The Effect of Acute and Prolonged Ethanol Treatment on the Contents of Coenzyme A, Carnitine and Their Derivatives in Rat Liver

By JENS KONDRUP and NIELS GRUNNET

Department of Biochemistry A, University of Copenhagen, Juliane Mariesvej 30,
DK-2100 Copenhagen, Denmark

(Received 10 August 1972)

1. CoA, acetyl-CoA, long-chain acyl-CoA, carnitine, acetylcarnitine and long-chain acylcarnitine were measured in rat liver under various conditions. 2. Starvation caused an increase in the contents of these intermediates, except that of carnitine. 3. A single dose of ethanol had no effect on CoA content, whereas those of acetyl-CoA, acetylcarnitine and carnitine were increased and those of long-chain acyl-CoA and acylcarnitine were decreased. 4. Four weeks' adaptation to ethanol consumption did not change the effect of ethanol administration on these metabolites. 5. It is suggested that ethanol directly increases hepatic fatty acid synthesis and esterification. It is also suggested that this change is reversible and limited to the period of ethanol oxidation. 6. It is demonstrated that ethanol-induced triglyceride accumulation is not related to carnitine deficiency.

The fraction of CoA and carnitine that is esterified is known to change according to the state of hepatic fatty acid metabolism, e.g. to increase during starvation and to decrease after carbohydrate feeding (Tubbs & Garland, 1964; Böhmer *et al.*, 1966; Pearson & Tubbs, 1967; Böhmer, 1967).

Ethanol is known to decrease fatty acid oxidation and to enhance fatty acid synthesis and esterification in liver (Lieber *et al.*, 1966).

The object of the present work was to examine whether the effect of ethanol on hepatic fat metabolism was reflected in the acylation state of hepatic CoA and carnitine.

Under some conditions triglyceride accumulation and depressed fatty acid oxidation in heart and liver is correlated to a decreased content of carnitine (Bressler & Wittels, 1965; Corredor *et al.*, 1967; Wittels & Spann, 1968). Therefore experiments with prolonged ethanol treatment were carried out to determine whether ethanol-induced triglyceride accumulation is also related to carnitine deficiency. These experiments also permit a comparison between the effects of acute and prolonged ethanol treatment on the CoA and carnitine intermediates of hepatic fat metabolism.

Materials and Methods

Chemicals and enzymes

NAD⁺, CoA, acetyl phosphate, acetylcarnitine, malate dehydrogenase, citrate synthase, phosphotransacetylase, carnitine acetyltransferase, α -glycerophosphate dehydrogenase and alcohol dehydrogenase were purchased from C. F. Boehringer und

Soehne G.m.b.H., Mannheim, Germany. Malate and dithiothreitol were obtained from Calbiochem, Los Angeles, Calif., U.S.A. 5,5'-Dithiobis(2-nitrobenzoate) was from Aldrich Chemical Co., Milwaukee, Wis., U.S.A., and L-carnitine was from Mann Research Laboratories, New York, N.Y., U.S.A. ⁶⁰Co(NO₃)₂ was purchased from AEK Forsøgsanlaeg, Risø, Denmark. Palmitoylcarnitine was synthesized as described by Bremer (1968). Acetyl-CoA was prepared by the method of Simon & Shemin (1953).

Animals

Six female Wistar rats weighing 130–140 g were used in each experiment. Animals used as fed controls had free access to an ordinary laboratory chow diet. Starved animals were deprived of food for 8 or 24 h in wire-bottomed cages, but had free access to drinking water. In experiments with acute ethanol treatment the rats were given 3 g of ethanol/kg body weight in an aqueous 13% solution by gastric tube, 2½–3 h before they were killed. The food was withdrawn after intubation.

