The absence of the soluble cytochrome *c*, however, does not prevent the oxidation of formaldehyde, formate, succinate or malate.

It is concluded from the characteristics of mutant PCT-76 that the soluble cytochrome *c* of *Pseudomonas* AM1 is not involved in the oxidation of the majority of substrates, but that it is essential for the oxidation of methanol and ethanol.

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The Involvement of Glycollate in the Pathway for Ethanol Assimilation in *Pseudomonas* AM1

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In *Pseudomonas* AM1 C_1 compounds are assimilated by the serine pathway (Large, Peel & Quayle, 1961); none of the enzymes of this pathway is involved in known routes of ethanol metabolism. A mutant (PCT-48) of *Pseudomonas* AM1, unable to grow on C_1 compounds or ethanol, has been isolated. This suggests a novel pathway for ethanol metabolism that has one or more enzymes common to the pathway for the assimilation of C_1 compounds.

Mutant PCT-48 has all of the known enzymes required for C_1 -unit assimilation. Further, wild-type ethanol-grown organisms have very low activities of serine-glyoxylate aminotransferase, an inducible enzyme of the serine pathway, indicating that ethanol is not metabolized by this route. Malate synthetase is the only enzyme of the glyoxylate by-pass found in ethanol-grown *Pseudomonas* AM1. The trace amount of the key enzyme isocitrate lyase (specific activity less than $0.1 \mu\text{mol/h per mg}$) is insufficient to account for the growth rate on ethanol (mean generation time 7h).

Acetate is likely to be an intermediate in ethanol assimilation; ethanol-grown organisms contain enzymes that oxidize ethanol to acetate as well as the enzymes of the tricarboxylic acid cycle. The results of short-term incubation of ethanol-grown *Pseudomonas* AM1 with $[U-^{14}\text{C}]$ acetate indicate that malate and glycollate are early intermediates in acetate metabolism. Very little label was present at early times in succinate, fumarate, citrate, glycine or serine.

Substrate amounts of glycollate allow growth of mutant PCT-48 on ethanol or acetate. This supports the suggestion that glycollate is an intermediate in ethanol and acetate assimilation. Further,

the hydroxypyruvate reductase mutant 20-BL (supplied by Professor J. R. Quayle) does not grow on ethanol even if glycollate is present. This mutant grows on ethanol plus glyoxylate indicating that the oxidation of glycollate to glyoxylate is involved in ethanol assimilation. Malate synthetase could provide a mechanism for malate formation from glyoxylate and acetate.

A possible sequence of intermediates in the assimilation of ethanol is as follows: ethanol, acetaldehyde, acetate (or derivative), glycollate, glyoxylate, malate.

Mutant PCT-48 has possibly lost an enzyme involved in the formation of glycollate from an acetate derivative. This enzyme may also be important for growth on C_1 compounds, because mutant PCT-48 can grow on methanol only in the presence of glycollate or glyoxylate but not acetate.

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In Search of Lactate Dehydrogenase from *Bacillus stearothermophilus*

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In the course of a multienzyme preparation from *Bacillus stearothermophilus* (Suzuki & Harris, 1971) by using fractionation with ammonium sulphate followed by column chromatography on DEAE-cellulose and hydroxyapatite, what appeared to be a pure protein possessing both lactate dehydrogenase and alcohol dehydrogenase activities was isolated. The elution profiles of the two activities from both columns coincided exactly and the purified enzyme also appeared to be homogeneous when examined by electrophoresis on gelatinized cellulose acetate (Cellologel) at different pH values, with and without 8M-urea, as well as on polyacrylamide gel in 0.1% sodium dodecyl sulphate. It also gave a single symmetrical peak in the analytical ultracentrifuge ($s_{20,w}$ 6.0S) and the molecular weight was estimated to be $135000 \pm 3\%$ by the sedimentation-equilibrium method. The molecular weight in 0.1% sodium dodecyl sulphate was estimated to be approx. 34000, and from these data it appeared that we had succeeded in isolating a tetrameric enzyme possessing both lactate dehydrogenase and alcohol dehydrogenase activities.

Subsequently, however, while attempting to purify phosphofructokinase from the same cell extracts it was found that this activity was also associated with the lactate dehydrogenase and