Influence of Ethanol on the Liver Metabolism of Fed and Starved Rats

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(Received 20 April 1964)

1. The influence of ethanol on the metabolism of livers from fed and starved rats has been studied in liver-perfusion experiments. Results have been obtained on oxygen consumption and carbon dioxide production, on glucose release and uptake by the liver and on changes in the concentrations of lactate and pyruvate and of β -hydroxybutyrate and acetoacetate in the perfusion medium. 2. Oxygen consumption and carbon dioxide production were lower in livers from starved rats than in livers from fed rats. Ethanol had no effect on the oxygen consumption of either type of liver. After the addition of ethanol to the perfusion medium carbon dioxide production ceased almost completely, the change being faster in livers from starved rats. 3. With livers from fed rats glucose was released from the liver into the perfusion medium. This release was slightly greater when ethanol was present. With livers from starved rats no release of glucose was observed, and when ethanol was added a marked uptake of glucose from the medium was found. A simultaneous release of glycolytic end products, lactate and pyruvate, into the medium occurred. 4. Acetate was the main metabolite accumulating in the perfusion medium when ethanol was oxidized. With livers from starved rats a slightly increased formation of ketone bodies was found when ethanol was present. 5. The lactate/pyruvate concentration ratio in the perfusion medium increased from 10 to 87 with livers from fed rats and from 20 to 171 with livers from starved rats when the livers were perfused with ethanol in the medium. The β -hydroxybutyrate/acetoacetate concentration ratio increased from 0.8 to 7.6 with livers from fed rats and from 1.0 to 9.5 with livers from starved rats when ethanol was added to the medium. 6. The effects of ethanol are discussed and related to changes in the redox state of the liver that produce new conditions for some metabolic pathways.

When ethanol is oxidized in the intact organism the breakdown of other substances decreases in proportion to the energy liberated from the ethanol (Atwater & Benedict, 1902). Carpenter & Lee (1937) observed that the oxidation of carbohydrate and fat was depressed by ethanol, whereas protein metabolism was very little affected. The effect of ethanol on liver metabolism is much greater than on the overall metabolism of the body (Atwater & Benedict, 1902). Thus it has been shown that the R.Q.* of the intact body decreases but little during the oxidation of ethanol, whereas the R.Q. of the liver decreases markedly. In one perfusion experiment on cat liver, Lundsgaard (1938) found that the R.Q. decreased from 0.69 to 0.37 when ethanol was added to the perfused blood. The theoretical R.Q. for total oxidation of ethanol is 0.67, and the low R.Q. found in the experiments was attributed to partial oxidation of ethanol in the liver. This has been confirmed by Leloir & Muñoz (1938) and later

by Forsander & Rähä (1960), using isotope techniques. Leloir & Muñoz (1938) have calculated that about three-quarters of the oxygen consumed by the liver is used for the oxidation of ethanol to acetate. Similar values have been reported by Lundquist, Tygstrup, Winkler, Møllemaard & Munck-Petersen (1962) in experiments on human subjects. Since the oxygen consumption of the liver is only slightly affected by the breakdown of ethanol the oxidation of other substrates must decrease in relation to the amount of ethanol oxidized. The R.Q. of livers from starved animals is known to be lower than that of livers from fed animals (Blixenkrone-Møller, 1938). This was thought to be due either to partial oxidation of fatty acids to ketone bodies or to other reactions in which oxygen is consumed without carbon dioxide production: by gluconeogenesis from fatty acids and protein or by desaturation of fatty acids.

In the present study the effect of ethanol on the oxygen consumption and carbon dioxide production of perfused livers from fed and starved rats has been

* Abbreviation: R.Q., respiratory quotient.

investigated. The effect of ethanol on the intermediary metabolism of the isolated liver has further been elucidated by studies on the release and uptake of glucose by the liver, and on the changes in the lactate, pyruvate, β -hydroxybutyrate and acetoacetate concentrations in the perfused medium.

MATERIAL AND METHODS

Experimental animals. Male albino rats from our own laboratory stock weighing between 200 and 250 g. were used in all experiments. The animals were kept on a normal laboratory diet. In the experiments with starved animals the time of starvation was 48 hr.

