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One-Carbon Compounds in the Biosynthesis of the "Biologically Labile" Methyl Group

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[Contribution from the Department of Biochemistry, Cornell University Medical College]

One-Carbon Compounds in the Biosynthesis of the "Biologically Labile" Methyl Group

By Vincent du Vigneaud, Walter G. L. Verly,[1a] John E. Wilson, Julian R. Rachele, Charlotte Ressler and John M. Kinney[1b]

Studies with methanol, formaldehyde and sodium formate labeled with C^{14} have shown that these substances can be utilized by the rat in the formation of the "biologically labile" methyl group. This has been demonstrated by the injection of these substances and the isolation of the choline from the animal tissues. In addition, the carbon of the formyl group of C^{14}-formyl-L-phenylalanine and of the methyl group of C^{14}-methyl stearate has been shown to appear in the methyl group of choline. Experiments with methanol doubly labeled with deuterium and C^{14} in the methyl group have indicated that the methyl group of methanol was not being utilized, at least to any great extent, directly in transmethylation but was being utilized through oxidation and subsequent reduction to the labile methyl group in the biosynthesis of the latter.

In 1945 it was reported[2] from this Laboratory that synthesis of "biologically labile" methyl groups occurred somewhere in the bodies of rats kept on an apparently adequate diet, although, as it was pointed out in the communication, the data did not distinguish between direct synthesis by the tissues and synthesis by intestinal bacteria with subsequent utilization of the methyl groups in the tissues. Indications of the synthesis of this grouping had been obtained earlier,[3a,b] when it was noted that some rats would grow with no known source of labile methyl group. While the crucial experiment in collaboration with Professor Reyniers, Dr. Luckey and their co-workers at LOBUND of the University of Notre Dame was under way to determine whether or not this synthesis of labile methyl groups could occur in germ-free rats,[4] we undertook a program of studying the possible precursors of the methyl group, for whether the synthesis took place in the tissues of the rat or through bacterial synthesis in the intestinal tract, the data would be of biological significance. As a result of the germ-free rat experiment[4] and of the *in vitro* work of Sakami and Welch[5-8] (*vide infra*), we now know that the labile methyl group can be synthesized by the tissues of the rat, and consequently our results in this paper on the one-carbon precursors of the labile methyl group are undoubtedly attributable in most part to synthesis of this grouping in the tissues.

The single carbon compounds labeled with C^{14} which we have investigated included methanol,[9a] bicarbonate,[9b] formaldehyde[9b] and formate.[9b] The bicarbonate was not found to act as a precursor of labile methyl groups. In this connection, it might be pointed out that Siekevitz and Greenberg[10] have shown that carbon dioxide is not reduced to formate by rat liver slices. In the case of methanol we found that the carbon was incorporated into the methyl groups of choline isolated from the tissues of the rat after the subcutaneous administration of C^{14}-labeled methanol.[9a] The earlier observation of Binkley and Watson[11] that methyl phosphate appears to be utilized in the methylation of guanidoacetic acid by rat liver homogenates is also of interest in this connection. We next turned to experiments with formaldehyde and formate and found that the carbon of these compounds likewise appeared in the methyl group of the choline isolated.[9b] The experimental results are summarized in Tables I, II and III.

While this work was under way, studies by Sakami[6] with C^{14}-methyl-labeled acetone showed that the C^{14} appeared in the methyl groups of choline and methionine, probably *via* the formation of "formate."[12] Furthermore, Welch and Sakami[7,8] demonstrated that the synthesis of the labile methyl group occurred in rat liver slices and in the whole rat from formate. Since the results of Sakami,[6] of Welch and Sakami,[7] and of our laboratory[9a,b] were reported, there have appeared confirmatory data regarding methanol and formate by Arnstein,[13] formaldehyde by Jonsson and Mosher,[14] and formate by Siekevitz and Greenberg.[15] Both isotopic[13-17] and growth studies[18-20] on the synthesis of the methyl group from metabolic precursors of "formate" such as glycine and serine likewise are in harmony with the utilization of "formate" in the synthesis of the methyl group.

Evidence from the work of Handler, Bernheim and Klein and from this Laboratory has shown that formaldehyde[21-23] and formic acid[22,23] are intermediates

(1) (a) Fellow of the Belgian American Educational Foundation; (b) A. E. C. Post-doctoral Fellow of the National Research Council.

