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Influence of Ethanol on the Metabolism of Perfused Normal, Fatty and Cirrhotic Rat Livers

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1. The influence of ethanol on the metabolism of perfused livers from normal rats and rats in various stages of development of dietary cirrhosis was studied. A choline-deficient, low-protein and high-fat diet was used. Results were 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 fatty and cirrhotic livers than in normal livers. Ethanol had no effect on the oxygen consumption of any of the various livers. After addition of ethanol to the perfusion medium carbon dioxide production ceased almost completely in normal livers. Only a slight decrease in the carbon dioxide production occurred in fatty and cirrhotic livers. 3. With every type of liver glucose was released from the liver into the perfusion medium during the initial control period. This release continued after the addition of ethanol to the perfusion medium in experiments with normal and fatty livers, whereas with cirrhotic livers a marked uptake of glucose from the medium was found. A simultaneous release of the glycolytic end products lactate and pyruvate into the medium occurred. 4. The production of ketone bodies was equal in normal and early fatty livers (6 weeks on the fat diet). It was smaller in late fatty livers (3–4 months on the fatty diet) and in cirrhotic livers. 5. The lactate/pyruvate concentration ratio in the perfusion medium increased from 11 to 67 with normal livers, from 12 to 16 with early fatty livers, from 13 to 26 with late fatty livers and from 21 to 55 with cirrhotic livers when the livers were perfused with a medium containing ethanol. The β-hydroxybutyrate/acetoacetate concentration ratio increased from 1·2 to 8·4 in normal livers, from 2·0 to 2·8 in early fatty livers, from 1·2 to 2·4 in late fatty livers and from 2·1 to 4·0 in cirrhotic livers when ethanol was added to the medium. 6. The effects of ethanol on liver metabolism during the development of dietary cirrhosis are discussed and related to human fatty liver and cirrhosis during chronic ethanol consumption.

The influence of ethanol on the liver metabolism of animals with hepatic injury has not been fully explored. Ethanol is often implicated, however, as a direct or indirect cause of cirrhosis (Best, Hartroft, Lucas & Ridout, 1949; Shorter & Baggenstoss, 1959; Klatskin, 1961). It is widely believed that the hepatic steatosis of alcoholics and cirrhosis may be related to subadequate food consumption, particularly of lipotropic agents (Klatskin, 1961; Popper, 1961). This is supported by studies on the long-term administration of ethanol, which demonstrate that ethanol increases the choline requirement (Kark, 1960). The fatty liver in this condition is readily corrected by administration of choline (Best *et al.* 1949). Nevertheless it is also evident that ethanol itself also plays an important role (Isselbacher & Greenberger, 1964).

It can be assumed that most of the changes in metabolism due to ethanol occur in the liver, which is the major site of oxidation of the administered ethanol (Masoro, Abramovitch & Birchard, 1953; Westerfeld, 1955). In a previous study (Forsander, Räihä, Salaspuro & Mäenpää, 1965) the effects of ethanol on liver metabolism were observed to be remarkable. The object of the present study was to compare the metabolic alterations produced in normal livers by ethanol with those occurring in fatty and cirrhotic livers during the development of dietary cirrhosis in rats.

The effect of ethanol on the oxygen consumption and carbon dioxide production of perfused rat livers of the three categories mentioned above was investigated. The effect of ethanol on the intermediary metabolism of these isolated livers was

further elucidated by studies on the release and uptake of glucose by the liver, and on changes in the lactate, pyruvate, β-hydroxybutyrate and acetoacetate concentrations in the perfused medium.

