

NOTE

**AUTORADIOGRAPHY WITH PHOTOGRAPHIC PAPER OF THIN-LAYER
CHROMATOGRAMS OF RADIOCARBON-LABELLED COMPOUNDS**

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SUMMARY

By exposing a thin-layer chromatogram of 4-¹⁴C-labelled steroids directly onto a sheet of photographic paper, evidence was obtained on the distribution of radioactivity on the chromatogram. This method offers a relatively easy way to make a copy for publication and recording.

Key words : Paper-autoradiogram, ¹⁴C-Labelled steroids, Thin-layer chromatogram, and Photographic paper.

INTRODUCTION

Macroautoradiography has been widely applied for detection of radioisotopes in the field of biological and medical sciences¹⁾. For metabolic study of steroid hormones at their physiological concentration, radiocarbon-labelled steroids have been employed as substrates. Among separation procedures, paper and thin-layer chromatography have been frequently used, and the radioactive spots on paper²⁾ and thin-layer³⁾ chromatogram are detected by autoradiography by exposing them onto medical X-ray film, because of its simplicity and substantially higher resolution than commercial radiochromatogram scanners. When the X-ray film-autoradiogram (hereafter, abbreviated as film-autoradiogram) is directly subjected to copy production by an electrostatic copy machine such as Xerox, the produced copy is not satisfactory,

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because the background of developed X-ray film is relatively dark and the quality of the copy is poor in contrast terms. In order to prepare a photographic copy from autoradiograms on X-ray film for publication, the following three procedures have been employed ;

Method (1). A direct positive copy (in purple) of film-autoradiogram is obtained by a blue-print (diazodye) copy machine. As the diazo-copy is relatively labile against sunlight, it is deteriorated during storage for a long time. Therefore, its black and white copy is used to be made by Xerox or other comparable copying machine.

Method (2). Negative of the film-autoradiogram is obtained by contact exposure of the autoradiogram to another sheet of X-ray film, and then its positive picture on photographic paper becomes available from the negative by contact printing procedure.

Method (3). Negative film is obtained by a camera from the film-autoradiogram on translucent glass lighted through the other side, and from the negative, a positive autoradiogram on photographic paper is available by enlarging it.

Among them, provided that a blue-print copy machine is available, Method (1) is the most simple and easy, because no further work in a dark room is necessary. In this communication, we would like to report a simple method of preparing **paper-autoradiogram** directly from thin-layer chromatogram of ^{14}C -labelled compounds.

MATERIALS AND METHODS

Metabolic study of steroid. 4- ^{14}C -Labelled testosterone was anaerobically incubated with hepatic homogenates of adult fowl in the presence of NADPH. After the incubation, radioactive metabolites together with the substrate were extracted with dichloromethane and an aliquot of the extract was subjected to silica gel thin-layer chromatography developed by mixture of benzene and acetone.

Preparation of autoradiogram. In the similar manner for preparing an autoradiogram with X-ray film, we put the commercial photographic paper for enlargement (black and white, for example, ILFOSPEED 3.1M Glossy) upon the thin-layer chromatogram, so that the emulsion side of the photographic paper is closely in contact

with the silica gel surface of the chromatogram. Then we allowed to expose for 4 weeks, taking into account that the photo-sensitive emulsion layer is thinner in the paper than in usual X-ray film. After the exposure, the paper was developed by regular developing procedures (D-72 as developer and F-5 as fixer, according to Kodak prescription or equivalents). The developed and fixed paper was dried after washing, and a paper-autoradiogram is obtained. In addition, the same thin-layer chromatograms were exposed to a sheet of medical X-ray film according to the usual technique as mentioned above.

RESULTS AND DISCUSSION

As shown in Fig. 1(A), the autoradiogram on the photographic paper indicated the radioactive spots over the white photographic paper instead of film. On the other hand, Fig. 1(B) was obtained according to Method (1). From the X-ray film-autoradiogram, a diazo-dye copy was prepared, from which an electrostatic copy in black and white was made. Among the three major spots, the steroid in the least polar spot was identified as androstenedione (6,484 dpm), and the most polar steroid was identified as 5 -androstane-3 ,17 -diol (21,919 dpm), while the middle between them was the mixture of 5 -dihydrotestosterone, 5 -androstane-3 ,17 -diol and testosterone (recovered substrate) (total 74,745 dpm), which were individually identified later.

A better result was to be obtained by paper-autoradiography for tracing weak radioactivity closer to background, because of less frequency of copying. According to our experience, the principle of preparing paper-autoradiogram was found readily applicable for polyacrylamide gel electrophoresis (PAGE) of radioiodine-labelled protein for less time of exposure. The direct production of autoradiogram on paper became available by this method and the radioactive images were distinctly detected, whereas one or two steps of image transfer were required for the above listed indirect methods. Also a paper-autoradiogram is accepted as direct evidence for distribution of radioactivity, and copies in appropriate size suitable for publication are easily prepared by any copy machine.

Recently, at our request, Fuji Photo Film Co. Ltd. (Tokyo, Japan) prepared a kind of "X-ray paper" for trial by coating the same photo-sensitive emulsion as

that of X-ray film onto paper. As a result, the exposure time required for the paper autoradiography was shortened to 7 days.

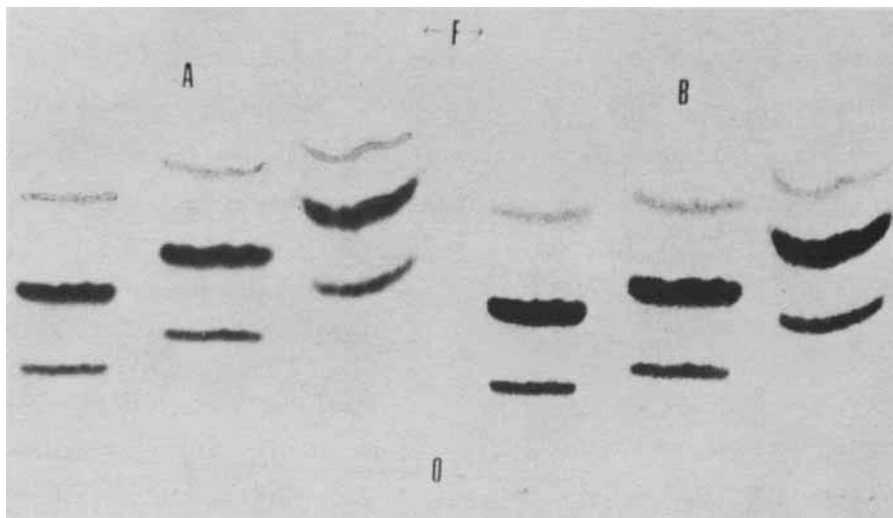


Fig. 1. Autoradiograms. (A) Paper-autoradiogram directly prepared by exposing photographic paper with thin-layer chromatograms for 4 weeks with radiocarbon-labelled steroids. The three chromatograms were obtained by developing with benzene : acetone (4 : 1, 3 : 1, and 2 : 1, v/v) system, from left to right, respectively. (B) Film-autoradiogram prepared by exposing X-ray film for a week with the same thin-layer chromatograms. And then black and white copy of its diazo copy was prepared by Method (I). O and F in the figure stood for the origin and the solvent front, respectively.

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