

[Reprinted from the Journal of the American Chemical Society, **72**, 2819 (1950).]

Incorporation of the Carbon of Formaldehyde and Formate into the Methyl Groups of Choline

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INCORPORATION OF THE CARBON OF FORMALDEHYDE AND FORMATE INTO THE METHYL GROUPS OF CHOLINE^{1,2}

Sir:

It has been recently demonstrated in this Laboratory that the carbon of methanol may be incorporated, *in vivo*, into the methyl groups of choline.³ Continuing work along these lines, we have found that the carbon of formaldehyde or of formate may also be utilized. The use of the carbon of bicarbonate for this purpose was not detected.

The compounds, labeled with C¹⁴, were injected subcutaneously into rats which were kept in an open circuit metabolism apparatus for the collection of the expired carbon dioxide. For a few days prior to the injections and during the experiments the animals were allowed free access to a diet of the following percentage composition: vitamin-free casein 20; cystine 0.4; sucrose 54.6; Osborne and Mendel salt mixture 4; fat (Covo) 19; corn oil (Mazola) 1, containing 4.0 mg. of α -tocopherol acetate and 0.1 mg. of 2-methyl-1,4-naphthoquinone, 750 I.U. of vitamin A and 125 I.U. of vitamin D; water-soluble vitamin mixture consisting of thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, nicotinic acid and *p*-aminobenzoic acid, 1 mg. each, calcium *d*-pantothenate 5 mg., inositol 10 mg., folic acid 0.4 mg., biotin 0.01 mg., and sucrose to make 1000 mg. Each animal received 3 micrograms of vitamin B₁₂ intraperitoneally at the beginning of the experiment.

Over a period of three days, rat no. 804 received daily, in three subcutaneous injections, 2 ml. of a 0.093 molar solution of C¹⁴-formaldehyde along with an equimolecular amount of ordinary bicarbonate. The animal was sacrificed three hours after the last injection. Of the 1.30×10^6 counts per minute injected as radioformaldehyde, 1.06×10^6 counts per minute were excreted in the expired

(1) While these experiments were underway, it was announced by Professor H. G. Wood in a Harvey Lecture, February 16, 1950, that Dr. W. Sakami and Professor A. D. Welch have been able to demonstrate the synthesis of "biologically labile" methyl groups from formate *in vivo* in the rat and by rat tissue *in vitro*.

(2) 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.

(3) du Vigneaud and Verly. THIS JOURNAL, 72, 1049 (1950)

carbon dioxide. The choline was isolated from the carcass as the choline chloroplatinate (*Anal.* Calcd. for $C_{10}H_{28}N_2O_2 \cdot PtCl_6$: Pt, 31.68. Found: Pt, 31.70). It was degraded to trimethylamine which was isolated as the chloroplatinate (*Anal.* Calcd. for $C_6H_{20}N_2 \cdot PtCl_6$: Pt, 36.96. Found: Pt, 37.08). The specific activities of these compounds were determined as previously described³ and are given in the table.

Rat no. 808, received daily, in three subcutaneous injections, 2 ml. of a 0.095 molar solution of sodium C^{14} -formate over a three-day period. The animal was sacrificed fourteen hours after the last injection. Of the 8.22×10^6 counts per minute injected as formate, 4.87×10^6 counts per minute were recovered in the expired carbon dioxide. The choline chloroplatinate was isolated (Found: Pt, 31.69) and converted to trimethylamine chloroplatinate (Found: Pt, 36.93). The specific activities are given in the table.

Rat no	Compound	Spec. activity counts/min/ millimole
804 ♂ wt., 195 g.	C^{14} -formaldehyde injected	2.33×10^6
	Choline chloroplatinate	2.37×10^4
	Trimethylamine chloroplatinate	2.19×10^4
808 ♂ wt., 194 g.	Sodium C^{14} -formate injected	1.44×10^7
	Choline chloroplatinate	1.55×10^5
	Trimethylamine chloroplatinate	1.42×10^5

These results have been confirmed in similar experiments.

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RECEIVED APRIL 3, 1950

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