

November 1, 1961

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TO: Committee on Use of Isotopes in Human Beings  
 FROM: Walton W. Shreeve, M.D., Ph.D.  
 SUBJECT: Request for Approval of Project Using T-labeled Organic Compounds

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1. Title of Project:

"Conversion of Tritium-labeled Glucose, Lactate, Pyruvate, and Acetate to Body Water, Plasma Triglycerides, and Fatty Acids"

2. Investigators:

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

REPOSITORY Records Holding Area Bldg. 494  
 COLLECTION Protocols - Clinical  
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3. General Statement:

## a) Purpose of Study

The purpose of this study is to investigate possible changes in the functioning of the hydrogen carrier system (1) via pyridine nucleotides in various metabolic (generally endocrine) abnormalities such as diabetes and obesity. It is well-known that in insulin-deficient diabetes there is a marked defect in fatty acid synthesis (2); on the other hand, in obese-hyperglycemic mice and possibly in some human types of obesity there is an excessive conversion of  $C^{14}$ -acetate to fatty acids (3). The diabetic lesion has been attributed to a deficiency of reduced TPN, which is required for fatty acid synthesis. Another possibility is a low efficiency of the TPN system for reductive synthesis, which may relate to the effect of steroids linking TPN to DPN through transhydrogenase (4). Conversely, in some obese states (which may display hypersecretion of insulin (3) as well as steroid abnormalities) there may be an unusually efficient TPNH reductive system. It is quite possible that hormones affect these carrier systems and coenzymes directly (4, 5, 6) and that tracing of hydrogen metabolism with tritium may provide sharper distinctions than have heretofore been shown with other tracer or balance studies in vivo.

Study of hydrogen transfer to plasma triglycerides or free fatty acids may give positive evidence of increased or decreased fat synthesis. Also it is postulated that tritium from the labeled compounds may be delivered to the body water as an inverse function of the fatty acid and other synthetic activity, thus indicating the latter indirectly. Such a correlation would be particularly useful because of the ease of measuring the tritium content of body fluids, e.g.,

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plasma, urine, or water vapor of the breath. A significant test with clinical applicability might thus be devised. The aim of such a test would be more sensitive diagnosis and more refined disease classification with consequently better prognosis and individualized control with diet, drug therapy, etc. A further value would be better understanding of basic hormonal mechanisms.

b) Type of Patients

Different standard types of diabetics, i.e., thin, juvenile and adult, mild obese, would be studied. Also, obese patients with different genetic and environmental histories would be included. In some special cases patients with overt steroid abnormalities (Cushing's disease, Addison's disease) may be included. Other diseases considered for later study are alcoholism and cancer. The former condition can cause a definite imbalance of the DPN:DPNH system (7) and perhaps secondarily through transhydrogenase the TPN:TPNH system. The findings of changes in conversion of  $C^{14}$ -glucose to  $CO_2$  in rats with carcinogenic predisposition (8) suggests early alterations in intermediary metabolism of the tumor host. Patients would not presently be selected rigidly for limitations on life expectancy or reproductive capacity.

c) Pertinent Information on Past Studies

The compounds to be used have a rapid distribution into extracellular space (9).  $C^{14}$  studies in humans have indicated that glucose has a half-time of turnover in plasma of about 2 hours (9), lactate about 15-20 minutes (10) and pyruvate about 1 to 2 minutes (11). Maximum appearance of  $C^{14}$  in the respired  $CO_2$  after  $C^{14}$ -glucose administration is about 90 to 120 minutes (9), after lactate and pyruvate about 60 to 90 minutes (10) and acetate within 30 minutes (12). Within 24 hours the fraction of these compounds converted to  $CO_2$  ranges from 25 to 75% (10, 12). A similar rate and extent of conversion of tritium to body water may be expected. Actually, in preliminary experiments on the measurement of expired water vapor in mice, we have estimated the appearance in body water in 2 hours of about one-third of an amount of tritium in glucose injected intraperitoneally. Foster and Bloom (13) found that rat liver slices converted to water about 8 to 9% of an amount of glucose-1- $H^3$  in the medium and 50 to 60% of glycerol-2- $H^3$ . Only .3% was found in the fatty acids. With similar conditions Lowenstein (14) showed about 1% uptake into fatty acids from lactate-2- $H^3$  and less from glucose-1- $H^3$ . We have observed about .2% in hepatic triglycerides and .6% in carcass triglycerides of obese mice (much less in non-obese) after injection of glucose-1- $H^3$ . Abraham et al (15) noted about 5 to 10% transfer to fatty acid of T from labeled DPN and TPN added to homogenates of lactating rat mammary gland under optimal conditions.