(2) du Vigneaud, Simmonds, Chandler and Cohn, *J. Biol. Chem.*, **159**, 755 (1945).

(3) (a) du Vigneaud, Chandler, Moyer and Keppel, *J. Biol. Chem.*, **131**, 57 (1939); (b) Toennies, Bennett and Medes, *Growth*, **7**, 251 (1943).

(4) This work demonstrated that the synthesis of the methyl group took place in the germ-free rat and hence was not dependent upon the presence of intestinal bacteria. A preliminary announcement of these results with a general discussion of the biological synthesis of methyl groups has been presented (du Vigneaud, Ressler and Rachele, *Science*, **112**, 267 (1950)).

(5) Harvey Lecture by H. G. Wood, February 16, 1950.

(6) Sakami, *Federation Proc.*, **9**, 222 (1950); *J. Biol. Chem.*, **187**, 369 (1950).

(7) Welch and Sakami, *Federation Proc.*, **9**, 245 (1950). In the oral presentation of this paper, they reported that formaldehyde was a precursor of labile methyl groups *in vitro*.

(8) Sakami and Welch, *J. Biol. Chem.*, **187**, 379 (1950).

(9) Preliminary reports of these results have already appeared: (a) du Vigneaud and Verly, This Journal, **72**, 1049 (1950); (b) du Vigneaud, Verly and Wilson, *ibid.*, **72**, 2819 (1950).

(10) Siekevitz and Greenberg, *J. Biol. Chem.*, **180**, 845 (1949).

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

(12) Sakami and Welch have used "formate" to indicate either formate itself or a one-carbon intermediate of formate metabolism.

(13) Arnstein, *Biochem. J.*, **47**, xviii (1950).

(14) Jonsson and Mosher, This Journal, **72**, 3316 (1950).

(15) Siekevitz and Greenberg, *J. Biol. Chem.*, **186**, 275 (1950).

(16) Weissbach, Elwyn and Sprinson, This Journal, **72**, 3316 (1950).

(17) Elwyn and Sprinson, *ibid.*, **72**, 3317 (1950).

(18) Stekol, Bennett, Weiss, Halpern and Weiss, paper presented before the American Society of Biological Chemists, Atlantic City, N. J., April 17, 1950.

(19) Stekol, Bennett, Weiss, Halpern and Joralemon, Abstracts of Papers, 118th Meeting of the American Chemical Society, Chicago, September 3, 1950.

(20) Stekol and Weiss, *J. Biol. Chem.*, **186**, 343 (1950).

(21) Handler, Bernheim and Klein, *ibid.*, **138**, 211 (1941).

(22) Mackenzie, Rachele, Cross, Chandler and du Vigneaud, *ibid.* **183**, 617 (1950).

(23) Mackenzie, *ibid.*, **186**, 351 (1950).

in the oxidation of the methyl group of sarcosine. Since Horner and Mackenzie[24] have demonstrated that the methyl groups of methionine and betaine are sources of the carbon of the methyl group of sarcosine, the intermediates in the oxidation of the methyl group of sarcosine become of significance to the general question of the oxidation of labile methyl groups.[22,25] Furthermore, the results obtained by Sakami[26] indicated that the methyl group of choline contributes to the formation of "formate" in the rat, and Siekevitz and Greenberg[15] have provided evidence that the methyl groups of methionine and choline are used in the formation of formate *in vitro*. Thus, with formaldehyde and formic acid as intermediates in the oxidation of the labile methyl group, the *biological* conversion of these oxidation products to the labile methyl group takes on particular significance.

The appearance of the carbon of methanol in the choline of the animal body allows several interpretations. It might be serving as a donor of intact methyl groups for the synthesis of one of the compounds in the transmethylation series. It is furthermore conceivable that methanol might be an intermediate in certain transmethylation reactions, that is, an actual metabolic intermediate in the transfer of the methyl group *in toto*. On the other hand, the methanol might be acting as a precursor of the methyl group through oxidation and subsequent reduction, and thus might arrive in the transmethylation series *via* this indirect route.

