

Changes in peroxisomes and mitochondria in liver of ethionine exposed rats: a biochemical and morphological investigation

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Administration of ethionine resulted in a dose- and time-dependent enhancement of the activities of peroxisomal β -oxidation, carnitine palmitoyltransferase and ω -oxidation, especially the 12-hydroxylation of lauric acid. The mitochondrial and, especially, the microsomal palmitoyl-CoA hydrolase activities were increased, whereas the peroxisomal and cytosolic activities were decreased. Ethionine administration decreased the catalase and urate oxidase activities in both a dose- and time-related manner. The liver cells and the volume fraction of cytoplasm decreased 40% in ethionine-exposed animals, whereas the average nuclei volume fraction increased ~50%. The volume fraction and the total number of mitochondria increased 1.5-fold after ethionine exposure and an accumulation of lipid in large droplets of the hepatocytes was observed. No proliferation of peroxisomes was observed after treatment; the volume fraction and the number of peroxisomes decreased. However, the size of peroxisomes in livers of ethionine-exposed rats tended to be greater than controls; a 1.5-fold increase in average size was observed. As there was no induction of the protein content of the bifunctional enoyl-CoA hydratase, an enzyme involved in peroxisomal β -oxidation, it is considered that ethionine selectively stimulates the peroxisomal β -oxidation due to increased peroxisome surface area rather than evoked a peroxisome proliferation capacity. Increased peroxisomal β -oxidation was also observed in the kidney of ethionine-exposed rats at a dose of 750 mg/day/kg body weight. At that dose the amount of reduced glutathione (GSH) was significantly increased in kidney. The amount of GSH and the level of peroxisomal β -oxidation were significantly increased in liver at an ethionine dose of 100 mg/day/kg body weight. These responses in liver were evident within 2 days of ethionine exposure and then leveled off whereas a significant increase in GSH and peroxisomal β -oxidation in kidney was observed within 12 days. Whether the acute H_2O_2 -generating peroxisomal oxidation of long-chain fatty acids in the liver may also make this organ susceptible to the long-term effects of low-dose ethionine and be an important step in the chain of events which eventually results in tumour development should be considered.

*Abbreviations: AdoEth, *S*-adenosylethionine; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine, Hcy, L-homocysteine; GSH, reduced glutathione.

Introduction

Ethionine is a structural analogue of the amino acid methionine. Investigations have shown that ethionine inhibits some methionine-dependent processes and replaces methionine in others (1).

The synthesis and accumulation of *S*-adenosyl-ethionine (AdoEth*) in the liver has received much attention. It has been postulated that ATP depletion occurs because the adenosyl-moiety of ATP is trapped as AdoEth (2). Hence, ATP deficiency, which is an early effect taking place within a few hours, may perturb several metabolic processes, including protein synthesis, ATP-requiring enzyme systems and choline metabolism (1-3). In addition, it has been assigned a role in the acute fatty metamorphosis of the liver (4). We recently found that the ATP-requiring enzyme involved in formation of long-chain acyl-CoA was not sensitive to a decrease in the ATP level (5). Ethionine exposure was found to increase the catabolism of fatty acids at the mitochondrial level, and increased the capacity for esterification of fatty acids at the endoplasmic reticulum (5).

Chronic ethionine feeding for several months produces liver tumours in rat (1-6) and mouse (7). There is no development of tumours in organs other than the liver (1). The mechanism by which ethionine induces liver cancer is not fully elucidated. Hypomethylation of DNA has been demonstrated in liver (8). It is conceivable that ethionine carcinogenesis may be due in part to altered AdoMet/*S*-adenosylhomocysteine (AdoHcy) ratio which may modulate gene expression (8-10).

A number of structurally unrelated compounds with hypolipidemic properties cause hepatocellular carcinomas in rat (11). A common characteristic of treatment with these compounds is a change in peroxisome morphology (11,12), a marked increase in the number of peroxisomes (11,12) and increased activities of enzymes involved in mitochondrial and peroxisomal fatty acid metabolism (13-15). The peroxisomal β -oxidation has received much attention with respect to the mechanism of peroxisome proliferator-induced cancer (16). Recently, we have found that some hypolipidemic peroxisome proliferating drugs show tumour promoter activity *in vitro* (17). As the peroxisome proliferators are neither mutagenic (18) nor DNA damaging (19), Reddy and his group (11,16,20) have proposed that their carcinogenicity is related to biologically active products of the proliferated peroxisome rather than to direct chemical effect on cellular macromolecules. An imbalance between H_2O_2 production and degradation due to high rates of the H_2O_2 generating peroxisome β -oxidation system can cause an intrahepatic oxidation stress which can modulate gene expression and induce neoplastic transformation.

The goal of the present study was to investigate possible changes in H_2O_2 generating β -oxidation enzymes and fatty acid metabolism in livers treated with the non-mutagenic hepatocarcinogenic agent ethionine (data to be published). In order to get a clearer idea of the effect of ethionine on the hepatocytes of male rats, the peroxisomes and mitochondria were studied by analytical differential centrifugation and analysis of marker

enzymes and morphometric analysis. It was also deemed of interest to consider the peroxisomal β -oxidation in relation to the cellular glutathione (GSH)-pool since this important thiol compound may be responsible for the defence against free radicals and H_2O_2 .

Materials and methods

Chemicals

L-ethionine was purchased from Aldrich-Chemie (Steinheim, FRG), [^{14}C]palmitoyl-CoA and [^{14}C]palmitoyl-L-carnitine were obtained from New England Nuclear (Boston, MA, USA). Palmitoyl-CoA, palmitoyl-L-carnitine, CoASH and Hepes were purchased from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals were obtained from commercial sources, and were of reagent grade.

Animals and diets

Male Wistar rats from Møllegaard Breeding Laboratory, Ejby, Denmark, weighing 180–220 g at the start of the experiment, were housed individually in metal wire cages in a room maintained at 12 h light/dark cycles and a constant temperature of $20 \pm 3^\circ C$. The animals were acclimatized for at least 5 days under these conditions before the start of the experiments. Ethionine was dissolved in physiological saline (24.5 mg/ml) and injected i.p. twice daily and the amount injected and duration of exposure are given in the tables and figures. Control rats were injected with saline. At the end of the experiment, the rats were weighed, and then killed by decapitation, and the liver and kidney quickly excised and rinsed in ice-cold homogenization buffer (0.25 M sucrose, 10 mM Hepes buffer, pH 7.4) (21).

