the isomerization reaction. The 3-hydroxyl group may be involved in donating a hydrogen bond to a receptor in the enzyme, so helping to hold the substrate in a conformation with the enediol hydroxyl groups *cis*.

The C-2-substituted D-glucose 6-phosphates cannot be substrates for glucose phosphate isomerase because the 2-hydroxyl group is involved in the isomerization. These compounds were, however, competitive inhibitors, with K_i values as follows: 2-chloro-2-deoxy-D-glucose 6-phosphate, 0.3 mM; 2-deoxy-2-fluoro-D-glucose 6-phosphate, 9 mM; 2-deoxy-D-*arabino*-hexose 6-phosphate, 2 mM; 2-amino-2-deoxy-D-glucose 6-phosphate, 3 mM. Since there is no correlation of inhibitor binding with the size of the substituent, steric factors may not be important. It is possible that the inductive effect of the substituent affects the rate of ring-opening to the acyclic form of the sugar and determines the ability of the inhibitor to bind to the enzyme.

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The Effect of Substitution at C-2 of D-Glucose 6-Phosphate on the Rate of Dehydrogenation by Yeast Glucose 6-Phosphate Dehydrogenase

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A number of halogen-substituted analogues of D-glucose 6-phosphate are now available (Bessell & Thomas, 1972). The C-2-substituted compounds have been used in a study of the effect of electron-withdrawing substituents on the rate of dehydrogenation by yeast glucose 6-phosphate dehydrogenase (EC 1.1.1.49).

The rates of reaction of D-glucose 6-phosphate, 2-deoxy-D-*arabino*-hexose 6-phosphate, 2-amino-2-deoxy-D-glucose 6-phosphate, 2-chloro-2-deoxy-D-glucose 6-phosphate and 2-deoxy-2-fluoro-D-glucose 6-phosphate were investigated. 2-Deoxy-2-fluoro-D-mannose 6-phosphate and 2,2-difluoro-D-*arabino*-hexose 6-phosphate were not substrates for the enzyme. K_m values for the sugar phosphates and NADP^+ were calculated from the double-reciprocal plot of Lineweaver & Burk (1934). The K_m value determined for NADP^+ was $2 \times 10^{-5} \text{ M}$ in all cases whereas the K_m values for the sugar phosphates varied. The order of $V_{\max}$ obtained experimentally

was $\text{OH} > \text{Cl} > \text{H} > \text{NH}_3^+ > \text{F}$ for the C-2-substituted sugars.

To comply with a mechanism involving transfer of a hydride ion from C-1 of the sugar to the nicotinamide ring of NADP^+ , the rate of hydride transfer should be inversely proportional to the inductive effect of the substituent on C-2. The dissociation constants of analogous substituted aliphatic carboxylic acids (Kortüm *et al.*, 1961) correlate with an order of electronegativity of $\text{H} < \text{OH} < \text{Cl} < \text{F}$. Thus an order of $V_{\max}$ of $\text{H} > \text{OH} > \text{Cl} > \text{F}$ would be expected. The order obtained experimentally differs from the expected order only in the position of H (2-deoxy-D-*arabino*-hexose 6-phosphate). However, substrate inhibition was observed with this compound and the maximum velocity obtained experimentally is therefore less than the theoretical $V_{\max}$. A theoretical $V_{\max}$ calculated for this compound is of the same order as that for D-glucose 6-phosphate. These observations are consistent with hydride ion transfer being the rate-determining step of the dehydrogenase reaction.

Investigations with 3-deoxy-3-fluoro-D-glucose 6-phosphate and 4-deoxy-4-fluoro-D-glucose 6-phosphate showed that each compound is a substrate for the dehydrogenase; the values of K_m ($1.25 \times 10^{-3} \text{ M}$) and $V_{\max}$ (0.12 with respect to D-glucose 6-phosphate) were identical.

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Effect of Diet and also Physiological State on some Enzymes of Carbohydrate Metabolism in the Liver of the Domestic Fowl

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Dietary lipid diminishes hepatic lipogenesis in the laying hen and the immature pullet (Balnave & Pearce, 1969; Pearce, 1971a). The present work was undertaken to examine the effects of dietary lipid on the activities of some of the key enzymes of glycolysis, gluconeogenesis and the pentose phosphate pathway.

Chemically defined diets (containing 0 or 10% lipid as corn oil) were fed to pullets (aged 3 weeks) for 7 days. The specific activity of phosphofructokinase was lower in liver extracts from birds fed on the

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.