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Received April 6, 1959. P.S.E.B.M., 1959, v101.

Rapid Reversal of Ethionine Inhibition of Enzyme Induction in *Pseudomonas aeruginosa* by L- and D-methionine. (24934)

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A number of amino acid analogues are incorporated into bacterial protein when the cells are grown in their presence (*cf.* 1 for review of literature). Presumably this also occurs when enzymes are induced in resting cell suspensions although proof is lacking. Enzyme induction is inhibited by amino acid analogues in such cells and the appropriate amino acid when added with the analogue prevents the inhibition. It was of interest to determine how rapidly an amino acid can reverse the analogue inhibition once it is established. This would give an indication of the rate the analogue can be displaced from the site or sites to which it is attached.

Methods. A strain of *Pseudomonas aeruginosa* was grown for 24 hours at 35° in 100 ml Difco nutrient broth. The cells were centrifuged, washed in water and centrifuged again. They were then suspended in 12 ml of 0.05 M Na-K-phosphate pH 7.6, and 0.5 ml of this suspension was used in each Warburg vessel which had a final fluid volume of 2 ml. Oxidation of 0.5 mg Na benzoate was measured. This proceeds by simultaneous induction of several enzymes(2). Because of the number of enzymes involved and the complexities of oxidative reactions no conclusion can be drawn about the locus of action of the analogue. But oxygen uptake measurements can accurately detect changes in enzyme activity and the rate at which they occur.

Results. Fig. 1 shows results with DL-ethionine. It was added with the benzoate at zero time and after 90 minutes produced a constant 63% inhibition of oxygen uptake. Oxidation of benzoate alone began after a 30 minute latent period and reached a linear rate after 90 minutes. After 120 minutes L- or D-methionine was added. When added to the vessels containing only benzoate the 2 isomers had no effect on the rate. Ethionine added at this time was also without effect. When the 2 isomers were added to the vessels in which ethionine had been present from the beginning rapid reversal occurred. For the L-isomer this became evident in 15 minutes, almost complete during the next 15 minutes. For the D-isomer, reversal was not apparent in the first 15 minutes but occurred rapidly during the next 15 minute period. The initial difference between the 2 isomers was small but consistent. Exactly the same results were obtained with 1.0 μg p-fluorophenylalanine and 0.2 μg of L- or D-phenylalanine. Phenylpyruvic acid is also an antagonist for this analogue and it behaved initially like the D-isomer. Addition of amino acids other than the specific ones failed to reverse the inhibitions.

Three other facts may be briefly mentioned. If the methionines were added 60 minutes before ethionine and benzoate no reversal was evident presumably because both the L- and D-isomers were metabolized. On the other hand, if the amino acids were incubated 60 minutes with ethionine before the addition of benzoate reversal occurred presumably because ethionine had inhibited the metabolism of both isomers. Finally, if the nitrogen pool was increased by a prior incubation of the cells with 30 μg $(NH_4)_2SO_4$ and 4.3 μg succinic acid, ethionine inhibited to the same extent as in the untreated cells. Similar results were obtained with p-fluorophenylalanine.

Discussion. No conclusions can be drawn from these experiments about the site of action of ethionine but several possibilities may

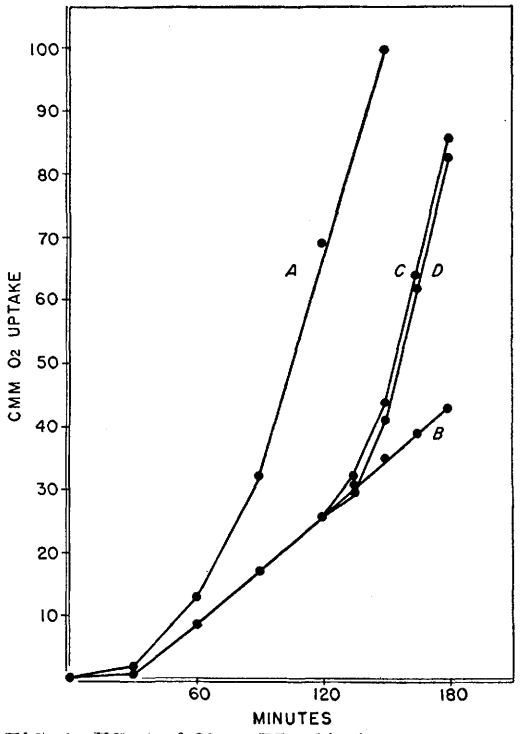

FIG. 1. Effect of 20 μg DL-ethionine and 2 μg of either L- or D-methionine on oxidation of 0.5 mg Na benzoate. Curve A: oxidation of benzoate. At 120 min. either L- or D-methionine or ethionine was added. There was no effect on oxidation rate. Curve B: oxidation of benzoate in presence of ethionine added at zero time. At 120 min. L-methionine (curve C) or D-methionine (curve D) was added.

be mentioned. After an initial period ethionine caused a constant inhibition. If it had merely delayed the entry of benzoate into the cells, *i.e.*, if oxidation rate was limited by amount of substrate available the inhibition should have decreased with time, because benzoate would be entering the cell throughout the entire experimental period. Since the inhibition remained constant, it seems probable that ethionine was not inhibiting a permease. Secondly, if upon addition of methionine the cell synthesized enzyme *de novo* and thus bypassed the ethionine inhibition, any amino acid should have been effective. The specificity of the reaction indicates that ethionine was displaced. Thirdly, if ethionine was incorporated into the enzyme protein during the 120 minute period, then methionine must have rapidly displaced it to convert an inactive into an active enzyme. Fourthly, the slightly slower action of the D-isomer may mean either that it was assimilated more slowly or that it was changed to the L-isomer by an isomerase.

Summary. 1) Ethionine inhibition of induction of enzymes involved in benzoate oxidation by washed cell suspensions of *Pseudomonas aeruginosa* is rapidly reversed by L-methionine and almost as rapidly by D-methionine. Neither amino acids nor ethionine have any effect once the enzymes have been induced. Similar results were obtained with p-fluorophenylalanine.

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Received April 10, 1959. P.S.E.B.M., 1959, v101.

Stimulation of Mitosis in Rat Marrow Cultures by Serum From Infected Rats.* (24935)

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Workers have reported substances in blood, urine, or inflammatory exudates which are capable of increasing the number of circulating leukocytes(1,2). However, the specific mechanism by which this effect is produced has not been determined. It has not been demonstrated, for instance, whether the observed leukocytosis results from increased mitotic activity in hematopoietic centers or from increased release of mature cells from these centers or other extravascular storage sites.

* This investigation supported by Senior Research Fellowship from P.H.S. and Grant from Nat. Cancer Inst., N.I.H., U.S.P.H.S.