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Ethionine-induced alterations of enzymes involved in lipid metabolism and their possible relationship to induction of fatty liver

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Changes of enzymes involved in the hepatic metabolism of long-chain fatty acids (palmitoyl-CoA synthetase (EC 6.2.1.3), carnitine palmitoyltransferase (EC 6.2.1.3), glycerophosphate acyltransferase (EC 2.3.1.15)) in the liver of male rats were examined after ethionine exposure. Ethionine administration resulted in a dose- and time-dependent enhancement of the palmitoyl-CoA synthetase activity both in the mitochondrial, peroxisomal and microsomal fractions. The total carnitine palmitoyltransferase activity in the mitochondrial fraction was enhanced. Ethionine administration was also associated with dose- and time-dependent changes of the microsomal glycerophosphate acyltransferase activity, whereas the mitochondrial enzyme activity was marginally affected. The hepatic triacylglycerol content of the ethionine-treated animals was increased. Hepatic lipids were accumulated in large droplets. Serum triacylglycerol and cholesterol were decreased. In particular, the serum HDL-cholesterol level was lowered. The concentration of ATP in the liver decreased. Accumulation of the metabolic product *S*-adenosylethionine (AdoEth) was observed for the first 2 days of exposure followed by a fall in *S*-adenosylmethionine (AdoMet) during the next 10 days. Linear regression analysis of ATP content versus AdoEth and AdoMet showed highly significant correlations. A significant correlation between the hepatic triacylglycerol and AdoEth content was also observed upon ethionine treatment. The data show that ethionine perturbs the hepatic lipid metabolism. Enhanced esterification of long-chain fatty acids, but not a simple reduction of their oxidation, might contribute to ethionine-induced fatty liver in addition to a block in secretion of lipoproteins and decreased protein synthesis.

Introduction

Ethionine, the ethyl homologue of methionine, is reported to be toxic in several species, including

rats [1–4]. Some of the effects after oral or parenteral exposure of ethionine are acute, occurring within hours or a few days. Other effects, such as liver cancer, appear only after chronic administration for several months [1,5]. Ethionine carcinogenesis is possibly due in part to an altered *S*-adenosylmethionine (AdoMet)/*S*-adenosylhomocysteine (AdoHcys) ratio, and thereby altered DNA methylation [6].

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One of the earliest lesions observed in rats fed ethionine is a rapid accumulation of hepatic triacylglycerols [7] and a depression of protein synthesis (at the RNA level) [2]. Evidence for the effects of ethionine in transmethylation reactions [1,8] first came from the observation that exposure of ethionine reduced ATP in the liver accompanying the formation of *S*-adenosylethionine (AdoEth) [3,4,9]. It has been postulated that ATP depletion occurs because AdoEth is accumulated in the liver, thereby trapping the adenosine moiety of ATP [1].

The accumulation of liver lipids has been suggested to be related to ATP deficiency and to inhibition of protein synthesis [1,6,10,11]. Administration of adenine or ATP is effective in removing the excess hepatic triacylglycerols [6] and prevents the decrease in serum lipid levels, which is characteristic of several forms of fatty liver, for example, choline deficiency [12,13].

Accumulation of triacylglycerols in the liver may be associated with an altered uptake or metabolic flux through pathways leading to synthesis or degradation of lipids. Non-esterified long-chain fatty acids from the blood are converted to the corresponding long-chain acyl-CoA through the action of long-chain acyl-CoA synthetases (EC 6.2.1.3) [14]. The long-chain acyl-CoA may either be oxidized or esterified with glycerol 3-phosphate, and this constitutes a branch-point connecting the synthesis and degradation of lipids.

The extramitochondrial fatty acyl-CoA is enzymatically transferred via carnitine to intramitochondrial long-chain acyl-CoA by carnitine palmitoyltransferase (EC 2.3.1.21), and this reaction appears to be rate-limiting in the transfer of activated fatty acids into the mitochondria [14].

Glycerophosphate acyltransferase (EC 2.3.1.15), which is localized to both the mitochondria and the endoplasmic reticulum [15], is probably the rate-limiting step in glycerolipid biosynthesis [16,17].

Hepatic accumulation of fat after ethionine exposure may not only be due to a reduction of ATP and inhibition of protein synthesis resulting in inhibition of lipoprotein secretion [1,6]. Increase in lipid biosynthesis, inhibition of lipid degradation or failure to transfer the newly synthesized triacylglycerol into plasma should also be considered.

The present paper deals with the subcellular distribution and activities of palmitoyl-CoA synthetase, carnitine palmitoyltransferase and glycerophosphate acyltransferase in liver of ethionine-fed rats. We also studied the ATP deficiency, changes in *S*-adenosylamino acids and altered liver and serum lipid levels following ethionine exposure.