We felt that this question might be approached by studying methanol doubly labeled in the methyl group with deuterium and C^{14}. It has already been demonstrated[27] that in transmethylation from methionine doubly labeled in this way, the methyl group can be transferred to choline without appreciable change in the ratio of deuterium to C^{14}.[28] If, in the case of doubly labeled methanol, the deuterium to C^{14} ratio in the methyl group of the choline isolated from the rat were the same as that in the methyl group of the injected methanol, the methanol must have played a role only in actual *transmethylation*. If, however, the ratio of deuterium to C^{14} in the methyl group of the choline were less than that in the methyl group of the injected methanol, then at least some of the methanol must have arrived in the choline *via* oxidation and reduction to a newly formed methyl group. The deuterium to C^{14} ratio in the methyl group of the choline isolated was considerably lower than that in the methyl group of the methanol injected, as shown in Table IV. It is apparent that these results demonstrate that the methyl radical in methanol is not used in the same way as the methyl group of methionine in transmethylation and indicate the strong possibility that the methyl radical of methanol is being utilized, at least in large part, as a precursor of a biologically labile methyl group *via* "formate" during which process deuterium is partially lost.

In the experiments already discussed the compounds were injected. We thought that it might be of interest to supply a source of labile methyl groups *via* the intestinal tract which would present to the body a continuous supply over a period of time in contrast to the injection of the labile methyl precursor which furnishes the source of labile methyl group over a shorter period. It was decided to use a methyl ester for this purpose and C^{14}-methyl stearate was employed. The degree of incorporation of C^{14} into the methyl group of choline in this instance, however, was approximately the same as in the case of the injected methanol, formaldehyde or formate.

In order to determine to what extent the formyl group of a formyl amino acid might act as a precursor of the labile methyl group, C^{14}-formylphenylalanine was injected. There was no pronounced difference between the results with this compound and those already described for formate.

Experimental

Labeled Compounds.—The C^{14}-labeled barium carbonate, C^{14}-labeled methanol, and the mixture of C^{14}-labeled methanol and formaldehyde were obtained from the Oak Ridge National Laboratory, on allocation from the United States Atomic Energy Commission.

Sodium C^{14}-Bicarbonate.—Barium C^{14}-carbonate was suspended in water and decomposed with phosphoric acid. The liberated C^{14}-carbon dioxide was trapped in aqueous sodium hydroxide. After an aliquot had been removed for radioactivity determination, the remaining solution was titrated with hydrochloric acid to a faint pink with phenolphthalein.

C^{14}-Methanol.—Sodium bisulfite was added to a mixture of radioactive formaldehyde and methanol in aqueous solution and the methanol was distilled *in vacuo*. After a second distillation the distillate was tested with dimedon (dimethyldihydroresorcinol) and found to be aldehyde-free.

C^{14}-Formaldehyde.—Ordinary methanol and formaldehyde were added as carriers to a mixture of radioactive methanol and formaldehyde in aqueous solution. Sodium bisulfite was then added and the solution was distilled to dryness *in vacuo*. The residual formaldehyde bisulfite compound and excess bisulfite were washed with methanol and then dissolved in water. After addition of about one-half volume of methanol the solution was distilled at reduced pressure. Formaldehyde was regenerated from the residue by adding hydrochloric acid and passing oxygen-free nitrogen through the solution for several hours to flush out the sulfur dioxide. The solution was neutralized with sodium hydroxide.

Sodium C^{14}-Formate.—This material was prepared from the barium C^{14}-carbonate by conversion to sodium bicarbonate and reduction of the bicarbonate by hydrogen with palladium as a catalyst by a method previously described for the reduction of potassium bicarbonate.[29,30] It was diluted with a suitable amount of carrier and used directly.

C^{14}-Deuteriomethanol.—An aqueous solution of methanol labeled in the methyl group with deuterium and C^{14} was obtained by mixture of ordinary water, deuteriomethanol and C^{14}-methanol. The C^{14} content of the mixture, as given in Table IV, was determined by combustion, precipitation of the carbon dioxide as barium carbonate and determination of the radioactivity. The deuterium content of the methanol was obtained by application of the falling drop method

(24) Horner and Mackenzie, *J. Biol. Chem.*, **187**, 15 (1950).

(25) Mackenzie, Chandler, Keller, Rachele, Cross, Melville and du Vigneaud, *ibid.*, **169**, 757 (1947); Mackenzie, Chandler, Keller, Rachele, Cross and du Vigneaud, *ibid.*, **180**, 99 (1949).

(26) Sakami, *ibid.*, **179**, 405 (1949).

(27) Keller, Rachele and du Vigneaud, *ibid.*, **177**, 733 (1949).

