Sex Difference in Ethionine Inhibition of Hepatic Protein Synthesis*†

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Ethionine, an ethyl analogue of methionine, was previously reported to induce a fatty liver in female but not in male rats (2, 3). This sex difference is probably a function, at least in part, of the presence of androgens in the male, since orchidectomy makes the male susceptible and the administration of androgens protects intact or ovariectomized females (3, 4). Ovariectomy or estrogen administration are without effect.

Ethionine also inhibits protein synthesis in the liver of rats, as evidenced by the inhibition of: (a) the incorporation in vitro of radioactive methionine and glycine into total liver protein (5); (b) the increase in tryptophan peroxidase activity in response to injected tryptophan (6); (c) the increase in xanthine oxidase after repeated injections of xanthine (7) and during repletion after the feeding of a protein-free diet (8); (d) the increase in threonine dehydrase in response to added threonine in the perfused liver (9); (e) the increase in 4-dimethylaminobenzene demethylase or reductase in response to injected methylcholanthrene (10); and (f) the increase in benzpyrene hydroxylase in response to injected benzpyrene (11). These results were observed in mature female or immature male rats.

In view of the sex difference in the induction of fatty liver by ethionine, it was of interest to determine whether a similar sex difference could be demonstrated in the effect of ethionine on hepatic protein metabolism. Three different methods of studying protein synthesis were used, namely, the incorporation in vitro of radioactive amino acids into total liver and plasma protein, the hepatic level of the labile enzyme system tryptophan peroxidase, and the incorporation of radioactive leucine into the protein of a liver microsome system in vitro. With all three methods it has been found that the administration of ethionine produces an inhibition of protein synthesis in the liver of female but not of male rats.

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1-C\textsuperscript{14}, (Isotopes Specialties Company), L-valine\textsuperscript{1} (randomly labeled with C\textsuperscript{14}), and glycine-2-C\textsuperscript{14}. The radioactive materials were obtained on allocation from the United States Atomic Energy Commission.

Tryptophan Peroxidase Studies—The animals were given food and water throughout the experiment. At zero time and at 1 hour they were given, by injection, one-half the dose of ethionine, ethionine-methionine, methionine, or saline. The animals were killed by a blow on the head at 5 hours. In one experiment the animals were given the total dose of ethionine or saline by injection at zero time, and some were killed at 1 hour and some at 3 hours. In another experiment the animals were treated by injection at zero time and at 1 hour, and were killed 12 hours later. The liver was rapidly removed, chilled, and weighed, and aliquots were homogenized with 7 volumes of cold 0.14 M KCl in a Potter-Elvehjem homogenizer. The homogenate was assayed for tryptophan peroxidase at two levels of tissue concentration by the method of Knox and Auerbach (13). In early trial experiments it was found that the length of homogenization of the liver was an important variable. In all subsequent experiments the liver was ground in the same homogenizer for a standard period of time (3 minutes). The results are expressed in both concentration (pmoles of kynurenine/gm. of wet liver/hour) and total activity (pmoles of kynurenine/liver/100 gm. of body weight/hour).

Amino Acid Incorporation in Vitro—The incorporation of L-leucine-C\textsuperscript{14} into protein in cell-free preparations of liver was measured by the method of Keller and Zamecnik (14), with minor modifications. In most experiments one experimental and one control animal were observed simultaneously. However, in a few experiments two experimental and two control rats were used. The animals were fasted overnight, treated by injection with one-half the dose of ethionine, ethionine-methionine, or saline at zero time and at 1 hour, and killed by a blow on the head at 5 hours. The liver was rapidly removed and chilled. In most experiments 3 gm. of liver were homogenized for 30 seconds in 2.3 volumes of cold Medium A of Keller and Zamecnik (14). The homogenate was centrifuged at 15,000 × g for 10 minutes in a Spinco model L preparative ultracentrifuge with No. 40 head. The first supernatant, excluding the fluffy coat, was carefully withdrawn with a needle and syringe and was diluted with 6 volumes of cold Medium B of Keller and Zamecnik, instead of with 3 volumes as recommended. This first supernatant was free of nuclei, mitochondria, and larger cell fragments but still contained microsomes in suspension. The purpose of the additional dilution was to obtain “cleaner” microsomes with over incorporating ability in the absence of added supernatant (see below). Previous experiments with 3 volumes of diluent frequently showed no enhancement of incorporation on addition of supernatant. With 6 volumes this was found less frequently but still occurred, especially in microsome preparations from male animals.

