The Metabolism of Ethionine in Rats

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SUMMARY

The L-[ethyl-L-14C]ethionine metabolites soluble in trichloroacetic acid were studied in rats by the use of column chromatography. After p.o. application of ethionine, its absorption from intestinal lumen was rapid and was complete in less than 2 hr. Any unabsorbed ethionine was later excreted in the feces. During the passage through the gastrointestinal tract, a portion of ethionine was metabolized. The chemical nature and biological significance of these metabolites is not yet known. The fate of absorbed ethionine was investigated in the small intestine, liver, blood, kidney, and urine as a function of time after application. A great part of ethionine was quickly oxidized to ethionine sulfoxide. In liver and kidney, the concentration of ethionine sulfoxide was higher than that of free ethionine. In all organs, the presence of N-acetylated ethionine sulfoxide was also demonstrated. Ethionine sulfoxide can be reduced and N-acetylated ethionine can be deacetylated in vivo as demonstrated by the formation of S-adenosylethionine from ethionine sulfoxide and N-acetylated ethionine. In urine, 4 main components were observed: N-acetylated ethionine sulfoxide, S-adenosylethionine, ethionine sulfoxide, and free ethionine. Some minor components, as yet unidentified, were also present in the urine and in different organs. The probable site of origin of urinary S-adenosylethionine is the kidney.

INTRODUCTION

Dyer (12) carried out the 1st study with ethionine, a methionine antimetabolite. In her experiments, ethionine, a synthetic ethyl homolog of methionine, could not substitute for methionine in supporting the growth of rats. She also observed that the toxicity of ethionine was reduced by simultaneous administration of methionine. This interesting discovery was followed by many other studies of its effect, both pathological and biochemical, on the liver and other organs of rats (see reviews in Refs. 15, 16, 42), which culminated in the discovery of liver carcinoma formation (14, 32). The resemblance of ethionine to methionine in chemical structure and cellular metabolism gives a study of ethionine carcinogenesis an advantage over other carcinogens. Since ethionine is carcinogenic, it provides an unusually important tool, otherwise unavailable, for investigating the possible mechanism of carcinogenesis.

The principal conclusions drawn from these classical studies were: (a) that the ethyl group of ethionine cannot physiologically replace the methyl group of methionine, and (b) that ethionine can be activated to the energy-rich S-adenosyl compound that can serve as an ethyl donor. From this background the attractive idea (17, 41) was formulated that hepatoma formation can be directly attributed to the alkylation of cellular macromolecules (6). Further important evidence of DNA ethylation has been offered by Swannet al. (43). The recently discovered facts cast doubts on the existence of a simple relationship between the alkylation of nucleic acids and carcinogenesis (10, 25). Furthermore, some skepticism about the exclusive role of S-AE3 as the ethyl donor in ethylation of RNA and DNA (27, 28) and the seemingly contradictory effects of some inhibitors of ethionine carcinogenesis (3-5) have demonstrated the need for a better understanding of the basic ethionine metabolism, including a continued search for other potential ethyl group donor(s). In this paper we present for the 1st time a profile of major ethionine metabolites in various organs.

MATERIALS AND METHODS

CFN female rats (Carworth Farms, New City, N.Y.) weighing 140 to 160 g were used in all experiments. Before the experiments, the rats were fed basic pellets (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.); any exceptions are specifically mentioned in the text. All the animals were housed in groups in steel cages with perforated bottoms in a room maintained at 22° and 50% humidity.

1- and D-ethionine, N-acetyl-L-ethionine, N-acetyl-D-ethionine, and N-acetyl-DL-ethionine were products of Cyclo Chemicals, Los Angeles, Calif. dl-Ethionine and Dl-ethionine sulfoxide were purchased from Nutritional Biochemicals, Cleveland, Ohio. N-Acetylethionine sulfoxide was synthesized by a method described by Smith and Beeman (39). L-[ethyl-L-14C]Ethionine was obtained from ICN, Cleveland, Ohio (specific activity, 1.34 mCi/mnmole). The purity of this compound was tested before the experiment by column chromatography (see below). Small amounts of ethionine sulfone and ethionine sulfoxide were always present. S-[ethyl-L-14C]Adenosylethionine (specific activity, 0.088 mCi/mnmole) was a product of New England Nuclear, Boston, Mass. The product contained small amounts of radioactive impurities and therefore it was rechromatographed.

1 Supported by USPHS Grant CA-11071 from the National Cancer Institute, NIH.
2 John J. Fomon Research Fellow of Florida Division of American Cancer Society.
3 The abbreviations used are: S-AE, S-adenosylethionine; DFFS, dry fat-free substance; TCA, trichloroacetic acid.
Ethionine Metabolism

on a AG 50W-X12 column (see below). Despite the purification, a small amount of free ethionine was present.

l[ethyl-1-14C]Ethionine was given to rats by means of a stomach tube. The dosage was 12.5 mg l-ethionine (containing 5 μCi l-ethionine) per 100 g body weight. This amount was based on chronic experiments in which rats on a diet containing 0.3% ethionine received 10 to 15 mg ethionine per 100 g body weight daily.

