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Utilization and Metabolic Effects of Acetaldehyde and Ethanol in the Perfused Rat Liver

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1. Removal of acetaldehyde and ethanol has been studied in perfused rat livers. 2. The maximum rate of ethanol oxidation was 2 μmol/min per g of liver, which was less than the calculated capacity of the ethanol-oxidizing system. The lactate/pyruvate ratio of the medium increased with the rate of ethanol removal. At low ethanol concentrations most of the acetaldehyde formed was oxidized further, but at ethanol concentrations above 16mM about 60% of the acetaldehyde left the liver unmetabolized. 3. At lower concentrations the greater part of added acetaldehyde was oxidized, but above 5mM, 50–60% of that removed was recovered as ethanol. 4. When the reduction of acetaldehyde was blocked by pyrazole, removal was strongly diminished. There was no effect on the lactate/pyruvate ratio during oxidation of low concentrations of acetaldehyde, even in the presence of pyrazole, but at higher concentrations a gradual increase occurred. 5. The results indicate that during ethanol oxidation the ethanol/acetaldehyde pair is not in redox equilibrium with the lactate/pyruvate pair. Ethanol oxidation was abolished by addition of acetaldehyde. Under these conditions the lactate/pyruvate ratio was 1.5–1.8 times the ethanol/acetaldehyde ratio, indicating equilibration of the alcohol dehydrogenase and lactate dehydrogenase systems. 6. The results support the view that ultimately the rate of mitochondrial oxidation of NADH limits the removal of ethanol in the liver.

It is now generally accepted that the shift in the redox state observed during ethanol combustion is the reason for the metabolic changes occurring in the liver during the two-step oxidation of ethanol via acetaldehyde to acetate. It is well established that the first step of this reaction, catalysed by alcohol dehydrogenase (EC 1.1.1.1), causes a shift of the redox pairs that are in equilibrium with the NAD^+/NADH ratio (Forsander *et al.*, 1965; Rawat, 1968; Krebs *et al.*, 1969; Lindros & Aro, 1969), but the metabolic effects of oxidation via the second step are not known. Büttner (1965) concluded that a cytosolic aldehyde dehydrogenase 4–5 times more active than alcohol dehydrogenase was responsible for most of the oxidation of acetaldehyde. Very low K_m values (about 10^{-6}M) for purified aldehyde dehydrogenases have been reported (Racker, 1949; Kraemer & Deitrich, 1968; Sheppard *et al.*, 1970).

These findings indicate that the oxidation of acetaldehyde is largely a cytoplasmic process and that if the rate of acetaldehyde removal exceeded its formation, the concentration of acetaldehyde during ethanol oxidation would remain low. This is not compatible with the view that ethanol and acetaldehyde form a redox couple that is in equilibrium with other NAD-coupled redox pairs, and also indicates that the concentration of acetaldehyde does not influence the rate of ethanol removal. Further, if NADH is produced in the cytoplasm in the second step too, this step must contribute as much to the shift in the redox state as the alcohol dehydrogenase-catalysed reaction.

Studies by Hedlund & Kiessling (1969) and by Hassinen *et al.* (1970) indicate, however, that a considerable part of acetaldehyde oxidation occurs in the mitochondria. In addition, rather high apparent K_m values for aldehyde oxidation have been found in crude liver homogenates (Deitrich, 1966; M. Pajari, personal communication).

In the present work we have studied the metabolism of acetaldehyde in the intact liver by using a non-recirculating perfusion system in which it is possible to measure the rate of ethanol removal and the formation and removal of acetaldehyde. The shift in the lactate/pyruvate ratio was observed at the same time. The results indicate that acetaldehyde oxidation influences the redox state of the cytoplasm to a much smaller degree than does ethanol oxidation and provide new information on the factors limiting the rate of the removal of ethanol.

Materials and Methods

Female albino rats of Wistar origin, weighing 200–300g and given a standard laboratory diet and water *ad libitum*, were used.

