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Incorporation *in vivo* of C^{14} from Labeled Methanol into the Methyl Groups of Choline

By Vincent du Vigneaud and Walter G. Verly

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INCORPORATION *IN VIVO* OF C^{14} FROM LABELED METHANOL INTO THE METHYL GROUPS OF CHOLINE

Sir:

An investigation has been undertaken to test whether methyl groups supplied in the form of methanol can enter into transmethylation reactions of the body. An earlier test of whether methanol could support the growth of animals on a diet free of "biologically labile" methyl groups and containing homocystine was negative.[1] In the present experiment the more sensitive tracer technique, utilizing C^{14}-labeled methanol, has been employed. The amount of radioactivity in the methyl groups of the choline isolated from the tissues of the rat after the administration of the labeled methanol was such as to indicate that methanol made available appreciable amounts of methyl groups which could be used in the transmethylation reactions of the body. The possible significance of this finding to the mechanism of transmethylation and even to the biological synthesis of "labile" methyl groups becomes of considerable importance and is being further investigated. In this connection the interesting observation of Binkley and Watson[2] may be pointed out, that methyl phosphate appears to be utilized in the formation of creatine from guanidoacetic acid by rat liver homogenates.

A total of 9 ml. of a 2.4% aqueous solution of C^{14}-labeled methanol with an activity of 5.33×10^6 counts per minute per ml. was injected subcutaneously in 1-ml. portions twice daily into a 161-g. rat over a five-day period. During this time the animal was kept in an open-circuit metabolism apparatus for the collection of the expired carbon dioxide. For fifteen days prior to injection and for the duration of the experiment the rat was allowed free access to a diet of the following composition (in g.): sucrose 54.85, vitamin-free casein 20, DL-methionine 0.15, fat (Covo) 19, Osborne and Mendel salt mixture 4, corn oil (Mazola) 1, containing 4.0 mg. of α-tocopherol acetate, 0.1 mg. of 2-methyl-1,4-naphthoquinone, 750 I. U. of vitamin A and 125 I. U. of vitamin D; water-soluble vitamins, ad-

(1) du Vigneaud, Chandler, Moyer and Keppel, *J. Biol. Chem.*, **131**, 57 (1939).

(2) Binkley and Watson, *ibid.*, **180**, 971 (1949).

ministered *per os* twice daily, in the following amounts (mg. per day): thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, nicotinic acid and *p*-aminobenzoic acid, 0.08 mg. each; calcium *d*-pantothenate 0.4, inositol 0.8, folic acid 0.02 and biotin 0.0008; 2 micrograms of vitamin B_{12} every other day.

During the five-day period a radioactivity of 22×10^6 counts per minute, out of the total injected radioactivity of 48×10^6 counts per minute, appeared in the expired carbon dioxide. The animal was then sacrificed; choline was isolated from the carcass as the chloroplatinate (*Anal.* Calcd. for $C_{10}H_{28}N_2O_2 \cdot PtCl_6$: Pt, 31.68. Found: Pt, 31.14), and creatine as the creatinine potassium picrate (purity determined by the Jaffe reaction, 100%). The choline was then degraded to trimethylamine, which was isolated as the chloroplatinate and recrystallized from water–ethanol (*Anal.* Calcd. for $C_6H_{20}N_2 \cdot PtCl_6$: Pt, 36.96. Found: Pt, 37.06). The specific activities of these compounds, determined after combustion and isolation of the carbon dioxide as barium carbonate, are given in the table, in terms of counts per minute per millimole of compound.

Compound	Specific activity
C^{14}-Labeled methanol injected	*ca.* 7×10^6
Choline chloroplatinate	7.18×10^5
Trimethylamine chloroplatinate	6.45×10^5
Creatinine potassium picrate	1.11×10^5

No exchange of methyl groups was found to occur between choline and C^{14}-labeled methanol, allowed to stand together for several days.

This work has been confirmed with another animal. Complete details of these experiments and related ones will be forthcoming shortly.

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