activities of the key enzymes of gluconeogenesis in the liver and kidney of the foetal guinea pig have been followed.

The foetal guinea pigs used had a gestational age of 68–72 days. Pyruvate carboxylase was present at very low activities in the kidney of the foetus and neonatal animal and also in the foetal liver until day 50, when it increased in activity to reach a value by day 60 of more than twice the maternal liver value. There was a rapid postnatal fall in activity. The foetal liver enzyme was largely mitochondrial, required acetyl-CoA for activity and, like the adult liver enzyme, had a K_m for pyruvate of approx. 0.7 mM.

Unlike the adult liver, most of the phosphopyruvate carboxylase was particulate, and the soluble-fraction enzyme of the foetal liver had the properties of the particulate enzyme of the adult and foetal liver. Particulate enzyme, which was largely mitochondrial, was present in significant amounts from day 35, and by day 65 its activity was approximately threefold greater in the foetal liver than the adult liver. In both the liver and the kidney soluble-enzyme activity was low during foetal life but showed a large rise during the first 2 days after birth.

Fructose 1,6-diphosphatase was present in low activity in the foetal liver and kidney until day 40, when its activity rapidly rose so that by day 45 it was just below the maternal enzyme activities. Foetal liver and maternal liver enzymes had similar properties, although the foetal liver enzyme was more sensitive to AMP and substrate inhibition and had a K_m for fructose 1,6-diphosphate of 1 μ M.

The changes in the activities of glucose 6-phosphatase were very similar to those for fructose 1,6-diphosphatase. In the foetal tissues the enzyme reached an activity of about half that found in the adult, had a K_m of 10 mM and was inhibited by glucose.

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Purification by Affinity Chromatography of Thermostable Glyceraldehyde 3-Phosphate Dehydrogenase from *Thermus aquaticus*

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The mechanisms that allow enzymes of thermophilic organisms to function at high temperatures are of considerable biochemical interest (cf. Brock, 1967). In this connexion a study of glyceraldehyde 3-phosphate dehydrogenase and other glycolytic-cycle enzymes from the obligate thermophile *Bacillus stearothermophilus* 1503 (grown at 60°C) has shown that enzymes from this source are considerably more stable towards heat and denaturing agents than are their counterparts from muscle and yeast (e.g. see Amelunxen, 1967; Suzuki & Harris, 1971). As an extension of this work we now report the isolation and properties of glyceraldehyde 3-phosphate dehydrogenase from the more extreme thermophile *Thermus aquaticus* (Brock & Freeze, 1969), which grows optimally at 70–75°C.

Cell extracts were treated with deoxyribonuclease and stirred with DEAE-cellulose equilibrated with 10 mM-tris-HCl buffer, pH 7.5 (containing β -mercaptoethanol and EDTA) (cf. Atkinson *et al.*, 1972). Material adsorbed on DEAE-cellulose was eluted by two washings with the same buffer containing successively 0.1 M- and 0.4 M-NaCl. The fraction eluted with 0.4 M-NaCl was adsorbed on hydroxyapatite in phosphate buffer, pH 6.5, and the hydroxyapatite was in turn washed successively with 0.06 M- and 0.2 M-phosphate buffer, pH 6.8. To the latter fraction was added solid $(\text{NH}_4)_2\text{SO}_4$ to 1.2 M, and the resulting supernatant, which contained most of the glyceraldehyde 3-phosphate dehydrogenase and phosphofructokinase, was now applied to a column (200 ml bed volume) of NAD^+ -Sephacrose prepared by the methods of Cuatrecasas (1970) and Weibel *et al.* (1971) (see also Lowe & Dean, 1971; Mosbach *et al.*, 1971). Phosphofructokinase activity was successfully eluted with 0.7 M-NaCl containing 1 mM-ATP, and pure glyceraldehyde 3-phosphate dehydrogenase (in 80% yield, representing 20–30-fold purification in this step) was subsequently eluted with 10 mM- NAD^+ . The yield of pure enzyme was 150–200 mg/500 g of frozen cells, and it was crystallized directly from this solution by dialysis against 1.7 M- $(\text{NH}_4)_2\text{SO}_4$. The crystalline enzyme, which contained 4 mol of firmly bound NAD^+ /mol, gave a single band when examined by gel electrophoresis with and without 0.1% sodium dodecyl sulphate. The molecular weight, on the basis of gel filtration on Sephadex G-200, was 150 000, and the subunit molecular weight in 0.1% sodium

dodecyl sulphate was 36000, indicating a tetrameric structure.

