- ¹ McAuslan, B. R., *Biochem. Biophys. Res. Commun.*, **19**, 15 (1965).
- ² McAuslan, B. R., P. Herde, D. Pett, and J. Ross, *Biochem. Biophys. Res. Commun.*, **20**, 586 (1965).
- ³ Jungwirth, C., and W. K. Joklik, *Virology*, **27**, 80 (1965).
- ⁴ Eron, L. J., and B. R. McAuslan, *Biochem. Biophys. Res. Commun.*, **22**, 518 (1966).
- ⁵ McAuslan, B. R., *Virology*, **21**, 383 (1963).
- ⁶ Fenner, F., *Virology*, **5**, 502 (1958).
- ⁷ Joklik, W. K., G. M. Woodroffe, I. H. Holmes, and F. Fenner, *Virology*, **11**, 168 (1960).
- ⁸ McAuslan, B. R., *Virology*, **20**, 162 (1963).
- ⁹ Penman, S., K. Scherrer, Y. Becker, and J. E. Darnell, these PROCEEDINGS, **49**, 654 (1963).
- ¹⁰ Shatkin, A. J., E. D. Sebring, and N. P. Salzman, *Science*, **148**, 87 (1965).
- ¹¹ Becker, Y., and W. K. Joklik, these PROCEEDINGS, **51**, 577 (1964).
- ¹² Salzman, N. P., *Virology*, **10**, 150 (1960).
- ¹³ Easterbrook, K. B., and C. I. Davern, *Virology*, **19**, 509 (1963).
- ¹⁴ Salzman, N. P., A. J. Shatkin, E. D. Sebring, and W. Munyon, in *Cold Spring Harbor Symposium on Quantitative Biology*, vol. 27 (1962), p. 237.
- ¹⁵ Eron, L. J., and B. R. McAuslan, *Biochim. Biophys. Acta*, **114**, 633 (1966).
- ¹⁶ Simon, E. D., *Virology*, **13**, 105 (1961).
- ¹⁷ Planterose, D. N., C. Nishimura, and N. P. Salzman, *Virology*, **18**, 294 (1962).
- ¹⁸ Marmur, J., *J. Mol. Biol.* **3**, 208 (1961).

POLYAMINES AND RNA SYNTHESIS IN A POLYAUXOTROPHIC STRAIN OF *E. COLI**

BY A. RAINA† AND SEYMOUR S. COHEN

DEPARTMENT OF THERAPEUTIC RESEARCH, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA

Communicated by Arthur Kornberg, April 28, 1966

Increasing evidence suggests that the naturally occurring polyamines, putrescine, spermidine, and spermine, play a role in the stability of cellular organelles, membranes, nucleic acids, and particles containing nucleic acid, e.g., ribosomes (see ref. 1). In addition to stabilizing cellular polyanions, polyamines also might affect directly the rate and extent of nucleic acid synthesis, probably by combining with nucleic acid products. Such a mechanism does appear to play a part in the stimulation by polyamines of bacterial DNA-directed RNA polymerase.²⁻⁴ Further, in regenerating rat liver there is a close correlation between the concentration of RNA and polyamines, as well as in the stimulation of synthesis of these compounds, as measured by incorporation of labeled precursors.⁵⁻⁷ A correlation in the accumulation of polyamines and nucleic acids has also been reported to occur in the developing chick embryo.⁸

In the present work we have used the polyauxotrophic *E. coli* strain 15 TAU⁹ to study the relationship between polyamines and nucleic acids in various nutritional conditions. It was shown earlier that by depriving this organism of an essential amino acid, the rate of RNA synthesis is reduced to about 10-15 per cent of normal.^{10, 11} Under these conditions the addition of chloramphenicol or streptomycin results in a stimulation in RNA synthesis.^{11, 12}

In this communication it will be shown that deprivation of an essential amino

acid as well as treatment with the above antibiotics not only changes the rate of RNA synthesis but also markedly changes the polyamine pattern of the cell. The possibility that the polyamine pattern itself might be a major controlling element in RNA synthesis was made more likely by the observation that the addition of spermidine to a growth medium lacking arginine causes a striking stimulation of RNA synthesis. Moreover, this stimulation is largely counteracted by a simultaneous addition of putrescine.