Preparation of total homogenate and different subcellular fractions

The livers and kidneys were homogenized in ice-cold homogenization buffer using a Potter–Elvehjem homogenizer at 720 r.p.m. and with two strokes of a loose-fitting Teflon pestle. Subcellular fractions of the livers were isolated by differential centrifugation as described (13,14).

The resulting nuclear plus post-nuclear fraction was used as the total homogenate. A mitochondrial enriched fraction (M-fraction) was prepared from the post-nuclear fraction using a time integral of $6.4 \times 10^8 \text{ min}^{-1}$. A peroxisome-enriched fraction (L-fraction) was prepared using a time integral of $4.3 \times 10^9 \text{ min}^{-1}$, this centrifugal 'window' is essential in preparing an optimal peroxisome-enriched fraction from normal rats (13). A microsomal-enriched fraction (P-fraction) was isolated from the post-peroxisomal fraction using a centrifugal effect of $7.4 \times 10^{10} \text{ min}^{-1}$. The remaining supernatant was collected as the cytosolic fraction (S-fraction).

Assay of marker enzymes and other analytical methods

The variation in the response from animal to animal was estimated separately for selected enzymes in the group of treated animals (four). The enzymatic activity of palmitoyl-CoA hydrolase (EC 3.1.2.2) (15,21), palmitoyl-L-carnitine hydrolase (EC 3.1.1.28) (22), palmitoyl-CoA dependent dehydrogenase (usually termed peroxisomal β -oxidation) (13), carnitine palmitoyltransferase (EC 2.3.1.21) (23), acid phosphatase (EC 3.1.3.2) (24), lactate dehydrogenase (EC 1.1.1.27) (24), catalase (EC 1.11.16) (23), urate oxidase (EC 1.7.3.3) (23), glutamate dehydrogenase (EC 1.4.1.2) (23), succinate: phenazine methosulphate (PMS) oxidoreductase (EC 1.3.99.1) (24) and rotenone-insensitive NADPH cytochrome *c* oxidoreductase (EC 1.6.2.4) (24) was determined as earlier described. Protein was determined by the Bio-Rad protein assay kit (Bio-Rad Laboratories, USA). All the spectrophotometric measurements were performed with a Varian 2300 spectrophotometer. Radioactivity was counted in a Packard Tricarb Liquid Scintillation Spectrometer, Model 3385.

SDS-PAGE was performed according to the method of Laemmli (25).

The reduced GSH content in liver and kidney was determined as earlier described (26).

Morphological methods

Rat liver pieces of the same animals were taken immediately for morphological examinations. They were cut into small blocks of 1 mm³ and fixed in 2.5% (v/v) glutaraldehyde in 0.25 M sucrose solution buffered to pH 7.4 with 10 mM Hepes buffer at 4°C for 30 min (22). The blocks were rinsed in buffer and post-fixed in 1% (w/v) OsO_4 in the same buffer at 4°C for 90 min. The tissue blocks were dehydrated in ethanol, embedded in epoxy resin and ultrathin sections were cut with an LKB ultratome III. The semithin sections, stained in toluidine, were studied using a light microscope, and exact drawings of the cells were made of 800 \times .

Electron micrographs were made from two or three blocks of liver tissue from each rat. The area of hepatocyte cytoplasm, the number of peroxisomes and mitochondria, and the area of the two organelles were measured in four micrographs per block of tissue with a Kontron MOP AM 03 Morphometric Analyzer. Calculations to estimate cytoplasmic volume, percent of cytoplasm occupied by peroxisomes or mitochondria, number of organelles per unit cytoplasmic volume, and average volume of peroxisomes and mitochondria were done either directly on electron micrographs or by projecting the images from negatives to the morphometric screen (magnification: 35 000 \times) (12,27).

Statistical methods

A two-way variance analysis was used for the comparison of values from the different groups. In these cases, comparison was made using Student's *t*-test and the *P* values were corrected using the Bonferroni correction.

Results

Effect on the total and specific activities of hepatic peroxisomal, mitochondrial and microsomal marker enzymes

There was a dose- and time-dependent decrease of the body weight and liver weight during ethionine exposure (5).

The distribution of protein and marker enzymes for mitochondria (glutamate dehydrogenase and succinate: PMS oxidoreductase), peroxisomes (catalase and urate oxidase), lysosomes (acid phosphatase) microsomes (rotenone-insensitive NADPH cytochrome *c* oxidoreductase) and cytosolic fraction (lactate dehydrogenase) was, for all groups of control and treated animals, both in the dose- and time-course studies, essentially similar to our previous findings for rat liver homogenates (11,25). The isolated cellular fractions appeared to be rather 'pure' based on these marker enzyme activities and in accordance with previous findings (14,25). Recovery of protein and enzyme activities was essentially the same for all feeding groups and in the range 96–105% (data not shown).

