00 β-galactosidase formation is also more sensitive to the presence of puromycin than is protein synthesis. In the presence of glycerol (but not in its absence) puromycin prevents the production of messenger RNA for β-galactosidase, presumably as a result of catabolite repression.*

Puromycin is a potent inhibitor of protein synthesis in microorganisms (*1*, *2*) and mammalian cells (*3*). In *Escherichia coli* 15T⁻, puromycin inhibits protein formation while allowing ribosomal and transfer RNA (*2*) to accumulate. The present investigation was initiated to determine whether puromycin would also allow messenger RNA (mRNA) to be formed in its presence. Thus the ability of puromycin to affect induced synthesis of β-galactosidase was studied. Although formation of this enzyme is inhibited by the antibiotic, evidence is presented indicating that puromycin does not suppress synthesis of mRNA for β-galactosidase.

Puromycin apparently inhibits β-galactosidase formation to a greater extent than it inhibits total protein synthesis. This preferential inhibition of the enzyme synthesis was demonstrated in the following way. Cell crops of *E. coli* 15T⁻ were grown overnight (17 to 19 hours) on a basal salt medium (*4*) containing glycerol (0.1 percent). After harvesting and resuspending the bacteria in fresh medium containing glycerol, two cultures, both containing puromycin (45 μg/ml), were prepared. To one, leucine-C^{14} was added; and to the other, thiomethylgalactoside (TMG) ($5 \times 10^{-4}M$), an inducer of β-galactosidase, was added. Total protein synthesis was determined by measuring the incorporation of leucine-C^{14}. Samples were taken at intervals and added to an equal volume of cold (0°C) 10 percent trichloroacetic acid (TCA). The precipitates were collected on Millipore filters (0.45-μ pore size), washed six times with 5 percent TCA (containing unlabeled leucine), glued to aluminum planchettes, dried, and counted in a Nuclear Chicago gas flow counter (*5*). Beta-galactosidase was measured in cells that had been treated with toluene by the method of Cohn and Torriani (*6*). Although leucine-C^{14} incorporation was inhibited by only 50 percent, the induced synthesis of β-galactosidase was suppressed more than 90 percent (Fig. 1, *a* and *b*). These results suggested that either the induction process or some step in the synthesis of the enzyme was particularly sensitive to the antibiotic.

As an explanation for the results obtained the induction process was ruled out by studying the effect of puromycin upon β-galactosidase formation and protein synthesis in *E. coli* 33.00, which produces the enzyme constitutively. In this case, also, leucine-C^{14} incorporation was inhibited to a lesser extent than was enzyme synthesis (Fig. 1, *c* and *d*). Thus, the selective effect produced by puromycin upon β-galactosidase formation is not due to a direct influence upon the process of induction. In order to examine the situation in more detail, the following experiments were performed.

Suspensions of *E. coli* 15T⁻ were harvested, washed, and resuspended in basal medium lacking Mg ion. The absence of this ion makes the cells more sensitive to puromycin (*2*). Thiomethylgalactoside was then added to cell suspensions, and the incubation was continued at 37°C. At intervals thereafter puromycin (20 μg/ml) was added, and β-galactosidase was measured at 5-minute intervals. After the addition of puromycin, enzyme activity increased for about 10 minutes and then remained constant (Fig. 2*a*). If the incorporation of tryptophan-C^{14} was measured under the same conditions, no significant amount of inhibition of protein synthesis was observed until after 10 minutes in the presence of the antibiotic (Fig. 2*b*). Thus, the effect upon β-galactosidase appears much greater than the effect on overall protein production. A possible explanation for these observations is that the inhibition of production of β-galactosidase which occurs after the addition of puromycin results from cessation of mRNA synthesis specific for this enzyme.

In order to eliminate the possibility that the constant amount of enzyme might only reflect an instability of the enzyme in the presence of puromycin, a suspension of *E. coli* was treated with TMG for 30 minutes, washed free of inducer, and resuspended in basal medium lacking magnesium. Puromycin was added to this suspension, and samples were removed for estimation of enzyme content. Under these conditions, puromycin did not alter the enzyme activity achieved during the treatment with TMG, and even after 60 minutes the degree of enzyme activity was still essentially unaffected by puromycin. Thus, puromycin does not

Table 1. Influence of puromycin upon the synthesis of mRNA specific for β-galactosidase in *E. coli* 15T⁻.

Incubation media*	Glycerol	Enzyme formed (units/ml)
Control	+	7.3
Puromycin	+	1.3
Control	0	7.4
Puromycin	0	7.3

* TMG ($5 \times 10^{-4}M$) was added to the incubation media 5 minutes after the addition of puromycin (22 μg/ml). Incubation continued for 2 minutes; the cells were then washed with cold buffer (0° to 4°C) on a Millipore filter. Cells were resuspended in warm media and then incubated for 18 minutes. Enzyme activity determined according to Cohn and Torriani (*6*).

