Influence of the Metabolism of Ethanol on the Lactate/Pyruvate Ratio of Rat-Liver Slices

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1. The influence of ethanol on the redox level of the redox pair lactate/pyruvate has been studied in experiments with rat-liver slices. 2. Ethanol had no effect on oxygen consumption but strongly depressed carbon dioxide formation. On the assumption that ethanol is oxidized to acetate in the liver slices, it could be calculated that most of the oxygen that disappeared was consumed in this reaction. 3. Addition of ethanol to the incubation medium increased the lactate/pyruvate ratio and when all the ethanol had been oxidized the redox value decreased to the normal again. Ethanol depressed the pyruvate concentration, whereas the lactate concentration was not much influenced. 4. Acetaldehyde in the concentrations present during ethanol oxidation did not influence the lactate/pyruvate ratio. Higher concentrations, however, increased the redox state. 5. Acetate in the concentrations present during ethanol oxidation in the experiments, and also in higher concentrations, did not influence the lactate/pyruvate ratio. 6. The mechanism by which ethanol influences the lactate/pyruvate ratio is discussed.

Ethanol has no appreciable effect on the oxygen consumption of man (Lundquist, Tygstrup, Winkler, Mellemgaard & Munck-Petersen, 1962b) or of the perfused rat liver (Forsander, Riih, Salaspuro & Menp, 1965). The redox state of the liver is shifted towards a more reduced state, however, when ethanol is oxidized. It has been shown that the ratio of the substrate pairs lactate/pyruvate and β -hydroxybutyrate/acetoacetate in the blood increases during ethanol oxidation. This takes place both in man (Bttner, 1961; Lundquist *et al.* 1962b) and in the perfused rat liver (Forsander *et al.* 1965). It has also been found that the ratio between the reduced and oxidized NAD of the rat liver changes towards a more reduced state when ethanol is oxidized (Smith & Newman, 1959; Riih & Oura, 1962).

Ethanol is broken down in the liver in two stages, first by oxidation of ethanol to acetaldehyde and then by further oxidation of this compound to acetate. These two reactions seem to be catalysed only by NAD-dependent dehydrogenases (Lundquist, Fugmann, Rasmussen & Svendsen, 1962a). The concentration of acetaldehyde in the blood is normally low but rises slightly during ethanol oxidation (Bttner, 1961). In the intact organism the acetate formed from ethanol is carried away from the liver by the blood stream and oxidized further extrahepatically (Forsander, Riih & Suomalainen, 1960; Lundquist *et al.* 1962b). In experiments with perfused liver, liver slices or sus-

pensions, however, acetate accumulates in the medium and only a small part is broken down to carbon dioxide (Leloir & Munoz, 1938; Forsander *et al.* 1960; Lundquist *et al.* 1962b). The present investigation is a study of whether the influence on the lactate/pyruvate redox value during ethanol oxidation is produced by ethanol or by its breakdown products acetaldehyde or acetate.

MATERIAL AND METHODS

Albino rats of Wistar origin and of both sexes, weighing between 200 and 300 g., were used for the experiments. Before the investigation the animals were kept on an ordinary laboratory diet available *ad lib*. The rats were killed by decapitation and bled, and the livers quickly removed and chilled in ice-cold saline. Liver slices were prepared with a Stadie-Riggs hand microtome.

The oxygen consumption and carbon dioxide production of the liver slices were measured in a Warburg apparatus by the technique of F. Dickens and F. Simer (Dixon, 1951). Krebs-Ringer bicarbonate buffer was used as medium (Umbreit, Burris & Stauffer, 1964). The respiratory quotient (R.Q.) and the total amounts of acids formed were calculated from the results of the experiments. The flasks containing the medium and liver slices (9:1, unless otherwise stated) were filled with oxygen-carbon dioxide (95:5) and shaken mechanically at 37. The ethanol content of the medium was estimated enzymically by the method described by Bcher & Redetzki (1951). Lactate and pyruvate were determined enzymically with test kits supplied by C. F. Boehringer und Soehne G.m.b.H., Mannheim.