Rats after a 16-hr period of fasting were used in these experiments. Every group (per time period) consisted of 4 rats. After administration of ethionine or other derivatives, the rats were killed at a given time under light ether anesthesia. The blood was obtained by cardiac puncture and the organs for analysis were quickly removed and extracted. For the chemical analysis, liver was homogenized in 10-fold volume (w/v) of cold 3% perchloric acid in a Waring Blender. The homogenates were then centrifuged in the cold for 10 min at 2000 r.p.m. The resulting precipitate was washed once with 3% perchloric acid, then with 95% ethanol (containing 10% potassium acetate), once with absolute ethanol, twice with ethanol:diethyl ether (3:1), once at 60° and once at room temperature, and twice with absolute ethanol and was then dried in vacuum to constant weight (DFFS). S-AE was determined in 1 aliquot of perchloric acid extract from Stekol's (41) modification of the method proposed by Schlenk and DePalma (34) for determination of S-AE in yeast. A 2nd aliquot of perchloric acid extract was neutralized by 5 N KOH, and ATP was determined by a method proposed by Cohn and Carter (8) and modified by Siekevitz and Potter (37). This method, introduced in this laboratory by J. A. Stekol, gives systematically lower ATP values than expected. We continued to use this method in order to compare our results with the previous detailed studies of Stekol. Also, the amount of obtained tissue is sufficient for column chromatographic determination of ATP, AMP, and ADP in the same specimen. The ATP values obtained by this method are directly proportional to those obtained after in situ freezing of liver from normal and ethionine-treated rats. Seromucoid concentration was determined by the method of Winzler (45). The urine was collected in a Bio-Nuclear glass metabolic cage. Before the experiments, 1 ml N HCl was added for preservation and to avoid the decomposition of S-AE. The volume of urine was adjusted to 50 ml and an aliquot was used for the chromatographic analysis. The analyses of ethionine metabolites were performed on 5% TCA extracts from different organs (perchloric acid treatment increased the amount of ethionine sulfoxide). An aliquot of urine or an aliquot of tissue TCA extract after removal of TCA by repeated ether extraction was applied on a column of AG 50W-X12 200 to 400 mesh (BioRad Laboratories, Richmond, Calif.) and then eluted with linear gradient of HCl (400 ml water + 400 ml 8 N HCl). The eluates were collected in 5-ml fractions and the aliquots were mixed with Bray's solution. The quenching of individual fractions was determined by internal standards.

N-Acetylethionine and N-acetylthionine sulfoxide fractions were hydrolyzed in nitrogen atmosphere (at 100° for 1 hr in 2 N HCl), and the presence of ethionine and ethionine sulfoxide was confirmed by the column chromatographic method. The main ethionine metabolites were identified by the aid of authentic substances and paper chromatography of Whatman 3 MM filter paper in the system of butanol:acetic acid:water (12:3:5, by volume). The radioactive substances on the paper chromatograms were detected by radioautography (Kodak No-screen X-ray film).

RESULTS

Differences in Overall Ethionine Metabolism after i.p. and p.o. Ethionine Application. The effect of i.p. and p.o. l[ethyl-1-14C]Ethionine administration on the metabolism is indicated in Table 1. Significant differences were found in the concentration of S-AE in liver 24 hr after application and in the amount of excreted total ethionine metabolites in urine, but not in the amount of respiratory carbon dioxide.

The excreted ethionine metabolites in urine were analyzed (Table 2). The effect of the route of ethionine application is slight; the difference probably demonstrates the fact that the main organ metabolizing ethionine is the liver.

Behavior of Ethionine in Gastrointestinal Tract and Its Absorption. In this experiment we sought to determine whether or not ethionine was completely absorbed from the intestinal lumen and the fate of any portion of ethionine remaining in the gastrointestinal tract. Ethionine was administered by stomach tube and the rats were sacrificed at different time periods following the application. About 60% of the ethionine was absorbed within 90 min, as shown in Table 3. The control experiments with i.p. applied ethionine have shown that the amount of ethionine metabolites excreted via bile is not responsible for the amount of the unabsorbed ethionine recovered from gastrointestinal tract (O.E. Matos, unpublished results).

These experiments were performed on rats fed the standard pellet diet and starved for 16 hr before ethionine application. Rats fed high-carbohydrate diet absorbed ethionine to a lesser extent (about 70%). A systematic study of the diet effect on ethionine absorption has not yet been done. The portion of ethionine remaining in the intestinal lumen is partly bound in solids and is partly in a soluble state.

The soluble fraction of the gastrointestinal contents prepared 2, 4, and 6 hr after ethionine administration was analyzed on an AG 50W column for ethionine metabolites. It was found that some new unidentified peaks of radioactivity were present in lumen during the passage of ethionine through the gastrointestinal tract that were not observed in TCA extracts from examined organs. The metabolites were distributed throughout the entire chromatographic profile and substantially increased the radioactivity of the background of the chromatogram.

