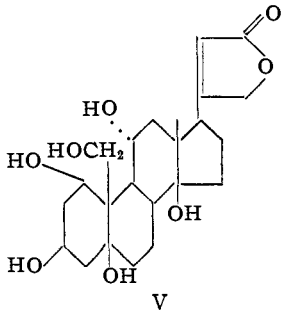
lishes C.11 as the position of the hydroxyl and keto



V

functions in I, II, and IV and, coupled with Tschesche's stereochemical results, permits assignment of structure V to ouabagenin.

DEPARTMENT OF CHEMISTRY
THE RICE INSTITUTE RICHARD B. TURNER
HOUSTON 1, TEXAS JOSEPH A. MESCHINO

RECEIVED AUGUST 20, 1956

A CRUCIAL TEST OF TRANSMETHYLATION *in Vivo* BY INTRAMOLECULAR ISOTOPIC LABELING

Sir:

In an earlier study of transmethylation[1] in the rat reported from this laboratory, use was made of methionine doubly labeled in the methyl group by mixing molecules labeled separately with deuterium and C^{14}, a type of labeling referred to as *intermolecular*. It was found in this experiment that the ratio of deuterium to C^{14} in the methyl groups of tissue choline and creatine was the same as that ratio in the labeled methionine administered in the diet. This result was taken as a demonstration that the methyl group of methionine is transferred as a unit in the biosynthesis of choline and creatine.

In later experiments[2,3] on the neogenesis of methyl groups in rats from methanol and formate triply labeled intermolecularly with deuterium, tritium and C^{14}, a hydrogen isotope effect was shown to occur in the cleavage of carbon–hydrogen bonds resulting in the retention of the heavier isotopes of hydrogen. Thus the disproportionation of $D:C^{14}$ values from precursors to methyl groups was due not only to metabolic changes but also to a hydrogen isotope effect. A similar isotope effect was found in the oxidation of the methyl group of methionine to carbon dioxide when the singly labeled methyl group, $C^{14}H_3-$, was compared to the doubly labeled methyl group, $C^{14}D_3-$, containing both deuterium and C^{14} bonded together in the same molecule, a type of double labeling referred to as *intramolecular*.[4]

It occurred to us that, in view of the extensive hydrogen isotope effects observed with intermolecularly multiply labeled compounds, our earlier transmethylation study[1] may have involved a fortuitous compensation of a metabolic loss of hydrogen from the methyl carbon of methionine by a retention of deuterium by this carbon through a hydrogen isotope effect, and thus the methyl group may only have appeared to be transferred as a unit.[5] It therefore became necessary to restudy transmethylation from methionine in such a way that isotope effects would be avoided. It has been shown in this laboratory[6] that it is possible to eliminate the effect of hydrogen isotope selection in the metabolism of formate by intramolecular double labeling with deuterium and C^{14}.

Thus, for the transmethylation study, L-methionine containing D and C^{14} in the same methyl group was prepared[4] and diluted so that the D content was about the same as in the earlier investigation.[1] The labeled methionine was administered in an amino acid diet to three male white rats, each of about 160 g., according to the plan used in the previous study,[1] with the exceptions that vitamin B_{12} at a level of 15 micrograms per cent. was added to the vitamin supplement and that the administration was for three days. The food intake was limited to 10 g. per day. At the end of the third day, the animals were killed by ether anesthesia, the livers removed, and choline and creatine were isolated from the remainder of the carcasses.[7] Choline was analyzed as the chloroplatinate for deuterium and C^{14}. Choline was then degraded to trimethylamine which was analyzed as the chloroplatinate for deuterium and C^{14}. Creatine, isolated as creatinine potassium picrate, was degraded without prior isotopic analysis to methylamine which was analyzed as the chloroplatinate for deuterium and C^{14}.

Table I lists the analytical results which indicate (a) that under the conditions of this experiment

TABLE I

ISOTOPE CONTENT OF THE METHYL GROUPS OF ADMINISTERED METHIONINE AND ISOLATED COMPOUNDS

Rat	Compound[a]	Deuterium, atom % excess	C^{14}, cpm/meq. methyl $\times 10^{-6}$	$D:C^{14}$ $\times 10^6$	$D:C^{14}$ isol. methyl / $D:C^{14}$ methionine methyl
	Methionine	64.5	70.1	9.20	
R-2	Choline[b]	5.20	5.56	9.34	1.01
	TMA[c]	5.18	5.70	9.08	0.99
	MMA[d]	3.60	4.80	8.83	0.96
R-5	Choline[b]	5.34	5.81	9.19	1.00
	TMA[c]	5.38	5.82	9.24	1.00
	MMA[d]	4.49	5.03	8.93	0.97
R-7	Choline[b]	5.21	5.87	8.88	0.97
	TMA[c]	5.27	5.87	8.98	0.98
	MMA[d]	4.89	5.45	8.97	0.98

[a] With the exception of methionine, all compounds were analyzed as the chloroplatinates. [b] It was assumed in the analysis of choline that the total isotope content resided in the methyl groups. [c] TMA = trimethylamine derived from choline. [d] MMA = monomethylamine derived from creatine.

(1) E. B. Keller, J. R. Rachele and V. du Vigneaud, *J. Biol. Chem.*, **177**, 733 (1947).

(2) J. R. Rachele, E. J. Kuchinskas, J. E. Knoll and M. L. Eidinoff, THIS JOURNAL, **76**, 4342 (1954).