Administration of increasing ethionine doses tended to decrease the glutamate dehydrogenase activity in the total liver homogenates and this decrease (~20%) was significantly revealed in the mitochondrial-enriched fraction, M, at a dose of 300 mg/day/kg body weight (Table I). A similar loss of the succinate: PMS oxidoreductase (inner mitochondrial membrane marker) activity was observed (data not shown). Acid phosphatase

Table I. Effect of ethionine exposure on the total and specific activities of marker enzymes in post-nuclear fraction (E), and in subcellular fractions (mitochondrial fraction, M; peroxisomal fraction, L; microsomal fraction, P; cytosolic fraction, S)

Ethionine dose (mg/kg/day)	Glutamate dehydrogenase		Acid phosphatase		Lactate dehydrogenase		Rotenone-insensitive NADPH cytochrome <i>c</i> oxidoreductase	
	E ^a	M ^b	E ^a	L ^b	E ^c	S ^b	E ^a	P ^b
0	21.2 \pm 3.1	524 \pm 11.2	6.0 \pm 0.1	188 \pm 30	45.0 \pm 4.1	470 \pm 20	82.3 \pm 1.2	352.5 \pm 14.2
100	23.7 \pm 4.2	492 \pm 25.6	5.5 \pm 0.7	103 \pm 6*	49.8 \pm 8.2	490 \pm 50	93.4 \pm 6.2*	359 \pm 4.2
300	19.3 \pm 0.6	434 \pm 1.8*	5.5 \pm 0.2	124 \pm 7*	30.6 \pm 3.1*	404 \pm 10	82.6 \pm 2.1	326 \pm 9.1
750	19.8 \pm 1.2	430 \pm 1.0*	5.5 \pm 0.3	101 \pm 10*	23.1 \pm 4.2	290 \pm 50	85.4 \pm 3.1	345 \pm 1.2

The total activity ($\mu\text{mol}/\text{min}/\text{g}$ liver^a or $\text{nmol}/\text{min}/\text{g}$ liver^c) was measured in the post-nuclear fraction, E.

The specific activity ($\text{nmol}/\text{min}/\text{mg}$ protein^b) was measured in the subcellular fractions M, L, P and S.

The tabulated values represent the means \pm SD of three animals in each experimental group.

*Significantly different from control, *P* < 0.05.

activity was marginally affected in the total liver homogenates of treated animals, but a decreased activity in the peroxisome-enriched fraction, L, was observed, resulting in a loss of ~50% at an ethionine concentration of 100 mg/day/kg body weight (Table I).

Hepatic lactate dehydrogenase activity decreased both in the total liver homogenates and the prepared cytosolic fraction. In the latter fraction, administration of ethionine doses of 300 and 750 mg/day/kg body weight decreased the lactate dehydrogenase activity ~15 and 40% respectively (Table I).

Increasing ethionine doses did not change the rotenone-insensitive NADPH cytochrome *c* oxidoreductase activity either in total liver homogenates or in the isolated microsomal fraction, P (Table I).

The activities of two peroxisomal marker enzymes, catalase and urate oxidase, were assayed in the total liver homogenates and isolated cellular fractions. In the dose- and time-course study, the decreases in catalase activity in the total liver homogenates were similar in magnitude to the changes in urate oxidase activity, except in the 2-day treated animals (Figure 1). The cytosolic catalase activity (Figure 2) and the peroxisomal-associated urate oxidase activity (Figure 1) were dramatically decreased with ethionine exposure: namely, at an ethionine dose of 300 mg/day/kg body weight the two enzyme activities were decreased ~85%. Notably, the catalase activity in the peroxisome fraction,

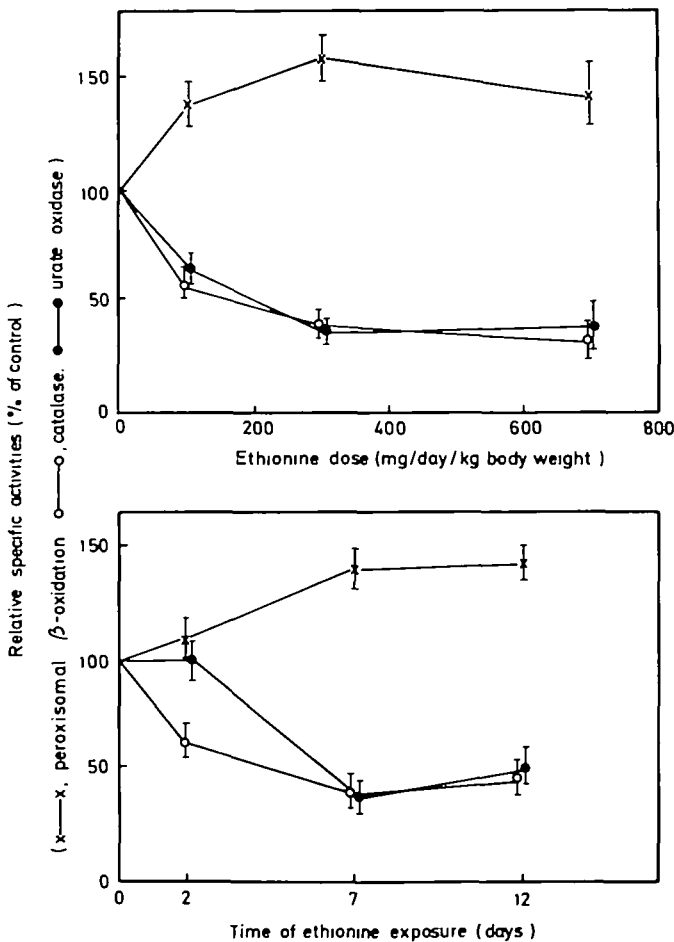


Fig. 1. Dose- and time-related effect of ethionine administration on the activities of peroxisomal β -oxidation (X), catalase (O) and urate oxidase (●) in total liver homogenates. (A) The activities as a function of ethionine dose, administered for 7 days. (B) The activities as a function of time with an ethionine dose of 300 mg/day/kg body weight.

L, was only marginally affected after ethionine exposure, resulting in a decreased activity of ~10% (Figure 2).

Effect on the total and specific activities of the hepatic long-chain fatty acid metabolizing enzymes

In contrast to a decrease in catalase and urate oxidase activities after ethionine administration, the peroxisomal β -oxidation was increased in a dose- and time-related manner in the total liver homogenates (Figure 1) and in the peroxisomal-enriched fraction (Figure 2). Furthermore, it should be noted that the increase in the peroxisomal-enriched fraction was more pronounced. The peroxisomal β -oxidation of the 7-day treated animals at a dose of ethionine of 300 mg/day/kg body weight was enhanced 2.3-fold (Figure 2).

The dose- and time-course studies show that the carnitine palmitoyl transferase activity was also increased on ethionine exposure (Figure 3); a 1.4-fold increase was revealed at a dose of 300 mg/day/kg body weight. This increase in the total liver homogenates was observed in the mitochondrial fraction (data not shown), in good agreement with previous findings (5).

