DPN/DPNH and Rate of Glycolysis in Erythrocyte Hemolysate

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Ethanol has been shown to have a marked effect on the intermediary metabolism of the liver^{1,2}. Studies on the mechanism of inhibition of galactose oxidation in the liver by ethanol³ have indicated that the DPN dependent UDP-galactose 4-epimerase reaction is markedly inhibited by the DPNH generated during ethanol oxidation. Many other effects of ethanol oxidation on liver metabolism have been ascribed to the change in DPN/DPNH ratio⁴. In the present study the effect of changes in the DPN and DPNH concentrations on the rate of glycolysis was studied.

Erythrocytes were chosen for study since they, in contrast to many other cell types, derive energy almost exclusively from glycolysis and since other metabolic pathways are restricted. Hemolysates of human erythrocytes were prepared from freshly drawn blood cells which were washed in cold 0.15 M KCl and hemolysed by rapid freezing and thawing.

The glycolytic activity was measured by the disappearance of glucose and the formation of lactic acid. Lactic acid was determined enzymatically⁵ and glucose by the method of Hultman⁶. The incubation medium contained the following components in a total volume of 2 ml: 20 μ moles of glucose, 10 μ moles of ATP, 10 μ moles of $MgCl_2$, 100 μ moles of potassium phosphate buffer (pH = 7.4) and 1 ml of hemolysate. The mixture was incubated for 2 h at 37°C. When increasing amounts of DPN was added to the reacting mixture the lactate production increased and reached an optimum at a DPN concentration of about 3×10^{-4} M. At this concentration the lactate production of the hemolysate was 12 μ moles/h/gHb which is similar to that found in intact erythrocytes⁷. When ethanol 0.11 M, ADH (Boehringer) 10 μ l, and semicarbazide hydrochloride 80 μ moles were added, an 80 % decrease of the lactate production was observed. The rate of glycolysis decreased to the level found without the DPN fortification of the medium. Semicarbazide was added to the system in order to couple the acetaldehyde formed, since at pH 7.4 the ADH catalyzed reaction is in favor for acetaldehyde reduction. Ethanol, ADH, or semicarbazide alone had no effect, and without semicarbazide the ethanol-ADH system had no effect. In the

experiments by Isselbacher³ an 80 % inhibition of galactose oxidation was produced in hemolysates with ADH and an ethanol concentration of 0.1 M, already without semicarbazide. Thus it seemed probable that the DPN-dependent triose phosphate dehydrogenase reaction was not as sensitive to a change in favor of DPNH. This assumption was strengthened by the experiments in which DPNH was added to the medium. Lactate production was not influenced when DPN and DPNH were added in equal amounts (10^{-3} M). When DPNH (10^{-3} M) alone was added to the reacting hemolysate mixture the lactate production was 70 % of that found with a similar DPN addition. In all experiments the glucose consumption followed the lactate production.

According to these results it seems unreasonable to assume that ethanol oxidation *in vivo* inhibits the rate of glycolysis.

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Initial Changes in the Level of Adenine Nucleotides By Glucose Addition in Ehrlich Ascites Tumor Cells

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In previous work on the inhibitory effect of glucose on the rate of respiration in Ehrlich ascites tumor cells (Crabtree effect), it has been shown that glucose initially gives rise to a transient decrease in the ATP concentration and a corresponding increase in the ADP concentration, but no change in the total amount of adenine nucleotides¹⁻³.

These initial changes of the ATP and ADP concentrations as well as the total concentration of adenine nucleotides have now been shown to be highly dependent of the total amount of glucose available per gram cells.