Identification of a Transaminative Pathway for Ethionine Catabolism

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ABSTRACT

The potential for the oxidation of ethionine by a transaminative route was studied in an attempt to elucidate the pathways whereby the ethyl carbons of ethionine are converted to carbon dioxide. Ethionine is transaminated based on the recovery of a radioactive phenylhydrazone derivative. Carbon 1 of the ethyl portion of ethionine is recovered as carbon dioxide when L-[ethyl-1-14C]ethionine is incubated in a rat liver homogenate system. The addition of pyruvate as an amino group acceptor for transamination stimulated oxidation by more than 10-fold based on carbon dioxide formation and on recovery of the α-keto acid of ethionine as the phenylhydrazone derivative. The addition of 3-ethylthiopropionate to the incubations resulted in complete inhibition of 14CO2 formation from 10 mM L-[ethyl-1-14C]ethionine. The expanded 3-ethylthiopropionate pool was isolated by anion-exchange chromatography to determine if it had become labeled during the incubation. Gas-liquid chromatography of the isolated products revealed a major radioactive peak with a retention time identical to that of a reference sample of 3-ethylthiopropionate. Mass spectral analysis of this radioactive peak obtained from liver homogenate incubations was identical to the spectra obtained from authentic 3-ethylthiopropionate. The same gas chromatograph peak and mass spectra were obtained when a boiled liver homogenate was incubated with L-[ethyl-1-14C]ethionine and an expanded 3-ethylthiopropionate pool; however, as expected, the peak recovered from the gas chromatograph was not radioactive. These results indicate that 3-ethylthiopropionate is formed, probably as a result of decarboxylation of the α-keto acid of ethionine and is thus an intermediate in ethionine catabolism. Rats fed a diet containing 1.5% of 3-ethylthiopropionate exhibited severe depressions in growth and food intake and displayed marked neuromuscular abnormalities. Mortality was 40% over the 2-week experimental period. The animal’s breath had an odor that was indistinguishable from ethanethiol. Experiments with the liver homogenate system demonstrated the formation of labeled ethanethiol in addition to 14CO2 from 10 mM [ethyl-1-14C]-3-ethylthiopropionate. This pathway appears to account for the majority of ethionine oxidation in a liver homogenate system. From these studies, it appears that this oxidative pathway may be intimately involved in the etiology of the many biochemical alterations that have been reported after ethionine administration.

INTRODUCTION

The oxidative metabolism of methionine has been studied extensively in recent years in an effort to explain why methionine is the most toxic of the dietary amino acids (18). Studies by Benevenga et al. (7–9, 26) showed that a pathway for methionine oxidation independent of S-adenosylmethionine formation was operative and that formaldehyde and formate were 2 intermediates in the oxidation of the methionine methyl group. It has since been shown that a transaminative pathway of methionine catabolism represented a major part of the catabolism of methionine in liver homogenate preparations of rats and monkeys (27, 37). Methionine was shown to be transaminated to its keto acid, α-keto-γ-methylbutyrate, and then decarboxylated to 3-methylthiopropionate (37). Rats fed diets with high levels of 3-methylthiopropionate exhibited growth and food intake depressions and darkened and enlarged spleens (36) similar to those seen in rats fed high levels of methionine (2). In rat liver, 3-methylthiopropionate was found to be catabolized to methanethiol, hydrogen sulfide, sulfate, and carbon dioxide (39). These results suggested that an intermediate(s) produced in this pathway was probably associated with the toxicity of methionine.

The metabolism of ethionine, the carcinogenic analog of methionine, is not well understood. Ethionine is known to be activated to an S-adenosyl derivative which has been found to act as an ethyl group donor in competition with some normal methylation reactions of S-adenosylmethionine (32). An abnormal alkylation of nucleic acids by S-adenosylethionine has been postulated to be involved in the hepatotoxicity of ethionine and thus to contribute to its carcinogenicity (15).

