

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

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 COLLECTION Protocols - Clinical
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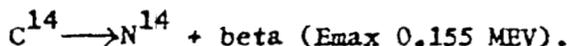
DATE: 27 July 1959

TO: Committee on Use of Radioactive Isotopes in Patients
 FROM: Lewis I. Gidez, Ph.D. *Lewis I. Gidez*
 I. L. Schwartz
 SUBJECT: Study of fatty acid transformations in the human. H-56

Request is made for the authorization for the use of doubly labeled palmitic acid in a specific patient. The acid will be labeled with C^{14} in the carboxyl carbon (carbon 1) and with H^3 on the ⁹ and ¹⁰ carbon atoms.

I. Physical Characteristics of the Isotopes

A. Carbon-14 has a half-life of about 5,000 years. The decay scheme is



B. H^3 (tritium) has a half-life of about 12.3 years. The decay scheme is

II. Metabolism of the Carrier (Palmitic Acid)

Since the proposed study involves tracing the metabolic pathways of two moieties of the palmitic acid molecule, i.e. C and H, the discussion of the metabolism of the compound will be presented in two parts.

A. Carbon Chain. Intestinal absorption of palmitic acid-1- C^{14} (administered with lipomul) can be expected to be about 90%. Oxidation of palmitic acid-1- C^{14} has been studied in dogs and rats after intravenous administration of tripalmitin. In the dog about 11% of the administered activity was expired after seven hours; in the rat 40-45% was expired in 24 hours. Studies with palmitic acid labeled in other carbon atoms along the chain indicated that the molecule is completely oxidized and stabilization of the original carbon chain (after loss of carbons 1 and 2, or 1, 2, 3 and 4) in shorter chain fatty acids is minimal. The palmitic acid which is not oxidized is incorporated into the lipids of the body, especially

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in plasma, liver, and probably depot fat. Incorporation into the lipides of other tissues will be variable: very low in the case of nerve lipids, higher in tissues such as intestine and kidney although not as much as in liver. Most of the C^{14} will be associated with the pool of palmitic acid, but there is conversion of palmitate to other fatty acids containing 16, 18, and 20 carbon atoms. It is the purpose of this study to investigate these conversions.

B. Hydrogen Atoms. The hydrogens which remain on the molecule (with one exception) undergo the same metabolic pathways as the carbon atom on the number one position. The one exception will be loss of hydrogens in the dehydrogenation of palmitic acid in the biosynthesis of unsaturated fatty acids. If 40-45% of the carbon activity is lost as $C^{14}O_2$, then one can expect to lose 40-45% of the tritium activity as HTO or short chain carbon intermediates containing readily exchangeable hydrogen atoms.

C. Turnover of Palmitic Acid-1- C^{14} , 9,10- H^3 . The turnover of fatty acids depends not only on the tissue in which it resides, but also on the lipid moiety in which it is incorporated. For example, free fatty acids in plasma have a T/2 of about 30 minutes. Esterified fatty acids in plasma have components of turnover varying from several hours to 3-4 days. In a previous study on a patient (, August 1956, BNL) who received palmitate-1- C^{14} the T/2 for total plasma fatty acids was estimated to be 3-4 days (slowest component of turnover). The mean T/2 for fat given in Handbook 52 is 35 days. Very little is known about the very slow component of carbon in bone, but this may be considered to be about 180 days.

The turnover of the tritiated portion of the fatty acid of course is identical with that of the entire molecule as discussed above. Probably 45-55% of the absorbed tritium activity will appear as HTO. The effective T/2 for this HTO which will be uniformly distributed is about 19 days.

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III. Radiation Exposure

In calculating the radiation exposure one may assume that the labeled palmitic acid has the following components of turnover.

<u>Carbon-14</u>	<u>Tritium</u>
15% has T/2 of 1 day	45% has T/2 of 19 days
83% has T/2 of 35 days	8% has T/2 of 1 day
2% has T/2 of 180 days	46% has T/2 of 35 days
	1% has T/2 of 180 days

The radiation dosages shown below in Tables I and II are based upon administration of 0.1 mc of C¹⁴ and 1.0 mc of H³ to a 65 Kg. man.

