Radioautography of ^{14}C- and ^{3}H-labeled steroids on thin-layer chromatograms*

In order to extend the usefulness of thin-layer chromatography to experiments with trace amounts of isotopically labeled compounds of high specific activity it is necessary to detect the radioactivity on the plate. Radioautography permits the radioactivity to be recovered from one portion of the plate and the remainder to be sprayed with a suitable reagent to develop unlabeled reference compounds which have been chromatographed at the same time. Although the use of radioautography with thin-layer chromatograms has been reported[1], we believe the method described below is convenient and that our results will be usefull to other workers.

In order not to injure the coated surface of such a chromatogram during radioautography a special cassette was devised (Fig. 1). In this an 8 × 8 in. plate, coated side up, fits into a well of the same depth as the thickness of the glass. The fit is

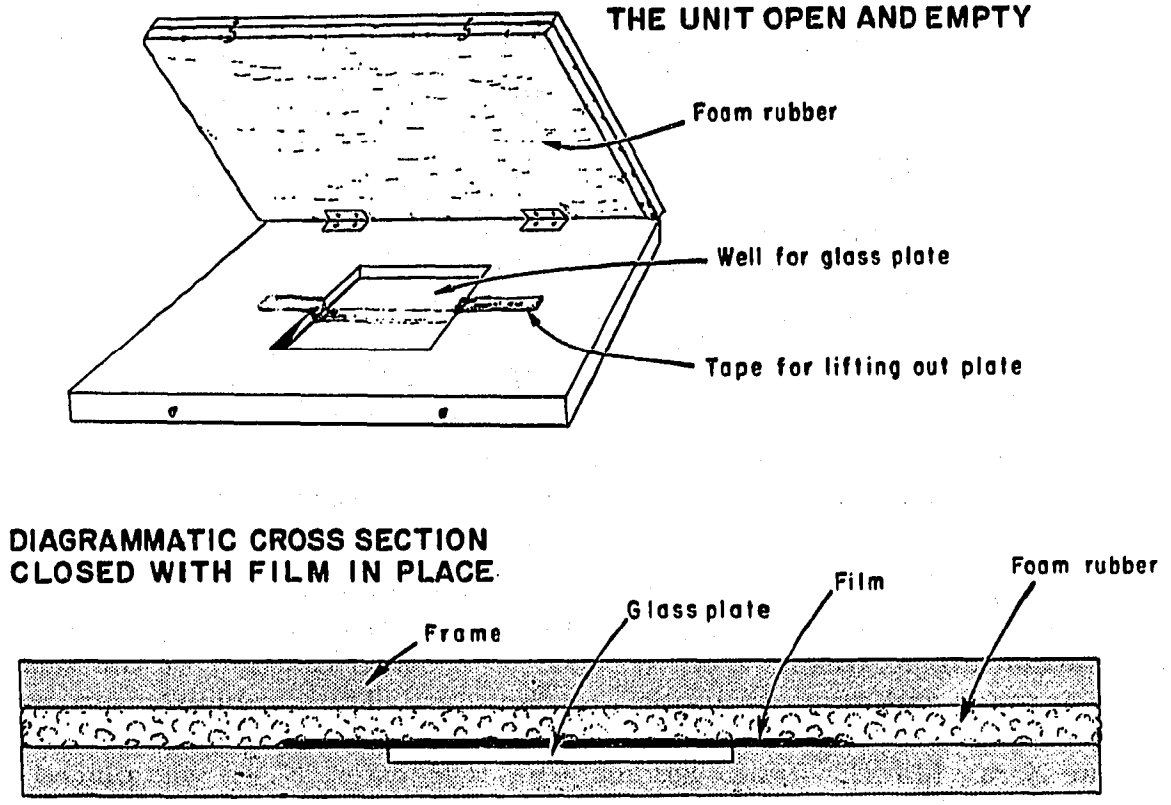

Fig. 1. Cassette for radioautography.

tight enough so that there can be no motion of the plate. (A strip of gauze laid under the plate so that the ends emerge on either side makes it easy to remove the plate from the well.) A layer of fine-texture foam rubber glued to the under surface of the lid of the cassette serves to hold the film against the plate in non-skid fashion with

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even pressure when the cassette is closed. This, plus a 3-in. margin in all directions around the plate, serves to exclude light.

An experiment with a ^{14}C-labeled compound was carried out and the resulting chromatogram is illustrated in Fig. 2. A solution of testosterone-4-^{14}C in 95 % aqueous