Distribution of Ethionine Metabolites in Various Organs within 24 Hr after Ethionine Application. The distribution of radioactive ethionine metabolites soluble in TCA in various organs as a function of time after ethionine application is presented in Chart 1, and the concentration of the metabolites is shown in Chart 2. The radioactivity in plasma and erythrocytes decreased with time. In all other organs studied, the maximum radioactivity was observed at or after 8 hr. Liver contained the largest portion of ethionine
Comparison of i.p. and p.o. application of L-[ethyl-1-\(^{14}\)C]ethionine to rats

L-[ethyl-1-\(^{14}\)C]Ethionine was administered in the dose of 12.5 mg/100 g body weight (specific radioactivity, 0.2 \(\mu\)Ci/mg) to rats after a 16-hr period of fasting.

<table>
<thead>
<tr>
<th>Ethionine metabolites</th>
<th>i.p.</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr after application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory CO(_2) in % of administered dose(^a)</td>
<td>6.10 ± 0.18(^b) (2)</td>
<td>6.33 ± 0.10 (2)</td>
</tr>
<tr>
<td>Excretion in urine in % of administered dose(^a)</td>
<td>75.82 ± 1.92 (2)</td>
<td>55.68 ± 2.13 (2)</td>
</tr>
<tr>
<td>S-AE concentration in liver(^a) in (\mu)moles/g DFFS</td>
<td>3.86 ± 0.22 (3)</td>
<td>10.29 ± 0.42 (4)</td>
</tr>
</tbody>
</table>

\(^a\) The respiratory CO\(_2\) was trapped in 2-aminoethanol/ethylene glycol monomethyl ether (1:1). \(^b\) The variability is given by S.D. of mean. The data were statistically analyzed by Student's \(t\) test.

Table 2
Comparison of the excretion of ethionine metabolites in urine after p.o. and i.p. L-[ethyl-1-\(^{14}\)C]ethionine application to rats

See Table 1 for the description of the experiment. An aliquot of the urine was analyzed by chromatography on an AG 50W column.

<table>
<thead>
<tr>
<th>Ethionine metabolites</th>
<th>i.p.(^a)</th>
<th>p.o.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr after application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylethionine sulfoxide</td>
<td>69.53 ± 1.48(^b)</td>
<td>75.32 ± 0.72(^c)</td>
</tr>
<tr>
<td>Ethionine sulfoxide</td>
<td>5.51 ± 0.16</td>
<td>4.03 ± 0.99</td>
</tr>
<tr>
<td>Ethionine (+ unidentified minor fractions)</td>
<td>6.84 ± 1.02</td>
<td>3.58 ± 0.58</td>
</tr>
<tr>
<td>S-AE</td>
<td>18.00 ± 0.58</td>
<td>16.78 ± 0.41</td>
</tr>
</tbody>
</table>

\(^a\) The figures represent the average of the experiment (3 rats in every group) and are given in percentage of the total radioactivity in urine. \(^b\) Mean ± S.D. \(^c\) Statistically different (\(p < 0.05\)).

Table 3
The absorption of L-[ethyl-1-\(^{14}\)C]ethionine from gastrointestinal tract after p.o. application

L-[ethyl-1-\(^{14}\)C]Ethionine (specific activity, 0.2 \(\mu\)Ci/mg) was applied by stomach tube (12.5 mg/100 g body weight) to rats that were fasted for 16 hr. The volume of ethionine solution was kept at 0.5 ml/100 g body weight.

<table>
<thead>
<tr>
<th>Time after</th>
<th>% radioactivity recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>96 ± 0.5(^a)</td>
</tr>
<tr>
<td>0.5</td>
<td>97 ± 0.7</td>
</tr>
<tr>
<td>1.0</td>
<td>76 ± 1.3</td>
</tr>
<tr>
<td>1.5</td>
<td>41 ± 2.4</td>
</tr>
<tr>
<td>2.0</td>
<td>12 ± 0.8</td>
</tr>
<tr>
<td>4.0</td>
<td>13 ± 0.9</td>
</tr>
<tr>
<td>8.0</td>
<td>11 ± 1.0</td>
</tr>
<tr>
<td>24.0</td>
<td>10 ± 0.7</td>
</tr>
</tbody>
</table>

\(^a\) The rats were sacrificed at the indicated time; the gastrointestinal tract was removed and rinsed out with cold 0.9% NaCl solution. The washings were combined and an aliquot was used for determination of radioactivity after the solids were dissolved in Hyamine.

Mean ± S.E.

Ethionine Metabolites in Mucosa of Small Intestine.