The diluted first supernatant was centrifuged at 100,000 × g (40,000 r.p.m.) for 1 hour. The supernatant from this centrifugation (final supernatant) was carefully withdrawn and the tube containing the sedimented microsomal pellet was carefully rinsed three times with 1 ml. portions of Medium B. The microsomes were suspended with the aid of a loose fitting, motor-driven, glass pestle in 1 volume of Medium A for every gm. of original liver. To each 10 ml. of the final supernatant was added from 0.8 to 0.85 ml. of acetate buffer, 0.06 M, pH 3.8, to give a final pH of 5.1 to 5.3. The resulting precipitate, called the pH 5 precipitate, was separated by centrifugation at 15,000 × g for 10 minutes, resuspended in Medium B, and recentrifuged. The precipitate was finally suspended in 2 volumes of Medium A for every gm. of original liver. Much of the precipitate appeared to dissolve, although some insoluble material always remained. All solutions were kept cold during the entire preparation. From 25 to 30 mg. of microsomal protein and 12 to 15 mg. of pH 5 precipitate protein was obtained from 3 gm. of liver. No consistent differences were found in the amounts of these fractions from the control and ethionine-treated animals.

Incubation flasks were set up containing the following materials: L-leucine-C\textsuperscript{14} (randomly labeled, Nuclear Instrument and Chemical Corporation), 6.1 × 10\textsuperscript{-8} to 9.2 × 10\textsuperscript{-9} pmoles, containing 80,000 to 100,000 c.p.m. (measured); potassium fructose-1, 6-diphosphate, 10 pmoles; ATP, 1 pmoles; guanosine di- or triphosphate, 0.25 pmoles; DPN, 0.5 pmoles; a dialyzed aqueous extract of an acetone powder of rabbit muscle extract (10 mg./ml.), 0.2 ml.; microsomes, 0.2 ml. (containing about 1.5 mg. protein); and pH 5 precipitate, 0.3 ml. (containing about 0.5 to 0.7 mg. protein); in a final volume of 1.0 ml. The incubation was carried out in 20 ml. beakers in the Dubnoff metabolic shaker under an atmosphere of 95 per cent N\textsubscript{2}-5 per cent CO\textsubscript{2} for 10 minutes. The reaction was stopped by washing the beaker contents into 10 per cent trichloroacetic acid to give a final trichloroacetic acid concentration of 5 per cent. Unincubated zero time controls were run in each experiment.

The resulting precipitate was washed four times with 4 per cent trichloroacetic acid (the last two washes containing 5 mg. each of nonradioactive carrier L-leucine), and twice with warm ethanol-ethyl ether (3:1). The precipitate was then extracted three times with hot 10 per cent trichloroacetic acid, twice with warm ethanol-ethyl ether mixture (3:1), and twice with ethyl ether. The precipitate was plated on filter paper (Whatman No. 50) and counted in a windowless flow counter to a precision of 3 per cent. The amount of protein on the plates was determined by the method of Lowry et al. (12). The values were corrected for self-absorption by an empiric curve whenever this was necessary because of significant differences in the amount of protein present.

Total Liver Protein—Liver protein content was measured by precipitating and homogenizing a suitable aliquot with trichloroacetic acid, washing once with 4 per cent trichloroacetic acid, twice with warm ethanol-ethyl ether (3:1), and once with ethyl ether. After solution in NaOH, protein was determined by the method of Lowry et al. (12), with the use of similarly prepared liver protein as a standard.

RESULTS

Amino Acid Incorporation in Vitro—The incorporation of labeled leucine and valine into total liver and plasma protein was definitely diminished by ethionine administration in female rats (Table I). These results confirm previous observations with radioactive methionine (S\textsuperscript{35}) and glycine (5). In contrast, mature male rats given ethionine showed very slight or no inhibition of incorporation of leucine, valine, or glycine into liver or plasma protein (Table I).

However, interpretation of data on amino acid incorporation in intact animals is subject to many uncertainties. Therefore,
it was considered desirable to see if other methods would show a similar sex difference in the effects of ethionine upon hepatic protein metabolism. The labile hepatic enzyme system tryptophan peroxidase was one system chosen for this purpose.

