on conditions of storage that degrade cytochrome P-450 to cytochrome P-420. As expected for a membrane-bound enzyme complex, it has proved resistant to solubilizing techniques (sonication, detergents, deoxycholate, trypsin), and treatment with deoxycholate, steapsin or papain markedly decreases aromatizing activity. Inhibition studies with classical inhibitors (0.1 mM) of hepatic microsomal drug-hydroxylating enzymes [SKF 525A (2-diethylamino-ethyl 2,2-diphenylvalerate hydrochloride), DPEA (2,4-dichloro-6-phenylphenoxethylamine), Lilly 18947 (2,4-dichloro-6-phenylphenoxyethyldiethylamine), ethyl isocyanide], competitive inhibitors of these enzymes {imipramine [10,11-dihydro-5-(3-dimethylaminopropyl)dibenz-[b,f]azepine], biphenyl, zoxazolamine (2-amino-5-chlorobenzoxazole), DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane], dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene)}, inhibitors of steroid hydroxylation {spiro-nolactone [β -(7 α -acetylthio-17 β -hydroxy-3-oxo-androst-4-en-17 α -yl)propionic acid lactone], betamethasone (9 α -fluoro-11 β ,17 α ,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione), metyrapone [2-methyl-1,2-bis-(3-pyridyl)propan-1-one]} and chelating agents (EDTA, $\alpha\alpha'$ -bipyridyl) were without effect on the aromatizing system. However, inhibition did occur at this concentration (0.1 mM) with testosterone (90%), [α -(p-aminophenyl)- α -ethylglutarimide] aminoglutethimide (60%), an inhibitor of desmolase, and with the thiol-blocking reagents p-hydroxy-mercuribenzoate (80% inhibition) and iodoacetamide (20%), but not with β -mercaptoethylamine. Slight inhibition also occurred at 0.1 mM with the fatty acids arachidonic acid (30%) and lauric acid (20%). It has been postulated that metyrapone inhibits steroid and drug hydroxylations by interaction with cytochrome P-450 (Hildebrandt *et al.*, 1969). We interpret this lack of inhibitory activity on the aromatizing enzyme, together with the absence of effect by CO and ethylisocyanide, as conclusive evidence that the conversion of androgens into oestrogens in the placenta is not mediated by cytochrome P-450.

These results from experiments *in vitro* indicate that, although the placenta has a poor ability to metabolize drugs, the presence of drugs and other xenobiotics in the placenta is unlikely to adversely affect the metabolism of androgens to oestrogens.

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The Deoxyfluoro-D-glucopyranose 6-Phosphates and their Effect on Yeast Glucose Phosphate Isomerase

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Glucose phosphate isomerase (EC 5.3.1.9) catalyses the isomerization of D-glucose 6-phosphate into D-fructose 6-phosphate. D-Glucose 6-phosphate binds in its α -pyranose form (Salas *et al.*, 1965) and is then converted into its acyclic form (Dyson & Noltmann, 1968), probably by a concerted ring-opening mechanism. Sugar phosphates that can only exist in an acyclic form (e.g. D-erythrose 4-phosphate and D-gluconic acid 6-phosphate) are good competitive inhibitors.

The deoxyfluoro-D-glucopyranose 6-phosphates were prepared from the corresponding deoxyfluoro-D-glucose and ATP by using hexokinase. 2-Deoxy-2-fluoro-, 3-deoxy-3-fluoro- and 4-deoxy-4-fluoro-D-glucose 6-phosphate were thus prepared. In addition, 2-chloro-2-deoxy-D-glucose 6-phosphate was obtained from 2-chloro-2-deoxy-D-glucose by this method.

Glucose phosphate isomerase was assayed by coupling to the phosphofructokinase reaction. The rate of proton release was followed spectrophotometrically by using Cresol Red. This method can provide a sensitive and convenient assay system. Both 3-deoxy-3-fluoro and 4-deoxy-4-fluoro-D-glucose 6-phosphate were substrates for glucose phosphate isomerase and, in addition, the products of this reaction, 3-deoxy-3-fluoro- and 4-deoxy-4-fluoro-D-fructose 6-phosphate respectively, were very good substrates for phosphofructokinase. The K_m values of the 3-deoxy-3-fluoro- and the 4-deoxy-4-fluoro-D-glucose derivatives for glucose phosphate isomerase were 6 and 7 mM respectively. The V_{max} values relative to D-glucose 6-phosphate were 0.006 and 0.31 respectively. The low relative V_{max} values for the 3-deoxy-3-fluoro derivative are not explained by the inductive effect of the fluorine substituent, since this should facilitate rather than hinder the formation of the proposed enediol intermediate. Although it appears that the 3-hydroxyl group in D-glucose 6-phosphate is not of vital importance in the binding of the substrate to the enzyme, it might be important in

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