The experiments with prolonged ethanol treatment were carried out by the techniques of Lieber & DeCarli (1970), by using pair-feeding with liquid diets. Three groups treated with different diets were examined: a glucose control diet containing, in percentage of total calories, 15% casein, 52% glucose and 33% fat; a high-fat diet containing 15% casein, 12% glucose and 73% fat; an ethanol diet containing 15% casein, 12% glucose, 41% fat and 32% ethanol. The composition of fat was, in percentage of

total fat: peanut oil 65%; corn oil 27%; cod liver oil 8%. Salts, vitamins, choline, methionine and cystine were added to all diets in the amounts used by Lieber & DeCarli (1970).

The rats were kept in single cages and fed twice a day from drinking tubes. During the last 24 h before being killed they were fed every 8 h by gastric tube. The last meal was given 8 h before death to minimize the blood ethanol concentration at the time of killing and thus permit a distinction between acute and prolonged ethanol treatment. The animals gained weight during the 4 weeks experimental period.

All animals were killed by cervical dislocation. The livers were quickly excised and freeze-clamped in an aluminium piston that had been pre-cooled in liquid N₂.

Extraction procedures

CoA and its derivatives were extracted as described by Williamson & Corkey (1969). The esterified derivatives of CoA were hydrolysed at pH 12.5 at room temperature (22–25°C) for 30 min. After hydrolysis the pH was adjusted with HClO₄ to 4.5.

Carnitine and its derivatives were extracted as described by Bøhmer *et al.* (1966). This method is known to extract labelled carnitine compounds completely. The butanol dissolved in the water phase inhibited the enzymic assay, and was therefore removed by washing with an equal volume of chloroform.

Water-soluble carnitine esters were hydrolysed in 0.1 M-KOH for 60 min at room temperature. Butanol-soluble carnitine esters (long-chain acyl-carnitine) were hydrolysed by adding 200 µl of 1 M-KOH to 2 ml of the butanol phase at 50°C. After 15 min of shaking, the sample was neutralized by adding 10 µl of 70% (v/v) HClO₄ and 100 µl of 0.8 M-Tris-HCl (pH 7.8). The water phase was separated from the butanol phase by centrifugation and washed with chloroform. Hydrolysis of palmitoyl-L-carnitine was tested by using 1, 2, 3 and 6 M-KOH; carnitine recovery was 86%, 93%, 87% and 43% respectively.

Assays

Acetyl-CoA. This was determined in the assay described by Wieland & Weiss (1963). The cuvette contained Tris-HCl (240 mM, pH 7.4), NAD⁺ (2 mM), malate (7 mM) and 1 ml of HClO₄ extract in a total volume of 1.1 ml. The reaction was started by addition of 2.5 units of malate dehydrogenase followed by 0.5 unit of citrate synthase. A correction was necessary since the formation of NADH is not stoichiometric with the disappearance of acetyl-CoA (Buckel & Eggerer, 1965).

CoA. This was converted into acetyl-CoA with

acetyl phosphate and phosphotransacetylase (Michal & Bergmeyer, 1970). The acetylation was carried out in a vessel containing Tris-HCl (240 mM, pH 7.4), acetyl phosphate (30 mM), dithiothreitol (650 µM), phosphotransacetylase (1 unit) and 1 ml of HClO₄ extract in a total volume of 1.17 ml. The vessel was incubated at 27°C for 30 min and afterwards the enzyme was inactivated by boiling for 1 min. This caused no loss of acetyl-CoA. Total acetyl-CoA was determined in the assay described above, and the tissue content of acetyl-CoA was subtracted, giving the amount of CoA.

Total water-soluble CoA compounds. These were determined by the same assay after alkaline hydrolysis of the HClO₄ extract. The hydrolysis never revealed an amount of CoA compounds that exceeded the sum of CoA and acetyl-CoA by more than 5%. Therefore the values for short-chain derivatives of CoA are not shown in the tables and the term acyl-CoA is used synonymously with long-chain acyl-CoA.

Acyl-CoA. This was determined as for CoA after hydrolysis of the acid-insoluble fraction.

