The Effect of Cupric Acetate on Ethionine Metabolism

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SUMMARY

The addition of cupric acetate, a potent inhibitor of ethionine carcinogenesis, to a diet containing ethionine increased the ethionine toxicity. The concentration of S-adenosylethionine in liver was found to be significantly higher when compared to animals fed only ethionine in the diet. Ethionine forms a complex(es) with cupric acetate that is insoluble at a pH higher than 4; however, this complex can be solubilized at a low pH. Ethionine, if administered p.o. in the form of this complex, was absorbed from the intestinal lumen in the same order of magnitude as when administered alone; however, as the body weight increased over 200 g, the portion of absorbed ethionine decreased. The absorption of ethionine bound in the complex was completed within 16 hr compared to 2 hr for free ethionine. This time delay was accompanied by a shift in the concentration maximum of ethionine metabolites in the liver from 8 to 24 hr. When ethionine was administered alone, it was metabolized in the intestinal lumen as demonstrated by the analysis of the soluble intestinal contents; the presence of cupric acetate inhibited this process. The chromatographic analysis of ethionine metabolites in urine of rats treated by the complex revealed an increased excretion of ethionine sulfoxide and other ethionine metabolites at the expense of N-acetylethionine sulfoxide. The increased concentration of S-adenosylethionine in the liver in chronic experiments may be, at least partly, a result of a diminished capacity of the rat to detoxify (acetylate) ethionine sulfoxide, which is considered the main reserve pool of ethionine for the maintenance of a high level of S-adenosylethionine.

INTRODUCTION

The use of inhibitors of chemical carcinogenesis that operate on different levels of metabolism of carcinogenic substances or on different levels of the pathological events produced by these substances can give new insights into the mechanism of carcinogenesis. Some systems were described (see review, Ref. 29) that emphasize mainly the role of nonspecific microsomal hydroxylases that participate in the metabolism of some carcinogenic substances. Ethionine is unique when compared to other carcinogens, since its metabolism is similar to that of methionine (see reviews in Refs. 6 and 26) and the hepatic microsomal enzymatic system apparently does not play an essential role in the ethionine action. The use of other inhibitors of ethionine action could provide insight into other possible mechanisms of carcinogenesis. CuAc applied together with some carcinogenic substances in food manifested itself as an effective inhibitor of liver carcinogenesis (10, 13). Kama-moto et al. (14) described a protective effect of CuAc on the formation of hepatomas in ethionine-fed animals. CuAc and ethionine form complex salts, and it is not clear what the biological and metabolic activity of these complexes is in comparison to free ethionine. The study of those questions is the subject of this paper.

MATERIALS AND METHODS

Female CFN rats were obtained from Carworth Farms, New City, N. Y. The rats were fed a semisynthetic C-24 diet (28) and water ad libitum. One group of rats was fed C-24 only, the 2nd group was fed C-24 + 0.30% dl-ethionine (Nutritional Biochemicals Corp., Cleveland, Ohio), the 3rd group received C-24 + 0.30% dt-ethionine + 0.30% CuAc [Cu(C2H4O2)·H2O] (Mallinckrodt analytical grade; Mallinckrodt Chemical Works, St. Louis, Mo.), and the 4th group received C-24 + 0.30% CuAc only. The animals were kept in screened stainless cages in an air-conditioned room at 22° and 50% relative humidity, with a regular light and dark regimen (12 hr light + 12 hr dark). The rats were weighed weekly and sacrificed at different stages of ethionine treatment.

In acute experiments t-ethionine (A grade; Calbiochem, Los Angeles, Calif.) was administered by garage (12.5 mg/100 g body weight) alone or together with CuAc solution (12.5 mg/100 g body weight). In all experiments the final volume of the administered solutions was adjusted with water and kept constant on the basis of body weight. Before the start of the experiment, the rats were fasted for 16 hr. The ethionine solution was labeled by t-[ethyl-1-14C]ethionine (New England Nuclear, Boston, Mass.) (2.5 μCi/25 mg ethionine). Ethionine was administered between 9 and 10 a.m. to minimize diurnal variations.

