SUPPRESSION BY ETHANOL OF ACETATE AND HEXANOATE OXIDATION STUDIED BY NONRECIRCULATING LIVER PERFUSION

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SUMMARY

The relationship between the effect of ethanol on the redox state and the tricarboxylic acid cycle has been studied by perfusion of hemoglobin-free medium through the liver without recirculation. The production of $^{14}CO_2$ from 1-^{14}C-acetate was suppressed 63 % by ethanol and that from 1-^{14}C-hexanoate by 54 % in normal livers. These suppressions were much weaker in livers from hyperthyroid rats and stronger in those from hypothyroid rats. Conditions where the ethanol-induced elevation of the lactate/pyruvate ratio was decreased led to less suppression of substrate oxidation by ethanol.

The oxidation of ethanol in the liver is known to lead to impairment of the activity of the citric acid cycle (1-3). This effect is generally believed to depend in some way on the shift towards reduction of both the extra- and the intramitochondrial redox state caused by ethanol (1-4), but the exact mechanism of action is still an open question. Since previous studies had shown that the ethanol-induced redox change is diminished in hyperthyroid and increased in hypothyroid rat livers (2,4,5), the possibility of a correlation between the suppression of the tricarboxylic acid cycle by ethanol and the redox change was investigated. In order to be able to follow the immediate and direct effect of ethanol on the production of CO_2 from different substrates, the nonrecirculating perfusion technique previously used by Exton and Park (6) was employed. This method also made it possible to follow the rate of ethanol removal and acetaldehyde output continuously, and to observe on the same liver the metabolic effects of alterations in the influent medium, which modified the ethanol-induced shift of the redox state.

MATERIAL AND METHODS

Male albino rats of the Wistar strain (300-400 g) were used. The animals were fed an ordinary laboratory diet and were fasted overnight before use. Induction of hyper- and hypothyroid states was performed by administration of triiodothyronine and propylthiouracil, respectively, as described previously (4).

The perfusion technique was modified from that described by Scholz and Bücher (7). The portal vein was catheterized and perfusion fluid immediately pumped through the liver in situ in order to avoid anoxia. The perfusion medium passed through the liver at a rate of 60 ml/min and consisted of Krebs-Ringer bicarbonate solution equilibrated with 95 % O_2/5 % CO_2 at 37°C and was supplemented with 11 mM glucose, 1.3 mM L-lactate and 0.1 mM pyruvate, if not otherwise stated. The liver was enclosed in a plastic envelope to prevent any evaporation of CO_2, ethanol or acetaldehyde. Samples were collected in tubes kept at 0°C from the medium entering or leaving the liver. The oxygen consumption was measured with a Clark type oxygen electrode (Radiometer), which registered the oxygen tension of the effluent medium.

$^{14}CO_2$ was determined from 15 ml samples after acidification and absorption of the liberated CO_2 in hyamine; the radioactivity of the solution was measured in an EKCO liquid scintillation counter. Lactate and pyruvate were assayed enzymatically (8) without deproteinization of the medium.

RESULTS AND DISCUSSION

Physiological levels of lactate (1.3 mM) and pyruvate (0.1 mM) were added to the medium in order to facilitate the registration of redox changes induced by the liver. It was found that although the fluid passed only once through the liver, the lactate/pyruvate ratio was maintained at

9-10 even when moderate alterations were made in the lactate or pyruvate concentration of the influent medium. The oxygen consumption and the rate of oxidation of acetate or hexanoate was also found to be constant as long as no alterations were made in the entering medium (Fig. 1). Metabolic changes induced by ethanol or other metabolites could be followed continuously and quantitatively by this method. New steady state output levels of metabolites were attained 2-3 minutes after such an addition (Fig. 1).

