EFFECT OF FOLIC ACID AND LEUCOVORIN ON SYNTHESIS OF THE LABILE METHYL GROUP FROM METHANOL IN THE RAT*

By WALTER G. VERLY,† JOHN M. KINNEY,‡ AND VINCENT DU VIGNEAUD

(From the Department of Biochemistry, Cornell University Medical College, New York, New York)

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Previous experiments from this Laboratory with C^{14}-methanol demonstrated that the carbon of methanol becomes incorporated into the methyl group of choline in the rat (1). This observation has been confirmed by Arnstein (2). In a subsequent investigation carried out in this Laboratory with methanol doubly labeled in the methyl group with deuterium and C^{14}, it was found that less than one-third of the hydrogens accompanied each carbon of the methanol when it made its appearance in the methyl group of choline (3). It was concluded that the methanol was being utilized, at least in large part, for the synthesis of a newly formed methyl group within the animal body, presumably through oxidation and subsequent reduction.

The present communication describes experiments undertaken with labeled methanol to determine whether folic acid[1] or its derivative, a synthetic preparation, Leucovorin (5), having *Leuconostoc citrovorum* factor (LCF) activity, had any effect on the utilization of methanol for methyl synthesis. The original dietary work of Bennett (6) and the recent contributions from various investigators have indicated that folic acid is in some way involved in labile methyl metabolism.

EXPERIMENTAL

Labeled Methanol—In these experiments C^{14}-methanol and methanol labeled with both deuterium and C^{14} in the methyl group were used. An aqueous solution of the latter was obtained by mixing ordinary water, deuteriomethanol, and C^{14}-methanol.

Diets—Four different diets, A, B, C, and D, were used. Diets C and D, which contained the metabolic antagonist methylfolic acid (7), were

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[1] A preliminary announcement of these results was made at a meeting of the American Society of Biological Chemists, Cleveland, April, 1951 (4).

fed only during the period prior to the injection of the methanol, as noted in the next section. The water-soluble vitamin mixture was identical with one described previously (8), except that folic acid was omitted. The percentage composition of the diets was as follows: Diet A, casein 20, sucrose 67.85, corn oil (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) 3, agar 2, Osborne and Mendel salt mixture[2] 4, sulfasuxidine 2, DL-methionine 0.15, water-soluble vitamin mixture 1; Diet B, casein 25, sucrose 65, corn oil (containing vitamins as given for Diet A) 1, Osborne and Mendel salt mixture 4, water-soluble vitamin mixture 1, and yeast (Anheuser-Bush) 4; Diet C, same as Diet A, except that the level of sulfasuxidine was changed to 1 per cent, and a 2 per cent level of methylfolic acid was included, with a suitable adjustment in the amount of sucrose; Diet D, same as Diet C, except that the casein was freed of vitamin B_{12} by extraction with alcohol.

Utilization of Methanol for Methyl Synthesis in Deficient and Vitamin-Treated Animals—Three groups of experiments were carried out under slightly different conditions.

Two male rats, Nos. 977 and 973, were fed on Diet A for 6 weeks and then on Diet C for 1 week, by which time they had stopped growing. The animals were then placed again on Diet A and the experiment was started. The rats were injected subcutaneously with 0.66 ml. of deuterio-C^{14}- methanol three times daily; the control animal, Rat 977, received *per os* 1 mg. of folic acid plus approximately 0.5 mg. present in the 14.5 gm. of food ingested during the experimental period. The animals were pair-fed.

Three male rats, Nos. 1, 4, and 6, were fed for 16 days on Diet D, by which time they had stopped growing. They were then placed on Diet B and received 10 γ of vitamin B_{12} intraperitoneally. Rat 4 received 1 mg. of folic acid *per os* and Rat 1 was subcutaneously injected with 1×10^7 units of crude Leucovorin. The injections of C^{14}-methanol were started on the following day, 0.33 ml. of C^{14}-methanol being given three times daily. During the injection period, Rat 1 received another supplement of 1.5×10^7 units of crude Leucovorin.

