notes on methodology

Detection of phospholipids on paper chromatograms by neutron activation

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SUMMARY Improvements in the detection of phospholipids on paper chromatograms by neutron activation (with the formation of P^{32}-compounds) have been made. Formaldehyde-treated papers were subjected to low neutron dosage after chromatographic development and the resulting radioactive spots were detected by automatic scanning and autoradiography.

KEY WORDS paper chromatography · corn phospholipids · detection · neutron activation · phosphatidyl inositol · phytoglycolipid · automatic scanning · autoradiography

RADIOACTIVE (P^{32}) PHOSPHOLIPIDS may be easily produced by bombardment of unlabeled phospholipids with thermal neutrons. Several workers (1, 2) have used such neutron activation to detect small amounts of phospholipids on paper chromatograms. The technique has been criticized on the grounds that the chromatography paper may disintegrate under severe neutron bombardment and may become difficult to handle (3). This is particularly inconvenient if the papers are to be subsequently scanned in a device through which the paper is passed under tension. In addition, impurities in the filter paper lead to the production of short-lived β- and γ-emitting isotopes which may interfere with the detection of P^{32} (2). This problem has been avoided by first irradiating the sample and then chromatographing the P^{32}-labeled compounds produced (2), but, unfortunately, even mild doses of thermal neutrons can lead to degradation and rearrangement of the compounds contained in the sample (2, 4).

We have found that irradiation of phospholipids on paper chromatograms for a relatively short time at low neutron flux can produce sufficient radioactivity for the detection of even small quantities of these lipids. Under the conditions employed, the paper retains its strength. Irradiation is carried out after development of the chromatograms to eliminate problems arising from irradiation-induced degradation of the lipids.

The production of radioactivity from impurities even with low neutron flux is relatively high in the case of both Whatman and Schleicher and Schüll filter papers, and the impurities are not removed by preliminary development of the paper with solvents. Hörhammer et al. (5) have used formaldehyde-treated papers for the separation of phospholipids. We have found that treatment with formaldehyde followed by overnight washing with water effectively removes impurities which would otherwise be activated during neutron irradiation.

Materials and Methods. Whatman No. 1 "filter paper for chromatography" was employed only for purposes of comparison. All actual analyses were carried out on Schleicher and Schüll paper 2043b Mgl previously treated with formaldehyde by the method of Hörhammer et al. (5). Samples of crude corn phosphatides (10–50 μg) were applied in aqueous pyridine as a series of 1.25-inch wide streaks across a large sheet of chromatography paper which was then developed 18–24 hr in redistilled *n*-butanol–acetic acid–water (4:1:5, upper phase). The paper was allowed to dry overnight and was then cut into $1^5/_8$-inch wide strips, this being the maximum width possible for use with the scanning device employed. The strips were rolled and placed in cylindrical plexiglass screw-capped irradiation vials which were approximately 4 inches long and 1 inch in diameter. The vials, each containing four strips, were irradiated in the University of Illinois Triga Mark II Reactor for 7 hr at 250 kw with a thermal neutron flux of 2×10^{12} neutrons/sec per cm^2 [contrast the conditions of Robinson (3): neutron flux of 5×10^{12} neutrons/sec per cm^2 for 4–5 hr at 1000 kw]. The irradiation vials became heavily contaminated and were used only once. The irradiated samples were allowed to decay within lead shielding for at least 48 hr after irradiation, before scanning for P^{32} on a linear-response Vanguard Model 800 Autoscanner. Successive strips for scanning were joined by narrow pieces of 3M masking tape; other adhesive tapes gave background counts and were avoided.

Autoradiography was carried out by placing 4-inch wide paper strips in direct contact with Kodak No-screen X-ray film for an appropriate length of time. The films were developed in Kodak rapid X-ray developer for 3 min at 20°, washed with water for 30 sec, fixed in Kodak X-ray fixer for 10 min, and finally washed in running water for 2 hr.