Carnitine. This was measured as CoA formed from acetyl-CoA after addition of carnitine acetyltransferase (Pearson *et al.*, 1970). The use of chloroform-methanol extraction makes determination of tissue carnitine by the dithionitrobenzoate method possible because high tissue blanks are avoided. Dithionitrobenzoate slowly inhibits carnitine acetyltransferase, but this is of no importance in a short-time assay, which is started by addition of the transferase. The cuvette contained Tris-HCl (130 mM, pH 7.8), dithionitrobenzoate (325 µM), acetyl-CoA (70 µM) and sample in a total volume of 800 µl; 1 unit of carnitine acetyltransferase was used.

Total water-soluble carnitine. The same assay was used to determine these compounds after hydrolysis. Under none of the conditions examined did this value exceed the sum of carnitine and acetylcarnitine by more than 10%, and therefore the contents of short-chain derivatives of carnitine are not shown in the tables, and the term acetylcarnitine is used to mean long-chain acetylcarnitine.

Acetylcarnitine. This was measured as carnitine after hydrolysis of the butanol phase.

Acetylcarnitine. This was measured in the assay described by Pearson *et al.* (1970). The cuvette contained Tris-HCl (115 mM, pH 7.8), CoA (260 µM), malate (9 mM), NAD⁺ (2.4 mM), dithiothreitol (3 mM) and 300 µl of chloroform-methanol extract in a total volume of 800 µl. The reaction was started with 3.5 units of malate dehydrogenase, followed by 0.4 unit of citrate synthase and 1.2 units of carnitine acetyltransferase. These measurements were also corrected as described by Buckel & Eggerer (1965).

Plasma contents of free fatty acids were determined as described by Ho & Meng (1969). Blood ethanol was analysed by the method of Bernt & Gutman (1970).

The method of Hohorst (1970) was used for measurement of α -glycerophosphate, and triglyceride was determined in the chloroform-methanol extract with a test combination from Boehringer.

Recovery and reproducibility

Livers from six rats were pooled, frozen, powdered and mixed. The powder was divided into 12 equal parts; six of these were submitted to the HClO_4 -extraction procedure and the other six to the chloroform-methanol extraction procedure (see under 'Extraction procedures'). CoA, acetyl-CoA, carnitine, acetylcarnitine and palmitoylcarnitine, added as internal standards in physiological amounts to three of the samples in each group at the first homogenization step, were recovered by 90–100%.

Based on estimations from the other three samples in each group reproducibility of the extraction and assay procedures, expressed as the largest percentage deviation from the mean, was: CoA, 2%; acetyl-CoA, 10%; acyl-CoA, 7%; carnitine, 4%; acetylcarnitine, 4%; acylcarnitine, 5%.

Results

The contents of CoA, acetyl-CoA, acyl-CoA, carnitine and acylcarnitine in fed control rats and the changes after 24h of starvation (Table 1) correspond to results of other workers (Garland, 1964; Tubbs & Garland, 1964; Pearson & Tubbs, 1967; Lumbers *et al.*, 1969).

The content of acetylcarnitine in these rats is three times higher than the value reported by Pearson & Tubbs (1967).

The amount of carnitine and its derivatives was also determined in livers from 200g female rats. These animals contained 250–300nmol of carnitine/g wet wt., 130–180nmol of acetylcarnitine/g wet wt., and about 10nmol of acylcarnitine/g wet wt. Thus it appears that the amount of carnitine in liver depends on the weight of the animal. In the 200g rats the percentage of total carnitine accounted for as acetylcarnitine corresponds to the results obtained by Bøhmer *et al.* (1966). Since we have used rats from the same origin and the same extraction procedure as Bøhmer *et al.* (1966), the high amount of acetylcarnitine in our rats compared with values found by Pearson & Tubbs (1967) may be due to differences in strain or extraction procedure.