**Tryptophan Peroxidase Studies**—In preliminary experiments an attempt was made to compare the increment in the activity of liver tryptophan peroxidase after the injection of tryptophan in ethionine-treated and control rats. The results confirmed those of Lee and Williams (6), who reported that pretreatment of female rats with ethionine partially inhibited the rise in tryptophan peroxidase activity that normally follows the injection of tryptophan. However, the findings in these experiments were not readily interpretable because ethionine diminished the level of the enzyme in animals not receiving tryptophan by injection. Therefore, the relative magnitude of the response of the experimental and control animals to administered tryptophan could not be validly compared.

Further work showed that a decrease in the basal level of hepatic tryptophan peroxidase activity was consistently produced in female rats by the administration of ethionine (Table II). When expressed both in concentration and total activity the basal level was about 58 per cent less in ethionine-treated females than in controls within 5 hours after the ethionine administration. In contrast, the ethionine-treated male rats had the same activity of the enzyme as did the control males (Table II). Incidentally, it was observed that in untreated animals the liver of males had more tryptophan peroxidase activity than that of females, whether the activity was expressed in concentration or in total quantity per liver corrected to constamt body weight. It was also found that the fall in enzyme activity induced by ethionine administration in females could be prevented by methionine, administered simultaneously with the ethionine. In fact the administration of ethionine and methionine in combination resulted in a large increase in enzyme activity over the control value (Table II). Methionine alone appeared to have much less effect. This finding is similar to the previous observation that the incorporation of radioactive glycine into liver protein in mice was greater after simultaneous methionine-ethionine administration than in controls given water or methionine alone (5).

The decreased enzyme activity in the ethionine-treated females could be due to a direct inhibitory effect of ethionine upon the enzyme, the loss of a normal activator, the production of an enzyme inhibitor, the interference with enzymes such as kynurenine formamidase or catalase which could affect the assay of tryptophan peroxidase (15), or to a decrease in enzyme protein. Several experiments were performed to test these possibilities. The direct effect of ethionine upon the activity of the enzyme in liver homogenates from normal animals was first determined. The addition of increasing amounts of ethionine up to 1.84 × 10⁻³ mmoles, containing 2.3 × 10⁵ c.p.m. (measured).

### Table I

<table>
<thead>
<tr>
<th>Radioactive amino acid administered, group, and sex</th>
<th>Specific activity of total protein</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>c.p.m./mg.</td>
<td>c.p.m./mg.</td>
</tr>
<tr>
<td></td>
<td>Difference from control%</td>
<td>Difference from control%</td>
</tr>
<tr>
<td>DL-Leucine-1-C-14*</td>
<td>51 ± 4†</td>
<td>78 ± 2†</td>
</tr>
<tr>
<td>Ethionine† (F)</td>
<td>40 ± 2</td>
<td>-22</td>
</tr>
<tr>
<td>L-Valine-C-14§</td>
<td>37 ± 2</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>Ethionine (F)</td>
<td>27 ± 1</td>
<td>-27</td>
</tr>
<tr>
<td>Glycine-C-14§†</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td>Ethionine (F)</td>
<td>31.6</td>
<td>-40</td>
</tr>
<tr>
<td>Ethionine (F)</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Control (F)</td>
<td>29.7</td>
<td>-33</td>
</tr>
<tr>
<td>Ethionine (F)</td>
<td>55.1</td>
<td></td>
</tr>
<tr>
<td>Control (F)</td>
<td>45.9</td>
<td>-17</td>
</tr>
<tr>
<td>DL-Leucine-1-C-14*</td>
<td>60 ± 3</td>
<td></td>
</tr>
<tr>
<td>Ethionine (M)</td>
<td>60 ± 5</td>
<td>0</td>
</tr>
<tr>
<td>L-Valine-C-14§</td>
<td>24 ± 1</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>Ethionine (M)</td>
<td>23 ± 1</td>
<td>-4</td>
</tr>
<tr>
<td>Glycine-C-2-C-14∥</td>
<td>22 ± 2</td>
<td>-28</td>
</tr>
<tr>
<td>Ethionine (M)</td>
<td>21 ± 1</td>
<td>-5</td>
</tr>
</tbody>
</table>

* 7.4 × 10⁻³ mmoles, containing 2.3 × 10⁵ c.p.m. (measured).
† Mean of the results from three animals ± standard error of the mean.
‡ See the text for dosage.
§ 15.8 × 10⁻³ mmoles, randomly labeled, containing 9.6 × 10⁴ c.p.m. (measured).
¶ Data of Simpson et al. (5).
∥ 0.27 × 10⁻³ mmoles, containing 2.78 × 10⁴ c.p.m. (measured).