Little information is available on the oxidation of ethionine to carbon dioxide. Early studies showed that only about 3% of the administered dose of L-[ethyl-1-14C]ethionine was recovered as 14CO2 after 24 hr. However, the oxidation of ethionine was apparently dose dependent because about 3 times more radioactivity was recovered in CO2 when the amount of ethionine administered was increased 10-fold (15). Stekol et al. (40) have confirmed these findings, and in addition they have found that male rats have a greater capacity to oxidize ethionine than do female rats. Recent studies by Brada et al. (4) found that about 6% of a p.o. or injected dose of L-[ethyl-1-14C]ethionine was recovered as 14CO2 after 24 hr.

The enzymatic mechanism for oxidation of the ethionine ethyl group to carbon dioxide is presently unknown. However, recent results from our laboratory (27) and others (11, 21) suggested that ethionine may be catabolized by the transaminative route that has been postulated for methionine catabolism. Mitchell and Benevenga (27) found ethionine to be a good substrate for transamination with pyruvate or α-keto-γ-methylbutyrate in the rat liver homogenate system. When leucine transaminase was purified to homogeneity from rat liver mitochondria by Ikeda et al. (21), it was found that ethionine transamination with α-ketoglutarate was twice that observed with leucine and α-ketoglutarate. Similarly, studies with purified preparations of glutamine transaminase from rat kidney showed ethionine also...
to be an excellent substrate for transamination by this enzyme (11).

In the experiments reported in this paper, we show that, in rat liver homogenate preparations, ethionine is transaminated to its a-keto acid derivative and catabolized to 3-ethylthiopropanic acid which yields ethyl mercaptan and an unknown product. In addition, we demonstrate the marked toxicity of 3-ethylthiopropionate in rats and speculate that this pathway may be important with respect to ethionine toxicity.

MATERIALS AND METHODS

Isotopes and Chemicals

D-[U-14C]Glucose was purchased from New England Nuclear, Boston, Mass., and was used without further purification. L-[ethyl-1-14C]Ethionine was purchased from ICN, Irvine, Calif. Radiochemical purity of the ethionine was determined by chromatography of an aliquot on a 0.6-x 125-cm analytical cation-exchange column (18). If necessary, the radioactive compounds were purified to greater than 98% purity the day before an experiment by cation-exchange chromatography on 0.6-x 12-cm glass columns packed with 400 mesh Dowex 50-X8 (sodium form) and then lyophilized as has been described previously (7). Synthesis of [ethyl-1-14C]-3-ethylthiopropionic acid from correspondingly labeled ethionine was accomplished by degradation with ninhydrin and oxidation of 3-ethylthiopropionaldehyde to 3-ethylthiopropionate by yeast aldehyde dehydrogenase (EC 1.2.1.5). The details of this procedure are reported elsewhere (38). Radiochemical purity of the isotope used in each experiment was checked by paper chromatography and was found to be greater than 98% in all cases.

Nonlabeled 3-ethylthiopropionate was kindly provided by Dr. Rudolf Fahnenstich of Degussa Chemical Company, Teterboro, N. J. Purity of this preparation was established by paper and thin-layer chromatography and also by gas-liquid chromatography using the system described below. All other chemicals were purchased from commercial sources and were of the highest grade available.

In Vivo Studies

Male Holtzman rats weighing about 75 g were used in these experiments. They were housed individually in suspended cages with wire mesh bottoms at 23° in a room with a 12-hr light-dark cycle. Water and diet in agar gel form were available ad libitum.

Rats were fed a basal diet of 10% casein for a 3-day adjustment period. The animals were then separated into 2 groups of the same average weight and fed either the basal diet or the basal diet supplemented with 1.5% of 3-ethylthiopropionate for 14 days. Supplementation of 3-ethylthiopropionate was made at the expense of the cerelose and cornstarch. Animals were weighed daily. After 14 days, all animals were anesthetized with ether, and blood was drawn by cardiac puncture for hematocrit and hemoglobin determination (22). Liver, spleen, kidneys, and heart were removed, blotted, and weighed.

In Vitro Studies

Animals and Diets. Male Holtzman rats weighing 150 to 300 g were used in all experiments. They were housed individually as described above. A commercial laboratory chow and water were offered ad libitum.