Materials and Methods

Animals and diets. Male Wistar rats (200–220 g) from Møllegaard Breeding Laboratory, Ejby, Denmark, were randomly selected for ethionine treatment or for control experiments. Each experimental group consisted of four rats housed in stainless steel cages at room temperature $23 \pm 2^\circ\text{C}$. Food and drinking water were available ad libitum [18]. In one experimental group, ethionine was injected intraperitoneally (i.p.) twice daily for 7 days corresponding to doses of 100, 300 and 750 mg/day per kg body weight. In an other group, 300 mg/day per kg body weight ethionine was injected i.p. twice daily for 2, 7 and 12 days. The control animals were injected with physiological saline.

At the end of the experiments, the animals were weighed and killed by decapitation, and blood as collected (for less than 30 s). A liver piece was rapidly removed, placed on ice, weighed and put in liquid nitrogen within 30 min. The rest of the liver was immediately chilled.

Preparation of total homogenates and subcellular fractions. The chilled liver and/or 'the less than 30 s piece' were homogenized in 0.25 M sucrose containing 10 mM Hepes buffer (pH 7.4) [19]. The postnuclear and nuclear fractions from individual animals were prepared as described [20]. The resulting postnuclear fraction plus the nuclear fraction were used as the total homogenate. In the group of animals injected with physiological saline (control group), the variation in the response from animal to animal was estimated separately for selected enzymes. The postnuclear fractions from four rats in each treated group were pooled, and the subcellular fractions including mitochondrial-enriched, peroxisome-enriched, microsomal-enriched and cytosolic fractions, were isolated by differential centrifugation [20].

Determination of ATP and AdoEth in liver. HPLC methods were used for quantitation of

ATP [21], AdoEth [22] and AdoMet [23] in ethionine-treated animals. The frozen liver pieces were homogenized in 0.4 M perchloric acid [24].

Determination of triacylglycerols and cholesterol in serum. Serum was prepared by centrifugation of whole blood and $1000 \times g$ for 10 min. Serum total cholesterol, and HDL cholesterol and triacylglycerol were measured using standard methods and commercial kits (Boehringer, Mannheim, F.R.G.).

Enzyme assays and other analytical methods. Palmitoyl-CoA synthetase [18,29,25], carnitine palmitoyltransferase [25] and glycerophosphate acyltransferase [25] were determined radiochemically as earlier described. Protein was determined by the Bio-Rad protein assay kit (Bio-Rad Laboratories). Liver triacylglycerol and cholesterol in whole homogenates were determined by the commercial kits (Boehringer Mannheim, F.R.G.).

Morphology. Rat liver pieces of the same animals were immediately taken for morphological examinations. They were cut into small blocks of 1 mm³ or smaller and fixed in 2% glutaraldehyde (v/v) in 0.25 M sucrose solution buffered to pH 7.4 with 10 mM Hepes buffer at 4°C for 30 min [26]. The blocks were rinsed in buffer and postfixed in 1% (w/v) OsO₄ in the buffer at 4°C for 90 min. The tissue blocks were dehydrated in ethanol and embedded in epoxy resin and ultrathin sections were cut with an LKB ultratome III. The semithin sections, stained in toluidine, were studied in a light microscope, and exact drawings of the cells were made at $\times 800$. The areas of the

hepatocytes were measured with a Leitz ASM morphometric analyzer.

Ultrathin sections were also stained in uranyl acetate and lead citrate. Electron microscopy was performed with a Jeol 100 CX electron microscope.

Statistical methods. A two-way analysis of variance and Student's *t*-test were used to evaluate the significance of differences in postnuclear fraction between population means; $P > 0.05$ was taken to be statistically nonsignificant.

Results

The effects of ethionine are reported in the following sections.

Body and liver weights

The body and liver weights of the rats and the relative liver weight (liver weight expressed as a percentage of the body weight) decreased gradually with increasing ethionine doses and exposure times (Table I). The concentration of liver protein (mg/g liver) was not changed in the ethionine-treated animals (Table I).

Hepatic triacylglycerols and cholesterol

The hepatic triacylglycerol content of the rats treated with ethionine for 7 days was increased 1.8-fold at a dose of 300 mg/day per kg body weight (Fig. 1A). At that concentration, the hepatic triacylglycerol level in male rats exposed for 2 days was increased even more, up to 3.7-fold, whereas exposure of ethionine for 12 days normal-

TABLE I

DOSE- AND TIME-RELATED EFFECT OF ETHIONINE ADMINISTRATION ON BODY AND LIVER WEIGHTS

Calculated as mean \pm S.D. of four animals in each group, the significance is: * $P < 0.01$ compared to the control group; ** $P < 0.05$ compared to the control group. *** Control at 2, 7 and 12 days.

