compared to specific tests for amino acids in that a generalized aminoacidemia can be readily detected.

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A novel charring technique for detection of lipids on thin-layer chromatograms

Lipids are frequently detected on thin layers of silica gel by spraying with a corrosive agent such as sulfuric or chromic acid, followed by charring at elevated temperatures. There are a number of inherent disadvantages to this procedure. The corrosive agents employed pose a health hazard if used in poorly ventilated areas, particularly since they are present in a finely dispersed form. Moreover, it is difficult to spray the plate evenly, especially when viscous reagents such as concentrated sulfuric acid are used. This factor is of prime importance when the plates are subsequently subjected to densitometry and uniform charring of the lipid fractions is desirable. JONES *et al.*[1] overcame the problem of uniform charring of the lipids by exposing the plate to volatile compounds such as sulfur trioxide or sulfuryl chloride; sulfuric acid was then generated *in situ* by exposing the plate to water vapor. However, this procedure is undesirable since volatile, acid-yielding materials are involved. The objections to the use of highly corrosive acids in fine dispersion can be overcome by taking advantage of the thermal instability of ammonium sulfate. At elevated temperatures this compound decomposes yielding ammonia, which is volatile, and sulfuric acid. Sulfuric acid can thus be generated *in situ* by spraying the plate with ammonium sulfate solution (10–20%) and heating. The present communication describes a variation of this procedure in which ammonium sulfate is incorporated into the silica gel layer so that the sulfuric acid generated on heating is evenly dispersed throughout the layer.

Preparation of plates

Thin-layer plates were prepared by slurrying 30 g of Silica Gel G (containing 14% $CaSO_4$ as binder) in 60 ml of water, 1% aqueous $(NH_4)_2SO_4$ or 10% aqueous $(NH_4)_2SO_4$ solution. The slurry was spread on the plate using commercially available equipment. The plates were dried for 10 min at room temperature and were then activated by heating for 30 min at 110°. After cooling, they were stored in a desiccator until required.

Fractionation of non-polar lipids

Fig. 1 represents the fractionation of non-polar lipids on the plain and $(NH_4)_2SO_4$-impregnated plates. The developing solvent was light petroleum–diethyl ether–acetic acid (90:10:1). The non-impregnated plate was sprayed with 20% $(NH_4)_2SO_4$ solution after development and all three plates were heated at 200° for 20 min. Differences in the ability of the plates to separate the non-polar lipids were small. The plain plate and that impregnated with 1% $(NH_4)_2SO_4$ solution were essentially the same, whereas the plate prepared with 10% $(NH_4)_2SO_4$ solution was slightly less polar than the others. All three procedures resulted in adequate charring of the lipids but the spots on the 1% $(NH_4)_2SO_4$ plate were less intense than those on the other