Chart 3A, showing the sequence of changes in concentration of different ethionine metabolites with time, demonstrates that the ethionine concentration after absorption from the small intestine decreases progressively from 4 to 24 hr, while the amount of ethionine sulfoxide reaches its maximum between 8 and 16 hr. The S-AE content remains low during ethionine absorption from intestinal lumen, but it increases when the absorption has just finished. A very similar trend is shown by the fraction not absorbed from neutral TCA extract on AG 50W. In urine, we identified this compound...
Chart 2. The amount of total L-ethionine metabolites soluble in TCA in different organs of rats after p.o. [ethyl-1-14C]ethionine application. •, liver; O, small intestine; □, kidney; △, plasma; and ▲, erythrocytes.

Chart 3. The amount of ethionine metabolites in different rat organs after p.o. L-[ethyl-l-14C]ethionine application. A, small intestine; B, total erythrocytes; C, kidney; D, total plasma. The rats were sacrificed at various times after ethionine application, and TCA-soluble substances were chromatographed on an AG 50W column. •, ethionine; O, ethionine sulfoxide; △, N-acetylethionine sulfoxide; □, S-AE; ▲, other minor fractions. The blood, erythrocytes, and plasma volumes were calculated on the basis of the measurement of Sharper et al. (35): 49.5 ml blood per kg body weight, plasma 54.4% of blood volume. The S-AE fraction in plasma (D) is shown, but, in relation to Chart 4, the quantity is hardly distinguishable from the base line radioactivity and no significance can be attributed to it.

as N-acetylethionine sulfoxide. In the small intestine this fraction was not studied in detail, and whether or not it is N-acetylethionine sulfoxide is yet to be confirmed. The ethionine metabolites present in small intestine mucosa represent not only ethionine passing from the small intestine lumen but also ethionine or ethionine metabolites passing from circulation into the intestinal mucosa.

Ethionine Metabolites in Blood. Plasma and erythrocytes were separated from the heparinized blood, and both components were analyzed separately. As expected, the plasma contained a large amount of ethionine metabolites. The amount of ethionine sulfoxide plotted against time after ethionine administration shows a maximum after 8 hr, followed by a decrease at a different rate in comparison to ethionine (Chart 3D). Chart 4 shows the chromatographic pattern of ethionine metabolites in plasma 4 and 24 hr after ethionine application. The chart also demonstrates the presence of some minor unidentified compounds, but no S-AE. The different kinetic pattern of ethionine and ethionine sulfoxide probably reflects the different fates of these 2 compounds. Ethionine is mostly metabolized by different tissues, but ethionine sulfoxide is excreted mostly unchanged or in the acetylated form into the urine. Ethionine metabolites in erythrocytes reflect roughly the concentration pattern described in plasma (Chart 3B) except for 1 important difference: the ratio of ethionine to ethionine sulfoxide is higher in plasma than in erythrocytes. The experiments show also that, if any S-AE is present in erythrocytes, its concentration is on the borderline of the sensitivity of our analytical methods.

Ethionine Metabolites in Liver. The main component of the TCA-soluble fraction from liver samples examined was S-AE. The maximum of S-AE concentration is between 8 and 16 hr. The concentration of ethionine and ethionine sulfoxide has a similar sharply decreasing pattern, and the amount of ethionine sulfoxide is higher than the amount of ethionine (Chart 5). N-Acetylethionine sulfoxide is also present and its concentration decreases slowly. After 16 hr there appear other, as yet unidentified, radioactive substances that represent direct ethionine metabolites or other ethylated low-molecular-weight compounds. It is also possible that those substances are present when ethionine is first taken by liver, but they cannot be detected because their peaks could be overshadowed by the large ethionine and ethionine sulfoxide zones, which are minimally 100 times greater than those of the minor compounds. Chart 6 shows the chromatographic profile of TCA extract from liver 4 and 24 hr after ethionine administration.

Ethionine Metabolites in Kidney. The concentration pattern of ethionine metabolites is very similar to that of the
Ethionine Metabolites in Liver. Chart 5 demonstrates the chromatographic profile of ethionine metabolites. The data are in agreement with observations in tissues, as described above. The relative amount of S-AE increased with time after ethionine application, and the N-acetyl ethionine sulfoxide slowly decreased; the biggest decrease was observed with ethionine sulfoxide. The decrease of the ethionine sulfoxide fraction could be due to the capacity of kidney or liver to acetylate it to N-acetyl ethionine sulfoxide. The percentage distribution of the main components was not influenced by the variation in the amount of ethionine administered (between 12.5 and 25.0 mg/100 g body weight).

The fraction not absorbed on the column of AG 50W was absorbed on column of Dowex 1 and eluted by an HCl gradient. Chart 6 presents the results. The chromatogram shows that the fraction contains almost exclusively N-acetyl ethionine sulfoxide. The minor compounds represent less than 1% of the total radioactivity of this fraction. The 1st compound was identified as N-acetyl ethionine and the 3rd fraction is yet to be identified. The position of the main peak is identical with chemically prepared N-acetyl ethionine sulfoxide.