After acute ethanol treatment the amount of CoA is unchanged compared with fed controls (Table 1) whereas acetyl-CoA is doubled ($P < 0.001$) and acyl-CoA is decreased by 30% ($P < 0.025$). The total content of CoA compounds is increased by 50% ($P < 0.001$). The contents of carnitine and acetylcarnitine are increased by 50% ($P < 0.01$ and < 0.02 respectively), whereas that of acylcarnitine is decreased by 50% ($P < 0.05$). The total content of

Table 1. Contents of CoA, carnitine and derivatives in female rat liver

Liver tissue was extracted and analysed as described in the text. See the text for details about dietary treatments. Six rats were used in each experiment; results are means $\pm$ S.D.; relevant P values are reported in the Results section.

	Content (nmol/g wet wt.)							
	CoA	Acetyl-CoA	Acyl-CoA	Total CoA	Carnitine	Acetyl-carnitine	Acyl-carnitine	Total carnitine
Fed controls	44.7 ± 9.4	35.6 ± 5.6	15.9 ± 2.9	99.3 ± 5.6	140 ± 15	138 ± 42	16.3 ± 5.6	290 ± 51
Starved 24h	89.7 ± 15	77.8 ± 10	33.8 ± 6.2	226 ± 35	116 ± 14	275 ± 63	49.5 ± 15	441 ± 83
Acute ethanol	53.6 ± 12	77.3 ± 6.0	11.3 ± 3.2	142 ± 12	213 ± 54	203 ± 31	8.3 ± 1.9	425 ± 76

carnitine compounds is increased by 50% ($P < 0.01$). The fraction of the CoA esterified with fatty acids ('CoA acylation ratio', defined in Table 3) is decreased by 30% ($P < 0.05$) and the corresponding carnitine acylation ratio is decreased by 60% ($P < 0.005$) (Table 3).

Hepatic α -glycerophosphate in rats starved for 8 and 24 h, and after acute ethanol treatment, was determined as 178 ± 22 , 362 ± 55 and 967 ± 154 nmol/g wet wt. (mean $\pm$ s.d.) respectively.

The plasma concentration of free fatty acids (μM , $\pm$ s.d.) was 199 ± 125 in fed controls and 568 ± 186 after acute ethanol treatment.

Experiments with liquid diets

The difference between fed controls and rats given the balanced glucose diet (Tables 1 and 2) are explicable by the 8 h of starvation preceding killing. This can be seen from the effects of 8 h starvation shown in Table 2. Contents of acetyl-CoA and acyl-CoA are doubled ($P < 0.001$) and CoA is slightly increased ($P < 0.05$). The rats given the balanced glucose diet contain 60% more acylcarnitine than do fed controls ($P < 0.005$), which is compatible with a condition of mild starvation (Bøhmer, 1967).

The effect of prolonged intake of a diet containing 70% of the total calories as fat is shown in Table 2. These rats differ from those on the glucose control diet only by slightly increased acylcarnitine ($P < 0.05$). Rats that had received 36% of their total calories as ethanol during 4 weeks had increased carnitine and acetylarnitine contents ($P < 0.02$ and < 0.01 respectively). The other results are similar to those for rats on the glucose control diet.

Hepatic triglyceride content, expressed as mg/g wet wt., $\pm$ s.d., was: glucose controls, 13.2 ± 3.3 ; high-fat group, 12.2 ± 4.0 ; prolonged-ethanol group, 22.4 ± 4.6 . The triglyceride accumulation in the ethanol-treated rats is less pronounced than in the reports of Lieber & DeCarli (1970). No explanation can be offered for this discrepancy.

At the time of killing blood ethanol concentration in the prolonged-ethanol group was 12.7 ± 5.1 (mM, $\pm$ s.d.).

Discussion

Acetylation and acylation states of CoA and carnitine

The degree of acylation of CoA and carnitine may reflect the state of fat metabolism, since these compounds mediate activation and transfer of acetyl and acyl units. Therefore acetylation and acylation ratios for CoA and carnitine have been calculated (Table 3). It might be expected that these ratios would vary in parallel since the equilibrium constants of the carnitine transferase reactions are near to 1 (Norum, 1964; Bremer & Aas, 1969).