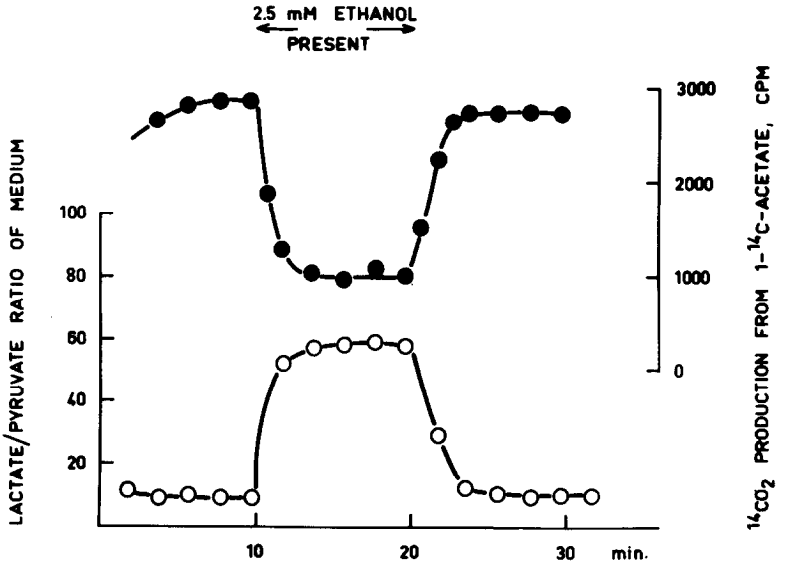

Fig. 1. Demonstration of the rapid adjustment of the steady state levels of the lactate/pyruvate ratios (O-O-O) and the rate of $^{14}CO_2$ formation from ^{14}C-acetate (●-●-●) in the presence of ethanol in nonrecirculating perfusion of rat liver. Samples were taken from the medium leaving the liver and assayed for its content of $^{14}CO_2$, lactate and pyruvate.

Addition of ethanol (2.5 mM) led to elevation of the lactate/pyruvate ratio from about 10 to 50-60. This corresponds to 0.45 µmol pyruvate reduced/g liver wet wt./min. In the presence of hexanoate the lactate/pyruvate ratio averaged 33 in the effluent medium. A similar elevation has been found during enhanced fatty acid oxidation in recirculating perfusion (3,9). This indicates that the transfer of reducing equivalents from the mitochondria to the cytoplasm must be

rapid enough to maintain a normal redox equilibrium in this perfusion system also. The subsequent addition of ethanol further increased the lactate/pyruvate ratio to about 80.

