DE 52) followed by gel filtration on Sephadex G-100. A second fractionation of the enzyme on Sephadex G-100 yielded an active preparation purified approx. 1200-fold from the high-speed supernatant. Samples of this preparation (20–200 μ g of protein) gave a single homogeneous enzymically active component on polyacrylamide-gel electrophoresis. The molecular weight (68000) determined by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (Weber & Osborn, 1969) was similar to the value (69000) estimated by gel filtration on Sephadex G-100.

The enzyme exhibited a sharply defined pH optimum (5.3) and was optimally active towards 4.0 mM-adenosine 5'-sulphatophosphate, above which concentration inhibition by excess of substrate was observed. The Michaelis constant (K_m) was determined to be 9.5×10^{-4} M.

The properties of the highly purified enzyme from bovine liver are therefore closely similar to those reported by Bailey-Wood *et al.* (1970) for the partially purified rat liver enzyme.

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Partial Purification and Characterization of Triokinase from Ox and Human Liver

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D-Glyceraldehyde may be incorporated into the glycolytic pathway in three main ways: by phosphorylation to glyceraldehyde 3-phosphate by triokinase (Hers & Kusaka, 1953); by conversion into dihydroxyacetone phosphate mediated sequentially by an alcohol dehydrogenase or glycerol dehydrogenase (Leuthardt & Wolf, 1954; Moore, 1959), glycerol kinase (Bublitz & Kennedy, 1954) and glycerophosphate oxidase (Ringler & Singer, 1959; Burch *et al.*, 1970); and by conversion into 2-phosphoglycerate through the action of aldehyde dehydrogenase (Lamprecht & Heinz, 1958; Holldorf *et al.*, 1959) and glycerate kinase (Ichihara & Greenberg, 1957; Lamprecht *et al.*, 1959).

Sillero *et al.* (1969) have claimed that, in the rat, D-glyceraldehyde is the centre of a metabolic cross-roads and that its incorporation into the glycolytic

pathway is mediated mainly by the enzyme triokinase. As part of a continuing study of the metabolism of D-glyceraldehyde in the mammal (Kormann *et al.*, 1972) we have investigated the properties of triokinase from ox and human liver.

Triokinase has been purified from both sources by $(\text{NH}_4)_2\text{SO}_4$ fractionation, chromatography on DEAE-cellulose and Sephadex G-200 and isoelectric focusing. Two enzymes capable of phosphorylating D-glyceraldehyde were found in ox liver. One of these had a high affinity for D-glyceraldehyde (K_m 0.01 mM), but significant amounts of aldehyde dehydrogenase activity remained closely associated with the enzyme even after extensive purification. The other enzyme from ox liver had a much lower affinity for D-glyceraldehyde (K_m 12 mM) and also had aldehyde dehydrogenase and adenosine triphosphatase activities associated with it. The triokinase from human liver has been purified extensively. The final preparation exhibited one major band and a few minor protein bands on disc gel electrophoresis and had no other enzyme activities associated with it. The enzyme has a fairly high affinity for D-glyceraldehyde (K_m 6 mM) and also for D-ribose. Throughout the purification procedures coupled assay systems involving the reduction of NAD^+ or oxidation of NADH were used to assay triokinase activity. Considerable interference by aldehyde dehydrogenase and/or alcohol dehydrogenase occurred, even in highly purified preparations, and several controls were employed for a single assay. The assay of triokinase was further complicated when it was found that ATP was a potent activator of aldehyde dehydrogenase.

Both the ox liver enzymes and human liver triokinase were found to require ATP and Mg^{2+} in a 1:1 ratio for maximal activity. When the ATP/ Mg^{2+} ratio was greater than 1.0 the reaction rate showed a sigmoidal dependence on the concentration of total Mg^{2+} . When the ATP/ Mg^{2+} ratio was less than 1.0 inhibition of triokinase activity occurred.