Tissue Preparation and Incubation Conditions

Rats were killed by decapitation, and the livers were excised, placed on ice, and then homogenized in 4 volumes of 0.25 M sucrose with a Potter-Elvejhem tube fitted with a Teflon pestle. Incubations were carried out in 50-ml Erlenmeyer flasks under a flowing oxygen atmosphere for 30 min at 37° in a shaking water bath. Except where noted, the incubation mixtures contained the following in a final volume of 5 ml: 2 ml of the 20% homogenate; 20 mM potassium phosphate buffer (pH 7.5); 1 mM NAD; 1 mM ATP; 5 mM MgCl2; and 10 mM labeled substrate. When the substrate was [ethyl-1-14C]Ethionine, 10 mM sodium pyruvate was included as an amino group acceptor for transamination. Water and 0.5 M sucrose were added to attain an osmotic pressure of 280 mOsmol/liter. Blank incubations were carried out after the reaction mixtures and enzymes were boiled for 1.5 min prior to the addition of the radioactive substrate. Values reported have been corrected for product formation in the blank incubations. Incubations were stopped by the addition of 1 ml of 2 N perchloric acid. Gassing was continued for 45 min to ensure complete recovery of volatile products. In experiments with ethionine as the substrate, the CO2 that evolved during the incubations was trapped in 3 ml of ethanolamine:methyl cellosolve (1:2, v/v). Radioactivity was determined in the traps by counting aliquots by liquid scintillation spectrometry with an organic scintillation fluid (23). Counting error of the samples was less than 1%, and counting efficiency of each sample was determined by using an automatic external standard.

When 3-ethylthiopropionate was the substrate, 2 additional trapping solutions were used to trap any potential volatile sulfur compounds. In some experiments, the gasses evolved were trapped in 8 ml of a 1% zinc acetate solution for identification and determination of hydrogen sulfide by the methylene blue method described by Siegel (34). Hydrogen sulfide concentrations were determined from a standard curve that was developed by using sodium sulfide as a standard. In other experiments, the gasses produced were first swept through a trap containing 5 ml of a 2% solution of mercuric acetate to remove any potential mercaptans before passage through the CO2 trap. Control experiments with sodium [14C]Carbonate release demonstrated that no 14CO2 was trapped in the mercuric acetate traps, and quantitative recovery of 14CO2 was obtained in the ethanolamine:methyl cellosolve trap. The mercuric acetate traps were lyophilized and analyzed for mercaptans by a procedure modified from Zieve et al. (10, 13). Approximately 100 mg of the lyophilized traps were placed in conical glass reaction vials. One hundred µl of toluene 3,4-dithiol were added, and the vials were closed with Teflon-lined septa. The reaction vials were heated in aluminum heating blocks at 250° for 5 min to initiate a flash exchange reaction. The resulting head space gasses produced were sampled with a gas syringe and injected into the gas chromatograph for analysis as described below. Samples inactivated with perchloric acid were centrifuged at 15,000 × g for 15 min. The supernatants were neutralized with 6 N potassium hydroxide, centrifuged, lyophilized, and stored at −80°. In some experiments, the α-keto-γ-ethylbutyrate
formed from ethionine during the incubations was recovered and quantitated as the phenylhydrazone derivative by techniques described by Mitchell and Benevenga (27).

Chromatographic Procedures

Ion-exchange chromatography of L-[ethyl-1-14C]ethionine and its degradation products produced during the incubations was performed by using a 0.6-x 34-cm glass column packed with 400 mesh Dowex 1-X8 (acetate form) and eluted with a water-hydrochloric acid (1 n) constant-volume (500 ml) gradient with a column flow rate of 0.5 ml/min. The eluate was monitored for radioactivity with a Packard flow cell and collected in a fraction collector. Fractions corresponding to selected radioactive areas were combined, neutralized, and lyophilized for further study.

Gas chromatography was performed on a Packard Model 419 gas chromatograph equipped with dual-flame ionization detectors. Column length and packing and the column, injection port, and detector temperatures are described in the chart legends. Nitrogen was used as the carrier gas at a flow rate of about 35 ml/min.

