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# Differential metabolic response of rat liver, kidney and spleen to ethionine exposure. S-Adenosylamino acids, homocysteine and reduced glutathione in tissues

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**Intraperitoneal injection of ethionine to male rats for up to 12 days caused a pronounced fall in S-adenosylmethionine (AdoMet) in liver, but did not or only slightly affect AdoMet in kidney and spleen. Liver and to a lesser degree kidney showed a dose-dependent, massive accumulation of the metabolic product, S-adenosylethionine (AdoEth), and this metabolic response was most pronounced within the first days of exposure. Trace amounts of AdoEth was demonstrated in the spleen. Both S-adenosylhomocysteine (AdoHcy) and homocysteine (Hcy) in the liver were markedly increased in a dose- and time-dependent manner. There was a moderate increase in Hcy content in spleen and kidney, whereas the AdoHcy levels in these tissues were not affected. The amount of reduced glutathione (GSH) was significantly increased in liver and kidney. This response in liver was evident within 2 days of ethionine exposure and then leveled off whereas there was a gradual increase in GSH in kidney. The GSH content in spleen was unaltered. In addition to a massive build-up of AdoEth, the unique features of the metabolic response of the liver are a pronounced decrease in the AdoMet/AdoHcy ratio (from 15 to 2) associated with an elevated Hcy content and a rapid increase in the amount of GSH. The possibility that the metabolic response of the liver could be assigned to the existence of isozymes or metabolic pathways unique to hepatic cells is discussed.**

## Introduction

Ethionine is a structural analogue of the amino acid methionine. Acute high doses of ethionine causes lesions in the liver and some other organs. Chronic feeding for several months produces liver tumors in the rat (1,2) and mouse (3). There is no tumor development in organs other than the liver.

The metabolic basis of the ethionine effects has been the subject of numerous investigations. Ethionine inhibits some methionine-dependent processes and replaces methionine in other reactions (1). Among these reactions, the synthesis of S-adenosylethionine (AdoEth*) (4) has received particular attention.

Accumulation of AdoEth in the liver is an early effect taking place within a few hours of its administration. There is a concurrent fall in the ATP content, which has been explained by trapping of the adenosyl-moiety of ATP as AdoEth (4). ATP deficiency may perturb several metabolic processes, including protein synthesis, and has been assigned a role in the acute fatty metamorphosis of the liver (5).

*Abbreviations: AdoEth, S-adenosylethionine; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; Hcy, L-homocysteine; GSH, glutathione; Eth, ethionine; Met, methionine.

AdoEth itself serves as an ethyl donor. Alkylation of both RNA, proteins (6), phospholipids (7) and DNA (8) has been demonstrated. In addition, AdoEth may inhibit S-adenosylmethionine- (AdoMet) dependent transmethylation reactions, including methylation of DNA (9), which may modulate gene expression. Hypomethylation of DNA has been demonstrated in liver and testis, but not in kidney and thymus of rats given ethionine (10). These effects of AdoEth may play a role in the hepatocarcinogenesis.

The susceptibility of the liver to ethionine suggests unique features of the metabolism of this agent in hepatic tissue. Furthermore, methionine antagonizes most of the deleterious effects of ethionine (1) and several properties of the metabolism of methionine and related compounds like AdoMet, S-adenylhomocysteine (AdoHcy) and homocysteine (Hcy), are specific to hepatic tissues (11). We therefore compared rat liver, kidney and spleen with respect to the amount of S-adenosylamino acids and Hcy in these organs following ethionine exposure. Reduced glutathione, a cellular detoxification agent (12), was also included since recent evidence suggests a link to the metabolism of AdoMet (13) and AdoHcy (14). Because the major object of this work was to uncover organ differences in the primary metabolic effects of ethionine, the agent was administered by parenteral injection, to avoid possible first-pass effects (15) of ethionine.

The metabolic pathways mentioned above and their relation to ethionine are depicted in Figure 1.

## Materials and methods

### *Chemicals*

DL-Hcy, AdoHcy and reduced GSH were purchased from Sigma Chemical Co. (St Louis, MO), and AdoMet was obtained from Koch-Light Laboratories (Colnbrook, England). L-Ethionine (95%) was from Aldrich Chemical Co. (Milwaukee, WI). The purity was evaluated by reversed-phase liquid chromatography following precolumn derivatization with *ortho*-phthaldialdehyde. A single peak accounts for 87% of the fluorescence yield.

Monobromobimane was purchased from Calbiochem–Behring (La Jolla, CA). Other chemicals were obtained from commercial sources, and were of reagent grade.

### *Animals*

Male Wistar rats, 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 ± 3°C. The animals were acclimatized for at least 5 days under these conditions before the start of the experiments.

