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 liver [1-3]. 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 [2,4], 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-^{14}C 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 [5,3]. 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, nicotinamide [6] 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 μmole 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 rat [5] 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-plot [1]. 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 Bull [2], except that + 40°C temperature was used. Ten points up to $P/P_o = 0.6$ were determined. The B.E.T. plots were quite straight, and the following constants were obtained from estimating equations:

original preparation Vm 11.3 ml/100 g, C 39;
depolymerized " 12.6 " /100 g, " 99.

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Hyaluronate adsorbs water more than collagen, but the difference is not large. The effect of depolymerization is seen in the change of the constant C.

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Purification and Properties of a Bacterial Glucose Dehydrogenase

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A bacterium posessing a glucose dehydrogenase was isolated from a secondary culture infection. It has provisionally been assigned to the genus *Alcaligenes*. 100 g quantities (dry wt.) of the organism could be grown in a simple 20 liter continuous culture apparatus in a normal work day. Resting cells oxidize glucose rapidly to the level of 1 atom oxygen consumed per molecule glucose, and from then on at a reduced rate. In this first period CO_2 evolution is slight. Cell-free extracts, prepared by grinding with Ballotini glass beads, also carry out oxidation of glucose with oxygen as acceptor. The primary dehydrogenation could be studied with 2,6-dichlorophenol indophenol as hydrogen acceptor. Using this assay method, the enzyme was purified 250 fold with protamine sulphate, ammonium sulphate, DEAE cellulose, and calcium phosphate gel. At this stage the enzyme catalyzes the reduction of 40 μmoles dye per min per mg protein.

Cytochrome *c* or DPN are not reduced at a significant rate, and reoxidation with oxygen is not measurable, unless phenazine methosulphate is also added. In the presence of phenazine methosulphate and oxygen, the immediate product of the reaction was shown to be gluconolactone. The absorption spectrum of the enzyme preparation shows the presence of a b-type hemoprotein, partially reducible by glucose. The spectrum also suggests the presence of functional flavin, and preliminary inhibition and fluoresence data support this evidence. This soluble dehydrogenase appears to represent a fragment of the particulate glucose oxidation system found in the cells and the extract.

Collagen Fractions in Experimental Lathyrism

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The collagen fractions of pooled skins of lathyritic and control rats were obtained by a modified procedure by Harkness *et al.*[1]. In the disodium phosphate-soluble fractions the collagen was estimated from the content of hydroxyproline [2], the acid-soluble and insoluble fractions from the nitrogen content. Four extraction series were performed in both groups.

The insoluble collagen was nearly same in lathyritic animals as in controls. The acid-soluble collagen was higher in lathyritic samples (content 0.48 % and 0.18 % from the air-dried skin samples of lathyritic and normal rats, respectively; $P>0.10$).

The most marked difference was in the disodium phosphate-soluble collagen. Calculated from the hydroxyproline contents of the extracts, the averages were 3.15 % and 0.62 % in the lathyritic and normal samples, respectively ($P<0.001$) and after precipitation the alkali-soluble collagen amounted to 1.07 % and 0.30 % ($P<0.01$). It is concluded that in experimental lathyrism the alkali-soluble collagen is increased but there is a defect in the fibre formation.

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A New Sensitive Detector for Gas Chromatography

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We constructed Lovelock's type of gas chromatography detector [1] of Pyrex-glass using the ^{90}Sr-covered silver plate as anode. Some incidental observations prompted us to replace the anode by a non-radioactive aluminium plate.