The animals were anaesthetized with 50mg of pentobarbital [Nembutal (R); Abbot S. A., Brussels, Belgium]/kg body wt., then the portal vein was catheterized *in situ* and oxygenated perfusion medium immediately pumped through the liver. All perfusions were performed without recirculation. The medium consisted of cell-free Krebs–Ringer bicarbonate solution (Umbreit *et al.*, 1964) equilibrated with O_2+CO_2 (95:5) at 37°C and supplemented with 11mM-glucose, 1.5mM-L-lactate and 0.2mM-pyruvate. The exact concentration of added lactate and pyruvate was assayed in each perfusion. The flow rate was 60ml/min. No swelling of the liver occurred. Evaporation of ethanol and acetaldehyde was prevented by enclosing the liver in a plastic envelope. Ethanol or acetaldehyde was added directly to the inflowing medium and rapidly mixed by stirring. Samples of both the inflowing and outflowing medium were collected in test tubes, kept at 0°C and analysed within a few hours. The rate of removal or formation of metabolites was calculated from the differences in input and output concentrations, and the flow rate and the weight of the liver as determined after the perfusion.

Approximately 2–3 min after a change in the inflowing medium had been made, the liver had adjusted its metabolism to this situation and new steady-state output concentrations of metabolites were registered. Samples were taken during the fourth and fifth minutes after such an alteration in the inflowing medium had been made, this being done in six to eight consecutive 5 min periods.

The O_2 concentration in the effluent was measured with a Clark-type O_2 electrode calibrated with medium saturated with O_2+CO_2 (95:5) at 37°C and with a borax solution freed of O_2 with sodium sulphite. As some O_2 diffused through the plastic part of the perfusion tubings, the actual maximum O_2 was determined by pre-perfusion without the liver. The O_2 consumption was calculated from the difference between this control value and the reading recorded during perfusion.

Ethanol and acetaldehyde were assayed with a Perkin–Elmer F 40 gas chromatograph, by using the head-space technique and t-butyl alcohol as internal standard. The temperature of the water bath was 65°C. Results of Truitt (1970) and of our laboratory (unpublished observation) indicate that some acetaldehyde is formed spontaneously from liver homogenates and from blood after deproteinization when ethanol is present, but this did not occur in the cell-free perfusion medium. No correction for non-enzymic acetaldehyde formation was therefore necessary. Lactate and pyruvate were assayed enzymically (Hohorst *et al.*, 1959) directly in the medium. Enzymes and coenzymes were obtained from C. F. Boehringer, Mannheim, Germany. The acetaldehyde used was from BDH Chemicals, Poole, Dorset, U.K., and was stated to be at least 99% pure. Sodium lactate was prepared by neutralization with NaOH of L(+)-lactic acid (purum), which was purchased from Fluka AG, Buchs, Switzerland.

Results

The rate of ethanol uptake, the output of acetaldehyde from the liver and the lactate/pyruvate ratio were recorded at input concentrations of ethanol that were varied between 0.5 and 32mM (Table 1). A maximum rate of ethanol utilization of about 2μmol/min per g of fresh wt. liver was found at ethanol concentrations above 2mM. From a Lineweaver–Burk plot the apparent $V_{max.}$ for ethanol oxidation in the intact liver was estimated to be 3.6μmol/min per g. The corresponding apparent K_m value for ethanol was found to be 1.6mM. This value is close to those reported with isolated alcohol dehydrogenase (Sund & Theorell, 1963; Büttner, 1965). It was not possible to determine the rate of ethanol disappearance accurately at ethanol concentrations higher than 4mM, because the difference between the input and output concentrations became too small. The concentration of acetaldehyde found in the effluent medium increased with the ethanol concentration and reached its maximum at about 16mM-ethanol (Table 1). Only 10–20% of the acetaldehyde formed during oxidation of 0.5–2mM-ethanol left the liver unmetabolized; the rest was further oxidized (Table 1). However, at higher concentrations of ethanol (above 8mM) 50% or more of the acetaldehyde formed passed out from the liver. The lactate/pyruvate ratio increased gradually with the ethanol concentration from 7 in the control experiments without ethanol to a maximum value of about 20 when the ethanol concentration exceeded 4mM. The lactate/pyruvate ratio and the output of acetaldehyde continued to increase with the concentration of ethanol beyond the point at which ethanol elimination was already maximum.

