OXIDATION IN VIVO OF THE METHYL GROUPS OF CHOLINE, BETAINE, DIMETHYLTHETIN, AND DIMETHYL-β-PROPIOTHETIN*

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(Received for publication, February 11, 1950)

It has recently been demonstrated in this laboratory (1–3) that “biologically labile” methyl groups can be oxidized to CO₂ in the rat. This has been established by feeding methionine containing radioactive carbon in the methyl group and demonstrating the presence of radioactive CO₂ in the expired air. In the present communication a comparison is made of the rates of oxidation of methyl groups administered as choline, betaine, dimethylthetin (4), and dimethyl-β-propiothetin (5). These compounds, labeled with C¹⁴ in one methyl group, have been prepared and injected intraperitoneally into rats. The radioactivity of the CO₂ in the expired air was measured at intervals during a 24 hour period.

EXPERIMENTAL

Synthesis of Labeled Compounds*—For the synthesis of choline, methyl iodide prepared from 3.88 mM of methanol containing 1.15 mc. of C¹⁴ was distilled in a stream of dry nitrogen into a solution of 346 mg. (3.9 mM) of dimethylaminoethanol in 5 ml. of ethanol, contained in a scrubber cooled in a solid CO₂ bath at −60°. After the distillation was complete, the cooling bath was removed and the mixture was allowed to stand at room temperature for 17 hours. The choline iodide was collected, the filtrate was evaporated to dryness, and the two fractions were recrystallized separately from boiling absolute ethanol. The two samples were then combined, dissolved in approximately 40 ml. of absolute ethanol, and the two fractions were recrystallized separately from boiling absolute ethanol. The two samples were then combined, dissolved in approximately 40 ml. of absolute ethanol, and shaken with a slight excess of AgCl. The AgI was removed by filtration and washed with ethanol and then with hot water. After evaporation of the filtrate and

* The authors wish to express their appreciation to the Lederle Laboratories Division, American Cyanamid Company, for a research grant which has aided greatly in this work.
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1 The starting material for all the preparations was radioactive methanol purchased from the Clinton Laboratories of the Monsanto Chemical Company on allocation from the United States Atomic Energy Commission; the values for the radioactivity are taken from data supplied by the company. The methanol was converted to methyl iodide by the method of Melville, Rachele, and Keller (6).
washings to dryness, the residue was recrystallized from ethanol-ether. The yield of choline chloride was 52 per cent of the theoretical amount on the basis of the methanol. A small sample was converted to the chloroplatinate for analysis.

\[ \text{C}_{10}H_{26}O_{2}N_{2} \cdot \text{PtCl}_{4} \]. Calculated, Pt 31.66; found, Pt 31.72

The radioactivity was determined after combustion of a small sample of the chloroplatinate and conversion of the CO\(_2\) to barium carbonate, according to the method described previously (7). The specific activities of the other labeled methyl compounds reported in this communication were also determined after oxidation according to this procedure. From the activity of the chloroplatinate, the specific activity of the choline chloride was calculated to be \(3.82 \times 10^6\) c.p.m. per mg.

For the preparation of betaine, methyl iodide made from 3.91 mm of methanol containing 1.23 mc. of C\(^{14}\) was distilled in a stream of nitrogen into a solution of 520 mg. (4.2 mm) of dimethylglycine sodium salt in 8 ml. of 75 per cent ethanol, contained in a scrubber cooled to \(-8^\circ\) to \(-10^\circ\). When the distillation was complete, the inlet tube was removed and the reaction vessel was tightly stoppered and heated in an oil bath at 70° for 85 minutes. The mixture was then evaporated \textit{in vacuo} to a small volume. 125 ml. of a saturated aqueous solution of ammonium reineckate were added and the mixture was acidified with HCl, cooled in ice overnight, and filtered. The precipitate was dissolved in 40 ml. of 0.1 N \(\text{NH}_{4}\text{OH}\) and shaken with excess silver oxide until all the color had been removed. The precipitate of silver reineckate was collected and washed with water; the filtrate and washings were combined, heated to 60°, and aerated for 4 hours to remove the ammonia. The solution was then concentrated to a volume of about 20 ml., acidified with \(\text{HCl}\), filtered, and evaporated to dryness. The residue was dissolved in the minimum amount of boiling ethanol, filtered, and cooled in ice. The first crop of crystals was collected; the filtrate was evaporated to a volume of 3 ml. and a second crop was obtained by cooling; the two crops were combined. After this material was dissolved in ethanol and reprecipitated with ether several times, the betaine hydrochloride, obtained in a yield of 30 per cent of the theoretical amount on the basis of the methanol, had a specific activity of \(3.30 \times 10^6\) c.p.m. per mg.

\[ \text{C}_{4}H_{14}O_{2}C\text{I}_{2}N \]. Calculated, Cl 23.08; found, Cl 23.02

For the preparation of dimethyl-\(\beta\)-propiothetin, methyl iodide prepared from 4.11 mm of methanol containing 1.23 mc. of C\(^{14}\) was distilled in a stream of dry nitrogen into a mixture of 525 mg. (4.37 mm) of \(\beta\)-methyl-mercaptopropionic acid, 3 ml. of formic acid, and 0.3 ml. of water, con-
tained in a U-shaped receiver which was cooled in a solid CO₂ bath. The
receiver was then stoppered securely and allowed to stand at room tem-
perature for 64 hours. The reaction mixture was dissolved in water and
shaken with 800 mg. of AgCl. The AgI was collected and washed well
with hot water. After evaporation of the filtrate and washings to dryness
the residue was recrystallized three times by dissolving in boiling ethanol
and precipitating with dry ether. The dimethyl-β-propiothetin hydro-
chloride weighed 512 mg. (73 per cent of the theoretical amount on the basis
of the methanol) and had a specific activity of 2.77 × 10⁶ c.p.m. per mg.