No further pertinent information is presently available on metabolism or distribution of tritium from labeled organic compounds of this general type. Since a major fraction of the tritium is converted to body water within hours or days, it is reasonable, however, to regard the long-term rate of this tritium as comparable to that of body water. The half time of turnover of water has been studied and is given as 10 days for man (16). Recent studies have indicated an equilibration of the

of the tritium in body water with the hydrogen of organic body structure within 5 to 10 days in rats and subsequent rates of decline with half lives of 7 to 10 days (17).

d) Course of Study

Patients will be given trace quantities of one of the labeled compounds intravenously in a fasting state. Samples of plasma will be collected at hourly intervals for 4 to 6 hours, urine less frequently. Breath water vapor will be collected at intervals similar to plasma and perhaps in time substituted for the latter for  $H_2^{30}$  analysis. Two or three of the blood samples will be large enough (50 to 100 ml.) for analysis of T in triglycerides and possibly free fatty acids. Tritium in water and in lipids will be analyzed with the Packard Tri-Carb spectrometer.

For obvious reasons patients will, in a preliminary test, be given .5 to 1.0 millicurie  $H_2^{30}$  for measurement of total body water. (Alternatively it may be possible to estimate sufficiently TBW by  $K^{40}$  content determined with the whole body counter). In certain cases the tritium-labeled compound will be given in a mixture with a  $C^{14}$ -labeled sample of the same or similar compound for purposes of comparison of carbon and hydrogen metabolism. Generally the activities of the two radioisotopes in terms of radiation dosage will be of the same order of magnitude and considered additive. It is anticipated that some patients will be studied repeatedly 2 to 5 times. Single administrations will not exceed 5 millicuries in quantity and total amount to a patient will not exceed 15 millicuries.

4. Isotope:

Tritium or  $H^3$  emits a negative  $\beta$  ray with a maximum energy of about .018 mev. It decays to  $He^3$  with a half-life of 11 or 12 years (18). The tritium-labeled compounds (glucose-1- $H^3$ , glucose-6- $H^3$ , lactate-2- $H^3$ , pyruvate-3- $H^3$ , acetate-2- $H^3$ ) will be obtained commercially from New England Nuclear Corporation and Volk Radiochemical Company. Paper chromatography and autoradiography will be used to establish purity. In some cases exchange resin chromatography may be used for purification. Specific activities of administered materials will range from 1 to 10 mc./mm. All samples will be assayed before use by liquid scintillation counting with the Tri-Carb spectrometer. Samples will be prepared in sterile, isotonic solution for intravenous injection by filtration through ultrafine sintered glass or Coors porcelain.

5. Radiation Dosage:

In addition to the observation of a biological half-life for water of 10 days in man (16), Bogdanov et al (17) have defined in mice and rats two components of body water turnover with half-lives of 2.5 and 12.5 days, respectively. These authors estimated a total body water distribution of 67% body weight, which is in agreement with values reported by Hevesy (19). Another report, however, seems to place the value from 50 to 60%, depending on age and sex (20). However, the findings of Bogdanov et al (17) suggest that the distribution of tritium from body water would be essentially whole body after a few days. The

organic compounds to be used (except acetate) may show some concentration in liver glycogen for a few days but probably at the most 10 to 20% of the administered material (10). Before oxidation they would be distributed mainly in extracellular space, but would be oxidized largely to body water within hours or days.

The dose rate in man from 1 mc. of tritium-labeled water can be estimated according to Hine & Brownell (21) to be:

$$51.2 \bar{E} C = \text{rad/day}$$

$$51.2 \times .0057 \times \frac{1}{70} = .00417 \text{ rad/day}$$

$$\text{where } \bar{E} = \text{mev and } c = \mu\text{c./gm.}$$

This calculation assumes uniform body distribution, complete retention, and an RBE of essentially 1 for tritium in body water (22). Liebman (16) figured the total infinite dose from 1 mc. according to the formula:

$$\frac{\text{dose rate} \times \text{biological half time}}{\ln 2} = \frac{.00417 \times 10}{.693} = .06 \text{ rad.}$$

Considering all the above evidence we might estimate that one of the organic labeled compounds would distribute during the first 10 days in half the body space. During this time the sustained dosage from 5 mc. would be:

$$\frac{1}{2} \times \frac{5}{35} \times 51.2 \times .0057 \times \frac{10}{.693} = .30 \text{ rad.}$$

Thereafter, the total body dose would be:

$$2.5 \times \frac{.00417 \times 10}{.693} = .150 \text{ rad.}$$

While distribution in body water space (first 10 days) is probably more innocuous than subsequent whole body distribution, we may nevertheless, consider a maximal whole body dose to be .30 + .150 = .45 rad. (or rem.) From 15 millicuries the total cumulative dosage would be 1.35 rem. spread over periods of weeks and months.

Five millicuries per 70 kg. man would provide a concentration of ca. .1  $\mu\text{c T/ml.}$  body water in man. It may be noted that, whereas this concentration of T in the form of labeled thymidine can produce inhibition of clonal growth (23) the concentration of  $\text{H}_2^3\text{O}$  required to produce radiation effects comparable to those caused by thymidine was about 1000 times greater (24).

#### 6. Health Physics:

It is not considered that the levels of tritium employed will present any radiation hazards for attendant or laboratory personnel. However, samples submitted to the clinical laboratories will be marked as tritium-containing for appropriate caution in pipetting.

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