Reversal of Fluoroacetate Inhibition by Vitamin B_{12} in a Strain of *Escherichia coli*. (22056)

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The addition of vit. B_{12} to aged cells of *Escherichia coli* strain 113-3, a mutant requiring vit. B_{12} or methionine for growth, stimulates the rate of oxidation of a number of substrates including acetate(1).

A study was conducted to investigate the cause of the stimulatory effect of vit. B_{12}. In the course of the work various metabolic inhibitors were used. Fluoroacetate was employed as an inhibitor of the tricarboxylic acid cycle, since it has been shown to be converted to fluorocitrate which acts as a competitive inhibitor of aconitase(2,3). The data reported here deal with the inhibitory effect of fluoroacetate on acetate and glucose oxidation in this mutant, and its reversal by vit. B_{12}.

Methods. The medium used was that of Davis and Mingioli(4) containing glucose and supplemented with 20 mg DL-methionine per liter. The organisms† were grown at 32°C for 18 hours without aeration in 18 liter pyrex bottles containing 16 liters of medium. The cells were harvested, washed twice, suspended in distilled water and aged in the refrigerator for a minimum period of 10 days. To adapt the cells to acetate, they were transferred in medium in which potassium acetate progressively replaced glucose. The acetate-grown mutant still required B_{12} or methionine for growth. The acetate-adapted cells were grown and treated in the same manner as the glucose-grown cells. The activity of the resting cell suspensions was measured at 37°C using conventional Warburg technics under air. All flasks contained potassium phosphate buffer, pH 7.0 (100 μM); vit. B_{12} (Cobione, Merck) was added at the same time as the substrate and inhibitor. The nitrogen content of the resting cell suspensions was determined by a micro-Kjeldahl method. Citric acid was determined by a modification of the method of Natelson, Lugovoy and Pincus(5).

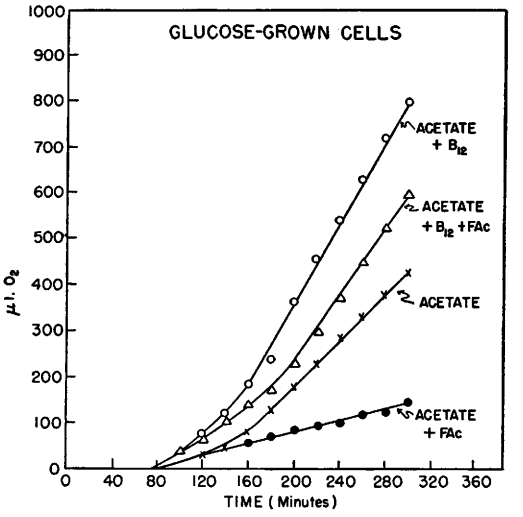

FIG. 1. Acetate oxidation in glucose-grown cells. *Flask contents:* 25 μM acetate, 3 μM fluoroacetate (FAc), 100 mμg B_{12}; N content, 1.09 mg. *Citric acid formed:* acetate ± B_{12}, 0; acetate + FAc, 0.1 μM; acetate + FAc + B_{12}, 0.3 μM.

Results. The effect of fluoroacetate‡ on acetate oxidation by glucose-grown cells is shown in Fig. 1. Acetate oxidation was inhibited and citric acid accumulated in the presence of fluoroacetate. No citrate was found in the absence of fluoroacetate. The addition of vit. B_{12} stimulated the oxidation of acetate as previously described(1), and decreased the inhibitory effect of fluoroacetate, although it did not affect the amount of citrate formed. The lag in acetate oxidation could be eliminated by the addition of malate. This may indicate that the main cause for the delay in oxidation of acetate is the need for the formation of a C_4-acid which is required for the oxidation of acetate via the tricar-