(28) In this earlier experiment the ratio of deuterium to C^{14} in the methyl group of the choline was calculated from the isotopic content of the isolated choline on the assumption that the deuterium (du Vigneaud, Cohn, Chandler, Schenck and Simmonds, *J. Biol. Chem.*, **140**, 625 (1941)) and C^{14} were located in the methyl group. In a comparable experiment recently completed, the choline was isolated and degraded to trimethylamine. The ratio of deuterium to C^{14} in the trimethylamine was the same, within experimental error, as that in the methyl group of the methionine fed. Thus the methyl group had been transferred *in toto*.

(29) Melville, Rachele and Keller, *J. Biol. Chem.*, **169**, 410 (1947).

(30) Bredig and Carter, *Ber.*, **47**, 541 (1914).

TABLE I

ADMINISTRATION OF LABILE METHYL PRECURSORS

Rat No.	Wt., g.	Diet	Compound injected	Molarity of injected solution	Number of subcutaneous injections	Millimoles injected	Duration[a] of expt., days
712 ♂	181	I	$NaHC^{14}O_3$	0.025	1	0.13	3
894 ♂	213	II[b]	$NaHC^{14}O_3$	.032	15	0.49	5
723 ♂	174	I	$C^{14}H_3OH$	.75	9	6.75	5
759 ♂	208	I	$C^{14}D_3OD$	2.58	4	9.8	2
968 ♂	210	II[b]	$C^{14}D_3OD$	3.45	15	34.5	5
969 ♂	223	II[b]	$C^{14}D_3OD$	3.45	15	34.5	5
816 ♂	180	II[c]	$C^{14}H_2O$	0.93	7	2.60	1.1[e]
804 ♂	194	II[d]	$C^{14}H_2O$	.093	9	0.56	3
808 ♂	194	II[d]	$HC^{14}OONa$	.095	9	.57	3
851 ♂	180	II[c]	$HC^{14}OONa$	.095	6	.38	2
943 ♂	189		C^{14}-Methyl stearate[f]			5.7	2
952 ♂	200	II[d]	$C^{14}HO$-L-Phenylalanine	.047	9	0.57	3

[a] Interval from first injection to time animal was sacrificed. [b] Received 15 micrograms vitamin B_{12} intraperitoneally at beginning of experiment. [c] Received 2 micrograms vitamin B_{12} intraperitoneally at beginning of experiment. [d] Received 3 micrograms vitamin B_{12} intraperitoneally at beginning of experiment. [e] Animal died 1 hour after last injection, showing dyspnea and muco-hemorrhagic diarrhea. Adhesions were observed between skin and muscles, with edematous infiltration at the sites of injections. [f] C^{14}-Methyl stearate was fed.

to the water of combustion of the mixture and correction for dilution by the water of the sample.

N-C^{14}-Formyl-L-phenylalanine.—The compound was prepared from L-phenylalanine essentially by the method of Fruton and Clarke[31] for the formylation of amino acids as applied to DL-phenylalanine,[32] with the exception that excess acetic anhydride as diluent replaced the excess formic acid usually employed as solvent.

0.31 millimole of sodium C^{14}-labeled formate with a specific activity of 2.28×10^5 counts per minute per millimole was diluted with 3.16 millimoles of formic acid and two drops of 85% phosphoric acid and the mixture was allowed to stand for four hours at room temperature. It was then allowed to distil slowly in a closed evacuated system into a test-tube cooled in Dry Ice. To the distillate 0.6 ml. of acetic anhydride was added and the mixture was allowed to stand for 2.5 hours. 3.4 millimoles of L-phenylalanine was then added with thorough mixing. After 2.5 hours, all volatile liquid was trapped *in vacuo* by distilling into a cooled receiver. The residue was washed twice with 1 *N* hydrochloric acid and once with water. Two recrystallizations from water gave a 55% yield of long white needles, m.p. 171–172°. A sample prepared in an identical trial run showed a rotation in 95% alcohol of $\alpha^{21.5}D$ +73.0 ± 1.4°, *c*, 3.02. The rotation reported[33] for the non-radioactive compound is $\alpha^{20}D$ +75.2°, *c*, 3.3.

C^{14}-Methyl Stearate.—The ester was prepared by treatment of stearyl chloride with C^{14}-methyl alcohol in the usual manner. It was recrystallized from methanol and then dried. The melting point was 37.8–38°.