MATERIALS AND METHODS

Experimental animals and diet. Male albino rats of the Wistar strain from our own laboratory stock were used in all experiments. At the beginning of the experiments the rats weighed 110–140g. and were 6–8 weeks old. They were kept in wire-bottomed cages, six in each. They received a diet consisting of 8% of casein, 48% of sucrose, 38% of lard, 4% of salt mixture and 2% of cod-liver oil. Each kg. of the ration was supplemented with 4·29g. of cystine, 7·15mg. of thiamine hydrochloride, 14·6mg. of calcium pantothenate, 14·6mg. of nicotinic acid and 2·92mg. of riboflavine. Every week each animal was given 5mg. of α-tocopheryl acetate. On the fifth and eleventh days after the commencement of the experiment each animal was given 20mg. of choline to prevent the renal lesions that may prove fatal in rats during the first few weeks on a diet that is deficient in lipotropic factors (Hoffbauer, 1959). This diet largely corresponds to the diet LVI of György & Goldblatt (1949) and can be characterized as a low-protein high-fat diet with a relatively low content of lipotropic factors. Some of the rats received a standard laboratory diet and were used as controls. The accumulation of fat and the development of cirrhosis in the livers of each of the experimental rats were checked by histological examination. The changes were found to be similar to those described by Ahlqvist (1960) in a study in which a comparable diet was used. The operative technique and the perfusion method were the same as described by Forsander *et al.* (1965).

The perfusion medium consisted of a mixture of bovine erythrocytes (washed twice with 0·9% NaCl solution) in a Krebs–Ringer bicarbonate solution (Umbreit, Burris & Stauffer, 1964) with added glucose (final concn. 5–6mM) and bovine albumin fraction V (final concn. 2·5g./100ml.). The amount of erythrocytes was adjusted to give a haemoglobin concentration of 15g./100ml. The total amount of circulating medium in the beginning of the experiment was 200ml. The medium corresponds closely to that used by Schimassek (1962*a*). The bovine blood was collected not more than 24hr. before the experiment, heparinized and treated with Terramycin (10mg./l.).

In all experiments the test period lasted for 2hr. and the flow through the liver was maintained at over 0·6ml./min./g. of liver, which is the level necessary for normal oxidative metabolism (Schimassek, 1962*b*). Before the test period the liver was control-perfused for 1hr. to correct the anoxia caused by its isolation and to ensure that a steady state had been reached (Brauer, Leong & Pessotti, 1953). 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 200mg./100ml.

The samples for O_2 and CO_2 determinations were collected from the cannulas entering and leaving the liver in Teflon tubes filled with liquid paraffin. All other samples were collected from the blood reservoir.

Analytical methods. Glucose was determined enzymically by the method described by Huggett & Nixon (1957). Lactate and pyruvate were determined enzymically with Biochemica Boehringer (Mannheim, W. Germany) test kits. The ketone bodies (acetoacetate and β-hydroxybutyrate) were determined as described by Forsander *et al.* (1965). The Van Slyke manometric apparatus was used for the determination of the blood gases (Van Slyke & Neill, 1924).

RESULTS

Oxygen consumption and carbon dioxide production. Over the test period the oxygen consumption of livers from normal rats was 2·12–2·40 μmoles/g. wet wt. of liver/min. (Table 1). The value is of the same order as that found by others in the perfused rat liver (Schimassek, 1963). The addition of ethanol to the perfusion medium had no effect on oxygen consumption. Livers from the rats maintained for 6 weeks or 3–4 months on a fat diet had an oxygen consumption 1·98–2·82 μmoles/g. wet wt. of liver/min. The cirrhotic livers had the lowest oxygen consumption, 1·12–1·32 μmoles/g. wet wt. of liver/min. It has been reported that the oxygen consumption of liver slices from choline-deficient rats is significantly lower than that of normal liver slices (Welch, Irving & Best, 1935; Rees & Kline, 1957). When the protein content of the choline-deficient diet is adequate no decrease in oxygen consumption can be observed (Mishkel & Morris, 1963). The decreased oxygen consumption of the cirrhotic liver is presumably due to the decreased hepatic circulation (Bradley, Ingelfinger, Bradley & Curry, 1945) and to the relatively smaller proportion of functional tissue because of the accumulation of fat. The formation of scar tissue in the livers after the rupture of fatty cysts may also play a part (Hartroft, 1950). As in the normal livers, ethanol had no effect on the oxygen consumption of fatty or cirrhotic livers.

