A STUDY OF THE RATE OF OXIDATION OF THE METHYL GROUP OF DIETARY METHIONINE*

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We have recently reported that the administration per os to a rat of a single 200 mg. dose of methionine labeled with C\(^{14}\) in the methyl group was followed by the appearance of one-quarter of the radioactive carbon in the expired carbon dioxide in 24 hours (1). This finding clearly demonstrated the ability of the animal organism to oxidize the biologically labile methyl group to carbon dioxide and water. It also posed the question as to the extent of oxidation of the smaller amounts of methionine methyl that are ingested in one feeding as a part of an average diet. Accordingly, a series of experiments was carried out in an attempt to answer this question.

The amino acid diets employed contained either 0.6 or 1.2 per cent methionine, levels of methionine equivalent to those provided by rations containing 17 and 34 per cent casein. These methionine levels covered the range most frequently encountered in adequate diets of both the purified and natural food types. Both of the diets contained 0.2 per cent choline chloride and 0.4 per cent cystine.

In studying the rate of oxidation of a dietary component in the whole animal with the use of isotopic carbon, it is desirable to determine not only the total amount oxidized during a given period of time, but also the actual changes in the rate of oxidation that occur within the same period, for the sharpness with which the oxidation curve rises and falls reflects the rapidity with which the animal is absorbing the exogenous material, destroying or excreting it, and integrating it with its endogenous counterparts already present in the body. When the experimental animal is given free access to the diet, the shape of the oxidation curve is difficult to interpret, since the amount of isotopic food ingested is not known or controlled for any time interval.

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Consequently, in the present study a single 2 gm. portion of the diet containing the radiomethionine was administered by stomach tube to rats which had free access, both before and after this treatment, to the same diet containing ordinary methionine. By determining the amount of radiocarbon dioxide in the expired air collected during hourly intervals following the ingestion of the "radioactive meal," the rise and fall in the rate of oxidation of the methyl group was obtained. The size of the "radioactive meal," approximately one-fifth of the daily food consumption, was dictated by the quantity of radiomethionine required to produce a concentration of radiocarbon dioxide in the expired air that could be measured accurately in the rats receiving the 0.6 per cent methionine diet.

In addition to measuring the per cent of the ingested methyl group converted to carbon dioxide and water, the amount of isotopic methyl carbon excreted in the urine and feces in 24 hours was determined. It was thus possible to compare the 24 hour elimination and retention of exogenous methyl carbon on the high and low methionine diets.

**Materials and Methods**

The l-methionine labeled with $^{14}$C in the methyl group that was used in these experiments had been previously synthesized in this laboratory by Melville, Rachele, and Keller (2). The barium carbonate prepared from 1 mg. of methionine gave approximately 125,000 c.p.m. when corrected for background and self-absorption.

The metabolism apparatus for the collection of expired $\text{CO}_2$, urine, and feces and the methods for determining the $^{14}$C content of these materials have been described in an earlier publication (1). Throughout this paper the $^{14}$C content of the urine, feces, and expired $\text{CO}_2$ is expressed as a per cent of the ingested methionine, methyl group, or $^{14}$C. Numerically, all three of these expressions are identical.

The composition of the 0.6 per cent methionine diet is shown in Table I. The 1.2 per cent methionine diet was the same except for the increase in methionine and the corresponding decrease in sucrose. The adequacy of the low methionine diet with respect to labile methyl groups and sulfur-containing amino acids was shown by the fact that male rats, weighing 130 gm., gained as rapidly in a 21 day period on this diet as on the 1.2 per cent methionine diet. At the end of this time the fat content of their livers was approximately 4 per cent.

In the metabolism experiments, young male rats, obtained from Rockland Farms, New City, New York, were fed one of the experimental diets containing non-radioactive L-methionine for a period of 6 to 10 days. When an animal weighed approximately 150 gm., it was given by stomach
tube, at 9.45 in the morning, a single 2 gm. portion of diet in which the ordinary methionine was replaced with L-methionine containing C\textsuperscript{14} in the methyl group.

The procedure employed in administering the radiomethionine diet was as follows. 400 mg. of the mixed fats (Covo and corn oil, Table I), previously chilled in the refrigerator, were fed to the rat on a small spatula. Next, a stomach tube, consisting of a piece of x-ray catheter with a hypodermic needle inserted in the proximal end, was introduced, and the non-

| Table I |
| Composition of 0.6 Per Cent Methionine Diet |
|-----------------|------------------|
| Sucrose          | 54.8             |
| Covo*            | 19.0             |
| Corn oil         | 1.0              |
| Salt mixture†    | 4.0              |
| Amino acid mixture‡ | 20.0          |
| Methionine       | 0.6              |
| L-Cystine        | 0.4              |
| Choline chloride | 0.2              |
| Vitamins§        |                  |