The reagents used were all analytically pure grade and

Table 1. *Oxygen consumption, carbon dioxide formation, respiratory quotient, acid production and ethanol oxidation of rat-liver slices*

The slices (200 mg.) were incubated in 2 ml. of Krebs–Ringer bicarbonate buffer for 60 min. at 37°. Experimental details are given in the text. Ethanol oxidation was analysed in different experiments, with identical incubation conditions. Ethanol to a final concentration of 17 mM was added to the flasks indicated. Results are given as the mean $\pm$ S.E.M. with the number of observations in parentheses.

	O ₂ (μ moles/g. wet wt./min.)	CO ₂ (μ moles/g. wet wt./min.)	R.Q.	Acid formation (μ moles/g. wet wt./min.)	Ethanol oxidation
Without ethanol	-1.64 ± 0.08 (16)	1.21 ± 0.07 (16)	0.74	0.51 ± 0.02 (16)	—
With ethanol	-1.63 ± 0.12 (16)	0.03 ± 0.08 (16)	0.02	1.37 ± 0.07 (16)	1.38 ± 0.02 (24)

the acetaldehyde was freshly distilled before use. The acetate used was as a sodium salt.

RESULTS

Ethanol had no effect on the oxygen consumption of the liver slices (Table 1). The carbon dioxide production was greatly depressed, however, by addition of ethanol to the medium. The R.Q. then decreased from the normal value of 0.74 to 0.02. As was to be expected, the production of acids was much greater in the experiments in which ethanol had been added to the medium than in the control experiments owing, presumably, to acetic acid production. The oxidation of ethanol was of the same order as the oxygen consumption.

During the preparation of the liver slices the tissue was in an anoxic state and 15 min. after incubation of the liver slices the lactate/pyruvate ratios were between 27 and 34 (Fig. 1). At this time ethanol was added to every second incubation flask and this produced a great increase in the ratio of this substrate pair. In the control flasks without addition of ethanol the ratio after 60 min. incubation was 12 and stayed at this value until the end of the experiment. After 105 min. incubation most of the ethanol added to the experimental flasks had disappeared and the lactate/pyruvate ratio had decreased, and after 150 min. was almost the same as in the control experiments. The lactate content of the medium increased at the beginning of the experiment but after 60 min. incubation reached a rather steady value (Table 2). The concentration of lactate and of pyruvate rose to about the same value as that given by Hohorst, Kreutz & Bücher (1959) for the intact rat liver. The shift of the lactate/pyruvate ratio with ethanol was mainly due to a decrease in the concentration of pyruvate. The lactate was not so much influenced. In this experiment ethanol had a slight effect on the lactate–pyruvate pool but this effect seems to depend on the nutritional condition of the liver. In a 'fasted' liver ethanol increases the pool (Forsander *et al.* 1965).

Fig. 1. Lactate/pyruvate ratio and ethanol concentration of the medium at different times during incubation of 2.0 g. of liver slices at 37° in 25 ml. of Krebs–Ringer bicarbonate buffer containing 11 mM-glucose. Ethanol (0.17 M-mole) was added at 15 min. to one series of flasks. Experiments ○, without ethanol, Δ, with ethanol (●, ethanol concentration).

Acetaldehyde is reported to be oxidized in the rat liver at a rate of about 66μ moles/g. of liver/hr. (Lundquist *et al.* 1962a). With optimum substrate concentration this would mean that the acetaldehyde concentration in the experiment described in Table 3 would decrease by 3.3 mM. The concentration of 1.78 mM used in one of the experiments should therefore decrease to about zero during the experiment. This concentration, however, increased the lactate/pyruvate ratio somewhat and higher concentrations increased the ratio further. The acetaldehyde had a greater effect on the pyruvate than on the lactate concentrations. With an acetaldehyde concentration of 17.8 mM a big increase in the ratio was observed. The total lactate–pyruvate pool was decreased by acetaldehyde.