For determination of the intestinal absorption, the rats were killed either 6 or 24 hr after the ethionine application. The gastrointestinal tract was removed and washed repeat-
edly with cold 0.90% NaCl solution. The washings were combined and analyzed. In the other experiments all animals were initially anesthetized with ether, and a cardiac blood sample was obtained. A portion of liver was quickly removed, frozen in liquid nitrogen, and homogenized in a 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 rpm. The resulting precipitate was washed once with 3% HClO₄ containing nonradioactive DL-ethionine (10 mg), once with 3% HClO₄ alone, once with 95% ethanol containing 10% potassium acetate, once with absolute ethanol, twice with ethanol/diethyl ether (3:1) (once at 60°C and once at room temperature), and twice with diethyl ether; it was then dried in vacuum to constant weight. For determination of the residual radioactivity, about 5 mg of the dried powder were dissolved in 1 ml of Hyamine mixed with 15 ml of Bray's scintillation cocktail. The assay of S-AE was done by the method proposed by Schlenk and DePalma (21) for determination of S-adenosylmethionine in yeast extracts that was modified for S-AE determination in rat liver by Stekol (27). The radioactivity distribution was determined in the unabsorbed part of the extract on a Dowex 50 column in 2 N HCl eluate and in 4 N HCl eluate. Radioactivity was counted in a mixture with Bray's solution in a Packard spectrometer after a proper quenching correction. 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 (5) and modified by Siekewitz and Potter (24) and Stekol. Liver ATP was separated on an AG 1-X8 column (Bio-Rad Laboratories, Richmond, Calif.) and hydrolyzed; the liberated adenine was isolated on the same column and its concentration was subsequently determined spectrophotometrically (about the details and limitations of this method, see Ref. 2).

The hemoglobin concentration in blood was determined spectrophotometrically as CN:ferrhemoglobin by the method of Heilmeyer (12), seromucoid concentration was determined by the method of Winzler (30), and total serum proteins were determined by the method of Lowry modified by Oyama and Eagle (17).

The urine was collected in a BioNuclear glass metabolic cage. Before the experiments 1 ml of 1 N HCl was added for preservation and to avoid the decomposition of S-AE. The volume of the urine was adjusted to 50 ml, and an aliquot was used for analysis.

The analysis of ethionine and its metabolites in urine and in combined washings from the gastrointestinal tract was performed chromatographically (2) on an AG 50W-X12 column, 200 to 400 mesh (Bio-Rad). We examined the chromatographic behavior of ethionine metabolites in the presence of CuAc (dissolved in perchloric acid) to exclude the possibility that the presence of CuAc complexes can influence the chromatographic ethionine and S-AE determination. No abnormalities were noted. For light microscopic observations the tissues were fixed in 10% neutral buffered formaldehyde solution, and routine paraffin sections were stained with hematoxylin and eosin. The data were statistically analyzed by Student's t test.

RESULTS

The Complex Formed between Ethionine and CuAc. The weight ratio between CuAc and the carcinogenic substances in diets used by previous investigators was kept at 1:1. To define this ratio, between ethionine and CuAc, an ethionine solution was titrated by increasing amounts of CuAc solution (Chart 1). The maximal precipitation of the insoluble complex produced was obtained at the molar ratio of ethionine to CuAc of 2:1. Ethionine sulfoxide and ethionine sulfone were not precipitated. The 10-fold dilution of ethionine and CuAc solutions (at concentrations used in vivo) did not prevent a precipitation of a substantial amount of the complex. This experiment was performed to demonstrate the possibility that, after the complex was dissolved in the stomach in vivo by gastric hydrochloric acid and was subsequently diluted, it could again precipitate after neutralization in the duodenum or in the small intestine.

Effect of CuAc on Rats Ingesting Ethionine. The growth curves of the rats in this experiment, in which ethionine, CuAc, and ethionine + CuAc were used, are shown in Chart 2. It is evident from the growth curves that both ethionine and CuAc produced distinct depression of growth. The combination of ethionine and CuAc depressed the growth more and produced a dramatic decrease in body weight after 8 weeks of feeding. The food intake per unit of body weight remained relatively constant (Table 1).