### *Treatment of animals*

The number of animals in each group was chosen so that each metabolite in the tissues of all the animals could be assayed within a single run. In this way the between-day variations were avoided.

L-Ethionine was dissolved in physiological saline (24.5 mg/ml) and injected i.p. twice daily, and the amounts injected and duration of exposure are given in the figures. Control rats were injected with saline.

At the end of the experiments, the animals were weighed, and then killed by decapitation, and liver, kidney and spleen rapidly removed and put into liquid nitrogen.

### *Determination of Hcy, AdoHcy and AdoMet in tissues*

Frozen tissue specimens were homogenized in 0.8 N perchloric acid. Acid-soluble Hcy was determined with a radioenzymic assay (16), whereas AdoHcy and AdoMet were determined directly in the acid extract using an HPLC method (17).

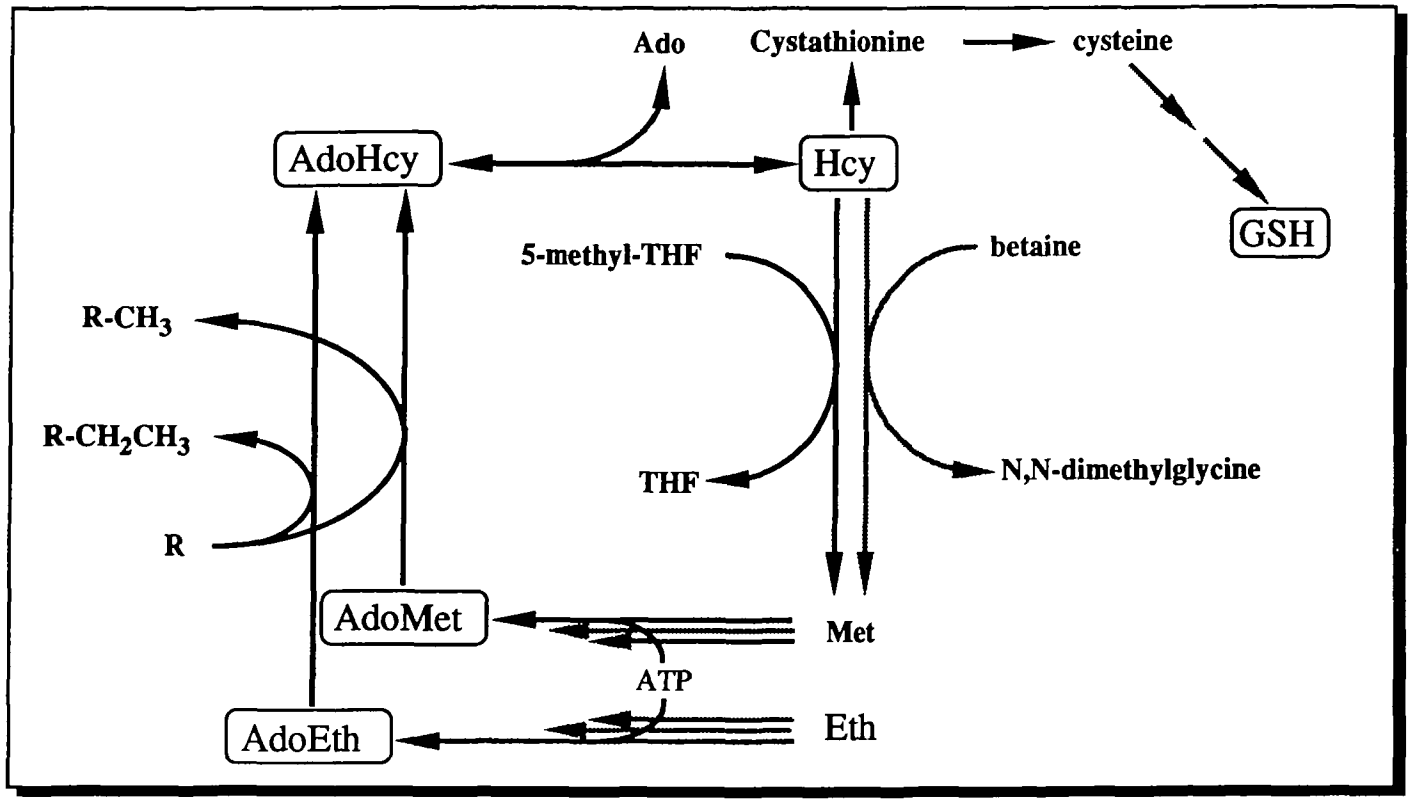


**Fig. 1.** The metabolism of Eth and its relation to AdoMet, GSH and related sulfur metabolites. The shaded arrows indicate metabolic pathways typical for hepatic tissue, and the shaded, parallel arrows pointing to AdoMet or AdoEth symbolize hepatic isozymes of AdoMet synthase. The metabolites measured in the present paper are encircled.

