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Notes on the paper chromatographic method for determination of phospholipids

In the method described by VOGEL *et al.*[1] a correction is made for 90 % recovery of phospholipid from the paper after staining with Rhodamine G. We also found that recovery was not quantitative, but varied between 60 and 90 %. The cause of the low recoveries has been traced to the staining with Rhodamine G. It was found that when the chromatogram was immersed in the Rhodamine G some of the phospholipid was lost. The present modification corrects this error.

Chromatography

The chromatographic separation can be done on silicic acid-impregnated papers prepared by the method of MARINETTI[2]. However, the commercially obtainable silica gel-loaded paper, Whatman Chromedia S.G. 81, was more convenient. The developing solvent was di-isobutylketone–acetic acid–water (40:30:7). The paper was first equilibrated in the vapour for 30 min before starting development which required about 4.5 h. Under such conditions a good separation of lysolecithin, $R_F = 0.30$, from the other phospholipids was obtained. (Sphingomyelin $R_F = 0.39$; lecithin $R_F = 0.44$; phosphatidyl serine $R_F = 0.53$; phosphatidyl ethanolamine $R_F = 0.58$.)

Staining

For quantitative work the best method for staining was light spraying with the Rhodamine G. The spots were then viewed under U.V. light 254 nm, marked, cut out, and the phospholipid eluted with 1 ml MeOH by refluxing the solvent through the paper for about 1 min. After evaporation of the MeOH the phosphorus was determined.

Determination of phosphorus

The method described by BERENBLUM AND CHAIN as modified by MARTIN AND DOTY[3] is reviewed by LINDBERG AND ERNSTER[4]. It is a very convenient method because of the stability of the colour intensity; however, the liberation of phosphate presented problems. Hydrolysis with concentrated sulphuric acid was unsatisfactory because the high acidity interfered with the extraction of phosphomolybdic acid. If the acid was neutralized with sodium hydroxide the high concentration of sodium sulphate prevented extraction of phosphomolybdic acid. Perchloric acid was found satisfactory for the digestion, the long time required (4 h) by the method of WAGNER, described by RANDERATH[5], being found to be unnecessary. The method used was as follows.

After evaporation of the methanol used for elution of the lipid spot, 0.4 ml of 60 % perchloric acid was added and the tube heated over a microburner or electric heater for 10 min, or till the solution went colourless again. Dilution of the tube contents with 3.2 ml of water brought the acid concentration to the correct range (1.0–1.1 *N*), and 0.5 ml of 10 % molybdate solution and 5 ml benzene–butanol mixture (1:1) were added. The mixture was shaken for 15 sec and then centrifuged to separate the layers completely. A suitable amount of the top layer (0.1 ml–2.5 ml containing

0.025–0.15 μmole P) was removed with an all glass syringe pipette and diluted with 3.2 % H_2SO_4 in absolute ethanol to 5 ml and 0.5 ml of freshly diluted stannous chloride solution (10 % $SnCl_2$ 2 H_2O in concentrated HCl freshly diluted 200 times with 0.5 *M* H_2SO_4) was added and mixed immediately. Colour intensity was measured immediately at 740 nm.

A standard curve was prepared with anhydrous A.R. disodium hydrogen phosphate dried over silica gel under vacuum overnight. This gave a straight line over the range 0–0.15 μmole P per aliquot of supernatant taken.

The above procedure gave essentially 100 % recoveries of phosphate from paper chromatograms of phospholipids and no correction factor was necessary.

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1 W. C. VOGEL, L. ZIEVE AND R. O. CARLITON, *J. Lab. Clin. Med.*, 59 (1962) 335.
2 G. V. MARINETTI, *J. Lipid Res.*, 3 (1962) 1.
3 J. B. MARTIN AND D. M. DOTY, *Anal. Chem.*, 21 (1949) 965.
4 O. LINDBERG AND L. ERNSTER, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. 3, Interscience, New York, 1956, p. 7.
5 K. RANDERATH, *Thin Layer Chromatography*, Academic Press, New York, 1963, p. 145.

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