Rats from each group were sacrificed on the 28th and 56th days of the experiment (Table 2). The action of CuAc alone was characterized by a slight decrease in the hemoglobin concentration. These changes were minimal after 28 days of feeding, but they became significant after 56 days. Changes produced by feeding only ethionine were in agreement with previous observations (3). The increase in liver weight after 56 days of ethionine feeding may be the result of hepatocellular proliferation. This increase was not observed in animals fed E-CuAc. In that case the relative liver weight remained unchanged (Table 2).

Biochemical changes directly connected with ethionine
metabolism are presented in Table 3. When CuAc was applied alone, the ATP value was slightly reduced. A striking observation is that the concentration of S-AE, after simultaneous CuAc feeding, was substantially increased.

The ATP concentration in this experiment was slightly decreased in comparison to a group of animals treated with ethionine alone.

The concentration of S-AE was measured in the liver at different times during the 24-hr cycle, and the results demonstrated that the increase in S-AE concentration was not directly related to the time of food intake (Chart 3).

The livers of rats fed only CuAc were normal on gross examination. Also no differences were observed between animals fed either ethionine or E-CuAc. Microscopic observations presented in Table 4 demonstrated some significant differences in groups treated with ethionine alone and with E-CuAc. These changes consisted of neutrophilic infiltrations and scattered hepatocellular necrosis. There was also an accumulation of a brownish-green pigment in Kupffer cells in both of these groups. The pigment has not been fully characterized, but it did not stain with Lilly's bile stain.

**Metabolism of E-CuAc in Acute Experiments.** We injected E-CuAc i.p. in order to compare the metabolism of free ethionine and ethionine in a complex with CuAc. The experiment demonstrated that the mixture that contained ethionine (1 mg/100 g body weight) was lethal to rats. This was probably caused by the presence of free CuAc. The isolated CuAc complex was demonstrated to be less toxic than the original mixture.

Rats tolerated relatively high amounts of E-CuAc when administered p.o., and no problems were noted with amounts even higher than the normal daily dose of ethionine, usually used in chronic experiments. In these experiments the rats were sacrificed 5, 24, and 72 hr after p.o. administration of ethionine. The highest S-AE concentration in liver was observed 5 hr after the administration of free ethionine and 24 hr after administration of E-CuAc (Table 5). The radioactivity of the S-AE fraction correlated with the concentration changes. The calculated specific activities of ethionine in the S-AE molecule were found to be in agreement with the specific activity of the administered ethionine. The determination of S-AE concentration and activity was omitted after 72 hr, since its concentration at that time was too low to give reliable results. The

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**Table 1**

Food intake in rats fed with ethionine and CuAc

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food intake (g/100 g body wt/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.28 ± 0.10 4.12 ± 0.30</td>
</tr>
<tr>
<td>Ethionine (0.3%)</td>
<td>4.45 ± 0.02</td>
</tr>
<tr>
<td>CuAc (0.3%)</td>
<td>3.31 ± 0.18</td>
</tr>
<tr>
<td>Ethionine (0.3%) + CuAc (0.3%)</td>
<td>3.14 ± 0.39</td>
</tr>
</tbody>
</table>

* A measurement of food intake was made on the 25th day of the experiment (8 rats/group).
A measurement of food intake was made on the 53rd day of the experiment (5 rats/group).

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**Table 2**

Effect of CuAc on ethionine-fed rats

The rats were sacrificed after a 16-hr period of fasting. The data were statistically analyzed by Student's *t* test. The assay and procedure for the treatment of the animals are described in "Methods and Materials."