*Determination of AdoEth in tissues*

This metabolite was determined in the perchloric acid extract by a modification of the procedure used for measurement of AdoHcy and AdoMet. The Partisil 10 SCX cation exchange column was eluted isocratically at a flow rate of 1.5 ml/min, with 0.42 M ammonium formate, pH 3.5, containing 2% propanol. AdoEth eluted at a retention time of 7.8 min.

*Determination of GSH in tissues*

Frozen tissue samples were rapidly homogenized (1:5 w:v) in 5% sulfosalicylic acid. The precipitated proteins were removed by centrifugation. Reduced GSH was determined in the acid extract by a slight modification of the method of Newton *et al.* (18) which involves derivatization of the free sulfhydryl groups in the presence of the monobromobimane (Kosower's) reagent. The GSH–bimane derivative is then quantitated by chromatography on a 3 μm ODS Hypersil column, which is equilibrated and eluted with 14.2% methanol and 0.25% glacial acetic acid adjusted to pH 3.9 with sodium hydroxide. The column is washed by increasing the methanol concentration to 90%. The retention time of the GSH–bimane derivative was 3.2 min.

*Statistical analysis*

The number of animals in each group (four) did not allow testing for normal distribution of the parameters. Therefore, the statistical evaluation of the data was based on nonparametric tests.

The Kruskal–Wallis test was used to evaluate whether the ethionine dose or time of exposure induced significant metabolic effects. In case differences between treatment groups were demonstrated ($P < 0.05$), a pairwise comparison between the control group and the separate groups of animals given ethionine was done using the Mann–Whitney rank-sum test. The $P$-values were adjusted using the Bonferroni correction for multiple comparisons.

## Results

*AdoEth and AdoMet*

There was a dose-dependent accumulation of AdoEth. This was most pronounced in liver, where AdoEth reached levels 4–8 times higher than that of AdoMet, and somewhat less marked in kidney, whereas spleen accumulated small but significant amounts of AdoEth (Figure 2A).

The time course for the accumulation of AdoEth showed some differences between the tissues. The AdoEth content in liver showed a maximal response within a few days of exposure. Then a decrease in the amount of AdoEth was observed. A similar response was observed in kidney. The peak concentration was about the same as that observed in the liver, but the AdoEth content declined to levels below that measured in the liver. The AdoEth content in spleen showed small variations as a function of time of ethionine exposure (Figure 2B).

There was a dose- and time-dependent decrease in the amount of AdoMet in liver, whereas the AdoMet content in kidney and spleen was slightly or not affected during ethionine exposure (Figure 2A,B).

*AdoHcy and Hcy*

Ethionine administration induced a pronounced, dose-dependent increase in both AdoHcy and Hcy in liver (Figure 3A). This effect was fully developed within 2 days (Figure 3B). There was a moderate increase in the amount of Hcy in kidney and spleen, but the AdoHcy content in these tissues was not altered (Figures 3A,B).

*GSH*

There was a significant increase in the amount of GSH in liver and kidneys of rats injected with ethionine. In both tissues there was a time-dependent increase in GSH content within the experimental period, and there seemed to be no further increase at doses above 300 mg/kg/24 h. The response in liver was observed at a lower dose of ethionine and at a shorter time of exposure than in the kidney. Ethionine did not affect the GSH content in spleen (Figure 4A,B).