Material and Methods.—Putrescine dihydrochloride and streptomycin sulfate were obtained from the Nutritional Biochemicals Corporation. Spermidine trihydrochloride and uracil-2- C^{14} were purchased from the California Corporation for Biochemical Research. Monoacetylputrescine was kindly supplied by Dr. Herbert Tabor of the National Institutes of Health. Chloramphenicol was a product of Parke-Davis and Co.

A polyauxotrophic mutant of *E. coli* strain 15, designated as strain TAU as a result of its growth requirement for thymine, arginine, and uracil,^{9, 13} was used in these experiments. Bacteria were grown in a mineral medium,¹¹ supplemented with 1 mg glucose, 2 μ g thymine, 20 μ g L-arginine, and 12 μ g uracil per ml, at 37°C with vigorous aeration. The mass doubling time was about 50 min under these conditions. The cells were harvested in exponential growth at $2-3 \times 10^8$ cells per ml and washed once with mineral medium. After resuspension in a small volume of mineral medium, the cells were inoculated in specified culture media and incubated for an additional 3 hr. The cell mass was estimated by determining the turbidity in a Klett-Summerson colorimeter at 420 m μ (a blue filter). At the logarithmic phase of growth, 10^3 turbidity units was equivalent to 9 mg wet weight, 1.9 mg dry weight, and 3.3×10^9 viable cells. Viable count was determined by plating on nutrient broth agar. Incorporation of uracil- C^{14} was determined after filtration of the bacteria on Millipore filters.¹¹ The portion of uracil incorporated into DNA was determined by digesting the acid-insoluble fraction with alkali and precipitating with ethanol as described earlier.¹¹

For the determination of polyamines, 150-ml aliquots were rapidly chilled in crushed ice. The cells were separated by centrifugation and extracted twice with 3% perchloric acid (PCA). The combined PCA extracts were neutralized with 4 N KOH, and $KClO_4$ was removed by centrifugation. The supernatant fluids were made strongly alkaline (pH ≥ 13), saturated with Na_2SO_4 , and extracted with *n*-butanol. The mean recovery in a single butanol extraction was 87% for putrescine, 89% for monoacetylputrescine, and 92% for spermidine and monoacetylspermidine. After evaporating the butanol to dryness, the residue was dissolved in a small volume of 0.1 N HCl. An aliquot of this final extract was hydrolyzed in 6 N HCl at 120°C for 12 hr,¹⁴ evaporated to dryness, and dissolved in the original volume of 0.1 N HCl. The polyamines were separated by paper electrophoresis in 0.1 M sodium citrate (pH 3.6), as described earlier.¹⁵ The paper strips were dried at 75°C for 10 min and stained with ninhydrin as described by Heilmann *et al.*¹⁶ The strips were rapidly drawn through the staining solution and immediately dried in an oven at 75°C for 90 min. The colored bands, along with a blank of equal size, were eluted with 5 ml of a mixture of water-glacial acetic acid-ethanol (1:5:4) containing 20 mg of cadmium acetate per ml. The extracted color, which has a maximum absorption at 505 m μ , is stable for several hours when kept in the dark. The amount of polyamine derivatives was calculated from the difference of the polyamine content of the hydrolyzed and unhydrolyzed extract. Monoacetylspermidine, which gives a color intensity with ninhydrin two thirds that obtained with free spermidine, can also be determined directly from the unhydrolyzed sample after paper electrophoresis, whereas monoacetylputrescine and basic amino acids migrate closely together. The monoacetyl derivatives of putrescine and spermidine were identified by paper chromatography¹⁴ and paper electrophoresis. For the determination of polyamines from the culture medium, an aliquot of 100 ml was first concentrated under vacuum to less than one tenth the original volume and, after addition of alkali, extracted with *n*-butanol as described above. RNA was determined in a hot trichloroacetic acid extract by Dische's modification of the orcinol reaction.¹⁷

