Influence of Ethanol on the Lactate/Pyruvate and β-Hydroxybutyrate/Acetoacetate Ratios in Rat Liver Experiments

OLOF A. FORSANDER,
PEKKA H. MÄENPÄÄ and
MIKKO P. SALASPURO

Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland

During ethanol oxidation the NAD^+/NADH ratio of the liver shifts towards a more reduced state.[1-3] At the same time the lactate level of the blood rises [4,5] and the lactate/pyruvate ratio increases.[3,6] The ratio of the redox pair β-hydroxybutyrate/acetoacetate of the blood is also increased during ethanol oxidation.[4,6]

For investigation of a possible correlation between the change of the lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios, liver perfusion experiments were performed in which the concentrations of these four metabolites in the perfused medium were measured during ethanol oxidation. Livers of male rats, four fed and four starved for 48 h, were used for the experiments. The perfusion was performed at 37°, using Schimassek's procedure.[7] Ethanol was added to the medium to a concentration of 35 mM after a 60 min preceding perfusion performed in order to equilibrate the perfusion system. Lactate and pyruvate were determined enzymically with the aid of Biochimica Boehringer's (Mannheim, W. Germany) test kits. Acetoacetate and β-hydroxybutyrate were measured as described earlier.[8]

At the end of the one-hour control perfusion the redox levels of the two substrate pairs showed the same values as has been found for the blood of intact rats. However, when ethanol was added to the perfusion medium the lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios started to rise (Figs. 1 and 2). In the fed livers the lactate/pyruvate ratio reached a new steady state about 60 min after the addition of ethanol, but in the starved livers the ratio rose steadily throughout the perfusion. The β-hydroxybutyrate/acetoacetate ratios of the medium rose in both the fed and the starved livers during the perfusion and faster in the starved than in the fed livers.

When the lactate/pyruvate ratios were plotted against the β-hydroxybutyrate/acetoacetate ratios (Fig. 3) it was found that an increase in the lactate/pyruvate

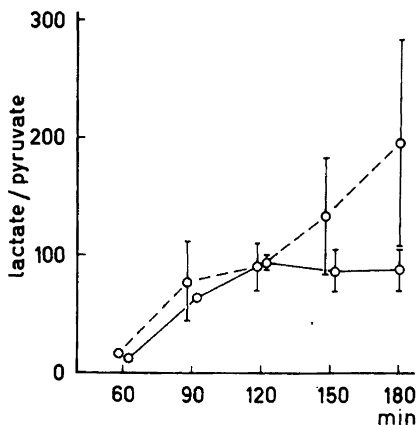

Fig. 1. The lactate/pyruvate ratios of the medium obtained during perfusion of isolated fed and starved rat livers plotted against time. — O — experiments with fed, - - - O - - - with starved livers. The bars represent the standard errors of the means.

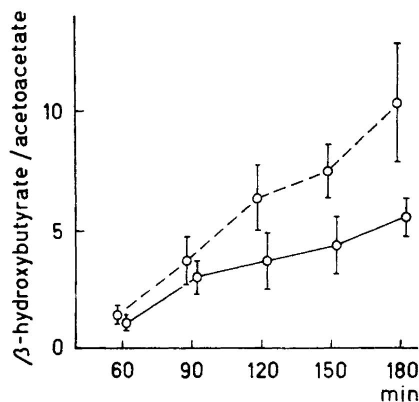

Fig. 2. The β-hydroxybutyrate/acetoacetate ratios of the medium obtained during perfusion of isolated fed and starved rat livers plotted against time. — O — experiments with fed, - - - O - - - with starved livers. The bars represent the standard errors of the means.

