Recording chromatograms of phosphatides on silicic acid-impregnated filter paper

Chromatography of phosphatides on silicic acid-impregnated filter paper is now a commonly used procedure. In this laboratory, while investigating the phosphatides of biological membranes, the need arose for a simple routine method to record chromatograms of different patterns. GROSSMAN[1], and CONDREA *et al.*[2] recently published photographs of rhodamine 6G stained chromatograms obtained by a camera, a method unsuitable for routine work. Photostating techniques, though simpler, need special light filters to sensitively record chromatograms of substances that either absorb[3] or fluoresce[4] in ultraviolet light. Moreover, phosphatides in the concentrations usually applied to paper chromatograms do not absorb sufficiently in the widely used range of 2537Å to enable their easy recording by these techniques.

It is found that the phosphatide spots on silicic acid paper chromatograms can be photostatically recorded when their absorption in ultraviolet light is increased by either of the following treatments.

(1). Treating the chromatograms with nitric acid to hasten the oxidation of the phosphatides by heating. This is carried out by immersing the chromatograms in 7 *N* nitric acid for 5 min. They are then hung in an oven at 100° for 45–60 sec, washed for 1 min in running tap water, and returned to the oven for 8 min. This treatment

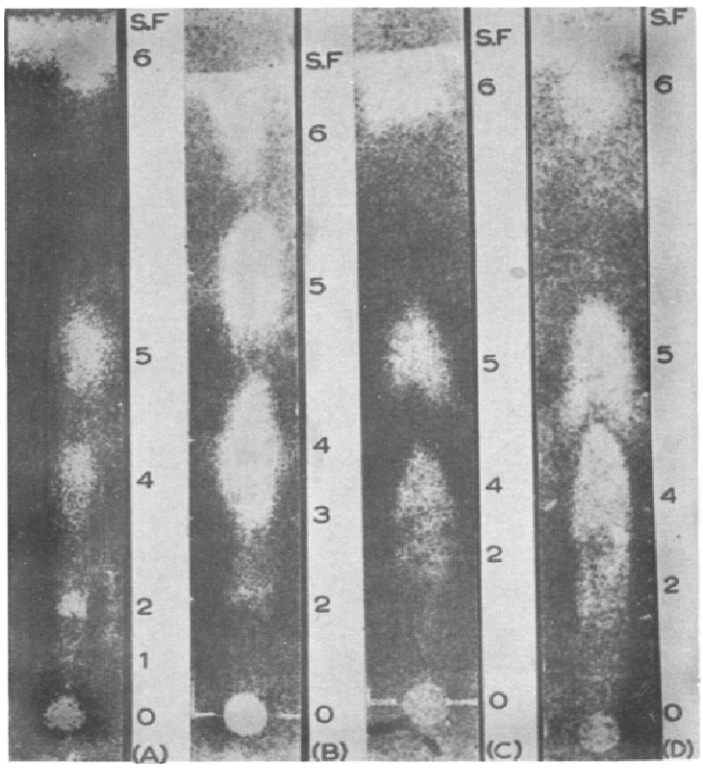

Fig. 1. Chromatograms A–D are treated with dinitrophenylhydrazine, *p*-nitrophenylhydrazine, phenylhydrazine, and by oxidation respectively. Total lipid phosphorus load is 7 μg, 14 μg, 18 μg, 11 μg in the above order. Abbreviations are: SF = solvent front; O = origin. Numbers indicate: 1 = diphosphoinositide; 2 = monophosphoinositide; 3 = sphingomyelin, 4 = phosphatidylcholine; 5 = phosphatidylethanolamine; 6 = non-phospholipids.

causes some loss of the phosphatide phosphorus, and chromatograms treated accordingly are therefore unsuitable for quantitative analysis.

(2). Immersing the chromatograms in an aqueous solution of an ultraviolet absorbing organic compound, such as 2,4-dinitrophenylhydrazine, *p*-nitrophenylhydrazine, and phenylhydrazine, (in 0.15 % solutions in 2 *N* HCl). These become adsorbed on the lipid spots and increase their ultraviolet absorption, within 1/2 h, 1 h, and overnight respectively. The chromatograms are then successively washed with 2 *N* HCl (10 min), running tap water (1 min), and finally dried. The adsorption treatment causes no loss of the phosphatide phosphorus, and the chromatograms are therefore suitable for purposes of quantitative analysis.

The dry chromatograms are imposed on a Kodak document paper (Duostat Rapid Reflex 23) and pressed between two thin plates of glass. Ultraviolet light (from a low pressure mercury resonance tube "Hanovia Chromatolite") is shone through for 5–10 sec, at a distance of 10 cm. It is found necessary to lower the fore-end of this lamp by 2 cm to shine a light of uniform intensity along the chromatograms. The prints shown in Fig. 1 are typical. The total lipid phosphorus load is indicated on each chromatogram after analysis of an identical but untreated chromatogram in the case of the first treatment, and a duplicate in the case of the second.

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Relative response of fatty acid methyl esters on the flame ionization detector

Considerable discussion has dealt with the relative response of the flame ionization detector for different organic substances. It was mentioned first by MCWILLIAM AND DEWAR[1] that for hydrocarbons, the relative molar responses seem to be directly proportional to the carbon number; detailed data on this subject were presented at the 1961 International Gas Chromatography Symposium, in East Lansing, Mich.[2]. However, only very few values are available regarding substituted organic compounds[3].

FARQUHAR *et al.*[4] reported in 1959 that when a beta-ray (Argon) ionization detector is used, for higher fatty acid methyl esters, the peak area per cent can be taken with good approximation as concentration by weight of the individual components. We could not, however, find any published data on the relative response of a flame ionization detector for fatty acid methyl esters. Therefore, we carried out some inves-