* A hydrogenated vegetable oil.
† Osborne-Mendel salt mixture, No. 1 (3), Eimer and Amend, New York.
‡ The proportion of individual amino acids per 185 parts of mixture is as follows: glycine 1, hydroxy-L-proline 1, L-proline 2, serine 2, L-aspartic acid 2, alanine 4, L-tryptophan 4, L-arginine hydrochloride 6, L-histidine-HCl-H\textsubscript{2}O 7, L-tyrosine 10, threonine 14, phenylalanine 15, isoleucine 18, valine 20, L-glutamic acid 20, leucine 26, L-lysine hydrochloride 19, NaHCO\textsubscript{3} 14.
§ The composition of the vitamin mixture in mg. or units per kilo of diet is 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.

lipide part of the diet (less methionine) suspended in 1.5 ml. of water was fed through the tube. This was followed immediately by the administration from a 1 ml. syringe of the radiomethionine (12 or 24 mg.) dissolved in 0.5 ml. of water. Finally, 0.5 ml. of water that had been used to wash out the methionine weighing bottle was given through the stomach tube. About 1 minute was required to give the non-lipide portion of the diet plus methionine.

After the stomach tube had been withdrawn, the animal was transferred at once to the metabolism apparatus and the urine, feces, and expired carbon dioxide were collected for the next 30 hours. During this time the
rat had continuous access to the diet containing non-radioactive methionine. No food was eaten during the day. Food consumption during the night was normal when the 2 gm. that had been given by stomach tube were taken into consideration.

**RESULTS AND DISCUSSION**

In the experiments reported in this paper, two rats were fed the 0.6 per cent methionine diet and four rats the 1.2 per cent methionine diet. In every case the oxidation of the methyl group of the labeled methionine began soon after the ingestion of the diet, as shown by the presence of C\textsubscript{14} in the carbon dioxide collected during the 1st hour. In two of the four animals receiving the high level of methionine the rate of oxidation reached its maximum in the 2nd hour and declined slightly during the 3rd hour. The other rats on this diet, as well as the two animals on the low methionine diet, attained their respective maximum rates of oxidation of the methyl group during the 3rd hour. After the 3rd hour the rate of oxidation in every animal declined sharply. Low and relatively constant rates of oxidation were reached at the 7th hour by the animals on the 0.6 per cent methionine diet, and at the 7th or 8th hour by the rats on the 1.2 per cent diet.

The average curves showing the rate of oxidation of the methyl groups of dietary methionine to carbon dioxide\textsuperscript{1} and water by the two sets of rats are given in Fig. 1. While differing considerably in magnitude, they possess the same general shape, and it is the significance of their shape with respect to the metabolism of the ingested methyl group that will be first discussed.

As indicated by the oxidation curves, the metabolism of the methyl group of dietary methionine falls into two phases; the first is characterized by a rapid rise and fall in the rate of oxidation, and the second by the establishment of a relatively steady state. For convenience the descriptive terms "period of assimilation" and "period of equilibrium" may be used to indicate these two phases. In the period of assimilation the rise

\textsuperscript{1} The sensitivity with which the oxidation curves of the methyl group mirror the actual rates of conversion of labeled methyl radicals to CO\textsubscript{2} in the body is indicated by the report of Gould and coworkers (4) that the maximum concentration of C\textsubscript{14}O\textsubscript{2} in the expired air occurs within 10 minutes after the injection of radioactive bicarbonate. The figures given in the present paper for the oxidation of the methyl group have not been corrected for the C\textsubscript{14}O\textsubscript{2} that was formed but subsequently entered into synthetic reactions in the body. According to Gould and coworkers (4) over 90 per cent of an injected dose of bicarbonate is eliminated as respiratory CO\textsubscript{2} in 4 hours. Since in our experiments the urine was collected in acid, any C\textsubscript{14}O\textsubscript{2} excreted as bicarbonate was collected along with the respiratory C\textsubscript{14}O\textsubscript{2}.
in the rate of oxidation is a measure of the rate of absorption of the exog-

METHIONINE DIET

-1.2 % METHIONINE DIET

---.6 % METHIONINE DIET

HOURS

FIG. 1. The rate of oxidation to CO₂ of the methyl group of methionine ingested in a single 2 gm. portion of diet. The diets containing L-methionine labeled with C¹⁴ in the methyl group were administered by stomach tube to 150 gm. male rats which, both before and after this treatment, had continuous access to the corresponding diets containing ordinary methionine. The oxidation curve for the 0.6 per cent methionine diet is an average of two experiments and the curve for the 1.2 per cent methionine diet is an average of four experiments.

translocations of the methyl group also contribute to the decline in the rate of oxidation.