Such a parallelism is not found, however. Compared with fed controls, the carnitine acetylation ratio is increased by 40% after starvation for 24 h ($P < 0.001$), whereas the CoA acetylation ratio is unchanged. After acute ethanol treatment, on the other hand, the carnitine acetylation ratio is unchanged, whereas the CoA acetylation ratio is increased by 25% ($P < 0.005$).

After starvation for 24 h the carnitine acylation ratio is increased 3-fold ($P < 0.001$), but the CoA acylation ratio remains unchanged. Since the carnitine transferases are usually supposed to have access to all compartments the lack of parallelism may reflect compartmentation, e.g. by specific protein binding of the substrates.

The suggestion by Bøhmer *et al.* (1966) that the carnitine acylation ratio may be taken as an indirect measure of the CoA acylation ratio is thus not supported by these results.

Apparently, the carnitine acylation ratio does reflect the direction of hepatic fat metabolism. This ratio is increased in starved animals, which have increased fat oxidation, and it is decreased in rats subjected to acute ethanol treatment, which have decreased fat oxidation and increased fat synthesis. The CoA acylation ratio, however, does not always parallel fat oxidation, since it remains unchanged after 24 h starvation. In contrast, Tubbs & Garland (1964) found the CoA acylation and acetylation ratios to be increased after 48 h starvation. They did not, however, find CoA to be lower in fed rats than in starved rats. Tubbs & Garland (1964) used sorbic acid and acyl-CoA synthetase for determination of CoA. Bergmeyer (1970) reports that acyl-CoA synthetase, unlike phosphotransacetylase, also reacts with dephospho-CoA, and it may be that dephospho-CoA is present in the fed state in relatively high amounts. After 8 h starvation (Table 2) the CoA acylation and acetylation ratios do increase because CoA is not yet increased to 'compensate' the increased acetyl-CoA and acyl-CoA.

Mass-action ratios. Table 4 shows that the mass-action ratios of the acetyl- and long-chain-carnitine acyltransferase reactions are close to K_{eq} in fed controls and glucose controls.

During starvation both mass-action ratios are increased owing to a relatively more increased acetylation and acylation of carnitine ($P < 0.005$ and < 0.001 respectively), but the ratio between the mass-action ratios is not significantly changed. After acute ethanol treatment both mass-action ratios are diminished because of increased carnitine ($P < 0.02$), but still the relationship between the mass-action ratios is unchanged. This constant relationship, which is near the theoretical value, may reflect a coupling between the two transferase reactions. However, the compartmentation of the compounds involved makes such a suggestion highly speculative.

Table 2. Contents of CoA, carnitine and derivatives in female rat liver after 4 weeks' treatment with liquid diets

See the text for experimental details. Six rats were used in each experiment; results are means $\pm$ s.d. Relevant *P* values are reported in the Results section. Dashes indicate quantities not determined.

	Content (nmol/g wet wt.)						
	CoA	Acetyl-CoA	Acyl-CoA	Total CoA	Carnitine	Acetyl-carnitine	Acyl-carnitine
Glucose controls	62.2 $\pm$ 8.6	69.4 $\pm$ 6.0	39.3 $\pm$ 6.0	171 $\pm$ 9	146 $\pm$ 15	157 $\pm$ 29	26.0 $\pm$ 1.0
High-fat diet	67.0 $\pm$ 15	73.8 $\pm$ 6.0	43.0 $\pm$ 10	184 $\pm$ 13	155 $\pm$ 40	132 $\pm$ 30	33.9 $\pm$ 9.0
Prolonged ethanol diet	53.2 $\pm$ 13	70.9 $\pm$ 8.2	31.1 $\pm$ 5.0	155 $\pm$ 18	235 $\pm$ 74	254 $\pm$ 67	35.0 $\pm$ 10
Starved 8 h	59.6 $\pm$ 13	81.0 $\pm$ 11	32.7 $\pm$ 4.9	171 $\pm$ 27	—	—	—
							Total carnitine
							329 $\pm$ 42
							321 $\pm$ 62
							577 $\pm$ 135

Table 3. Acetylation and acylation ratios of CoA and carnitine in female rat liver

The ratios are based on the same experiments as in Table 1 and were calculated for each of the six animals. The results are means $\pm$ s.d.

