The Galactose Utilization Rate in Liver Slices from Man and Rat and Its Relation to the Lactate/Pyruvate Ratio of the Medium

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The effect on the galactose utilization rate of changes in the lactate/pyruvate concentration ratio produced by addition of lactate, pyruvate, and ethanol was studied in liver slices from rat and man. The galactose utilization rate of human liver slices was twice as great as that of rat liver slices. Addition of pyruvate increased the utilization rate, lactate caused no significant change, and ethanol decreased it to about 20 per cent. Combinations of the additions caused intermediate changes, irrespective of the resulting lactate/pyruvate ratio. Evidence for a dissociation between cytoplasmic NADH/NAD ratio and lactate/pyruvate concentration ratio in the medium is produced.

Key-words: Ethanol metabolism; galactose elimination; lactate; liver slices; pyruvate

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Galactose utilization is decreased by ethanol both in vitro (9) and in vivo (13, 14, 15). According to Isselbacher & McCarthy (8) this decrease is due to inhibition of the epimerase reaction caused by the increased cytoplasmic $NADH_2/NAD$ ratio during ethanol oxidation. The cytoplasmic $NADH_2/NAD$ ratio cannot be determined directly but it is assumed to be in equilibrium with the lactate/pyruvate ratio (7, 10). This raises the question whether the galactose utilization rate is correlated to the lactate/pyruvate ratio and whether other substances which change this ratio also affect the galactose utilization rate. In the present work the effect of added pyruvate and lactate, alone and in combination with ethanol, on galactose utilization of liver slices was studied in relation to the lactate/pyruvate ratio in the medium. The results obtained in experiments with human and rat liver were compared.

METHODS

Samples from normal human liver tissue obtained during abdominal surgery in halothane-O_2-N_2O anesthesia were immediately cut into slices (0.3 mm thick) with a McIlwain-Buddle tissue chopper and incubated for 60 minutes in 10 ml phosphate buffer at pH 7.4 (12) under aerobic conditions at 37 °C. Each flask contained 0.1-0.3 g of liver. The medium contained 1.7 mM galactose but no glucose. Samples of the incubation medium were removed prior to and after incubation and deproteinized by the addition of $HClO_4$; the galactose content was determined enzymatically (5) and

used for the calculation of the utilization rate. Control flasks without liver slices showed a small increase in galactose concentration due to the evaporation of water, and a correction for this was applied. The deproteinized incubation medium was also used for the determination of lactate (6) and pyruvate (1).

The same liver was used in parallel assays in which ethanol (10 mM), L-lactate (20 mM), pyruvate (20 mM), and both ethanol and lactate or pyruvate were added to the incubation medium.

In experiments with rat liver, fed Wistar rats weighing about 200 g were used. The rats were decapitated and the liver was rapidly removed, sliced, and incubated in the same way as samples from human liver. Rat liver slices were also incubated with acetaldehyde (10 mM).

RESULTS

The galactose utilization rate by the slices is shown in Table I. In human liver tissue ethanol decreases the galactose utilization rate to 22 per cent of the control value. Addition of L-lactate (20 mM) to the medium causes a slight, insignificant rise in the galactose utilization rate and addition of pyruvate (20 mM) gives a significant increase ($P<0.01$). Experiments with ethanol and pyruvate (20 mM). and with ethanol and L-lactate (20 mM) show a significantly higher galactose utilization rate than experiments with ethanol alone. In control experiments, and when pyruvate is present in the medium, the rate of galactose utilization is almost constant throughout the incubation period (Fig. 1).

In rat liver the galactose uptake is about half of that of human liver under all experimental conditions which have been examined. Acetaldehyde, only examined in rat liver slices, inhibits galactose uptake almost completely.