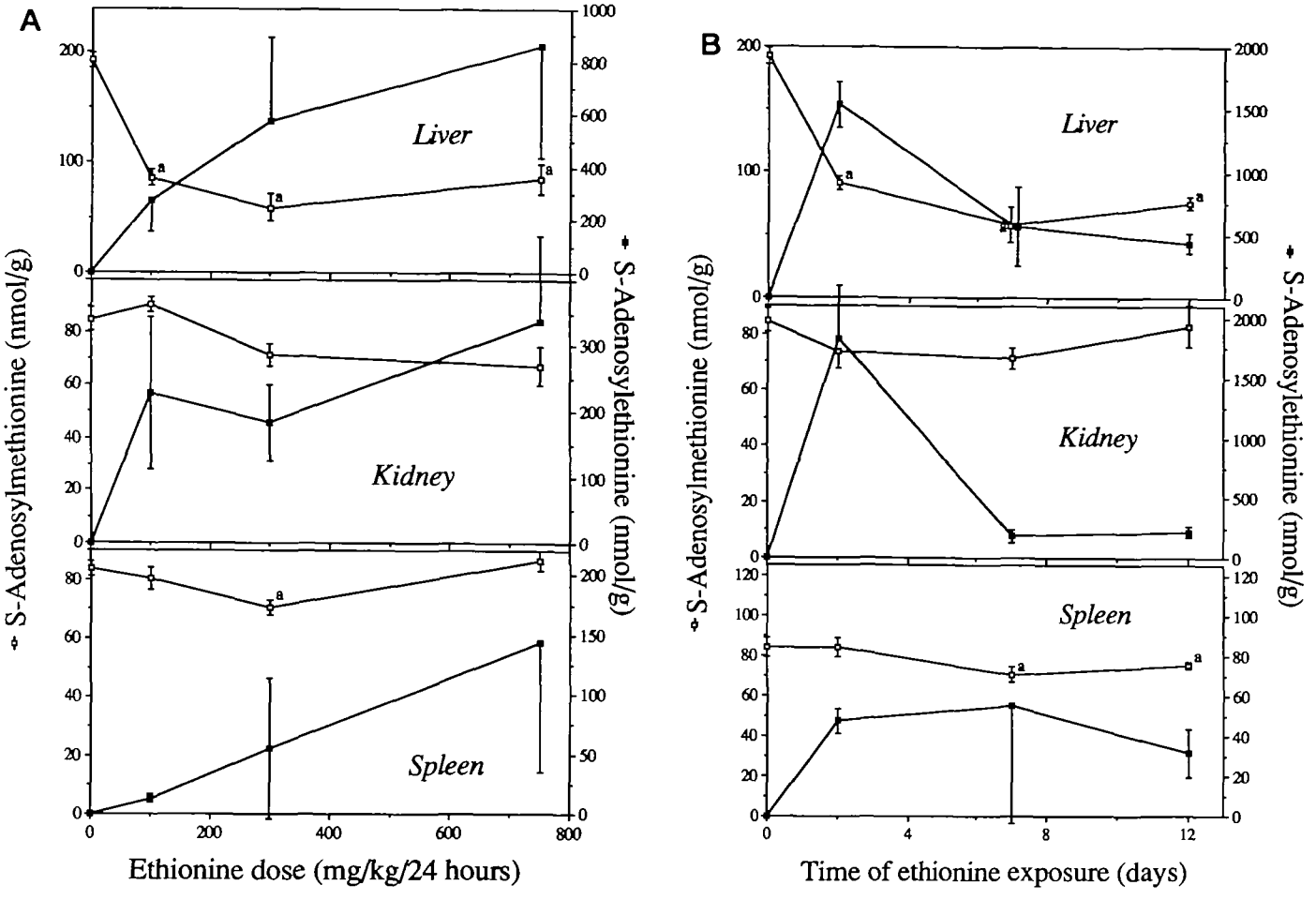


**Fig. 2.** Dose- and time-related effect of Eth administration on AdoMet and AdoEth contents in liver, kidney and spleen. A shows the concentrations of these metabolites as a function of Eth dose, administered for 7 days; B shows these parameters plotted versus time of Eth exposure, dose is 300 mg/kg/24 h. Values are given as mean of four determinations ± SD. [a]Significantly different from control ($P < 0.05$).

## Discussion

The aim of the present study was to compare the metabolic response to ethionine exposure of liver, kidney and spleen. Various doses of ethionine were injected intraperitoneally for up to 12 days. This route of administration and not oral ingestion was used for two reasons. Firstly, the ethionine dose could be controlled. Secondly, after peroral intake, ethionine reaches the systemic circulation via the portal vein which drains to the liver. Ethionine may undergo first-pass metabolism in the liver (15), and the ethionine exposure of the liver may become quite different from that of other organs.

The amounts of metabolites are given per g of tissue weight. Ethionine may increase liver size due to cellular accumulation of fat and water (5). Therefore, an increase in metabolite content would be slightly underestimated, whereas a decrease may be overestimated.

We observed that ethionine administration caused a marked decrease in AdoMet content of the liver and induced build-up of copious amounts of the congener, AdoEth, especially in liver but also in kidney and spleen. A similar metabolic response was observed by others in liver and non-hepatic tissues following administration of ethionine in the diet to rats for 3–6 weeks (19,20).

Several additional features described here serve to distinguish the metabolic response of the liver from that of the other tissues investigated (kidney and spleen). Taken together, the response of the liver is characterized by (i) a pronounced and sustained decrease in the AdoMet content and accumulation of AdoEth; (ii) a marked increase in the amount of AdoHcy and Hcy. Accordingly, the AdoMet/AdoHcy ratio decreases from about 15 to about 2; (iii) an increase in the GSH content, which is significant after a short time of exposure (2 days, 300 mg/kg/24 h) and at a moderate dose (100 mg/kg/24 h, 7 days) of ethionine.