The decreased enzyme activity in the ethionine-treated females could be due to a direct inhibitory effect of ethionine upon the enzyme, the loss of a normal activator, the production of an enzyme inhibitor, the interference with enzymes such as kynurenine formamidase or catalase which could affect the assay of tryptophan peroxidase (15), or to a decrease in enzyme protein. Several experiments were performed to test these possibilities. The direct effect of ethionine upon the activity of the enzyme in liver homogenates from normal animals was first determined. The addition of increasing amounts of ethionine up to 1.84 × 10⁻³ mmoles, containing 2.3 × 10⁵ c.p.m. (measured).

In a few experiments in which female animals were killed at different time intervals after the administration of ethionine or saline, the following per cent differences between the enzyme activity of experimental and control animals were found: 1 hour, 0 per cent; 3 hours, -43 per cent; and 12 hours, -82 per cent.

**Amino Acid Incorporation in Vitro**—In order to study the mechanism of action of ethionine upon hepatic protein metabolism, a system was required which would show in vitro the same sex difference as was observed in intact rats. In an attempt to find this, the liver microsome-supernatant system of Zamcnik and Keller (14, 16), capable of incorporating radioactive amino acids into protein, was first tested. In preliminary experiments the reported requirements of the system for ATP, guanosine di- or triphosphate, and an energy-generating system

* J. Botero and E. Farber, unpublished results.
Effect of ethionine and methionine administration to male and female rats upon the tryptophan peroxidase activity of the liver

**Table II**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Sex</th>
<th>Number of animals</th>
<th>Liver tryptophan peroxidase activity</th>
<th>Difference from control*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>moles kyurenine/gm. wet liver/hr.</td>
<td>moles kyurenine/liver/100 gm. body weight/hr.</td>
</tr>
<tr>
<td>Control...</td>
<td>F</td>
<td>21</td>
<td>2.1 ± 0.2</td>
<td>7.8 ± 0.6</td>
</tr>
<tr>
<td>Ethionine...</td>
<td>F</td>
<td>22</td>
<td>0.9 ± 0.1</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Control...</td>
<td>M</td>
<td>10</td>
<td>3.1 ± 0.3</td>
<td>11.7 ± 1.1</td>
</tr>
<tr>
<td>Ethionine...</td>
<td>M</td>
<td>10</td>
<td>3.0 ± 0.3</td>
<td>11.2 ± 1.4</td>
</tr>
<tr>
<td>Ethionine-methionine...</td>
<td>F</td>
<td>4</td>
<td>2.1 ± 0.1</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>Control...</td>
<td>F</td>
<td>2</td>
<td>2.0 ± 0.3</td>
<td>8.6 ± 1.4</td>
</tr>
<tr>
<td>Methionine...</td>
<td>F</td>
<td>2</td>
<td>2.7 ± 0.6</td>
<td>10.9 ± 1.4</td>
</tr>
</tbody>
</table>

* See the text for dosage.
† Based upon results expressed/liver/100 gm. of body weight.
‡ Mean ± standard error of the mean.
§ P < 0.01 (highly significant).
¶ P > 0.05 (not significant).
|| P < 0.05 (significant).

**Table III**

Incorporation of radioactive L-leucine into the protein of liver microsomal system from ethionine-treated and control female and male rats

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of animals</th>
<th>Origin of liver microsomes and supernatant*</th>
<th>Specific activity of protein**</th>
<th>Difference from control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>12</td>
<td>MC + SC</td>
<td>24 ± 3</td>
<td>-76</td>
</tr>
<tr>
<td>F</td>
<td>12</td>
<td>ME + SE</td>
<td>91 ± 4</td>
<td>-9</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>MC + SE</td>
<td>25 ± 5</td>
<td>-75</td>
</tr>
<tr>
<td>M</td>
<td>11</td>
<td>MC + SC</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>11</td>
<td>ME + SC</td>
<td>95 ± 6</td>
<td>-5</td>
</tr>
<tr>
<td>M</td>
<td>8</td>
<td>ME + SC</td>
<td>115 ± 6</td>
<td>+15</td>
</tr>
<tr>
<td>M</td>
<td>9</td>
<td>ME + SC</td>
<td>85 ± 10</td>
<td>-15</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>MC + SC</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>ME + SC</td>
<td>35</td>
<td>-65</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>MEM + SC</td>
<td>101</td>
<td>+1</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>MM + SC</td>
<td>104</td>
<td>+4</td>
</tr>
</tbody>
</table>