Liver-perfusion experiments. The perfusion apparatus used was a modification of that described by Miller, Bly, Watson & Bale (1951). Under pentobarbital anaesthesia the portal vein and the bile duct were cannulated. Also, the inferior caval vein was cannulated through the right atrium and the inferior caval vein ligated above the junction of the renal veins. The liver was kept intact in its normal position, and after the cannulas were placed in position and perfusion was started the whole operating table with the rat was lowered into a thermostat containing 0.9% NaCl solution at 37°. The perfusion apparatus with the circulating medium was kept in an air-warmed thermostat at 37°. The perfusion medium was oxygenated with O₂ + CO₂ (95:5).

The anoxic phase during the operation did not exceed 3 min. Before the test period the liver was control-perfused for 1 hr. to correct the anoxia caused by its isolation and to ensure that a steady state had been reached (Brauer, Leong & Pessotti, 1953). At the start of the perfusion the liver was in an anaerobic state, but after the control perfusion the lactate/pyruvate concentration ratio had decreased to that reported for blood *in vivo* (Hohorst, Kreutz & Bücher, 1959), which shows that the operation had been performed properly and that the flow through the liver was adequate. Liver function was also tested by continuous collection of bile and by estimation of ketone-body formation. In all experiments the actual test perfusion period lasted for 2 hr. and the flow through the liver was maintained at over 0.6 ml./min./g. of liver, which is necessary to maintain normal oxidative metabolism (Schimassek, 1962).

The perfusion medium consisted of a mixture of equal amounts of heparin-treated bovine blood and Krebs-Ringer bicarbonate solution (Umbreit, Burris & Stauffer, 1964) with added glucose (final concn. about 5–6 mM). The haematocrit value of the medium was 25 ± 3 . The bovine blood was collected not more than 8 hr. before the experiment. In the experiments in which ethanol was used this was added to the medium after the control perfusion period to give a final ethanol concentration of 200 mg./100 ml.

The samples for O₂ and CO₂ determinations were collected from the cannulas entering and leaving the liver in Teflon tubes filled with paraffin oil. All other samples were collected from the blood reservoir.

Analytical methods. Ethanol was determined enzymically by the method described by Bücher & Redetzki (1951), and glucose was determined by the enzymic method of Huggett & Nixon (1957). Lactate and pyruvate were determined enzymically with Biochimica Boehringer (Mannheim, W. Germany) test kits. The ketone bodies (acetoacetate and β -hydroxybutyrate) were determined by a modification of

the method described by Lyon & Bloom (1958). The reagents and amounts used were exactly the same as used by these authors. The size of the distillation tubes was 18 mm. external diam. $\times$ 190 mm. The colour reagent was pipetted into a 60 mm. side tube as described by Ahola & Somersalo (1963). With the exception of the size of the tubes the analysis did not differ from that described by Ahola & Somersalo (1963). Owing to the interference of ethanol with the ketone-body determination all ethanol present in the samples was evaporated *in vacuo*. The Van Slyke manometric apparatus was used for the determination of blood gases (Van Slyke & Neill, 1924). Organic acids were determined chromatographically as reported by Forsander & Riih (1960). This method does not quite quantitatively reveal the amounts of acids formed, since acetate is partially evaporated in the oxygenator and some acids are partially destroyed during the preparation of the samples.

RESULTS

Oxygen consumption and carbon dioxide production. At the beginning of the test period the oxygen consumption of livers from fed rats was about 2.5 μ moles/g. wet wt. of liver/min. (Table 1). During continued perfusion the oxygen consumption decreased somewhat. The values are of the same order as those found by others in perfused rat liver (Schimassek, 1963). The addition of ethanol to the perfusion medium had no effect on oxygen consumption. Livers from starved rats had a much lower oxygen consumption, about 1.5 μ moles/g. wet wt. of liver/min., than did those from fed rats. Decreased oxygen consumption by livers from starved animals has been reported for rat-liver slices (Leloir & Muoz, 1938; Weinhouse & Millington, 1951) and for perfused cat liver (Blixenkrnll, 1938). Some variations in the initial oxygen consumption during the 1 hr. control perfusion were observed in the experiments. This was probably due to differences in the effects of starvation on the animals. As with the livers from fed rats, ethanol had no effect on the oxygen consumption of the livers from starved rats.

