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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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

exhibit a linear increase of luminosity as a function of concentration with up to 50 ppm of calcium ion in the presence of 5000 ppm of EDTA and 6000 ppm of KOH.

The applications of protective chelation with the use of internal standards for the flame-photometric determination of calcium and other cations in biological samples are under investigation.

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Evaluation of Chloroform Activation of Human Plasminogen

The presence of spontaneous proteolytic activity in human blood was first recognized 60 years ago (*1*). The observations were extended when it was discovered that chloroform treatment of human serum increased the proteolytic activity (*2*). Recently it was found that the production of proteolytic activity of human plasma or, in later experiments, of fraction III isolated from human plasma by cold ethanol fractionation (*3*) was more effectively achieved by reaction with streptokinase, a metabolic fermentation product of certain streptococci (*4*). It was of even more interest, however, that the reaction mixture of fraction III and streptokinase possessed considerable fibrinolytic activity.

In contrast, treatment of human fraction III with chloroform did not produce measurable fibrinolytic activity. The difference in behavior of the two activators has created considerable confusion; we have made an attempt to explain these divergences.

An aqueous acid extract of human fraction III was adjusted to a *p*H of 7.8, shaken with one-fifth its volume of chloroform, and left in contact at room temperature. At intervals, samples were analyzed for proteolytic activity by measurement of casein hydrolysis according to the method of Kunitz (*5*).

Proteolytic activity developed slowly but measurably over a period of several days. Addition of minute amounts of streptokinase-activated human fraction III (that is, human activator) increased the proteolytic activity during the first days of incubation, whereas after about 10 days of standing in contact with chloroform, no additional proteolytic activity was produced.

Concurrently, the determination of fibrinolytic activity of the reaction mixture was carried on according to our modification (*6*) of the procedure of Christensen (*4*). No measurable activity could be detected. However, addition of streptokinase brought forth rapid lysis of the standard fibrin clot.

These experiments may readily be explained in view of the recently proposed mechanism for the "activation of human plasminogen by streptokinase" (*7*). Only the proteolytic precursor is converted in contact with chloroform to the active proteolytic enzyme. The same conversion can be achieved by catalytic amounts of human activator which is produced by the reaction of streptokinase with human fraction III. This latter path of conversion is catalyzed by human activator to the extent of the presence of proteolytic precursor. After its exhaustive conversion in contact with chloroform, addition of human activator will not increase the proteolytic activity (Fig. 1).

The proteolytic component of human fraction III did not effect this conversion. Human fraction III that had been activated by streptokinase was heated at *p*H 2.0. The mixture contained 50 percent of the original proteolytic activity, but it did not possesss fibrinolytic activity or the ability to catalyze the conversion of the proteolytic precursor to the active proteolytic enzyme.

The proactivator component of human fraction III was not affected by treatment with chloroform and could be converted to the activator by reaction with streptokinase regardless of the length of time of prior incubation with chloroform. The resulting fibrinolytic activity is a direct measure of the activator activity and can be demonstrated only on a fibrin substrate that contains the proteolytic precursor (*8*). The therapeutic efficacy of streptokinase-activated human fraction III depends therefore on its activator content, since the clot-dissolving activity of the proteolytic enzyme is very small.

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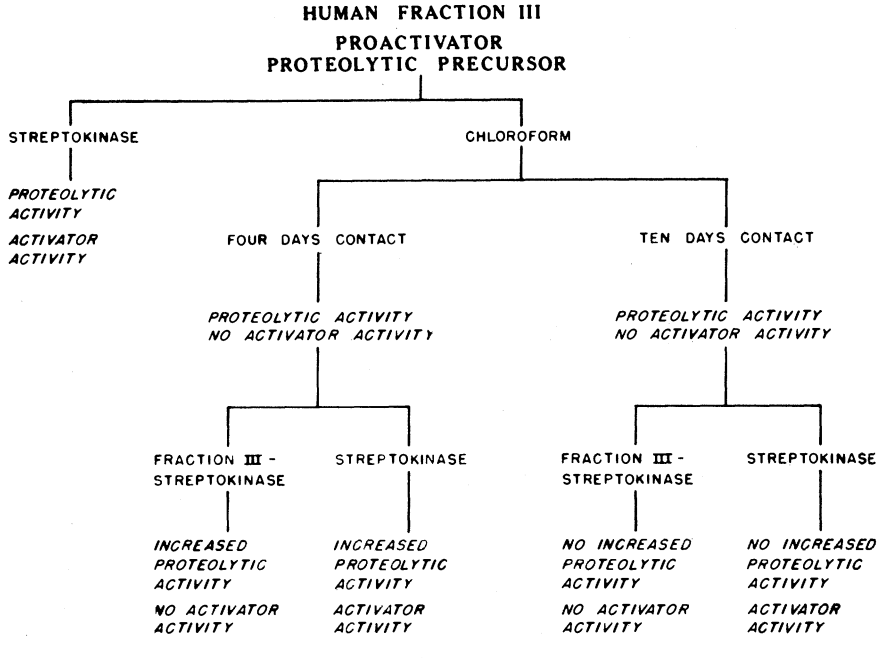

Fig. 1. Activation of human fraction III by chloroform or streptokinase.

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