The formation of carbon dioxide was lower in the fatty and cirrhotic livers than in the normal ones. As shown in Table 1, the respiratory quotients of the livers from normal rats varied in the range 0·75–1·03, whereas they were slightly lower in fatty livers, about 0·62–0·81. This conforms with the results reported by Mishkel & Morris (1963). Ethanol had a marked effect on the carbon dioxide production of normal livers. After the addition of ethanol to the medium, carbon dioxide production ceased almost completely. This confirms the results obtained by Forsander *et al.* (1965). However, the decrease of carbon dioxide production was slight in the fatty and cirrhotic livers.

Changes in the glucose concentration of the perfusion medium. During the 1hr. control perfusion period the glucose concentration of the medium increased markedly in all experiments (Table 2). The increase was greatest in the experiments with normal livers. During the test period there was a

Table 1. *Oxygen consumption, carbon dioxide production and respiratory quotient during perfusion of normal, fatty and cirrhotic livers of the rat*

Experimental details are given in the text. Ethanol was added to the perfusion medium immediately after sampling at 60 min. The results for O_2 consumption and CO_2 production are given as means ± S.E.M. of three experiments.

Nature of liver	Ethanol present in perfusion medium	O_2 consumption (μmoles/g. wet wt. of liver/min.)			
Sampling time (min.)		60	90	120	180
Normal	−	2·40±0·42	2·28±0·22	2·35±0·30	2·12±0·28
	+	2·69±0·26	2·21±0·22	2·38±0·40	2·19±0·50
Fatty	−	2·42±0·16	2·82±0·41	2·31±0·52	1·98±0·19
(6 weeks on the diet)	+	2·17±0·21	1·81±0·08	2·03±0·18	1·85±0·17
Fatty	−	2·02±0·17	2·73±0·23	2·38±0·35	2·20±0·15
(3–4 months on the diet)	+	1·67±0·14	1·77±0·13	1·72±0·10	1·52±0·25
Cirrhotic	−	1·12±0·32	1·32±0·14	1·28±0·11	1·22±0·22
(6–8 months on the diet)	+	1·09±0·28	1·08±0·19	1·22±0·09	1·17±0·26
		CO_2 production (μmoles/g. wet wt. of liver/min.)			
Normal	−	1·81±0·19	1·98±0·12	2·05±0·32	2·18±0·09
	+	2·37±0·20	0·80±0·51	0·14±0·04	0·31±0·16
Fatty	−	1·94±0·18	1·75±0·20	1·73±0·12	1·33±0·17
(6 weeks on the diet)	+	1·22±0·20	0·74±0·12	0·62±0·07	0·64±0·11
Fatty	−	1·57±0·11	1·86±0·21	1·76±0·09	1·78±0·14
(3–4 months on the diet)	+	0·92±0·17	0·54±0·04	0·66±0·11	0·56±0·15
Cirrhotic	−	0·72±0·11	0·69±0·14	0·75±0·19	0·74±0·10
(6–8 months on the diet)	+	0·75±0·21	0·64±0·22	0·65±0·16	0·53±0·16
		Respiratory quotient			
Normal	−	0·75	0·87	0·87	1·03
	+	0·88	0·36	0·06	0·14
Fatty	−	0·80	0·62	0·75	0·67
(6 weeks on the diet)	+	0·56	0·41	0·31	0·27
Fatty	−	0·78	0·68	0·74	0·81
(3–4 months on the diet)	+	0·55	0·31	0·38	0·27
Cirrhotic	−	0·64	0·52	0·59	0·61
(6–8 months on the diet)	+	0·69	0·59	0·53	0·45

Table 2. *Changes in the concentrations of glucose in the perfusion medium during perfusion of normal, fatty and cirrhotic livers of the rat*

Experimental details are given in the text. Ethanol was added to the perfusion medium immediately after sampling at 60 min. The concentrations of glucose are given as means ± S.E.M. of three experiments.