In contrast, in spleen and kidney, there are essentially no alterations in the AdoMet and AdoHcy content, and the quantitative relations between these metabolites remain stable. In kidney, prolonged ethionine exposure or high doses are required to induce a significant elevation of GSH.

Several metabolic pathways handling sulfur compounds are catalyzed by enzymes or isoenzymes which occur solely in hepatic tissue (see Figure 1). This may explain the metabolic response of the liver to ethionine exposure.

The decrease in AdoMet content and the sustained accumulation of AdoEth in liver (Figure 2A,B) may reflect the occurrence of isoenzymes of AdoMet synthase unique to the liver (21). Tissue specificity of ethionine carcinogenesis has recently been assigned to the hepatic $\beta$-isozyme (22), and the activity of another liver isozyme (type $\alpha$) is increased during AdoEth accumulation (23).

The accumulation of AdoHcy is associated with a parallel increase in Hcy (Figure 3A,B). Elevation of Hcy may offer a clue to the mechanism behind the increase in AdoHcy, since metabol-

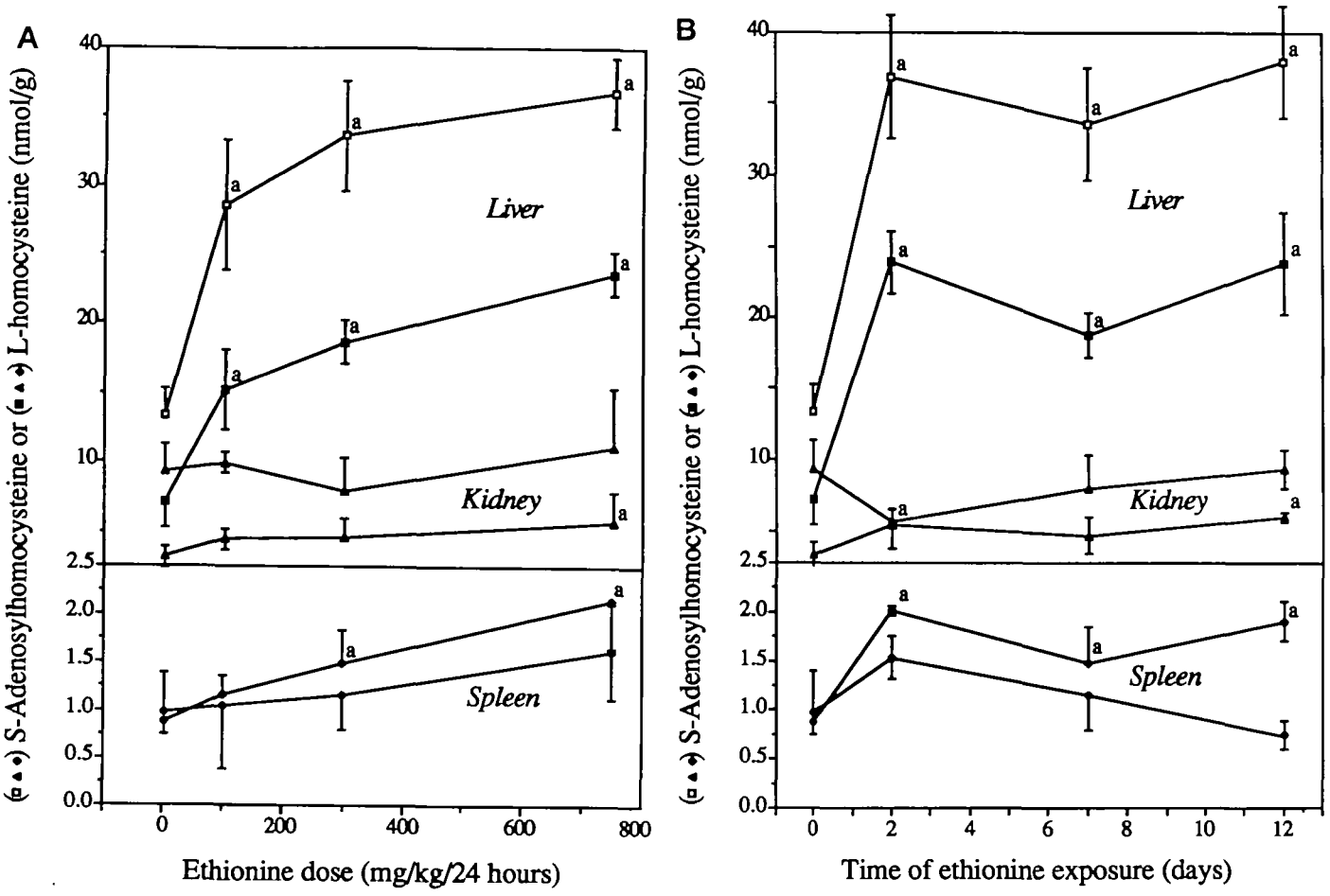


**Fig. 3.** Dose- and time-related effect of Eth administration on AdoHcy and Hcy contents in liver, kidney and spleen. **A** shows the concentrations of these metabolites as a function of Eth dose, administered for 7 days; **B** shows these parameters plotted versus time of Eth exposure, dose is 300 mg/kg/24 h. Values are given as mean of four determinations ± SD. [a]Significantly different from control ($P < 0.05$).