Feeding periods (days)	Ethionine dose (mg/kg per day)	Changes of body weight (g)	Liver weight (g)	Liver/body weight (%)	Protein (mg/g liver)
2,7,12 ***	(0) Control	+35.2 \pm 3.9	11.3 \pm 0.8	4.6 \pm 0.2	154.6 \pm 4.8
7	100	+21.0 \pm 3.6 *	9.8 \pm 0.5 **	4.1 \pm 0.1 **	162.7 \pm 7.1
7	300	+6.8 \pm 4.9 *	8.5 \pm 0.2 *	3.7 \pm 0.4 *	159.5 \pm 10.5
7	750	-16.5 \pm 3.5 *	8.3 \pm 0.2 *	3.6 \pm 0.1 *	155.6 \pm 4.2
2	300	+3.5 \pm 2.0 *	9.4 \pm 0.6 *	4.3 \pm 0.2	153.5 \pm 9.7
12	300	+15.3 \pm 3.3 *	8.8 \pm 0.6 *	4.0 \pm 0.2 *	158.7 \pm 3.9

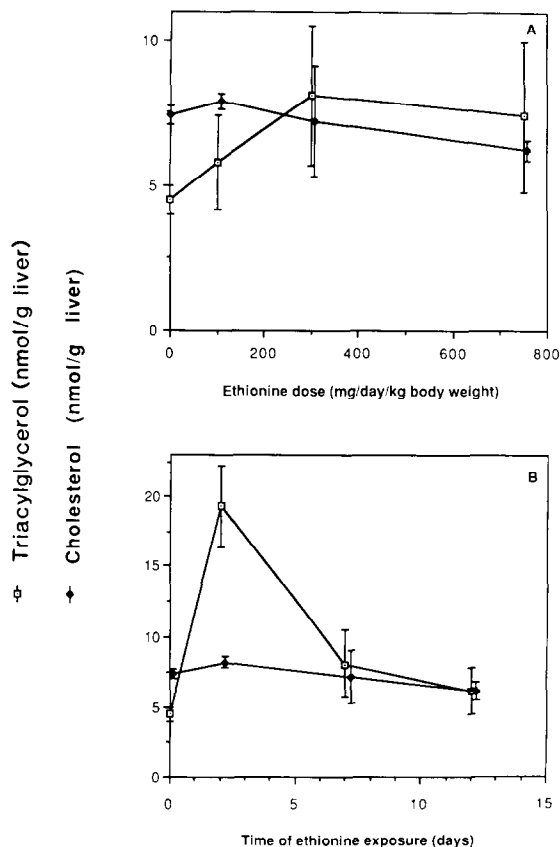


Fig. 1. Effect of increasing amounts of ethionine for 7 days on the hepatic lipids (A) and changes in lipids during the course of ethionine exposure (300 mg/day per kg body weight) (B). The bars (measured in postnuclear fraction) represent the means \pm S.D. of four animals.

ized the liver triacylglycerol content (Fig. 1B). The hepatic cholesterol content was not changed in animals treated for either 2 or 7 days at 300 mg ethionine/day per kg body weight (Fig. 1A and B). At a level of 750 mg ethionine/day per kg body weight, a slight, but significant decrease of cholesterol was observed.

Morphology

After 7 days at a dose of 300 mg/day per kg body weight, the average hepatocyte size of the treated animals was little affected. Light (Fig. 2A and B) and electron microscopy study after ethionine administration (Fig. 2C) clearly showed aggregates of fat droplets lying in the cytoplasm and often abutting onto different organelles. Morphometric analyses of randomly selected paren-

chymal cells from control rats (Fig. 2B) revealed means number of fat vacuoles per cell of 0.32 (230 cells were measured), whereas in ethionine-exposed rats (Fig. 2A and B), the mean number of fat droplets per cell was 2.69 (270 cells were measured).

Serum lipids

The serum triacylglycerol level of the animals exposed for 7 days was not changed up to a dose of 300 mg ethionine/day per kg body weight (Fig. 3A). At a dose of 750 mg/day per kg body weight, however, the serum triacylglycerol content was decreased by about 70% when compared with controls. No significant changes of the serum triacylglycerols were observed as a function of time exposure (Fig. 3B).

The total serum cholesterol concentration of the animals treated for 7 days was decreased about 40% at the lowest dose of ethionine, but was almost at pretreatment level when the dose was increased to 750 mg/day per kg (Fig. 3A). The time of ethionine exposure also changed the cholesterol content. At an ethionine dose of 300 mg/day per kg body weight, the cholesterol concentrations of the rats treated for 2 and 12 days decreased by about 50 and 20%, respectively, compared to that of the controls.

The greatest changes of the serum lipids on ethionine exposure were observed on HDL cholesterol. Fig. 3A shows that the HDL cholesterol content of serum gradually decreased with the dose of ethionine. At a dose of ethionine of 750 mg/day per kg weight, the HDL cholesterol content decreased by about 85%. Furthermore, low HDL cholesterol was an early response, as the serum content decreased about 70% in animals treated for 2 days (Fig. 3B).