In some experiments, a stream splitter was connected to the column before the detector so that 90% of the effluent was diverted and collected with a Packard Model 852 gas fraction collector. The inlet tube that led to the fraction collector was maintained at the temperature of the detector to prevent premature condensation of the eluted compounds. The effluent was condensed on glass wool-filled cartridges inserted into a turntable which was manually advanced. A well in the turntable was filled with dry ice in ethanol to ensure complete condensation of the compounds in the effluent onto the glass wool. The glass wool was expelled from the cartridges into scintillation vials; water and aqueous scintillation fluid were added, and the radioactivity was determined as described above.

Gas chromatography was carried out with a Varian 2780 gas chromatograph connected to a Du Pont 21-491 B mass spectrometer. The column length and packing and column, injection port, and detector temperatures used are described in the chart legends. Helium was used as a carrier gas at 30 ml/min. The analytical system was of all glass construction and used a jet separator. Peaks were detected by plots of total ion current versus time, and spectra were taken manually during the elution of the samples.

RESULTS

The results of initial studies on ethionine ethyl group catabolism in the rat liver homogenate system are shown in Table 1. In a similar system, the recovery of the α-keto acid of methionine as the phenylhydrazone derivative was 54 ± 4% (S.D.) (1). However, since only relative rates of transamination were of interest in these studies, no corrections for recovery of the phenylhydrazone derivatives were made. With no added cofactors, little ethionine was transaminated to α-keto-γ-ethylbutyrate, and even less of the labeled ethyl carbon was recovered as carbon dioxide. However, the addition of 10 mM pyruvate to the incubations resulted in a 20-fold increase in oxidation of L-[ethyl-1-14C]ethionine to carbon dioxide. These results are in good agreement with recent studies by Mitchell and Benevenga (27) who showed that, in addition to the α-keto acid of methionine, pyruvate was also an excellent cosubstrate for ethionine transamination. The effect of other α-keto acids as cosubstrates for the transamination of ethionine in the liver homogenate system has not been investigated.

The addition of 2.5 mM 3-ethylthiopropionate to the incubations resulted in an 85% decrease in recovery of 14CO2 from L-[ethyl-1-14C]ethionine; an effect which was similar to but more pronounced than the dilution of 14CO2 production from L-[methyl-1-14C]methionine by 3-methylthiopropionate (37). Based on recovery of radioactivity in the phenylhydrazone derivative, the addition of 3-ethylthiopropionate had essentially no effect on transamination of ethionine.

The effect of 3-ethylthiopropionate on ethionine ethyl carbon oxidation to carbon dioxide was studied in more detail. Chart 1 shows the effect of varying levels of 3-ethylthiopropionate on L-[ethyl-1-14C]ethionine oxidation to 14CO2. Essentially, complete blockage of 14CO2 production from ethionine occurred with the addition of 10 mM 3-ethylthiopropionate to the liver homogenate incubations. The values shown for CO2 production from ethionine and the degree of inhibition by 3-ethylthiopropionate are in agreement with the data reported in Table 1.

Because the effect of 3-ethylthiopropionate on ethionine oxidation could be explained as well by an effect on cellular metabolism, its addition on the conversion of glucose carbon (10 mM [U-14C]glucose) to CO2 was studied in the same rat liver homogenate system. Chart 1 shows that glucose oxidation was not affected by 3-ethylthiopropionate at any concentration studied. These observations indicate that the depression in ethionine oxidation is specifically associated with ethionine metabolism and not with general cellular oxidation.

The results of these studies indicated that ethionine is quite possibly oxidized by a transaminative pathway similar to the one suggested for methionine catabolism (37), and that the decrease in recovery of 14CO2 from L-[ethyl-1-14C]ethionine by 3-ethylthiopropionate is due to expansion of an intermediate.
tions were stopped with perchloric acid, and the supernatants were recovered, neutralized, and lyophilized. Aliquots of concentrated supernatants from active homogenates and heat-inactivated homogenates were chromatographed on a Dowex 1 column and eluted with a water:hydrochloric acid gradient as described previously (37). Areas of the elution profile corresponding to 3-ethylthiopropionate, determined by prior chromatography of [ethyl-1-14C]-3-ethylthiopropionate, were collected, neutralized, lyophilized, and used to determine the radioactivity of the 3-ethylthiopropionate pool. The lyophilized samples were dissolved in acid and extracted with 5 volumes of peroxide-free diethyl ether. The ether extract was carefully concentrated under nitrogen and analyzed by gas chromatography.