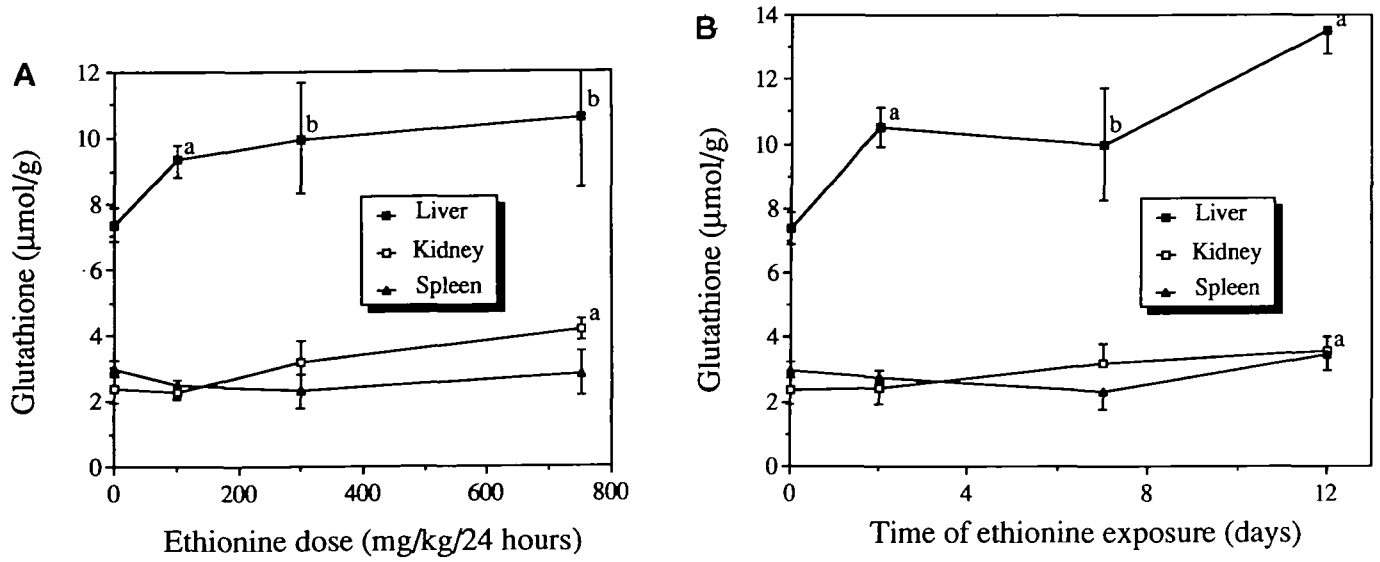


**Fig. 4.** Dose- and time-related effect of Eth administration on the GSH content in liver, kidney and spleen. **A** shows the concentrations of GSH as a function of Eth dose, administered for 7 days; **B** shows this parameter plotted versus time of Eth exposure, dose is 300 mg/kg/24 h. Values are given as mean of four determinations ± SD. [a]Significantly different from control ($P < 0.05$); [b]$0.1 > P > 0.05$.

ism of the former compound shows several features specific to hepatic tissue. Hcy is either remethylated to methionine, and this reaction is catalyzed by either 5-methyltetrahydrofolate:homocysteine methyltransferase or by betaine:homocysteine methyltransferase. Alternatively, Hcy is converted irreversibly to cystathionine (11, and Figure 1). This reaction and the betaine-

dependent remethylation are confined to the liver. Notably, Finkelstein and Martin have recently reported that the betaine: homocysteine methyltransferase is inactivated by AdoEth, at least *in vitro* (24). Inactivation of this enzyme may lead to accumulation of Hcy which in turn retards AdoHcy catabolism.

AdoEth is an inhibitor of DNA methyltransferase (9), and AdoHcy is an established inhibitor of numerous AdoMet-dependent methyltransferases (25), including the methylation of DNA, phospholipids and histone in the intact rat liver (26). It has been hypothesized that the ratio between AdoMet and AdoHcy in normal tissue may regulate the ability of the cell to carry out transmethylation reactions (25). Thus, it is conceivable that alteration of this ratio from 15 to about 2 in the liver following ethionine exposure may have an impact on transmethylation reactions particularly sensitive to the inhibitory effect of AdoHcy. The contribution of this metabolic effect to ethionine toxicity may be difficult to assess because accumulation of AdoEth, ATP depletion and ethylation of tissue constituents as well as inhibition of protein synthesis (5) probably have substantial effects on enzymes involved in AdoMet metabolism and methyl transfer reactions.