Nature of liver	Ethanol present in perfusion medium	Concn. of glucose (mM)				
Sampling time (min.)		0	60	90	120	180
Normal	−	5·1±0·4	12·8±0·7	13·0±0·7	13·2±0·8	13·3±1·0
	+	5·7±0·3	11·5±0·5	12·7±0·5	13·3±1·0	15·8±0·5
Fatty	−	4·7±0·4	7·0±0·5	7·8±0·2	8·1±0·9	8·3±1·1
(6 weeks on the diet)	+	4·9±0·4	7·7±0·7	8·0±0·7	7·9±0·8	8·2±1·0
Fatty	−	4·7±0·6	7·8±0·5	8·1±0·8	8·9±1·2	9·4±1·1
(3–4 months on the diet)	+	4·5±0·1	8·6±0·6	8·5±0·7	8·8±1·0	9·6±1·4
Cirrhotic	−	5·0±0·4	7·2±0·3	7·8±0·9	7·9±0·5	8·1±1·2
(6–8 months on the diet)	+	6·0±0·7	8·1±0·3	5·8±0·9	5·4±0·8	4·2±1·0

further slight increase in the experiments with normal and fatty livers. This confirms previous observations on normal cat liver (Blixenkrone-Møller, 1938) and normal rat liver (Field, Williams & Mortimore, 1963). In the experiments with livers from cirrhotic rats a slight rise in the glucose con-

centration in the medium was observed in the control perfusion, whereas in the test perfusion the ethanol markedly increased glucose uptake from the medium.

Changes in metabolic products of the liver. In the experiments with normal livers the concentration of lactate in the medium decreased slowly during the perfusion and was not affected by ethanol (Table 3), which confirms the results obtained by Forsander *et al.* (1965). This occurred in the perfusions with fatty livers too, whereas with cirrhotic livers a marked increase in the lactate concentration was observed. With livers from cirrhotic rats the individual lactate concentrations during the experiments showed greater variations than with livers from normal rats. This might reflect differences in the degree of cirrhosis. In the perfusions with fatty livers the lactate and pyruvate concentrations were smaller than with normal or cirrhotic livers, probably indicating a slower glycolysis. In normal livers ethanol produced a marked decrease in the pyruvate concentration. In fatty or cirrhotic livers only a slight decrease in the pyruvate concentration was observed.

Without ethanol in the perfusion medium the lactate/pyruvate concentration ratios in normal and fatty livers were almost equal (Table 3). The ratio was similar to the ones found by Schimassek (1962*b*) and Hohorst, Kreutz & Bücher (1959) in normal livers. As far as we know, no values for the lactate/pyruvate ratio in fatty or cirrhotic livers have been reported. A higher lactate/pyruvate ratio was observed in experiments with cirrhotic livers. This may indicate a slight degree of hypoxia owing to the decreased hepatic circulation of the cirrhotic liver (Bradley *et al.* 1945; Kessler, Liebler, Bronfin & Sass, 1954).

Ethanol strongly increased the lactate/pyruvate concentration ratio in experiments with normal livers. By contrast, the increase was very slight in experiments with fatty livers. A twofold increase was observed in experiments with cirrhotic livers.

The initial concentrations of ketone bodies in the medium varied somewhat in the different experiments, but a gradual increase in the concentrations of β-hydroxybutyrate and acetoacetate was observed in all experiments without ethanol (Table 4). After the addition of ethanol the increase of acetoacetate ceased in experiments with normal livers, whereas in experiments with

Table 3. *Changes in the concentrations of lactate and pyruvate in the perfusion medium during perfusion of normal, fatty and cirrhotic livers of the rat*

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 ± S.E.M. of three experiments.