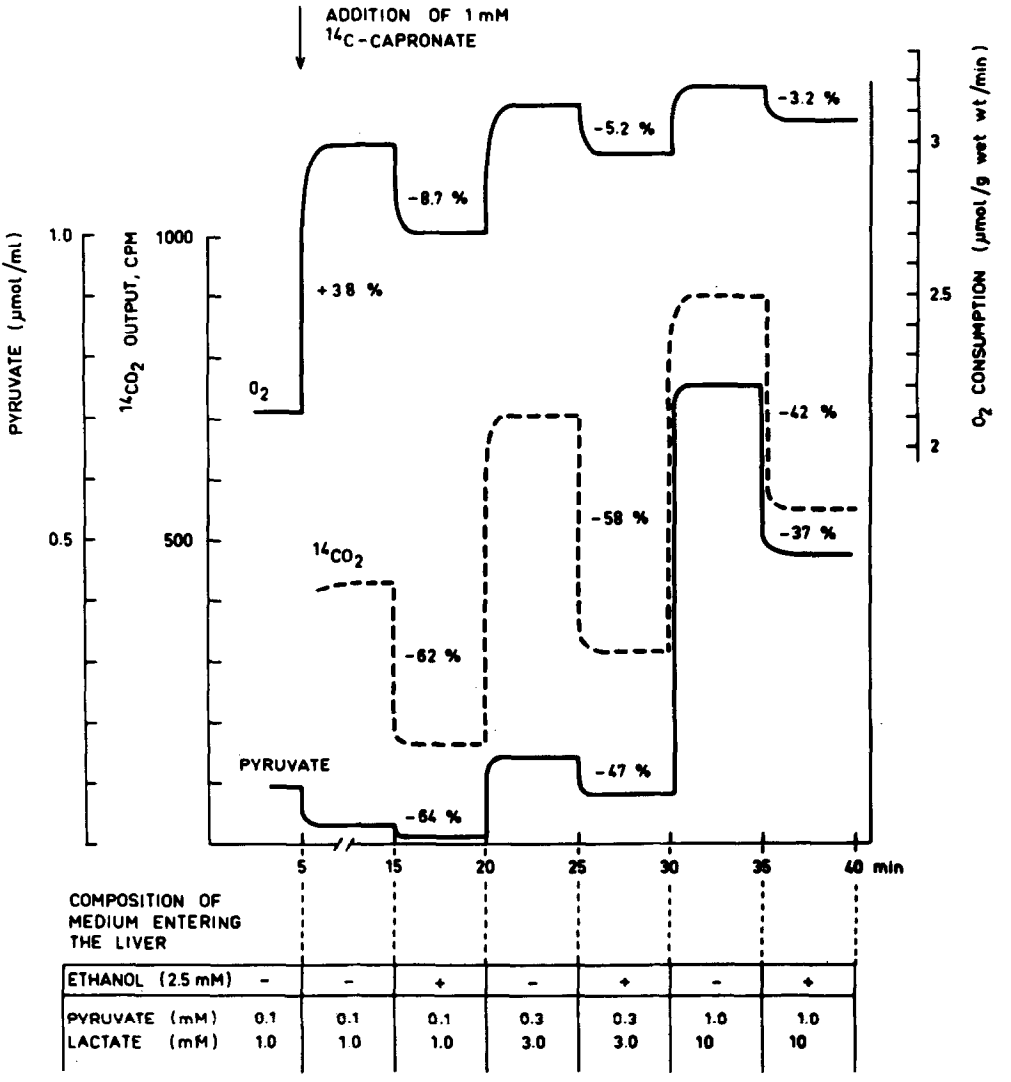

Fig. 2. Oxygen consumption, production of CO_2 from capronate and levels of pyruvate as influenced by ethanol in livers perfused once through with medium supplemented with three different levels of lactate and pyruvate. Lactate and pyruvate were added in the same proportion in all instances so that the lactate/pyruvate ratio of the medium entering the liver was kept at 10. The levels of pyruvate are those of the medium leaving the liver. The presentation is slightly schematic for the sake of clarity. It is based on the mean values of three perfusions with identical patterns of directional changes. The numbers indicate the suppression induced by ethanol.

The average oxygen consumption of livers from rats fasted overnight and perfused in standard conditions was 2.0 µmoles/g wet wt./min. Hyperthyroid livers showed increased rates and hypothyroid livers decreased rates of oxygen consumption. The addition of 1 mM hexanoate stimulated oxygen consumption by almost 40 % (Fig. 2). When the pyruvate level was elevated to 0.5 mM the oxygen consumption also increased, in both the presence and absence of hexanoate (Fig. 2). Addition of ethanol (2.5 mM) regularly led to a small decrease in oxygen consumption (Fig. 2). Most of the previous investigations indicate either no influence or a slightly stimulating effect by ethanol on hepatic oxygen consumption (1,2,3,10).

The production of $^{14}CO_2$ from 1-^{14}C- acetate (5 mM, specific activity 0.5 µCi/mmol) was suppressed 63 % by ethanol (Table 1). The isotope dilution due to ethanol-derived acetate is negligible under these

Table 1. Suppression by ethanol of production of carbon dioxide from 1-^{14}C-acetate (5 mM; specific activity 0.5 nCi/µmol) and 1-^{14}C-hexanoate (1 mM; specific activity 5 nCi/µmol) during nonrecirculating perfusion of livers from hyper-, hypo-, and euthyroid rats. Mean values ± S.D. of 3-5 perfusions are given.

