Influence of some Aliphatic Alcohols on the Metabolism of Rat Liver Slices

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The influence of some aliphatic alcohols on oxygen uptake, carbon dioxide production, acid formation and lactate and pyruvate concentrations of rat liver slices was studied. At the concentrations used, none of the alcohols was found to influence oxygen uptake. Of the alcohols that are not oxidized by liver alcohol dehydrogenase, methanol increased carbon dioxide production, propan-2-ol decreased it and 2-methylpropan-2-ol was without influence. All the alcohols that are oxidized by the enzyme strongly decreased carbon dioxide production. The alcohols that are not oxidized had no marked effect on the lactate/pyruvate concentration ratio, whereas the other alcohols strongly increased the ratio. A highly significant correlation was found between the effects of the alcohol on pyruvate concentration and carbon dioxide production. It is assumed that the shift in the redox potential inhibits the function of the tricarboxylic acid cycle of the liver.

Liver alcohol dehydrogenase is a non-specific enzyme that catalyses the oxidation or reduction of a variety of alcohols or aldehydes (Winer, 1958; Merritt & Tomkins, 1959). The oxidation of ethanol in the liver is followed by dramatic changes in the metabolic pathways. This is manifested from the decrease in the respiratory quotient (Lundsgaard, 1937; Forsander, Rih, Salaspuro & Menp, 1965b) and the shift in the redox state of the liver that take place when ethanol is oxidized (Forsander, Rih & Suomalainen, 1958). Results obtained in studies with the intact organism (Bttner, 1961; Tygstrup, Winkler & Lundquist, 1965), in liver perfusion experiments (Forsander *et al.* 1965b) and with liver slices (Forsander, 1966) all show a similar pattern.

Since many aliphatic alcohols are rapidly oxidized in the liver, the present study was undertaken to investigate the extent to which these alcohols alter the liver metabolism as observed by their influence on oxygen uptake and carbon dioxide production and on the redox state of the substrate pair lactate and pyruvate.

MATERIAL AND METHODS

Albino rats of the Wistar strain and of both sexes, weighing 200–300 g., were used for the experiments. Before the investigation the animals were kept on an ordinary laboratory diet available *ad libitum*. The rats were killed by decapitation and bled, and the livers were quickly removed and chilled in ice-cold 0.9% NaCl. Liver slices were prepared with a Stadie–Riggs hand microtome.

The O₂ uptake and CO₂ production of the liver slices were measured in a standard Warburg apparatus by the method of Dickens & Simer (Dixon, 1951). Krebs–Ringer bicarbonate buffer was used as medium (Umbreit, Burris & Stauffer, 1964). The respiratory quotient (R.Q.) and the total amount of acids formed were calculated from the results of the experiments. In the experiments in which lactate and pyruvate concentrations were estimated, liver slices were incubated in flasks containing Krebs–Ringer bicarbonate buffer filled with an O₂+CO₂ (95:5) gas mixture and shaken mechanically in a water bath at 37°. After incubation the reaction was stopped by addition of ice-cold perchloric acid. The lactate and pyruvate in the medium were determined enzymically with test kits supplied by C. F. Boehringer und Soehne G.m.b.H., Mannheim, W. Germany. The reagents were all of A.R. grade.

RESULTS

During preparation of the liver slices the tissue was in an anoxic state. Since the medium contained lactate, the lactate/pyruvate concentration ratio during the early stages of incubation was high. Only after 60 min. did the ratio reach a steady-state value of about 10, which is the same as that reported for intact rat liver (Hohorst, Kreutz & Bcher, 1959). When ethanol was added to the medium the lactate/pyruvate concentration ratio began to rise and continued to do so throughout the incubation period (Fig. 1). The increase in the ratio was mainly a function of a decrease in the pyruvate concentration of the medium. The lactate concentration was not influenced. At the lowest pyruvate concentrations the analytical method used was not

Fig. 1. Lactate/pyruvate concentration ratio of the medium at different times during incubation of 1 g. of rat liver slices at 37° in 10 ml. of Krebs-Ringer bicarbonate buffer containing 11 mm-glucose and 10 mm-lactate. Ethanol (22 m-moles) was added at 20 min. to one series of flasks. ○, Without ethanol; ●, with ethanol. The points represent average values of two determinations.

sufficiently sensitive to give accurate results and therefore the lactate/pyruvate concentration ratios calculated give only a rough picture of the influence on the redox state.