Nature of liver	Ethanol present in perfusion medium	Sampling time (min.).....	60	90	120	180
Normal	−	Concn. of lactate (μM)	3500±200	2922±164	2610±212	2140±240
	−	Concn. of pyruvate (μM)	271±21	289±11	291±14	231±14
	−	Lactate/pyruvate concn. ratio	13	10	9	9
Normal	+	Concn. of lactate (μM)	3013±259	2641±508	2480±638	2082±564
	+	Concn. of pyruvate (μM)	285±35	57±5	36±5	31±4
	+	Lactate/pyruvate concn. ratio	11	47	69	67
Fatty	−	Concn. of lactate (μM)	2070±231	1992±412	1858±514	1692±442
(6 weeks on the	−	Concn. of pyruvate (μM)	188±42	181±21	206±31	169±17
diet)	−	Lactate/pyruvate concn. ratio	11	11	9	10
Fatty	+	Concn. of lactate (μM)	2055±89	1844±77	1759±98	1737±31
(6 weeks on the	+	Concn. of pyruvate (μM)	171±5	142±5	136±3	109±5
diet)	+	Lactate/pyruvate concn. ratio	12	13	13	16
Fatty	−	Concn. of lactate (μM)	2564±322	2342±390	2186±141	1982±286
(3–4 months on	−	Concn. of pyruvate (μM)	197±21	146±27	219±41	180±16
the diet)	−	Lactate/pyruvate concn. ratio	13	18	10	11
Fatty	+	Concn. of lactate (μM)	1944±312	1615±290	1967±504	1645±302
(3–4 months on	+	Concn. of pyruvate (μM)	154±21	120±27	90±13	64±10
the diet)	+	Lactate/pyruvate concn. ratio	13	13	22	26
Cirrhotic	−	Concn. of lactate (μM)	3614±569	4123±691	4998±814	5962±912
(6–8 months on	−	Concn. of pyruvate (μM)	198±26	204±16	213±15	202±18
the diet)	−	Lactate/pyruvate concn. ratio	18	20	23	30
Cirrhotic	+	Concn. of lactate (μM)	3759±513	3959±604	4622±770	5604±832
(6–8 months on	+	Concn. of pyruvate (μM)	182±13	143±21	131±22	102±22
the diet)	+	Lactate/pyruvate concn. ratio	21	28	35	55

fatty or cirrhotic livers it continued. Ethanol increased the β-hydroxybutyrate/acetoacetate concentration ratio in normal livers, whereas there was no increase in the ratio in fatty livers and only a slight rise in cirrhotic livers. The increase followed much the same pattern as the lactate/pyruvate concentration ratio.

Rate of ethanol oxidation. Ethanol is oxidized in the liver by the enzyme alcohol dehydrogenase and the maximal rate of oxidation is reached even at a low ethanol concentration (less than 5 mM; Larsen, 1963). Therefore only one ethanol concentration was used in this experiment. A correct estimate 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 rates of oxidation of ethanol were almost equal in experiments with normal and fatty livers, whereas in experiments with cirrhotic livers the rate was somewhat lower.

Mikata, Dimakulangan & Hartroft (1963) observed that rats with fatty livers and minimal cirrhotic changes maintained almost normal rates of ethanol metabolism. Ethanol metabolism was progressively impaired as the rats developed cirrhosis, until finally both oral and intravenous administration of ethanol was followed by significantly decreased rates of clearance in the cirrhotic rats. In the same study hepatic alcohol-dehydrogenase activity was found to be decreased only in cirrhotic rats, which confirms the results reported by Figueroa & Klotz (1962) with human subjects. In the light of this, it can be assumed that the

Table 4. *Changes in the concentrations of β-hydroxybutyrate and acetoacetate in the perfusion medium during perfusion of normal, fatty and cirrhotic livers of the rat*

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 ± S.E.M. of three experiments.