Elution profiles of a standard solution of 3-ethylthiopropionate in ether and of the ether extract from the homogenate incubations are shown in Chart 2. A major peak was eluted at approximately 9.5 min in both samples and accounted for about 80% of the total peak area, excluding the ether solvent peak at the beginning of the elution. The peak that was eluted at about 3 min appears to be due to contamination or breakdown since it was also observed upon chromatography of the reference sample of 3-ethylthiopropionate. The elution profile of standard 3-ethylthiopropionate and that isolated from the incubation were qualitatively and quantitatively identical.

During gas chromatography of these samples, a portion of the effluent was diverted with a stream splitter so that the distribution of the radioactivity injected onto the column could also be determined. Table 2 shows the distribution of radioac-

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**Chart 1.** Effect of increasing concentrations of 3-ethylthiopropionate on the conversion of 10 mM L-[ethyl-1-14C]ethionine to 14CO2 and of 10 mM [U-14C]-glucose to 14CO2 in rat liver homogenate incubations. Incubations were carried out for 30 min at pH 7.5 as described under "Materials and Methods." Each point represents the mean of 4 observations. Coefficients of variation for each point were less than 5%. The amount of CO2 produced was calculated from the specific activity of the added labeled substrate. △, [ethyl-1-14C]ethionine; □, [U-14C]glucose.

**Chart 2.** A, gas chromatography elution profile of radioactive compounds isolated by anion exchange chromatography of supernatants from liver homogenate incubations with L-[ethyl-1-14C]ethionine. B, elution profile from a reference sample of 3-ethylthiopropionate. A 5-ft column packed with 10% SP-1200/1% H3PO4 on 80 to 100 mesh Chromosorb W AW was used with a flame ionization detector. Temperature parameters were: column, 150°; injection port, 175°; and detector, 180°.

**Table 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>0–2 min</th>
<th>3–5 min</th>
<th>6–8 min</th>
<th>9–11 min</th>
<th>11–13 min</th>
<th>% of recoverya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>10.3 ± 1.6b</td>
<td>5.8 ± 0.9</td>
<td>7.5 ± 0.6</td>
<td>68.3 ± 3.8</td>
<td>8.8 ± 1.1</td>
<td>73.2 ± 3.8</td>
</tr>
<tr>
<td>Blankc</td>
<td>29.3 ± 0.7b</td>
<td>16.3 ± 2.2</td>
<td>18.9 ± 1.8</td>
<td>20.8 ± 1.0</td>
<td>14.7 ± 2.4</td>
<td>102.3 ± 3.3</td>
</tr>
</tbody>
</table>

a Total dpm recovered x 100 dpm injected
b Mean ± S.E. for 6 observations (unknown) and 3 observations (blank).
c The blank samples were prepared from incubations which were boiled for 1.5 min before the addition of labeled substrate.
tivity in injected samples prepared from standard incubations and from blank incubations in which the incubation mixture had been boiled for 1.5 min prior to the addition of \( l\)-ethanethiol-1,14C\]. Ethionine. In incubations conducted with labeled ethionine, about 70% of the radioactivity that was recovered from the column was associated with the peak detected at 9.5 min (Chart 2). The radioactivity recovered from the column when samples from the blank incubations were injected was evenly spread across the elution profile. Because little 3-ethylthiopropionate would be expected to be produced in the blank, the total amount of radioactivity injected was also only 10% of the amount injected in the other samples. Thus, while the peak at 9.5 min was still detected in about the same relative amount as the test samples, there was no radioactivity associated with it. Attempts to divorce the radioactivity from the peak at 9.5 min by altering gas flows or changing column temperature were not successful. Therefore, production of labeled 3-ethylthiopropionate is enzyme dependent.