The Origin of S-AE in Urine. When [ethyl-1-14C]S-AE was administered to a rat i.p., almost 100% of the radioactivity was recovered in the urine in 48 hr. The majority was in the form of unchanged S-AE, and only 20% was present in the form of N-acetyl ethionine sulfoxide (identified by liver except that ethionine sulfoxide and N-acetyl ethionine sulfoxide in kidney represent a higher portion of the total TCA-soluble compounds than was observed in liver, and S-AE reached its maximum between 16 and 24 hr. Furthermore, the levels of ethionine and ethionine sulfoxide decreased more slowly in kidney than in liver (Chart 3C). The presence of some minor compounds (Chart 7) is also a matter of interest.

Ethionine Metabolites in Urine. Chart 8 demonstrates the chromatographic profile of ethionine metabolites. The data are in agreement with observations in tissues, as described above. The relative amount of S-AE increased with time after ethionine application, and the N-acetyl ethionine sulfoxide slowly decreased; the biggest decrease was observed with ethionine sulfoxide. The decrease of the ethionine sulfoxide fraction could be due to the capacity of kidney or liver to acetylate it to N-acetyl ethionine sulfoxide. The percentage distribution of the main components was not influenced by the variation in the amount of ethionine administered (between 12.5 and 25.0 mg/100 g body weight).

The fraction not absorbed on the column of AG 50W was absorbed on column of Dowex 1 and eluted by an HCl gradient. Chart 9 presents the results. The chromatogram shows that the fraction contains almost exclusively N-acetyl ethionine sulfoxide. The minor compounds represent less than 1% of the total radioactivity of this fraction. The 1st compound was identified as N-acetyl ethionine and the 3rd fraction is yet to be identified. The position of the main peak is identical with chemically prepared N-acetyl ethionine sulfoxide.
therefore it was assumed that those compounds could be ethionine were reported to produce fatty liver (20, 26), and ethionine (12.5 mg/100 g body weight). Under these circumstances, the formation of liver S-AE is strongly suppressed (Table 4). When labeled ethionine was applied in the same manner, the amount of radioactivity in urine (collected for 48 hr) was not significantly changed, but the proportions of compounds present were considerably altered. Table 5 compares the relative amounts of ethionine metabolites in urine after application of carbon tetrachloride. The previously published studies (18, 23) showed only the total acid-soluble radioactivity in various organs after i.p. injection. Our curves obtained after p.o. application were similar, with 1 important difference: the higher radioactivity soluble in TCA was observed in liver, while Levine and Tarver (23) observed a higher concentration in kidney. Also, the concentration in blood 4 and 8 hr after p.o. application in our experiments was higher than in kidney. In agreement with previous observations (23), TCA-soluble ethionine metabolites appeared to persist longer in kidney than in several other examined organs. The observed difference in the concentration of ethionine and its metabolites in liver is in agreement with our findings on

DISCUSSION

The basic data on the metabolism of ethionine were presented many years ago and were based almost exclusively on acute experiments after i.p. applications (18, 23, 36). Comparing the i.p. and p.o. applications of ethionine, we can see significant differences (Table 1). After p.o. application of the same amount of ethionine, the excretion of ethionine metabolites in urine during a 24-hr period was decreased and the concentration of S-AE in liver was almost 3 times higher. The increase of S-AE concentration is due probably to anatomical differences; ethionine goes from the intestinal lumen directly to the liver in portal venous blood.

The absorption of ethionine from the intestinal lumen in intact animals was not complete, with only about 70 to 90% being absorbed. In this respect the behavior of ethionine is similar to the behavior of methionine (19, 24). Chromatographic analysis of the soluble part of the intestinal lumen contents revealed that ethionine was partially metabolized during the passage.

The distribution of L-[ethyl-1-14C]ethionine is not uniform; some organs have higher levels of acid-soluble radioactivity (Chart 2). The previously published studies (18, 23) showed only the total acid-soluble radioactivity in various organs after i.p. injection. Our curves obtained after p.o. application were similar, with 1 important difference: the higher radioactivity soluble in TCA was observed in liver, while Levine and Tarver (23) observed a higher concentration in kidney. Also, the concentration in blood 4 and 8 hr after p.o. application in our experiments was higher than in kidney. In agreement with previous observations (23), TCA-soluble ethionine metabolites appeared to persist longer in kidney than in several other examined organs. The observed difference in the concentration of ethionine and its metabolites in liver is in agreement with our findings on

chart 8: Chromatographic pattern of ethionine metabolites in urine collected at different times after p.o. L-[ethyl-1-14C]ethionine application. Diluted urine, 10 ml, was applied on an AG 50W column and eluted by linear hydrochloric acid gradient (from 200 ml water to 200 ml n HCI). AcE, N-acetylethionine sulfoxide; EO, ethionine sulfoxide; E, ethionine.

