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To: Committee on Use of Radioactive Isotopes in Patients

From: Walton W. Shreeve

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Metabolism of I-Inositol-2-C^{14}

By: L. Anderson (Univ. of Wisconsin), L. V. Hankes and W. W. Shreeve (B.N.L.) and O. Touster (Vanderbilt Univ.).

Inositol is a component of phosphatidyl inositol. This glycolipid appears to play some role in both biosynthetic and transport phenomena. For instance, it is involved in the activation of certain enzymes and in the secretion of some proteins (1). It is required for synthesis of cell wall polysaccharides (2). Fibroblasts and other human cells in tissue culture require inositol as a growth constituent (3). Phosphatidyl insositol is a ubiquitous compound which may be formed from cytidine diphosphate diglyceride and free inositol (1). Therefore, the metabolism of free inositol will affect the maintenance of this phospholipid.

Myoinositol, a stereoisomer of hexahydroxy-cyclohexane, is sometimes classed as a vitamin. Its role in human nutrition is unsettled, but under some circumstances it is like choline, another constituent of a phospholipid, in being a "lipotropic" agent (3). Inositol may have a marked effect in reducing the amount of cholesterol in the liver (4).

A study in humans of conversion of C^{14}-labeled inositol to carbohydrates (glucose, glucuronic acid, and pentoses) and to expired CO_2 may help elucidate abnormalities associated with development of

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"fatty liver" in disease states such as cirrhosis and alcoholism. It is proposed that patients over 40 with histories of alcoholism with actual cirrhosis or varying degrees of liver involvement be used in this study. The Suffolk County Home will provide a source of such patients. Patients may have normal life expectancy and reproductive status and be of either sex.

There is a special point of interest in regard to catabolism of inositol via L-xylulose, the study of which will be aided by the inclusion in the patient group of one or more individuals with pentosuria. It may be anticipated that the study will improve understanding of the latter condition, also.

Moscatelli and Larner (5) have recently provided some information on the major metabolic fate of inositol. Using intraperitoneal injection of uniformly-labeled C^{14}-inositol in rats, they have observed the appearance in the expired CO_2 of about 25% of the injected C^{14} within 12 hours when using the fasted animal and about 10% when the animal is fed. About 3% of the dose was found in liver glycogen of the fed rat, as observed also by Anderson and Coots (6), and only 1/20 as much in the fasted rats. Total incorporation into lipids of liver and kidney was about .25% in each organ and one-third as much in lipids of brain and heart. Recoveries of radioactivity in the urine were 23% and 56% for the fasted animals and 6% for the fed animal. Anderson and Coots (6) noted only 2% in the 24-hour urine of fed rats. 50% of the administered radioactive dose was found in the carcass of the fed rat (5).

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From the above information it would appear that in a fasted patient about half of an injected amount of C^{14} in inositol would leave the body within 24 hours and in the fed patient about one-fourth. The retained portion would evidently be distributed rather evenly in the body and not so far as presently known concentrated to any extent in any organ or tissue. Since feeding markedly augments the incorporation into glycogen (5) and catabolism via carbohydrates is of major interest in this study, at least some of the patients will probably be studied in the fed state.

Nothing is presently known about the long-term retention of inositol or products of its metabolism. Isotopic studies indicate that the inositol component of phosphatidyl inositol turns over or exchanges at an appreciable rate (1). A large fraction of inositol may be converted to pentoses (7) which are in general metabolized rapidly. For instance, Segal and Foley (8) showed that 48% of a tracer dose of 1-C^{14}-ribose in humans is excreted as $C^{14}O_2$ within 6 hours after administration.

In the proposed study the conversion to pentoses will be studied by collection of L-xylulose, which is excreted in large quantities by pentosuric subjects. In non-pentosuric patients conversion to pentose may also be studied by isolation from the urine of a complex of ribose and imidazoleacetic acid. Although evidence from study of the intermediate, u-C^{14}-glucuronolactone, (9) indicates that much less C^{14} from 2-C^{14}-inositol will be excreted as $C^{14}O_2$ in the pentosuric than in other patients, a large fraction of the tracer radioisotope should nevertheless be excreted early as xylulose.

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Assuming that the 2-C^{14}-inositol will have approximately the same fate as C^{14} from u-C^{14}-inositol (5), it appears that in the fed subject the rate of excretion based upon data for the first 24 hours (5) will be about half that noted when compounds such as 2-C^{14}-glycine (10) and 2-C^{14}-pyruvate (11) are given to humans. Since 100 microcuries C^{14} in either of the latter two compounds are presently allowed (Authorization H-48 and (10)) for use in patients with normal life expectancy, then 50 microcuries of 2-C^{14}-inositol should be allowable. While it is presently estimated that 25 microcuries will be sufficient to carry out the planned study, permission to use up to 50 microcuries per patient is requested. Although no repetition or extension of a study in the same individual is currently planned, this authorization would give latitude for one repeat dose of 25 μc.

A simple formula for beta-dosimetry, assuming uniform distribution in a volume large in relation to the range of beta particles (12) is:

$$d_{\beta(day)} = 51.2\ C\ \bar{E}_{\beta}\ \text{rads}$$

where C = concentration of isotope in $\mu c/gm$.

$\bar{E}_{\beta}$ = average energy per disintegration in MEV

substituting,

$$d_{\beta} = 51.2 \times \frac{50}{70{,}000} \times .05 = .0018\ \text{rads/day}$$

This dose of 1.8 m rads/day or .66 rads/year would be sustained if there were complete retention indefinitely and is therefore a maximum estimate for whole-body dosage. The known metabolism of inositol

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would not suggest any critical organs except liver and kidney, and even in these cases the finding of $<5\%$ of an injected dose in glycogen and total lipids of liver and $<1\%$ in lipids of kidney (5,6) does not indicate any organ to be more critical than the whole body.

The patients will be given the 2-C^{14}-inositol in .9% NaCl solution intravenously in specific activity of approximately 1 millicurie/mM. The labeled compound is synthesized in the laboratory of Dr. L. Anderson at the University of Wisconsin (6) and has no radioactive components other than the C^{14}. The material will be sterilized by filtration through ultrafine sintered glass. An aliquot will be assayed at Brookhaven before use by addition of carrier inositol and combustion by Van Slyke-Folch technique to CO_2 for gas counting. No pharmacologic effect of this amount of inositol is to be expected.

From the use of 25 microcuries of C^{14} there will be no radiologic hazard to attendant personnel (cf. Authorization H-48 and H-41). However, it may be noted thatas part of the study the patient's breath will be collected for analysis during the first few hours and continuously evacuated. No special disposal of blood, urine, or feces as radioactive waste will need to be done; however, samples on the first day for the clinical labs will be marked as C^{14}-containing. No monitoring procedure, except in case of accident, or plan of isotope accountability is considered necessary.

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Anderson, Hankes, Shreeve, Touster
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Approved:

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

Victor P. Bond, M.D., Ph.D.

Samuel Fine, M.D.

Elmer E. Stickley, Ph.D.

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

Lee E. Farr, M.D.
Ex Officio

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