Nature of liver	Ethanol present in perfusion medium	Sampling time (min.).....	60	90	120	180
Normal	−	Concn. of β-hydroxybutyrate (μM)	172±29	272±29	414±82	986±113
	−	Concn. of acetoacetate (μM)	124±14	259±41	389±39	1043±121
	−	β-Hydroxybutyrate/acetoacetate concn. ratio	1·4	1·1	1·1	0·9
Normal	+	Concn. of β-hydroxybutyrate (μM)	331±114	904±269	1373±357	2036±507
	+	Concn. of acetoacetate (μM)	268±101	229±43	232±46	241±50
	+	β-Hydroxybutyrate/acetoacetate concn. ratio	1·2	3·9	5·9	8·4
Fatty (6 weeks on the diet)	−	Concn. of β-hydroxybutyrate (μM)	770±71	780±49	910±86	1550±104
	−	Concn. of acetoacetate (μM)	200±15	370±23	360±28	510±29
	−	β-Hydroxybutyrate/acetoacetate concn. ratio	3·9	2·1	2·5	3·0
Fatty (6 weeks on the diet)	+	Concn. of β-hydroxybutyrate (μM)	616±39	856±73	1117±110	1327±75
	+	Concn. of acetoacetate (μM)	302±85	302±14	399±34	478±6
	+	β-Hydroxybutyrate/acetoacetate concn. ratio	2·0	2·8	2·8	2·8
Fatty (3–4 months on the diet)	−	Concn. of β-hydroxybutyrate (μM)	410±51	520±76	570±51	820±101
	−	Concn. of acetoacetate (μM)	180±14	260±22	280±48	380±39
	−	β-Hydroxybutyrate/acetoacetate concn. ratio	2·2	2·0	2·0	2·2
Fatty (3–4 months on the diet)	+	Concn. of β-hydroxybutyrate (μM)	197±46	192±32	313±50	556±59
	+	Concn. of acetoacetate (μM)	162±37	198±29	194±14	236±30
	+	β-Hydroxybutyrate/acetoacetate concn. ratio	1·2	1·0	1·6	2·4
Cirrhotic (6–8 months on the diet)	−	Concn. of β-hydroxybutyrate (μM)	118±41	278±73	346±91	586±65
	−	Concn. of acetoacetate (μM)	59±12	79±13	121±34	136±19
	−	β-Hydroxybutyrate/acetoacetate concn. ratio	2·0	3·5	2·8	4·3
Cirrhotic (6–8 months on the diet)	+	Concn. of β-hydroxybutyrate (μM)	146±68	309±80	319±48	511±79
	+	Concn. of acetoacetate (μM)	70±25	84±17	116±18	128±8
	+	β-Hydroxybutyrate/acetoacetate concn. ratio	2·2	3·7	2·8	4·0

metabolic changes caused by ethanol in fatty livers compared with the ones in normal livers, and presented in this study, cannot be due to alterations in the rate of ethanol metabolism.

DISCUSSION

Influence of ethanol on oxygen consumption and carbon dioxide production. Ethanol did not influence the oxygen consumption of any of the types of liver. This conforms with results obtained in experiments with normal human subjects (Lundquist, Tygstrup, Winkler, Mellemgaard & Munck-Petersen, 1962), with normal liver slices (Fondal & Kochakian, 1951) and with perfused livers of normal and starved rats (Forsander *et al.* 1965). The marked decrease in carbon dioxide production evoked by ethanol has been discussed by Forsander *et al.* (1965). It was suggested that the citric acid cycle is inhibited during the oxidation of ethanol. The low respiratory quotient of fatty livers in the perfusions without ethanol is peculiar to the endogenous oxidation of fatty acids to ketone bodies in partial inhibition of the citric acid cycle. In late fatty and cirrhotic livers this seems not to be the case (Table 4), because lower concentrations of ketone bodies are found than with normal livers. In fatty and cirrhotic livers ethanol caused only a slight additional decrease in the respiratory quotient, in contrast with its effect on normal livers. Maintenance on the high-fat low-protein diet has altered the metabolic response of livers to ethanol. This change appears to be related to a metabolic adaptation to the fatty diet. The present results are not sufficient to elucidate the nature of this adaptation.

Influence of ethanol on metabolic pathways. Table 5 shows the quantitative changes of glucose, lactate + pyruvate and β-hydroxybutyrate + acetoacetate in the medium during the perfusion of the four types of livers. Great differences exist, especially in the release and uptake of glucose and glycolytic end products. In experiments with normal livers glucose was released rapidly into the perfusion medium, probably from the glycogen depots during the control perfusion period (Table 2). The increase in the glucose concentration was not so marked in experiments with fatty or cirrhotic livers, and this is presumably due to the diminished glycogen content of the fatty and cirrhotic livers.

