lipid-supplemented diet (111.13 ± 28.89 units, mean $\pm$ S.E.M.) (1 unit of enzyme activity being 1 nmol/min per mg of protein) than in those fed on the lipid-free diet (169.03 ± 31.32 units). Fructose diphosphate aldolase, which functions in both glycolysis and gluconeogenesis, did not vary in activity with dietary treatment (10% lipid, 201.48 ± 14.94 units; 0% lipid, 234.11 ± 30.20 units). The gluconeogenic enzyme fructose diphosphatase had a greater activity in birds fed on the lipid-supplemented diet (10% lipid, 54.34 ± 5.44 units; 0% lipid, 36.16 ± 3.13 units). These results suggest that dietary lipid, as well as diminishing hepatic lipogenesis, also decreases glycolysis and increases gluconeogenesis.

In mammals the pentose phosphate pathway is an important source of NADPH for lipogenesis (Masoro, 1962), but studies of the dissimilation of glucose by chick liver indicate that this pathway does not function to any great extent in the chick (Duncan & Common, 1967). In the present work, although dietary lipid decreased lipogenic enzyme activity, there was little variation in the activities of glucose 6-phosphate dehydrogenase (10% lipid, 7.94 ± 0.90 units; 0% lipid, 6.26 ± 0.59 units) and 6-phosphogluconate dehydrogenase (10% lipid, 19.31 ± 1.59 units; 0% lipid, 20.05 ± 2.35 units). Further evidence that this pathway is not involved in lipogenesis was obtained with mature laying hens and cockerels. In the hen there is a demand for lipid for egg production, and lipogenic enzyme activity is greater than in the cockerel (Pearce, 1971b). Similar activities were found in the hen and cockerel for glucose 6-phosphate dehydrogenase (hens, 7.09 ± 1.37 units; cockerels, 5.96 ± 0.72 units) and 6-phosphogluconate dehydrogenase (hens, 17.38 ± 2.38 units; cockerels, 22.96 ± 0.86 units), confirming that this pathway is not involved in lipogenesis in the chick.

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Preliminary Investigations on Bovine Hepatic Fructose 1,6-Diphosphatase

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Several procedures for the purification of mammalian hepatic fructose 1,6-diphosphatase (EC 3.1.3.11) have been reported (Pontremoli *et al.*, 1965;

Sarnagadharan *et al.*, 1970; Byrne *et al.*, 1971; Traniello *et al.*, 1971, 1972). However, it has been shown that the properties of fructose 1,6-diphosphatase are susceptible to modification, owing to limited proteolysis, during purification (Nakashima & Horecker, 1971; Traniello *et al.*, 1971, 1972). These modifications include alteration in the pH optimum of the enzyme (from neutral pH in the 'native' enzyme to alkaline pH in the 'modified' form), partial desensitization to inhibition by AMP, a small decrease in molecular weight (from 136000 to 128000), the loss of all tryptophan residues from the enzyme and the appearance of an additional subunit type on gel electrophoresis in sodium dodecyl sulphate.

Fructose 1,6-diphosphatase has been purified from ox liver by a process involving homogenization in 0.15M-KCl, brief treatment at pH 4.5, $(\text{NH}_4)_2\text{SO}_4$ fractionation and chromatography on CM-cellulose and Sephadex G-200. The final preparation is homogeneous on polyacrylamide-gel electrophoresis and analytical ultracentrifugation. The pH optimum and AMP-sensitivity of the enzyme do not alter throughout the purification. In the presence of 1 mM-MgSO₄ the enzyme is optimally active at pH 7.4. AMP is a non-competitive inhibitor with respect to fructose 1,6-diphosphate, with K_i $14 \mu\text{M}$ at 30°C. At fixed concentrations of fructose 1,6-diphosphate the response to AMP is sigmoidal with n (the Hill coefficient) being 1.9 ± 0.1 . The molecular weight measured by the sedimentation-diffusion method is 139000. On gel electrophoresis in sodium dodecyl sulphate (Weber & Osborn, 1969) the enzyme gives only one major band, with a molecular weight of 36000. The enzyme contains 12 mol of tryptophan/mol (assayed by the method of Edelhoch, 1967). Like other liver fructose 1,6-diphosphatases, the enzyme requires EDTA for maximal activity.