Results.—*Polyamine patterns in growing and nongrowing cells:* Putrescine, spermidine, and their monoacetyl derivatives, identified by paper electrophoresis and paper chromatography, were the main polyamines in *E. coli* strain TAU when grown in a glucose-mineral medium at pH 7.4, supplemented with thymine, arginine,

FIG. 1.—The effect of arginine deprivation and chloramphenicol on the polyamine and RNA content of *E. coli* strain TAU. The cells were harvested in exponential growth and incubated for 3 hr in media with various additions as indicated in the graph. At zero time the concentration of cells was 2.9×10^8 cells per ml. Aliquots were taken at 60 and 180 min and analyzed as described in *Methods*. The polyamine contents are expressed as μmoles and RNA-P as μmoles per 10^3 Klett units (approx. 9 mg wet weight). T = thymine, A = arginine, U = uracil, CM = chloramphenicol, 20 μg per ml.

and uracil. In growing cells the ratio of total polyamine nitrogen to RNA phosphorus was found to vary within fairly narrow limits, i.e., 0.21–0.23 (see Fig. 1 and legend to Fig. 2). No spermine was detected in this organism (less than 0.5 μmole per μmole RNA-P could have been detected).

In a complete system there was only little increase in the polyamine content per cell during exponential growth (Fig. 1). The average intracellular concentration of total putrescine was 10.8 μmoles and that of total spermidine 4.7 μmoles per gram wet weight. About 20 per cent of the total putrescine and 60 per cent of the total spermidine within the cells occurred as monoacetyl derivatives under these conditions. Elimination of arginine from the growth medium distinctly changed the polyamine pattern of the cells, under conditions in which the total RNA content was not extensively altered. The putrescine content increased by about 60 per cent within 1 hr and remained elevated during further incubation, whereas the concentration of spermidine remained at the initial level. This also resulted in a significant increase in the ratio of putrescine to spermidine. A similar accumulation of putrescine, without any essential change in spermidine, was also found when the growth was stopped by exhaustion of glucose in a complete medium. In both the above cases, in which the putrescine content of cells was markedly increased, there was also extrusion of large amounts of putrescine into the medium, mainly in the unconjugated state. The amount of putrescine found in the medium was several times in excess of that recovered from the cells at the end of the 3-hr incubation period. Much less spermidine, almost entirely as the monoacetyl derivative, was found in the medium.

Effect of chloramphenicol: The addition of chloramphenicol produced a very considerable increase in both free and total spermidine, but only moderately increased the putrescine content of the cells (Fig. 1). At the end of the 3-hr incubation period, the chloramphenicol-treated cells contained roughly twice as much spermidine as those without chloramphenicol, approaching a concentration of total spermidine close to 10 mM. This increased accumulation of spermidine within the cells was seen both in the presence and absence of arginine. In the latter case there was also a significant stimulation by chloramphenicol in the net increment of spermidine. Although the cellular changes in RNA and spermidine were parallel in the first hour, it can be seen in Figure 1 that chloramphenicol also produced marked increases in free and total spermidine long after accumulation of RNA had stopped.

FIG. 2.—The effect of streptomycin (60 $\mu\text{g/ml}$) on the relative ratio of polyamine to RNA in *E. coli* strain TAU. The experimental conditions were comparable to those indicated in Fig. 1, except that 3 mg of glucose (instead of 1 mg) per ml was added to the growth medium. The actual values for zero time, i.e., 65.5 μmoles of free putrescine, 11 μmoles of free spermidine, and 26.4 μmoles of total spermidine per μmole of RNA phosphorus, were taken to be 1.0. T = thymine, A = arginine, U = uracil, SM = streptomycin.

