EFFECT OF CHOLINE AND CYSTINE ON THE OXIDATION OF THE METHYL GROUP OF METHIONINE*

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(Received for publication, October 31, 1951)

Several years ago it was reported from this laboratory that the methyl group of methionine is oxidized to carbon dioxide in the animal body (1). Subsequent experiments showed that the rate of oxidation of the methyl group of dietary methionine is markedly increased by increasing the level of methionine in the diet (2). Since methionine is known to be a source of the methyl group of choline (3, 4) and of the sulfur of cystine (5, 6), we have studied the effect of these compounds on the rate of oxidation of the methyl group of dietary methionine.

In the present investigation the basal diet was identical with the 0.6 per cent methionine diet used in our earlier studies (2), except that choline and cystine were omitted. This level of methionine is equivalent to that provided by a 17 per cent casein ration. Under these conditions it should be noted that methionine is supplying the entire sulfur requirement and is the sole source of exogenous labile methyl groups. Young male rats were fed either the basal diet, the basal diet plus choline, or the basal diet plus choline and cystine for a period of 10 days. At the end of this time each rat received by stomach tube a single 2 gm. portion of its respective diet in which radioactive methionine replaced the ordinary methionine previously fed. The animal was then placed in a metabolism apparatus, and the C\textsuperscript{14} eliminated in the expired carbon dioxide, urine, and feces during the next 24 hours was determined. During this time the rat had continuous access to the non-isotopic diet. Growth on all of the diets was good (Table I), and none of the rats had fatty livers or gross signs of kidney damage.

In rats fed the basal diet alone approximately 3.5 per cent of the methyl groups ingested as methionine were converted to carbon dioxide in 24 hours. As is shown in Fig. 1, the oxidation of the radiomethyl group fell into two phases, the first characterized by a rise and fall in the rate of oxidation, and the second by the establishment of a relatively steady state. The terms “period of assimilation” and “period of equilibrium” have been used to indicate these two phases (2).

* The authors wish to express their appreciation to the Lederle Laboratories Division, American Cyanamid Company, for a research grant that has aided greatly in these experiments.
The addition of choline to the diet had a striking effect on the oxidation of the methyl groups of methionine. 0.2 per cent of choline chloride produced a 3-fold increase in the quantity of radiomethionine methyl groups converted to carbon dioxide in 24 hours (Table II). By far the greatest increase in the rate of oxidation of methionine methyl occurred during the period of assimilation (Fig. 1). There was a 10-fold increase in the rate of oxidation during the 1st hour following the ingestion of the radioactive meal.

![Graph showing the rate of oxidation to CO₂ of the methyl group of methionine ingested in a single 2 gm. portion of diet containing 0.6 per cent methionine. The diets containing L-methionine labeled with C₁⁴ in the methyl group were administered by stomach tube to 150 gm. male rats, which, before and after this treatment, had continuous access to the corresponding diets containing ordinary methionine.](http://www.jbc.org/)

When, in addition to choline, 0.4 per cent of L-cystine was added to the diet, the rate of oxidation during the period of assimilation was restored to the level that prevailed on the methionine basal diet (Fig. 1). In an experiment in which cystine alone was added to the basal diet, there was no decrease in the rate of oxidation of the methyl group of methionine (Table II).

The addition to the diet of choline or cystine or of choline and cystine had little or no effect on the per cent of radiomethyl carbon excreted in the urine or feces in 24 hours (Table II). Consequently the shifts in the rate of oxidation of the exogenous methyl group that were produced by altering the diet were not due to changes in the rate of excretion of the methionine methyl group or its oxidation products.
It may be instructive to view the experimental results from the standpoint of withdrawing cystine from the diet containing methionine plus.

**Table I**

**Growth and Liver Fat**

The basal diet contained 0.6 per cent methionine. Choline chloride and L-cystine were added at levels of 0.2 and 0.4 per cent, respectively. Male rats weighing approximately 120 gm. were maintained on the diets for 2.5 weeks.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Supplement to basal diet</th>
<th>Average gain per day (gm.)</th>
<th>Per cent liver fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>None</td>
<td>3.4</td>
<td>5.8</td>
</tr>
<tr>
<td>5</td>
<td>Choline</td>
<td>3.7</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>Cystine</td>
<td>3.6</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>Choline + cystine</td>
<td>3.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Table II**