The most striking effects of ethanol occurred in livers of cirrhotic rats. A marked uptake of glucose from the medium was seen (Table 2), and simultaneously glycolytic end products, lactate and pyruvate, appeared in the perfusion medium. The impaired circulation of the cirrhotic liver and the slight hypoxia may at least partly be responsible for the observed changes in glucose uptake. No reports could be found on the influence of ethanol on glycolysis in the liver. In a previous study (Forsander *et al.* 1965) the same kind of uptake of glucose and increase in glycolytic end products was observed in perfused starved rat livers, and it can be assumed that the aetiology of the two cases is the same.

The total production of ketone bodies was almost equal in experiments with normal and with fatty livers from rats that had been 6 weeks on the fat diet (Table 2). This confirms results obtained from perfusion of livers from choline-deficient rats (Mishkel & Morris, 1963). The production of ketone bodies decreased in the experiments with fatty livers (3–4 months on the fat diet) and with cirrhotic livers. This is probably partly due to adaptation during the fat diet (McClellan & Du Bois, 1930; I. El Rawi & E. Geiger, unpublished work cited by

Table 5. *Net production or consumption of metabolites by normal, fatty and cirrhotic livers of the rat during perfusion* (120 *min.*) *with ethanol added to the perfusion 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 value represents the mean value of three experiments.

Nature of liver	Ethanol present in perfusion medium	Production or consumption of metabolites (μmoles/g. wet wt. of liver)		
		Glucose	Lactate + pyruvate	Ketone bodies
Normal	−	+16·2	−3·6	+5·9
	+	+26·0	−4·2	+7·8
Fatty (6 weeks on the diet)	−	+5·6	−1·1	+6·1
	+	+5·1	−2·7	+6·5
Fatty (3–4 months on the diet)	−	+5·4	−1·4	+2·8
	+	+4·6	−2·1	+2·3
Cirrhotic (6–8 months on the diet)	−	+4·8	+13·9	+2·9
	+	−36·6	+12·5	+3·3

Deuel, 1957) and partly to the diminished metabolic capacities of the late fatty and cirrhotic livers.

Influence on the redox state. In experiments with human subjects ethanol has been observed to raise the normal lactate/pyruvate concentration ratio (Büttner, 1961; Seligson, Stone & Nemir, 1959). Liver perfusion experiments with rats have confirmed this (Forsander *et al.* 1965).

In normal livers, when ethanol is available as substrate, a shift in the redox state of the cytoplasm occurs, which is manifested as a decrease in the liver NAD/$NADH_2$ concentration ratio (Räihä & Oura, 1962). On the other hand, Schimassek (1963) has shown in perfusion experiments 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 extramitochondrial cytoplasmic compartment of the liver cell. In fatty livers the shift in the redox state of the cytoplasm to a more negative condition caused by ethanol was greatly decreased (Table 3). Maintenance on the high-fat, low-protein and choline-deficient diet altered the capacities of fatty liver cells to metabolize the extramitochondrial reducing equivalents.

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). It can be assumed that these alterations in liver metabolism caused by ethanol do not occur in fatty livers. Because fatty liver and dietary cirrhosis induced by choline deficiency are regarded as the experimental duplicate of alcoholic fatty liver and cirrhosis in man (Hartroft, 1964), these observations may throw some new light on the interpretation of galactose tolerance tests in human alcoholics.

REFERENCES

Ahlqvist, J. (1960). *Acta path. microbiol. scand.* **50**, suppl. 142, 1.

Best, C. H., Hartroft, W. S., Lucas, C. C. & Ridout, J. H. (1949). *Brit. med. J.* **2**, 1001.

Blixenkrone-Møller, N. (1938). *Hoppe-Seyl. Z.* **252**, 117.

Bradley, S. E., Ingelfinger, F. J., Bradley, G. P. & Curry, J. J. (1945). *J. clin. Invest.* **24**, 890.

Brauer, R. W., Leong, G. F. & Pessotti, R. L. (1953). *Amer. J. Physiol.* **174**, 304.