A comparison of the unknown radioactive compound and a reference sample of 3-ethylthiopropionate was also made utilizing gas chromatography-mass spectrometry. A series of mass spectra were taken during the elution of a reference sample of 3-ethylthiopropionate and during the elution of the samples isolated from the rat liver homogenate incubations. Analysis of the spectra obtained from the reference sample indicated that the peak that eluted from the column at 9.5 min was 3-ethylthiopropionate (Chart 3). Spectral analysis also showed that the radioactive peak at 9.5 min (Chart 2) recovered from the rat liver homogenate incubations was also 3-ethylthiopropionate. A number of spectra were taken during the beginning, the middle, and at the end of the radioactive peak eluting at 9.5 min and were all identical, indicating that the product was homogeneous and consisted of only a single compound, 3-ethylthiopropionate.

Because marked effects of feeding rats diets containing 1.5% of 3-ethylthiopropionate were observed, additional studies were conducted with the rat liver homogenate system to isolate metabolites of 3-ethylthiopropionate catabolism. The possibility that radioactive ethanethiol could be recovered after incubation of [ethyl-1,14C]-3-ethylthiopropionate was investigated. Incubations were carried out, and the gasses evolved were trapped in a mercuric acetate solution. Gas chromatography of the head space gasses produced after the flash exchange reaction of the mercuric acetate traps resulted in elution profiles as shown in Chart 4A along with a chromatogram obtained with a reference sample of ethanethiol (Chart 4B). A major peak was detected with the same retention time as a standard sample of ethanethiol. About 40% of the injected radioactivity was recovered as ethanethiol when the stream splitter system was installed so that the distribution of radioactivity could be determined. The nature of the remaining 60% of radioactivity is not known since it apparently did not elute from the column. Another possibility is that the efficiency of collection of ethanethiol may be low, thus accounting for incomplete recovery. Nevertheless, a radioactive peak that cochromatographed with ethanethiol was observed, indicating that ethanethiol is an intermediate of 3-ethylthiopropionate catabolism and therefore of ethionine catabolism.

Additional experiments were conducted to determine if hydrogen sulfide was produced during 3-ethylthiopropionate oxidation as was observed during the oxidation of 3-methylthiopropionate (39). Gasses produced from the incubation of 3-ethylthiopropionate at pH 6.5 (39) in the rat liver homogenate system were trapped in a zinc acetate solution and analyzed for hydrogen sulfide by the methylene blue method (34). This assay will detect as little as 50 nmol of hydrogen sulfide (39). The test for the formation of methylene blue was negative indicating that hydrogen sulfide, if formed, was further catabolized at a rate sufficient to prevent its accumulation.

A final experiment was conducted to determine the extent of oxidation of [ethyl-1,14C]-3-ethylthiopropionate to 14CO2 in the liver homogenate. The gasses evolved during the incubations were first swept through a solution of mercuric acetate to
remove [ethyl-1-14C]ethanethiol and other volatile organic sulfur compounds followed by an ethanolamine:methyl cellosolve (1:2, v/v) trap and counted for radioactivity. The oxidation of 10 μmol [ethyl-1-14C]-3-ethylthiopropionate to CO2 at pH 7.7 in the rat liver homogenate system resulted in the recovery of 1.83 ± 0.07 (N = 5) μmol CO2 per g wet tissue per hr. This was approximately twice the recovery observed when ethionine was incubated with pyruvate (Table 1). This coupled with the recovery of the ethylcarbon of ethionine as the keto acid (16.38 μmol/g/hr) when pyruvate was supplemented (Table 1) indicates that the decarboxylation of α-keto-γ-ethiolbutyrate to 3-ethylthiopropionate might be the limiting step in the transaminative pathway for ethionine catabolism.

With the identification of 3-ethylthiopropionate and ethanethiol as intermediates in the oxidative catabolism of ethionine, it was decided to feed 3-ethylthiopropionate to rats to determine if it possessed any of the toxic properties of ethionine or their biological analogs, methionine or 3-methylthiopropionate. The effects of feeding rats for 2 weeks a 10% casein diet supplemented with 1.5% of 3-ethylthiopropionate are shown in Chart 5. The body weights of animals consuming the 3-ethylthiopropionate diet were depressed after only 1 day and remained so for the remainder of the 2-week experimental period. Food intakes were reflective of body weight changes (data not shown). Whereas the control animals approximately doubled their weight over the 2-week period, the animals fed 3-ethylthiopropionate did not maintain their initial weight.