Effect of streptomycin: Earlier studies from this laboratory¹² have shown that streptomycin, like chloramphenicol, can stimulate RNA synthesis in the absence of arginine, although the stimulation by streptomycin appears only after a certain lag period. The relative ratio of cellular polyamines to RNA in bacteria incubated in the presence and absence of streptomycin is plotted in Figure 2. In the presence of arginine, streptomycin at 60 μg per ml caused an almost immediate decrease in the putrescine content of the cells. At 1 hr in the presence of the antibiotic the medium was observed to contain twice as much putrescine as that without streptomycin, although in that time the sums of intra- and extracellular putrescine in the two systems were essentially identical. In the absence of arginine this extrusion of putrescine from the cells was prevented for an hour, but at 3 hr was almost as complete as that observed in the complete medium. In sharp contrast to the fate of putrescine, the spermidine content of the streptomycin-treated cells significantly exceeded that without streptomycin. The net effect was a marked decrease in the ratio of intracellular putrescine to spermidine. In the absence of arginine both the increase in free spermidine and the stimulation of uracil incorporation by streptomycin¹² do not take place until after 1 hr incubation.

The changes in the total amount of polyamines, comprising both free amines and their butanol-soluble derivatives, isolated from the same volume of culture are presented in Figure 3. In the absence of arginine, streptomycin reduced total putrescine production but did not affect spermidine production, as did chloramphenicol. In the presence of arginine, such an inhibition did not occur, if the increment of polyamines is calculated per cell.

FIG. 3.—Total polyamines recovered from the cells and medium of 150-ml aliquots of culture. The cultures are the same as in Fig. 2.

Effect of polyamines on uracil- C^{14} incorporation in the absence of arginine: The observations that chloramphenicol and streptomycin, both of which stimulated RNA synthesis in the absence of arginine, also had a similar effect on the cell polyamines, i.e., they caused an increase in the cellular concentration of spermidine and a decrease in the ratio of putrescine to spermidine, prompted us to study the direct effect of these polyamines on incorporation of radioactive uracil in the absence of amino acid. In experiments not shown in the graphs, it was found that a simultaneous addition of putrescine and spermidine in a molar ratio 2:1 at a concentration of 10 mM putrescine and 5 mM spermi-

dine, or less, had no effect on uracil incorporation. RNA synthesis was, however, clearly stimulated in a medium containing 50 mM putrescine and 25 mM spermidine. At a concentration of 200 mM putrescine and 100 mM spermidine, the uracil incorporation was inhibited for the first hour, followed by a significant stimulation.

In a subsequent experiment only a single polyamine was added to the medium (Fig. 4). Putrescine alone at a concentration of 150 mM slightly stimulated RNA synthesis, but had no effect at 50 mM or less. Spermidine alone at a concentration of 20 mM or higher markedly increased uracil incorporation into RNA.

Extending these results, increasing amounts of putrescine were added to media containing 80 mM spermidine (Fig. 5). In 80 mM spermidine alone, the rate of uracil incorporation was about sevenfold that of the control; such a treatment was also lethal. While the viability at 1 hr was essentially unchanged, at 3 hr it was reduced by 70 per cent. Increasing amounts of putrescine gradually abolished both the stimulation of RNA synthesis and lethality. In 160 mM putrescine, the uracil incorporation was reduced at first even below the control. It can be seen in Figure 5 also that during the first 30 min the stimulation by 20 μ g per ml of chloramphenicol was of the same magnitude as obtained with spermidine. The chloramphenicol effect, however, largely shut off after 1 hr, unlike that of spermidine.

Discussion.—Our data for the polyamine content of *E. coli* strain 15 TAU are in general agreement with those reported for *E. coli* B grown in mineral medium.¹⁴ The polyamine pattern, however, is dependent on the composition and pH of the culture medium. For instance, cells of *E. coli* B grown in triethanolamine buffer have a lower ratio of putrescine to spermidine than the cells grown in mineral medium.¹⁴ Increase in pH results in a decrease in putrescine and an increase in spermidine, especially in the acetyl derivatives of the latter.^{14, 18} Addition of exogenous polyamines to the growth medium also enhances the rate of acetylation.¹⁴

During normal growth, large amounts of putrescine and small amounts of acetyl-spermidine are leaked into the medium. In many cases this necessitates the inclusion of the medium in analysis. On the other hand, it has been reported that

FIG. 4.—The effect of polyamines on uracil incorporation in *E. coli* strain TAU in the absence of arginine. The cells grown in a complete medium were harvested at a concentration of 2×10^8 cells per ml and incubated in media containing uracil- C^{14} and lacking arginine. The concentrations of the polyamines added into the media are given in the graph. T = thymine, U = uracil- $2-C^{14}$, Pu = putrescine, Spd = spermidine. $\circ$ — $\circ$, T + U; Δ — Δ , T + U + Spd; ∇ — ∇ , T + U + Pu.

