the assay system of shaking cultures aerobically for 2 days, and extracting the steroid substrate from the broth. Activity was demonstrated with five out of 12 strains of lecithinase-negative clostridia and relatively weakly with five out of 11 strains of lecithinase-positive clostridia but not with nine strains of enterobacteria, nine strains of enterococci or 16 strains of the non-sporing strictly anaerobic bacteria.

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The Glyceraldehyde 3-Phosphate Dehydrogenase of *Azotobacter beijerinckii* and its Possible Significance in Poly- β -hydroxybutyrate Biosynthesis

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Azotobacter beijerinckii can accumulate the storage polymer poly- β -hydroxybutyrate up to 70% of its dry weight when grown on glucose as the sole source of carbon and energy in the absence of a fixed nitrogen source. The enzymes β -ketothiolase, acetoacetyl-CoA reductase and polyhydroxybutyrate polymerase effect the synthesis of one hydroxybutyrate monomer from 2 molecules of acetyl-CoA, with the concomitant release of 1 CoA molecule and the reoxidation of 1 molecule of either NADPH or NADH. An acyl-carrier protein is not involved in polyhydroxybutyrate biosynthesis (Ritchie & Dawes, 1969). The reductive step is catalysed by acetoacetyl-CoA reductase, which reacts with NADPH at five times the rate with NADH (Ritchie, 1968). Our interest in the glyceraldehyde 3-phosphate dehydrogenase of the organism stems from a survey of reactions generating the large amounts of NADPH necessary for the massive synthesis of polyhydroxybutyrate which occurs when the organism is fixing nitrogen, a process that itself makes considerable demands on the production of reducing equivalents.

A glyceraldehyde 3-phosphate dehydrogenase has been isolated and partially purified by ammonium sulphate fractionation and ion-exchange cellulose chromatography. The enzyme reacts preferentially with NADPH ($V_{\max}$ 63.1 and 11.1 nmol/min with NADPH and NADH respectively) and thus resembles that more commonly encountered in plants,

which, however, operates in the direction of reduction. Johnson & Johnson (1961) recorded a similar coenzyme preference by the enzyme from *Azotobacter agilis*. The *A. beijerinckii* enzyme requires a thiol group for activity, as indicated by the inhibitory action of *p*-chloromercuribenzoate, *N*-ethylmaleimide, iodoacetate and mersalyl. The pH optimum is 7.5 and with phosphate concentrations higher than 100 mM there is an inhibitory effect at pH values above 6.5. The equilibrium of the reaction at pH 7.5 and 30°C greatly favours oxidation (K_{eq} approx. 10^7) and we believe that this reaction may play an important role in furnishing reducing equivalents for polyhydroxybutyrate biosynthesis.

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Conversion of Pyruvate into Citrate by Mitochondria Isolated from Rat Epididymal Fat-Cells

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From a previous study of the total activities and location of enzymes in isolated fat-cells and the permeability properties of intact fat-cell mitochondria (Martin & Denton, 1970) we have concluded that acetyl units for cytoplasmic fatty acid synthesis are transferred from mitochondria as citrate (as postulated by other workers largely on the basis of the parallelism between ATP citrate lyase activity and fatty acid synthesis rates), that transfer of citrate requires malate, presumably for counter-transport, and that the intramitochondrial metabolism of citrate and malate is restricted to allow transfer of acetyl units to the cytoplasm and to limit the loss of cytoplasmic reducing power into mitochondria.

Support for these conclusions has come from an investigation of the metabolism of pyruvate by isolated fat-cell mitochondria. The mitochondria were incubated with pyruvate, ADP and P_i and the effects of additions of malate, hydroxymalate (a non-oxidized malate analogue) and bicarbonate on the time-courses of pyruvate uptake, citrate output, malate uptake (or output) and oxygen consumption were determined. On addition of bicarbonate alone there was an appreciable lag in citrate production presumably because in the absence of