Preparation of Tritiated 7,12-Dimethylbenz(a)anthracene

The preparation of 7,12-dimethylbenz(a)anthracene (DMBA) containing tritium was undertaken (*1*) with the purpose of having available a strong carcinogen that might be used in studying its metabolism in mouse skin. The carcinogen was labeled with tritium by a direct-exchange method. The tritiated compound had a specific activity of 1.62×10^5 disintegration/min mg. Labeling of a carcinogenic polycyclic aromatic hydrocarbon with tritium has not been previously reported.

Replacement on the aromatic nucleus of hydrogen atoms with tritium atoms was accomplished by means of direct exchange in the presence of a catalyst. The exact mechanism of the exchange reaction is not known. However, it has been used successfully in labeling benzene, cholesterol, and Δ^4-androstene-3,17-dione with tritium, and cholesterol with deuterium (*2*).

Two samples of tritiated DMBA were prepared, the temperature of the reaction being the only variable. Seven hundred seventy-five milligrams of DMBA was weighed into a heavy-walled Pyrex tube. Seventy-five milligrams of reduced platinum dioxide in a solution of 9.2 ml of glacial acetic acid, 2.0 ml of glass-distilled water, and 2.0 ml of tritiated water (containing 4.0 mc/ml) (*3*) were added to the reaction tube. The tube was evacuated, flame-sealed, and then placed inside a length of iron pipe that was capped at both ends. The reaction mixture for the first sample was shaken at room temperature for 14 days; for the second sample, the mixture was placed in a shaker oven at 100° C for 14 days.

Following the reaction period the liquid was distilled off in a vacuum at 50° C, and the crystalline product was dissolved in redistilled petroleum ether (Skellysolve B) and chromatographed in the dark on Florisil (100 to 200 mesh) with petroletum ether as the eluant. The progress of the chromatography was observed by occasional momentary exposure to ultraviolet light. Two fluorescent bands were observed. The main band was bright blue and was rapidly eluted with petroleum ether and collected. Just beneath the platinum, which remained as a black deposit on the surface of the Florisil column, a narrow, yellow fluorescent band was observed; this band did not change position during development of the column.

The crystalline product was recovered from the petroleum ether eluate by slow evaporation under a nitrogen stream. The crystals were very pale yellow and melted at 123.0° to 123.8°C (recorded mp of nonradioactive DMBA: 122.8°–123.5°C, corrected, *4*). When dissolved in 95 percent ethanol, the material showed an ultraviolet absorption spectrum identical with that of the nonradioactive carcinogen.

The tritium-labeled carcinogen was dissolved in toluene containing 0.3 percent 2,5-diphenyloxazole, and the radioactivity was determined with a liquid scintillation counter (*5*). Corrections of the counts were made for internal quenching by using a standard DMBA solution containing known added radioactivity.

After corrections for internal quenching and for counting efficiency had been made, the specific activity of the sample prepared at room temperature was 2.98×10^3 disintegration/min mg, and that of the sample prepared at 100°C was 1.62×10^5 disintegration/min mg. The application of heat to the reaction mixture produced a 54-fold increase in the radioactivity of the sample.

Although it is recognized that the method described here produced samples of relatively low radioactivity, it is probable that samples with a much greater specific activity might well be obtained by using water containing a high percentage of tritium.

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References and Notes

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3. Obtained from Abbott Laboratories on allocation from the U.S. Atomic Energy Commission.
4. J. A. Miller and C. A. Baumann, *Cancer Research* **3**, 217 (1943).
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Suppression of Radiation Interference in Flame Photometry by Protective Chelation

Radiation interference is the enhancement or suppression of the light emitted by an excited ion when other species of ions are present in solution. This phenomenon was first observed in the determination of cations in urine by emission spectroscopy (*1*). It was found that the radiation interference was proportional to the amount of interfering ion present until a limiting value was approached. Correction was attempted by the use of synthetic standards containing predicted amounts of interfering substances or by a "method of excess" in which both samples and standards were made up to contain amounts of interfering ions sufficient to cause maximum suppression.

Table 1. Effects of radiation interfering substances on luminosity of calcium at 554 mμ.

Interfering substance (ppm)	Net luminosity of 48-ppm calcium samples determined as $CaCl_2$	
	Control	In the presence of 5000 ppm EDTA and 6000 ppm KOH
None	54	49
Sulfate (500)	26	49
Nitrate (500)	38	50
Phosphate (500)	13	29
Magnesium (20)	50	51

The development of the flame photometer (*2*) made possible the rapid, accurate analysis of many cations. However, radiation interferences made difficult the determination of calcium and magnesium from biological and geologic sources. The radiation interferences of sodium and potassium may be eliminated by the use of an internal standard of additional calcium (*3*). The method of excess does not yield accurate results in the case of interference by phosphate in biological samples (*4*). Of the anions studied here (*5*), nitrate, sulfate, and phosphate produce an increasing degree of suppression (Table 1).

Ethylenediaminetetraacetic acid (EDTA) may be used to chelate polyvalent metal ions in a solution and thus protect the calcium from the radiation interference of other ions (*6*) (Table 1). The correction of the interference of sulfate, nitrate, and magnesium on calcium is demonstrated within the precision of the method (± 1 unit). The interference of the phosphate is markedly reduced compared with the external standard for the series.

However, by a combination of the protective chelate and internal-standard techniques, accurate determinations may be obtained of up to 25 ppm of calcium ion in the presence of 300 ppm of phosphate solutions containing 5000 ppm of EDTA and 6000 ppm of KOH. The chelate functions to prevent a radical change in the amount of calcium that is free to interact with phosphate within the limits of the calcium-EDTA equilibrium. Solutions of calcium chloride