When lactate present in the liver tissue at the beginning of the experiments (mean 6.19 ± 0.85 mmoles per g tissue wet weight, n = 4) is subtracted, only a small amount of

Table I. Metabolism of galactose, lactate and pyruvate in liver slices of man and rat incubated for 60 min

Medium	n	Galactose uptake μmol/g	Lactate output μmol/g	Pyruvate output μmol/g	Lactate concentration mM	Pyruvate concentration mM
			Human liver tissue			
Control	11	5.64 ± 0.41	11.9 ± 1.2	0.16 ± 0.04	0.33 ± 0.04	0.004 ± 0.0009
Ethanol (10 mM)	11	1.25 ± 0.14	14.1 ± 1.2	0.08 ± 0.02	0.39 ± 0.06	0.002 ± 0.0005
Lactate (20 mM)	6	6.11 ± 0.35	5.4 ± 5.1	6.8 ± 1.3	21.5 ± 0.3	0.148 ± 0.013
Pyruvate (20 mM)	5	8.38 ± 0.83	33.2 ± 2.1	−68 ± 22	1.11 ± 0.19	17.6 ± 0.8
Ethanol (10 mM) Lactate (20 mM)	6	1.93 ± 0.24	− 6.5 ± 6.9	1.4 ± 1.3	21.8 ± 0.4	0.031 ± 0.005
Ethanol (10 mM) Pyruvate (20 mM)	5	4.46 ± 0.78	59.4 ± 5.1	−78 ± 13	2.09 ± 0.44	17.1 ± 0.7
			Rat liver tissue			
Control	16	2.47 ± 0.16	15.8 ± 0.7	0.56 ± 0.04	0.78 ± 0.05	0.028 ± 0.002
Ethanol (10 mM)	16	0.82 ± 0.11	15.2 ± 0.7	0.09 ± 0.03	0.74 ± 0.05	0.005 ± 0.002
Lactate (20 mM)	6	2.47 ± 0.24	3.2 ± 4.8	4.6 ± 0.5	21.0 ± 0.7	0.244 ± 0.040
Pyruvate (20 mM)	6	3.74 ± 0.41	55.6 ± 4.7	−141 ± 16	2.39 ± 0.30	12.8 ± 1.1
Ethanol (10 mM) Lactate (20 mM)	6	1.10 ± 0.14	− 0.9 ± 2.2	1.2 ± 0.2	21.1 ± 0.6	0.052 ± 0.011
Ethanol (10 mM) Pyruvate (10 mM)	6	1.95 ± 0.33	77.0 ± 6.0	−149 ± 18	3.50 ± 0.41	12.2 ± 1.1
Acetaldehyde (10 mM)	4	0.23 ± 0.20	16.3 ± 2.6	0.08 ± 0.01	0.96 ± 0.16	0.005 ± 0.0006

The values are given as mean ± S.E.M.

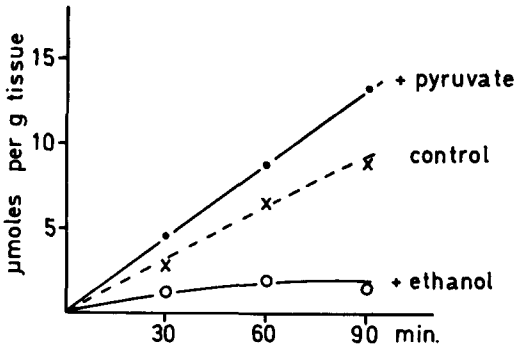

Fig. 1. Time course of galactose uptake by human liver slices. Incubated in phosphate medium pH 7.4 containing 1.7 mM galactose at 37° C. (x - - - - - X) control, (○——○) plus 10 mM ethanol, and (●——●) plus 20 mM pyruvate.

lactate is formed during the incubation period in human liver slices. Lactate release is increased about three times over the control value by addition of pyruvate (20 mM) and about four times by adding pyruvate (20 mM) together with ethanol (10 mM). No release of lactate is observed if lactate alone (20 mM) is present in the medium, and an uptake of lactate occurs when the incubation medium contains both lactate (20 mM) and ethanol (10 mM).

As shown in Table I, the production of pyruvate is strongly increased by lactate. The production is lower when lactate is added together with ethanol but still considerably above the control value. Ethanol in the medium almost eliminates pyruvate production, if the pyruvate present in the tissue at the beginning of the experiments (mean 0.03 ± 0.05 mmoles per g tissue wet weight, n = 4) is taken into consideration. There is a considerable uptake of pyruvate when pyruvate is present in the medium. This uptake is not influenced by the addition of ethanol.