Ethanol between 43 and 172 mm had no effect on the oxygen uptake of the liver slices (Table 1). Carbon dioxide production, however, was almost totally suppressed at these concentrations of ethanol. In the experiment in which the highest concentration of ethanol was used, carbon dioxide was taken up from the medium, making the respiratory quotient negative. The s.e.m. values show that rather wide variations were found in experiments with livers from different rats. There were no differences in the size of the lactate pyruvate pool at any of the concentrations of ethanol used (Table 2). Addition of ethanol to the incubation mixture, however, decreased the pyruvate concentration strongly, making the lactate/pyruvate concentration ratio high. The ratio rose with increasing ethanol concentration, but owing to inexact pyruvate analyses the increase is not significant at low pyruvate concentrations.

In the experiments in which the effects of different aliphatic alcohols were investigated it was observed

Table 1. Influence of different concentrations of ethanol on the oxygen uptake, carbon dioxide production, acid production and respiratory quotient of rat liver slices

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 was added before the start of the experiments. Results are given as means $\pm$ s.e.m. for these experiments.

Concn. of ethanol (μ moles/ml. of incubation mixture)	Uptake or production (μ moles/g. wet wt. of liver/min.)			R.Q.
	O ₂ uptake	CO ₂ production	Acid production	
0	1.97 $\pm$ 0.13	1.43 $\pm$ 0.04	0.69 $\pm$ 0.07	0.73
43	1.83 $\pm$ 0.04	0.05 $\pm$ 0.08	1.58 $\pm$ 0.14	0.03
86	2.07 $\pm$ 0.12	0.22 $\pm$ 0.09	1.59 $\pm$ 0.04	0.11
172	1.76 $\pm$ 0.19	-0.12 $\pm$ 0.04	1.63 $\pm$ 0.12	Negative

Table 2. Influence of different concentrations of ethanol on lactate and pyruvate concentrations

Liver slices (500 mg.) were incubated at 37° in Krebs-Ringer bicarbonate buffer containing 11 μ moles of glucose/ml. Ethanol was added to the medium after a preincubation period of 15 min. and analyses were made 60 min. later. Results are given as means $\pm$ s.e.m. for three experiments.

Concn. of ethanol (μ moles/ml. of incubation mixture)	Concn. (μ moles/ml. of incubation mixture)			Lactate/pyruvate ratio
	Lactate	Pyruvate	Lactate + pyruvate	
0	0.980 $\pm$ 0.038	0.123 $\pm$ 0.07	1.041	7.5
69	0.930 $\pm$ 0.028	0.008 $\pm$ 0.002	0.938	116
172	0.816 $\pm$ 0.078	0.006 $\pm$ 0.003	0.872	136
430	0.918 $\pm$ 0.030	0.006 $\pm$ 0.001	0.924	143

Table 3. *Influence of different aliphatic alcohols on oxygen uptake, carbon dioxide production, acid production and respiratory quotient of rat liver slices*

Slices (200 mg.) were incubated in 2 ml. of Krebs-Ringer bicarbonate buffer for 60 min. at 37°. A 25 μ l. sample of each alcohol was added to the medium before the start of the experiments. Results are given as means $\pm$ s.e.m. with the numbers of experiments in parentheses.

Alcohol added	Uptake or production (μ moles/g. wet wt. of liver/min.)				R.Q.
	O ₂ uptake	CO ₂ production	Acid production		
None	1.25 $\pm$ 0.08 (24)	0.940 $\pm$ 0.090 (24)	0.36 $\pm$ 0.10 (24)		0.75
Methanol	1.18 $\pm$ 0.10 (9)	1.358 $\pm$ 0.142 (9)	0.25 $\pm$ 0.10 (9)		1.15
Ethanol	1.30 $\pm$ 0.14 (6)	0.201 $\pm$ 0.082 (6)	1.02 $\pm$ 0.14 (6)		0.15
Propan-1-ol	1.34 $\pm$ 0.12 (6)	0.112 $\pm$ 0.060 (6)	1.16 $\pm$ 0.11 (6)		0.08
Propan-2-ol	1.04 $\pm$ 0.07 (7)	0.515 $\pm$ 0.104 (7)	0.46 $\pm$ 0.12 (7)		0.50
Butan-1-ol	1.30 $\pm$ 0.16 (7)	0.351 $\pm$ 0.157 (7)	0.91 $\pm$ 0.10 (7)		0.27
2-Methylbutan-1-ol	1.09 $\pm$ 0.12 (8)	0.455 $\pm$ 0.095 (8)	0.69 $\pm$ 0.14 (8)		0.42
2-Methylpropan-2-ol	1.19 $\pm$ 0.07 (6)	0.896 $\pm$ 0.179 (6)	0.43 $\pm$ 0.14 (6)		0.75
Pentan-1-ol	1.07 $\pm$ 0.12 (7)	0.381 $\pm$ 0.060 (7)	0.77 $\pm$ 0.09 (7)		0.36
3-Methylbutan-1-ol	1.10 $\pm$ 0.16 (8)	0.485 $\pm$ 0.082 (8)	0.90 $\pm$ 0.08 (8)		0.44