The formation of carbon dioxide was fairly constant during the perfusion of both types of liver when ethanol had not been added to the medium. As shown in Table 1, carbon dioxide production of the livers from starved rats was much lower than that of livers from fed rats. The R.Q. values of livers from fed rats varied between 0.77 and 1.02, but were between 0.30 and 0.61 in the experiments on livers from starved rats. Ethanol had a marked effect on the carbon dioxide production of both types of liver. After the addition of ethanol to the medium, carbon dioxide production almost completely ceased. The change was maximal after 1½ hr. with livers from fed rats and after ½ hr. with livers from starved rats. In the experiments with livers from fed rats carbon dioxide production rose somewhat at the

Table 1. *Oxygen consumption, carbon dioxide production and respiratory quotient during perfusion of livers from fed and starved rats*

Experimental details are given in the text. Ethanol was added to the perfusion medium immediately after sampling at 60 min. The results for O₂ consumption and CO₂ production are given as means $\pm$ s.e.m.

Source of liver	Ethanol present in perfusion medium	No. of rats	O ₂ consumption (μ moles/g. wet wt. of liver/min.)				
			Sampling time (min.) ... 60	90	120	150	180
Fed rat	—	4	2.51 $\pm$ 0.25	2.26 $\pm$ 0.17	2.29 $\pm$ 0.21	2.23 $\pm$ 0.33	2.07 $\pm$ 0.30
	+	4	2.50 $\pm$ 0.39	2.35 $\pm$ 0.31	2.41 $\pm$ 0.55	2.20 $\pm$ 0.26	2.24 $\pm$ 0.30
Starved rat	—	3	1.14 $\pm$ 0.21	1.35 $\pm$ 0.14	1.43 $\pm$ 0.18	1.77 $\pm$ 0.03	1.54 $\pm$ 0.14
	+	4	1.83 $\pm$ 0.40	1.71 $\pm$ 0.34	1.38 $\pm$ 0.16	1.49 $\pm$ 0.19	1.51 $\pm$ 0.15
CO ₂ production (μ moles/g. wet wt. of liver/min.)							
Fed rat	—	4	1.94 $\pm$ 0.12	2.03 $\pm$ 0.08	2.03 $\pm$ 0.22	2.00 $\pm$ 0.17	2.12 $\pm$ 0.24
	+	4	2.38 $\pm$ 0.34	0.54 $\pm$ 0.60	0.16 $\pm$ 0.39	0.01 $\pm$ 0.30	0.32 $\pm$ 0.24
Starved rat	—	3	0.55 $\pm$ 0.08	0.83 $\pm$ 0.23	0.55 $\pm$ 0.17	0.53 $\pm$ 0.19	0.74 $\pm$ 0.15
	+	4	0.81 $\pm$ 0.03	0.00 $\pm$ 0.22	0.54 $\pm$ 0.23	0.24 $\pm$ 0.19	—0.02 $\pm$ 0.25
R.Q.							
Fed rat	—	4	0.77	0.90	0.89	0.90	1.02
	+	4	0.95	0.23	0.07	0.01	0.14
Starved rat	—	3	0.48	0.61	0.38	0.30	0.48
	+	4	0.44	0.00	0.39	0.16	—0.01

end of the experiment, but no clear rise was observed with livers from starved rats.

Changes in glucose concentration of the perfusion medium. During the 1 hr. control perfusion the glucose concentration of the medium increased markedly in the experiments in which livers from fed rats were used (Fig. 1). During the test period there was a slight further increase. This confirms previous observations on cat liver (Blixenkrone-Møller, 1938) and rat liver (Field, Williams & Mortimore, 1963). In the experiments on livers from starved rats in which the glycogen depots are small, a very slight rise of the glucose concentration in the medium was observed during perfusion without ethanol. The presence of ethanol in the medium somewhat increased the release of glucose from livers from fed rats. With livers from starved rats, however, ethanol markedly increased glucose uptake from the medium.