In rat liver the same pattern is found as in human liver regarding lactate and pyruvate metabolism under the different experimental conditions, except for a greater lactate output and pyruvate uptake after the addition of pyruvate.

DISCUSSION

An inhibition of the epimerase reaction by $NADH_2$ (8) will cause an accumulation of galactose-1-phosphate which in turn will inhibit the galactokinase (3) and thus the galactose utilization rate. Since the UDP-galactose-4-epimerase is located in the cytosol of the liver cell, the utilization rate of galactose may be a function of the $NADH_2/NAD$ ratio, as suggested by several workers (4, 12). Furthermore lactate dehydrogenase is claimed to establish equilibria between its substrates and the free $NADH_2/NAD$ in the cytoplasm (7, 10). Thus the galactose elimination rate and the lactate/pyruvate concentration ratio should both reflect the magnitude of the $NADH_2/NAD$ ratio. Even if ethanol reduces the galactose utilization rate and increases the L/P ratio in all experiments, no constant relation between the lactate/pyruvate ratio and the galactose utilization rate is found (Fig. 2).

One explanation of this lack of correlation may be that the lactate/pyruvate ratio of the medium fails to reflect the $NADH_2/NAD$ ratio of the cytosol when lactate or pyruvate is added. Chance (2) has stressed that the L/P ratio is quite useful for detecting changes in the cytoplasmic redox ratio, but its correlation with the $NADH_2/NAD$ ratio depends on all the elements involved in the equilibrium and this information is not readily available in a biochemical system.

The experiments with acetalehyde confirm that the inhibition of galactose uptake is not specifically associated with the action of alcoholdehydrogenase.

The utilization of pyruvate, both by gluconeogenesis and by carboxylation to oxaloacetate, will increase the formation of ADP which may accelerate $NADH_2$ oxidation through the respiratory chain. This may account for the increased utilization of galactose following the addition of pyruvate. Furthermore the addition of pyruvate will increase the NAD formation. The addition of lactate will not influence the net formation of NAD or NADH. This may explain the difference between galactose utilization in experiments with the addition of