Chart 9: The chromatographic pattern of N-acetylethionine sulfoxide fraction from urine. The 1st fraction from previous chromatography (see Chart 8) was applied on a Dowex 1-X4 column (1 x 10 cm) and eluted by linear hydrochloric acid gradient (from 200 ml water to 200 ml n HCI). The main fraction represents N-acetylethionine sulfoxide, the 1st fraction represents N-acetylethionine.
Table 4
Influence of carbon tetrachloride pretreatment on concentration of S-AE in rat liver

The rats were sacrificed 5 hr after ethionine application. The data were statistically analyzed by Student's t test.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>S-AE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats</td>
</tr>
<tr>
<td>CCl₄, a and 17 hr later, ethionine b</td>
<td>3</td>
</tr>
<tr>
<td>Ethionine a only</td>
<td>3</td>
</tr>
</tbody>
</table>

a Carbon tetrachloride was applied by stomach tube (0.25 ml/100 g body weight) in the form of a mixture (1:1) with sesame oil.
b Ethionine (12.5 mg/100 g body weight) was administered by stomach tube.
c Mean ± S.D.
d Statistically significant difference.

Table 5
The effect of carbon tetrachloride pretreatment on the excretion of ethionine metabolites in urine

The figures represent an average of 2 independent experiments and are given in percentage of the total radioactivity in urine. The urine was collected within a period of 48 hr. The total amount of radioactivity excreted into the urine was the same in both groups. Two rats were used in every group.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>N-Acetyl ethionine sulfoxide (%)</th>
<th>Ethionine sulfoxide (%)</th>
<th>Ethionine unidentified compounds (%)</th>
<th>S-AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionine a</td>
<td>74.8 (73.3–76.3) b</td>
<td>5.1</td>
<td>4.7</td>
<td>15.3</td>
</tr>
<tr>
<td>CCl₄ + ethionine a</td>
<td>35.0 (27.4–42.6) a</td>
<td>25.2</td>
<td>8.0</td>
<td>31.3</td>
</tr>
</tbody>
</table>

a [ethyl-1-14C] Ethionine was administered in the dose of 12.5 mg/100 g body weight (specific radioactivity, 0.2 μCi/mg) to rats after a 16-hr period of fasting.
b The results of individual determinations are given in parentheses.
c Carbon tetrachloride was applied by stomach tube (0.25 ml/100 g body weight) in the form of a mixture with sesame oil (1:1) 16 hr before ethionine application.

d Table 6
S-AE concentration in liver in rats after i.p. administration of ethionine sulfoxide

Ethionine derivative, 613 μmoles/100 g body weight, dissolved in water, was injected. The data were statistically analyzed by Student's t test.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of rats</th>
<th>Body wt (g)</th>
<th>Liver wt (g)</th>
<th>DFFS (%)</th>
<th>μmoles/g fresh liver</th>
<th>μmoles/g DFFS</th>
<th>S-AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Ethionine sulfoxide, 4 hr</td>
<td>3</td>
<td>132 ± 3 e</td>
<td>3.72 ± 0.10</td>
<td>21.55 ± 0.11</td>
<td>0.80 ± 0.02 d</td>
<td>3.67 ± 0.02 d</td>
<td>0.92 ± 0.20 d</td>
</tr>
<tr>
<td>DL-Ethionine sulfoxide, 24 hr</td>
<td>3</td>
<td>130 ± 0</td>
<td>3.64 ± 0.07</td>
<td>20.14 ± 0.15 e</td>
<td>0.76 ± 0.01 d</td>
<td>2.87 ± 0.05 d</td>
<td>1.71 ± 0.09 d</td>
</tr>
</tbody>
</table>

a The hours indicate the period of time between the injection and sacrifice.
b Mean ± S.E.
c Statistically significant difference was noted when compared with DL-ethionine after 4 hr (see Table 8).
d Statistically significant difference was noted when compared with DL-ethionine after 24 hr (see Table 8).
and may well limit further the rate of absorption. The relatively high concentration of ethionine in the intestine during the absorption process could produce certain changes in the intestinal mucosa that may affect its transport rate, but these factors have not been studied as yet.

The concentration of TCA-soluble ethionine metabolites in the small intestine is relatively low and is probably due to a very quick transport of ethionine from the intestinal mucosa into the circulation. These metabolites in the intestine represent a mixture of free ethionine and some ethionine metabolites, and their quantitative ratio undergoes certain changes during the time after ethionine application. The delayed increase of the concentration of N-acetylethionine sulfoxide could depend on the availability of ethionine sulfoxide, as the variation of the levels of both substances with time is very similar.

In blood, contrary to other organs studied, free ethionine represents the main component. The presence of ethionine in blood was studied by Shull et al. (36), who considered blood an important ethionine pool for the maintenance of a high level of S-AE in liver. We can confirm this assumption.