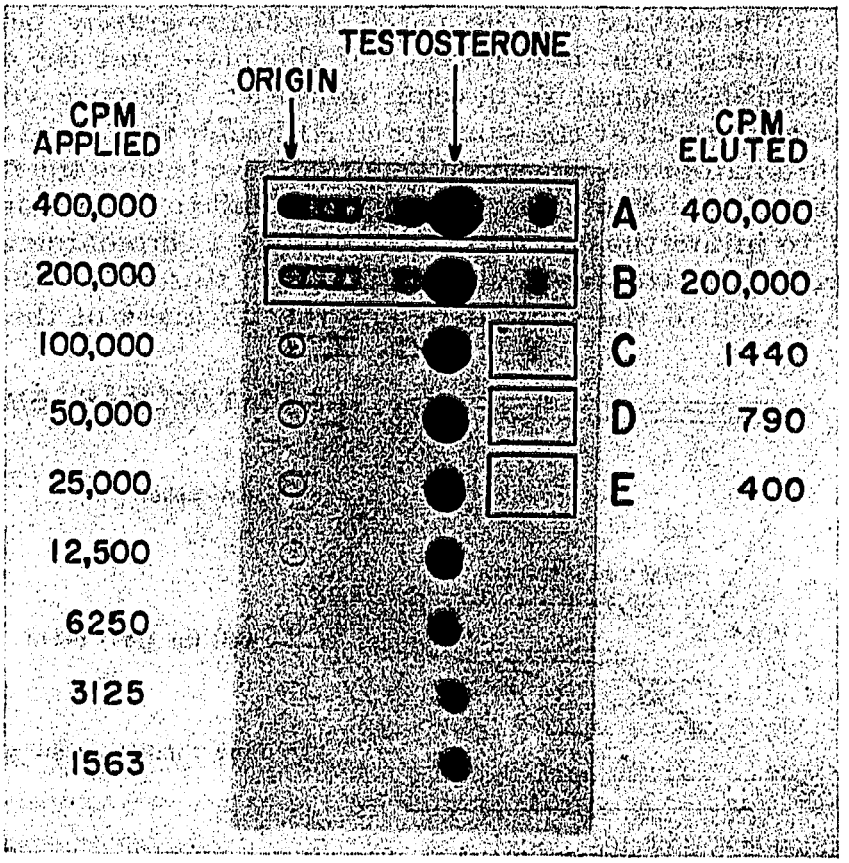

Fig. 2. Radioautograph of a chromatogram of a sample of testosterone-4-^{14}C.

ethanol containing 800,000 c.p.m./ml was prepared (1 μC equivalent to $1.1 \cdot 10^6$ c.p.m.). A thin layer (250 μ) of silica gel G (E. Merck, Darmstadt, Germany) on glass was prepared according to the method of STAHL[2]. A serial dilution of the testosterone solution was made and aliquots applied to the plate. The chromatogram was developed in ether–benzene (2:1, v/v). After evaporation of the solvent at room temperature Royal Blue X-ray film (Eastman Kodak, Co., Rochester, N.Y.) was placed in direct contact with the silica gel layer in the cassette.

At the end of 9 days the film was removed and developed in the prescribed manner. Radioactive areas on the plate were located by placing it over the film on a horizontal X-ray viewer. These areas were marked with a needle and then transferred from the plate into fluted filter papers. For this a nichrome spatula with one end ground to a straight, sharply beveled edge was used. The steroid was eluted with 20 ml of absolute methanol and aliquots of the eluates were counted. Areas A and B of Fig. 2 include the entire track as detected on the film. From each of these areas the same amount of material was recovered as had been applied at the origin, or 400,000 and 200,000 c.p.m. respectively. (In general we have been able to recover 90 %

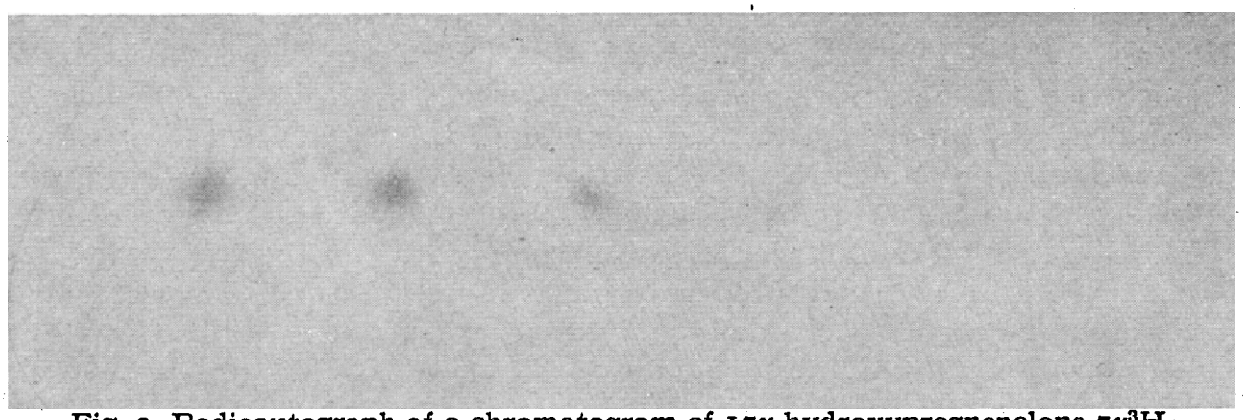

Fig. 3. Radioautograph of a chromatogram of 17α-hydroxypregnenolone-7α^{3}H.

or more of applied radioactivity.) Areas C, D and E, corresponding to the least exposed areas on the film, contained 1440, 790, and 400 c.p.m. respectively. The sites of application of material at the origin averaged 0.4 cm^2 in area and the less well defined areas of the spots at C, D and E averaged roughly 0.5 cm^2. The radioautograph, then, detects 400 c.p.m. ($4 \cdot 10^{-4}$ μC) of ^{14}C in 0.5 cm^2 in 9 days, and can be expected to detect 7200 c.p.m. ($7.2 \cdot 10^{-3}$ μC) per cm^2 in one day, or in more general terms $5 \cdot 10^6$ accumulated c.p.m./cm^2.

