Autoradiography of tritium chromatograms

Good autoradiographic pictures of many radio-isotopes can easily be obtained from paper chromatograms by pressing the chromatogram against an X-ray film for a suitable exposure time. With tritium, however, this technique is unsatisfactory because of the very low energy of the emitted beta particle, which has a mean penetration range of less than one micron (*cf.* ref. [1]) when the density of the medium exceeds 1.

ROGERS has described a method to overcome this difficulty by submerging the chromatogram in a liquid nuclear emulsion[2]. In this way an intimate contact between the isotope and the silver bromide is established. However, many substances would be extracted from the chromatogram by this technique. In the present paper a modification of ROGERS' procedure is described which permits autoradiographic analysis also of highly soluble tritiated substances in chromatograms. Instead of submerging the chromatogram in the liquid nuclear emulsion, the emulsion is sprayed on the chromatogram. Also some additional data are given here, which might be of experimental value.

Fig. 1 illustrates the application of the technique to a tritium and ^{14}C chromato-

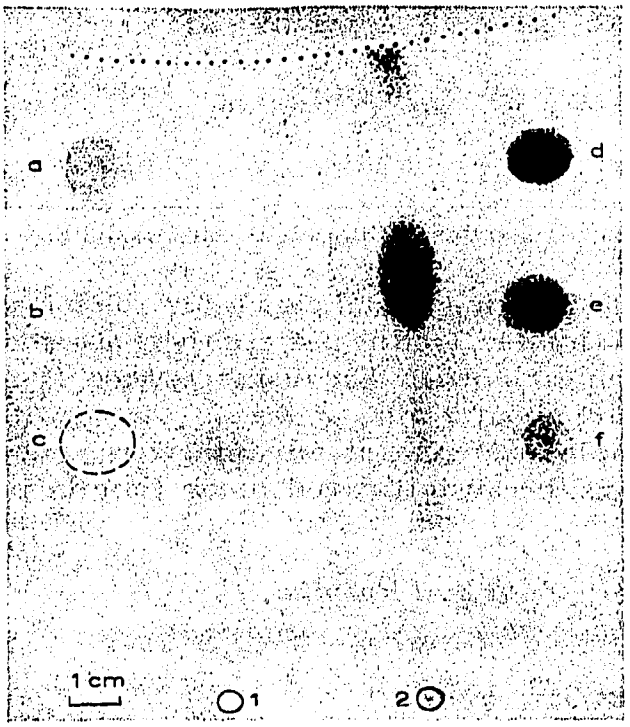

Fig. 1. Paper chromatogram of (1) $8 \cdot 10^{-3}$ μC of ^{14}C-valine and (2) 10 μC of ^{3}H-leucine run for 6 h in a mixture of butanol, acetic acid, and water (780:50:170). It was sprayed with a G5 emulsion, exposed for 4 days, and processed for 2 min in D–19b. The reference spots a–f were applied after chromatography: a–c contained $6 \cdot 10^{-3}$ μC, $2 \cdot 10^{-3}$ μC, and $7 \cdot 10^{-4}$ μC of ^{14}C-leucine (Radiochemical Centre, Amersham); d–f contained 5 μC, 1.7 μC, and 0.6 μC of ^{3}H-leucine (New England Nuclear Corp.).

gram. Further experimental data pertaining to this method are given in Table I. The chromatographic paper used in these experiments was Whatman No. 1. This

TABLE I

AMOUNT OF ISOTOPE AND EXPOSURE TIME IN AUTORADIOGRAPHY OF CHROMATOGRAMS

Isotope	*Amount of isotope to give a spot just visible after 10 days of exposure*		*Grain yield per disintegration in just visible spot* *
	μC per cm²	*Number of disintegrations per min and cm²*	
^{3}H**	$3 \cdot 10^{-2}$	$7 \cdot 10^{4}$	0.3
^{14}C***	$1 \cdot 10^{-3}$	$2 \cdot 10^{3}$	10

* The calculations were based on the observation that 50 grains per 100 μ^2 give a just visible spot in ordinary autoradiograms with G5 emulsions.
** ^{3}H-DL-Leucine (New England Nuclear Corp., Boston, Mass.).
*** ^{14}C-L-Leucine (Radiochemical Centre, Amersham, England).

paper was, however, found to desensitize the nuclear emulsion for beta particles, an effect that was overcome by treating the paper before use with diluted (0.3 *M*) hydrochloric acid for a few hours. The paper was then washed for 1 h in running tap water and finally rinsed several times in distilled water.

The nuclear emulsion used was Ilford G5. It was melted by heating to 50° and diluted with 7 volumes of distilled water. The chromatogram was then sprayed with the emulsion using an all-glass spray and nitrogen or carbon dioxide for the gas stream. 10 ml of emulsion was found sufficient for 400 cm² of paper. Drying of the chromatogram was completed in about 1 h in a box with an electric fan and calcium chloride as desiccant. The dry chromatogram was carefully wrapped in aluminium foil to prevent light coming into contact with the emulsion. All these operations were carried out in a dark room illuminated with a 25 Watt bulb, and using a Wratten filter No. 1.

The exposure was carried out in a refrigerator. A suitable exposure time might be calculated from the data in Fig. 1 and Table I. The exposed chromatogram was processed in Kodak D–19b developer for 2 min followed by a stop bath. It was then fixed in an acid hypo for 15 min, and rinsed for at least 1 h in running tap water.

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