* MC = microsomes from control rats; ME = microsomes from ethionine-treated rats; MEM = microsomes from ethionine-methionine-treated rats; MM = microsomes from methionine-treated rats; SC = pH 5 precipitate from the supernatant fraction from control rats; SE = pH 5 precipitate from the supernatant fraction from ethionine-treated rats (see the text for dosage). See the text for the composition of the incubation medium.
† In each experiment the specific activity of the microsome-supernatant system from the control rats is considered as 100 and the specific activity of each of the other systems is expressed relative to the control values. The values are the means from all the animals for the system ± the standard error of the mean.

The decrease in incorporation in preparations from ethionine-treated females could be due to consistent differences in the amount of preformed tissue leucine diluting the added labeled leucine. Increases in the levels of free amino acids in the livers of ethionine-treated female and male rats have been reported (17, 18). In order to test this possible explanation, the effect of adding different amounts of radioactive leucine upon incorporation was determined in several experiments. In all instances, increasing the level of the labeled leucine from 1 to 3 μg had no effect upon either the absolute amount of incorporation or the relative amount in preparations from ethionine-treated females as compared to that from controls. These results indicate that an optimal amount of leucine was used in the experiments and that the large decrease in incorporation in the system from the experimental females is not the result merely of increased dilution of the added radioactive leucine.

It is apparent from the results presented in Table III that, in the liver amino acid incorporation system from female rats, the extent of incorporation into protein is mainly a function of the source of the microsome fraction and not of the supernatant fraction. For example, the incorporation is consistently low when microsomes from ethionine-treated females are used, regardless of the origin of the supernatant fraction. In like manner, with microsomes prepared from control females, the incorporation is essentially the same with the pH 5 precipitate from the supernatant fraction of either control or experimental females. From these results it would seem that one important biochemical lesion affecting the incorporation in vitro of radioactive leucine into protein in the liver of ethionine-treated females is localized in the microsomes. However, since some incorporation always occurs into microsomal protein in the absence of added pH 5 precipitate from the supernatant fraction, the microsome fraction may very well be contaminated by components from the supernatant fraction. The localization of one defect to the microsomes is therefore at best tentative.

In contrast to the results with females, the incorporation of leucine into protein was equal in preparations from ethionine-
treated and control males (Table III). However, in the male the relative incorporation varied with the origin of the supernatant fraction. With the supernatant fraction from ethionine-treated males, the uptake into protein in preparations from both control and ethionine-treated animals was greater than with the supernatant fraction from control animals. Unfortunately, in these experiments, the enhancement of incorporation by the addition of supernatant was not consistently found. In those experiments in which the enhancement was present, the incorporation in the presence of supernatant from ethionine-treated males was 30 to 40 per cent larger than that with supernatant from controls. The results in Table III are the mean values of these experiments and therefore show only a 15 per cent increase in incorporation.

In one experiment, the injection of methionine along with ethionine completely protected against the inhibitory effect of ethionine administration on the activity of female microsomes (Table III). The administration of methionine alone had no obvious effect on incorporation. Methionine added in vitro to preparations from ethionine-treated females did not alter the low level of incorporation. In agreement with the results of Zamecnik and Keller (16), the addition of \( \text{L-ethionine in vitro} \) had no inhibitory effect upon the incorporation of leucine in preparations from control females.

**Protein Content of Liver**—In view of the deleterious effects of ethionine upon protein metabolism in female rats, it was of interest to determine whether any differences between the total liver protein of the ethionine-treated and control females would be observed within the same time intervals as were used in the above experiments. In fed animals, the liver protein in mg./liver/100 gm. of body weight was 442 ± 16 in controls and 443 ± 1 in ethionine-treated animals. In fasted animals the corresponding values were 392 ± 4 in controls and 341 ± 10 in ethionine-treated females. Thus, under both nutritional conditions, no significant differences were found in the values of total liver protein in experimental and control rats.