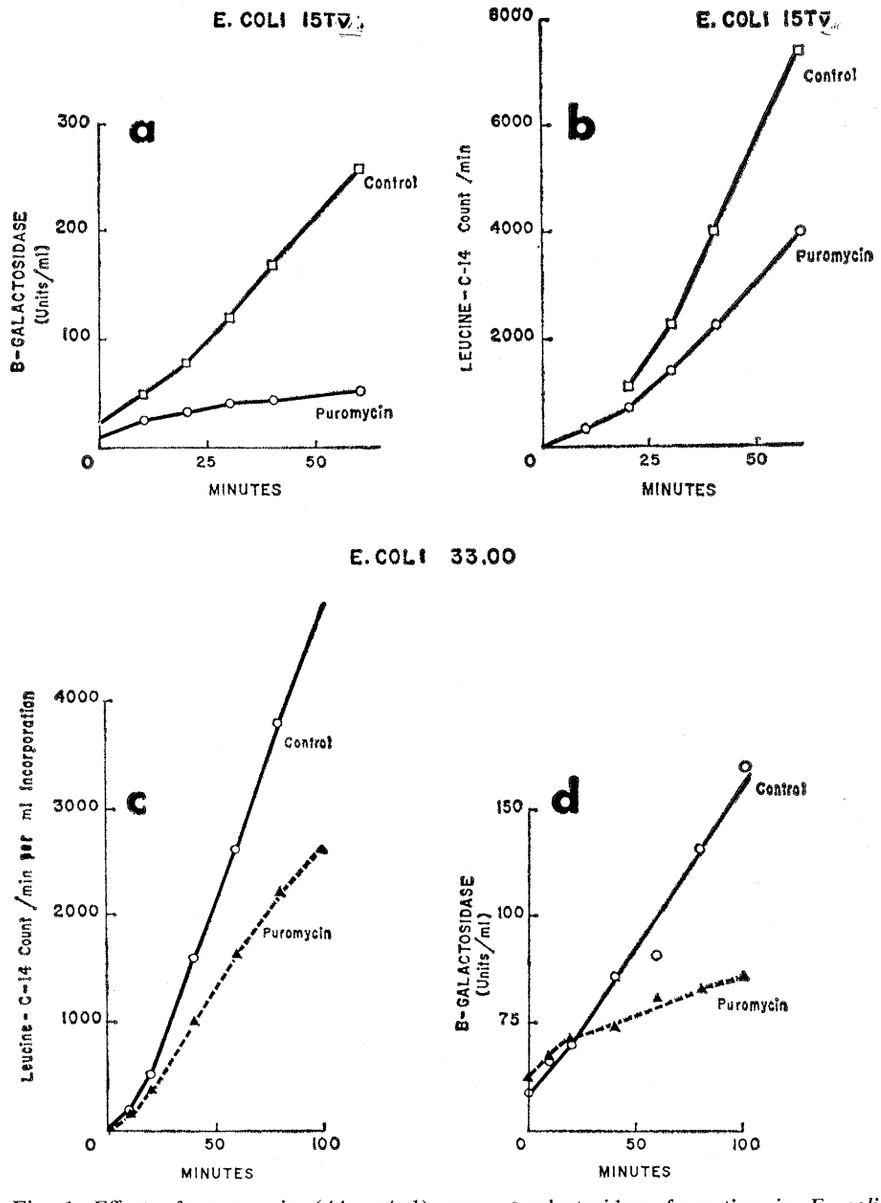

Fig. 1. Effect of puromycin (44 μg/ml) upon β-galactosidase formation in *E. coli* 15T⁻ (*a*) and *E. coli* 33.00 (*d*). Leucine-C^{14} (0.005 μc and 12 μg/ml) incorporation in *E. coli* 15T⁻ (*b*) and *E. coli* 33.00 (*c*) after addition of puromycin.