Four male rats, Nos. 60, 61, 63, and 66, were fed for 22 days on Diet A, then on Diet C for 7 days, then again on Diet A. If the animals resumed growth upon being returned on Diet A, they were placed again on Diet C; this procedure was repeated until the animals did not resume growth on being placed on Diet A. They were then injected intraperitoneally with 10 γ of vitamin B_{12}. On the day before the injections of methanol were started, intraperitoneal injection of 100 γ of folic acid was given to Rat 60, 1.5×10^6 units of crude Leucovorin were given to Rat 63, and 240 γ of the calcium salt of Leucovorin were given to Rat 66. The four animals

[2] Osborne-Mendel salt mixture No. 1 (9), Eimer and Amend, New York.

were then injected subcutaneously with 0.66 ml. portions of a solution of C^{14}-methanol daily.

The animals were sacrificed after the time periods indicated in Table I

TABLE I

Administration of Labeled Methanol

Rat No.	Diet during experimental period	Supplement	Duration of experiment	Weight of rat at end of experiment	No. of injections	mM injected
			days	*gm.*		
977	A	1.5 mg. folic acid	4	172	12	27.6
973	"	None	4	154	12	27.6
4	B	1 mg. folic acid	3	70	9	0.93
6	"	None	3	73	9	0.93
1	"	2.5×10^7 units crude Leucovorin	3	58	9	0.93
60	A	100 γ folic acid	1.4	142	5	1.02
61	"	None	1.4	133	5	1.02
63	"	1.5×10^6 units crude Leucovorin	1.4	174	5	1.02
66	"	240 γ Leucovorin	1.4	178	5	1.02

TABLE II

Isotopic Content of Methanol and Trimethylamine

Rat No.	Supplement	Methanol		Trimethylamine chloroplatinate				
					C^{14}		Deuterium	
		C^{14}	Deuterium in methyl group	Pt content*	In compound	In methyl group	In compound	In methyl group
		c.p.m. per mM	*atom per cent excess*	*per cent*	*c.p.m. per mM*	*c.p.m. per mM*	*atom per cent excess*	*atom per cent excess*
977	Folic acid	2.77×10^5	88.4	36.9	5.08×10^4	8.45×10^3	0.45	0.50
973	None	2.77×10^5	88.4	36.9	1.93×10^4	3.22×10^3	0.13	0.15
4	Folic acid	2.11×10^7		37.2	6.68×10^5	11.13×10^4		
6	None	2.11×10^7		36.9	1.87×10^5	3.22×10^4		
1	Leucovorin (crude)	2.11×10^7		37.2	4.27×10^5	7.12×10^4		
60	Folic acid	2.11×10^7		37.2	2.93×10^5	4.88×10^4		
61	None	2.11×10^7		36.7	0.75×10^5	1.25×10^4		
63	Leucovorin (crude)	2.11×10^7		36.8	6.09×10^5	10.15×10^4		
66	Leucovorin	2.11×10^7		37.2	4.04×10^5	6.73×10^4		

* Theoretical Pt content, 37.0 per cent.

and the choline was isolated and degraded to trimethylamine. The C^{14} and deuterium contents of the trimethylamine chloroplatinates and the administered methanol, determined by methods described previously (3), are given in Table II.

DISCUSSION

The results of these experiments show that the synthesis of the methyl group of choline from methanol is decreased in the folic acid-deficient rat; an increase in the degree of synthesis results from the administration of either folic acid or Leucovorin. In this connection it is to be noted that Sakami and Welch (10) have observed that the addition of folic acid to liver slices from folic acid-deficient rats increased the amount of methyl synthesis from radioformate, and Stekol, Weiss, and Weiss (11) have reported an effect of folic acid on methyl synthesis from radioformate in the rat. It should also be recalled that Plaut, Betheil, and Lardy (12), in a study on the relationship of folic acid to formate metabolism with C^{14}-formate in the rat, noted that folic acid-treated rats fixed about 10 times as much C^{14} into liver protein and 3 times as much into viscera protein as did the folic acid-deficient rats.

It should be reemphasized that the isolation of choline with the C^{14} in its methyl group does not necessarily mean that it is in the synthesis of choline that the newly formed methyl group occurred. What it does mean is that some compound possessing a "biologically labile" methyl group has been synthesized in the body. The labile methyl group might well first be formed in the synthesis of another labile methyl compound and then reach choline through transmethylation.

SUMMARY

It has been demonstrated that the amount of the C^{14} carbon of labeled methanol appearing in the methyl group of tissue choline is less in the case of the folic acid-deficient rat than in one receiving folic acid or Leucovorin.

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