When acetaldehyde alone was added, it was found to be rapidly removed from the perfusate (Table 2). The rate of removal continued to increase with increased addition of acetaldehyde. At the highest concentration present, 25.6mM, the rate of uptake was 15.4μmol/min per g of liver, but this rate was still submaximal. Part of the disappearance was due to reduction to ethanol. At lower acetaldehyde concentrations only a small part was reduced, but at higher concentrations the reduction to ethanol accounted for more than 50% of the total uptake. Addition of up to 3mM-acetaldehyde did not influence the lactate/pyruvate ratio appreciably; at still higher concentrations a gradual increase occurred. The O_2 consumption was slightly stimulated by low concentrations of acetaldehyde, but was gradually inhibited when the concentration was approx. 10mM or higher.

Table 1. *Effect of ethanol concentration on the rate of its utilization and on the concentration of acetaldehyde, lactate and pyruvate in the effluent medium of perfused rat livers*

Livers from fed rats were perfused in a non-recirculating system with a cell-free Krebs–Ringer bicarbonate medium supplemented with 11 mM-glucose, 1.5 mM-L-lactate and 0.2 mM-pyruvate. Ethanol was added to the perfusate and the rate of its disappearance calculated from the flow rate and the concentration difference between the inflowing and outflowing medium. The rate of acetaldehyde formation has been calculated on the assumption that the rate of ethanol removal is unchanged at higher ethanol concentrations. Mean values ± S.E.M. of six perfusions are given.

Ethanol concn. in inflowing medium (mM)	Ethanol removed (μmol/min per g of liver)	Output of acetaldehyde (μmol/min per g of liver)	Liver output of acetaldehyde (% of acetaldehyde formed from ethanol)	Lactate concn. in effluent (μmol/ml of effluent)	Pyruvate concn. in effluent (μmol/ml of effluent)	Lactate/pyruvate ratio of effluent	Ethanol/acetaldehyde ratio of effluent
0	—	—	—	1.47±0.05	0.195±0.009	7.0±0.4	—
0.5	0.88±0.04	0.11±0.01	13	1.51±0.06	0.125±0.005	11.6±0.4	29.1±3.5
1.0	1.38±0.06	0.28±0.02	20	1.54±0.07	0.111±0.009	13.1±0.6	25.0±2.9
2.0	2.04±0.09	0.66±0.03	25	1.51±0.05	0.095±0.006	15.9±1.9	29.0±3.6
4.0	2.04±0.09	0.80±0.05	37	1.51±0.06	0.082±0.006	19.2±1.6	40.5±3.9
8.0	—	1.05±0.05	50	1.51±0.06	0.075±0.008	20.6±1.6	61.2±3.8
16.0	—	1.24±0.06	61	1.50±0.05	0.072±0.007	20.5±1.8	102.3±4.9
25.0	—	1.29±0.07	—	1.50±0.07	0.072±0.008	20.3±1.6	—
32.0	—	1.26±0.07	60	1.54±0.06	0.073±0.010	19.8±1.5	208.5±6.2

Table 2. *Effect of acetaldehyde concentration on the rate of its removal, on the formation of ethanol, on oxygen consumption and on the lactate/pyruvate ratio in the effluent of perfused livers*

The perfusions were performed as described in Table 1. Acetaldehyde was added to the inflowing medium and the rate of its disappearance and reduction to ethanol was calculated from the rate of flow and the concentration difference between the inflowing and outflowing medium. Mean values ± S.E.M. for three perfusions are given.