Rate of ethanol oxidation. Since the maximal rate of oxidation of ethanol is reached at a low ethanol concentration (less than 1 μ M; Larsen, 1963), only one concentration was used in the experiments. During the perfusion the ethanol concentration in the medium decreased, partly owing to evaporation in the oxygenator and partly owing to oxidation in

the liver. A correct estimation of the amount of ethanol oxidized could not be made, since the amount disappearing in the oxygenator was not constant and could not be measured exactly. It was possible, however, to observe that the rate of oxidation of ethanol was greater in livers from fed rats than in livers from starved rats. This observation is in accordance with previous reports on intact animals (Owens & Marshall, 1955) and on liver slices (Leloir & Muñoz, 1938; Smith & Newman, 1959).

Changes in metabolic products of the liver. Acetate is the main product formed from ethanol in the liver, but small amounts of acetoacetate and β -hydroxybutyrate are also produced (Forsander & Råihä, 1960). In addition to these three acids, pyruvate and lactate are always present in blood. Paper-chromatographic studies on the present perfusion experiments confirmed that acetate was the main intermediate formed from ethanol.

In experiments with livers of fed rats the concentration of lactate in the medium decreased slowly during the perfusion and was not affected by ethanol (Table 2). In the experiments with livers of starved rats, however, the lactate concentration decreased somewhat faster in the control experiments and rose when ethanol was added to the

Fig. 1. Glucose concentrations of the medium during perfusion of livers from fed and starved rats, with and without ethanol added to the perfusion medium. Ethanol was added to the medium after a 60 min. control perfusion (marked by arrow). Each point represents the mean value of four experiments and the bars represent standard errors. ○, Livers from fed rats, ethanol absent; ●, livers from fed rats, ethanol present; □, livers from starved rats, ethanol absent (standard errors too small to be drawn); ■, livers from starved rats, ethanol present.

medium. In the experiments without ethanol the pyruvate concentration in the medium at first increased and later decreased during the perfusion in both types of liver. In both types of liver ethanol produced a marked decrease in the pyruvate concentration.

At the beginning of the 1 hr. control perfusion the high lactate/pyruvate concentration ratio indicated post-operative anoxia of the liver. During the control perfusion of livers from fed rats the lactate/pyruvate concentration ratio decreased to values similar to those found by Schimassek (1962) and Hohorst *et al.* (1959). With livers from starved rats, however, the ratio remained somewhat higher. With livers from starved rats the individual lactate concentrations at the end of the control perfusion showed greater variations than with livers from fed rats. This might reflect variations in the effects of starvation. Ethanol strongly influenced the lactate/pyruvate concentration ratios (Table 2). In the experiments with livers from fed rats the ratio increased during the test period and reached a steady state after 120 min. The effect on livers from starved rats was still greater and the ratio steadily increased during the whole perfusion.

The initial concentration of ketone bodies in the medium varied somewhat in the different experiments, but after the 1 hr. control perfusion the values became stable. In the experiments without ethanol the concentrations of both β -hydroxybutyrate and acetoacetate increased at the same rate during the perfusion (Table 3). Ethanol

Table 2. Changes in the concentrations of lactate and pyruvate in the perfusion medium during perfusion of livers from fed and starved rats

Experimental details are given in the text. Ethanol was added to the perfusion medium immediately after sampling at 60 min. The concentrations of lactate and pyruvate are given as means $\pm$ s.e.m. of four experiments.

