

The Enzymatic Reduction of Uracil to Dihydrouracil in Rat Liver

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The reduction of uracil to dihydrouracil is the rate limiting step in the breakdown of uracil to β -alanine in rat liver¹⁻³. This fact indicates the central role of this reaction in the uracil-catabolizing system. The present work describes a procedure for the assay of dihydrouracil dehydrogenase in rat liver. The quantitative relation between enzymatic activity and factors which influence the reduction, has been investigated.

Spectrophotometric studies were carried out with an enzyme purified by ammonium sulphate fractionation of the particle-free supernatant. Optimal rate of reduction was in the pH-range 7.0 to 7.6. The reaction is TPNH-dependent^{3,4}, and the optimal concentration was found to be 7×10^{-5} M. The Michaelis constant was 1.7×10^{-5} M. The optimal uracil concentration was very small and less than 1×10^{-5} M. The reversible reduction of uracil was demonstrated, but the very slow rate suggested that this reaction is without physiological significance.

The quantitative determination of dihydrouracil dehydrogenase was carried out by anaerobic incubation of homogenate or the soluble fraction with uracil-6-¹⁴C in the presence of TPNH, with G-6-P and G-6-P dehydrogenase as a TPN reducing system. ATP was found to have a stimulating effect on the reaction under these conditions^{5,2}. The effect decreased, however, when the TPNH concentration was increased, indicating that ATP had a sparing action on the cofactor rather than a stimulating influence on the reduction as such. When appropriate amounts of ATP, nicotinamide⁶ and fluoride were included in the incubation mixture, a linear relation between the rate of reduction and the amount of homogenate added, was obtained. The enzymatic activities of the homogenate and the soluble fraction were identical, indicating that dihydrouracil dehydrogenase is situated entirely in the soluble fraction. The enzymatic activity per g

of liver, wet weight, corresponded to the reduction of about 0.7 μ mole of uracil in 10 min.

The TPNH level in normal liver^{7,8} is probably greater than that required for optimal enzymatic activity. Hence, the capacity to degrade uracil may be limited only by the amount of enzyme. The rate of uracil breakdown in the intact rat⁵ support this view.

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Adsorption of Water Vapour by Tissue Polysaccharide

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Since "water-binding" is considered as a special rôle of the connective tissue polysaccharides, the affinity of water vapour to their surface was determined using the Brunauer-Emmett-Teller-plot¹. The preparation was isolated from human umbilical cords (using CaCl_2 -extraction, precipitation with 66 % ethanol and repeated Sewag-procedure) and contained nitrogen 0.07 mg/ml and glucosamine 0.32 mg/ml (corresponding to about 76 % polysaccharide and 24 % protein). The solution was lyophilized. Part of this dry residue was depolymerized with hyaluronidase, and the digest was dialyzed and lyophilized. The combustion residues were about 15-17 % and the results are correlated accordingly.

The practical procedure was adopted from Bull², except that +40°C temperature was used. Ten points up to $P/P_0 = 0.6$ were determined. The B.E.T. plots were quite straight, and the following constants were obtained from estimating equations:
original preparation V_m 11.3 ml/100 g, C 39;
depolymerized " 12.6 " /100 g, " 99.

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