Radiochemical Purity of Tritium Exchange Labeled Barbital

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Barbital (diethylmalonylurea) was isotopically labeled with tritium by the Wilzbach gas exposure procedure. The specific activity of the tritiated barbital was 266 mc./Gm. after removal of labile tritium. The following criteria of homogeneity were employed: recrystallization to constant specific activity using 2 solvent systems; column liquid-liquid partition and paper chromatography; and evidence for stability in a biological system. Final specific activity was 253 mc./Gm.

THE ADVANTAGES associated with the use of tritium as a tracer isotope prompted our studies with barbital (diethylmalonylurea) tritiated according to the Wilzbach gas exposure procedure (1). Our purpose was to determine radiochemical purity as well as biological stability of this exchange labeled tritiated compound. Criteria of homogeneity included recrystallization to constant specific activity using two solvent systems; column liquid-liquid partition and paper chromatography; and evidence for stability in a biological system.

EXPERIMENTAL

Analytical.—Radioactivity measurements were carried out using the Tri Carb Beta liquid scintillation spectrometer.[1] Five-dram vials made from glass of low potassium content were used to hold radioactive samples.[2] Aqueous solutions containing tritium radioactivity were incorporated into a xylene:dioxane:ethanol (5:5:3) system containing in each liter, 80 Gm. naphthalene, 5 Gm. 2,5-diphenyloxazole (PPO), and 0.1 Gm. 1,4-di[2-(5-phenyloxazolyl)] benzene (POPOP) (2). Toluene containing 0.3% PPO and 0.01% POPOP was used for counting radioactive samples miscible with it. Barbital was determined spectrophotometrically in 0.5 *N* sodium hydroxide at 255 mμ (3).

Tritiation, Exchange, and Recrystallization.—Barbital sodium was tritiated according to the Wilzbach (1) procedure by the New England Nuclear Co., Boston, Mass. The compound was exposed to 10 c. of carrier-free tritium gas for 2 weeks at room temperature and 0.39 Atm. pressure. The tritiated product was repeatedly equilibrated with water by the New England Nuclear Co. and in our laboratory to remove labile tritium. Specific activity of the free acid following the equilibration steps was 266 mc./Gm. The tritiated barbital was successively crystallized from ethanol-water, three times from toluene, and finally from water, yielding a product which retained approximately 96% of the radioactivity present after removal of labile tritium. Unlabeled barbital was added before final recrystallization from water, however, so that the actual specific activity was reduced to 166 mc./Gm.

Paper Chromatography.—Unlabeled barbital and barbital-t were chromatographed on Whatman No. 1 paper using *n*-butanol:5% ammonium hydroxide (1:1) (4). Radioactive areas were visualized autoradiographically. The papers were sprayed with 0.1 *N* aqueous silver nitrate:10% ammonium hydroxide (1:1) for detection of the barbital (5). A substance with very low specific activity and which was more polar than barbital was detected in the first recrystallization product. For all other cases, the darkening of the film was coincident with the barbital-t area and parallel to unlabeled barbital chromatographed concurrently.

Column Liquid-Liquid Partition Chromatography.—Barbital-t was chromatographed on a diatomaceous earth[3] column (6) using borate buffer 0.1 *M*, pH 9.2 as the internal phase and chloroform:butanol (93:7) as the external phase. Following the third recrystallization from toluene, barbital-t exhibited a homogeneous peak followed by a minor peak containing less than 5% of the radioactivity. The homogeneity of the major component was confirmed by analyzing the specific activities of fractions throughout the peak by the method of Baggett and Engel (7). The slope of specific activities for successive tubes through the peak was not significantly different from zero. After recrystallization from water, the barbital-t exhibited a single peak as illustrated in Fig. 1. The specific activity of six combined peak fractions was the same before and after column partition and the minor peak was no longer evident.

Biological Stability.—A suitable dilution of the final product was injected intravenously into a rabbit. Two hours later barbital was recovered from the serum by the method of Askevold and Løken (8). Table I shows the amount of barbital and radioactivity injected and recovered from the serum. Specific activities shown in the last column agree within 5%. This is within the range of experimental error associated with the analytical procedure.

DISCUSSION

Three types of evidence have been presented to demonstrate the degree of purity of exchange-labeled tritiated barbital. First, there was negligible loss of tritium radioactivity upon successive

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[1] Model 314-X, Packard Instrument Co., La Grange, Ill.
[2] Five-dram crystallite vial No. A-3305, T. C. Wheaton Co., Millville, N. J.
[3] Celite 545, Johns-Manville Products, New York, N. Y.

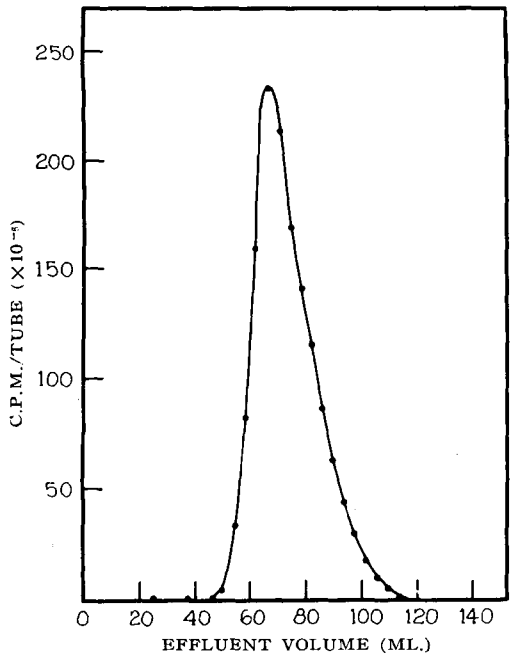

Fig. 1.—Celite column partition chromatogram of barbital-t.

recrystallization from toluene and water. Second, chromatographic methods indicated that a single substance, identical with barbital, constituted most of the detectable radioactivity. On paper chromatography of the first crystalline product, and in one column chromatogram of the third toluene recrystallization product, a minor radioactive component was present. The minor component of the column chromatogram constituted less than 5% of the radioactivity. After subsequent recrystallization from water, this secondary peak did not appear. Third, barbital-t lost little, if any, of its tritium after injection into an experimental animal and recovery after 2 hours. This latter observation nearly eliminates the possibility of the presence of high specific activity contaminants in barbital-t inasmuch as we would not expect, if contaminants were present, that their distribution, metabolic degradation, sojourn in the body, or chloroform extractability from serum would be identical with that of barbital. We have concluded from these results that a product with a high degree of radiochemical purity and stability suitable for pharmacologic studies was obtained. After removal of labile tritium by exchange with water, the barbital-t retained approximately 96% of the remaining isotope through recrystallization and chromatography. This is the highest yield yet reported for the Wilzbach exchange-labeling method. If the barbital-t had been subjected to the procedures outlined without dilution with unlabeled barbital, its specific activity would have been 253 mc./Gm. This represents the presence of approximately 1 tritium atom per 610 molecules of barbital. The results with this compound are unusual since there was little evidence for the presence in the labeled material initially of a high specific activity contaminant.

TABLE I.—BARBITAL AND RADIOACTIVITY INJECTED AND RECOVERED FROM RABBIT SERUM

Barbital-t	Radioactivity, C/M	Sample Weight, mcg.	Specific Activity C/M per mcg.
Administered to rabbit	2.04×10^9	6.22×10^5	3280
Isolated from rabbit serum[a]	1.125×10^7	3.6×10^3	3125

[a] Two hours following intravenous injection.

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