Büttner, H. (1961). *Dtsch. Arch. klin. Med.* **207**, 1.

Deuel, H. J., jun. (1957). *The Lipids*, vol. 3, p. 159. New York: Interscience Publishers Inc.

Field, J. B., Williams, H. E. & Mortimore, G. E. (1963). *J. clin. Invest.* **42**, 497.

Figueroa, R. B. & Klotz, A. P. (1962). *Gastroenterology*, **43**, 10.

Fondal, E. & Kochakian, C. D. (1951). *Proc. Soc. exp. Biol., N.Y.*, **77**, 823.

Forsander, O. A., Räihä, N., Salaspuro, M. & Mäenpää, P. (1965). *Biochem. J.* **94**, 259.

György, P. & Goldblatt, H. (1949). *J. exp. Med.* **89**, 245.

Hartroft, W. S. (1950). *Anat. Rec.* **106**, 61.

Hartroft, W. S. (1964). In *The Liver*, vol. 2, p. 477. Ed. by Rouiller, Ch. New York: Academic Press Inc.

Hoffbauer, F. W. (1959). *A.M.A. Arch. Path.* **68**, 160.

Hohorst, H. J., Kreutz, F. H. & Bücher, Th. (1959). *Biochem. Z.* **332**, 18.

Huggett, A. St G. & Nixon, D. A. (1957). *Biochem. J.* **66**, 12P.

Isselbacher, K. J. & Greenberger, N. J. (1964). *New Engl. J. Med.* **270**, 402.

Isselbacher, K. J. & Krane, S. M. (1961). *J. biol. Chem.* **236**, 2394.

Kark, R. M. (1960). *Gastroenterology*, **39**, 643.

Kessler, B. J., Liebler, J. B., Bronfin, G. J. & Sass, M. (1954). *J. clin. Invest.* **33**, 1338.

Klatskin, G. (1961). *Gastroenterology*, **41**, 443.

Larsen, J. A. (1963). *Acta physiol. scand.* **57**, 209.

Lieber, C. S. & Schmid, R. (1961). *J. clin. Invest.* **40**, 394.

Lundquist, F., Tygstrup, N., Winkler, K., Mellemgaard, K. & Munck-Petersen, S. (1962). *J. clin. Invest.* **41**, 955.

McClellan, W. S. & Du Bois, E. F. (1930). *J. biol. Chem.* **87**, 651.

Masoro, E. J., Abramovitch, H. & Birchard, J. R. (1953). *Amer. J. Physiol.* **173**, 37.

Mikata, A., Dimakulangan, A. A. & Hartroft, W. S. (1963). *Gastroenterology*, **44**, 159.

Mishkel, M. A. & Morris, B. (1963). *Quart. J. exp. Physiol.* **48**, 202.

Popper, H. (1961). *Acta hepato-splenol.* **8**, 279.

Räihä, N. C. R. & Oura, E. (1962). *Proc. Soc. exp. Biol., N.Y.*, **109**, 908.

Rees, E. D. & Kline, D. L. (1957). *Amer. J. Physiol.* **190**, 446.

Schimassek, H. (1962*a*). *Life Sci.* **1**, 629.

Schimassek, H. (1962*b*). *Life Sci.* **1**, 635.

Schimassek, H. (1963). *Biochem. Z.* **336**, 468.

Seligson, D., Stone, H. H. & Nemir, P., jun. (1959). *Surg. Forum*, **9**, 85.

Shorter, R. G. & Baggenstoss, A. H. (1959). *Amer. J. clin. Path.* **32**, 422.

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964). *Manometric Techniques*, 4th ed., p. 132. Minneapolis: Burgess Publishing Co.

Van Slyke, D. D. & Neill, J. M. (1924). *J. biol. Chem.* **61**, 523.

Welch, M. S., Irving, L. & Best, C. H. (1935). *Amer. J. Physiol.* **113**, 136.

Westerfeld, W. W. (1955). *Tex. Rep. Biol. Med.* **13**, 559.