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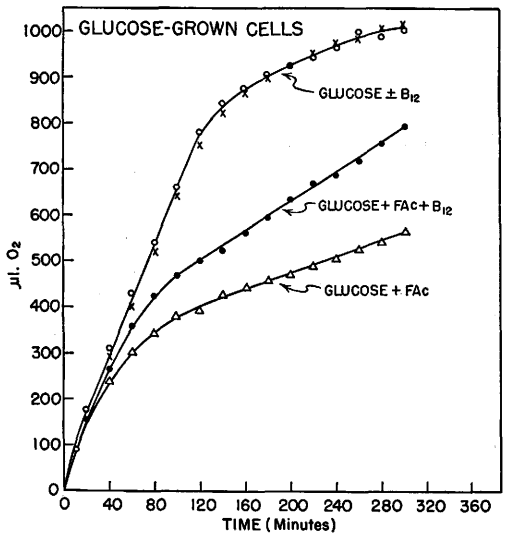

FIG. 2. Glucose oxidation in glucose-grown cells. *Flask contents:* 11 μM glucose, 5 μM fluoroacetate (FAc), 100 mμg B_{12}; N content, 1.77 mg. *Citric acid formed:* glucose $\pm$ B_{12}, 0; glucose + FAc, 0.3 μM; glucose + FAc + B_{12}, 0.4 μM.

boxylic acid cycle. Vit. B_{12} had no effect on this lag.

Since Ling and Chow(6) reported that vit. B_{12} was involved in glucose oxidation by erythrocytes, the influence of vit. B_{12} and fluoroacetate on glucose oxidation in this mutant was investigated. The results of this study are presented in Fig. 2. Vit. B_{12} had no effect on either the rate or extent of glucose oxidation. A marked inhibition of oxidation resulted from the addition of fluoroacetate, an effect which could be partially prevented by the addition of vit. B_{12} (Fig. 2).

The oxidation of acetate by acetate-grown cells and the effect of fluoroacetate and vit. B_{12} are shown in Fig. 3. No lag was observed in the oxidation of acetate, and vit. B_{12} had no effect on the rate of oxidation. The inhibitory effect of fluoroacetate was not as great as that found in the glucose-grown cells, and the addition of vit. B_{12} completely overcame it. Glucose oxidation was also inhibited by fluoroacetate, but it was only partially reversed by vit. B_{12} (Fig. 4).

The presence of fluoroacetate during the oxidation of glucose and acetate caused the accumulation of citric acid in both the acetate- and glucose-grown cells. Vit. B_{12} had no effect on the amount accumulated. In some cases the amount of citric acid found was slightly greater in the presence of vit. B_{12}. (See Fig. 1, 2, 3, 4).

Discussion. Fluoroacetate decreased the rate of oxidation of acetate and glucose by aged cells of *E. coli,* strain 113-3, which had been grown on glucose or acetate. The extent of inhibition was less in the acetate-adapted cells. Vit. B_{12} prevented this inhibition partially or entirely. The nature of the action of vit. B_{12} is not known. It would be possible to overcome partially the fluoroacetate inhibition by increasing the oxalacetate level or by introducing an alternate pathway of acetate oxidation. In this work malate, which would form oxalacetate on oxidation, did not replace vit. B_{12} in relieving the inhibition. If an alternate pathway is visualized, then acetate oxidation would by-pass the tricarboxylic acid cycle, although fluoroacetate would continue to form fluorocitrate which would accumulate and be determined as citrate. In these studies, although vit. B_{12} prevented some of the decrease in oxidation of acetate by fluoroacetate, it had no effect on the accumulation of citrate. It is unlikely, therefore, that vit. B_{12} affects directly or indirectly the enzyme systems of the tricarboxylic acid cycle.