The palmitoyl-CoA hydrolase is shown to have multiple subcellular localization (mitochondria, peroxisomes, microsomes and cytosol) (13,14,23). An ethionine dose of 100 mg/day/kg body weight caused a significantly increased (18%) palmitoyl-CoA hydrolase activity in the total liver homogenates, and a moderate increase was observed with increasing time of ethionine exposure (Figure 3). Figure 4 shows that this increase was

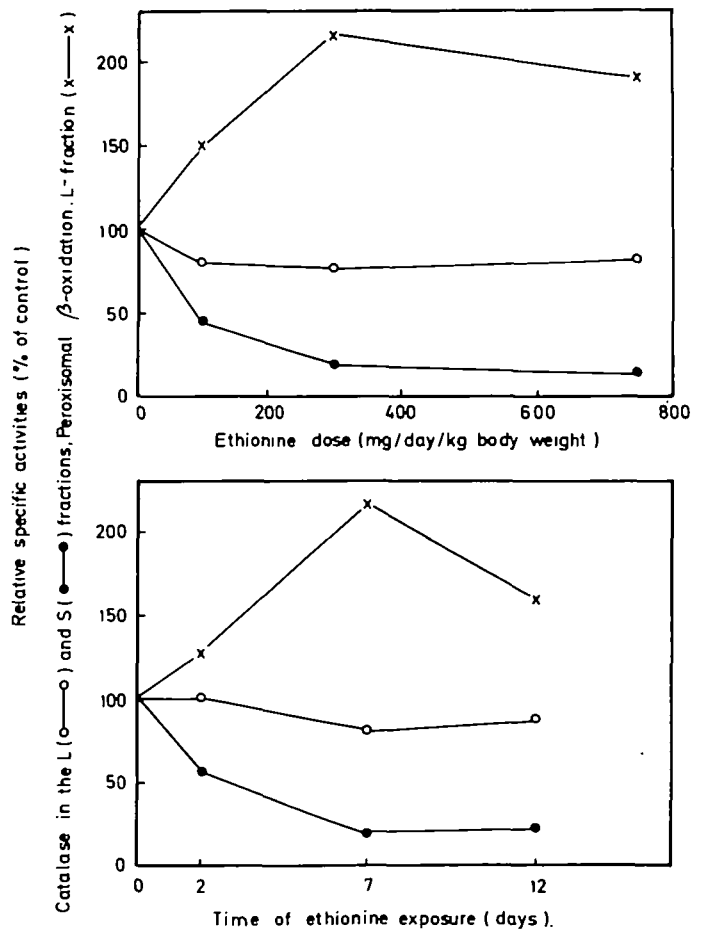


Fig. 2. Dose- and time-related effect of ethionine administration on the peroxisomal β -oxidation (X) in the peroxisome-enriched fraction (L) and catalase activity in the L-fraction (O) and cytosolic (S)-fraction (●). For more details see legend to Figure 1.

attributed both to increased mitochondrial and microsomal palmitoyl-CoA hydrolase activities (~1.5-fold increase) but not to peroxisomal activity (data not shown) or cytosolic palmitoyl-CoA hydrolase activity. The cytosolic palmitoyl-CoA hydrolase activity was rather decreased in both a dose- and time-related manner.

The palmitoyl-L-carnitine hydrolase is shown to be localized to the microsomal fraction (24,28). The enzyme activity in the total homogenates (Figure 3) and in the microsomal fraction (Figure 4) was dramatically decreased as a function of dose and time after ethionine administration. The decreases in palmitoyl-L-carnitine hydrolase activity in the total liver homogenates were similar in magnitude to the changes observed in the peroxisomal fraction; ~80% loss of activity was observed at a dose of ethionine of 300 mg/day/kg body weight (Figure 4).

The 1.5-fold increase in the total hydroxylation of lauric acid after ethionine exposure at a dose of 300 mg/day/kg body weight resulted from the stimulation of only 12-hydroxylation (Figure 5). The consequence was that the ratio 12-/11-hydroxy lauric acid increased ~2.3-fold at that ethionine dose. After a 2-day lag-time, the time-course study showed an increasing stimulation of both 11- and 12-hydroxylation of lauric acid (Figure 5). Again

the 12-hydroxylation was mostly increased. In the 12-day treated rats the 12-hydroxylation of lauric acid was enhanced 4-fold whereas the 11-hydroxylation was elevated 1.9-fold (Figure 5).

Morphological studies

Administration of ethionine at a dose of 300 mg/day/kg body weight decreased the liver weight ~25% (Table II). Morphometric analysis of randomly selected parenchymal cells from the 7-day ethionine-treated animals at that dose showed that the cell size was markedly decreased; a 40% decrease resulted (Table II). In contrast, ethionine exposure increased the average nuclear size 1.4-fold (Table II). Therefore, the area fraction of nuclei increased by a factor of 2.3.

Ethionine exposure showed increased aggregates of fat-droplets lying in the cytoplasm and often abutting on to different organelles (Figure 6). Morphometric analyses revealed that in treated animals the volume fraction of fat-droplets and the number of fat-droplets increased 17- and 15-fold respectively (Table II). This is in agreement with increased hepatic accumulation of triglycerides after ethionine administration (5).

