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THE EFFECT OF ACUTE ETHANOL INGESTION ON *IN VITRO* METABOLISM OF CHOLINE AND ETHANOLAMINE DERIVATIVES IN RAT LIVER

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Abstract—The effect of acute ethanol ingestion on hepatic phospholipid metabolism has been investigated in the rat. No significant increase in the content of either lecithin or phosphatidylethanolamine occurred in the liver after 12 hr from treatment. The triglyceride content of the liver increased three-fold. An increase of lecithin and phosphatidylethanolamine synthesis by the Kennedy pathway was found *in vitro* in the homogenates and microsomal fractions prepared from the ethanol-treated rats. A higher rate of conversion of phosphorylcholine and phosphorylethanolamine to CDP-choline and CDP-ethanolamine was also found in the experimental animals. The breakdown of CDP-choline to phosphorylcholine was noticeably decreased in the liver fractions of the ethanol-treated rats. No changes in the sequential methylation pathway for lecithin synthesis in the liver were observed after acute ethanol ingestion.

IT IS WELL known that ethanol ingestion in the rat results in increased hepatic triglyceride content and alterations in lipid metabolism. However, the manner by which the ingestion of ethanol, either in acute or chronic conditions, leads to fatty infiltration of the liver is not clear, although the lipid accumulation due to acute ethanol treatment has been postulated by many authors to constitute a direct effect of ethanol itself on the molecular structure of the membranes. Particularly, a lipoperoxidation process has been thought to play a starting causative role in the outbreak of acute ethanol-induced liver alterations.[1–3]

Chronic ethanol ingestion produces several side-effects, including interference with the hepatic release of triglycerides.[4,5] Phospholipids are known to be involved in this release under normal conditions. The mechanism of the removal is as yet unknown, although it is clear that the plasma phospholipids which constitute the lipoproteins containing the triglycerides released from the liver are formed and metabolized in the liver.[6] Popular opinion at the moment indicates that the removal of triglycerides from the liver, which occurs by lipoprotein release to the plasma,[7] is impaired at least during chronic ethanol treatment. It was thought by several authors in the past that ethanol induced fatty liver would contain more triglycerides owing to the lack of production of phospholipids. However, this hypothesis has been proved unreliable, since the system of phospholipid synthesis in fatty liver produced by chronic ethanol treatment is efficient,[8] and the hepatic phospholipid content is even increased.[9,10]

Although studies *in vitro* and *in vivo* have been carried out by many workers on hepatic phospholipid synthesis and metabolism during chronic ethanol ingestion,[9–12] little attention has been given to acute ethanol treatment in this aspect. A quantitative study was undertaken therefore on the *in vitro* metabolism of choline and ethanolamine, and their respective phosphate and cytidine diphosphate esters, in homogenates and purified microsomal membranes prepared from fatty livers of rats undergoing acute ethanol treatment. A parallel study was carried out in addition on phospholipid synthesis under comparable conditions.

MATERIALS AND METHODS

Animals and diets. Female Wistar rats weighing 120–150 g body wt (8–10 weeks old) were used throughout and caged individually. All groups were matched according to weight at the start of the experiments. Experimental and control rats were fed a diet (Piccioni, S.p.A., Brescia, Italy) containing 20·4% protein, 3·6% fat, 66·1% mixed starches, 7% ash and small amounts of cellulose by weight. The diet was composed of soy-flour powder containing 55% protein (37%), rice starch (20%), sucrose (32·4%), olive oil (3%) and pure cellulose (2·6%). Salts and minerals (4·5%), and additional vitamins and cofactors were added and the diet mixture thoroughly mixed before use. Water was given *ad lib.* to both groups of animals. The rats, all kept in the same environment were fed this diet for 7–10 days before treatment.