The activities of palmitoyl-CoA synthetase, carnitine palmitoyltransferase and glycerophosphate acyltransferase

The distribution of protein and marker enzymes for mitochondria, peroxisomes and microsomes was for all groups of animals essentially similar to our previous findings for rat liver homogenates [18,19,25]. The nuclear fraction was contaminated by whole cells, cell debris and different organelles but the other fractions appeared to be

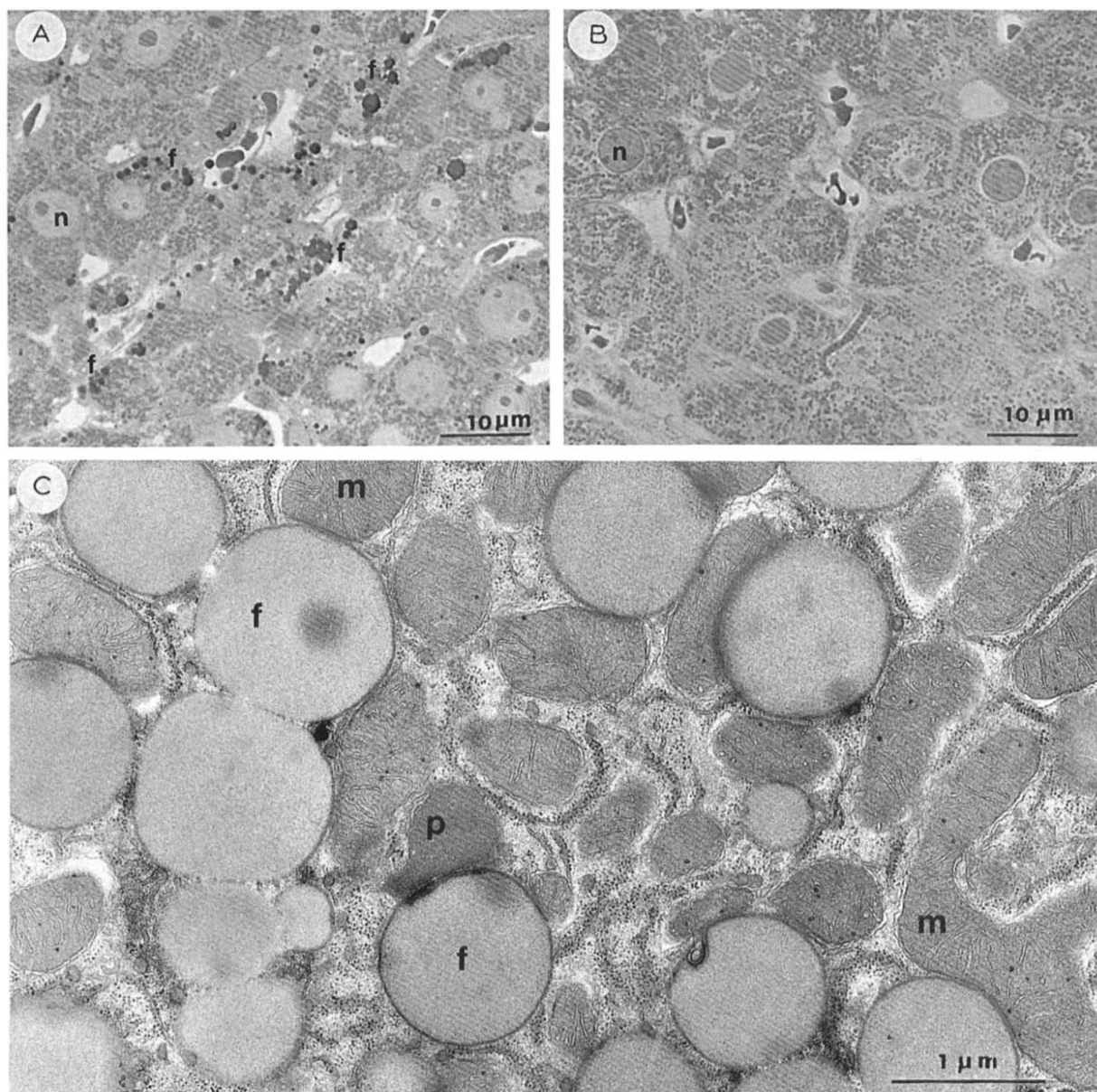


Fig. 2. Light micrographs of liver sections (A and B) and transmission electron micrographs of a hepatocyte (C) from ethionine-treated rats (300 mg/day per kg body weight), n, nuclear fraction. Notice the accumulation of fat vacuoles (f), and the mitochondria (m) and peroxisomes (p) adjacent to these. $\times 24\,000$. Control hepatocytes with virtual absence of fat vacuoles (B).

rather pure, with low contamination based on marker enzymes (data not shown). The amount of glutamate dehydrogenase, cytochrome-*c* oxidase, catalase and urate oxidase suggested a 3–6% contamination of mitochondria and peroxisomes in

the microsomal fraction. Based on the total activities, the amount of NADPH-cytochrome-*c* reductase, glutamate dehydrogenase and cytochrome-*c* oxidase suggested a 1–10% contamination with mitochondrial and microsomal marker