Specific Activity Determinations.—C^{14}-Methanol and C^{14}-deuteriomethanol were burned in oxygen to carbon dioxide which was trapped in sodium hydroxide and diluted with inert sodium carbonate as a carrier. Barium carbonate was prepared from an aliquot of this solution, and the radioactivity was determined under a thin mica window Geiger–Müller counter, in connection with a scaling circuit. The results were corrected for background and self-absorption.

An aliquot of the formaldehyde solution was treated with dimedon and the radioactivity of the resulting dimedon derivative of formaldehyde was determined after combustion as described above.

In the case of sodium formate, an aliquot of the solution was dried in a desiccator and oxidized with the Van Slyke oxidizing mixture.[34] The radioactivity of the carbon dioxide formed was determined on barium carbonate prepared as in the cases of methanol and formaldehyde.

The radioactivity of the N-formyl-L-phenylalanine and methyl stearate was determined in a manner similar to that used for the sodium formate.

(31) Fruton and Clarke, *J. Biol. Chem.*, **106**, 667 (1934).
(32) du Vigneaud and Meyer, *ibid.*, **98**, 295 (1932).
(33) Fischer and Schoeller, *Ann.*, **357**, 1 (1907).
(34) Van Slyke and Folch, *J. Biol. Chem.*, **136**, 509 (1940).

Tests for Methyl Precursor Activity

The animals were given free access to one of two very similar diets (I and II). Diet I was identical with that reported previously.[9a] Diet II was of the same composition as Diet I except that the methionine supplement was replaced by 0.4% cystine, the sucrose content was reduced to 54.6% and the water-soluble vitamins were incorporated in the diet.[9b] In the case of the feeding experiment with C^{14}-methyl stearate, Diet II was used and the fat content was reduced to 12.4%, the other 6.6% being in the form of C^{14}-methyl stearate.

For the duration of the experiment, the animals were kept in an open-circuit metabolism apparatus for the collection of the expired carbon dioxide.[35] The radioactive compounds were then injected subcutaneously over the time periods indicated in Table I. In the case of the methyl stearate, the compound was administered in the diet. Each animal was killed by placing it in an atmosphere containing chloroform. After post-mortem examination the animals were frozen in Dry Ice. No gross pathological changes were observed, unless otherwise stated. Table I outlines the procedure for each experiment.

The methods of isolation of the choline as choline chloroplatinate, of creatine as creatinine potassium picrate, and the

TABLE II

ANALYSES OF ISOLATED COMPOUNDS

Rat No.	Administered compound	Choline chloroplatinate ($(C_5H_{14}NO_2)_2 \cdot PtCl_6$ Pt = 31.68)	Trimethylamine chloroplatinate ($(C_3H_{10}N)_2 \cdot PtCl_6$ Pt = 36.96)	Creatinine potassium picrate
712	$NaHC^{14}O_3$	31.31		103.9
894	$NaHC^{14}O_3$	31.55	36.83	99.6
723	$C^{14}H_3OH$	31.48	37.12	100.0
759	$C^{14}D_3OD$	31.72	36.67	
968	$C^{14}D_3OD$	31.59	36.84	
969	$C^{14}D_3OD$	31.64	36.86	
816	$C^{14}H_2O$	31.46	37.13	99.0
804	$C^{14}H_2O$	31.70	37.08	100.4
808	$HC^{14}OONa$	31.69	36.93	99.8
851	$HC^{14}OONa$	31.61	36.71	99.3
943	C^{14}-Methyl stearate	31.35	36.77	101.0
952	$C^{14}HO$-L-Phenylalanine	31.90	36.63	99.5

(35) Mackenzie, Chandler, Keller, Rachele, Cross and du Vigneaud, *ibid.*, **180**, 99 (1949).