Table 4. *Influence of different aliphatic alcohols on lactate and pyruvate concentrations*

Liver slices (500 mg.) were incubated at 37° in Krebs-Ringer bicarbonate buffer containing 11 μ moles of glucose and 1.4 μ moles of lactate/ml. A 25 μ l. sample of each alcohol was added to the incubation mixture after a pre-incubation period of 45 min. and analyses were made 60 min. later. Results are given as means $\pm$ s.e.m. with the numbers of experiments in parentheses.

Alcohol added	Concn. (μ moles/ml. of incubation mixture)			Lactate/pyruvate ratio
	Lactate	Pyruvate	Lactate + pyruvate	
Methanol	1.10 $\pm$ 0.26 (4)	0.099 $\pm$ 0.016 (4)	1.20	11
Ethanol	0.92 $\pm$ 0.10 (4)	0.008 $\pm$ 0.002 (4)	0.93	115
Propan-1-ol	1.24 $\pm$ 0.11 (4)	0.025 $\pm$ 0.004 (4)	1.27	50
Propan-2-ol	1.38 $\pm$ 0.26 (4)	0.048 $\pm$ 0.005 (4)	1.43	29
Butan-1-ol	1.21 $\pm$ 0.27 (4)	0.017 $\pm$ 0.009 (4)	1.23	71
2-Methylbutan-1-ol	1.31 $\pm$ 0.14 (6)	0.014 $\pm$ 0.002 (6)	1.32	94
2-Methylpropan-2-ol	1.14 $\pm$ 0.22 (4)	0.079 $\pm$ 0.004 (4)	1.22	14
Pentan-1-ol	1.45 $\pm$ 0.13* (4)	0.007 $\pm$ 0.002 (4)	1.46	207
3-Methylbutan-1-ol	1.99 $\pm$ 0.22** (4)	0.025 $\pm$ 0.005 (4)	2.02	80

* $P < 0.02$ from ethanol value; ** $P < 0.005$ from ethanol value.

that they had no significant effect on oxygen uptake when added to final concentrations of 140–320 μ moles/ml. (Table 3). Many alcohols, but not all, had a strong influence on carbon dioxide production. The differences in respiratory quotients are mainly a function of differences in carbon dioxide production. In the experiments with methanol, carbon dioxide production increased, and this led to an increase in the respiratory quotient from 0.75 to 1.15. 2-Methylpropan-2-ol had no effect and all the iso-alcohols had less depressing effects than the normal alcohols. In these experiments, in which older animals were used, the respiration was lower than in the experiments described in Table 1. In all experiments except that with methanol, the production of acids was higher

when an alcohol was present in the medium than it was in the control.

Only methanol and 2-methylpropan-2-ol had no effect on the lactate/pyruvate concentration ratio. All the other alcohols tested increased the ratio strongly. The pentanols increased the size of the lactate pools to above the level obtained in the experiment with ethanol (Table 4). The size of the pool was also elevated by propan-2-ol and 2-methylpropan-1-ol.

DISCUSSION

Purified horse liver alcohol dehydrogenase oxidizes butan-1-ol faster than ethanol, propan-1-ol and pentan-1-ol (Winer, 1958; Merritt & Tomkins,

1959). The enzyme also oxidizes 2-methylpropan-1-ol and 3-methylbutan-1-ol fairly rapidly, but cannot utilize methanol, propan-2-ol or 2-methylpropan-2-ol as substrate. The rate of oxidation of the alcohols in the intact tissue may, however, be very different from that found in experiments with purified alcohol dehydrogenase (Barron & Levine, 1952).