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days fed</th>
<th>No. of rats</th>
<th>Body wt (g)</th>
<th>Liver wt in % of body wt</th>
<th>Dry fat-free substances (%)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>Seromucoid (mg/100 ml)</th>
<th>Serum protein (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>3</td>
<td>257 ± 13</td>
<td>2.83 ± 0.12</td>
<td>21.94 ± 0.54</td>
<td>15.25 ± 0.47</td>
<td>20.39 ± 1.98</td>
<td>6.56 ± 0.20</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>28</td>
<td>3</td>
<td>200 ± 13</td>
<td>3.61 ± 0.78</td>
<td>18.87 ± 0.37</td>
<td>12.53 ± 1.25</td>
<td>14.97 ± 0.78</td>
<td>5.93 ± 0.39</td>
</tr>
<tr>
<td>0.3% ethionine + CuAc</td>
<td>28</td>
<td>3</td>
<td>192 ± 10</td>
<td>2.66 ± 0.15</td>
<td>18.21 ± 0.43</td>
<td>13.34 ± 1.25</td>
<td>16.12 ± 1.27</td>
<td>5.51 ± 0.53</td>
</tr>
<tr>
<td>0.3% CuAc</td>
<td>28</td>
<td>3</td>
<td>227 ± 3</td>
<td>2.95 ± 0.35</td>
<td>20.45 ± 0.70</td>
<td>14.43 ± 0.47</td>
<td>22.65 ± 1.84</td>
<td>6.24 ± 0.40</td>
</tr>
<tr>
<td>Control</td>
<td>56</td>
<td>5</td>
<td>299 ± 35</td>
<td>2.68 ± 0.26</td>
<td>20.43 ± 0.35</td>
<td>15.03 ± 0.73</td>
<td>19.56 ± 3.49</td>
<td>6.76 ± 0.22</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>56</td>
<td>5</td>
<td>212 ± 29</td>
<td>3.21 ± 0.45</td>
<td>16.81 ± 0.68</td>
<td>13.40 ± 0.93</td>
<td>14.82 ± 2.18</td>
<td>5.97 ± 0.37</td>
</tr>
<tr>
<td>0.3% ethionine + CuAc</td>
<td>56</td>
<td>5</td>
<td>133 ± 15</td>
<td>2.82 ± 0.26</td>
<td>15.58 ± 0.60</td>
<td>12.75 ± 1.24</td>
<td>15.71 ± 1.54</td>
<td>4.87 ± 0.09</td>
</tr>
<tr>
<td>0.3% CuAc</td>
<td>56</td>
<td>5</td>
<td>232 ± 5</td>
<td>2.67 ± 0.13</td>
<td>19.82 ± 0.33</td>
<td>13.56 ± 0.45</td>
<td>20.33 ± 1.33</td>
<td>6.54 ± 0.34</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
Statistically significant difference was noted when compared with control group.
Statistically significant difference was noted when compared with ethionine group.
Table 3

Effect of CuAc on ATP and S-AE concentration in liver of ethionine-fed rats

The assay and procedure for the treatment of animals are described in “Methods and Materials.”

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days fed</th>
<th>No. of rats</th>
<th>ATP (µmoles)</th>
<th>S-AE (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>g fresh liver</td>
<td>g dry fat-free substances</td>
</tr>
<tr>
<td>Control</td>
<td>28</td>
<td>3</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>28</td>
<td>3</td>
<td>0.62 ± 0.03</td>
<td>3.29 ± 0.23</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% CuAc</td>
<td>28</td>
<td>3</td>
<td>0.52 ± 0.06</td>
<td>2.86 ± 0.43</td>
</tr>
<tr>
<td>0.3% CuAc</td>
<td>28</td>
<td>3</td>
<td>0.58 ± 0.07</td>
<td>4.53 ± 0.28</td>
</tr>
<tr>
<td>Control</td>
<td>56</td>
<td>5</td>
<td>1.11 ± 0.05</td>
<td>5.47 ± 0.19</td>
</tr>
<tr>
<td>0.3% ethionine</td>
<td>56</td>
<td>5</td>
<td>0.61 ± 0.06</td>
<td>3.56 ± 0.40</td>
</tr>
<tr>
<td>0.3% ethionine + 0.3% CuAc</td>
<td>56</td>
<td>5</td>
<td>0.59 ± 0.08</td>
<td>3.81 ± 0.65</td>
</tr>
<tr>
<td>0.3% CuAc</td>
<td>56</td>
<td>5</td>
<td>0.92 ± 0.01*</td>
<td>4.65 ± 0.10*</td>
</tr>
</tbody>
</table>

* The rats were sacrificed after a 16-hr period of fasting. The data were statistically analyzed by Student’s t test.
* ND, not determined; NA, not applicable.
* Mean ± S.D.
* A statistically significant difference when compared with ethionine group.
* A statistically significant difference when compared with control group.

Radioactivity of the total TCA-soluble substances from liver is given in Chart 4 and shows the same pattern as does S-AE. No differences were observed between ethionine and the E-CuAc group after 72 hr.

