THE EFFECT OF TEMPERATURE ON THE ACETYLATION OF SPERMIDINE

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Previous publications have reported that E. coli contains, in addition to 1,4-diaminobutane and spermidine, large amounts of their acetylated derivatives (Dubin and Rosenthal, 1966; Raina and Cohen, 1966). Similarly, after uptake of ^{14}C-spermidine, a large percentage of the labeled amine was acetylated (Dubin and Rosenthal, 1960; Tabor and Tabor, 1966). These findings have recently had renewed interest because of the possible importance of the amines and their acetylated derivatives in the control of RNA synthesis (Raina and Cohen, 1966; Dykstra and Herbst, 1965; Raina et al, 1966; Raina et al, 1967); as part of the latter studies, measurements have been made of both the free and acetylated amine levels of E. coli.

Our present study indicates that the methods used for harvesting cells in the older studies, namely, cooling the culture in ice and centrifuging the cells in the cold, may have resulted in striking changes in the degree of acetylation of the amines. We have found that significant acetylation of tracer amounts of ^{14}C-spermidine by E. coli B occurred only in the cold. No acetylation (<1%) was observed when the culture was maintained at 37° and harvested without exposure to cold. These findings are in marked contrast to the large amount of acetylation which we and others have reported. On the other hand, when cells which had been prelabeled with ^{14}C-spermidine at 37° were cooled to 4° and stored at that temperature for 2.5 hours, 59% of the ^{14}C-amine was converted to monoacetylspermidine.

METHODS. E. coli B was grown at 37° with vigorous aeration in 250 ml of a glucose-salts medium (Vogel and Bonner, 1956) to a density of 0.5-1.0 x 10^8 cells per ml. ^{14}C-spermidine (specific activity 4.4 x 10^6 cpm/μmole) was then added to give a final concentration of 3.3 x 10^{-8} M, and the cells were allowed to grow at 37° for an additional 10 minutes. During this interval over 85% of the ^{14}C-spermidine added to the medium was taken up by the cells. The cultures were then maintained at either 37° with vigorous aeration or 4° without aeration. Periodically 2 ml aliquots of the cultures were filtered through millipore filters at room temperature; the filters were immersed in a dioxane phosphor (Davidson and Feigelson, 1957), and total cellular radioactivity was determined in a scintillation counter. At the same periods 25 ml aliquots were rapidly (<60 seconds) filtered through 47 mm millipore filters at room temperature; the entire filter was then immediately extracted with 1 M trichloracetic acid. This extract was subjected to paper chromatography, using the methods previously described for the determination of the percent of total counts in free spermidine and in monoacetylspermidine (Tabor and Tabor, 1966). The areas of the chromatogram which coincided with known marker compounds were cut out and the radioactivity determined in a scintillation counter. This method was sufficiently sensitive to detect 1% conversion of ^{14}C-spermidine to ^{14}C-monoacetylspermidine.

RESULTS. One aliquot of the cells containing ^{14}C-spermidine was allowed to grow at 37° with aeration for another 4 generations (180 minutes). The cells were rapidly harvested at room temperature by millipore filtration without cooling. No ^{14}C-monoacetylspermidine was found in the trichloracetic acid extracts of these cells (Fig. 1).

Another aliquot of the cells containing ^{14}C-spermidine was rapidly cooled to 4° in an ice slush and kept at this temperature for 130 minutes. At the end of this experiment, 59% of the radioactivity was present as monoacetylspermidine, while only 41% remained as spermidine (Fig. 1). A smaller

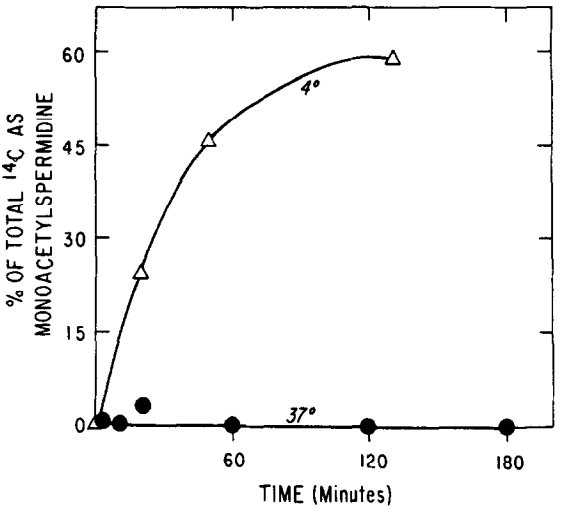

Fig. 1. Effect of temperature on the conversion of ^{14}C-spermidine to ^{14}C-monoacetylspermidine. Conditions of growth, harvesting, and assays of radioactivity are described in "Methods." Essentially no counts were lost from the cells incubated at 37° for 180 minutes; less than 20% of the total cellular ^{14}C was lost from the cells stored at 4° for 130 minutes. ●——●, incubation at 37°; Δ——Δ, incubation at 4°.

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percentage of the total amines was acetylated during 2 to 3 hours' storage at 0° (33%) or at 11° (35%).

DISCUSSION. Under the experimental conditions used in this study, essentially none of the ^{14}C-spermidine taken up by labeled E. coli B was converted to ^{14}C-monoacetylspermidine during logarithmic growth. In the past in our laboratory and in others, care was taken to cool cultures and to centrifuge the cells in the cold to prevent metabolic changes from occurring during harvesting. These precautions resulted in the conversion of a significant percentage of ^{14}C-spermidine to ^{14}C-monoacetylspermidine. The striking effects due to the method of harvesting on the polyamine content of E. coli make it difficult to use data in the literature to evaluate the role of spermidine or its derivatives in bacterial metabolism until the various analytical studies are repeated. Furthermore, the experiments presented in this paper point up the possible complications that may result from harvesting the cells

in the cold in other studies of bacterial chemistry.

Among the possible explanations of the very striking increased acetylation which occurs in the cold are an increased availability of acetyl-Co A, a diminished binding of spermidine to cellular polyacids, a cold-activation of the acetylating enzyme, and a markedly diminished turnover of acetyl spermidine. Further studies are needed to determine the mechanism involved.

REFERENCES

Davidson, J. D., and Feigelson, P., Intern. J. Appl. Radiation and Isotopes, 2, 1 (1957).
Dubin, D. T., and Rosenthal, S. M., J. Biol. Chem., 235, 776 (1960).
Dykstra, Jr., W. G., and Herbst, E. J., Science, 149, 428 (1965).
Raina, A., and Cohen, S. S., Proc. Nat. Acad. Sci., 55, 1587 (1966).
Raina, A., Jänne, J., and Siimes, M., Biochim. Biophys. Acta, 123, 197 (1966).
Raina, A., Jansen, M., and Cohen, S. S., J. Bacteriol., 94, 1684 (1967).
Tabor, C. W., and Tabor, H., J. Biol. Chem., 241, 3714 (1966).
Vogel, H. J., and Bonner, D. M., J. Biol. Chem., 218, 97 (1956).