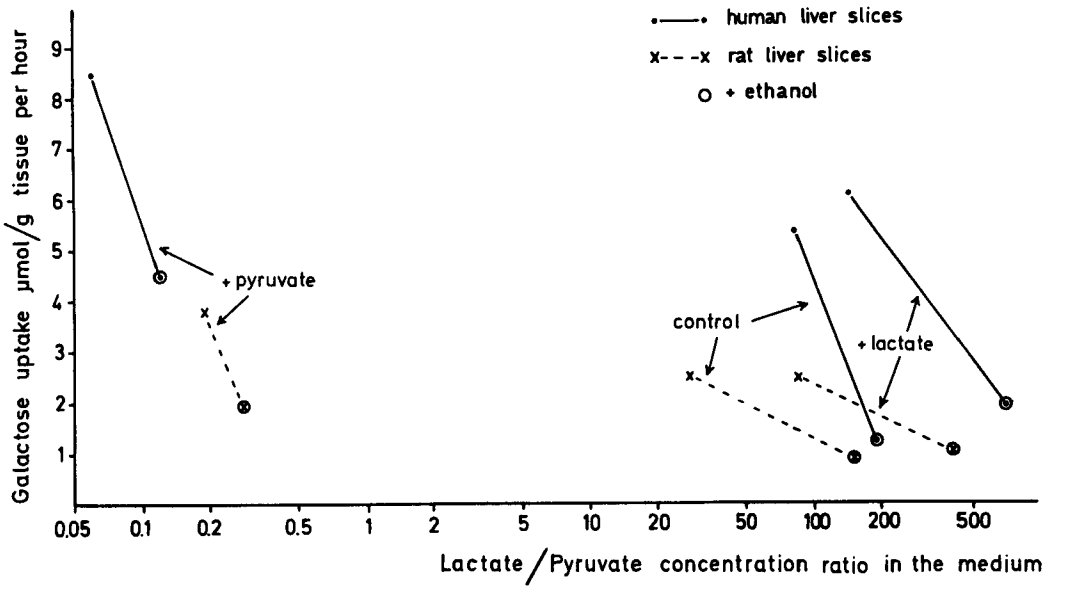

Fig. 2. Galactose uptake by liver slices versus lactate/pyruvate concentration ratio in the medium. The concentration of lactate and pyruvate was determined after the liver slices were incubated in phosphate medium pH 7.4 at 37° C for 1 h. The results of experiments with added ethanol (10 mM) are circled and connected with corresponding experiments on the same tissue by unbroken (human liver) or broken (rat liver) lines.

pyruvate, and in those with the addition of lactate

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REFERENCES

1. Bücher, T., Czok, R., Lamprecht, W. & Latzko, E. Pyruvate. pp. 253-259 in Bergmeyer, H.-U. (ed.) *Methoden der enzymatischen Analyse.* Verlag Chemie, Weinheim/Bergstr., 1963.
2. Chance, B. General discussion with Krebs, H. A. The redox state of nicotinamide adenine dinucleotide in the cytoplasm and mitochondria of rat liver. pp. 435-436 in Weber, G. (ed.) *Advances in Enzyme Regulation.* Pergamon Press, 1967.
3. Cuatrecasas, P. & Segal, S. Mammalian galactokinase. *J. biol. Chem. 240,* 2382, 1965.
4. Forsander, O. A. The galactose tolerance test as a measurement of the redox potential of the liver. *Scand. J. clin. Lab. Invest. 18,* Suppl. 92, 143, 1966.
5. Hjelm, M. & deVerdier, C.-H. A methodological study of the enzymatic determination of glucose in blood. *Scand. J. clin. Lab. Invest. 15,* 415, 1963.
6. Hohorst, H. J. L-(+)-Lactat, Bestimmung mit Lactat-Dehydrogenase und DPN. pp. 266-270 in Bergmeyer, H.-U. (ed.) *Methoden der enzymatischen Analyse.* Verlag Chemie, Weinheim/Bergstr., 1963.
7. Hohorst, H. J., Kreutz, F. H. & Bücher, T. Über Metabolitgehalte und Metabolit-Konzentrationen in der Leber der Ratte. *Biochem. Z. 332,* 18, 1959.
8. Isselbacher, K. J. & Krane, S. M. Studies on the mechanism of the inhibition of galactose oxidation by ethanol. *J. biol. Chem. 236,* 2394, 1961.
9. Isselbacher, K. J. & McCarthy, E. A. The influence of pyridine nucleotides on galactose-1-C^{14} oxidation to $C^{14}O_2$. *Biochem. biophys. Res. Commun. 1,* 49, 1959.
10. Krebs, H. A. & Veech, R. L. Regulation of the redox state of the pyridine nucleotides in rat liver. pp. 413-438 in Sund, Horst (ed.) *Pyridine Nucleotide Dependent Dehydrogenases.* Springer Verlag, Berlin, Heidelberg, New York, 1970.

11. Robinson, J. R. Some effects of glucose and calcium upon metabolism of kidney slices from adult and newborn rats. *Biochem. J. 45,* 68, 1949.
12. Salaspuro, M. P. Ethanol inhibition of galactose oxidation as related to the redox state of the fatty liver. *Scand. J. clin. Lab. Invest. 18,* Suppl. 92, 145, 1966.
13. Stenstam, T. Effect of ethyl alcohol on galactose metabolism. *Acta med. scand.,* Suppl. 177, 89, 1946.
14. Tygstrup, N. & Lundquist, F. The effect of ethanol on galactose elimination in man. *J. Lab. clin. Med. 59,* 102, 1962.
15. Wagner, F. Klinische Untersuchungen über die Bedeutung der verschiedenen Zuckerproben für die Beurteilung der Leberfunktion. *Z. Klin. Med. 80,* 174, 1914.

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