Results and Discussion. Different "backgrounds" due to impurities, obtained with various types of paper with

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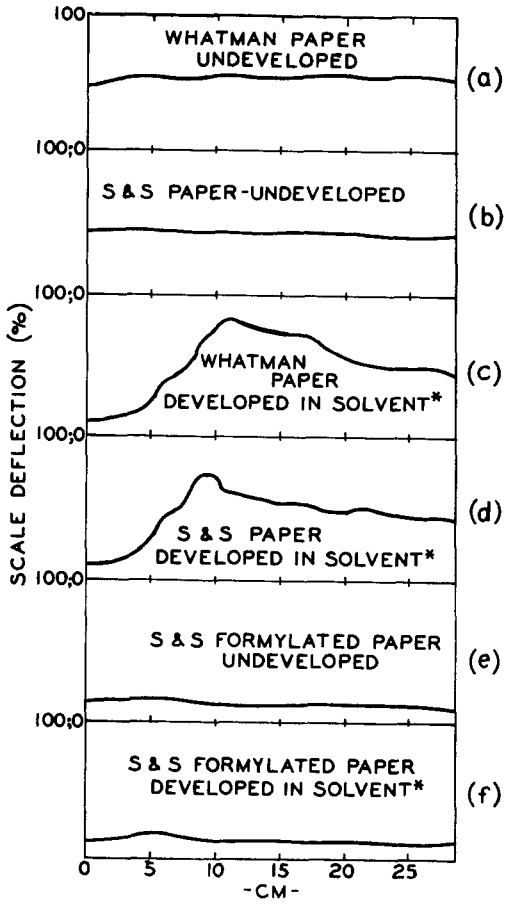

FIG. 1. Radioactivity scans of different kinds of blank paper, 4 days after neutron irradiation, with and without prior solvent development. Maximum scale deflection = 60,000 cpm with background set for unactivated paper at 0 cpm.

* Solvent, *n*-butanol–acetic acid–water (4:1:5, upper phase).

or without solvent development, are shown in Fig. 1, together with the effect of formaldehyde treatment. Prior treatment of the Schleicher and Schüll paper with formaldehyde followed by overnight washing reduces the background level of radioactivity more than two-thirds. This background is at a level low enough to allow very small amounts of phospholipids to be detected (10^{-4} μg P). Peaks from chromatographed samples can be measured weeks after irradiation, as shown by the separation of phytoglycolipid and phosphatidyl inositol from a mixture of corn phosphatides in Fig. 2.

Since papers had to be cut into narrow strips for scanning, it was difficult to obtain a good over-all picture of a large chromatogram. Autoradiography has therefore been used on 4-inch strips. Activation of larger papers depends upon the availability of reactor facilities. An interesting finding is that autoradiograms can be conveniently identified on the film by writing in pencil on the chromatograms prior to their irradiation. For several days after irradiation the resulting radioactivated pencil marking is clearly recorded by X-ray film after a few hours' exposure to the chromatogram. Other pencil marks on the chromatogram are to be avoided.

The possible radiation-induced degradation of phospholipids is not of importance if one is analyzing simply by R_F on the paper. When phospholipids are to be eluted after chromatography, parallel samples may be run on one large paper and only one sample strip utilized for neutron activation analysis.

The method has been used thus far only for qualitative detection of phospholipids on chromatograms. Extension of the method to quantitative measurement is obviously desirable, and further studies are in progress.

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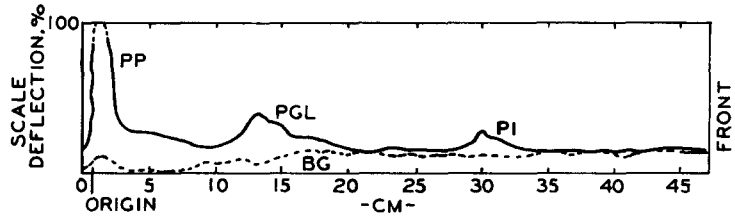

FIG. 2. Scan of P^{32} radioactivity of chromatographed corn phosphatides 27 days after neutron irradiation. *PP*, polyphosphates; *PGL*, phytoglycolipid; *PI*, phosphatidyl inositol; *BG*, background for the activated paper. Maximum scale deflection = 3000 cpm with background for unactivated paper set at 0 cpm. Developing solvent: *n*-butanol–acetic acid–water (4:1:5, upper phase).

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