Treatment. Both experimental and control rats were fasted 12 hr before either saline or ethanol administration. At the desired time, ethanol was given by stomach tube as a 50 per cent solution, in a single dose of 6 g/kg. Control rats received by the same route a 0·9% saline solution, in a single dose of 7·5 mg NaCl/100 g body wt. All animals, fasted after dosing, were killed by decapitation 12 hr from treatment, the livers promptly removed, rinsed with cold isotonic saline and treated as follows.

Tissue preparation and subcellular fractions. Livers were chopped into small pieces and fractions for quantitative triglyceride determinations taken before further operations and frozen, if not immediately used. The remaining liver pieces were pooled, and a homogenate was prepared as described previously.[13] Microsome and supernatant fractions were prepared according to Binaglia *et al.*[13] The microsomal pellet was resuspended manually in a small glass-to-glass round-bottomed homogenizer for few sec, to give a final suspension in 0·25 M sucrose of about 20–30 mg protein/ml. The original post-microsomal supernatant which was used directly contained about 4 mg protein/ml. The biochemical purity of microsomes was examined according to Porcellati *et al.*[14]

Incubations. The incubations with the following substrates were performed according to previously published methods;[13] the products of the reactions are shown in brackets : choline (phosphorylcholine, CDP-choline, phosphatidylcholine), phosphorylcholine (choline, CDP-choline, phosphatidylcholine), CDP-choline (choline, phosphorylcholine, phosphatidylcholine), ethanolamine (phosphorylethanolamine, CDP-ethanolamine, phosphatidylcholine, phosphatidylethanolamine), phosphorylethanolamine (ethanolamine, CDP-ethanolamine, phosphatidylcholine, phosphatidylethanolamine) and CDP-ethanolamine (ethanolamine, phosphorylethanolamine, phosphatidylcholine, phosphatidylethanolamine). In addition the amounts of the remaining substrate were determined. The components of the incubation mixtures

and other experimental details of the incubations are given under the appropriate tables.

Extraction, separation and identification of the water-soluble labelled components. The reactions were stopped by placing the flasks in ice and then boiling for 3 min in a water-bath without shaking.[15] The subsequent operations as well as the various identification criteria which have been adopted throughout the work were similar to those reported by Binaglia *et al.*[13]

Isolation and analysis of hepatic lipids. Lipids were extracted from the washed, precipitated material according to Porcellati *et al.*[14] Separation and identification of ethanolamine phosphoglycerides and choline phosphoglycerides were done by the procedures described by Binaglia *et al.*[13]

Analytical methods. Radioactivity of hydrosoluble compounds was measured according to Binaglia *et al.*,[13] using calibration curves with labelled hydrosoluble compounds of known radioactivity content. Similarly, labelled lipids were determined as reported by Binaglia *et al.*[13] with recovery values of about 95 per cent of radioactivity.

Phosphorus content of the hydrosoluble compounds, phospholipid P content of t.l.c. spots, and protein were determined as previously described.[13] Triglyceride was estimated by the method of Van Handel and Zilversmit.[16]

Calculation of results. Recovery values and correction factors were calculated for each lipid class and hydrosoluble product,[13] and used to compile all the data of this communication. Unless otherwise stated, isotope incorporation was converted into nmole of product formed by dividing the estimated nCi by the specific activity of the incubated precursor. The activity was then expressed as nmoles mg protein^{-1} hr^{-1} $\pm$ S.E.M. The per cent conversion of the incubated precursor into product(s) was calculated for the whole incubation mixture on the basis of the nCi found related to the incubated nCi of precursor.