Ethionine is an inhibitor of various methionine-dependent processes (1), and inhibits GSH efflux from hepatocytes (27). Furthermore, hepatocytes (28) but not spleen lymphocytes (29) utilize methionine via the trans-sulfuration pathway for GSH synthesis (Figure 1). It therefore seemed warranted to investigate whether ethionine may inhibit hepatic GSH synthesis leading to GSH depletion which in turn may contribute to hepatotoxicity. This is certainly not the case. The increased GSH content in some tissues probably reflects enhanced GSH synthesis during ethionine exposure.

There is no quantitative relationship between the GSH content in liver or other tissues (Figure 4) and other primary metabolic effects of ethionine, including AdoEth accumulation (Figures 2 and 3). For example, there was a sustained increase in GSH in liver and kidney whereas the amount of AdoEth showed a maximal concentration during the first days. There were individual variations in GSH among animals within a group, but no relation to the other metabolic effects (AdoMet, AdoEth, AdoHcy or Hcy content in tissues) could be demonstrated (data not shown). Furthermore, increased Hcy content was observed in liver and spleen whereas GSH increased in liver and kidney.

Elevation of the GSH content is an acute response in liver and increases more slowly in kidney. These data support the idea of differential metabolic response of various tissues to ethionine exposure. Obviously, GSH elevation is not intimately related to other primary metabolic derangements investigated here. Thus, the increased GSH content may be a cytoprotective response to other acute lesion(s) induced by ethionine, including ATP deficiency. Notably, most of the acute toxic effects as well as ATP depletion following ethionine exposure are reversed by administration of adenine or an adenine-precursor, but the AdoEth elevation persists (1). Thus, AdoEth accumulation can be dissociated from some acute effects of ethionine, including GSH elevation.

In conclusion, altered disposition of S-adenosylamino acids and Hcy in tissues observed within 12 days of ethionine exposure may play a role in the general acute cytotoxicity of ethionine. Unique features of the metabolic response of the liver to ethionine exposure, such as reduction in AdoMet content, pronounced and sustained accumulation of AdoEth, increased AdoHcy and Hcy and a rapid increase in the detoxification agent, GSH, should be related to the hepatic lesions during acute ethionine exposure. In addition, the acute metabolic response of the liver may also reflect properties of the hepatic metabolism of sulfur compounds, which make this organ susceptible to the long-term effects of low-dose ethionine, like tumor development.