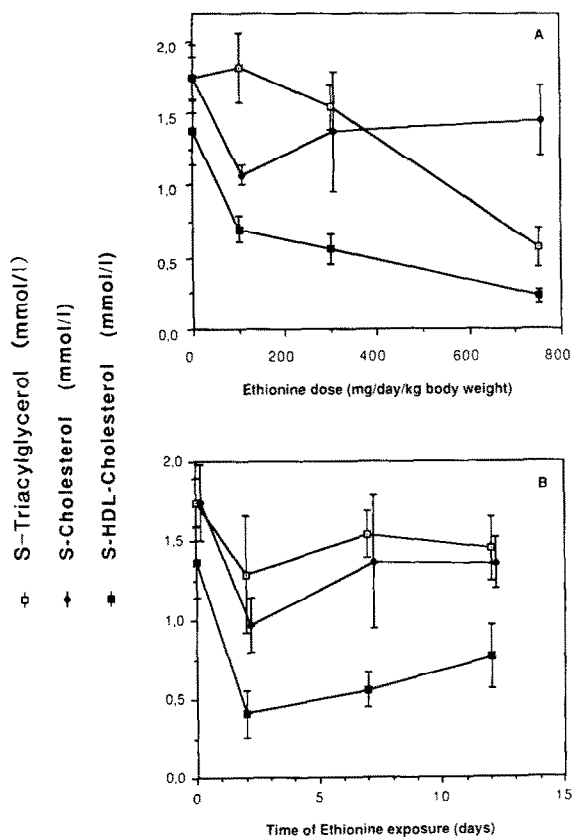


Fig. 3. Changes of serum lipids with increasing amount (A) and during the course (B) of ethionine exposure. Data are expressed as the mean \pm S.D. of four individual animals. In (B), 300 mg/kg body weight were used.

enzymes in the peroxisome-enriched fraction. The mitochondrial fraction, however, contained peroxisomes (10–15%) based on the peroxisomal marker enzymes, catalase and urate oxidase (data not shown).

Palmitoyl-CoA synthetase activity in the whole homogenates of rat liver increased 1.4-fold and reached a maximum with a dose of 100–300 mg ethionine/day per kg body weight (Fig. 4A). In the time-course study with 300 mg/kg per day, the specific activity of the enzyme gradually increased up to 7 days exposure (Fig. 4B).

The mitochondrial and peroxisomal palmitoyl-CoA synthetase activities tended to increase at a dose of ethionine of 100 mg/day per kg body weight. The microsomal palmitoyl-CoA synthetase

activity was slightly increased at a dose of 300 mg ethionine/day per kg body weight (Fig. 4A).

Fig. 4B shows that both the mitochondrial and microsomal palmitoyl-CoA synthetase activities gradually increased with time of ethionine administration. The microsomal enzyme activity of the animals treated for 12 days was enhanced by a factor of 1.5, whereas the mitochondrial enzyme activity increased 1.2-fold.

The carnitine palmitoyltransferase activity in the whole homogenates of liver was almost unaffected by ethionine administration (Fig. 5A and

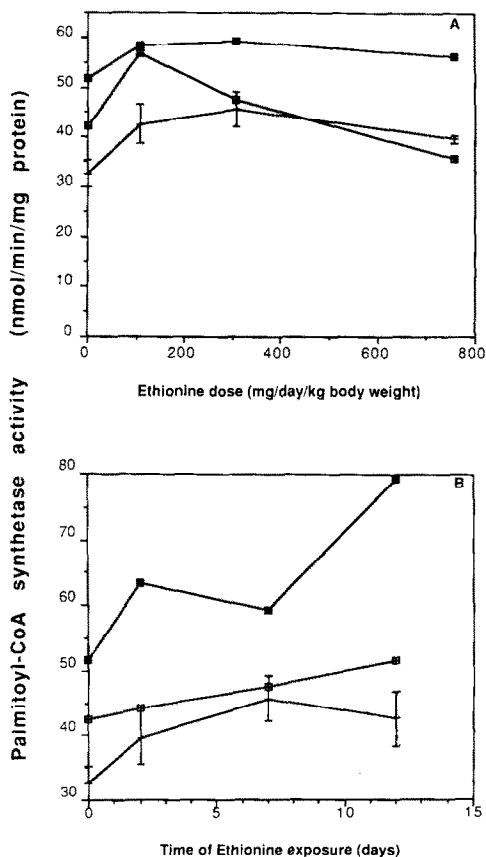


Fig. 4. Changes of palmitoyl-CoA synthetase activities in subcellular fractions with increasing doses (A) and during the course (B) of ethionine exposure. Enzyme activity in the postnuclear fraction (—○—) (data are expressed as the means \pm S.D. of four individual animals); in the mitochondrial fraction (—□—) and in the microsomal fraction (—■—). The data in the mitochondrial and microsomal fractions represent the means of four animals of two feeding experiments.