Acetaldehyde concn. in inflowing medium (mM)	Acetaldehyde removed (μmol/min per g of liver)	Ethanol formed (μmol/min per g of liver)	Lactate concn. in effluent (μmol/ml of effluent)	Pyruvate concn. in effluent (μmol/ml of effluent)	Lactate/pyruvate ratio of effluent	O_2 consumption (μmol/min per g of liver)
0	—	—	1.50±0.08	0.205±0.008	7.0±0.4	2.25±0.10
0.4	2.32±0.09	0.48±0.03	1.45±0.08	0.200±0.009	7.1±0.4	2.56±0.12
0.8	3.04±0.12	0.54±0.04	1.46±0.08	0.191±0.007	7.8±0.4	2.70±0.11
1.6	4.40±0.14	1.43±0.06	1.45±0.08	0.180±0.008	8.2±0.5	2.75±0.12
3.2	6.56±0.16	2.46±0.10	1.49±0.09	0.159±0.007	9.1±0.5	2.76±0.13
6.4	9.92±0.27	5.21±0.13	1.50±0.08	0.102±0.007	14.5±0.7	2.74±0.12
12.8	12.72±0.37	7.31±0.19	1.49±0.07	0.090±0.006	16.6±1.0	2.20±0.10
25.6	15.36±0.43	9.02±0.28	1.50±0.08	0.084±0.006	17.8±1.3	1.68±0.10

In control experiments 1mM-pyrazole inhibited the reduction of acetaldehyde to ethanol almost totally and limited the rate of acetaldehyde oxidation (Table 3). The maximum rate of oxidation, 3.2μmol/min per g, was found when the acetaldehyde concentration was 3–4mM or higher. The apparent K_m value for acetaldehyde in the acetaldehyde-oxidizing system was 0.71mM and the apparent $V_{max.}$ was 3.7μmol/min per g. When the reduction of acetaldehyde was blocked by pyrazole, there was almost no effect on the lactate/pyruvate ratio at lower concentrations of acetaldehyde. At concentrations above 1.6mM the lactate/pyruvate ratio gradually increased and at 6.4mM the shift in the lactate/pyruvate ratio was almost the same as that caused by ethanol. The effect of acetaldehyde on O_2 consumption was similar to that found in the absence of pyrazole. The influence of ethanol and acetaldehyde on each other's oxidation was studied by simultaneous addition of ethanol and acetaldehyde to the perfusate. The results in Table 4 show the effect of increasing concentrations of acetaldehyde on the oxidation of 2.5mM-ethanol. Even a low concentration of acetaldehyde retarded the oxidation of ethanol. When the concentration of acetaldehyde was further increased, the rate of ethanol oxidation was lowered by the enhanced acetaldehyde reduction. At the same time the shift in the lactate/pyruvate ratio caused by ethanol was gradually diminished. When approx. 0.8mM-acetaldehyde was added, the alcohol dehydrogenase reaction was in true equilibrium; that is, ethanol was neither oxidized nor formed. In this situation the ethanol-induced shift of the lactate/pyruvate ratio was strongly diminished and approached the value obtained in the absence of ethanol. The rate of acetaldehyde oxidation was 3.3μmol/min per g.

In a corresponding series of experiments, 0.8mM-acetaldehyde was present in the perfusate throughout the perfusion and the effect of increasing concentrations of ethanol was tested (Table 5). The rate of acetaldehyde uptake was diminished by ethanol. The reduction of acetaldehyde was estimated to be zero when 1.4mM-ethanol was present. In this situation there was almost no effect on the lactate/pyruvate ratio, although the rate of acetaldehyde oxidation was 2.8μmol/min per g.

Discussion

Non-recirculation perfusion allows the rates of uptake and output of metabolites to be more accurately determined than by the recirculation system.