The chromatographic profile of ethionine metabolites in the urine collected within 48 hr basically confirms the observations described by Smith et al. (39, 40), showing N-acetylethionine sulfoxide as the main ethionine metabolite. The 2nd largest component was S-AE and the 3rd

### Table 7

S-AE concentration in liver in rats after i.p. administration of N-acetylethionine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of rats</th>
<th>Survival (%)</th>
<th>Body wt (g)</th>
<th>Liver wt (% body wt)</th>
<th>DFFS (%)</th>
<th>ATP μmoles/g fresh liver</th>
<th>S-AE μmoles/g fresh liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl-L-ethionine (4 hr)</td>
<td>3</td>
<td>66</td>
<td>133 ± 4 *</td>
<td>3.42 ± 0.01</td>
<td>21.38 ± 0.12</td>
<td>0.39 ± 0.01</td>
<td>1.82 ± 0.01</td>
</tr>
<tr>
<td>N-Acetyl-L-ethionine (24 hr)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-Acetyl-D-ethionine (4 hr)</td>
<td>2</td>
<td>100</td>
<td>133 ± 2</td>
<td>3.50 ± 0.13</td>
<td>23.00 ± 0.31</td>
<td>0.61 ± 0.06</td>
<td>2.69 ± 0.29</td>
</tr>
<tr>
<td>N-Acetyl-D-ethionine (24 hr)</td>
<td>2</td>
<td>100</td>
<td>135 ± 0</td>
<td>3.40 ± 0.00</td>
<td>20.14 ± 0.23</td>
<td>0.78 ± 0.03</td>
<td>4.00 ± 0.17</td>
</tr>
<tr>
<td>N-Acetyl-DL-ethionine (4 hr)</td>
<td>3</td>
<td>66</td>
<td>135 ± 4</td>
<td>3.26 ± 0.15</td>
<td>21.89 ± 0.20</td>
<td>0.52 ± 0.02</td>
<td>2.48 ± 0.17</td>
</tr>
<tr>
<td>N-Acetyl-DL-ethionine (24 hr)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The hours indicate the period of time between injection and sacrifice.
* Mean ± S.E.
* Significant difference was noted when compared with D-ethionine after 4 hr (see Table 8).
* Significant difference was noted when compared with D-ethionine after 24 hr (see Table 8).
* Significant difference was noted when compared with L-ethionine after 4 hr (see Table 8).

### Table 8

S-AE concentration in liver in rats after i.p. administration of ethionine isomers

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of rats</th>
<th>Body wt (g)</th>
<th>Liver wt (% body wt)</th>
<th>DFFS (%)</th>
<th>ATP μmoles/g fresh liver</th>
<th>S-AE μmoles/g fresh liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Ethionine</td>
<td>3</td>
<td>132 ± 3 *</td>
<td>3.34 ± 0.09</td>
<td>21.66 ± 0.31</td>
<td>0.41 ± 0.02</td>
<td>2.04 ± 0.15</td>
</tr>
<tr>
<td>24 hr</td>
<td>3</td>
<td>132 ± 25</td>
<td>3.77 ± 0.05</td>
<td>16.97 ± 0.38 *</td>
<td>0.42 ± 0.04</td>
<td>2.38 ± 0.07</td>
</tr>
<tr>
<td>D-Ethionine</td>
<td>4 hr</td>
<td>3</td>
<td>132 ± 4</td>
<td>3.36 ± 0.32</td>
<td>21.55 ± 0.57</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>24 hr</td>
<td>3</td>
<td>132 ± 4</td>
<td>4.07 ± 0.25</td>
<td>18.79 ± 0.30</td>
<td>0.38 ± 0.03</td>
<td>2.14 ± 0.09</td>
</tr>
<tr>
<td>DL-Ethionine</td>
<td>4 hr</td>
<td>3</td>
<td>133 ± 3</td>
<td>3.25 ± 0.07</td>
<td>22.01 ± 0.05</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>24 hr</td>
<td>3</td>
<td>135 ± 5</td>
<td>4.08 ± 0.23</td>
<td>17.93 ± 0.31</td>
<td>0.45 ± 0.05</td>
<td>2.54 ± 0.24</td>
</tr>
</tbody>
</table>

* The hours indicate the period of time between injection and sacrifice.
* Mean ± S.E.
* Statistically significant difference was noted when compared with D-ethionine after 4 hr.
* Statistically significant difference was noted when compared with D-ethionine after 24 hr.
* Statistically significant difference was noted when compared with L-ethionine after 24 hr.
component was ethionine sulfoxide. A small amount of free ethionine and a minute amount of N-acetylethionine were also present. Smith and Beeman (39) identified ethionine sulfoxide in the urine of rats fed ethionine for a long time. Our results show that this substance was also present in urine in acute experiments, independent of the route of application. The presence of a substantial amount of S-AE in urine brings up the question of its origin. When liver function was considerably impaired by previous application of carbon tetrachloride, the formation of S-AE in liver was considerably decreased. However, the amount of excreted S-AE in urine was doubled (Table 5). This increase was accompanied by a lower excretion of N-acetylethionine sulfoxide and a higher excretion of ethionine sulfoxide. We may assume that the increased amount of excreted S-AE is a result of its enhanced synthesis in kidney, due to the higher availability of free ethionine or ethionine sulfoxide. An alternative explanation may be the damage of kidney tubules, which is caused by carbon tetrachloride as well as by ethionine. The failure to demonstrate the presence of S-AE in plasma eliminated the extrarenal origin of urinary S-AE. After i.p. injection of S-AE, we have not detected in urine any expected degradation products of S-AE such as S'-ethylidenosine and S'-ethylioribose. The only metabolic product formed from S-AE in vivo was N-acetylethionine sulfoxide, showing the possibility of release of ethionine from this molecule.

