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.

1. Lieber, C. S. and Davidson, C. S. *Am. J. Med.* **33** (1962) 319.
2. Forsander, O. and Räihä, N. *In press*.
3. Isselbacher, K. J. and Krane, S. M. *J. Biol. Chem.* **236** (1961) 2394.
4. Räihä, N. and Oura, E. *Proc. Soc. Exptl. Biol. Med.* **109** (1962) 908.
5. Scholz, R., Schmitz, H., Bücher, Th. and Lampen, J. O. *Biochem. Z.* **331** (1959) 71.
6. Hultman, E. *Nature* **33** (1962) 319.
7. Bernstein, R. E. *J. Clin. Invest.* **38** (1959) 1572.

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.

Using 150 mg cells/ml suspension and 5 μ mole glucose/ml only a transient change between ATP and ADP is observed. If a more diluted suspension and the same glucose concentration is used, the cells initially loose up to 60 % of their adenine nucleotides as described in the following experiment.

In a cell suspension containing 45 mg cells/ml (55 mM phosphate, Locke's solution pH 7.35) the addition of glucose (5 μ mole/ml) gives rise during the first 60 sec to a fall in ATP of about 60 % of its initial value and a corresponding rise in the ADP concentration. In the following 3 min, however, the ADP almost disappears from the cell, while the ATP concentration is not changed, resulting in a 60 % decrease in the total concentration of the adenine nucleotides.

During the disappearance of ADP a corresponding amount of decomposition products (hypoxanthine, inosine and adenosine) is observed to accumulate in the medium.

In the following 50 to 60 min the hypoxanthine, inosine and adenosine disappear from the medium and during this period the lost adenine nucleotides are resynthesized and the size of the adenine nucleotide pool restored to its initial value.

From these experiments it appears, therefore, that the amount of glucose available per cell is of great importance for evaluating its effect on the adenine nucleotide level in Ehrlich cells.

1. Ibsen, K. H., Coe, E. L. and McKee, R. W. *Biochim. Biophys. Acta* **30** (1958) 384.
2. Hess, B. and Chance, B. *J. Biol. Chem.* **236** (1961) 239.
3. Wu, R. and Racker, E. *J. Biol. Chem.* **234** (1959) 1036.

Studies on Active Calcium Transport in Mitochondria

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Addition of calcium ions to a mitochondrial suspension results in a number of changes; activation of ATPase activity, uncoupling,

swelling, abolition of permeability barriers, and loss of mitochondrial nucleotides and other substances. By using small calcium additions, 50–200 μ A/L, and short time spans, the effect of calcium can be studied in unaltered mitochondria without the complication of secondary changes. Under these conditions similar changes are observed as after small ADP additions: a burst of respiration, a cycle of oxidation and reduction of DPN (pyridine nucleotide) and other respiratory carriers. This cycle can be repeated a few times until carriers stay oxidized, swelling sets in, and the mitochondria are completely uncoupled.

Data are presented to show that in a complete system containing an oxidizable substrate, small additions of calcium ions marked with ^{47}Ca result in a complete uptake within 20–30 sec at 20°. In the absence of a substrate the uptake is smaller. Uncoupling either by aging or by uncoupling agents like 2,4-dinitrophenol greatly diminish the uptake. Azide and cyanide also inhibit calcium uptake. Magnesium has no influence on the uptake.

In a medium containing ATP but no substrate ATPase activity after small calcium additions was followed by inorganic phosphate estimations. The ATPase activity was found to be triphasic: first a high activity during the calcium uptake, then a leveling off or cessation of activity, and finally an accelerating rate of ATPase activity. With large calcium additions the high ATPase activity in the third phase will completely overlap the preceding phases.

The calcium uptake was found to be accompanied by the building up of a pH-gradient between the mitochondria and the medium, a pH-drop being recorded in the medium. When swelling sets in, the pH-gradient disappears.

These results are interpreted as indicating the presence in mitochondria of a calcium transporting mechanism. The bearing of these results on the mechanism of calcium induced aging is discussed.

In this study rat liver mitochondria were used but a similar mechanism was found to operate in other mitochondria tested, e.g. rat kidney and pigeon heart mitochondria.

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