Morphometric analyses of cells from control rats (Table II) revealed a volume fraction of 1.0% for the peroxisomes and 14.8% for the mitochondria, in good agreement with previously published data (24). Notably, the proportion of cytoplasmic

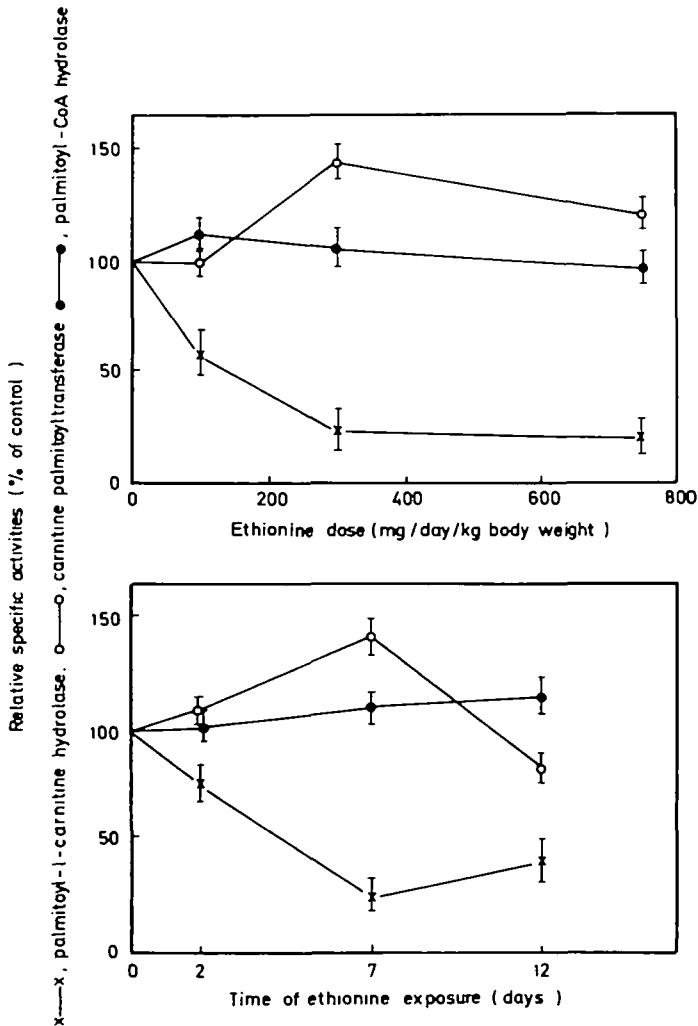


Fig. 3. Dose- and time-related effect of ethionine on the activities of carnitine palmitoyltransferase (O), palmitoyl-L-carnitine hydrolase (X) and palmitoyl-CoA hydrolase (●) in total liver homogenates. An ethionine dose of 300 mg/day/kg body weight was used in the time-course study.

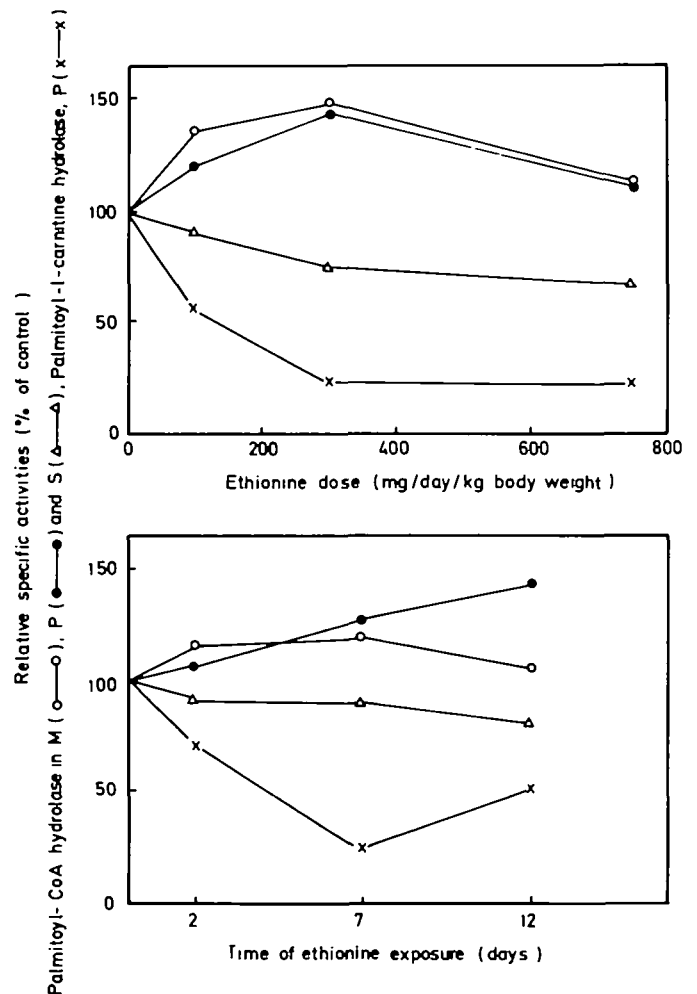


Fig. 4. Dose- and time-related effect of ethionine administration on the palmitoyl-CoA hydrolase activities in mitochondrial fraction (M) (O), microsomal fraction (P) (●) and cytosolic fraction (S) (Δ) and on palmitoyl-L-carnitine hydrolase activity in the microsomal (P) fraction (X).

volume occupied by peroxisomes in the liver was decreased ~35% in rats treated with ethionine. The mean number of peroxisomes in the liver of these rats was also decreased (~55%), whereas the size of peroxisomes tended to be greater than controls: i.e. ~1.5-fold increase of the mean peroxisome volume was observed (Table II).

Changes in the mitochondrial population during ethionine exposure was also observed and the most characteristic effect was a 1.5-fold increase in the volume fraction and more than 1.4-fold increase in the number of mitochondria (Table II). No change in the mitochondrial size was observed after ethionine administration. If the population density of mitochondria is estimated by the volume ratio of mitochondria to peroxisomes it is seen from Table II that this parameter increased from 15:1 to 33:1 during ethionine administration.