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## References

1. Farber,E. (1963) Ethionine carcinogenesis. *Adv. Cancer Res.*, **7**, 383–474.
2. Brown,J.D., Wilson,M.J. and Poirier,L.A. (1983) Neoplastic conversion of rat liver epithelial cells in culture by ethionine and S-adenosylethionine. *Carcinogenesis*, **4**, 173–177.
3. Hoover,K.L., Hyde,C.L., Wenk,M.L. and Poirier,L.A. (1986) Ethionine carcinogenesis in CD-1, BALB/c and C3H mice. *Carcinogenesis*, **7**, 1143–1148.
4. Shull,K.H., McConomy,J., Vogt,M., Castillo,.A and Farber,E. (1966) On the mechanism of induction of hepatic adenosine triphosphate deficiency by ethionine. *J. Biol. Chem.*, **241**, 5060–5070.
5. Farber,E. (1967) Ethionine fatty liver. *Adv. Lipid Res.*, **5**, 119–183.
6. Farber,E., McConomy,J., Franzen,B., Maroquin,F., Stewart,G.A. and Magee,P.N. (1967) Interaction between ethionine and rat liver ribonucleic acid and protein *in vivo*. *Cancer Res.*, **27**, 1761–1772.
7. Stekol,J.A., Weiss,S. and Somerville,C. (1963) A study of the comparative metabolism of ethionine and methionine in the male and female rat. *Arch. Biochem. Biophys.*, **100**, 86–90.
8. Swann,P.F., Pegg,A.E., Hawks,A., Farber,E. and Magee,P.N. (1971) Evidence for ethylation of rat liver deoxyribonucleic acid after administration of ethionine. *Biochem. J.*, **123**, 175–181.
9. Cox,R. and Irving,C.C. (1977) Inhibition of DNA methylation by S-adenosylethionine with the production of methyl-deficient DNA in regenerating rat liver. *Cancer Res.*, **37**, 222–225.
10. Shivapurkar,N., Wilson,M.J. and Poirier,L.A. (1984) Hypomethylation of DNA in ethionine-fed rats. *Carcinogenesis*, **5**, 989–992.
11. Mudd,S.H. and Levy,H.L. (1983) Disorders of transsulfuration. In Stanbury,B. (ed.), *Metabolic Basis of Inherited Diseases*. McGraw–Hill, New York, pp. 522–559.
12. Meister,A. and Anderson,M.E. (1983) Glutathione. *Annu. Rev. Biochem.*, **52**, 711–760.
13. Feo,F., Pascale,R., Garcea,R., Daino,L., Pirisi,L., Frassetto,S., Ruggiu,M., Di Padova,C. and Stramentinoli,G. (1986) Effect of variations of S-adenosyl-L-methionine liver content on fat accumulation and ethanol metabolism in ethanol-intoxicated rats. *Toxicol. Appl. Pharmacol.*, **83**,331–341.
14. Brodie,A.E. and Reed,D.J. (1985) Glutathione changes occurring after S-adenosylhomocysteine hydrolase inhibition. *Arch. Biochem. Biophys.*, **240**, 621–626.
15. Wilkinson,G.R. (1987) Clearance approaches in pharmacology. *Pharmacol. Rev.*, **39**, 1–48.
16. Ueland,P.M., Helland,S., Broch,O.J., Schanche,J.-S. (1984) Homocysteine in tissues of the mouse and rat. *J. Biol. Chem.*, **259**, 2360–2364.
17. Ueland,P.M., Refsum,H., Male,R. and Lillehaug,J.R. (1986) Disposition of endogenous homocysteine by mouse fibroblast C3H/10T1/2 Cl 8 and the chemically transformed C3H/10T1/2 MCA Cl 16 cells following methotrexate exposure. *J. Natl. Cancer Inst.*, **77**, 283–289.
18. Anderson,M. (1985) Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol.*, **113**, 548–555.
19. Hyde,C.L. and Poirier,L.A. (1982) Hepatic levels of S-adenosylethionine and S-adenosylmethionine in rats and hamsters during subchronic feeding of DL-ethionine. *Carcinogenesis*, **3**, 309–312.
20. Shivapurkar,N. and Poirier,L.A. (1985) Levels of S-adenosylmethionine and S-adenosylethionine in four different tissues of male weanling rats during subchronic feeding of DL-ethionine. *Biochem. Pharmacol.*, **34**, 373–375.
21. Hoffman,J.L. and Sullivan,D.M. (1982) Differential properties and regulation of methionine adenosyltransferase isoforms from the rat. In Usdin,E., Borchardt,R.T. and Creveling,C.R. (eds), *Biochemistry of S-Adenosylmethionine and Related Compounds*. Macmillan, London, pp. 683–686.
22. Cox,R. and Goorha,S. (1986) Tissue specificity of ethionine carcinogenesis is due to the $\beta$-isozyme of methionine adenosyltransferase. *Prc. Am. Assoc. Cancer Res.*, **27**, 78.
23. Matsumoto,C., Suma,Y. and Tsukada,K. (1984) The $\alpha$-form of S-adenosylmethionine synthetase isozymes from liver is principally functional. *J. Biochem.*, **95**, 1223–1226.
24. Finkelstein,J.D. and Martin,J.J. (1984) Inactivation of betaine-homocysteine

methyltansferase by adenosylmethionine and adenosylethionine. *Biochem. Biophys. Res. Commun.*, **118**, 14–19.
25. Cantoni,G.L. (1986) The centrality of S-adenosylhomocysteinase in the regulation of the biological utilization of S-adenosylmethionine. In Borchardt,R.T., Creveling,C.R. and Ueland,P.M. (eds), *Biological Methylation and Drug Design.* Humana Press, Clifton, NJ, pp. 227–238.
26. Hoffman,D.R., Marion,D.W., Cronatzer,W.E. and Duerre,J.A. (1980) S-Adenosylmethionine and S-adenosylhomocysteine metabolism in isolated rat liver. *J. Biol. Chem.*, **255**, 10822–10827.
27. Aw,T.Y., Ookhtens,M. and Kaplowitz,N. (1984) Inhibition of glutathione efflux from isolated rat hepatocytes by methionine. *J. Biol. Chem.*, **259**, 9355–9358.
28. Beatty,P.W. and Reed,D.J. (1980) Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. *Arch. Biochem. Biophys.*, **204**, 80–87.
29. Brodie,A.E., Potter,J. and Reed,D.J. (1982) Unique characteristics of rat spleen lymphocytes, L1210 lymphoma cells and HeLa cells in glutathione biosynthesis from sulfur-containing amino acids. *Eur. J. Biochem.*, **123**, 159–164.

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