	'CoA acetylation ratio'	'CoA acylation ratio'	'Carnitine acetylation ratio'	'Carnitine acylation ratio'
	Acetyl-CoA (CoA + acetyl-CoA)	Acyl-CoA (CoA + acyl-CoA)	Acetyl-carnitine (Carnitine + acetyl-carnitine)	Acyl-carnitine (Carnitine + acyl-carnitine)
Fed controls	0.48 $\pm$ 0.08	0.27 $\pm$ 0.06	0.49 $\pm$ 0.08	0.10 $\pm$ 0.03
Starved 24 h	0.47 $\pm$ 0.05	0.27 $\pm$ 0.03	0.70 $\pm$ 0.03	0.30 $\pm$ 0.06
Acute ethanol	0.59 $\pm$ 0.06	0.18 $\pm$ 0.06	0.49 $\pm$ 0.05	0.04 $\pm$ 0.01

Table 4. *Mass-action ratios of carnitine transferase reactions*

The ratios are based on the same experiments as in Tables 1 and 2, and were calculated for each of the six animals. The results are means $\pm$ s.d.

	$\frac{[\text{Acylcarnitine}][\text{CoA}]}{[\text{Acyl-CoA}][\text{Carnitine}]}$ (a)	$\frac{[\text{Acetylcarnitine}][\text{CoA}]}{[\text{Acetyl-CoA}][\text{Carnitine}]}$ (b)	(a)/(b)
Fed controls	0.31 ± 0.06	1.18 ± 0.32	0.28 ± 0.11
Glucose controls	0.29 ± 0.07	0.96 ± 0.14	0.31 ± 0.11
Starved 24 h	1.31 ± 0.31	2.77 ± 0.99	0.40 ± 0.08
Acute ethanol	0.17 ± 0.05	0.65 ± 0.12	0.28 ± 0.11
K_{eq} *	0.45	1.67	0.27

* Norum (1964); Bremer & Aas (1969).

Effect of acute ethanol treatment

Acute ethanol treatment causes a decrease in hepatic acyl-CoA, acylcarnitine and the CoA and carnitine acylation ratios (Tables 1 and 3) (for statistical significance see the Results section). Hepatic ethanol oxidation depresses fatty acid oxidation and increases fatty acid synthesis and esterification (Lieber *et al.*, 1966, 1967). Carbohydrate administration also leads to lowered contents of acyl-CoA and acylcarnitine, low CoA and carnitine acylation ratios, and to depressed fatty acid oxidation concomitant with increased fatty acid synthesis and esterification. However, carbohydrate administration lowers, whereas ethanol increases, plasma contents of fatty acids (see the Results section). Increased plasma contents of fatty acids after starvation, diabetes and fat-feeding leads to increased fat oxidation, increased acyl-CoA and acylcarnitine contents and increased CoA and carnitine acylation ratios (Tables 1 and 3).

Thus it appears that ethanol has a carbohydrate-like effect on hepatic fat metabolism that counteracts the usual effect of increased inflow of fatty acids to the liver.

During ethanol oxidation cytoplasmic reducing equivalents are accumulated (Williamson *et al.*, 1969), α -glycerophosphate is increased, the inflow of fatty acids to the liver is increased and acetyl-CoA is doubled (see Table 1 and the Results section). By these changes precursors and energy for synthesis, elongation and esterification of fatty acids are more available than in the fed state.