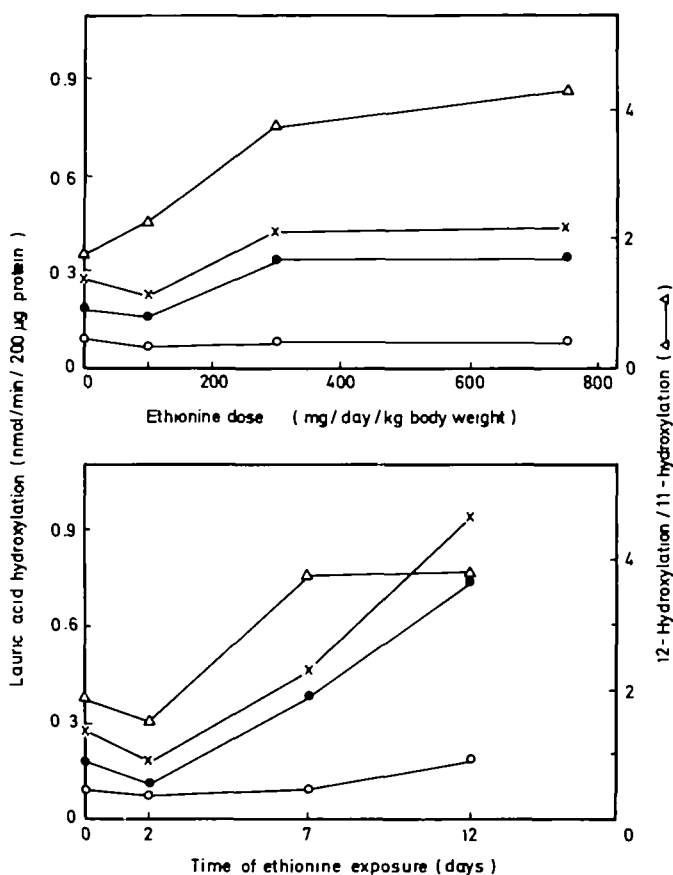


Fig. 5. Dose- and time-related effect of ethionine administration on total lauric acid hydroxylation (X), 11-hydroxylation (O), 12-hydroxylation (●) and the ratio 12-hydroxy/11-hydroxy of lauric acid (Δ) in the microsomal (P) fraction.

Table II. Morphometric analysis of peroxisomes, mitochondria, nuclei and fat droplets in hepatocytes of normal and ethionine treated rats (300 mg/day/kg body weight, 7 days)

	Liver weight (g)	Hepatocytes			Fat droplets Volume fraction (%)	Mitochondria			Peroxisomes		
		Cell volume (μm^3)	Nuclei volume (μm^3)	Cytoplasm volume (μm^3)		Volume fraction (%)	Number/ μm^3 cytoplasm	Mean volume (%)	Volume fraction (%)	Number/ μm^3 cytoplasm	Mean volume (%)
Control	11.3	7238	288	6950	0.045	14.8	0.71	0.218	1.03	0.26	0.040
Ethionine	8.3*	4189*	429*	3760*	0.77*	22.2*	1.03*	0.225	0.67*	0.12*	0.058*

The analysis was performed on eight micrographs from each of four rats per treatment group.

*Significantly different from control, $P < 0.05$.

Effect on SDS-PAGE patterns

SDS-PAGE was carried out with the peroxisomal-enriched fraction to examine the induction of the 80 kd protein, which is referred to as the bifunctional enoyl-CoA hydratase (20). The induction of 69 kd protein and 80 kd protein is known to be closely associated with peroxisome proliferation. Figure 7 shows that there was no induction of these protein bands by increasing ethionine doses and time of ethionine exposure.

Effect on GSH and peroxisomal β -oxidation in kidney and liver

The peroxisomal β -oxidation in the total kidney homogenates was significantly increased at the highest dose of ethionine (Figure 1 and Table III). In both tissues there was a time-dependent increase in GSH content within 12 days, and there seemed to be no further increase at doses above 300 mg/day/kg body weight. Notably, increased peroxisomal β -oxidation and GSH content in liver were observed at lower doses of ethionine and at a shorter time of exposure than in the kidney (Figure 2 and Table III).

Discussion

ATP deficiency, an early effect that occurs within a few hours in the liver of ethionine-exposed rats, perturbs several metabolic processes, including protein synthesis and ATP-requiring enzyme systems (1-3). Notably, the activities of the ATP-requiring enzyme, palmitoyl-CoA synthetase, localized to mitochondria, peroxisomes and endoplasmic reticulum (24) and the glycerophosphate acyltransferase, localized in both mitochondria and endoplasmic reticulum, were increased in ethionine-exposed rats (5). The present study has shown that the activities of carnitine palmitoyltransferase (Figure 3) and peroxisomal β -oxidation (Figures 1 and 2) were also increased after ethionine exposure, whereas the activities of the cytosolic palmitoyl-CoA hydrolase and the microsomal palmitoyl-L-carnitine hydrolase, which both carry out degradative reactions, were dramatically decreased (Figure 4). It should be considered that enzyme activities involved in degradative, non-energy requiring reactions of lipids appear to be especially sensitive in ethionine-induced ATP-deficient rats whereas enzymes involved in synthetic and ATP-requiring reactions of lipid metabolism are not. Increased fatty acid oxidation (mitochondrial and peroxisomal), which is linked to ATP-generating processes, probably reflects a compensating response for the ATP-depletion under ethionine administration.

The enzymes in the different cell-compartments are synthesized either on membrane-bound or -free polysomes.

Results presented here demonstrated that the activities of marker enzymes for the different cellular organelles were all decreased, but to different extents in ethionine-exposed rats, except for the endoplasmic reticulum marker which was not changed (Table I). The activity of the palmitoyl-L-carnitine