Materials. The labelled and unlabelled substrates, organic solvents, cofactors and chromatographic reagents were obtained from commercial sources or prepared, as reported elsewhere.[13]

RESULTS

Effect of ethanol on hepatic glycerolipids. Acute ethanol administration increased the hepatic triglyceride content. Already two hours after its dosing the hepatic triglyceride concentration was slightly increased in the experimental rats, while a significant increase was observed only 6 hr after treatment, with maximum values at 8 hr. At the maximum increase, however, the data was scattered and it was found more useful to adopt a 12 hr-interval from dosing since a more reproducible and still very consistent increase was noticed, as shown in Table 1. It is clear from this table that the hepatic triglyceride content increased more than 3-fold in these conditions ($P < 0{\cdot}001$). There were no substantial differences in triglyceride levels in different areas of the liver of treated and control rats. In preliminary experiments it was also observed that a dose of glucose, isocaloric to ethanol, gave the same results as sodium chloride administration with regard to liver triglyceride content for the 12 hr examined. Unlike chronic ethanol treatment,[9,10] the acute administration did not produce significant changes in the phosphatidylcholine and phosphatidylethanolamine content of either liver homogenates or microsomal membranes (results not shown in the

TABLE 1. CONCENTRATION OF HEPATIC TRIGLYCERIDES AFTER ACUTE ETHANOL INTOXICATION

Hours after treatment	Treatment	Triglycerides (mg/g wet wt)
12	Saline	9·4 ± 0·72 (18)
12	Ethanol	29·8 ± 4·52 (27)

Ethanol was given by gastric intubation (6 g/kg), as a 50% solution. Controls received saline by the same route (see the text).

Results are expressed as means ± S.E.M. Number of experiments in brackets.

table). Similarly, the wet weight and the protein content of liver from the ethanol-treated rats did not change significantly as compared to the control levels.

General results. Before giving details of the results of subsequent sections, it must be mentioned that the incubations were always performed in the presence of saturating substrate concentrations[13] and for time intervals that ensure zero-order kinetic rates, as determined in preliminary experiments and with reference to previous results.[13] It was observed also in preliminary experiments that dosing the control rats with glucose, isocaloric to ethanol, in place of saline solution, produced similar results in respect to any substrate examined. Isotonic sodium chloride control was therefore adopted throughout. All the tables in this paper report absolute values of conversion, expressed as nmoles of product formed per mg of incubated protein per hr, only for those reactions which involve a direct transformation of substrate to product, e.g. choline into phosphorylcholine, or CDP-ethanolamine into lipid, etc. It was thought misleading to express the other conversion data in absolute values as above, owing to the change in specific activity of direct precursor due to the amounts of endogenous material. All other data are expressed therefore as per cent of conversion of the incubated precursor into product(s), calculated for the whole incubation mixture on the basis of the nCi found related to the incubated nCi of precursor.

Effect of ethanol on the conversion of choline and ethanolamine into water-soluble and lipid material. Table 2 shows the results obtained by incubating choline or ethanolamine with liver microsomes and homogenates. No differences exist between production of phosphorylcholine from choline and of phosphorylethanolamine from ethanolamine in normal and fatty liver, either in homogenates or in microsomal fractions. There were no significant variations in the formation of CDP-choline, whereas a valuable increase of the rate of production of CDP-ethanolamine was observed during ethanol intoxication (70 per cent change in the microsomes). From the same table it appears that a higher conversion of lipid precursor into lipid material (phosphatidylcholine) occurred in fatty liver by ethanol. The per cent of this conversion in liver homogenate, which is about 0·32 per cent in the controls, increased to about 0·8 per cent in fatty liver. The increase, though less evident, also occurred with liver microsomes. Probably CDP-choline, once formed, is more readily transformed into lipid material in fatty liver. Recovery of unreacted choline or ethanolamine was 92–95 per cent in both normal and fatty liver preparations.