Source of liver	Ethanol present in perfusion medium		Sampling time (min.) ... 60	90	120	150	180
Fed rat	—	Concn. of lactate (μ M)	3590 $\pm$ 230	2980 $\pm$ 170	2700 $\pm$ 270	2450 $\pm$ 290	2260 $\pm$ 280
	—	Concn. of pyruvate (μ M)	279 $\pm$ 27	292 $\pm$ 26	308 $\pm$ 24	254 $\pm$ 12	239 $\pm$ 20
	—	Lactate/pyruvate concn. ratio	13	10	9	10	10
Fed rat	+	Concn. of lactate (μ M)	3230 $\pm$ 56	3440 $\pm$ 200	3220 $\pm$ 260	2700 $\pm$ 440	2610 $\pm$ 560
	+	Concn. of pyruvate (μ M)	280 $\pm$ 30	55 $\pm$ 2	36 $\pm$ 4	33 $\pm$ 4	30 $\pm$ 4
	+	Lactate/pyruvate concn. ratio	12	63	89	82	87
Starved rat	—	Concn. of lactate (μ M)	2920 $\pm$ 210	2440 $\pm$ 340	2160 $\pm$ 340	1890 $\pm$ 160	1610 $\pm$ 110
	—	Concn. of pyruvate (μ M)	111 $\pm$ 13	119 $\pm$ 16	120 $\pm$ 15	105 $\pm$ 15	82 $\pm$ 12
	—	Lactate/pyruvate concn. ratio	26	21	18	18	20
Starved rat	+	Concn. of lactate (μ M)	2940 $\pm$ 700	3080 $\pm$ 710	2920 $\pm$ 780	3920 $\pm$ 1170	4970 $\pm$ 1280
	+	Concn. of pyruvate (μ M)	194 $\pm$ 57	54 $\pm$ 19	34 $\pm$ 8	31 $\pm$ 5	29 $\pm$ 6
	+	Lactate/pyruvate concn. ratio	15	57	86	126	171

Table 3. *Changes in the concentrations of β -hydroxybutyrate and acetoacetate in the perfusion medium during perfusion of livers from fed and starved rats*

Experimental details are given in the text. Ethanol was added to the perfusion medium immediately after sampling at 60 min. The concentrations of β -hydroxybutyrate and acetoacetate are given as means $\pm$ S.E.M. of four experiments.

Source of liver	Ethanol present in perfusion medium		Sampling time (min.) ... 60	90	120	150	180
Fed rat	—	Concn. of β -hydroxybutyrate (μ M)	142 $\pm$ 26	200 $\pm$ 27	254 $\pm$ 56	278 $\pm$ 94	400 $\pm$ 63
	—	Concn. of acetoacetate (μ M)	119 $\pm$ 18	187 $\pm$ 32	248 $\pm$ 33	410 $\pm$ 59	521 $\pm$ 103
	—	β -Hydroxybutyrate/acetoacetate concn. ratio	1.2	1.1	1.0	0.7	0.8
Fed rat	+	Concn. of β -hydroxybutyrate (μ M)	288 $\pm$ 94	495 $\pm$ 118	594 $\pm$ 142	722 $\pm$ 168	1168 $\pm$ 306
	+	Concn. of acetoacetate (μ M)	246 $\pm$ 39	149 $\pm$ 14	162 $\pm$ 34	192 $\pm$ 35	153 $\pm$ 19
	+	β -Hydroxybutyrate/acetoacetate concn. ratio	1.2	3.3	3.7	3.8	7.6
Starved rat	—	Concn. of β -hydroxybutyrate (μ M)	164 $\pm$ 51	181 $\pm$ 53	220 $\pm$ 19	338 $\pm$ 33	353 $\pm$ 30
	—	Concn. of acetoacetate (μ M)	106 $\pm$ 23	164 $\pm$ 26	229 $\pm$ 21	310 $\pm$ 29	369 $\pm$ 16
	—	β -Hydroxybutyrate/acetoacetate concn. ratio	1.5	1.1	1.0	1.1	1.0
Starved rat	+	Concn. of β -hydroxybutyrate (μ M)	181 $\pm$ 36	465 $\pm$ 75	689 $\pm$ 62	830 $\pm$ 74	1110 $\pm$ 135
	+	Concn. of acetoacetate (μ M)	134 $\pm$ 19	135 $\pm$ 15	117 $\pm$ 15	117 $\pm$ 11	117 $\pm$ 15
	+	β -Hydroxybutyrate/acetoacetate concn. ratio	1.4	3.4	5.9	7.1	9.5

produced an increase in the β -hydroxybutyrate concentration with livers from both fed and starved rats. The concentration of acetoacetate either remained unchanged or decreased somewhat. The ratio between the reduced and oxidized forms was fairly constant in the perfusions without added ethanol and there was no significant difference between the two types of liver. Ethanol increased the β -hydroxybutyrate/acetoacetate concentration ratio and the increase mainly followed the same pattern as the lactate/pyruvate concentration ratio.

