A DEVIATION FROM THE CONVENTIONAL TRICARBOXYLIC ACID CYCLE IN *PSEUDOMONAS AERUGINOSA**

by

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It is now accepted that various strains of bacteria possess several pathways of glucose degradation that have not yet been demonstrated in animal tissue. In spite of this there is a strong tendency amongst biochemists to accept the conventional tricarboxylic acid cycle as the sole means of terminal respiration in aerobic microorganisms.

CAMPBELL AND STOKES[1] showed that dried cell preparations of *Pseudomonas aeruginosa* 9027 had the ability to oxidize citrate, *cis*-aconitate, *iso*citrate, ketoglutarate, succinate, fumarate, malate and oxalacetate. It has now been shown that this organism has the condensing enzyme, as evidenced by its ability to convert pyruvate to citrate. Also that the oxidation of succinate, fumarate and malate proceeds by way of oxalacetate. This would seem to be good evidence in favor of a conventional tricarboxylic acid cycle. However, employing cell-free extracts of *P. aeruginosa* obtained by subjecting a heavy cell suspension to a 10 kc Raytheon sonic oscillator for 15 minutes and using citrate as the substrate all attempts to detect ketoglutarate in the fermentation liquor failed. The paper chromatographic method of CAVALLINI[2], which can be used for the identification of the 2,4-dinitrophenylhydrazones of aldehydes or ketones, was followed in these experiments. While trying to detect the 2,4-dinitrophenylhydrazone of ketoglutarate it was noted that two unidentified spots were always present in the chromatographed fermentation liquors. On further investigation it was found that these spots corresponded to glyoxylic acid. In that in the conventional tricarboxylic acid cycle citrate is in equilibrium with *cis*-aconitate and *iso*citrate it was quite possible that *cis*-aconitate or *iso*citrate, and not citrate, was the immediate source of glyoxylate. On using these compounds as substrates it was found that *cis*-aconitate was dissimilated to glyoxylate whereas *iso*citrate was not. If glyoxylate was produced directly from citrate or from *cis*-aconitate it would appear that a 4–2 split is in operation. The organism under study is an obligate aerobe and the experiments outlined were carried out under aerobic conditions. However, if a 4–2 split is operative then the reaction could readily be a hydrolysis resulting in the production of one mole of glyoxylate and one of succinate. Anaerobic experiments with citrate or *cis*-aconitate as substrate did result in the production of large quantities of glyoxylate and succinate. Succinate was determined both by acid-base chromatography and by the succinic dehydrogenase method of KREBS[3]. In confirmation of the absence of *iso*citrate from this system, no succinate was formed from it under either aerobic or anaerobic conditions. In addition, if "aconitase" is present in this organism *iso*citrate should be in equilibrium with *cis*-aconitate and citrate and the addition of *iso*citrate to the cell extract should result in the production of citrate. However, no citrate could be detected on incubation of the cell extract with *iso*citrate. In contrast to this incubation of the cell extract with *cis*-aconitate or with succinate plus glyoxylate resulted in the production of large amounts of citrate.

It would appear, therefore, that these reactions are readily reversible and that *iso*citrate is not involved. It would also appear either that this organism has an unusual type of "aconitase" or that "aconitase" is two enzymes and our organism lacks the fraction which takes *cis*-aconitate to *iso*citrate.

All indications are that this pathway is a major one in this organism for in at least one anaerobic experiment 1 mole of succinate was determined per mole of citrate utilized. The cycle is similar to the conventional cycle with the exception of the steps from *cis*-aconitate to succinate. The production of glyoxylic acid is also of interest from the point of view of its possibility as a precursor of glycine.

REFERENCES

1 J. J. R. CAMPBELL AND F. N. STOKES, *J. Biol. Chem.*, 190 (1951) 853.
2 D. CAVALLINI, N. FRONTALI AND G. TOSCHI, *Nature*, 163 (1949) 568.
3 H. A. KREBS, *Biochem. J.*, 31 (1937) 2095.

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