T. aquaticus glyceraldehyde 3-phosphate dehydrogenase (the apo- as well as holo-enzyme) is stable for several hours at 90°C (the *B. stearrowthermophilus* enzyme is totally inactivated within 10 min at this temperature) but is rapidly inactivated above 100°C. Moreover, like the *B. stearrowthermophilus* enzyme (K. Suzuki & J. I. Harris, unpublished work), it retains its activity fully after exposure to 8M-urea and 1% sodium dodecyl sulphate. Some chemical, physico-chemical and kinetic parameters of the enzyme will be presented.

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Metabolic Regulation in the C₄ Dicarboxylic Acid Pathway of Photosynthesis

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We have suggested (Baldry *et al.*, 1971) that in the tropical grasses the C₄ dicarboxylic acid pathway of photosynthesis (Hatch & Slack, 1970) is located in the cytoplasm and acts as a mechanism for concentrating CO₂ at the site of reductive assimilation in the chloroplasts of the mesophyll cell layer. The initial incorporation of CO₂ into C₄ organic acids is catalysed by phosphopyruvate carboxylase (EC 4.1.1.31). Investigations of cytoplasmic phosphopyruvate carboxylase partially purified from leaves of the plant *Pennisetum purpureum*, which utilizes the C₄ dicarboxylic acid pathway, suggest that this enzyme may be regulated by photosynthetic activity.

Leaf tissue (100g) was homogenized in 200ml of chloroplast isolation medium [as described by Baldry *et al.* (1968), with the omission of thiols, which

were not necessary since this plant contains very low *o*-diphenol oxidase (EC 1.10.3.1) activity]. Subcellular particles were removed by centrifugation and cytoplasmic protein was precipitated with (NH₄)₂SO₄ (60% saturation). The dialysed freeze-dried precipitate was dissolved in 10mM-tris-HCl buffer, pH 7.0, applied to a column of DEAE-cellulose and eluted with a linear gradient of tris-HCl buffer, pH 7.0. Incorporation of ¹⁴C into oxaloacetate, trapped as the 2,4-dinitrophenylhydrazone, was measured in reaction mixtures containing phosphoenolpyruvate, Mg²⁺, H¹⁴CO₃⁻ and enzyme in 200 μl of 50mM-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) buffer, pH 8.0.

Variation of rate of reaction as a function of limiting concentrations of HCO₃⁻ or of Mg²⁺ approximated to first-order kinetics. However, sigmoid kinetics were observed when the rate of reaction was determined as a function of phosphoenolpyruvate concentration. Addition of intermediates of the photosynthetic carbon-reduction cycle (5 mM) stimulated (ribose 5-phosphate, fructose 1,6-diphosphate, fructose 6-phosphate, glucose 6-phosphate) or inhibited (phosphoglycerate) the reaction. The effects of glucose 6-phosphate were investigated further. Enzyme activity increased when the concentration of phosphoenolpyruvate exceeded that of glucose 6-phosphate, but was decreased when the concentration of glucose 6-phosphate exceeded that of phosphoenolpyruvate. The enzyme was also inhibited by oxaloacetate (50% at 1.5 mM) and malate (50% at 6.0 mM). In the presence of malate dehydrogenase (EC 1.1.1.37) NADH increased the rate of reaction, presumably owing to reduction of oxaloacetate to the less inhibitory malate. The increased enzyme activity observed when NADH was included in the reaction mixture was additive to that caused by glucose 6-phosphate. Activation by glucose 6-phosphate did not occur in the presence of thiols (β-mercaptoethanol, thioglycollate, dithiothreitol, GSH, at 0.1–10 mM) or 1.5M-urea.

These results suggest that the cytoplasmic phosphopyruvate carboxylase of *P. purpureum* is an allosteric enzyme regulated by intermediates of the photosynthetic carbon-reduction cycle. In this case the initial assimilation of CO₂ into oxaloacetate in plants that utilize the C₄ dicarboxylic acid pathway could be regulated by the photosynthetic activity of the mesophyll chloroplasts.

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