50[7,8] followed by paper chromatography of the hydrolyzate in an ethanol-ammonia-water system,[9] sulfamic acid could be identified by its R_f value (0.34–0.37) and by its specific reaction with a nitrous acid–benzidine spray devised especially for this purpose.

The antibiotic has pK_a 9.3 as an acid, too high for a free sulfonic acid group, but of the same order of magnitude as the pK_a of a sulfonamide. Treatment of nucleocidin with barium nitrite in dilute acid at room temperature produced barium sulfate where treatment with barium chloride under the same conditions leaves the antibiotic unaffected. From the foregoing evidence we conclude that nucleocidin is not an amine sulfonate but an ester of sulfamic acid.

Nucleocidin, $C_{11}H_{16}N_6SO_8$,[1a] should, therefore, be formulated as II. Except for a few simple synthetic examples[10] no esters of N-unsubstituted sulfamic acid have been described previously. The unusual structure of the carbohydrate moiety of nucleocidin and the total structure of the antibiotic will be reported in a separate communication.

(7) J. X. Khym, *et al.*, THIS JOURNAL, **75**, 1262 (1953).

(8) J. X. Khym and W. E. Cohn, *ibid.*, **76**, 1818 (1954).

(9) A. G. Long, J. Quayle and R. J. Stedman, *J. Chem. Soc.*, 2197 (1951).

(10) Even the claims for simple alkyl sulfamates seem open to question. For references in this confused field, see: (a) R. Behrend, *Ann.*, **222**, 116 (1884); (b) W. W. Binkley and E. F. Degering, *Proc. Indiana Acad. Sci.*, **47**, 122 (1938); (c) **53**, 122 (1943); (d) R. Kuhn and W. Brydowna, *Ber.*, **70**, 1333 (1937); (e) W. Traube, H. Zander and H. Gaffron, *ibid.*, **57**, 1045 (1924).

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CHOLESTEROL—A PRECURSOR OF ESTRONE *IN VIVO*

Sir:

Following the report by Heard and O'Donnell[1] of the inability of the pregnant mare to synthesize estrone from C^{14}-labeled cholesterol, it was assumed that this sterol was not a precursor of the estrogen hormones.[2] Recently, however, evidence to the contrary has accumulated. The conversion of C^{14}-labeled testosterone[3–5] and 19-hydroxy-Δ^4-androstene-3,17-dione[6] to the estrogens have strongly implicated cholesterol as a precursor. We wish to record an experiment that demonstrates this conversion. A pregnant woman[7] was given 87.4 μc. of cholesterol-4-C^{14} (2.35 μc./mg.) over a six-day period during which time daily urine samples were collected and assayed for radioactivity (0.6% found). The urinary steroid conjugates were hydrolyzed with β-glucuronidase and extracted with ether at *p*H 5. The crude phenolic fraction, weighing 338 mg. and with 0.012% of the administered radioactivity, was obtained by using the procedure of Engel.[8] Carrier estrone, 3.875 mg. was added to the toluene before the extraction of the estrogens.[8] The ketonic residue from a Girard separation was chromatographed for 3 hours on 15 sheets of Whatman no. 1 paper in the methanol–water–benzene–petroleum ether system of Migeon, *et al.*[9] The area with an R_f corresponding to estrone was cut out and eluted with methanol. Using the Kober assay, 4.5 mg. were found. The material did not separate from authentic estrone in mixed paper chromatography in two solvent systems.[10] Estrone, 110.9 mg., was added as carrier and the acetate was prepared in the usual manner. The acetate was purified over charcoal and crystallized twice from aqueous methanol. The crystals were dissolved in 15 ml. of toluene containing 30 mg. of 2,5-diphenyloxazole (DPO) and the solution was counted in a Packard Tri-Carb Liquid Scintillation Counter.[11] The toluene was removed *in vacuo* and the acetate was freed from DPO by washing twice with 0.5 ml. of petroleum ether. After two crystallizations from aqueous methanol, the acetate was recounted. The toluene was then removed, the acetate hydrolyzed with 5% methanolic potassium hydroxide, and the estrone converted to the benzoate derivative.[3] The benzoate was crystallized from 95% ethanol twice (15 ml. each time) and was counted as described above for the acetate. After the toluene was removed and the crystallization steps again carried out, the counting was repeated. The counting data are shown in Table I. It is unlikely that the estrone was synthesized from a degradation product of cholesterol-4-C^{14} since this sterol does not appear to be degraded *in vivo* to any appreciable extent.[12]

TABLE I

SPECIFIC ACTIVITY OF ESTRONE ISOLATED BY CARRIER TECHNIQUE

Derivative	No. times crystallized	M.p.°[a]	Mg. counted	DPM[b]	DPM/mg. free estrone
Acetate	2	125.5–127.0	117.4	1615	15.9
Acetate	4	120.5–121.5	96.1	1335	16.0
Benzoate	2	217.0–219.5	84.7	890	14.7
Benzoate	4	216.5–218.5	77.0	806	14.5
Benzoate	6	216.0–218.0	68.9	761	15.3

[a] Taken on a Fisher-Johns apparatus, uncorrected. [b] Corrected for quenching effects (23–30%) on the counting. The counting efficiency was 61.5% and the background was 35 c.p.m.

When the placenta was removed the venous plasma free cholesterol specific activity was 1871 DPM/mg., while that of the placenta cholesterol (free + ester) was 816 DPM/mg. Although the data do not permit a determination of the fraction

(1) R. D. H. Heard and V. J. O'Donnell, *Endocrinology*, **54**, 209 (1954).

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(3) R. D. H. Heard, P. H. Jellnick and V. J. O'Donnell, *Endocrinology*, **57**, 200 (1955).

(4) H. W. Wotiz, J. W. Davis, H. M. Lemon and M. Gut, *J. Biol. Chem.*, **222**, 487 (1956).

(5) B. Baggett, L. L. Engel, K. Savard, and R. I. Dorfman, *ibid.*, **222**, 931 (1956); *Fed. Proc.*, **14**, 175 (1955).

(6) A. S. Meyer, *Biochem. Biophys. Acta*, **17**, 441 (1955).

(7) Six days before a scheduled therapeutic abortion.

(8) L. L. Engel, *Recent Progress Hormone Research*, **5**, 343 (1950).

(9) C. J. Migeon, W. R. Slaunwhite, Jr., R. Aldous, R. Fox, R. Hardy, D. Johnson and W. Perkins, *J. Clin. Endocrinol. & Metab.*, **15**, 775 (1955).

(10) Toluene–propylene glycol[8] and that described by Migeon *et al.*[9]

(11) Commercially available from Packard Instrument Corp., LaGrange, Illinois.

(12) H. Werbin, D. M. Bergenstal, R. G. Gould and G. V. LeRoy, *J. Clin. Endocrinol. & Metab.*, in press.

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.