Lieber *et al.* (1966) suggested that the ethanol-induced triglyceride accumulation may be a consequence of decreased fatty acid oxidation owing to decreased tricarboxylic acid-cycle activity. However, an isolated decrease in fatty acid oxidation combined with increased inflow of free fatty acids would not be expected to decrease acyl-CoA and acylcarnitine (Table 1). The results of the present paper indicate that ethanol has a direct effect in facilitating hepa-

tic fatty acid synthesis (*de novo* or elongation) and esterification.

Zakim (1965) also observed lowered contents of acyl-CoA after ethanol feeding. He suggested that the usual inhibition of hepatic fatty acid synthesis after increased inflow of fatty acids is turned off by ethanol through a decrease in acyl-CoA whereby acetyl-CoA carboxylase is activated.

In contrast to the present investigation, Bode *et al.* (1970) reported elevated contents of acyl-CoA and acylcarnitine after treating male rats with ethanol. This discrepancy may be due to the sex difference, since male rats are reported to exhibit less triglyceride accumulation after ethanol treatment (Lieber *et al.*, 1966; Zakim, 1965). Bode *et al.* (1970) also administered a higher dose of ethanol, in a 35% (v/v) solution preceded by food deprivation for 2 h, and they used ether anaesthesia. This procedure may have stimulated secretion of adrenaline, which is known to cause a marked increase in hepatic acyl-CoA (Zakim, 1965), and ether anaesthesia has been reported to increase the acylcarnitine and acyl-CoA contents of rat liver (Pearson & Tubbs, 1967).

In agreement with Forsander & Lindros (1967), Bode *et al.* (1970) and Breen *et al.* (1971), acute ethanol treatment is shown to increase acetyl-CoA (Table 1). This acetyl-CoA may be derived from β -oxidation after increased inflow of free fatty acids, from activation of acetate, or from decreased utilization of acetyl-CoA.

In perfusion experiments Williamson *et al.* (1969) also found that ethanol increased acetyl-CoA, if oleate was present in the perfusion medium.

The increase in acetyl-CoA is paralleled by increased acetylcarnitine, as is the case with starved animals. In the starved state, however, the carnitine acylation ratio is increased, whereas it is unchanged after acute ethanol treatment (Table 3). This may reflect the different metabolic fate of acetyl units in the two states, since carbohydrate feeding, and thus a state of fat synthesis, is related to low carnitine acetylation ratios (Böhmer, 1967).

The content of acetyl-CoA reaches the same value, about 75 nmol/g wet wt., after highly different dietary treatments (Tables 1 and 2). Thus it appears that there is a definite limit to accumulation of acetyl-CoA. Acetylcarnitine may take part in a buffer mechanism for acetyl-CoA, but increased acetyl-CoA contents are not always followed by increased acetylcarnitine contents (Table 2).

Effect of prolonged ethanol treatment. Prolonged ethanol treatment causes triglyceride to accumulate (see the Results section).

The difference in the concentration of CoA and carnitine compounds between fed controls and rats given the prolonged ethanol diet may be explained by a combined effect of the last ethanol-containing meal and the 8 h starvation preceding killing (Tables 1 and 2). The increase in carnitine and acetylcarnitine corresponds to the effect of acute ethanol treatment and persists 8 h after ethanol feeding, when the blood concentration of ethanol is low (see the Results section). Increased acetyl-CoA, acyl-CoA and acylcarnitine correspond to the values for the group on the glucose control diet, and thus probably reflect the reversion from a state of ethanol oxidation to a state of mild starvation. It appears that the results may be interpreted without implicating effects of the prolonged treatment *per se*.

Fatty liver due to choline deficiency was suggested to be a consequence of secondary carnitine deficiency by Corredor *et al.* (1967). This work illustrates that triglyceride accumulation may develop in spite of largely increased hepatic contents of carnitine.

The increase in triglyceride and carnitine is not solely due to increased inflow of fatty acids to the liver, since the rats treated with the high-fat diet do not exhibit these changes.