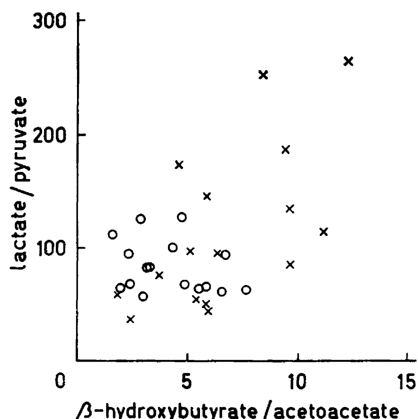

Fig. 3. The lactate/pyruvate ratios of the medium obtained during perfusion of isolated fed and starved rat livers plotted against the β-hydroxybutyrate/acetoacetate ratios. O experiments with fed, × with fasted livers.

ratio was usually followed by an increase in the β-hydroxybutyrate/acetoacetate ratio. There was no significant correlation between these two ratios, however.

The lactate/pyruvate substrate pair of the perfusion medium is in equilibrium with the NAD-dependent redox systems of the extramitochondrial cytoplasmic compartment of the liver cell,[9,10] while the β-hydroxybutyrate/acetoacetate substrate pair is assumed to be in equilibrium with the NAD-dependent redox systems of the mitochondria.[11] In the liver ethanol is first oxidized to acetaldehyde and further to acetate.[1] The alcohol dehydrogenase is located mainly in the extramitochondrial compartment of the liver cell [12] and at least a part of the aldehyde dehydrogenase [13] responsible for the second oxidation stage [14] is located in the same compartment. It is therefore to be assumed that during ethanol oxidation the primary change in the redox potential occurs in the extramitochondrial compartment of the liver cell, the mitochondrial change being secondary. But it seems that a change in the extramitochondrial redox potential is not always followed by a corresponding change in the mitochondrial redox potential. The way in which hydrogen is transferred from the ethanol to the mitochondria is not known, but it might be expected that the α-glycerophosphate shuttle is the carrier. Krebs *et al.*[15] and likewise Klingenberg and v. Häfen [11] have found that succinate also produces an increase in the β-hydroxybutyrate/acetoacetate ratio. These investigators do not agree on the mechanism by which the ratio is influenced. It has been shown that succinate and α-glycerophosphate are oxidized in the mitochondria by enzymes located in close structural proximity at the respiratory chain.[16] It might therefore be expected that succinate and α-glycerophosphate, and hence indirectly ethanol, would influence the β-hydroxybutyrate/acetoacetate ratio by the same mechanism.

1. Forsander, O., Räihä, N. and Suomalainen, H. *Z. physiol. Chem.* **312** (1958) 243.
2. Smith, M. E. and Newman, H. W. *J. Biol. Chem.* **234** (1959) 1544.
3. Büttner, H. *Deut. Arch. Klin. Med.* **207** (1961) 1.
4. Lundquist, F., Tygsstrup, N., Winkler, K., Mellemgaard, K. and Munch-Petersen, S. *J. Clin. Invest.* **41** (1962) 955.
5. Himwich, H. E., Nahum, L. H., Rakieten, N., Fazikas, J. F., Du Bois, D. and Gildea, E. F. *J. Am. Med. Assoc.* **100** (1933) 651.
6. Seligson, D., Stone, H. E. and Nemir, P., Jr. *Surgical Forum* **9** (1959) 85.
7. Schimassek, H. *Life Sciences* **1** (1962) 635.
8. Forsander, O., Räihä, N., Salaspuro, M. and Mäenpää, P. *Biochem. J.* **94** (1965) 259.
9. Schimassek, H. *Biochem. Z.* **336** (1963) 468.
10. Hohorst, H. J. *Thesis*, Univ. Marburg 1960.
11. Klingenberg, M. and v. Häfen, H. *Biochem. Z.* **337** (1963) 120.
12. Nyberg, A., Schuberth, J. and Änggård, L. *Acta Chem. Scand.* **7** (1953) 1170.
13. Glenn, J. L. and Vanko, M. *Arch. Biochem. Biophys.* **82** (1959) 145.
14. Lundquist, F., Fugmann, U., Rasmussen, H. and Svendsen, I. *Biochem. J.* **84** (1962) 281.
15. Krebs, H. A., Eggleston, L. V. and D'Alessandro, A. *Biochem. J.* **79** (1961) 537.
16. Ringler, R. L. and Singer, T. P. *J. Biol. Chem.* **234** (1959) 2211.

Received August 19, 1965.