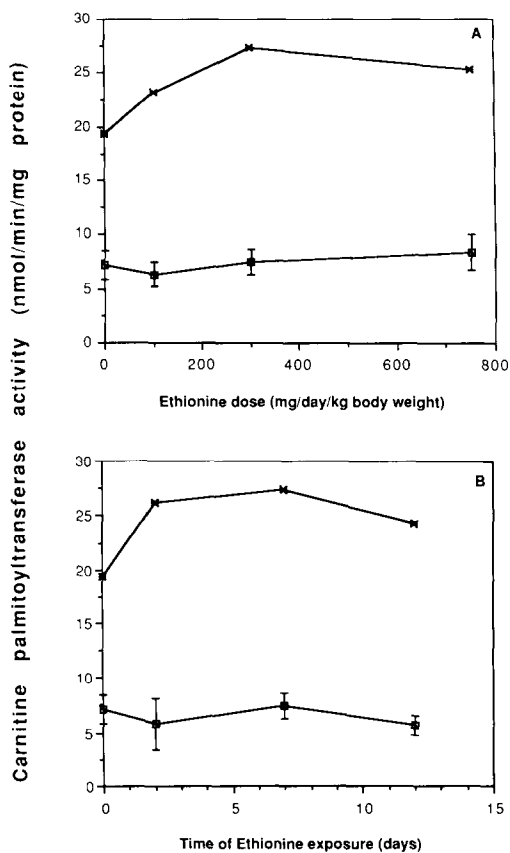


Fig. 5. Effect of ethionine exposure on carnitine palmitoyltransferase activity as a function of increasing doses (A) and times (B). The enzyme activity in the postnuclear fraction (■) and in the mitochondrial fraction (×). Data are expressed as the means \pm S.D. and only means (see legend to Fig. 4).

B). After subcellular fractionation, over 80% of the total carnitine palmitoyltransferase activity was localized to the mitochondrial fraction. The mitochondrial enzyme activity of the rats treated for 7 days was increased 1.4-fold at ethionine concentrations up to 300 mg/day per kg body weight. Changes of the mitochondrial carnitine palmitoyltransferase activity appear to be an early event, as an increase was observed already after 2 days of treatment (Fig. 5B).

The changes of glycerophosphate acyltransferase activity in whole homogenates of rat liver, in subcellular fractions at increasing doses of ethionine and of time are shown in Fig. 6. The total glycerophosphate acyltransferase activity of

the animals treated for 7 days was slightly increased. The increase was statistically significant at the highest ethionine dose used (Fig. 6A). A dose of 300 mg/day per kg body weight caused a statistically significant increase in this enzyme activity of the rats exposed for 12 days compared with the animals treated for 2 days (Fig. 6B).

Like palmitoyl-CoA synthetase, glycerophosphate acyltransferase is a multiorganelle-localized enzyme [17]. The mitochondrial glycerophosphate acyltransferase activity was not affected by increasing doses of ethionine or as a function of time (Fig. 6A and 6B). The microsomal glycerophosphate acyltransferase activity, however, tended to increase at the lowest dose, 100 mg ethionine/day per kg body weight. No changes were seen at higher ethionine doses. At a dose of

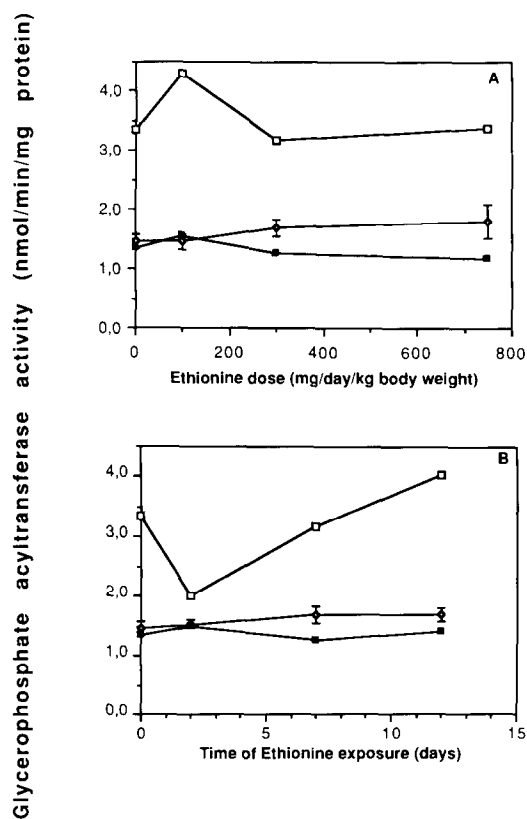


Fig. 6. Dose- (A) and time- (B) dependent changes of glycerophosphate acyltransferase activity in the postnuclear fraction (◇), in the mitochondrial fraction (■) and in the microsomal fraction (□) during ethionine exposure. Data are expressed as means \pm S.D. and only means (see legend to Fig. 4).