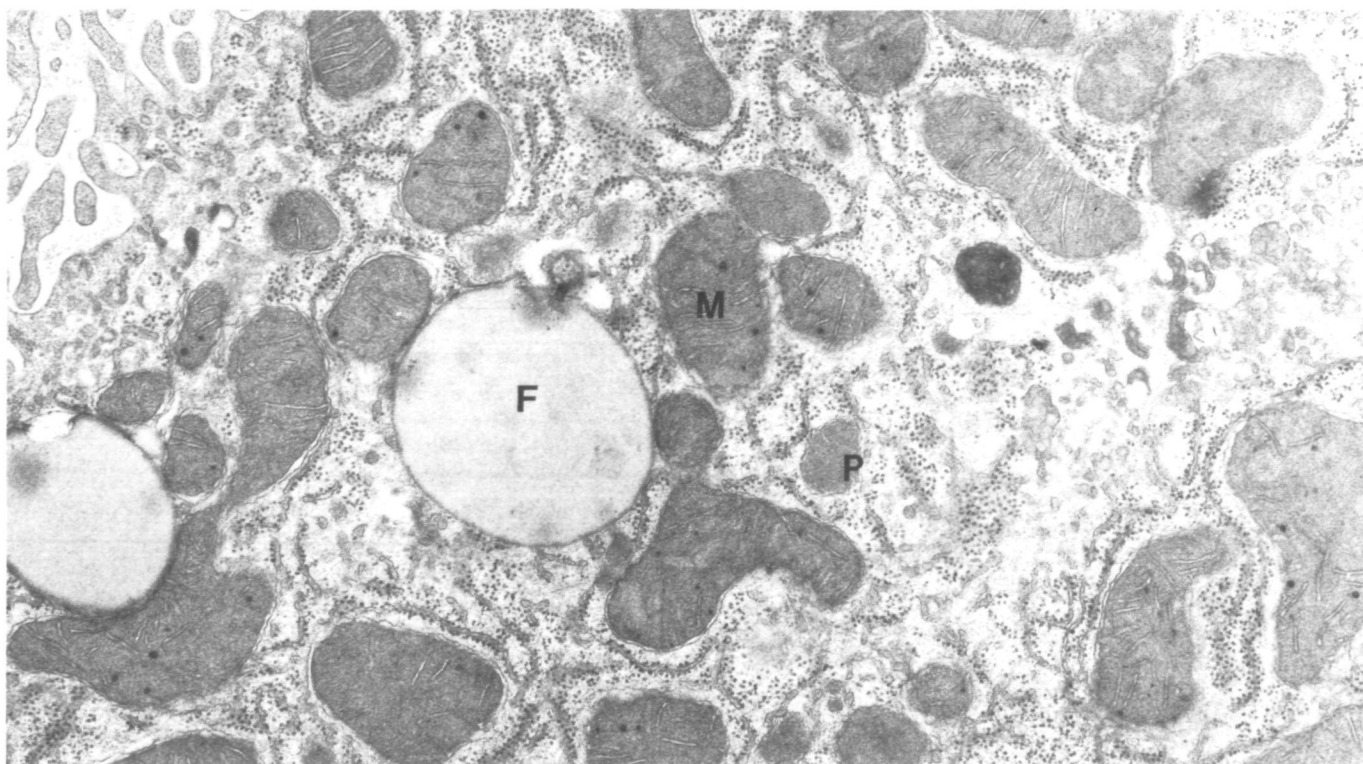


Fig. 6. Representative electron micrographs of a hepatocyte from ethionine-treated rats (300 mg/day/kg body weight for 7 days). M, mitochondrion; F, fat vesicle; P, peroxisome. Magnification: 24 000 \times .

hydrolase, a microsomal-associated enzyme (28) localized to the luminal side of endoplasmic reticulum (22) was dramatically decreased after ethionine exposure (Figures 3 and 4). Ethionine perturbs the protein synthesis (1). Whether the changes of enzyme activities in the subcellular fraction of ethionine-induced ATP-deficient rats (1) are a reflection of the existence of their intracellular compartmentalization and topography, should be considered.

The biogenesis of the cell organelles, especially the peroxisomes has been the subject of controversy (29). It is now established that both catalase and urate oxidase are synthesized at their final size on free polysomes and enters peroxisomes *in vivo* post-translationally without detectable processing (29). Recently, it has also been shown that the RNAs coding for the three proteins involved in the peroxisomal β -oxidation system are found predominantly on free polysomes (29). The catalase activity was decreased both in the peroxisome-enriched fraction and especially in the cytosolic fraction of liver of ethionine-treated rats (Figure 2). The peroxisomal β -oxidation, however, was stimulated (Figure 2). These results suggest that the changes of enzyme activities in ethionine-induced ATP-deficient rats cannot be ascribed to the site of protein synthesis, namely on membrane-bound or free polysomes.

The present study has confirmed previous findings that ethionine induces fatty liver and perturbs the hepatic lipid metabolism including the mitochondrial fatty acid oxidation (5). The results of the present experiments clearly demonstrate that ethionine exerts biochemical and morphological effects on mitochondria and peroxisomes. The slightly increased mitochondrial palmitoyl-CoA synthetase activity (5), carnitine palmitoyltransferase activity (Figure 3) and mitochondrial palmitoyl-CoA hydrolase activity (Figure 4) may be ascribed to

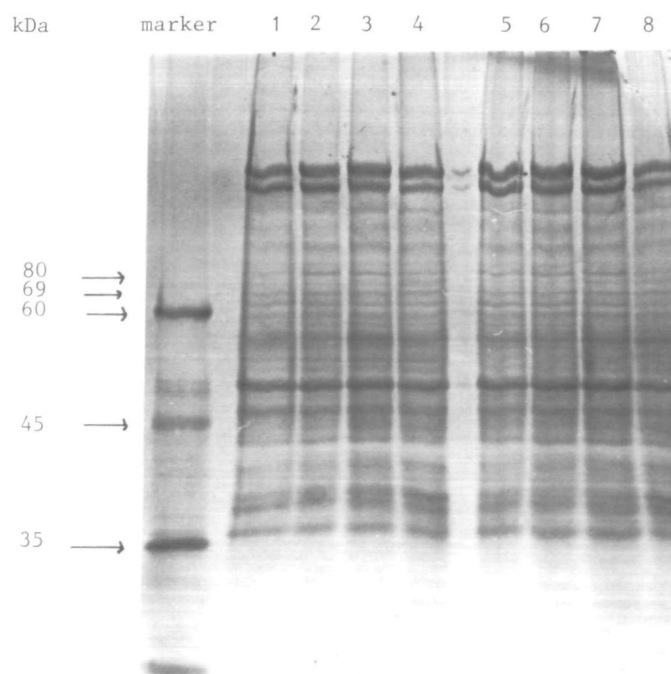


Fig. 7. SDS-PAGE (9%) of the polypeptides obtained from peroxisome-enriched fractions of animals treated with ethionine. The dose related effect of ethionine; animals treated for 7 days (lane 1, lane 5), 100 (lane 2), 300 (lane 3) and 750 (lane 4) mg/day/kg body weight i.p. ethionine. The time related effect of ethionine administration; animals treated with 300 mg/day/kg body weight i.p. ethionine for 2 days (lane 6), 7 days (lane 7) and 12 days (lane 8). The arrows indicate the position of the mol. wt markers of 66, 45 and 35 kd and the position of the peroxisomal membrane polypeptides 69 kd and 80 kd. The polypeptides are visualized by Coomassie brilliant blue.