**DISCUSSION**

It is obvious from the results of this study that ethionine, when administered to intact rats, has different effects upon hepatic protein metabolism in males and in females. In the ethionine-treated females there is a decrease in the uptake of some radioactive amino acids into liver and plasma protein in vivo, in the basal level of liver tryptophan peroxidase, and in the incorporation in vitro of radioactive leucine into the protein of the liver microsome-supernatant system of Keller and Zamecnik (14). In contrast, ethionine has none of these effects in the male.

This sex difference corresponds to that observed in the induction of fatty liver in rats by ethionine (2, 3). However, no comparable sex difference has been demonstrated in other biochemical and morphological effects of ethionine, such as (a) inhibition of transmethylation from methionine to choline in vivo (19, 20); (b) inhibition of the oxidation of the methyl group of methionine to formate (21); (c) elevation of the level of free amino acids in the liver (17, 18); (d) inhibition of hepatic choline oxidase (22); (e) increase in pancreatic protein synthesis (23); and (f) production of pancreatic acinar degeneration (24, 25).

The sex difference in the ethionine-induced fatty liver is probably a function, at least in part, of the presence of androgens in the male, since orchidectomy makes the male susceptible and the administration of testosterone propionate or of other androgens protects intact or ovariectomized females (3, 4). On the other hand, ovariectomy or the administration of estrogens to castrate females is without effect upon the production of fatty liver by ethionine (3, 4). In view of the correlation between the occurrence of the fatty liver and the inhibition of hepatic protein synthesis, it is probable that the sex difference in the effects of ethionine upon hepatic protein metabolism is also mediated by the presence of androgens in the male.

The exact mechanism of action of ethionine upon protein metabolism, as well as upon other metabolic systems, is not clear. From the available evidence, it is probable that the effects of ethionine are in some way related to the metabolism of methionine. The administration of adequate amounts of methionine has been found to protect animals against most of the effects of ethionine (2, 5, 6, 10, 11, 21, 23, 24, 26), including the long term carcinogenic effects on the liver (27). It has been found that this protective action is dependent upon the continuous presence of sufficient amounts of methionine in such a manner as to suggest an intimate interaction between the analogue and the natural amino acid (2, 24). No protective action has been demonstrated for certain other amino acids (2, 5, 24) or for choline in acute experiments (2, 24). Also, some of the effects of ethionine have recently been reproduced by dietary methionine deficiency. When rats were force-fed methionine-free diets that were adequate in choline, they developed a fatty liver with the same sex difference and lobular distribution pattern of the excess lipide as is seen with ethionine (28).

However, the nature of the interaction of ethionine and methionine, especially in regard to protein metabolism, is problematical. Since the results obtained on incorporation in vivo of leucine into protein in the microsome-supernatant system appear to parallel those obtained in vitro, with regard to the effects of ethionine on hepatic protein metabolism, the further study of the action of ethionine upon the suggested components (29) of the system in vitro may give new insight into the mechanism of this interaction of ethionine with methionine.

**SUMMARY**

1. The effect of the administration of ethionine upon protein metabolism in the liver of female and male rats was investigated by three different methods: (a) incorporation of radioactive amino acids into total liver and plasma protein in vivo; (b) the basal level of tryptophan peroxidase activity; and (c) the incorporation in vitro of radioactive leucine into the protein of the microsome-supernatant system of Zamecnik and coworkers.

2. With all three methods, it was found that ethionine administration inhibits protein synthesis in the liver of female rats but not of male rats. This sex difference coincides with that previously described for the induction of fatty liver by ethionine. Since the sex difference in the production of fatty liver is a function, at least in part, of the presence of androgens in the male, it is probable that the sex difference in the effects of ethionine upon hepatic protein metabolism is also mediated by androgens.

3. The administration of methionine was found to counteract the inhibitory effects of ethionine upon the activity of liver tryptophan peroxidase and upon the incorporation in vitro of leucine into protein.

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\footnote{R. Levy and E. Farber, unpublished results.
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Sex Difference in Ethionine Inhibition of Hepatic Protein Synthesis
Emmanuel Farber, Magruder S. Corban and With the technical assistance of Arthur Castillo


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