300 mg/day per kg body weight, the microsomal glycerophosphate acyltransferase activity was lower in the rats treated for 2 days (Fig. 6B). After 12 days of exposure, the activity again was increased, and was slightly higher than the control values.

Relationship between ATP, S-adenosylamino acids and hepatic triacylglycerol accumulation

The ATP and AdoEth of whole liver after ethionine exposure is shown in Fig. 7. The AdoEth concentration was gradually increased with increasing doses of ethionine, whereas a decrease of the ATP level was observed up to a dose of 300 mg ethionine/day per kg body weight (Fig. 7A). At this dose, the liver ATP concentration of the animals treated for 7 days was decreased about 70%.

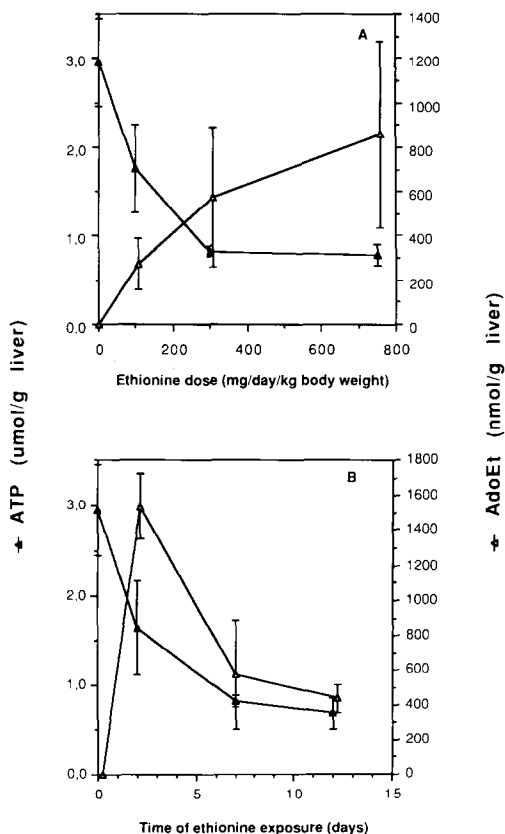


Fig. 7. Dose- (A) and time- (B) dependent changes of the amount of ATP (▲) and AdoEth (△) in the liver upon ethionine administration. Data are expressed as the means \pm S.D. of four animals.

The concentration of ATP of the animals treated for 2 days was decreased about 50%. The decrease continued with ethionine exposure up to 7 days (Fig. 7B). Accumulation of liver AdoEth was highest after 2 days of treatment (Fig. 7B). An inversed correlation, with a correlation coefficient of 0.93 ($P < 0.01$) was found between ATP and AdoEth (data not shown). A positive significant correlation was found between triacylglycerol and AdoEth, $r = 0.79$ ($P < 0.01$) (data not shown). No correlation between hepatic triacylglycerol and ATP was found (data not shown).

During ethionine exposure, the AdoHcys content was increased, whereas the AdoMet concentration was decreased [22]. A positive correlation was found between ATP and AdoMet ($r = 0.76$; $P < 0.01$) and between ATP and the ratio of AdoMet/AdoHcys ($r = 0.80$; $P < 0.01$, data not shown). No relation between triacylglycerol and AdoMet and AdoHcys could be demonstrated (data not shown).

Discussion

It has recently been reported that some changes occur in hepatic fatty acid metabolism, notably at the level of mitochondria, peroxisomes and endoplasmic reticulum on feeding male rats a choline-deficient diet [27].

As choline deficiency and ethionine exposure both induce fatty liver, we decided to evaluate the effect on activities of enzymes involved in activation of fatty acids, in esterification and mitochondrial oxidation of long-chain fatty acids after ethionine exposure. The key enzymes of these pathways are palmitoyl-CoA synthetase, glycerophosphate acyltransferase and carnitine palmitoyltransferase. The two former enzyme activities have been shown to have a multiorganelle localization, i.e., palmitoyl-CoA synthetase (mitochondria) [14], (peroxisomes) [28], and (microsomes) (endoplasmic reticulum) [14] and glycerophosphate acyltransferase (mitochondria and microsomes) [15].

Although many liver enzymes and several plasma proteins have been found to be decreased in ethionine-exposed animals [1,6], the present study shows that the ATP-requiring enzyme, palmitoyl-CoA synthetase, was moderately stimu-

lated in a dose- and time-dependent manner by ethionine exposure, at least in the microsomal fraction (Fig. 4). In contrast, the activities of catalase, urate oxidase and lactate dehydrogenase were decreased over 60% at an ethionine dose of 250 mg/day per kg (unpublished data).