The perfusion medium was deficient, as only glucose, lactate and pyruvate had been added to the buffer solution. Essential liver metabolites, such as amino acids, would be expected to leak out from the liver cells, leading to gradual depletion of freely exchangeable compounds. The adequacy of the

Table 3. *Effect of acetaldehyde concentration on the rate of its removal, on oxygen consumption and on the lactate/pyruvate ratio of the effluent of livers perfused in the presence of* 1mM-*pyrazole*

The perfusions were performed as described in Table 1. 1mM-Pyrazole completely blocked the reduction of acetaldehyde to ethanol. The rate of acetaldehyde removal was calculated from the flow rate and the concentration difference between the inflowing and outflowing medium. Mean values ± S.E.M. of four perfusions are given.

Acetaldehyde concn. in inflowing medium (mM)	Acetaldehyde removed (μmol/min per g of liver)	Lactate concn. in effluent (μmol/ml of effluent)	Pyruvate concn. in effluent (μmol/ml of effluent)	Lactate/pyruvate ratio of effluent	O_2 consumption (μmol/min per g of liver)
0	—	1.48 ± 0.06	0.212 ± 0.012	7.0 ± 0.5	2.29 ± 0.12
0.4	1.42 ± 0.08	1.48 ± 0.06	0.207 ± 0.009	7.1 ± 0.5	2.58 ± 0.14
0.8	1.80 ± 0.14	1.45 ± 0.05	0.190 ± 0.008	7.6 ± 0.6	2.67 ± 0.13
1.6	2.72 ± 0.15	1.50 ± 0.06	0.131 ± 0.006	12.6 ± 0.8	2.68 ± 0.11
3.2	3.23 ± 0.24	1.52 ± 0.04	0.084 ± 0.007	17.6 ± 1.4	2.68 ± 0.11
6.4	3.24 ± 0.23	1.50 ± 0.04	0.082 ± 0.005	18.8 ± 1.5	2.57 ± 0.11
12.8	3.20 ± 0.21	1.52 ± 0.05	0.080 ± 0.004	19.7 ± 1.8	2.42 ± 0.12
25.6	3.16 ± 0.20	1.51 ± 0.06	0.062 ± 0.006	18.7 ± 1.7	1.93 ± 0.12

Table 4. *Influence of acetaldehyde on the metabolism of ethanol by perfused livers*

The concentration of ethanol in the inflowing medium was 2.5 mM throughout. Removal or formation of ethanol and acetaldehyde was calculated from the flow rate and the concentration difference between the inflowing and outflowing medium. The lactate/pyruvate ratio of the effluent medium was 7.40±0.5 before addition of ethanol. Other details of the perfusion are described in Table 1. Mean values±S.E.M. of four perfusions are given.

Acetaldehyde concn. in inflowing medium (mM)	Ethanol removed (−) or formed (+) (μmol/min per g of liver)	Acetaldehyde formed (+) or removed (−) (μmol/min per g of liver)	Lactate concn. in effluent (μmol/ml of effluent)	Pyruvate concn. in effluent (μmol/ml of effluent)	Lactate/pyruvate ratio of effluent
0	−2.08±0.12	+0.20±0.05	1.41±0.05	0.075±0.007	19.2±1.9
0.04	−1.92±0.10	+0.17±0.03	1.39±0.05	0.086±0.003	16.1±1.8
0.08	−1.56±0.10	+0.04±0.01	1.33±0.06	0.102±0.006	13.3±1.5
0.20	−1.11±0.09	−0.57±0.05	1.42±0.08	0.139±0.004	10.5±1.1
0.40	−0.82±0.05	−1.26±0.12	1.38±0.06	0.167±0.004	8.4±0.8
0.80	−0.12±0.03	−2.81±0.38	1.39±0.08	0.180±0.003	7.7±0.4
1.00	+0.34±0.03	−3.41±0.56	1.35±0.05	0.184±0.005	7.3±0.6
2.00	+1.03±0.09	−4.76±0.55	1.36±0.06	0.158±0.006	8.6±0.7
4.00	+2.10±0.09	−6.18±0.52	1.36±0.08	0.145±0.005	9.3±0.9

Table 5. *Influence of ethanol on the metabolism of acetaldehyde by perfused livers*

The acetaldehyde concentration of the inflowing medium was 0.8 mM throughout. Removal or formation of acetaldehyde or ethanol was calculated from the flow rate and the concentration difference between the inflowing and outflowing medium. The lactate/pyruvate ratio of the effluent was 7.65±0.4 before addition of acetaldehyde. Other details of the perfusion are indicated in Table 1. Mean values±S.E.M. of four perfusions are given.