On reaction with small quantities of *p*-chloromercuribenzoate the activity of the enzyme assayed with Mg^{2+} decreases slightly; however, the activity with Mn^{2+} increases. These effects do not depend on the pH of the assay medium. Maximal stimulation (to 160% of the control) is observed at a ratio of 4 mol of *p*-chloromercuribenzoate/mol of enzyme.

The enzyme is subject to inhibition by high concentrations of fructose 1,6-diphosphate. This is not due to chelation of metal ion, and does not affect sensitivity to AMP. When native enzyme is used this inhibition is partially non-competitive (Dixon & Webb, 1964) with respect to Mg^{2+} : it plateaus at 55% inhibition. However, with *p*-chloromercuribenzoate-modified enzyme inhibition by high concentrations of fructose 1,6-diphosphate is fully non-competitive, and so a linear Dixon plot is obtained. Inhibitory concentrations of fructose 1,6-diphosphate specifically prevent the activation of the enzyme by *p*-chloromercuribenzoate.

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Some Properties of Guinea-Pig Liver Fructose 1,6-Diphosphatase in Relation to the Control of Gluconeogenesis

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The control of gluconeogenesis in the guinea-pig liver differs from that in the rat liver since it is not stimulated by fatty acids despite elevated acetyl-CoA concentrations (Söling *et al.*, 1971). Therefore pyruvate carboxylase may not be an important site of control in gluconeogenesis in the guinea pig.

Guinea-pig liver fructose 1,6-diphosphatase, prepared by acid precipitation (Underwood & Newsholme, 1965), had a pH optimum at 7.5–8.0 and was not stimulated by prior preincubation with citrate or EDTA (see Taketa *et al.*, 1971a). The K_m for fructose 1,6-diphosphate was 2.4–3.0 μM with a maximum rate at about 30 μM , the enzyme being inhibited by higher substrate concentrations. The enzyme was also inhibited by ATP above 0.05 mM, and strongly by AMP (activity diminished to 50% at 15 μM , mean Hill coefficient 1.8). Although 1 mM-3-phosphoglycerate partially overcame the inhibition by ATP (see Taketa *et al.*, 1971b), citrate had a more striking effect on the enzyme. Citrate at 0.4 mM and above increased the effectiveness of AMP inhibition (10 mM-citrate decreased the Hill coefficient by 70%). However, citrate also increased the K_m of fructose 1,6-diphosphatase for, and diminished the effectiveness of the inhibition by, its substrate. The net effect was that at low AMP concentrations citrate increased fructose 1,6-diphosphatase activity by acting on substrate inhibition, but at AMP concentrations above 5 μM citrate enhanced the inhibitory effects of AMP and the enzyme activity was decreased. Thus changes in citrate con-

centration brought about large changes in the activity of guinea-pig fructose 1,6-diphosphatase. Only small effects on activity were produced by acetyl-CoA, palmitate, oleate and alanine.

In the rat liver the appearance of gluconeogenesis is associated with a rise in the concentrations of acetyl-CoA, long-chain acyl-CoA, AMP and fructose 1,6-diphosphate and a fall in those of ATP and cytoplasmic citrate (Greenbaum *et al.*, 1971). If these changes occur in the guinea-pig liver it seems unlikely that changes in ATP concentration would have more than a small effect on fructose 1,6-diphosphatase, and this is likely to be counteracted by the rise in the concentrations of AMP and fructose 1,6-diphosphate. However, the 50% fall in cytoplasmic citrate concentration, particularly as guinea-pig liver citrate concentrations are comparatively high (Haeckel & Haeckel, 1971), could be one of the important factors controlling the rate of gluconeogenesis.

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The Development of some Enzymes of Gluconeogenesis in the Liver and Kidney of the Foetal Guinea Pig

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The foetal rat liver has been shown to be largely incapable of glucose synthesis (Ballard & Oliver, 1963; Yeung & Oliver, 1967), and this is, in part, the result of gluconeogenic enzymes being either present at low activities or absent until birth (Yeung *et al.*, 1967; Greengard & Dewey, 1967). However, the livers of foetal sheep (Ballard & Oliver, 1965) and foetal guinea pigs (C. T. Jones, unpublished work) have significant rates of gluconeogenesis *in vitro*. Also, the human foetal liver has significant activities of gluconeogenic enzymes early in gestation (Räihä & Lindros, 1969; Auricchio & Rigillo, 1960). As part of a study on the importance of foetal glucose synthesis the changes occurring in the properties and