The effects of feeding rats 3-ethylthiopropionate were not confined to changes in body weight and food intake. The breath of the animals had the very distinctive smell of ethyl mercaptan after only 1 day of feeding. Gross neurological changes were observed in each animal with a marked lethargic condition being the most obvious. Mortality during the 2-week experimental period was 40%. Of the surviving animals, 2 rats exhibited partial paralysis, and one showed evidence of moderate nasal hemorrhage. The hematocrits of the surviving animals at the end of the experimental period were decreased significantly as compared to control rats (34 versus 38%, p < 0.01). The kidneys were enlarged substantially (1.31 versus 0.71 g/100 g body weight, p < 0.001), and the spleens were very dark but not enlarged as was reported for rats fed 3-methylthiopropionate (36).

**DISCUSSION**

These experiments demonstrate clearly that ethionine may be oxidized by a transaminative pathway similar to the pathway that has been described for methionine catabolism (27, 35, 37, 39). Based on the results reported here and of others (11, 21, 27), ethionine may be transaminated to α-keto-γ-ethiolbutyrate and then apparently decarboxylated to carbon dioxide and 3-ethylthiopropionate. This is the first demonstration of 3-ethylthiopropionate formation in a mammalian system. However, studies with *Escherichia coli* have demonstrated the formation of 3-ethyl[35S]thiopropionate from either [35S]sulfide or [35S]-ethionine (14).

The complete metabolic fate of 3-ethylthiopropionate is not known, but carbon-sulfur bond cleavage does occur at some point yielding ethanethiol and an unknown product, possibly acrylic acid. Brown and Scholefield (5) have observed the formation of 14CO2 from 3-ethylthio-[1-14C]propionate in rat liver slices. Kidney and heart also showed significant activities, and a small degree of oxidative capacity was observed in spleen and lung preparations. Ethanethiol is apparently catabolized further since 14CO2 was recovered from incubations with either [ethyl-1-14C]-3-ethylthiopropionate or ethionine. Recent evidence by Farber (16) on the mechanism of S-dealkylation supports our conclusions. Ethanethiol was found to be an excellent substrate for a soluble deethylating enzyme and yielded acetaldehyde as a product. Natori and Tarver (28) had earlier demonstrated the formation of acetate from ethionine. Thus, the fate of the ethyl carbons of ethanethiol is likely to be oxidation to CO2 via the intermediate formation of acetaldehyde and acetate. The existence of ethanol as an additional intermediate in the oxidation series is possible also since labeled ethanol was isolated from rats given injections of L-[ethyl-1-14C]ethionine (28).

The fate of the sulfur atom of ethionine and hence ethanethiol is not documented. However, based on our earlier observations with methionine catabolism and methanethiol oxidation (39), we suspect that the sulfur atom of ethanethiol is probably oxidized to sulfate via hydrogen sulfide, thiosulfate, and sulfite formation.

The results in Table 1 and Chart 1 indicate that this transaminative pathway for ethionine catabolism probably represents the major route for ethionine oxidation *in vitro*, since 14CO2 production from [ethyl-1-14C]ethionine can be totally suppressed by expansion of the 3-ethylthiopropionate-trapping pool. The significance of this pathway *in vivo* has not been investigated. The appearance of the α-keto acid of ethionine in the urine of animals fed ethionine, however, suggests that this pathway is operative *in vivo* (3, 33).

Ethionine administration to animals has resulted in a number of biochemical alterations in addition to the development of...
hepatic carcinoma. Some of these observations include depletion of liver glutathione (17, 20), decreased levels of hepatic ATP (29, 43), disaggregation of liver polysomes (42), inhibition of RNA synthesis (12, 41), microsomal membrane alterations (24, 25), and ethylation of tRNA (30, 31). It is not known whether all of the changes observed after ethionine administration can be accounted for solely through formation of S-adenosylmethionine since complete inhibition of S-adenosylmethionine formation by methionine or 1-aminocyclopentane-carboxylic acid did not completely inhibit ethylation of tRNA by ethionine (30, 31). Whether the transaminative pathway of ethionine metabolism reported here is involved in any of these metabolic changes due to ethionine administration appears worthy of further investigation.

REFERENCES

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