The Absorption of Ethionine from Its Copper Complex in Vivo. Previous results concerning differences in the concentration of ethionine metabolites in liver as a function of time after administration of free ethionine and E-CuAc suggest the possibility of a differential absorption rate from the gastrointestinal tract. The absorption of ethionine was studied by a direct measurement of the remaining radioactive ethionine in the gastrointestinal contents, and the results are presented in Table 6.

The results demonstrate that the absorption of ethionine from E-CuAc was significantly decreased in the 1st hrs after administration. Further, this experiment showed that a significant portion of ethionine administered in its free form was not absorbed and was later excreted in feces. This observation is in agreement with our previous findings (2). With free ethionine, the absorption was completed within 2 hr after administration; when E-CuAc was administered, the absorption process took almost 24 hr. In this experiment the final unabsorbed amount of ethionine was the same,

Table 4

Histopathological changes in the liver of rats fed ethionine and CuAc

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days fed</th>
<th>Hepatocytes</th>
<th>Hyperplasia</th>
<th>Pleomorphism</th>
<th>Fatty change</th>
<th>Multinucleation</th>
<th>Necrosis</th>
<th>Neutrophilic infiltration</th>
<th>Oval cells</th>
<th>Bile duct proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionine</td>
<td>28</td>
<td>+*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ethionine + CuAc</td>
<td>28</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CuAc</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethionine</td>
<td>56</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Ethionine + CuAc</td>
<td>56</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>CuAc</td>
<td>56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* For detailed description of the experiment see Tables 2 and 3.
* +, minimal change; ++, mild change; ++++, marked change.
Table 5
Concentration of S-AE in liver after p.o. application of ethionine and E-CuAc complex(es) in acute experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>S-AE (μmoles/g DFFS)</th>
<th>dpm × 10⁻³ in S-AE fraction/g DFFS</th>
<th>Specific activity of S-AE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 hr</td>
<td>24 hr</td>
<td>5 hr</td>
</tr>
<tr>
<td>Ethionine</td>
<td>3</td>
<td>9.97 ± 1.38ª</td>
<td>5.41 ± 0.52</td>
<td>56.9 ± 6.6</td>
</tr>
<tr>
<td>E-CuAc</td>
<td>4</td>
<td>1.92 ± 0.37ª</td>
<td>8.82 ± 0.34ª</td>
<td>9.0 ± 0.7</td>
</tr>
</tbody>
</table>

ª DFFS, dry fat-free substances.
ª The specific activity of S-AE is given in dpm × 10⁻³/μmole S-AE.
ª Mean ± S.D.
ª A statistically significant difference was noted when compared with ethionine group.

In our experiments, there was no dependence of the percentage of unabsorbed free ethionine on the amount of administered ethionine per unit of body weight. The same results were obtained when a constant amount of free ethionine per unit of body weight was administered to rats of various body weights. Chart 6 presents the ratio between unabsorbed ethionine from E-CuAc and from free ethionine plotted against body weight. The volume of applied free ethionine and E-CuAc per 100 g body weight was the same in all experiments. Despite the decreased absorption of ethionine from E-CuAc in animals with a higher body weight regardless of whether free or bound ethionine was applied. When a larger amount of the E-CuAc was administered, the emptying of the stomach was delayed. In some cases, a part of the complex remained in an undissolved state in the small intestine and in the cecum. Data concerning the distribution of ethionine radioactivity in the gastrointestinal tract after administration of 2 different amounts of the E-CuAc mixture to rats of the same body weight are presented in Chart 5.

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Chart 4. Radioactivity of TCA-soluble material from liver of rats treated with L-[ethyl-l-1⁴C]Ethionine (12.5 mg/100 g body weight) (○) and by its copper complex (12.5 mg ethionine + 12.5 mg CuAc per 100 g body weight) (□). Each point represents an average of determinations in 4 rats. The variability is given by standard deviation of mean. DFFS, dry fat-free substances.

Chart 5. Distribution of the unabsorbed ethionine from E-CuAc (12.5 mg ethionine + 12.5 mg CuAc) in different parts of the gastrointestinal tract (24 hr after administration). □, radioactivity of solids; □, radioactivity of supernatants. Body weight of rats, 320 ± 18 g. The results represent an average of 3 rats/group. Int., intestine.