FIG. 5.—The effect of putrescine on the stimulation of RNA synthesis caused by spermidine. The concentration of chloramphenicol was 20 μ g per ml; the concentrations of putrescine and spermidine are given in the graph. T = thymine, U = uracil- $2-C^{14}$, CM = chloramphenicol, Pu = putrescine, Spd = spermidine.

essentially no putrescine is detected in culture media of *E. coli* strain W.¹⁸ Despite the possibility of turnover of the polyamines, *E. coli* does not readily obtain nitrogen or carbon from putrescine,^{19, 20} nor is there indication of degradation of spermidine in this organism.¹⁴

Treatment with streptomycin resulted in an almost immediate extrusion of putrescine from the cells in a complete medium; omission of arginine delayed the release of putrescine. In contrast, there was an increase in both total and free spermidine, the latter stimulation being particularly marked in the absence of the amino acid. The loss of putrescine may be due to altered permeability of the cell membrane, as in the leakage of amino acids²¹ and potassium.²² In our later experiments (unpublished) it has been shown that chloramphenicol, added together with streptomycin, prevents the leakage of putrescine, as it also protects against the lethality of streptomycin. Neither putrescine nor spermidine added to the growth medium simultaneously with streptomycin protected against the lethality of the latter.

The cause of the accumulation of spermidine in cells treated with chloramphenicol is not known; this was observed both in the presence and absence of arginine and must arise in part from stimulation of spermidine synthesis. Both chloramphenicol and streptomycin treatment modified the polyamine pattern of cells in the same direction. In both cases the concentration of spermidine was increased and the ratio of putrescine to spermidine decreased. A question arises of whether the change in the polyamines is the cause of stimulation of RNA synthesis observed with those antibiotics, whether an excess of RNA induced by the antibiotics stimulates spermidine synthesis, or whether polyamine and RNA are independently stimulated. However, the fact that in the absence of arginine, exogenous spermidine even at a concentration of 20 mM caused a significant increase in uracil incorporation suggests that a relatively high intracellular spermidine content can itself stimulate RNA synthesis. Stimulation of RNA synthesis has also been observed in tissue culture in the presence of exogenous spermine.²³ The present observation that putrescine, which in itself only slightly stimulated RNA synthesis, can abolish the stimulation by spermidine, indicates that both the concentration and the relative amounts of the several polyamines are of significance.

Summary.—Polyamine patterns in *E. coli* were studied in their relation to RNA synthesis during either normal growth, amino acid deprivation, or treatment with chloramphenicol and streptomycin. Putrescine, spermidine, and their monoacetyl derivatives are the main polyamines in *E. coli* strain 15 TAU. Deprivation of arginine causes accumulation of putrescine within the cells, whereas the spermidine and RNA content remain essentially unchanged. Addition of chloramphenicol markedly increases the biosynthesis and accumulation of spermidine within the cells and also increases the RNA content. On the other hand, treatment with streptomycin results in a moderate increase in intracellular spermidine, but causes a dramatic leakage of putrescine into the medium; the latter effect is delayed in the absence of arginine.

Addition of spermidine to a medium devoid of arginine caused up to a sevenfold increase in uracil incorporation into RNA, and was slowly lethal. The stimulation of RNA synthesis, as well as lethality, was gradually abolished by increasing amounts of putrescine.

* This investigation has been supported by a USPHS grant 7005 from the National Institute of Allergy and Infectious Diseases and by a grant from the Sigrid Juselius Foundation, Helsinki, Finland.

† Postdoctoral fellow of National Institutes of Health, U.S. Public Health Service (1 F05-TW-887-01).