Effect of ethanol on the conversion of phosphorylcholine and phosphorylethanolamine into water-soluble and lipid material. Production of CDP-choline from phosphorylcholine and of CDP-ethanolamine from phosphorylethanolamine increased slightly

TABLE 2. INCORPORATION OF LABELLED CHOLINE INTO PHOSPHORYLCHOLINE (PC) CDP-CHOLINE (CDPC) AND PHOSPHATIDYLCHOLINE (PHC), AND OF LABELLED ETHANOLAMINE INTO PHOSPHORYLETHANOLAMINE (PE) AND CDP-ETHANOLAMINE (CDPE) BY HOMOGENATES AND MICROSOMAL FRACTIONS OF NORMAL AND ETHANOL INDUCED FATTY LIVER (FL)*

	Fraction examined			
	Homogenate		Microsomes	
Products formed	N	FL	N	FL
PC†	0·18 ± 0·02 (7)	0·20 ± 0·03 (9)	2·51 ± 0·31 (5)	2·20 ± 0·33 (5)
CDPC‡	0·070 (8)	0·067 (9)	0·050 (5)	0·051 (5)
PhC‡	0·32 ± 0·071 (8)	0·79 ± 0·13 (7)¶	0·18 ± −0·032 (5)	0·40 ± 0·046 (5)‖
PE†	0·40 ± 0·04 (5)	0·37 ± 0·04 (8)	1·67 ± 0·14 (5)	1·81 ± 0·13 (6)
CDPE‡	0·15 ± 0·021 (4)	0·18 ± 0·030 (6)§	0·06 ± 0·0010 (6)	0·10 ± 0·0012 (9)¶

* The following incubation mixture was used: 19·2 mM sodium pyruvate, 1·25 mM sodium malate, 1·63 mM ATP, 0·48 mM CTP, 1·63 mM CMP, 19·2 mM Tris–HCl buffer (pH 7·50), 0·40 mM 1,2-^{14}C-choline (5–6 μCi/μmoles) or 0·83 mM 1,2-^{14}C-ethanolamine (5–6 μCi/μmoles), 0·19 mM CoA, microsomes (about 2 mg protein), supernatant fraction (about 4 mg protein) and 4·8 mM $MgCl_2$. Final volume 1·25 ml. The components of the incubation system were added at 4° in the indicated order, and the mixture incubated for 60 min at 40° in a thermo-regulated shaker at about 80 strokes/min. In the homogenate experiments, 8–9 mg of homogenate protein replaced the microsomal plus supernatant fractions. The incubation mixture for ethanolamine experiments was similar to that for choline, except that ethanolamine replaced choline.

† nmoles/mg protein/60 min, mean ± S.E.M. (number of experiments in brackets).

‡ Per cent conversion of incubated precursor into product calculated for the whole incubation mixture, mean ± S.E.M. (number of experiments in brackets).

§ $P < 0·1$.

‖ $P < 0·05$.

¶ $P < 0·01$.

TABLE 3. CONVERSION OF LABELLED PC INTO CDPC, PhC AND CHOLINE, AND OF LABELLED PE INTO CDPE AND ETHANOLAMINE BY HOMOGENATES AND MICROSOMAL FRACTIONS OF NORMAL (N) AND ETHANOL INDUCED FATTY LIVER (FL)*

	Fraction examined			
	Homogenate		Microsomes	
Products formed	N	FL	N	FL
CDPC†	2·21 ± 0·30 (10)	3·09 ± 0·46 (10)§	7·91 ± 0·84 (5)	17·21 ± 1·02 (5)‖
PhC‡	0·10 ± 0·02 (11)	0·18 ± 0·03 (11)‖	0·12 ± 0·01 (6)	0·17 ± 0·03 (7)§
Choline†	15·01 ± 1·07 (10)	15·96 ± 1·13 (10)	107·1 ± 7·4 (5)	116·2 ± 9·1 (6)
CDPE†	1·36 ± 0·12 (4)	1·87 ± 0·17 (9)§	11·12 ± 0·96 (6)	20·51 ± 1·33 (9)¶
Ethanolamine†	1·48 ± 0·11 (5)	1·56 ± 0·12 (9)	21·12 ± 1·68 (6)	19·86 ± 1·40 (7)