Carnitine palmitoyltransferase and glycerophosphate acyltransferase are competing for the available long-chain acyl-CoA. Administration of ethionine moderately increased the carnitine palmitoyltransferase activity (Fig. 5), whereas the mitochondrial glycerophosphate acyltransferase activity was unchanged. Adaptive changes in the activities of these key enzymes may reflect alterations in the metabolic flux through the corresponding pathways. Changes in enzyme activities are either caused by an altered enzyme content in the liver or changes in enzyme activity modulated by metabolites. The activity of carnitine palmitoyltransferase runs in parallel with the mitochondrial oxidation of fatty acids (unpublished data). The small increase in carnitine palmitoyltransferase may reflect an increase in oxidation of fatty acids, thereby decreasing phosphatidic acid/di- and triacylglycerol formation. Thus, at the mitochondrial level of the ethionine-treated animals, the increased ratio of carnitine palmitoyltransferase activity to glycerophosphate acyltransferase activity may lead a greater part of activated fatty acids towards oxidation and less towards triacylglycerol formation.

It was of interest, however, that the microsomal glycerophosphate acyltransferase activity was increased in the ethionine-treated animals. It is conceivable that the ethionine response, at the endoplasmic reticulum, is such that a greater part of activated fatty acids are directed from oxidation towards triacylglycerol esterification, where excess diacylglycerol is converted to triacylglycerol. This response is in accordance with the observation that ethionine exposure increased the hepatic lipid level (Fig. 1) and caused a massive accumulation of small droplets of fat, lying in clusters in the cytoplasm and often abutting on the endoplasmic reticulum (Fig. 2).

Assuming that changes in enzyme activities mean that the flux through the pathways will be affected, the accumulation of triacylglycerols in the liver of the ethionine-treated animals is prob-

ably not only due to an interference with the synthesis of the protein moiety [11] and the lipid moiety of the lipoproteins, but also to an increased flux through pathways providing phosphatidic acid/diacylglycerol for triacylglycerol synthesis. The enzymological data suggest that hepatic fat accumulation of ethionine-treated rats may be related to an enhanced esterification of long-chain fatty acids, but cannot be attributed simply to a reduction of their oxidation.

The stimulation of three key enzymes involved in lipid metabolism by ethionine, in particular the palmitoyl-CoA synthetase activity, which uses ATP as a cofactor, is somewhat paradoxical. Ethionine has been shown to inhibit many liver enzymes, especially those which are localized in the cytosol [13] and mitochondria [29], and to decrease the concentration of ATP [6]. The reduced concentration of ATP in the liver upon ethionine treatment (Fig. 7) confirms well-known results. Moreover, the ethionine-induced ATP depression appears to be a rapid biological response (Fig. 7A).

Results presented here show that decreased serum lipid content (Fig. 3), concomitant with an increased hepatic lipid level (Figs. 1 and 2) are also biological responses attributed to ethionine. This is in agreement with previous findings [1,6] suggesting that ethionine increased the liver glycerolipid biosynthesis while lipoprotein secretion was impaired [10]. Concomitant with an ATP decrease, an increased accumulation of AdoEth occurs (Fig. 7). The inverse correlation of ATP and AdoEth up to a dose of 300 mg ethionine/kg per body weight (Fig. 7) suggests that the induction of ATP deficiency by ethionine depends on the conversion of ethionine to AdoEth. The concentration of ATP may decrease because it reacts with ethionine to form AdoEth at a rate faster than the cell can synthesize the nucleotide *de novo* from available precursor.

Whether the decrease of ATP may be the underlying basis also for induction of fatty liver should be considered. In addition to a positive correlation between triacylglycerol and AdoEth (data not shown), the time-course study of ethionine showed that the hepatic triacylglycerol level (Fig. 1) and the AdoEth (Fig. 7) content changed in a parallel manner. This effect further strengthens the interpretation that the develop-

ment of fatty metamorphosis in the liver appears to be related in some fashion to lowering of ATP via the ATP-trapping reaction.

It was of interest to observe that the serum HDL cholesterol concentration was so dramatically lowered (Fig. 3). Since the HDL plasma lipoproteins contain a high content of protein (about 50%) and phospholipid (about 30%) and to a lesser degree, cholesterol and triacylglycerol [29,30], it is possible that the effect on HDL cholesterol may be secondary to a more fundamental disturbance in protein and phospholipid synthesis.

We have recently found that a choline-deficient diet and also methotrexate exposure, which both induce fatty liver, reduce the AdoMet content in liver [27]. A similar effect has been observed after ethionine exposure [22]. Low AdoMet favors accumulation of fat in the liver [30–32]. Decreased methylation capacity for phosphatidylcholine synthesis due to lowering of AdoMet may interfere with the synthesis of the phospholipid moiety, and in this way may affect the assembly and secretion of lipoproteins. This in turn indicates a relationship not only between hepatic ATP deficiency and fatty liver and between inhibition of protein synthesis and fatty liver, but also between the latter and a limited methylation capacity to form phosphatidylcholine from phosphatidylethanolamine.

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