The determination of ethionine sulfoxide demonstrated that the oxidation of ethionine to ethionine sulfoxide was very rapid and it probably occurred in all tissues. The system that oxidizes ethionine to ethionine sulfoxide has not been studied as yet, and therefore we are limited in our considerations to the available data on oxidation of methionine. Methionine sulfoxide was recognized as a normal methionine metabolite present in small amounts in urine of normal man (7) and in elevated quantities in some pathological conditions (11, 29, 30). Also, in normal rats, the methionine sulfoxide was excreted in urine when the rats were fed a diet containing 1 to 3% methionine (9). The formation of the sulfoxides of alkylated amino acids is a prevalent pathway for the metabolism of these compounds in the rat (13, 21, 38). Methionine sulfoxide exists as 4 possible diastereoisomers (22). Toennies and Kolb (44) suggested that there might be differences in the biological effects among these isomers. Similar to methionine sulfoxide, ethionine sulfoxide can exist as 4 optical isomers that may be products of the oxidation of 2 racemic modifications. No data are available as yet about the presence of these stereoisomers (+ and −) in biological systems. This can be of importance, considering the function of methionine sulfoxide and ethionine sulfoxide. Ethionine sulfoxide injected i.p. produced fatty liver (20, 26). Generally accepted at this time is Farber's hypothesis (16) that fatty liver production is linked directly to ATP depletion and to the subsequent protein synthesis inhibition as a result of the ATP-trapping effect of ethionine (through the S-AE formation). As ethionine sulfoxide cannot be directly activated to S-AE, the biological activity of ethionine sulfoxide depends on its reduction to ethionine. In our study (Table 6), 4 hr after the i.p. DL-ethionine sulfoxide application, the S-AE concentration in liver represented only about 20%, and after 24 hr, represented about 60% of the amount obtained after DL-ethionine injection (Table 8). The different time curves of S-AE concentration in liver after ethionine and ethionine sulfoxide application suggest that the ethionine sulfoxide, which is formed immediately after ethionine application, could have a protracted effect on the maintenance of the high level of S-AE concentration during the 24-hr cycle. The investigation of the role of particular stereoisomers of ethionine sulfoxide was limited to the use of DL-ethionine sulfoxide only because of its commercial availability. Concerning the mechanism of ethionine sulfoxide reduction, we can only speculate that it could proceed in ways similar to those for methionine sulfoxide. Black et al. (2) isolated 3 enzymes that were all required for the reduction of L(−)-methionine sulfoxide to methionine in the presence of reduced triphosphopyridine nucleotide from yeast. Although the purified enzyme system was active only with L(−)-methionine sulfoxide, the crude extracts of yeast catalyzed the reduction of the L(+) and D(+) isomers. This fact indicates the existence of other methionine sulfoxide-reducing systems.

The main pathway of ethionine sulfoxide is its acetylation to N-acetylethionine sulfoxide. In all examined organs we found a detectable level of this compound. Acetylation is a potential biological reaction of all types of amino groups. Therefore, we can consider the formation of N-acetylethionine sulfoxide as a normal detoxification reaction of this type of compound. Smith and Beeman (39) injected N-acetylethionine sulfoxide i.p. and recovered almost the entire radioactivity in the form of the original substance. N-Acetylethionine, a substance that has now been identified as the ethionine metabolite in vivo, can be deacetylated. N-Acetylethionine produced fatty liver in acute experiments (16, 26). Our experiments show (Tables 7 and 8) that the formation of S-AE from these compounds depends on their stereoconfiguration. N-Acetyl-L-ethionine is a very good source of ethionine, but this substance is very toxic. The rats do not survive 24 hr. N-Acetyl-D-ethionine is less efficient, but it is also less toxic. The racemic mixture represents an average in reactivity and toxicity. The stereospecificity of N-acetylethionines for the deacetylating enzymes (1) is not surprising because N-acetyl-DL-methionine can also be split to its antipods (31, 33) by this method. The inductive value of this experiment is limited by the lack of information on the transport of acetylated and oxidized derivatives of ethionine through cellular membranes. The rate of S-AE formation and the development of pathological changes (fat content in liver and seromucoid concentration) were slightly different when stereoisomers of ethionine were used (Table 8). In all chronic experiments previously reported, the racemic mixture of DL-ethionine has been used exclusively, and the different metabolic behavior of the isomers may be of importance.

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The Metabolism of Ethionine in Rats

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