* PC was incubated in the following incubation mixture : 5·6 mM 1,2-*sn*-diacyl glycerol prepared from soybean lecithin, 50 mM Tris–HCl buffer (pH 7·50), 6·1 mM ATP, 4·1 mM CTP, 12·3 mM cysteine, microsomes (about 1 mg of protein), supernatant fraction (2 mg of protein) and 20·4 mM $MgCl_2$. The labelled precursor (2·66 mM 1,2-^{14}C-choline phosphate, S.A. of 0·43 μCi/μmole) was added just before the enzymes. In the experiments with PE, 2·66 mM 1,2-^{14}C-ethanolamine phosphate, S.A. of 0·55 μCi/μmole), replaced the phosphorylcholine. Final volume 0·90 ml. The mixtures were incubated for 60 min at 40°, as indicated in Table 2. In the homogenate experiments 8–9 mg of homogenate protein replaced the microsomal and supernatant protein. For abbreviations see Table 2.

† nmoles/mg protein/60 min, mean ± S.E.M. (number of experiments in brackets).

‡ Per cent conversion of incubated precursor into products calculated for the whole incubation mixture, mean ± S.E.M. (number of experiments in brackets).

§ $P < 0·1$.

‖ $P < 0·01$.

¶ $P < 0·005$.

TABLE 4. CONVERSION OF LABELLED CDPC INTO CHOLINE, PC AND PHC, AND OF LABELLED CDPE INTO ETHANOLAMINE, PE, PHOSPHATIDYLETHANOLAMINE (PHE) AND PHC BY HOMOGENATES AND MICROSOMAL FRACTIONS OF NORMAL (N) AND ETHANOL INDUCED FATTY LIVER (FL)*

	Fractions examined			
	Homogenate		Microsomes	
Products formed	N	FL	N	FL
Choline‡	1·23 ± 0·11 (12)	1·28 ± 0·13 (13)	0·98 ± 0·12 (7)	0·90 ± 0·14 (9)
PC†	44·5 ± 3·8 (12)	26·3 ± 1·8 (13)**	137·2 ± 15·1 (6)	101·6 ± 12·0 (6)¶
PhC†	14·29 ± 1·40 (11)	19·31 ± 1·81 (12)‖	42·20 ± 5·51 (8)	63·71 ± 6·22 (9)**
Ethanolamine‡	0·95 ± 0·12 (5)	1·01 ± 0·14 (9)	1·06 ± 0·09 (5)	0·98 ± 0·10 (6)
PE†	62·23 ± 5·86 (5)	59·80 ± 5·21 (9)	148·8 ± 12·8 (6)	155·1 ± 14·4 (9)
PhE†	5·15 ± 0·62 (5)	6·81 ± 0·66 (9)¶	17·21 ± 1·66 (6)	25·69 ± 1·98 (9)¶
PhC§	0·71 ± 0·08 (5)	0·76 ± 0·09 (9)	0·40 ± 0·05 (6)	0·36 ± 0·05 (9)

* Incubation mixture: 5·6 mM 1,2-*sn*-diacyl glycerol prepared from soybean lecithin, 50 mM Tris–HCl buffer (pH 7·90), 12·3 mM sodium fluoride, 12·3 mM cysteine, 1·53 mM labelled CDP-choline (CMP-1,2-^{14}C-choline phosphate, S.A. of 0·35 μCi/μmole), microsomal protein (about 1 mg) and 20·4 mM $MgCl_2$. Components were added in the order indicated. Homogenate (about 8–9 mg of protein) replaced microsomes when indicated. Final volume 0·90 ml. Incubation was carried out for 60 min at 40°. In the experiments with incubated CDPE, 1·34 mM labelled CDP-ethanolamine (CMP-1,2-^{14}C-ethanolamine phosphate, S.A. of 0·55 μCi/μmole) replaced labelled CDP-choline. For abbreviations, see Table 2.