Table I - 0.1 mc palmitic acid-1-C¹⁴

<u>Component (T/2 days)</u>	<u>Uniform body distribution</u>	<u>Distribution in blood. Liver system only</u>
Rads at infinite time		
1	0.001	0.005
35	0.170	0.969
180	0.020	0.114
Total	0.191	1.088
Rads in first week	0.021	0.120

The values in Table I may be about 1.5 times too high when one takes into account the activity unabsorbed and lost in the respiratory gases during the first 24 hours.

Table II - 1.0 mc palmitic acid-^{4,10}H³

<u>Component (T/2 days)</u>	<u>Uniform body distribution</u>	<u>Distribution in blood. Liver system only</u>
Rads at infinite time		
19 (HTO)	0.0556	(0.0556)
1	0.0005	0.0028
35	0.1046	0.5960
180	0.0117	0.0667
Total	0.1724	0.7211
Rads in first week	0.0268	0.0940

Thus for 0.1 mc of C¹⁴ and 1.0 mc of H³ the probable maximum radiation at infinite time would be 1.088 rads and 0.721 rads, respectively.

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IV. Specific Proposal

It is proposed to administer orally 100-150 microcuries of palmitate-1-C¹⁴ and 1 mc of palmitate-^{9,10}8,9-H³ by mouth in a fat emulsion to one or more human subjects. The radioactive palmitic acid and a small amount of carrier palmitic acid will be dissolved in ether. To this solution will be added liposul. An aliquot of this solution will then be assayed for Carbon-14 and tritium. The ether will be removed and the resulting radioactive lipid will be administered to the patient. The experiment is being carried out to measure the conversion of palmitic acid to other saturated and unsaturated fatty acids. A previous study revealed that when plasma fatty acids from a patient who had received palmitate-1-C¹⁴ were separated by means of gas chromatography and subsequently counted, there was appreciable radioactivity in certain acids that are in very low concentration in plasma and in acids that have been termed "essential," i.e. linoleic acid. The results suggest that such essential acids may indeed be synthesized by man from palmitic acid, one of the most abundant and ubiquitous of the fatty acids in the normal diet. Since minute amounts of acids are isolated by the gas chromatographic technique it is virtually impossible to determine specific activities. However, by the use of both C¹⁴ and H³ one can measure the ratio H³/C¹⁴ which is independent of the amount of material collected. Since the palmitate is labeled with tritium in only two positions, any dehydrogenation which takes place on carbons 8 or 9 will result in loss of tritium activity, thereby altering the H³/C¹⁴ ratio. By comparing ratios of different fatty acids one may be able to elucidate the mechanisms of palmitate transformations.

Most of these reactions take place within 12 hours from the time of administration of the isotope. Therefore, frequent small samples of blood will be obtained during the first day. The tritium in plasma water will also be measured. Probably a minimum of 8-10 ml. of plasma for each time period will be necessary.

If the patient is kept in a well-ventilated room, there need be no special precautions regarding C¹⁴O₂ unless it is possible to collect the expired gases. The stools and urine can be disposed of safely in the regular plumbing system.

It is requested, therefore, that permission be given to administer 100-150 microcuries of carbon- and 1 millicurie of tritium-labeled palmitic acid.

The responsible physician will be Dr. Irving Schwartz.

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Request approved:

Elmer E. Stickley, Ph.D., Chairman

Walton W. Shreeve
Walton W. Shreeve, M.D., Ph.D.

Donald C. Borg
Donald C. Borg, M.D.

J. S. Robertson
James S. Robertson, M.D., Ph.D., Ex Officio

Lee E. Farr - for moment only - Special
Lee E. Farr, M.D., Ex Officio

approval for such
patient to be used
must be sought and
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Addendum

The carboxyl-labeled palmitic acid was prepared by a Grignard reaction. The starting material was the 15 carbon hydrocarbon. The C¹⁴ can be only in the carboxyl position. The labeled palmitic acid was recrystallized from ether as the ammonium salt. The only possible impurities are myristic and stearic acids. The 9,10 tritiated palmitic acid was prepared by reduction of palmitic acid obtained from yeast lecithin.

The solution of lipids to be administered will be counted in a Packard Liquid Scintillation Counter. Correction for any quenching due to the lipid will be made.

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