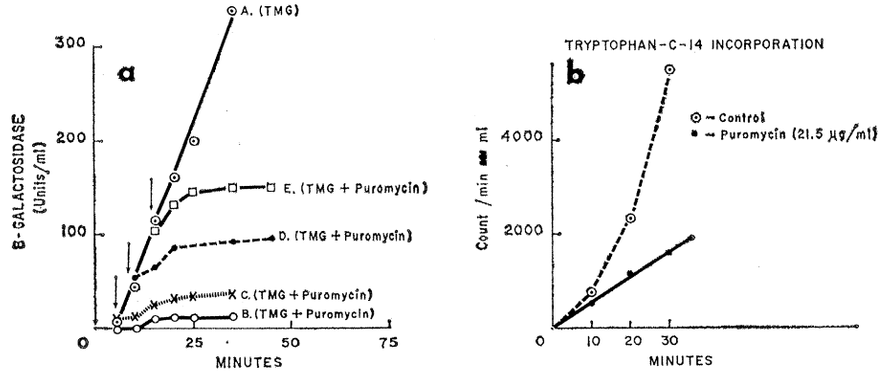

Fig. 2. *a*, Effect of addition of puromycin (21.5 μg/ml) at various intervals (arrows) after TMG ($5 \times 10^{-4} M$) administration upon β-galactosidase synthesis. *b*, Tryptophan-C^{14} (0.04 μc and 4 μg/ml) incorporation in the presence of puromycin (21.5 μg/ml).

affect either the stability or the activity of the enzyme.

To measure the influence of puromycin upon mRNA, experiments were performed to separate the protein synthesizing step from the formation of mRNA. Since extensive inhibition of protein synthesis in *E. coli* K12 in the presence of glycerol allows suppression of mRNA synthesis for β-galactosidase formation (*7*), an examination was made of the influence of puromycin (in the presence and absence of glycerol) upon the first stage of transcription during a time when protein synthesis is not inhibited. Suspensions of *E. coli* 15T⁻ were grown in basal medium lacking magnesium in the presence and absence of glycerol (0.1 percent). Each group of cells was treated with puromycin while the controls were incubated in the absence of antibiotic. The effect of TMG was examined in these various systems for its ability to produce messenger RNA. After addition of TMG the cells were incubated for 2 minutes and then washed with cold media on Millipore filters to remove inducer and inhibitor. The filtered bacteria were resuspended in warmed media containing glycerol, and the incubation was continued for an additional 18 minutes. During the second incubation, the existing messenger expresses itself as enzyme. After resuspension of the bacteria, no further messenger can be formed because of the absence of inducer. All enzyme which is formed, consequently, is due to the accumulation of messenger material made only in the presence of the inducer. Puromycin does not influence the production of mRNA in the first phase of transcription if glycerol is absent from the medium (Table 1). If glycerol is added to the medium, the presence of puromycin allows suppression of mRNA formation although protein synthesis is only slightly affected during this period (Fig. 2*b*). These results suggest that puromycin, in the presence of glycerol: (i) allows the accumulation of a substance which suppresses the synthesis of mRNA specific for β-galactosidase, or (ii) allows greater than normal destruction of mRNA.

Thus the suppression of the β-galactosidase synthesis in the puromycin-treated cells results from an inhibition of the first step in transcription, namely mRNA formation. The complete inhibition of β-galactosidase

messenger synthesis may result from catabolite repression (*8*) and is manifest before significant inhibition of protein formation has occurred. These data with puromycin are in accord with the findings of Paigen (*9*), who reported that chloramphenicol suppressed induced enzyme formation while protein synthesis was unaffected.

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References and Notes

1. Y. Takeda, S. Hayashi, H. Nakagawa, F. Suzuki, *J. Biochem.* (Tokyo) **48**, 169 (1960).
2. B. H. Sells, *Biochim. Biophys. Acta* **80**, 230 (1964).
3. G. C. Mueller, K. Kajiwara, E. Stubblefield, R. Rueckert, *Cancer Res.* **22**, 1084 (1962).
4. S. S. Cohen and J. Arbogast, *J. Exptl. Med.* **91**, 619 (1950).
5. B. H. Sells, *Biochem. Pharmacol.* **2**, 255 (1959); D. B. Roodyn and H. G. Mandel, *Biochim. Biophys. Acta* **41**, 80 (1960).
6. G. Cohn and A. M. Torriani, *Biochim. Biophys. Acta* **10**, 280 (1953).
7. D. Nakada and B. Magasanik, *J. Mol. Biol.* **8**, 105 (1964).
8. D. E. Kennel and B. Magasanik, *Biochim. Biophys. Acta* **55**, 139 (1962).
9. K. Paigen, *ibid.* **77**, 318 (1963).
10. Supported by the Damon Runyon Memorial Fund and the American Lebanese Syrian Associated Charities.

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Fatty Acids in Blue-Green Algal Mat Communities

Abstract. *The concentrations of the major fatty acids were determined for the varied layers of an algal mat community. The living mat contains substantial amounts of the unsaturated acids, while the underlying layers become progressively depleted in unsaturated molecules. A similar progressive increase in the ratio of saturated to unsaturated acids was detected in a sediment core from a hypersaline lagoon.*

The fatty acids are major components of all living organisms. As such they may be an important source material for petroleum and perhaps for the ubiquitous kerogen. Fatty acids have been found in recent and ancient sediments by Cooper and Bray (*1*) and by Abelson *et al.* (*2*). However, the chemical procedure of Cooper and Bray excluded unsaturated acids. The algal mats and their associated sediments provide a natural laboratory in which to test the relative survivability of different fatty acids.