Ethanol concn. in inflowing medium (mM)	Acetaldehyde removed (μmol/min per g of liver)	Ethanol formed (+) or removed (−) (μmol/min per g of liver)	Lactate concn. in effluent (μmol/ml of effluent)	Pyruvate concn. in effluent (μmol/ml of effluent)	Lactate/pyruvate ratio of effluent
0	−3.42±0.31	+0.58±0.05	1.52±0.07	0.198±0.010	7.4±0.5
0.5	−3.34±0.32	+0.56±0.05	1.54±0.05	0.200±0.009	7.7±0.5
1.0	−3.12±0.33	+0.27±0.03	1.44±0.05	0.195±0.009	7.4±0.4
1.5	−3.09±0.30	−0.10±0.02	1.45±0.05	0.191±0.008	7.4±0.4
2.0	−2.98±0.27	−0.24±0.03	1.45±0.07	0.181±0.007	7.9±0.5
3.0	−2.57±0.17	−0.49±0.04	1.52±0.08	0.163±0.008	9.3±0.6
4.0	−2.34±0.22	−0.58±0.06	1.56±0.06	0.152±0.008	10.0±0.9
5.0	−2.20±0.20	−0.82±0.05	1.55±0.06	0.144±0.007	10.5±0.9

technique was therefore tested in preliminary experiments. From the parameters measured (O_2 consumption, $^{14}CO_2$ output from labelled substrates, the lactate/pyruvate ratio, etc.) it was concluded that the metabolism of the liver was constant as long as no changes in the perfusate were made.

It is assumed that the lactate/pyruvate ratio of the effluent medium reflects that of the liver cell, which in turn is in equilibrium with the free NADH/free NAD^+ ratio of the cytoplasm (Williamson *et al.*, 1967). The validity of this assumption was tested in experiments in which the lactate/pyruvate ratio was determined simultaneously in the medium and in the freeze-clamped liver tissue. In these experiments, the lactate/pyruvate ratio in controls was 11.2 ± 2.2 (S.D.) in the medium and 8.2 ± 1.8 in the liver ($n = 4$), and during ethanol oxidation 32 ± 13 in the medium and 26.8 ± 8 in the liver ($n = 4$). Thus a reasonably good correlation was found between liver and effluent medium, indicating no significant gradient for lactate or pyruvate between the extra- and intra-cellular space.

Acetaldehyde metabolism

Acetaldehyde at low concentrations was removed mainly by oxidation. This indicates that the affinity for the acetaldehyde-oxidizing enzyme(s) is greater than that for the backward alcohol dehydrogenase-catalysed reaction. The K_m for acetaldehyde as substrate for alcohol dehydrogenase is reported to be 0.16mM (Sund & Theorell, 1963). The K_m values reported for purified aldehyde dehydrogenases (Racker, 1949; Sheppard *et al.*, 1970; Kraemer & Deitrich, 1968) are about two orders of magnitude lower. On the other hand, the values found in a crude liver homogenate are much higher (Deitrich, 1966; M. Pajari, personal communication) and in good agreement with the apparent K_m value found by us in the intact liver (0.71 mM). The possible interference of various aldehyde oxidases, which have still higher K_m values (see Lundquist *et al.*, 1962), cannot be excluded.

The unchanged lactate/pyruvate ratio of the medium during oxidation of lower concentrations of acetaldehyde shows that the redox state of the NAD couple in the cytosol is influenced much less than at comparable rates of production of reduced equivalents during ethanol oxidation. This finding does not support the view that a NAD-coupled cytoplasmic dehydrogenase is the chief enzyme involved in the removal of acetaldehyde.