In order to make accurate recoveries of material from chromatograms having multiple radioactive and standard spots we have usually placed the chromatogram over a light source, covered it with a protective layer of cellophane, then placed the

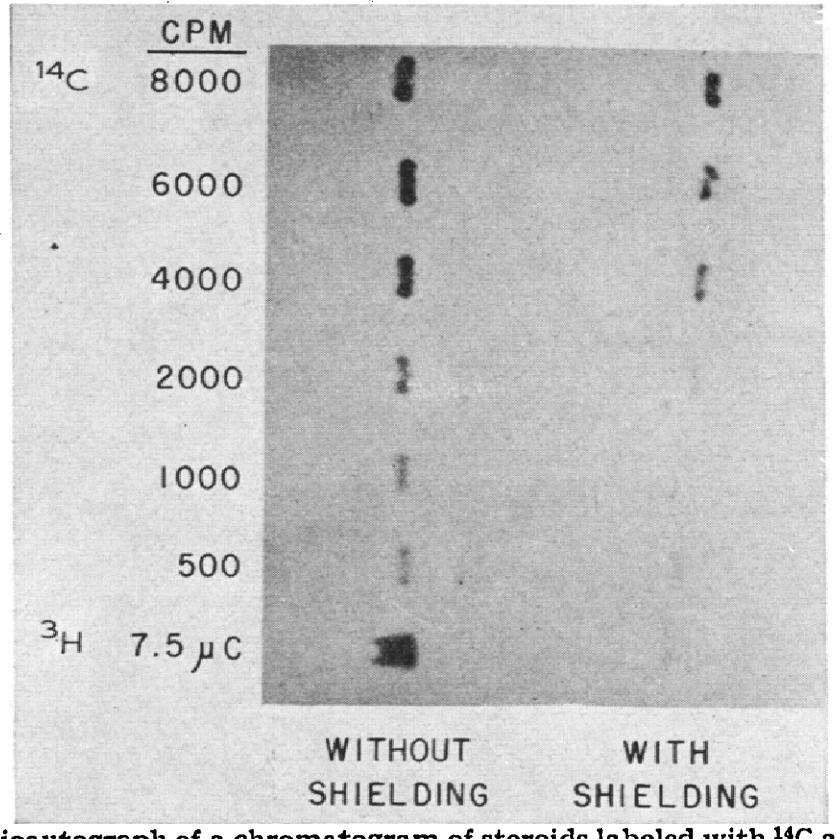

Fig. 4. Radioautograph of a chromatogram of steroids labeled with ^{14}C and tritium.

radioautograph over it, centering it by means of radioactive dye markers. If the exposed areas on the film are then traced with a pencil or similar instrument the outline is carried through to the plate without otherwise marking the surface. The areas so marked are removed, and the remainder of the chromatogram is sprayed to develop standards. The film is again placed on the chromatogram with a protective layer of cellophane in place and the outlines of the standard spots traced onto it in pencil. The X-ray film thus serves as the permanent record of the chromatogram.

The method applied to tritiated material is illustrated in Fig. 3 which shows a radioautograph of a chromatogram of 17α-hydroxypregnenolone-7α^3H. (New England Nuclear Corporation). From left to right 1.0 μC, 1.0 μC, 0.5 μC, 0.3 μC, and 0.2 μC, were applied at the origin. The chromatogram was run in ether–benzene (2:1, v/v) and radioautographed for 24 h.

The marked difference in energy level and range of ^{14}C and ^{3}H make it possible to differentiate between the two on a chromatogram by comparing a radioautograph with interposed shielding with another made without it. For ^{14}C the maximum energy is 0.156 meV and the range in aluminum is 25 mg/cm^2. For ^{3}H the corresponding figures[3] are 0.018 meV and 0.7 mg/cm^2. Approximately half of ^{14}C is adsorbed by 3 mg/cm^2 while ^{3}H is completely absorbed in such a layer. Fig. 4 shows a comparison of radioautographed spots containing the same amount of activity with and without shielding. In this instance Cellophane (Dupont C.), weighing 3.35 mg/cm^2., was used. It will be seen that the intensity of radiation as reflected by the density of the spot was reduced by about one-half in the case of ^{14}C, while in the case of ^{3}H only the faintest exposure of the film occurred on the shielded side even though 7.5 μC was present.

We have found that estradiol which is completely free of radioactivity as demonstrated by scintillation counting produces a faint autograph on X-ray film when present in amounts of 20 μg or more. While this is the only steroid thus far found to do this, it emphasizes the need for controls in radioautographing any new compound.

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