- ¹ Tabor, H., and C. W. Tabor, *Pharmacol. Rev.*, **16**, 245 (1964).
- ² Krakow, J. S., *Biochim. Biophys. Acta*, **72**, 566 (1963).
- ³ Fox, C. F., and S. B. Weiss, *J. Biol. Chem.*, **239**, 175 (1964).
- ⁴ Fox, C. F., W. S. Robinson, R. Haselkorn, and S. B. Weiss, *J. Biol. Chem.*, **239**, 186 (1964).
- ⁵ Raina, A., J. Jänne, and M. Siimes, in *Abstracts*, Second Meeting, Federation of European Biochemical Societies, Vienna, 1965.
- ⁶ Raina, A., J. Jänne, and M. Siimes, *Biochim. Biophys. Acta*, in press.
- ⁷ Dykstra, W. G., Jr., and E. J. Herbst, *Science*, **149**, 428 (1965).
- ⁸ Calderera, C. M., B. Barbiroli, and G. Moruzzi, *Biochem. J.*, **97**, 84 (1965).
- ⁹ Kanazir, D., H. D. Barner, J. G. Flaks, and S. S. Cohen, *Biochim. Biophys. Acta*, **34**, 341 (1959).
- ¹⁰ Cohen, S. S., M. Sekiguchi, J. L. Stern, and H. D. Barner, these PROCEEDINGS, **49**, 699 (1963).
- ¹¹ Stern, J. L., M. Sekiguchi, H. D. Barner, and S. S. Cohen, *J. Mol. Biol.*, **8**, 629 (1964).
- ¹² Stern, J. L., and S. S. Cohen, these PROCEEDINGS, **51**, 859 (1964).
- ¹³ Barner, H. D., and S. S. Cohen, *J. Bacteriol.*, **74**, 350 (1957).
- ¹⁴ Dubin, D. T., and S. M. Rosenthal, *J. Biol. Chem.*, **235**, 776 (1960).
- ¹⁵ Raina, A., *Acta Physiol. Scand.*, **60**, Suppl. 218 (1963).
- ¹⁶ Heilmann, J., J. Barrolier, and E. Watzke, *Z. Physiol. Chem.*, **309**, 219 (1957).
- ¹⁷ Ashwell, G., in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan (New York: Academic Press, 1957), vol. 3, p. 73.
- ¹⁸ Tabor, H., S. M. Rosenthal, and C. W. Tabor, *J. Biol. Chem.*, **233**, 907 (1958).
- ¹⁹ Gale, E. F., *Biochem. J.*, **36**, 64 (1942).
- ²⁰ Kim, K., *J. Bacteriol.*, **86**, 320 (1963).
- ²¹ Anand, N., and B. D. Davis, *Nature*, **185**, 22 (1960).
- ²² Dubin, D. T., R. Hancock, and B. D. Davis, *Biochim. Biophys. Acta*, **74**, 476 (1963).
- ²³ Goldstein, J., *Exptl. Cell Res.*, **37**, 494 (1965).

**DIFFERENTIAL SYNTHESIS OF RIBONUCLEIC ACID
IN PROSTATIC NUCLEI: EVIDENCE FOR
SELECTIVE GENE TRANSCRIPTION INDUCED BY ANDROGENS***

BY SHUTSUNG LIAO, R. W. BARTON,† AND ALICE H. LIN

THE BEN MAY LABORATORY FOR CANCER RESEARCH AND THE
DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CHICAGO

Communicated by Charles B. Huggins, April 25, 1966

It is now well established that RNA synthesis in the cell nucleus plays an important role in the hormonal control of biological functions.^{1, 2} Rapid increases of RNA synthesis, demonstrated by pulse-labeling RNA with radioactive precursors *in vivo*^{2, 3} or by measuring RNA polymerase activity *in vitro*,⁴⁻¹⁰ have been observed in hormone-sensitive tissues shortly after the administration of either steroidal or nonsteroidal hormones to hormone-deficient animals. The findings that several hormones induce the synthesis of specific proteins¹¹ also prompted the concept that certain hormones may regulate gene expression at the sites of RNA synthesis. Sev-