Acetate in concentrations between 1.0 and 10.0 mM had no appreciable effect on the lactate/pyruvate ratio (Table 4). With the higher acetate

Table 2. *Lactate and pyruvate concentrations of the medium at different times*

Liver slices (2.0g.) were incubated in 25 ml. of Krebs-Ringer bicarbonate buffer at 37°, containing 11 μ moles of glucose/ml. Ethanol (0.17 m-mole) was added to one series of flasks after 15 min. Values given are means $\pm$ s.e.m. for three experiments.

Sampling time (min.).....	15	60	105	150
Lactate (μ moles/ml. of incubation mixture)				
without ethanol	0.586 $\pm$ 0.050	1.300 $\pm$ 0.044	1.694 $\pm$ 0.228	1.762 $\pm$ 0.210
with ethanol	0.660 $\pm$ 0.088	1.196 $\pm$ 0.126	1.104 $\pm$ 0.116	1.584 $\pm$ 0.010
Pyruvate (μ mole/ml. of incubation mixture)				
without ethanol	0.022 $\pm$ 0.008	0.114 $\pm$ 0.032	0.163 $\pm$ 0.026	0.160 $\pm$ 0.017
with ethanol	0.019 $\pm$ 0.007	0.008 $\pm$ 0.004	0.026 $\pm$ 0.014	0.109 $\pm$ 0.003
Lactate + pyruvate (μ moles/ml. of incubation mixture)				
without ethanol	0.608 $\pm$ 0.044	1.414 $\pm$ 0.052	1.857 $\pm$ 0.250	1.922 $\pm$ 0.228
with ethanol	0.679 $\pm$ 0.084	1.204 $\pm$ 0.130	1.130 $\pm$ 0.104	1.693 $\pm$ 0.012

Table 3. *Influence of acetaldehyde on the lactate and pyruvate concentrations*

Liver slices (500mg.) were incubated for 60 min. at 37° in 5 ml. of Krebs-Ringer bicarbonate buffer containing 11 μ moles of glucose/ml. and different concentrations of acetaldehyde as indicated. Results are given as the means $\pm$ s.e.m. with the number of observations in parentheses.

Acetaldehyde (μ moles/ml. of incubation mixture)	Lactate (μ moles/ml. of incubation mixture)	Pyruvate (μ mole/ml. of incubation mixture)	Lactate + pyruvate (μ moles/ml. of incubation mixture)	Ratio lactate/ pyruvate
0	1.480 $\pm$ 0.100 (8)	0.106 $\pm$ 0.010 (8)	1.586 $\pm$ 0.108 (8)	13.1
1.78	1.134 $\pm$ 0.036 (3)	0.054 $\pm$ 0.018 (3)	1.188 $\pm$ 0.051 (3)	19.0
8.90	1.152 $\pm$ 0.050 (3)	0.044 $\pm$ 0.031 (3)	1.196 $\pm$ 0.018 (3)	24.0
17.8	0.978 $\pm$ 0.054 (8)	0.008 $\pm$ 0.002 (8)	0.986 $\pm$ 0.052 (8)	119.0

Table 4. *Influence of acetate on the lactate and pyruvate concentrations*

Liver slices (500mg.) were incubated for 60 min. at 37° in 5 ml. of Krebs-Ringer bicarbonate buffer containing 11 μ moles of glucose/ml. and different concentrations of acetate. Results are expressed as the means $\pm$ s.e.m. with the number of observations in parentheses.

Acetate (μ moles/ml. of incubation medium)	Lactate (μ moles/ml. of incubation medium)	Pyruvate (μ mole/ml. of incubation medium)	Lactate + pyruvate (μ moles/ml. of incubation medium)	Ratio lactate/ pyruvate
0	0.924 $\pm$ 0.156 (6)	0.087 $\pm$ 0.018 (6)	1.011 $\pm$ 0.168 (6)	10.7
1.0	1.062 $\pm$ 0.104 (6)	0.089 $\pm$ 0.008 (6)	1.151 $\pm$ 0.112 (6)	12.0
4.0	0.916 $\pm$ 0.088 (5)	0.079 $\pm$ 0.008 (5)	0.995 $\pm$ 0.091 (5)	11.6
10.0	0.796 $\pm$ 0.162 (5)	0.061 $\pm$ 0.008 (5)	0.857 $\pm$ 0.167 (5)	13.5

concentrations a slight decrease in the lactate-pyruvate pool could be observed.