**Effect of Choline and Cystine on Oxidation and Excretion of Methyl Group of Dietary Methionine**

The basal diet contained 0.6 per cent methionine. Choline chloride and L-cystine were added at levels of 0.2 and 0.4 per cent, respectively. Male rats (150 gm.) were fed by stomach tube a single 2 gm. portion of diet in which L-methionine labeled with C¹⁴ in the methyl group replaced the ordinary methionine otherwise employed.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Radioactive methionine ingested (mg.)</th>
<th>Supplement to basal diet</th>
<th>Per cent C¹⁴ oxidized to CO₂</th>
<th>Per cent C¹⁴ excreted* in 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>6-12 hrs.</td>
<td>12-24 hrs.</td>
</tr>
<tr>
<td>260</td>
<td>12.8</td>
<td>None</td>
<td>2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>395</td>
<td>12.0</td>
<td>&quot;</td>
<td>2.3</td>
<td>0.6</td>
</tr>
<tr>
<td>442</td>
<td>12.4</td>
<td>&quot;</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>255</td>
<td>12.4</td>
<td>Choline</td>
<td>10.8</td>
<td>1.4</td>
</tr>
<tr>
<td>322</td>
<td>12.1</td>
<td>&quot;</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>426</td>
<td>12.4</td>
<td>&quot;</td>
<td>7.6</td>
<td>1.3</td>
</tr>
<tr>
<td>3498†</td>
<td>11.9</td>
<td>&quot; and cystine</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>3592†</td>
<td>12.3</td>
<td>&quot;</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>195</td>
<td>12.3</td>
<td>Cystine</td>
<td>4.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Not all of the excreted C¹⁴ is present as methyl groups; a portion represents their oxidation products.
† Rats used in concomitant experiments on the effect of the level of methionine on the oxidation rate.

choline and cystine. From this standpoint it becomes obvious that the absence of cystine resulted in a great increase in the oxidation of the methyl groups. Then the further withdrawal of choline, making methionine the
sole source of exogenous labile methyl groups and sulfur amino acids, resulted in a decrease in the oxidation of the methyl groups.

EXPERIMENTAL

Materials and Methods

The percentage composition of the basal diet was as follows: sucrose 55.4, Covo 19.0, corn oil 1.0, amino acid mixture 20.0 (2), salt mixture 4.0 (2), methionine 0.6, and vitamin mixture. The composition of the vitamin mixture in mg. or units per kilo of diet was as follows: thiamine hydrochloride 10, riboflavin 10, pyridoxine hydrochloride 10, nicotinic acid 10, calcium pantothenate 50, inositol 100, p-aminobenzoic acid 10, folic acid 1, biotin 0.1, α-tocopherol acetate 40, 2-methyl-1,4-naphthoquinone 1, vitamin A 7200 units, vitamin D 1200 units. Supplements of choline chloride and l-cystine were added at levels of 0.2 and 0.4 per cent, respectively. These supplements replaced an equal weight of sucrose.

Young male rats of the Rockland Farms strain, weighing 115 to 130 gm., were fed the experimental diets for 2.5 weeks. The growth of animals fed the diet devoid of choline and cystine compared favorably with the growth of animals fed the diet supplemented with these compounds (Table I). At the end of 2.5 weeks the fat content of the livers of the unsupplemented rats was 5.8 ± 0.6 per cent, a value somewhat higher than that found in rats receiving choline or cystine or both (Table I) but within the "normal" range for the rat (7). Liver fat was determined by thoroughly extracting the homogenized liver with hot alcohol and ether and freeing these extracts of ether-insoluble material.

The rats used in the metabolism experiments weighed between 150 and 153 gm. They were selected from groups of animals fed one of the experimental diets for 10 days. Each animal was given by stomach tube a 2 gm. portion of diet (2) in which 12 mg. of L-methionine labeled with C¹⁴ in the methyl group (8) replaced the non-isotopic methionine. The barium carbonate from 1 mg. of the radiomethionine gave 1.25 × 10⁶ c.p.m., corrected for self absorption.

After the administration of the "radioactive meal" the animal was immediately placed in a metabolism apparatus. The apparatus for the collection of expired CO₂, urine, and feces, and the methods for determining the C¹⁴ content of these materials have been described in an earlier publication (1). The C¹⁴ content of the expired air, urine, and feces has been expressed as per cent of the ingested radiomethyl group or as per cent of the ingested C¹⁴. Numerically these expressions are identical. The results of the metabolism experiments are summarized in Table II.

The authors wish to acknowledge the technical assistance of Mr. Harry L. Isrow.
SUMMARY

The relation of dietary choline and cystine to the rate of oxidation of the methyl group of radioactive dietary methionine to C\textsuperscript{14}O\textsubscript{2} has been studied in rats maintained on a 0.6 per cent methionine diet.

In the presence of choline the rate of oxidation of the methyl group of dietary methionine was increased. This increase was most pronounced during the first 6 hours following the ingestion of the radiomethionine, i.e. during the period of assimilation.

When cystine was added in addition to the choline, the rate of oxidation during the period of assimilation was restored to the level observed on the methionine basal diet.

In neither instance was there an appreciable change in the per cent of C\textsuperscript{14} excreted in the urine and feces in 24 hours.

BIBLIOGRAPHY

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