of the urinary estrone derived from cholesterol, the relatively high number of counts in the estrone indicates that this may be an important biosynthetic pathway. Since cholesterol can serve as a precursor for estrone it is no longer necessary to assume a pathway of estrogen biosynthesis independent of cholesterol *in vivo*, although such a pathway may exist. The data lend support to the scheme of estrogen biosynthesis from cholesterol recently proposed by Solomon, *et al.*[13]

[13] S. Solomon, R. V. Wiele and S. Lieberman, THIS JOURNAL, **78**, 5453 (1956).

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TRITIUM-LABELING BY EXPOSURE OF ORGANIC COMPOUNDS TO TRITIUM GAS[1]

Sir:

An ingenious method for labeling organic compounds with tritium by the action of recoil tritons has recently been proposed.[2] Although this method has wide applicability, radiation damage to compounds from the gamma flux and from recoiling alpha particles and tritons is rather extensive and limits the activities attainable.

It has now been found that exposure of organic compounds to tritium gas yields tritiated products of high activity without extensive radiation damage. Data summarized in Table I reveal that concentrations of tritium ranging from 1 to 90 millicuries per gram have been obtained in such diverse materials as *n*-heptane, toluene, benzoic acid, sucrose, cholesterol and digitoxin.

TABLE I

PRODUCTS LABELED BY EXPOSURE TO TRITIUM GAS

Compound exposed	Wt., g.	Time, days	Gas used, curies	Tritium incorporated Total, mc.	Labile, mc.	In pure, product, mc./g.
Toluene	0.86	2.9	7.5	42.7	None	22.2
n-Heptane	1.37	9.8	6.9	17.5[c]	None	1.3
Benzoic acid	1.31	5.0	6.4	156	40	14.0
Sucrose[a]	4.0	6.7	14.0	593	480	5.0
Cholesterol	1.88	4.8	7.2	335	90	64.3
Digitoxin[b]	0.50	5.8	7.5	438	182	90

[a] In collaboration with Dr. P. Latimer, Reynolds Tobacco Co., Winston-Salem, North Carolina. [b] In collaboration with Dr. G. Okita, Argonne Cancer Hospital, University of Chicago, Chicago, Illinois. [c] Exclusive of 150 millicuries of organically bound tritium in recovered gas.

The exchange of hydrogen induced by tritium radiation when organic compounds are exposed to tritium gas leads to organic bonding of as much as one per cent. of the tritium per day. As in the triton-recoil method of labeling, an appreciable fraction of this tritium appears in labile positions and in trace amounts of highly tritiated by-products. Even in the absence of gross chemical damage, therefore, rigorous purification is required to obtain a radiochemically pure compound. Also as in triton-recoil method,[3] the distribution of tritium in the product is not completely random. In the exposure of toluene to tritium gas, for example, a marked preference for aromatic bonding was demonstrated, 95% of the tritium being retained upon oxidation of the toluene to benzoic acid.

The self-induced exchange reactions occur at room temperature with sub-atmospheric pressures of tritium. The hydrogen content of the tritium gas preferably should be low, but removal of helium-3 formed by decay is not necessary. The tritium recovered from an exposure may be reused, but increased formation of by-products might occur unless the gas were purified, as by absorption on and regeneration from uranium. The amount of tritium incorporated for a given exposure will vary, of course, with the compound; in cases investigated so far the amount of tritium incorporated into purified reactant per curie-day exposure has ranged from 0.02 to 2.2 millicuries. Although there are no limits to the quantity of organic material which may be exposed, use of the smallest amount which can completely absorb the radiation offers some advantages. Since the β-particle from tritium has a range[4] of 0.7 mg./cm.^{2}, efficient absorption in solids or liquids can be achieved by distributing the material over the walls of a small vessel. Glass tubes 1 cm. in diameter and 10 cm. long have proved convenient for quantities of solids up to 1 g. Larger vessels are necessary to obtain efficient absorption in a vapor; bulbs of 100 ml. volume, maintained at 40° to permit a higher vapor density, were used in irradiation of heptane and toluene.

After exposure, materials containing labile hydrogen should first be treated to remove readily exchangeable tritium. The methods of purification employed depend, of course, on the individual compound. Although considerable purification can be effected by sublimation or recrystallization, multistage processes, such as fractional distillation, counter current extraction and chromatography, should be employed where possible.

The availability[5] of tritium gas at low cost and the high levels of activity attainable, even in materials of complex structure, combine to make exposure to tritium gas an attractive method for the preparation of tritium-labeled compounds. In addition, the technique provides a tool for study of radiation chemistry: identification of the products of radiation decomposition is facilitated by the presence of a tritium label, and information concerning the activation of different positions in a reactant is obtainable from the distribution of tritium in the molecule.

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[1] Work performed under the auspices of the United States Atomic Energy Commission; presented before the Division of Organic Chemistry at the 130th Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 16–21, 1956.

[2] (a) R. Wolfgang, F. S. Rowland and C. N. Turton, *Science*, **121**, 715 (1955); (b) F. S. Rowland and R. Wolfgang, *Nucleonics*, **14**, no. 8, 58 (1956).

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

[4] L. E. Glendenin, *Nucleonics*, **2**, no. 1, 12 (1948).

[5] Tritium gas can be obtained by licensed users at a cost, exclusive of handling charges, of $2.00 per curie from Oak Ridge National Laboratory, Oak Ridge, Tennessee.