THE ABNORMAL PATTERN OF PROTEIN SYNTHESIS IN *PSEUDOMONAS AZOTOGENSIS* IN THE PRESENCE OF HEXETIDINE[1]

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In previous studies we have shown that the ratio of Δ protein/Δ nucleic acid in *Pseudomonas azotogensis* is doubled during exponential growth in the presence of hexetidine [3]. The inhibitor leads to a 50 per cent increase in the mass per cell which is entirely accounted for by increased protein while the nucleic acid per cell is unaltered. These increases could be attributed to (*a*) the synthesis of specific protein(s) in response to hexetidine, or (*b*) an increase in the differential rate of general protein synthesis.

One method by which the functionality of the newly synthesized protein can be examined is to determine whether, in the presence of hexetidine, the development of individual enzymes was in the same ratio to one another and to the newly synthesized cell mass as they were in normal cultures. The results of direct measurements of the effect of hexetidine on the synthesis of three enzymes are shown in Table I. In all three cases, hexetidine not only inhibited enzyme biosynthesis but also the enzyme levels/ml of culture. The latter reductions were variable, suggesting that the entire phenomenon could not be attributed to cell lysis. Of the three enzymes examined, only DPNH cytochrome c reductase increased in the presence of the inhibitor (12 per cent of the $\Delta E/\Delta OD$ of the control). Alpha-glucosidase synthesis was completely inhibited and a slight decrease in the level of 6 PG dehydrogenase was observed.

TABLE I. *Effect of hexetidine on enzyme biosynthesis.*

Enzyme	Reference	Control	Hexitidine		
		$\Delta E/\Delta OD$	At addition	Δ OD of 0.087	$\Delta E/\Delta OD$
DPNH cytochrome *c* reductase	2	14.9×10^{-3}	1.10×10^{-3}	1.26×10^{-3}	1.83×10^{-3}
6 PG dehydrogenase	7	30×10^{-3}	2.97×10^{-3}	2.52×10^{-3}	-0.45×10^{-3}
α-Glucosidase	5	11×10^{-4}	1.48×10^{-4}	1.43×10^{-4}	-0.05×10^{-4}

Cells were grown with or without 6 μg/ml hexetidine [3]. Aliquots (200 ml) were removed, washed, resuspended in 0.1 *M* glycylglycine buffer, pH 7.2, and ruptured (1 hr.) in a Raytheon 10 kc sonic oscillator at 1°C. The rates are recorded as μM/min/ml.

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On the premise that selective protein synthesis may occur in the presence of hexetidine, a protein fractionation of $^{35}SO_4^{2-}$ grown cells was undertaken. The gradient elution pattern of radioactivity extracts of control and inhibited cultures from a DEAE cellulose column is shown in Fig. 1. The elution curves show a striking difference between the proteins formed in the control and in the hexetidine-containing cultures. In the inhibited culture a larger proportion of the incorporated ^{35}S is found in the second large peak. Incorporation of radioactivity into the proteins eluting between these two peaks and after the second is largely abolished by hexetidine. Although the proteins found in the latter case have not been identified the results are in agreement with the determination of enzyme biosynthesis in suggesting a selective protein synthesis in the presence of hexetidine.

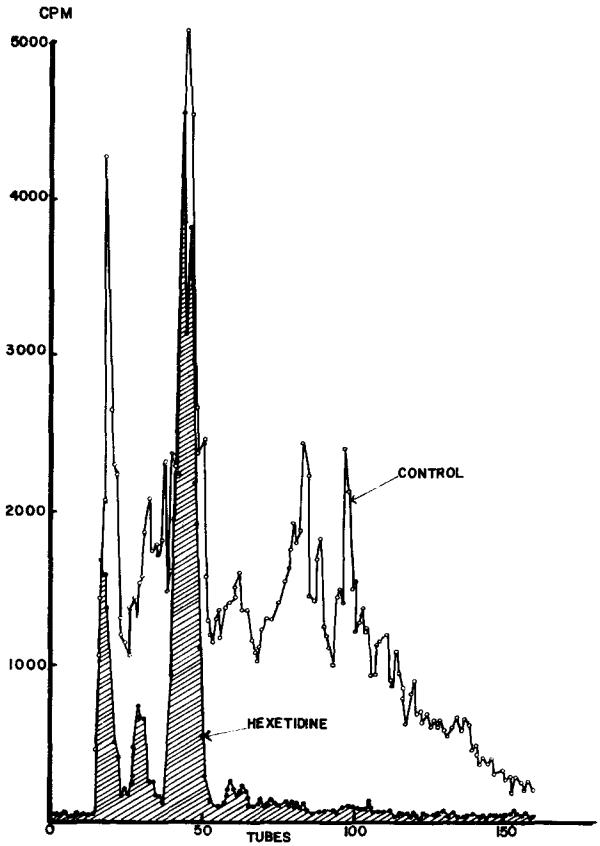

Fig. 1.—Elution patterns of cell extracts grown in $^{35}SO_4^{2-}$. Control cells were totally labeled with $^{35}SO_4^{2-}$. The inhibited cultures were harvested after an Δ OD of 0.495 in the presence of 6 μg/ml hexetidine and $^{35}SO_4^{2-}$. The cells were harvested, washed, disrupted in a Hugh's press and the supernate applied to a DEAE cellulose column [8]. An increasing NaCl gradient was applied by means of two 100 ml reservoirs; (*a*) 0.5 *M* NaCl and (*b*) 0.01 *M*. Tris and 0.005 *M* Mg acetate, pH 7.5. One ml fractions were collected.

The dramatic effect of growth in the presence of hexetidine is to double the protein content per bacterium without affecting the differential synthesis of nucleic acids or carbon precursor pools. In microbial and mammalian systems hexetidine has been shown to be an inhibitor of pyruvate metabolism [6] and an uncoupling agent for oxidative phosphorylation [4] respectively. If hexetidine regulates the energy-yielding reactions of the cell, these findings would appear paradoxical since the syntheses of proteins, nucleic acids and their precursors all involve ATP coupled reactions. However, a selectivity among biosynthetic reactions based on restriction of energy supply would permit such changes in the differential rates of biosynthesis. Although our knowledge of the exact synthesis is as yet incomplete, the recent demonstration of selective amino acid biosynthesis in the presence of an uncoupling agent of oxidative phosphorylation [1] provides an explanation for changes in cellular composition based on a limitation of energy supply.

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ISOPYKNOTIC CUSHIONING FOR DENSITY GRADIENT CENTRIFUGATION

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RECENTLY Holter and Møller [1] in a short note to this journal described a specially synthesized substance, "Ficoll", for aqueous density gradients. Despite the fact that this substance, a water-soluble, polymolecular, neutral colloid appears to be particularly suited for gradient centrifugation of cells, its use may be limited because of its availability only from Denmark.

For the past six years in the laboratory of cellular physiology at New York University we have been using a substance which has been highly satisfactory in meeting

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