Extensive algal mat communities in the Texas lagoons and mud flats develop in water from 1 to 50 centimeters deep. Many organisms are present in the mat but the biomass is largely made up of the filamentous blue-green algae, *Lyngbya confervoids* (*3*). Well-developed communities have a structure which is of use to the geochemist. The living mat covers the mud bottom like a sheet of leather. Directly under the living mat is a layer of black mud at times a few centimeters thick. The remains of a former mat are under the first mud layer, and they too have a mud layer under them. This pattern of mat and mud repeats itself. In the samples taken for study, the living mat, two former mats, and two mud layers were distinct. All samples were frozen at the time of collection and kept frozen until used.

The analytical procedure was designed to detect fatty acids containing 12 to 20 carbon atoms. Care was taken to minimize decomposition of the unsaturated acids. The frozen sample was treated with dilute HCl to remove carbonate. After filtration the moist sample was treated with methanol for 15 minutes while being stirred and subjected to ultrasonic vibrations. The methanol was recovered by filtration. The sample was treated with chloroform and again stirred and subjected to ultrasonic vibrations. The recovered chloroform was combined with the methanol, and the mixture almost completely dried with a rotating evaporator. This residue was taken up in chloroform and washed with 1*N* HCl to remove inorganic salts. The chloroform was dried under a stream of dry nitrogen. After saponification with potassium hydroxide, impurities were removed by extracting the basic alkaline solution with chloroform. Finally the alkaline solution was acidified, and the fatty acids were extracted into chloroform. The methyl esters were prepared with BF_3 (*4*).

The methyl esters were identified and measured by gas chromatography on a diethelyene glycol succinate column (*5*). Either an Aerograph 202 or an F & M model 400 instrument was used. The chemical yields for the procedure were good, 75 to 95 percent as determined with the use of carbon-14–labeled palmitic acid. The organic carbon content of the sediments was determined by combustion with CuO on a vacuum line, freezing out the CO_2, and allowing it to expand against a calibrated manometer.

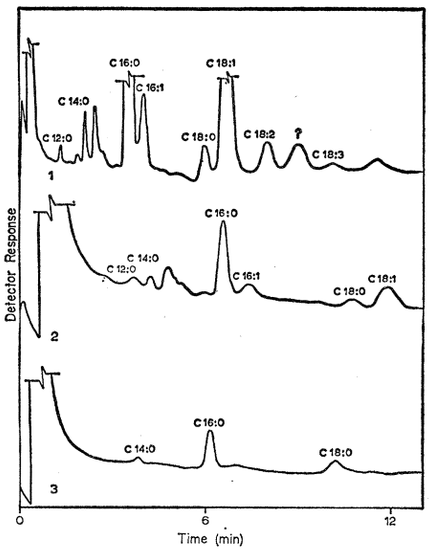

Fig. 1. Gas chromatogram of methyl esters of fatty acids from algal mat: 1, the living mat; 2, first mud layer; 3, second mud layer.

All recent sediments examined thus far are rich in fatty acids (Table 1). All the molecules identified are those which have even numbers of carbon atoms, and which are usually found in organisms. A few unidentified peaks have been encountered, but they do not correspond to any straight-chain fatty acids with even or odd carbon numbers. In the algal mat acids the most striking observation is the systematic change in the ratio of saturated to unsaturated acids (Fig. 1).

The living mat is rich in the unsaturated acids, especially oleic acid. The relative survivability of the different acids is proportional to the degree of unsaturation. This suggests that chemical interactions, such as polymerization, among the organic com-

Table 1. Concentration of the major fatty acids in recent sediments expressed as parts of fatty acid per million parts of organic carbon of each sediment. n.d., not detected.

Location	Organic carbon (%)	C_{16} (0)*	C_{16} (1)	C_{18} (0)	C_{18} (1)
Harbor Island algal mat					
Living mat †	32	3100	560	130	1200
1st mud layer	1.1	1200	330	180	330
2nd mud layer	0.84	200	23	97	n.d.
Baffin Bay core					
0–10 cm	2.0	149	53	53	89
37–41	0.85	192	35	35	50
60–64	1.1	154	6	58	20

* Numbers in parentheses indicate the number of double bonds. † The living mat contained 296 ppm $C_{18}(2)$ and 31 ppm $C_{18}(3)$.