The period of equilibrium was initiated by the establishment of a low and relatively constant rate of oxidation. This indicates that the major (most rapid) chemical conversions and anatomical translocations of the

2 *In vitro* studies in this laboratory have shown that the methyl group provided by methionine is oxidized by liver and kidney cortex, but not by heart muscle or testes. There is at present no evidence to indicate whether the initial step in the oxidation can occur while the methyl group is attached to the S of methionine, or whether it takes place only after the methyl group has been transferred to some other molecule.
radiomethyl group have occurred, and that it and its oxidation products have merged with their counterparts already present in the body. However, as we have previously shown (1), the concentration of the ingested methyl carbon in the tissues and organs is far from uniform even after 52 hours. Presumably a continuous slow redistribution of the radiomethyl group (and its oxidation products) follows this initial rapid but uneven distribution.

8 We have isolated radioformaldehyde from liver homogenates incubated with sarcosine labeled with C\textsuperscript{14} in the methyl group (5). Moreover, as we reported at the meeting of the American Society of Biological Chemists at Detroit, 1949, the presence of radioactive formic acid has been indicated by the evolution of C\textsuperscript{14}O\textsubscript{2} when the distillate from the formaldehyde-free homogenate is oxidized with HgCl\textsubscript{2}.

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**Fig. 2.** The effect of oral and intraperitoneal administration on the rate of oxidation to CO\textsubscript{2} of the methyl group of methionine labeled with C\textsuperscript{14} in the methyl radical. The oral dose was given by stomach tube as a component of 2 gm. of a 1.2 per cent methionine diet. The injected dose was given immediately after the administration of 2 gm. of a methionine-free diet. Before and after treatment both animals (150 gm. male rats) had continuous access to a diet containing 1.2 per cent of ordinary methionine.
In view of the prompt and rapid rise of oxidation during the period of assimilation, the possibility that the intestinal flora contributed significantly to the initial degradation of the exogenous methyl group was investigated. A rat on the 1.2 per cent methionine diet was fed 2 gm. of the ration without methionine and then injected intraperitoneally with 23.9 mg. of radiomethionine. The oxidation curve is shown in Fig. 2. The curve for Rat 3501 (see also Table II), fed 25.6 mg. of radiomethionine in

**Table II**

Oxidation and Excretion of Methyl Group of Dietary Methionine

The experimental diet contained 0.4 per cent cystine, 0.2 per cent choline chloride, and 0.6 or 1.2 per cent methionine. Male rats (150 gm.) were fed by stomach tube a single portion of diet in which L-methionine labeled with C\(^{14}\) in the methyl group replaced the ordinary methionine otherwise employed. The oxidation and excretion of the radiomethyl carbon was measured for 24 hours.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Radioactive methionine ingested</th>
<th>Per cent C(^{14}) oxidized to CO(_2)</th>
<th>Per cent C(^{14}) excreted*</th>
<th>Per cent C(^{14}) eliminated (A+B+C)</th>
<th>C(^{14}) retained†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>0-6 hrs. 6-12 hrs. 12-24 hrs. Total (A)</td>
<td>Urine (B)</td>
<td>Feces (C)</td>
<td>mg.</td>
</tr>
<tr>
<td>3498</td>
<td>11.9</td>
<td>1.5 1.0 2.6 5.1</td>
<td>3.70</td>
<td>0.32</td>
<td>9.12</td>
</tr>
<tr>
<td>3592</td>
<td>12.3</td>
<td>3.2 1.2 2.1 6.5</td>
<td>5.04</td>
<td>0.31</td>
<td>11.85</td>
</tr>
<tr>
<td>3500</td>
<td>24.2</td>
<td>23.4 2.1 3.3 28.8</td>
<td>4.02</td>
<td>0.29</td>
<td>27.01</td>
</tr>
<tr>
<td>3501</td>
<td>25.6</td>
<td>18.8 1.6 2.3 22.7</td>
<td>4.71</td>
<td>0.50</td>
<td>28.51</td>
</tr>
<tr>
<td>3507</td>
<td>24.4</td>
<td>17.6 2.4 3.3 23.3</td>
<td>4.91</td>
<td>0.34</td>
<td>31.45</td>
</tr>
<tr>
<td>389</td>
<td>25.3</td>
<td>21.6 2.0 2.8 26.4</td>
<td>4.71</td>
<td>0.34</td>
<td>31.45</td>
</tr>
<tr>
<td>3497‡</td>
<td>23.9</td>
<td>27.1 1.8 3.4 32.3</td>
<td>4.56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not all of the excreted C\(^{14}\) is present as methyl groups; a portion represents their oxidation products.
† Expressed as mg. of methionine.
‡ In this rat the radiomethionine was injected intraperitoneally immediately after the administration by stomach tube of 2 gm. of diet from which methionine was omitted.