We are much indebted to Professor F. Lundquist for his inspiring criticism during the work, to Mr. R. Bredahl for excellent handling of the animals, to Dr. S. Keiding, Rigshospitalet, Copenhagen, for preparation of the liquid diets, to Mrs. L. Stummann for skilful technical assistance and to Statens Laegevidenskabelige Forskningsråd for a Unicam SP.8000 spectrophotometer.

References

- Bernt, E. & Gutman, I. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H. U., ed.), pp. 1457–1460, Verlag Chemie, Weinheim/Bergstr.
- Bode, Ch., Stähler, E., Kono, H. & Goebell, H. (1970) *Biochim. Biophys. Acta* **210**, 448–455
- Breen, K. J., Shaw, J., Levinson, J. D. & Schenker, S. (1971) *Proc. Soc. Exp. Biol. Med.* **138**, 1096–1100
- Bremer, J. (1968) *Biochem. Prep.* **12**, 69–73
- Bremer, J. & Aas, M. (1969) *Mitochondria: Struct. Funct., FEBS Meet. 5th* **17**, 127–135
- Bressler, R. & Wittels, B. (1965) *Biochim. Biophys. Acta* **104**, 39–45
- Buckel, W. & Eggerer, H. (1965) *Biochem. Z.* **343**, 29–43
- Bøhmer, T., Norum, K. R. & Bremer, J. (1966) *Biochim. Biophys. Acta* **125**, 244–251
- Bøhmer, T. (1967) *Biochim. Biophys. Acta* **144**, 259–270
- Corredor, C., Mansbach, C. & Bressler, R. (1967) *Biochim. Biophys. Acta* **144**, 366–374
- Forsander, O. A. & Lindros, K. O. (1967) *Acta Chem. Scand.* **21**, 2568
- Garland, P. B. (1964) *Biochem. J.* **92**, 10c–12c
- Ho, R. J. & Meng, H. C. (1969) *Anal. Biochem.* **31**, 426–436
- Hohorst, H.-J. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H. U., ed.), pp. 1379–1383, Verlag Chemie, Weinheim/Bergstr.
- Lieber, C. S. & DeCarli, L. M. (1970) *Amer. J. Clin. Nutr.* **23**, 474–478
- Lieber, C. S., Spritz, N. & DeCarli, L. M. (1966) *J. Clin. Invest.* **45**, 51–62
- Lieber, C. S., Lefèvre, A., Spritz, N., Feinman, L. & DeCarli, L. M. (1967) *J. Clin. Invest.* **46**, 1451–1460
- Lumbers, J., Threlfall, C. J. & Stoner, H. B. (1969) *Anal. Biochem.* **31**, 21–32
- Michal, G. & Bergmeyer, H. U. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H. U., ed.), pp. 1892–1921, Verlag Chemie, Weinheim/Bergstr.
- Norum, K. R. (1964) *Biochim. Biophys. Acta* **89**, 95–108
- Pearson, D. J. & Tubbs, P. K. (1967) *Biochem. J.* **105**, 953–963
- Pearson, D. J., Tubbs, P. K. & Chase, J. F. A. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H. U., ed.), pp. 1710–1723, Verlag Chemie, Weinheim/Bergstr.
- Simon, E. J. & Shemin, D. (1953) *J. Amer. Chem. Soc.* **75**, 2520
- Tubbs, P. K. & Garland, P. B. (1964) *Biochem. J.* **93**, 550–557
- Wieland, O. & Weiss, L. (1963) *Biochem. Biophys. Res. Commun.* **10**, 333–339
- Williamson, J. R. & Corkey, B. E. (1969) *Methods Enzymol.* **13**, 434–513
- Williamson, J. R., Scholz, R., Browning, E. T., Thurman, R. G. & Fukami, M. H. (1969) *J. Biol. Chem.* **244**, 5044–5054
- Wittels, B. & Spann, J. F. (1968) *J. Clin. Invest.* **47**, 1787–1794
- Zakim, D. (1965) *Arch. Biochem. Biophys.* **111**, 253–256