DISCUSSION

Effect on oxygen consumption and carbon dioxide production. Ethanol did not influence the oxygen consumption of livers from fed or starved rats. This confirms results obtained from experiments with human subjects (Lundquist *et al.* 1962) and with liver slices (Fondal & Kochakian, 1951). The marked decrease in carbon dioxide production evoked by ethanol indicates that a big change has taken place in the intermediary metabolism of the liver. The tricarboxylic acid cycle, which is probably responsible for most of the carbon dioxide liberated, seems to be inhibited. In this respect livers from starved rats differ from those from fed rats, even without the oxidation of ethanol, since the R.Q. is much lower. This indicates a partial oxidation of substrate, but the present experiments

do not reveal what type of partial oxidation occurs in livers from starved rats.

Influence of ethanol on metabolic pathways. The primary source of energy for hepatic metabolism is normally fatty acids, which are broken down to carbon dioxide (Fritz, 1961). The contribution of glucose to the total carbon dioxide production of the liver is small, indicating the quantitatively minor role played by glucose in hepatic oxidation (Majchrowicz & Quastel, 1963). The glycolytic pathway of the liver seems to be more important for the synthesis of glycogen and glucose than for the breakdown of the carbohydrates (Pesch & Topper, 1963).

Fig. 2 shows the quantitative changes of glucose, lactate + pyruvate and β -hydroxybutyrate + acetoacetate in the medium during the perfusion of livers from fed and starved rats. Great quantitative differences exist between the different types of experiments, especially in the release and uptake of glucose and of glycolytic end products. The total production of ketone bodies is fairly constant, but in livers from starved rats ethanol produces a significant increase in ketone-body formation. This confirms a report by Forsander & Rih (1960), but is in contrast with the observations by Lundquist *et al.* (1962) on human liver. The discrepancy in results is most probably due to the fact that the livers in the present experiments were starved for 48 hr., in contrast with the 12 hr. period used by Lundquist

Fig. 2. Net production or consumption of metabolites by livers from fed and starved rats during perfusion (120 min.), with and without ethanol added to the perfusion medium. Ethanol was added to the medium after a 60 min. control perfusion. The values are calculated from the concentrations of substrates in the perfusion medium, from the volume of medium and from the amounts of metabolites taken out as samples. Each column represents the mean value of four experiments. A, Glucose; B, lactate + pyruvate; C, ketone bodies. □, Livers from fed rats, ethanol absent; ▨, livers from fed rats, ethanol present; ▤, livers from starved rats, ethanol absent; ▩, livers from starved rats, ethanol present.

et al. (1962) in the human subjects. With livers from fed rats glucose was released rapidly, probably from the glycogen depots during the first 60 min. into the perfusion medium (Fig. 1). During the subsequent periods the glucose release decreased. This was presumably due to the diminished glycogen content of the liver (Field *et al.* 1963). More glucose was released in our experiments by the ethanol-perfused liver than by the control liver, in contrast with the observation made by Field *et al.* (1963).

The most striking changes were in ethanol-perfused livers from starved rats. In the control perfusion the glucose output was minimal owing to the low glycogen reserves after starvation, but when ethanol was added a marked uptake of glucose from the medium was observed. Simultaneously glycolytic end products, lactate and pyruvate, appeared in the perfusion medium. The high glucose uptake from the medium with ethanol-perfused livers from starved rats could be explained by an increased intensity of glycogenesis or by increased glycolysis in the liver. The first possibility is unlikely, since ethanol decreases the incorporation of glucose and pyruvate (Field *et al.* 1963) and of glycerol (Nikkilä & Ojala, 1963a) into liver glycogen. The appearance of increased amounts of lactate and pyruvate in the medium is in favour of the second possibility. As shown in Fig. 2, the glycolytic end products do

not account for the total uptake of glucose. However, during ethanol oxidation the α -glycerophosphate concentration in liver increases, apparently owing to increased reduction of dihydroxyacetone phosphate (Nikkilä & Ojala, 1963b) and increased breakdown of glucose. This α -glycerophosphate is partly utilized for the increased triglyceride synthesis that occurs in the ethanol-metabolizing liver (Nikkilä & Ojala, 1963b). No reports could be found on the influence of ethanol on glycolysis in the liver. In erythrocyte haemolysate with added alcohol dehydrogenase Rähä (1963) was not able to detect any effect of ethanol on the rate of glycolysis.