† nmoles/mg protein/60 min, mean ± S.E.M. (number of experiments in brackets).

‡ Per cent conversion of incubated precursor into product calculated for the whole incubation mixture, mean ± S.E.M. (number of experiments in brackets).

§ This lipid is formed *via* the stepwise methylation of preformed PhE (see the text). The value is based on the incubated CDPE and is expressed as per cent of product based on CDPE. Absolute values in nmoles/mg protein/60 min are for normal liver homogenate and microsomes 0·74 and 2·51, respectively.

‖ P 0·1.

¶ P 0·01.

** P 0·001.

in the fatty liver homogenate (Table 3). This increase was more evident and statistically significant in both cases in the experiments using microsomes, where the per cent of variations were from 85 to 117 per cent. The successive conversion of phosphorylcholine into lipid material (phosphatidylcholine) also increased under conditions of fatty liver *in vitro*, but not to a noticeable extent. The breakdown of the two phosphoric esters to choline and ethanolamine was not affected. The main result arising from Table 3 points to a faster utilization of incubated phosphorylcholine and phosphorylethanolamine in the fatty liver for the synthesis of correspondent nucleotides and lipid material. The recovery of incubated substrates averaged always 85–90 per cent in both normal and fatty liver. This result, together with those of Table 3, indicated that loss of material had not occurred and that neither substrate entered other material.

Effect of ethanol on the conversion of CDP-choline and CDP-ethanolamine into water-soluble and lipid material. CDP-choline and CDP-ethanolamine were converted to a higher extent into correspondent phospholipids in fatty liver homogenate and microsomes, compared with control values (Table 4), with significant and strikingly similar results (35–36 per cent increase in the homogenates and 50 per cent in the microsomes). CDP-choline was hydrolyzed to phosphorylcholine at a reduced rate in the liver homogenate of the treated rats (44·5 nmoles of phosphorylcholine produced per mg of protein per hr against 26·3 nmoles in the fatty liver experiments, a decrease of about 40 per cent). A decrease of the rate of breakdown of CDP-choline took place also at the microsomal level (Table 3), although to a lesser extent (25–28 per cent). Contrary to these last findings, the breakdown of CDP-ethanolamine to phosphorylethanolamine was high both in normal and fatty liver. Choline and ethanolamine were produced in similar amounts in normal and fatty liver, as shown in the same Table. The amounts of labelled phosphatidylcholine arising from the stepwise methylation of the preformed phosphatidylethanolamine was also estimated.[13] The amount of this lipid, which is produced in the range of about 12–15 per cent of the phosphatidylethanolamine, did not change during fatty liver production (Table 4). The recovery of the unreacted CDP-choline and CDP-ethanolamine was always satisfactory, and the sum of the unreacted nucleotides together with the produced hydrosoluble and lipid compounds often approached 90–95 per cent.

DISCUSSION

Few studies on the effects of acute ethanol ingestion on hepatic phospholipid metabolism have been described, and the results from these investigations refer chiefly to alteration in the fatty acyl profile of membrane phospholipids due to the treatment. The present study reveals significant changes in the rate of phospholipid synthesis.

On the other hand, the importance of phospholipids for the structural integrity and biochemical function of cellular and subcellular membranes is of such magnitude to suggest that some of the changes in phospholipid metabolism found by us during the course of acute ethanol intoxication may be caused by alteration in the molecular architecture of hepatic membranes which is known to constitute the primary effect of ethanol itself. Variations in the molecular structure of liver membranes, such as those which occur at the level of the fatty acid profile of microsomal phospholipids during ethanol administration,[17,18] are probably the main factors responsible for the

changes in phospholipid metabolism, since it is known that this profile will affect to some extent enzymic activities related to phospholipid metabolism itself.[19,20]

The results of our experiments demonstrated that 8–12 hr after ethanol administration the incorporation of some lipid precursors into either phosphatidylcholine or phosphatidylethanolamine of fatty liver is significantly enhanced. These changes are accompanied by triglyceride infiltration of the liver. It has been frequently observed during our experiments that the higher the elevation of triglyceride content of the liver, the higher were the changes in the rate of synthesis of the two mentioned phospholipids. It can be postulated therefore that an increased rate of synthesis of phospholipids may reflect a rise in triglyceride and vice versa. It is impossible to state to what extent these relationships could be relevant for the biochemical damage of fatty liver induced by acute ethanol treatment.