It is well known that acetaldehyde is toxic at higher doses. It might interfere with the normal metabolism by binding free CoA (Ammon *et al.*, 1969). The decrease in O_2 consumption observed on addition of high concentrations of acetaldehyde (above 10mM) indicates that the metabolic integrity of the liver is impaired and this may contribute to the increase of the lactate/pyruvate ratio also observed.

When high concentrations of acetaldehyde are added, its rapid removal through simultaneous oxidation and reduction indicates that an acetaldehyde dismutation process similar to that observed in a crude homogenate (Lundquist *et al.*, 1959) and with purified enzymes (Abeles & Lee, 1961; Dalziel & Dickinson, 1965) also functions in the intact liver. The rate of acetaldehyde oxidation (total removal minus reduction to ethanol) was twice the maximum rate found when reduction was blocked with pyrazole. However, from the present results it is not possible to decide whether alcohol dehydrogenase can remove acetaldehyde by acting as a mutase, as suggested by Dalziel & Dickinson (1965), or whether an acetaldehyde-oxidizing enzyme is alone responsible for the part of acetaldehyde removed by oxidation.

Ethanol oxidation

At ethanol concentrations above 4mM, 50% or more of the acetaldehyde formed was not further oxidized by the liver. If the liver behaves similarly *in vivo*, this would mean that a considerable part of the acetaldehyde formed during oxidation of ethanol is oxidized extrahepatically.

The lactate/pyruvate ratio of the effluent was increased, as expected, during ethanol oxidation. The increase was dependent on the rate of ethanol removal. The same conclusion can be made from the results in Table 4, where the concentration of ethanol was kept constant during the experiment. The lactate/pyruvate ratio does, however, continue to increase with the concentration of ethanol after ethanol removal is already maximum (Table 1). This may be explained by assuming that there is a tendency for ethanol and acetaldehyde to equilibrate with the lactate/pyruvate pair. When more ethanol is added, the ethanol/acetaldehyde ratio is increased, causing the lactate/pyruvate ratio to increase too.

Ethanol and acetaldehyde as a redox couple

Ethanol and acetaldehyde are in equilibrium with the NAD^+/NADH couple in yeast cells (Holzer *et al.*, 1956). Scholz (1968) and Krebs (1969) presented results which suggest that ethanol and acetaldehyde are also in redox equilibrium in the liver during ethanol oxidation. From the known equilibrium constants of the lactate dehydrogenase (Williamson *et al.*, 1967) and alcohol dehydrogenase systems (Bäcklin, 1958), it can be estimated that if these are in equilibrium with the NAD couple the lactate/pyruvate ratio should be around 1.7 times the ethanol/acetaldehyde ratio. If it is assumed, as discussed above, that the effluent approximately reflects the intracellular lactate/pyruvate ratio, it follows that the

Table 6. *Ethanol/acetaldehyde ratio and the lactate/pyruvate ratio in the perfusion medium when equilibrium of the alcohol dehydrogenase-catalysed reaction is achieved by simultaneous addition of ethanol and acetaldehyde*

The ratios have been calculated from Tables 4 and 5 by extrapolation to the point at which ethanol is neither formed nor removed, i.e. the ethanol concentrations are equal in the inflowing and outflowing medium. Mean values ± S.E.M. of four perfusions are given.