In their model of the glyceraldehyde cross-roads Sillero *et al.* (1969) have assumed that no control is exercised *in vivo* on the enzymes involved. The sigmoidal response obtained by varying the Mg^{2+} /ATP ratio and the activation of aldehyde dehydrogenase by ATP suggests that some form of control may be exerted. Further, the presence of two enzymes capable of phosphorylating D-glyceraldehyde in ox liver and the presence in human liver of a triokinase with a lower affinity for D-glyceraldehyde than that of rat liver triokinase indicates that the model of the glyceraldehyde cross-roads proposed by Sillero *et al.* (1969) for rat liver may not apply to either ox or human liver.

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Studies on the Functional Specificity of Cortisone Reductase

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Crystalline preparations of cortisone reductase (EC 1.1.1.53) have certain 3- and 20-hydroxy steroid-NAD⁺ oxidoreductase activities that may possibly depend on one active centre with complex steroid-binding properties (Gibb & Jeffery, 1971). Studies are now described in which a series of test substrates was used to elucidate certain aspects of the specificity of these reactions. It was shown that there is no absolute requirement for the hydrogen added to or removed from C-3 to be α , β , axial or equatorial, or for the chirality at C-3 to be *R* or *S*. In the 5 α -androstan-3-one compounds studied, the hydrogen added to or removed from C-3 was β and equatorial, but C-3 had *R* or *S* chirality. The 5 β -androstan-3-one compounds studied were very poor substrates, and among these compounds preference was found for utilization of 3 β -hydrogen, which was axial and C-3 *R*, rather than 3 α -hydrogen, which was equatorial and C-3 *S*, though the latter was observed. These poor substrates were utilized so inefficiently that it was not possible to establish kinetically the relationship between the slight 3 β -hydroxy steroid dehydrogenase activity and either the 3 α - or the 20-hydroxy steroid dehydrogenase activity.

The non-steroid adamantanone was a substrate, undergoing a reaction clearly analogous to the 3-hydroxy steroid dehydrogenase activity towards

steroids, but achiral, and equally related to 3 α , 3 β , 3-axial and 3-equatorial activity. This activity was not kinetically independent of the 20-hydroxy steroid dehydrogenase activity (Gibb & Jeffery, 1972).

The hydrogen added to or removed from C-20 was *R* or *S*, but was consistently 20 α in the compounds so far investigated.

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Studies on the Active Centre for the 3-Hydroxy Steroid-Nicotinamide-Adenine Dinucleotide Oxidoreductase Activity of Cortisone Reductase

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Kinetic studies were carried out with 17 β -hydroxy-5 α -androstan-3-one (I), a series of related steroids differing in structure only in ring A, and the analogous non-steroid adamantanone. The 1 α -methyl and 2 α -methyl derivatives of compound (I) had apparent K_m and apparent V values similar to those of compound (I). The 1 β -methyl and 4 α -methyl derivatives of compound (I) were not substrates, but were moderately effective inhibitors. The 1 α -hydroxy derivative of compound (I) was a very poor substrate with high apparent K_m and low apparent V . These findings would allow the view that the binding site is hydrophobic in the region around the A-ring; that it is possibly of somewhat more restricted dimensions (or lower flexibility) in the direction across the A-ring (1 β –4 α), and somewhat bigger than a steroid (or highly flexible) in the region out of the plane of the A-ring (i.e. from above the β -side to below the α -side). This would lead to an estimate of roughly 0.60×0.52nm for the minimum dimensions of the site where the A-ring binds. Such a view could accommodate in a general way the further findings that 17 β -hydroxy-5 β -androstan-3-one and the non-steroidal but analogous adamantanone were poor substrates; and even that 17 β -hydroxy-1 α ,2 α -methylene-5 α -androstan-3-one and the corresponding 1 β ,2 β -methylene compound were moderately effective inhibitors, though it is less clear why these two compounds were not substrates. Certain other