Table 6
Effect of CuAc on ethionine absorption from gastrointestinal tract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of experiment (hr)</th>
<th>No. of rats</th>
<th>Body wt (g)</th>
<th>Ethionine applied (dpm × 10⁻³)</th>
<th>Ethionine recovered (dpm × 10⁻³)</th>
<th>Ethionine unabsorbed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionine</td>
<td>6</td>
<td>3</td>
<td>122 ± 4.4ª</td>
<td>2518 ± 91</td>
<td>883 ± 57</td>
<td>34.99 ± 0.98</td>
</tr>
<tr>
<td>Ethionine</td>
<td>24</td>
<td>4</td>
<td>115 ± 0.0</td>
<td>2359 ± 0</td>
<td>746 ± 56</td>
<td>31.63 ± 2.38</td>
</tr>
<tr>
<td>E-CuAc</td>
<td>6</td>
<td>3</td>
<td>122 ± 1.7</td>
<td>2871 ± 39</td>
<td>2111 ± 219</td>
<td>73.61 ± 10.00</td>
</tr>
<tr>
<td>E-CuAc</td>
<td>24</td>
<td>4</td>
<td>123 ± 3.5</td>
<td>2949 ± 83</td>
<td>912 ± 49</td>
<td>30.89 ± 1.08</td>
</tr>
</tbody>
</table>

ª Mean ± S.D.
weight (in rats weighing 300 g, only about 50% of the administered ethionine was absorbed in 24 hr), the resulting radioactivity of the perchloric acid extracts from liver and the TCA extracts from blood was higher in all cases than in rats receiving ethionine only.

Effect of CuAc on the Metabolism of Ethionine. After p.o. administration of labeled ethionine, about 50% of the applied radioactivity was excreted in the urine within 48 hr independent of the weight of the rats. When ethionine was administered in the form of E-CuAc, small rats excreted less labeled compounds during the 1st 24 hr. However, the deficit was compensated for by an increased excretion during the 2nd 24-hr period. In rats of a higher body weight, the total amount excreted during 48 hr was significantly decreased (Table 7), which is in accord with the observation of a decreased intestinal absorption.

The quantitative distribution of excreted ethionine metabolites in urine after p.o. administration of ethionine and of E-CuAc is summarized in Table 8. The excretion of acetylated ethionine sulfoxide is decreased after application of E-CuAc, while the excretion of ethionine sulfoxide, S-AE, and some unidentified components is increased. The main component of the complex fraction of the unidentified substances was recently identified as unchanged ethionine (2).

The Nature of Ethionine Metabolites in the Gastrointestinal Tract Contents. The chemical nature of the unabsorbed ethionine metabolites was studied in washings from the gastrointestinal lumen obtained after varying periods of time following ethionine and E-CuAc administrations. The radioactivity was found in both the solid and liquid portions. Some of the radioactivity in the solid portion was extractable with 5% TCA. The radioactivity distribution between the solid and liquid portion in both groups of animals was similar, except that in animals with a higher body weight there was an increase in the radioactivity in the solid portion probably due to the presence of the undissolved E-CuAc (Table 9). The liquid portions obtained from rats at 0.5, 2, 4, 8, and 16 hr after the application of ethionine were analyzed chromatographically. The results have shown

---

**Table 7**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Av. body wt (g)</th>
<th>1st 24 hr (%)</th>
<th>2nd 24 hr (%)</th>
<th>Total in 48 hr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionine</td>
<td>3</td>
<td>150 ± 4*</td>
<td>44.03 ± 2.80</td>
<td>5.72 ± 0.49</td>
<td>49.74 ± 2.75</td>
</tr>
<tr>
<td>Ethionine</td>
<td>3</td>
<td>291 ± 18</td>
<td>46.32 ± 3.01</td>
<td>5.43 ± 1.09</td>
<td>51.75 ± 2.50</td>
</tr>
<tr>
<td>E-CuAc</td>
<td>3</td>
<td>140 ± 3</td>
<td>28.91 ± 3.01</td>
<td>21.22 ± 1.18</td>
<td>50.13 ± 2.99</td>
</tr>
<tr>
<td>E-CuAc</td>
<td>3</td>
<td>280 ± 14</td>
<td>27.40 ± 2.36</td>
<td>10.59 ± 2.32</td>
<td>37.99 ± 1.34</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