Influence on the redox state. In human subjects the normal lactate/pyruvate concentration ratio in the blood has been reported to be 13.5 (Büttner, 1961) and 15 (Seligson, Stone & Nemir, 1959). After ethanol consumption the ratios rose to 23 and 82 respectively. In the present experiments ethanol increased the lactate/pyruvate concentration ratio, which with livers from fed rats reached a steady state after perfusion for 1 hr. With livers from starved rats, however, the ratio rose continuously throughout the experiment; 2 hr. after the addition of ethanol the ratio in the experiments with livers from fed rats was 87 and with livers from starved rats 171 (Table 2). The β -hydroxybutyrate/acetoacetate concentration ratio in blood from the hepatic vein of human subjects has been found to be 2.3 and during ethanol oxidation 4.2 (Lundquist *et al.* 1962); Seligson *et al.* (1959) found values of 1.2 and 2.0–2.3 respectively. In our experiments the ratio between these two components without addition of ethanol was about 1 in experiments on livers from both fed and starved rats, but rose in the presence of ethanol to 9.5. As with the lactate/pyruvate concentration ratio a steady state was reached with livers from fed rats but not with those from starved rats (Table 3). No direct comparison of the perfusion experiments with experiments on human subjects can be made, owing to the existence in the intact organism of an interaction between the liver and the extrahepatic tissue, which is eliminated in the perfused liver.

In perfusion experiments Schimassek (1963) has shown that the lactate/pyruvate concentration ratio in the perfusion medium is equal to that in the liver and thus reflects the redox state of the cytoplasmic compartment of the cell. Alcohol dehydrogenase, which is responsible for the breakdown of ethanol, is mainly located in the liver cytoplasm (Nyberg, Schubert & Ånggård, 1953). When ethanol is available as substrate for this enzyme a shift in the redox state of the cytoplasm occurs, which is manifested as a change of the liver NAD/NADH₂ concentration ratio (Rähä & Oura, 1962) and of the lactate/pyruvate concentration ratio in the perfusion medium (Table 2).

Regulation of liver metabolism in the presence of

ethanol. The oxidation of ethanol in the liver accounts for more than 75% of the total amount of oxygen consumed (Lundquist *et al.* 1962). But the rate of oxygen consumption by livers from fed or starved rats is not increased by ethanol (Table 1). The overall regulation of metabolic intensity thus remains intact. The rate of ethanol oxidation must also submit to this regulation. Oxygen consumption proceeds at a lower rate in livers from starved rats than in livers from fed rats, and this may explain why the rate of oxidation of ethanol is different in the two types of liver.

The only metabolite accumulating in any great amount during the oxidation of ethanol in the liver is acetate. Its further oxidation, which proceeds in the tricarboxylic acid cycle, must have been blocked. It has been hypothesized that a change in the redox state of the liver towards a more reduced state results in the inhibition of the tricarboxylic acid cycle, which can also be seen during intensive fatty acid oxidation (Wieland, Matschinsky, Löffler & Müller, 1961). Since ethanol oxidation strongly increases the reduced state of the liver, this would explain the inhibition of the oxidation of ethanol beyond the acetate level.

As mentioned above, the primary source of energy for hepatic metabolism is the oxidative breakdown of fatty acids (Fritz, 1961). If the initial breakdown of fatty acids were to proceed at a normal rate during the oxidation of ethanol ketone bodies would accumulate. This is not the case with livers from fed rats, and with livers from starved rats with intensive fatty acid oxidation only a fairly small accumulation of ketone bodies occurs. It is thus probable that the oxidation of ethanol inhibits not only the tricarboxylic acid cycle but also the early stages of fatty acid oxidation in the liver.

The change of the redox state of the liver during the oxidation of ethanol will produce new conditions for the NAD-dependent reactions. Thus the breakdown of galactose decreases (Isselbacher & Krane, 1961), urea production decreases (Field *et al.* 1963) and fatty acid synthesis increases during the oxidation of ethanol in the liver (Lieber & Schmid, 1961).

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