Some investigators have observed that low concentrations of ethanol and propan-1-ol increase the oxygen uptake of liver slices and unstimulated brain slices (Fondal & Kochakian, 1951; Wallgren, 1961). Other workers have not observed such an increase (Beer & Quastel, 1958; Wartburg, Röthlisberger & Eppenberger, 1961), but all have found that these two alcohols and also other alcohols decrease the oxygen uptake when the alcohol concentration rises above a certain level. The alcohol concentrations used in the present work were lower than the values producing an inhibition, but no increases in oxygen uptake were found either.

Lundsgaard (1937) showed that the respiratory quotient of the liver decreased when ethanol was oxidized. This has since been confirmed by many other workers (cf. e.g. Majchrowicz & Quastel, 1963; Forsander *et al.* 1965b). Using liver slices, Majchrowicz & Quastel (1963) showed that carbon dioxide production was also decreased by other alcohols and that the effect increased with increasing alcohol concentrations. In the present experiments the effects of the alcohols were much stronger than in those described by Majchrowicz & Quastel (1963), and may depend on the incubation medium used. The Canadian workers used phosphate buffer, in contrast with the bicarbonate buffer used in the present experiments. The author has found (unpublished work) that the influence of ethanol on carbon dioxide production was much stronger in a bicarbonate medium than in a phosphate medium.

It has been shown that it is the first step in the breakdown of ethanol, the reaction catalysed by alcohol dehydrogenase, that produces the shift of the lactate/pyruvate concentration ratio of the liver (Forsander, 1966). Accordingly, it might be assumed that alcohols that are not oxidized by alcohol dehydrogenase would not influence the redox state. As expected, methanol, propan-2-ol and 2-methylpropan-2-ol, which are not oxidized by the enzyme, did not influence the lactate/pyruvate concentration ratio. On the other hand, the normal alcohols, which are excellent substrates for the enzyme, influenced the ratio strongly.

Fatty acids are the main substrate for the liver (Fritz, 1961) and consequently it is to be expected that the tricarboxylic acid cycle is the main carbon dioxide producer there. The influence of ethanol on carbon dioxide production can thus be related to a decreased function of this pathway. As a result of the block in the cycle the acetate produced from

Fig. 2. Pyruvate concentration plotted against CO_2 production of rat liver slices incubated in Krebs-Ringer bicarbonate buffer containing different aliphatic alcohols. Incubation temperature was 37° and time 60 min. Pyruvate concentration and CO_2 production were measured in separate experiments. The data used are taken from Tables 3 and 4.

ethanol is not oxidized further but accumulates (Leloir & Munoz, 1938). Likewise, when other aliphatic alcohols inhibit the function of the pathway the organic acids formed from the alcohols are not oxidized further but accumulate, as shown by the increase in acid production (Table 3.)

The block in the tricarboxylic acid cycle is assumed to be a consequence of the shift produced by the alcohols on the redox state of the liver. The observed lactate/pyruvate concentration ratio is a function of the redox state of the cytoplasm, and a shift in this redox state is followed by a similar shift in the NAD-dependent dehydrogenase systems of the mitochondria (Forsander, Mäenpää & Salaspuro, 1965a). This would lead to a shift in the equilibrium of the intramitochondrial oxaloacetate/malate concentration ratio in favour of malate whereby the concentration of oxaloacetate would decrease and the turnover of the citric acid cycle would diminish. It has been proposed that the same mechanism inhibits the cycle during ketosis (Wieland, Weiss & Eger-Neufeldt, 1964). If this hypothesis is correct a correlation should exist between the influence of the alcohols on the concentration of the oxidized partner of the redox pair and their influence on carbon dioxide production. Fig. 2 shows a plot of the pyruvate concentration of the liver (taken from Table 4) against carbon dioxide production (from Table 3) during incubation of the slices in a medium

containing aliphatic alcohols. The correlation between the two parameters is highly significant ($P < 0.001$).

The pharmacological effect of aliphatic alcohols on the central nervous system (Lindbohm & Wallgren, 1962) and the stabilizing effect on erythrocyte membranes (Seeman, 1966) depends on the physicochemical properties of these alcohols. No correlation can be found between these properties and the effect on the redox potential or on the carbon dioxide production of the liver.

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