**Table 8**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N-Acetylation (%)</th>
<th>Ethionine sulfoxide (%)</th>
<th>S-AE (%)</th>
<th>Other (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionine</td>
<td>75.0 (73.2–76.8)*</td>
<td>9.7 (9.4–10.0)</td>
<td>9.4 (7.8–11.0)</td>
<td>5.8 (5.3–6.4)</td>
</tr>
<tr>
<td>E-CuAc</td>
<td>56.0 (54.5–57.5)</td>
<td>22.3 (21.4–23.3)</td>
<td>15.4 (14.1–16.7)</td>
<td>6.2 (5.5–7.0)</td>
</tr>
</tbody>
</table>

* The figures represent the average of 2 independent experiments.

Numbers in parentheses, results of individual determinations.
that the label pattern changed characteristically depending on the time after ethionine administration. These changes were probably produced by the formation of new metabolites, which appeared as some new peaks and partially increased the background label across the chromatogram. The possible origin of a part of these metabolites from the bile remains to be established. These effects were almost totally absent in the presence of CuAc.

DISCUSSION

There are many reports concerning the protective effect of copper salts on liver damage and on carcinogenesis produced by different chemical carcinogens (4, 9, 10, 13, 18, 22). The reports about the mechanism of the copper action were rather conflicting (7, 8, 11, 13, 15); only some of them confirmed the role of a direct interaction between copperic salt and hepatotoxic substances. As shown by Kamamoto et al. (14) CuAc also inhibited the induction of liver tumors in rats ingesting ethionine. The copper content in the whole liver increased more in rats treated with ethionine and CuAc than in rats treated with CuAc alone. The authors suggested that copper was bound to ethionine and deposited in the liver nuclei.

As mentioned above, CuAc inhibits liver carcinogenesis in several experimental systems. Although the final biological effect of this compound in all systems was similar, it is not clear whether the protective mechanism is the same. The toxic substances producing various pathological lesions in the liver have a different chemical nature, and their direct chemical interaction with CuAc can also be different. Only when we accept the previously suggested hypothesis (10, 14) that the effect is at the level of cellular receptors of carcinogens and when we assume that the decisive receptors in different experimental models are the same or at least that all are reacting with copper salts, might we then consider the same or similar mechanism in all cases. Because there is no available evidence that would permit such conclusions, we will limit our discussion only to the interaction of CuAc with ethionine.

The preliminary study of the formation of an insoluble complex between ethionine and CuAc demonstrated that at the molar ratio of 1:1, which was used in the previous studies in vivo, the amount of copper is higher than that necessary for the maximal production of an insoluble complex(es). On the other hand, it is possible that ethionine is competing with other components present in the diet reacting with copper; therefore we preserved this ratio in our study, although the maximum of the insoluble complex formation in vitro was reached at a considerably lower molar ratio of copper to ethionine (1:2).

Before starting this study we anticipated that, due to the low solubility of E-CuAc, the absorption of ethionine from the intestinal lumen could be prevented by the presence of copper. However, quantitative measurements of S-AE concentration in liver in chronic experiments did not corroborate this assumption. The actual concentration of S-AE in the liver was significantly higher in animals receiving E-CuAc than in animals receiving ethionine only.

The difference between S-AE concentrations in the 2 groups can be attributed to the timing of the determination. CuAc can change the time pattern of ethionine absorption from intestinal lumen and consequently modify the concentration curve of liver S-AE plotted against time. Although a 2nd alternative is that CuAc can influence the food intake pattern of rats, data from a 24-hr cycle demonstrate (Chart 3) that, whatever causes the increase in concentration, the increment is significant at any time of the cycle. This is the 1st recorded observation of the inhibition of ethionine carcinogenesis accompanied by a distinct rise of S-AE concentration in liver. This rise can be explained by an increased synthesis of this compound or by a decreased catabolism. Both alternatives may be a consequence of the formation of copper complex(es). These complexes could have a different metabolic behavior than free ethionine and other free ethionine metabolites do. On the other hand, the administration of CuAc could influence the reactivity of rat tissues, particularly that of liver, which can result in modified ethionine metabolism.