TABLE III

ACTIVITIES OF COMPOUNDS INJECTED AND ISOLATED

Rat	Administered compound	Injected activity: Specific activity (*A*) counts/minute/millimole	Injected activity: Total activity counts/minute	Creatinine potassium picrate counts/minute/millimole	Choline chloroplatinate counts/minute/millimole	Trimethylamine chloroplatinate (*B*) counts/minute/millimole	$\frac{B}{6A}$
712	$NaHC^{14}O_3$	3.03×10^5	3.82×10^7	2.88×10^3	0	...	0
894	$NaHC^{14}O_3$	3.61×10^5	1.75×10^5	4.22×10^3	450	350	
723	$C^{14}H_3OH$	7.1×10^6	48.0×10^6	1.11×10^5	7.59×10^5	6.43×10^5	1.51×10^{-2}
759	$C^{14}D_3OD$	7.04×10^6	69.2×10^6	.....	.....	4.55×10^5	1.07×10^{-2}
968	$C^{14}D_3OD$	2.77×10^5	95.6×10^6	.....	.....	7.40×10^4	4.44×10^{-2}
969	$C^{14}D_3OD$	2.77×10^5	95.6×10^5	.....	.....	6.91×10^4	4.15×10^{-2}
816	$C^{14}H_2O$	2.33×10^4	6.05×10^6	4.22×10^3	4.75×10^4	4.96×10^4	3.55×10^{-3}
804	$C^{14}H_2O$	2.33×10^6	1.30×10^6	3.10×10^2	2.37×10^4	2.19×10^4	1.57×10^{-3}
808	$HC^{14}OONa$	1.44×10^7	8.22×10^5	1.22×10^4	1.55×10^5	1.42×10^5	1.64×10^{-3}
851	$HC^{14}OONa$	1.44×10^7	5.48×10^6	7.87×10^3	1.29×10^5	1.19×10^5	1.38×10^{-3}
943	C^{14}-Methyl stearate	1.67×10^6	9.45×10^6	1.89×10^4	8.84×10^4	8.34×10^4	8.32×10^{-3}
952	$C^{14}HO$-L-Phenylalanine	1.84×10^7	1.05×10^7	2.97×10^4	1.54×10^5	1.47×10^5	1.33×10^{-2}

TABLE IV

EXPERIMENTS WITH DEUTERIO-C^{14}-METHANOL

Rat No.	Compounds	Deuterium content: Compound Atom % excess	Deuterium content: Methyl group (*A*) Atom % excess	C^{14} content: Compound counts/minute/millimole	C^{14} content: Methyl group, (*B*) counts/minute/millimole	$\frac{A}{B}$ (*C*)	*X*[a]
759	Injected $C^{14}D_3OD$	87.5	87.5	7.04×10^6	7.04×10^6	12.4×10^{-6}	
	Trimethylamine chloroplatinate (TMCP)	0.26	0.29	4.55×10^5	7.58×10^4	3.81×10^{-6}	0.31
968	Injected $C^{14}D_3OD$	88.4	88.4	2.77×10^5	2.77×10^5	31.9×10^{-5}	
	TMCP	0.869	0.965	7.40×10^4	1.23×10^4	7.85×10^{-5}	0.25
969	Injected $C^{14}D_3OD$	88.4	88.4	2.77×10^5	2.77×10^5	31.9×10^{-5}	
	TMCP	0.887	0.986	6.91×10^4	1.15×10^4	8.57×10^{-5}	0.27

[a] *X* is obtained by dividing the ratio of deuterium to C^{14} in the methyl group of the trimethylamine chloroplatinate by the ratio of deuterium to C^{14} in the injected methanol. The D:C^{14} ratios are expressed as (*C*) in the preceding column.

degradation of choline to trimethylamine, isolated as trimethylamine chloroplatinate, have been described.[36] Each chloroplatinate sample was analyzed for platinum, and each creatinine potassium picrate sample was analyzed for purity by the Jaffé colorimetric reaction. The analytical data are summarized in Table II.

The compounds were oxidized with the Van Slyke oxidizing mixture and the resulting carbon dioxide was precipitated as barium carbonate. In the case of the trimethylamine chloroplatinates from the experiments with doubly labeled methanol, the compounds were burned in oxygen and the resulting carbon dioxide was precipitated as barium carbonate. The activity was determined on this precipitate with a thin mica window Geiger–Müller counter and a scaling circuit. The results were corrected for background and self-absorption.

In the case of the trimethylamine chloroplatinates isolated after injection of C^{14}-deuteriomethanol, the water obtained by combustion was reduced with hot zinc and the deuterium content was determined with the mass spectrometer.[37]

The results for the compounds labeled with C^{14} are summarized in Table III and those for the doubly labeled methanol are given in Table IV. It seems probable that the slight activity found in the creatinine potassium picrate from the rats injected with C^{14}-bicarbonate was caused by the presence of a small amount of C^{14} in the amidine moiety of the creatinine.

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

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(36) du Vigneaud, Cohn, Chandler, Schenck and Simmonds, *J. Biol. Chem.*, **140**, 625 (1941).

(37) Rittenberg, private communication.