	Liberation of $^{14}CO_2$ (cpm/g liver/min.)					
Substrate	Acetate		Suppression by ethanol	Hexanoate		Suppression by ethanol
Presence of ethanol	-	+	%	-	+	%
Type of liver						
Hyperthyroid	84±41	72±25	- 14	228±89	145±73	- 35
Euthyroid	101±28	37±14	- 63	195±33	89±33	- 54
Hypothyroid	71±20	13±3	- 81	224±53	86±24	- 62

conditions. The corresponding suppression by ethanol of the oxidation of 1-^{14}C-hexanoate (1 mM, specific activity 5 μCi/mmol) was 54 % (Table 1).

In livers from hyperthyroid rats the ethanol- induced suppression of the oxidation of both acetate and hexanoate was much weaker and in hypothyroid livers stronger than in normal livers (Table 1). In view of the previous findings that the ethanol-induced shift towards reduction of both extra- and intramitochondrial redox pairs was diminished in hyperthyroid but enhanced in hypothyroid rats (2,4,5), these results afford further evidence of a relation between the redox state and the flux through the citric acid cycle.

A different approach to the study of the relation between the redox state and the citric acid cycle was made by introducing different levels of lactate and pyruvate but keeping their ratio constant (Fig. 2). At higher levels, the ethanol-induced redox change is diminished and at the same time a weaker suppression of the oxidation of hexanoate is observed. A relation between the decreases caused by ethanol in the pyruvate levels and in the $^{14}CO_2$ production rates can be seen (Fig. 2). Also, the fluctuations in oxygen consumption seem to bear a relation to the pyruvate levels. It is suggested that this relation reflects the activating effect of pyruvate on the tricarboxylate cycle by providing oxaloacetate, since simultaneously the oxidation of capronate is stimulated by pyruvate. From the differences in the levels of metabolites in the fluid entering and leaving, it was calculated that at the highest pyruvate level employed in this experiment (1 mM) addition of ethanol leads to a reduction of pyruvate at the rate of 1.8 μmol/g/min. The high pyruvate level increased the rate of ethanol elimination and the output of acetaldehyde (unpublished results). It is therefore anticipated that part of the pyruvate disappearance is due to interaction between the

alcohol dehydrogenase and lactate dehydrogenase systems, the $NADH_2$ formed during the oxidation of ethanol being rapidly reoxidized when pyruvate is reduced to lactate.

REFERENCES

1. Forsander, O.A., Räihä, N., Salaspuro, M., and Mäenpää, P., Biochem.J. 94, 259 (1965).
2. Ylikahri, R.H., Mäenpää, P.H., and Hassinen, I.E., Ann.Med.Exp. Biol.Fenn. 46, 137 (1968).
3. Williamson, J.R., Scholz, R., Browning, E.T., Thurman, R.G., and Fukami, M.H., J.Biol.Chem. 244, 5044 (1969).
4. Lindros, K.O., Eur.J.Biochem. 13, 111 (1970).
5. Rawat, A.K. and Lundqvist, F., Eur.J.Biochem. 5, 13 (1968).
6. Exton, J.H. and Park, C.R., J.Biol.Chem. 242, 2622 (1967).
7. Scholz, R. and Bücher, Th., in Control of Energy Metabolism, ed. by B. Chance, R.W. Estabrook and J.R. Williamson. Academic Press, New York 1965 p. 393.
8. Hohorst, H.J., Kreutz, F.H., and Bücher, Th., Biochem.Z. 332, 18 (1959).
9. Williamson, J.R., Kreisberg, R.A., and Felts, P.W., Proc.Nat. Acad.Sci. 56, 247 (1966).
10. Scholz, R., in Stoffwechsel der isoliert perfundierten Leber, hrsgg. W. von Staib und R. Scholz. Springer Verlag, Berlin 1968 p. 25.