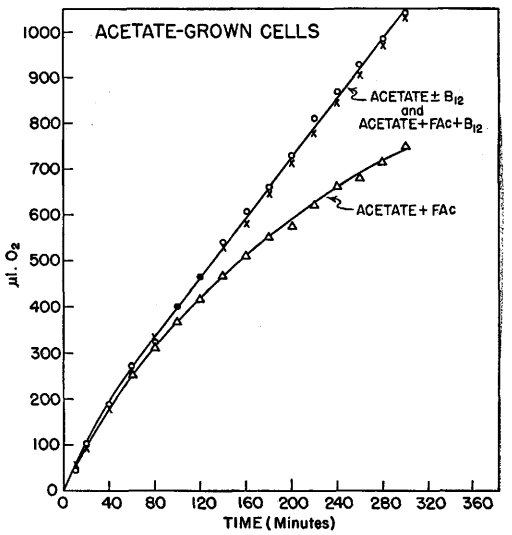

FIG. 3. Acetate oxidation by acetate-grown cells. *Flask contents:* 25 μM acetate, 3 μM fluoroacetate (FAc), 100 mμg B_{12}; N content, 0.7 mg. *Citric acid formed:* acetate $\pm$ B_{12}, 0; acetate + FAc, 0.6 μM; acetate + FAc + B_{12}, 0.5 μM.

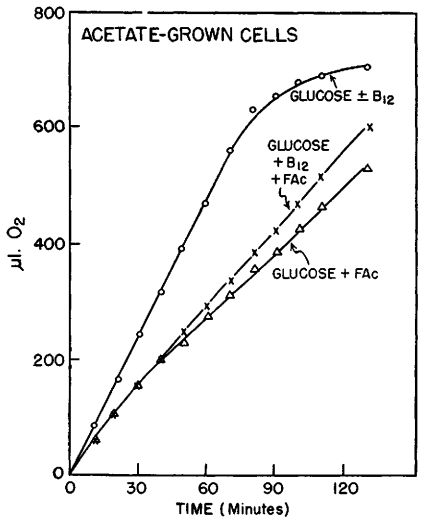

FIG. 4. Glucose oxidation by acetate-grown cells. *Flask contents:* 10 μM glucose, 5 μM fluoroacetate (FAc), 100 mμg B_{12}; N content, 0.7 mg. *Citric acid formed:* glucose $\pm$ B_{12}, 0; glucose + FAc, 0.6 μM; glucose + FAc + B_{12}, 0.5 μM.

The alternate pathway may be utilized to a greater extent in acetate-adapted cells, since there is less inhibition by fluoroacetate of acetate oxidation in these cells than in the glucose-grown cells. Vit. B_{12} may function by shifting acetate oxidation into the alternate pathway, either by stimulating the activity of an enzyme system or by stimulating the formation of the enzyme system, or one of its components. Since vit. B_{12} has no effect on acetate oxidation by mutant cells that are not aged, the aging process may cause a destruction or inactivation of some part of the alternate pathway. In acetate-adapted cells in which vit. B_{12} does not affect oxidation, less inactivation must occur.

Thus, as a possible explanation of the influence of vit. B_{12} on fluoroacetate inhibition, it is suggested that vit. B_{12} partially shunts the oxidation of acetate into an alternate terminal oxidative pathway.

Summary. 1. The rate of acetate oxidation in aged, glucose-grown cells of *Escherichia coli* strain 113-3 was stimulated by addition of vit. B_{12}. Stimulation of glucose oxidation was not observed. Upon addition of fluoroacetate with either glucose or acetate as substrate, oxidation was inhibited. This inhibition was partially prevented by the simultaneous supplementation with vit. B_{12}. 2. Acetate-adapted cells of this mutant failed to show a stimulatory effect of vit. B_{12} on oxidation of acetate. However, in the presence of fluoroacetate, oxidation of acetate and glucose was stimulated by vit. B_{12}.

1. Oginsky, E. L., Smith, P. A., Tonhazy, N. E., Umbreit, W. W., Lichstein, H. C., and Carson, S. F., *J. Bact.*, 1951, v61, 581.

2. Lotspeich, W. D., Peters, R. A., and Wilson, T. H., *Biochem. J.*, 1952, v51, 20.

3. Morrison, J. F., and Peters, R. A., *ibid.*, 1954, v56 (Proc.) xxxvi.

4. Davis, B. D., and Mingioli, E. S., *J. Bact.*, 1950, v60, 17.

5. Natelson, S., Lugovoy, J. K., and Pincus, J. B., *J. Biol. Chem.*, 1947, v170, 597.

6. Ling, C. T., and Chow, B. F., *Fed. Proc.*, 1954, v13, 253.

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