	Ethanol/acetaldehyde ratio of the effluent medium	Lactate/pyruvate ratio of the effluent medium	$\frac{\text{Lactate/pyruvate}}{\text{Ethanol/acetaldehyde}}$
Table 4 (ethanol constant, acetaldehyde varied)	5.13 ± 0.48	7.0 ± 0.3	1.46 ± 0.36
Table 5 (acetaldehyde constant, ethanol varied)	4.38 ± 0.44	7.4 ± 0.4	1.79 ± 0.42

ethanol/acetaldehyde ratio exceeds by more than one magnitude that expected if equilibrium with the lactate/pyruvate ratio were to occur. It seems that at higher ethanol concentrations the acetaldehyde concentration ceases to increase and the increase of ethanol raises the ethanol/acetaldehyde ratio. Similar results were obtained in our laboratory in a previous study *in vivo* (Forsander *et al.*, 1969), in which the ethanol/acetaldehyde ratio was more than ten times as high as the corresponding lactate/pyruvate ratio. The same study also showed roughly similar acetaldehyde concentrations in liver and hepatic venous blood, which excludes the existence of a concentration gradient for acetaldehyde between liver and blood. These findings therefore do not indicate near-equilibrium between the ethanol/acetaldehyde pair and the lactate/pyruvate pair *in vivo*. The turnover rate of the reaction catalysed by alcohol dehydrogenase is probably not sufficiently high to cope with the rapid elimination of the acetaldehyde formed.

The addition of suitable concentrations of both ethanol and acetaldehyde to the inflowing medium enabled determination of the ethanol/acetaldehyde ratio at the point when ethanol was neither removed nor formed, when the alcohol dehydrogenase-catalysed reaction may be considered to be in true equilibrium (Table 6). The lactate/pyruvate ratio was approx. 7 in these circumstances and the ethanol/acetaldehyde ratio was 4–5. The relation between the two pairs is very close to the theoretical (1.7), taking into account the approximations made in the calculations.

Rate-limiting factors in ethanol oxidation

There have been numerous attempts to correlate the rate of ethanol removal with the maximum activity of the alcohol dehydrogenase (for a discussion of this, see Videla & Israel, 1970), but most of them have been unsuccessful. From our results we calculated an apparent $V_{max.}$ for the capacity of the whole liver to oxidize ethanol. A Lineweaver–Burk plot from the results in Table 1 gave a maximum rate of 3.6 μmol/min per g, which is nearly twice the actual maximum value obtained in the perfusion (2 μmol/min per g). This indicates that the capacity of the alcohol dehydrogenase system is not fully utilized.

Krebs (1969) suggests that the acetaldehyde concentration may limit the rate of the conversion of ethanol into acetate. According to him, the rate of oxidation of acetaldehyde may be determined by the concentration of acetaldehyde, which in turn will be dependent on the redox state of the cytoplasm. This proposal presupposes a high K_m for the acetaldehyde-oxidizing enzyme and that the ethanol/acetaldehyde pair is in redox equilibrium in the cytoplasm. As discussed above, the values for the affinity for acetaldehyde are conflicting and do not allow any conclusions to be made. However, even if a low affinity is assumed, the role of the acetaldehyde concentration would nevertheless be small if the ethanol/acetaldehyde pair is far from equilibrium, as indicated in this study.

We believe that the capacity of the liver to reoxidize NADH normally limits ethanol removal. This was also concluded by Videla & Israel (1970), who found that the uncouplers 2,4-dinitrophenol and arsenate accelerated ethanol metabolism (see also Israel *et al.*, 1970; Scholz *et al.*, 1971). In our experiments the maximum rate of ethanol removal was about 2 μmol/min per g (Table 1). As about half of the acetaldehyde formed is not oxidized by the liver, about 3 μmol of NADH/min per g will be produced. The corresponding amount of NADH produced during maximum acetaldehyde oxidation (Table 3) is 3.2 μmol/min per g. Calculations indicate that roughly the same amount of reduced equivalents are also liberated during the simultaneous removal of ethanol and acetaldehyde (Tables 4 and 5). Under these circumstances the average O_2 consumption is 2.6 μmol/min per g. Thus about 60% of the O_2 is

utilized for reoxidation of the amount of NADH formed. The rate of oxidation of these metabolites therefore seems to be connected with the general control of the energy metabolism. Ultimately, the rate of mitochondrial reoxidation of NADH seems to be the most important of the factors limiting the rate of hepatic ethanol metabolism.

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