The methionine content of the diet had a profound effect on the amount of dietary methyl groups converted to carbon dioxide. On the low methionine diet 6 per cent and on the high methionine diet 25 per cent of the ingested methyl carbon appeared in the expired air as radioactive carbon dioxide in 24 hours. This increase in the proportion of dietary methyl
groups oxidized daily was accomplished almost entirely by a great increase in the rate of oxidation during the period of assimilation, as is shown in Table II. Doubling the methionine content increased the rate of oxidation approximately 9-fold during the first 6 hours. On the other hand, the increase in the rate of oxidation during the period of equilibrium was relatively slight.

The relation between the previous methionine intake and the high rate of oxidation in the period of assimilation on the 1.2 per cent methionine diet was investigated. A rat which had been maintained on the 0.6 per cent methionine regimen was given a 2 gm. portion of the 1.2 per cent radiomethionine diet by stomach tube and then returned to the lower level of methionine. The rate of oxidation (Rat 389, Table II) was just as high in this animal as in those maintained on 1.2 per cent methionine for 7 days before treatment. Consequently, the ability to oxidize the methyl groups at a faster rate on the higher level of methionine was not due to an adaptive increase in the concentration of the oxidative enzymes.

Probably the ingestion of the 24 mg. dose of radiomethionine as contrasted to the 12 mg. dose resulted in the saturation of the synthetic enzymes, thus increasing the proportion of methyl groups available for destruction by the oxidative enzymes. From this point of view, the lower rate of oxidation in rats fed 0.6 per cent methionine was the result of competition for the ingested methyl groups between synthetic and degradative (oxidative) enzyme systems.

An appreciable quantity of the ingested methyl groups was eliminated in the urine in 24 hours in the form of methyl compounds or the oxidation products of methyl groups.\(^4\) On the 0.6 per cent methionine diet the amount of \(^{14}C\) present in the urine was nearly as high as the amount of \(^{14}C\) present in the expired carbon dioxide (Table II). Much smaller amounts were present in the feces. However, doubling the level of methionine had little effect on the per cent of exogenous methyl carbon excreted in either the urine or feces. This is in contrast with the considerable increase in the rate of oxidation during the period of assimilation produced by doubling the methionine intake, and it further emphasizes the contribution of oxidation, in this phase of metabolism, to the regulation of the methyl balance of the body.

Nevertheless, the higher rate of oxidation of the methyl group on the 1.2 per cent methionine diet fell short of reducing the amount of ingested

\(^4\) One oxidation product is the \(CO_2\) which has been used in the synthesis of urea. The carbon of urea possesses the same specific activity as the carbon of the expired \(CO_2\) (6). Since the amount of urea carbon equals about one-thirty-third of the carbon present in the expired \(CO_2\), the per cent of ingested \(^{14}C\) present as urea may be approximated by multiplying the per cent present as expired \(CO_2\) by 0.03.
methyl carbon retained by the body in 24 hours to the level that prevailed on 0.6 per cent methionine. The retention of C^{14} was 10.8 mg. (expressed as methionine) on the low methionine diet and 17.8 mg. on the high methionine diet (Table II).

Since both rations contained an adequate supply of labile methyl groups, and since the animals on both rations gained at the same rate, it does not seem likely that the increased retention of C^{14} was due to an over-all increase in the storage of methyl groups or their oxidation products. More probably, the ingestion of the high methionine diet increased the oxidation and excretion of non-radioactive methyl groups already present in the body, thus increasing the rate of turnover. Also, raising the methionine level may have increased the oxidation of the methyl groups simultaneously consumed in the form of choline. In either event the retention of newly ingested methyl groups would be increased without appreciably changing the concentration of total methyl groups in the body.

SUMMARY

The rate of oxidation of the methyl group of dietary methionine has been measured in rats fed diets containing choline and cystine, and either 0.6 or 1.2 per cent methionine. This was accomplished by determining the amount of radiocarbon dioxide expired following the ingestion of a single meal in which C^{14}-labeled methionine replaced the ordinary methionine otherwise present in the diet.

On both diets the oxidation of the radiomethyl group fell into two phases. During the first phase, or period of assimilation, there was a rapid rise in the rate of oxidation to a maximum attained during the 3rd hour, followed by a decline until the 7th or 8th hour. The second phase, or period of equilibrium, was initiated at this time by the establishment of a low and relatively constant rate of oxidation.

Doubling the methionine content of the diet produced a 9-fold increase in the per cent of radiomethyl groups oxidized during the period of assimilation. It had relatively little effect on the rate of oxidation during the period of equilibrium, or on the per cent of radiomethyl carbon excreted in the urine or feces in 24 hours.

The implications of these results are discussed.

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