(3) J. R. Rachele, E. J. Kuchinskas, J. E. Knoll and M. L. Eidinoff, presented at a meeting of the New York Section of the American Chemical Society, March 16, 1956.

(4) J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, *J. Biol. Chem.*, **215**, 593 (1955).

(5) We wish to acknowledge a personal communication from Dr. H. R. V. Arnstein of the National Institute for Medical Research, London, expressing a similar opinion with respect to hydrogen isotope effects on transmethylation.

(6) J. R. Rachele and H. Aebi, *Federation Proc.*, **15**, 333 (1956).

(7) V. du Vigneaud, C. Ressler, J. R. Rachele, J. A. Reyniers and T. D. Luckey, *J. Nutrition*, **45**, 361 (1951).

the methyl group of methionine contributes insignificantly to the ethanolamine moiety of choline and (b) that the methyl groups of choline and creatine have within experimental limits the same ratio of deuterium to C^{14} as the administered methionine. The present demonstration of the latter point with intramolecular labeling of the methionine methyl groups with deuterium and C^{14} confirms the conclusions drawn in the earlier study[1] with regard to the transfer of methionine methyl as a unit and further shows that hydrogen isotope effects played no significant role in the prior experiment.

(8) This work was supported in part by a grant (H-1675) from the National Heart Institute of the Public Health Service, for which we wish to express our appreciation.

DEPARTMENT OF BIOCHEMISTRY VINCENT DU VIGNEAUD[8]
CORNELL UNIVERSITY MEDICAL COLLEGE
JULIAN R. RACHELE
NEW YORK, NEW YORK ALAN M. WHITE

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PRODUCTION OF LABELED ORGANIC MATERIAL WITH ACCELERATED TRITIUM

Sir:

Tritium ions when accelerated to an energy of not over 100 e.v. will react to combine with organic material placed in its path. This has led to the production of radioactively labeled organic material and suggests that low energy beams of radioactive atoms may be used as a convenient agent for the practical synthesis of tracer compounds.

These experiments are a consequence of studies of the reactions of recoil tritium atoms produced in nuclear reactions. Recoil tritium will displace hydrogen atoms in organic compounds to substitute in their place and can be used for convenient one-step syntheses of tracer compounds of moderate specific activity. Recent experiments on the mechanism[1,2] of the recoil tritium labeling reaction indicate that the tritons possess only a few electron volts kinetic energy when they undergo reaction to enter organic combination. Thus the 2.7 Mev. possessed by recoil tritons from the $Li^6(n,\alpha)T$ reaction, though useful in enabling the triton to penetrate the material which it is to activate, is expended almost entirely in causing gross radiation damage. To prevent total destruction of the sample by this radiation damage it is necessary to limit the number of recoil tritons and thereby the possible specific activity. Thus by using low energy accelerated tritium rather than high energy recoil tritons it should in principle be possible to produce very high specific activity tracers.

(1) F. S. Rowland, C. N. Turton, and R. Wolfgang, THIS JOURNAL, **78**, 2354 (1956).

(2) R. Wolfgang, J. Eigner and F. S. Rowland, *J. Phys. Chem.*, **60**, 1137 (1956).

A simple discharge tube has been used as the source of accelerated tritium. A cylindrical vessel with electrodes of about 50-mm. diameter about 25 mm. apart was used. About 20 mg. of the material to be activated was spread on the cathode in a thin layer and approximately 0.03–0.06 mm. of T_2 introduced. A discharge of about 100 microamp. and about 0.5 hour duration was then passed using a potential difference of the magnitude of 500 volts. The sample which had thus been bombarded with T^+ and T_2^+ was then removed, subjected to chemical purification to remove gross decomposition products, reduced to gas and counted in a proportional counter. Following this, rigorous further purifications were performed on most of the samples.

Eleven organic compounds ranging in molecular weight from benzoic acid to bovine albumin have been irradiated using this technique. In each case specific activities of the order of 0.1 millicurie/mg. ($\sim 2 \times 10^8$ dis. p. min./mg.) were produced. This tritium is in non-volatile, soluble, non-labile (in water) organic combination. Chromatography indicates that a wide range of labeled species is formed. Some of the activity was incorporated in the compound irradiated but this amount varied erratically from run to run and is less than 10% in most cases and apparently negligibly small in some.

In a control experiment benzoic acid and tritium were placed together but no discharge passed. This yielded some organically bound activity but the amount was smaller by at least a factor of 10^3 to 10^4 than if a discharge had been passed.

The incorporation of such a large fraction of the activity in degradation products is probably not anomalous in spite of the expectation that low energy tritium should cause only little total radiation damage. The bombarding tritium has very little penetrating power. This means that all the labeling reactions and all the energy dissipation must proceed within a few monolayers of the surface which may thus become severely damaged. Present work is directed at modifying this effect. Especially if this difficulty is overcome, the use of low energy accelerated radionuclides appears to offer promising possibilities as a simple and broad method for the production of high specific activity tracer compounds of tritium and other radioactive isotopes, *e.g.*, C^{14}. It may also provide a fundamental approach to the study of "hot" atoms[3] and ions.

(3) J. Willard, *Ann. Rev. Nuclear Sci.*, **3**, 193 (1953); *Ann. Rev. Phys. Chem.*, **6**, 141 (1955).

CHEMISTRY DEPARTMENTS RICHARD WOLFGANG
FLORIDA STATE UNIVERSITY
TALLAHASSEE, FLORIDA THOMAS PRATT
PRINCETON UNIVERSITY
PRINCETON, NEW JERSEY F. S. ROWLAND

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