The greatest increase of enzyme activity noticed in our *in vitro* studies were observed at the level of the incorporation of phosphorylcholine and phosphorylethanolamine into corresponding phospholipids by the Kennedy pathway. This increase was clearly accompanied by significantly elevated levels of radioactive CDP-choline and CDP-ethanolamine, respectively. Probably one of the most interesting effects of the ethanol treatment is the increase in the rate of reaction of the cytidylyltransferases (EC 2.7.7.14 and EC 2.7.7.15), which produce the respective cytidine nucleotides. These enzymic steps are known to be rate-limiting stages in the Kennedy pathway of phospholipid metabolism,[19] and the activating effect of ethanol upon them may be interesting in this connection. The activity of the diacyl glycerol cholinephosphotransferase and diacylglycerol ethanolaminephosphotransferase (EC 2.7.8.2) is also increased during the experimental period, as observed by the increased rate of incorporation of CDP-choline and CDP-ethanolamine into corresponding phospholipids. This result may explain also the increased rate of incorporation of the phosphoric ester of choline into lipid material. However, it cannot be excluded that a modification of the endogenous diglyceride pool due to ethanol administration might be responsible for the observed modifications, since it is not proven yet that the exogenously added diglycerides are undistinguishable from the endogenous diglycerides for the active site(s) of the phosphotransferases.

Although chronic ethanol treatment would also cause an increased rate of synthesis of hepatic phospholipid *in vitro* and *in vivo*,[9,10] there are however some relevant differences between the effect of acute and chronic treatment upon phospholipid synthesis. While long-term ethanol ingestion by rats produces an increase of lecithin synthesis by the sequential methylation pathway and no changes in the CDP-choline pathway,[9] the reverse is true for acute ethanol ingestion (Table 4). It must be added in this connection that ethanol ingestion for 2 weeks only does not affect the biosynthesis of phosphatidylcholine by the methylation of phosphatidylethanolamine.[12] There are probably intermediate stages of ethanol intoxication, depending on duration of treatment, in which enzymic activities are specifically affected in different ways. It is interesting to recall, in this connection, that another type of acute liver injury and fatty infiltration, e.g. by CCl_4, does not similarly affect the methylation pathway.[21]

It is known that the liver is the main source of phospholipids in plasma. Therefore any biochemical change of phospholipid metabolism in the liver is reflected by parallel variations in the plasma. The finding that two independent pathways for phospha-

tidylcholine synthesis in the liver (the Kennedy pathway and the stepwise methylation pathway) are differently affected during acute ethanol intoxication may be important in this respect, because the two pathways provide different lecithin molecules in the liver.[22,23] The different degree of response of the two pathways to ethanol treatment could constitute an efficient means of controlling the availability of specific molecular species of lecithins for the lipoproteins to transport triglycerides to the plasma.

The variations in enzymic activities of phospholipid metabolism noticed during the present work do not seem to be related to enzyme induction due to ethanol. This consideration is substantiated by the finding that among the enzyme activities examined some were unaffected and some even decreased, such as the CDP-choline hydrolyzing enzyme. Moreover, the acute administration of ethanol, contrary to the chronic treatment, produces a decrease in the smooth endoplasmic reticulum,[24] and no variations of cytochrome P-450 and other microsomal enzymatic activities.[24,25] The changes in enzyme activities observed during acute ethanol treatment probably reflect the development of the fatty liver.

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