DISCUSSION

The concentrations of lactate and pyruvate in the circulating blood are in equilibrium with those of the cytoplasmic compartment of the liver and the ratio between this substrate pair is the same

inside the liver and in the blood (Hohorst *et al.* 1959). In the liver cytoplasm the substrate pair is reported to be in redox equilibrium with the other NAD-dependent redox pairs (Hohorst *et al.* 1959). When rat-liver slices were incubated in the Krebs-Ringer bicarbonate buffer the lactate and pyruvate contents of the medium rose (Table 2) until a value was reached which was approximately the same as that reported for intact rat-liver tissue (Hohorst

et al. 1959). The lactate/pyruvate ratio was also identical with that for the liver. It can therefore be assumed that, at least in respect of this substrate pair, the incubation medium acts as an extended cytoplasmic compartment.

In man the arterial blood lactate/pyruvate ratio has been found to rise to about two to three times the normal value when ethanol has been consumed (Büttner, 1961). In rat-liver perfusion experiments the ratio may increase eight- to nine-fold. In the experiments with liver slices (Fig. 1) ethanol raised the ratio to about 12-fold that of the controls.

The redox level of the lactate/pyruvate substrate pair of the liver has been found to be influenced by inadequate oxygen supply (Schimassek, 1962*a,b*), by starvation and by some hormones (Hohorst, Kreutz, Reim & Hübener, 1961). Since the oxygen consumption is not depressed by ethanol, it cannot be assumed that lack of oxygen is the cause of the shift in the redox value (Table 1). The effects of starvation and of hormones can also be excluded in these experiments *in vitro*.

It has been shown that acetaldehyde inhibits the mitochondrial oxidation of certain substrates (Kiessling, 1963). If in fact it does retard the reoxidation of a reduced intermediate component carrying hydrogen from the cytoplasm to the mitochondria it is understandable that the oxidation/reduction state of the cytoplasm will change. During ethanol oxidation the concentration of acetaldehyde is very low, less than $10\mu\text{M}$ (Lundquist *et al.* 1962*b*), and therefore the aldehyde cannot be the cause of the increased redox ratio. Since the shift in the lactate/pyruvate ratio is not influenced by acetate, either, which is the end product of ethanol breakdown in the liver, the breakdown of ethanol to acetaldehyde must be the cause of the shifted redox state.

When ethanol is present in the medium the oxidation of the substrate to acetate will almost completely monopolize the oxygen consumption (Table 1). In earlier experiments Leloir & Munoz (1938) and Lundquist *et al.* (1962*b*) have found that about 75% of the oxygen consumed is utilized for the oxidation of ethanol to acetate. This means that a big shift has taken place in the metabolic pathways. Fritz (1961) has calculated that the liver utilizes 77% of the oxygen for the breakdown of fatty acids to carbon dioxide and ketone bodies, whereas the rest is consumed in several other minor oxidation reactions. The fatty acids are oxidized

in the mitochondria and hence the extramitochondrial oxygen consumption is low during normal conditions. When ethanol is oxidized, the oxidation of fatty acid is totally inhibited, which can be seen from the fact that no carbon dioxide is produced and also that ketone-body production is not appreciably influenced by ethanol (Lundquist *et al.* 1962*a*; Forsander *et al.* 1965). By contrast, the extramitochondrial oxidation increases since the alcohol dehydrogenase, which oxidizes ethanol to acetaldehyde, is mainly located in the cytoplasm of the liver cell (Nyberg, Schubert & Änggård, 1953; Rähä & Koskinen, 1964). The rate of oxidation of ethanol by alcohol dehydrogenase seems to be faster than the regulated reoxidation of NADH in the respiratory chain of the mitochondria. This would explain why the NADH/NAD⁺ ratio of the cytoplasm increases during ethanol oxidation, an increase which can also be seen in the increased lactate/pyruvate ratio.

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