Table III. Dose (A)- and time (B)-related effect of ethionine administration on the GSH content in liver and kidney and on peroxisomal activities in total kidney homogenates

Ethionine (mg/day/kg body weight, i.p.)	Days of treatment	Liver		Kidney	
		GSH nmol/g liver	nmol/mg protein	GSH nmol/g liver	Peroxisomal β -oxidation nmol/min/mg protein
A					
0	7	7.4 \pm 0.5	48.7 \pm 3.8	2.35 \pm 0.44	0.70 \pm 0.04
100	7	9.3 \pm 0.5*	59.3 \pm 1.6*	2.29 \pm 0.24	0.67 \pm 0.04
300	7	10.0 \pm 1.6*	76.0 \pm 6.7*	3.19 \pm 0.66	1.18 \pm 0.42
750	7	10.6 \pm 2.0*	76.2 \pm 6.7*	4.16 \pm 0.34*	1.26 \pm 0.07*
B					
300	2	10.5 \pm 0.5*	71.7 \pm 6.8*	2.42 \pm 0.43	0.69 \pm 0.12
300	12	13.5 \pm 0.7*	89.1 \pm 2.3*	3.6 \pm 0.49*	0.87 \pm 0.07

*Significantly different from control ($P < 0.05$).

the increased number of liver mitochondria and not to alteration of the mitochondrial size (Table II).

In the peroxisome-enriched fraction of rat liver, the activity of the peroxisomal β -oxidation was enhanced more than 2-fold (Figure 2). The catalase specific activity in the same fraction tended to decrease but the most dramatic inhibition of the catalase activity was found in the cytosolic fraction (Figure 2). The urate oxidase activity in the total liver homogenates was decreased by a similar magnitude as the catalase activity (Figure 2). Urate oxidase is located in the 'core' of peroxisomes whereas catalase is a matrix protein (29,30). The enzymes involved in peroxisomal β -oxidation are mostly membrane bound (30). Whether the increase in peroxisomal β -oxidation, concomitant with decreased catalase and urate oxidase activities, may be due to the increased surface area of peroxisomes rather than to increased number (Table II) in ethionine-induced rats, should be considered.

The subcellular distribution of palmitoyl-CoA hydrolase activity was changed as a result of ethionine exposure (Figure 4). The mitochondrial and, in particular, the microsomal palmitoyl-CoA hydrolase activities were increased, whereas the peroxisomal palmitoyl-CoA hydrolase activity was marginally affected. Notably, ethionine did not stimulate the cytosolic palmitoyl-CoA hydrolase activity (Figure 4). Rather a decreased activity was observed both in a dose- and time-related manner. Thus, the cellular distribution of palmitoyl-CoA hydrolase in ethionine exposed animals is not similar to the picture obtained with various peroxisome proliferators, i.e. hypolipidemic drugs (14,15), high-fat diet (24), choline deficiency (31) or to modulations in responses to altered physiological status, e.g. starvation (21) where there is an increased palmitoyl-CoA hydrolase activity.

The induction of the 80 kd protein is known to be closely associated with peroxisome proliferation (20,29). Increased cytosolic palmitoyl-CoA hydrolase (13,15,21) is a characteristic feature shared by peroxisome proliferators. No changes of these parameters were observed in the ethionine-treated rats. Thus, based on biochemical markers and in accordance with the morphological data ethionine shows no peroxisome-proliferating effect in rat liver, but rather may selectively stimulate the rate-limiting enzymes for fatty acid and glycerolipid metabolism, including a stimulation of peroxisomal β -oxidation and ω -oxidation. The effect of ethionine exposure on hydroxylation of lauric acid as detailed here has, to the best of our knowledge, not been reported previously.

The present data have demonstrated that ethionine exposure

can induce an increase in the synthesis of H_2O_2 -generating peroxisomal β -oxidation enzyme system in liver accompanied by a decreased catalase activity. Accordingly, the peroxisomal β -oxidation/catalase ratio in total liver homogenates increased from 1 to 5. This response is similar to the picture obtained with various peroxisome proliferators (13,14). Notably, in kidney of ethionine-treated rats the peroxisomal β -oxidation/catalase ratio was increased 1.5-fold (data not shown). Furthermore, high doses of ethionine were required to enhance a significant elevation of peroxisomal β -oxidation in the kidney. From our previous data (26) and the results described here, the GSH response of the liver is characterized by an increase in the GSH content which was significant after a short time of exposure and at a moderate dose of ethionine. Notably, in kidney high doses and prolonged ethionine exposure were required to enhance a significant elevation of GSH (Table IV).

An excess production of H_2O_2 and genesis of free radicals due to enhanced H_2O_2 -generating peroxisomal β -oxidation and uncontrolled lipid peroxidation (20), may lead to early DNA damage. This effect may be an important event leading to initiation of carcinogenesis in the liver of rats treated with ethionine. It was also of interest to consider the peroxisomal β -oxidation in relation to the cellular GSH pool since this thiol is regarded as an important scavenger of H_2O_2 and free radicals (20). The GSH level was increased by ethionine exposure—a phenomenon which is also observed after hypolipidemic peroxisome proliferators (20). Furthermore, the GSH content parallels the peroxisomal β -oxidation levels in liver of ethionine administered rats. No such correlation has been found between GSH and other metabolic effects (AdoMet, AdoEth, AdoHcy or Hcy) (26). However, methyl group deficiency alone has been shown to produce a significant incidence of liver tumours in rats (32–34). Whether the excess production of H_2O_2 generated through peroxisomal oxidation of fatty acids may be an important step in a chain of events which eventually results in tumour formation and hepatocellular carcinomas by long-term effect of low-dose ethionine, should be considered.

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