On the basis of liver copper determinations (14), we
anticipate that the E-CuAc complex could represent only a small fraction of the total ethionine and its metabolites present in body tissues and fluids. To achieve the protective effect of CuAc, simultaneous applications of cupric salts and carcinogens were required in some cases (15). Therefore, in those cases the biochemical changes produced by CuAc alone cannot be considered as essential. Since no such studies have been performed as yet with ethionine, we must consider this possibility open.

In our experiments the combination of ethionine with CuAc produced a dramatic drop in body weight. Amino acids are a transporting vehicle for copper ions (20) in plasma, and therefore the complex formation of ethionine with CuAc could increase the copper transport. The consequence of this increase would be a higher copper toxicity. A similar effect should also be observed in the combination of CuAc with methionine. However, our observations (not published here in detail) did not confirm this prediction. Therefore, we anticipate that the toxicity enhancement depends at least partially on the toxic effect of ethionine.

When E-CuAc was administered in an acute experiment, the absorption of ethionine was distinctly delayed and still continued, while the absorption of free ethionine in a control experiment had already been completed. The ultimate absorption values measured 24 hr after E-CuAc administration did not substantially differ from those of free ethionine (Table 7) in rats weighing 100 to 200 g. Gross examination of the contents in the gastrointestinal lumen containing ethionine or E-CuAc has shown a decrease in the rate of the emptying of the stomach and a decrease of passage time for the complex through the intestinal tract. This observation revealed that the insoluble E-CuAc in the stomach was gradually dissolved and that the neutralization of the fluid in the duodenum did not produce any subsequent precipitation. In large rats (over 200 g) the insoluble complex was released from the stomach essentially unchanged, and that increased the TCA-soluble amount of ethionine in the intestinal solids (Table 9).

The ratio of excreted ethionine metabolites in urine was changed in the presence of copper. The excretion of ethionine sulfoxide and some other metabolites was increased at the expense of N-acetylethionine sulfoxide (Table 8). This observation points to the decreased capacity of the body to acetylate ethionine sulfoxide. Since N-acetylethionine sulfoxide is considered (2, 25) an ultimate detoxification product of ethionine, the diminished acetylation could increase the ethionine toxicity. This is in agreement with our histological findings and with observed growth curves in chronic experiments. The increased S-AE content in liver can be a result of a higher ethionine sulfoxide content in the body. In our previous paper we suggested (2) that the ethionine sulfoxide might be responsible for the maintenance of a high S-AE level in liver during the 24-hr cycle.

Alternatively, the increase of the S-AE concentration in liver can be a result of the diminished catabolism. Thus far no data are available on the degradative pathway of this substance with the exception of the formation of S-adenosylhomocysteine after the transfer of the ethyl group. Whether this reaction is the only existing pathway of S-AE in the mammalian system is unknown. The possible breakdown products predicted on the basis of observations in microbiological systems have not yet been detected in rats. After an i.p. injection of [ethyl-1-14C]S-AE, most of the radioactivity was excreted into urine and the only product formed from S-AE in vivo was N-acetylethionine sulfoxide (20% of excreted radioactivity), showing the possibility of the release of ethionine from this molecule (2). S-AE was considered as a key substance in the transfer of the ethyl group into cellular macromolecules during the ethionine carcinogenesis (6). In recent years, its dominant position as an exclusive donor of the ethyl group was challenged (1, 16, 19). Whether the S-AE concentration increase demonstrates the decreased transthelylation or the limited importance of this substance in the process of ethionine carcinogenesis still remains obscure. The possible effect of CuAc on transthelylation has not been studied, and this may be a promising subject for future investigation. Likewise, as in the case of some other inhibitors of ethionine carcinogenesis studied by Sidransky et al. (23), the protective effect of CuAc on tumor formation can be explained in general pathological terms by the increased toxicity of E-CuAc, which could change the reactivity of liver tissue to such an extent that it would result in a modification of the quality of the toxic effect.

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REFERENCES

10. Fare, G., and Howell, J. S. The Effect of Dietary Copper on Rat Carcinogenesis by 3-Methoxy Dyes. I. Tumors Induced at Various Sites by Feeding 3-Methoxy-4-aminoazobenzene and Its N-Methyl
Z. Brada et al.

The Effect of Cupric Acetate on Ethionine Metabolism

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