C₅H₁₀O₂ClS. Calculated, Cl 20.78; found, Cl 20.63

The procedure employed for the synthesis of the dimethylthetin was
exactly the same as that described for dimethyl-β-propiothetin. The
starting materials in this case were methyl iodide, prepared from 4.13 mm
of methanol containing 1.26 mc. of C¹⁴, and S-methylthioglycolic acid
(465 mg., 4.37 mm). The dimethylthetin hydrochloride obtained after
three recrystallizations from ethanol-ether weighed 374 mg. (58 per cent
of the theoretical amount on the basis of the methanol) and had a specific
activity of 2.90 × 10⁶ c.p.m. per mg.

C₅H₁₀O₂ClS. Calculated, Cl 22.64; found, Cl 22.36

Oxidation Experiments in Vivo—Four male rats of the Rockland strain
(Rats 1 to 4) weighing 100 to 120 gm. were placed on an amino acid diet
identical with one described previously (Diet II (8)) except that the level
of dl-methionine was 0.6 per cent. The animals were maintained on this
diet, which was choline-free and contained 0.4 per cent of cystine, until
they had reached a weight of 140 ± 4 gm. Aqueous solutions (0.7 ml.)
of the labeled methyl compounds were then injected intraperitoneally at
approximately the same time of day in each case (9.30 to 11 a.m.). The
compounds were administered in the following amounts, providing equiva-

ten amounts of methyl groups (0.21 to 0.22 mm CH₃—): choline chloride
10.0 mg. (Rat 1), betaine hydrochloride 10.9 mg. (Rat 2), dimethylthetin
hydrochloride 16.7 mg. (Rat 3), and dimethyl-β-propiothetin hydrochloride²
18.0 mg. (Rat 4). The animals were placed immediately in the open
circuit metabolism apparatus described previously (3) and allowed free
access to food. The food consumption over the 24 hour period was as
follows: 11 gm. (Rat 1), 9 gm. (Rat 2), 8 gm. (Rat 3), and 12 gm. (Rat 4).
The expired CO₂ was collected continuously in two absorbers, each con-
taining 400 ml. of 2.5 N NaOH, which were changed every hour for the
first 7 or 8 hours and again 24 hours after administration of the labeled

² In this case the administered compound was a 59:41 per cent mixture of labeled
and ordinary dimethyl-β-propiothetin hydrochloride.
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compounds. Aliquots of these solutions were converted to BaCO₃ for determination of the radioactivity. The precipitation and counting procedures were identical with those previously described (3, 7).

The per cent of the injected radioactivity which was expired as CO₂ during various time intervals after administration of the C¹⁴-labeled choline, betaine, dimethylthetin, and dimethyl-β-propiothetin was determined. The results are presented in Fig. 1. These experiments were repeated on another set of animals with results which corresponded closely for each compound with those shown in Fig. 1.

![Graph showing the percentage of injected radioactivity expired as CO₂ at various time intervals after injection of choline, betaine, dimethylthetin, and dimethyl-β-propiothetin labeled with C¹⁴ in one methyl group.](http://www.jbc.org)

**DISCUSSION**

It will be noted that, when betaine, dimethylthetin, dimethyl-β-propiothetin, and choline are administered intraperitoneally, at levels providing equivalent amounts of methyl groups, the percentage of methyl groups oxidized is greater in the case of the first three compounds than with the choline. The highest level of radioactivity in the expired CO₂ occurred some time during the first 4 hours after administration of the compound in all cases.

The fact that the methyl groups supplied in the form of choline are converted to CO₂ more slowly than the methyl groups administered as betaine or the two sulfonium compounds may be due to the dilution of the radioactive choline by body choline or may be simply a reflection of the rapid
diversion of the choline into reactions other than those involving oxidation of the methyl groups. Nevertheless, the data would seem to indicate that oxidation of the methyl groups of betaine, dimethylthetin, and dimethyl-β-propiothetin does not require that these groups be first transferred to choline.

It is known, for rats on a labile methyl-free diet containing homocystine, that dimethylthetin and dimethyl-β-propiothetin have growth-promoting activity which closely approximates that of choline and betaine (4, 5, 8). The thetins are also active methyl donors for the methylation in vitro of homocysteine by liver and kidney homogenates (9). The close similarity between the rates of oxidation of the methyl groups of betaine and the two thetins provides another example of the rather remarkable metabolic similarity between betaine and its sulfur analogues.

The authors wish to express their appreciation for many helpful suggestions during the course of the work from Dr. C. G. Mackenzie, Dr. J. R. Rachele, and Dr. D. B. Melville. They also wish to thank Mrs. Josephine T. Marshall for the microanalyses and for assistance with some of the barium carbonate precipitations.

SUMMARY

The synthesis of choline, betaine, dimethylthetin, and dimethyl-β-propiothetin labeled with C\(^{14}\) in one methyl group has been described. The percentage of the methyl groups of these compounds which was expired as CO\(_2\) by the rat over a period of 24 hours was measured. The methyl groups administered in the form of betaine, dimethylthetin, and dimethyl-β-propiothetin were found to be oxidized to a greater extent than the methyl groups administered as choline. A high degree of similarity was observed in the rates of oxidation of the methyl groups of betaine and the two thetins.

BIBLIOGRAPHY

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