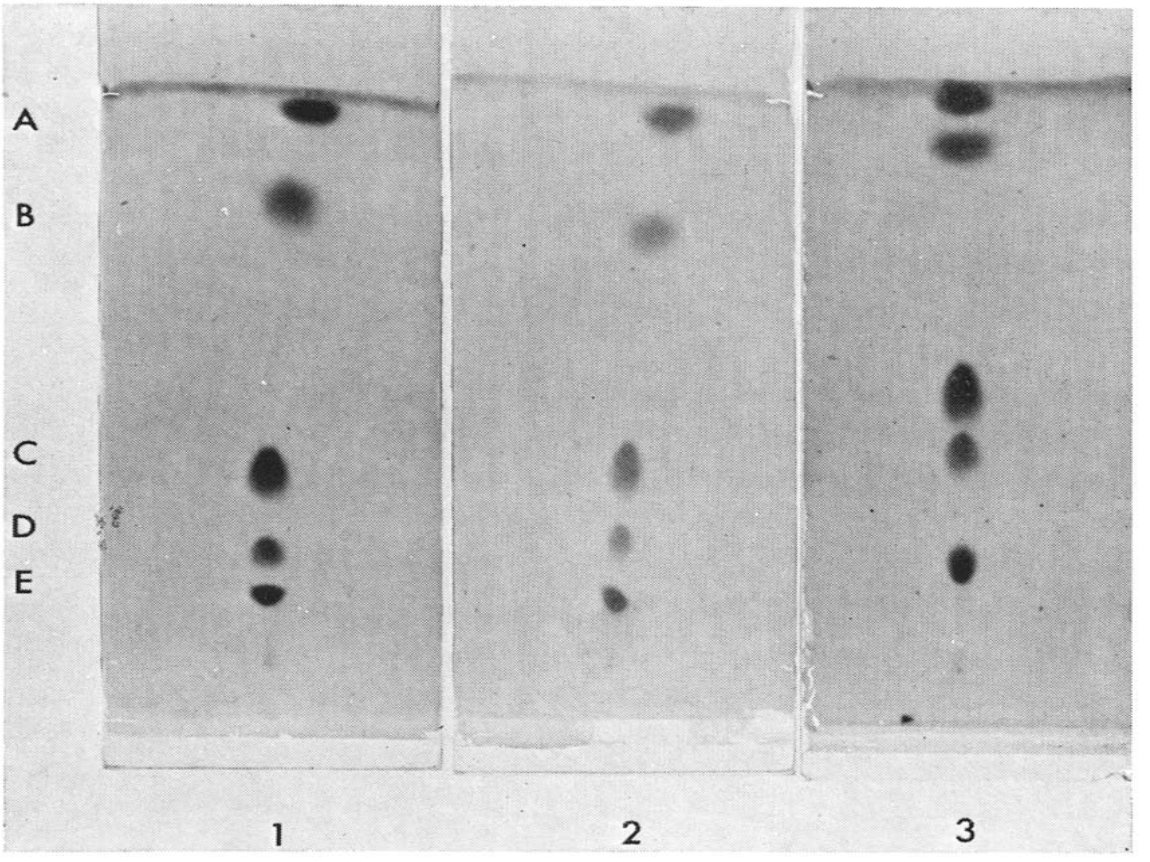

Fig. 1. Thin-layer chromatography of non-polar lipids. Plates: 1, Silica Gel G–water; 2, Silica Gel G–1% $(NH_4)_2SO_4$; 3, Silica Gel G–10% $(NH_4)_2SO_4$. Lipids: A, cholesterol ester; B, methyl oleate; C, triglyceride; D, free fatty acid; E, cholesterol. Solvent: light petroleum–diethyl ether–acetic acid (90:10:1).

two plates. A uniform darkening was evident on the 10% $(NH_4)_2SO_4$ plate. This intensified on prolonged heating and was apparently due to impurities in the gel, since development of the plate in diethyl ether prior to application and separation of the lipids eliminated this problem. By applying different concentrations of the non-polar lipids on the plate and developing as described, discernable spots were obtained with 0.5 μg or less of the individual lipids on the plate impregnated with 1% $(NH_4)_2SO_4$. With the plain plate and the plate impregnated with 10% $(NH_4)_2SO_4$ solution, most lipids were still discernable at concentrations as low as 0.1 μg. Cholesterol oleate was an exception in this instance, the lower detection limit being approached at 0.4 μg. This was probably due to the larger area of this spot in comparison with those of the other components on the plate, a consequence of the high R_F value of the cholesterol ester.

Fractionation of polar lipids

The ability of the $(NH_4)_2SO_4$-impregnated plates to separate the polar lipids was investigated. The results are presented in Fig. 2. The solvent system employed was chloroform–methanol–water (65:25:3.5), and although this separated phosphatidylethanolamine, phosphatidylcholine and sphingomyelin (lane a), the choline and inositolglycerophosphatides had the same mobility, and phosphatidylserine streaked badly (Fig. 2, plate 1, lane c). Addition of ammonium sulfate to the plate eliminated the streaking problem noted with the serine phosphatide (plates 2 and 3, lane c). This

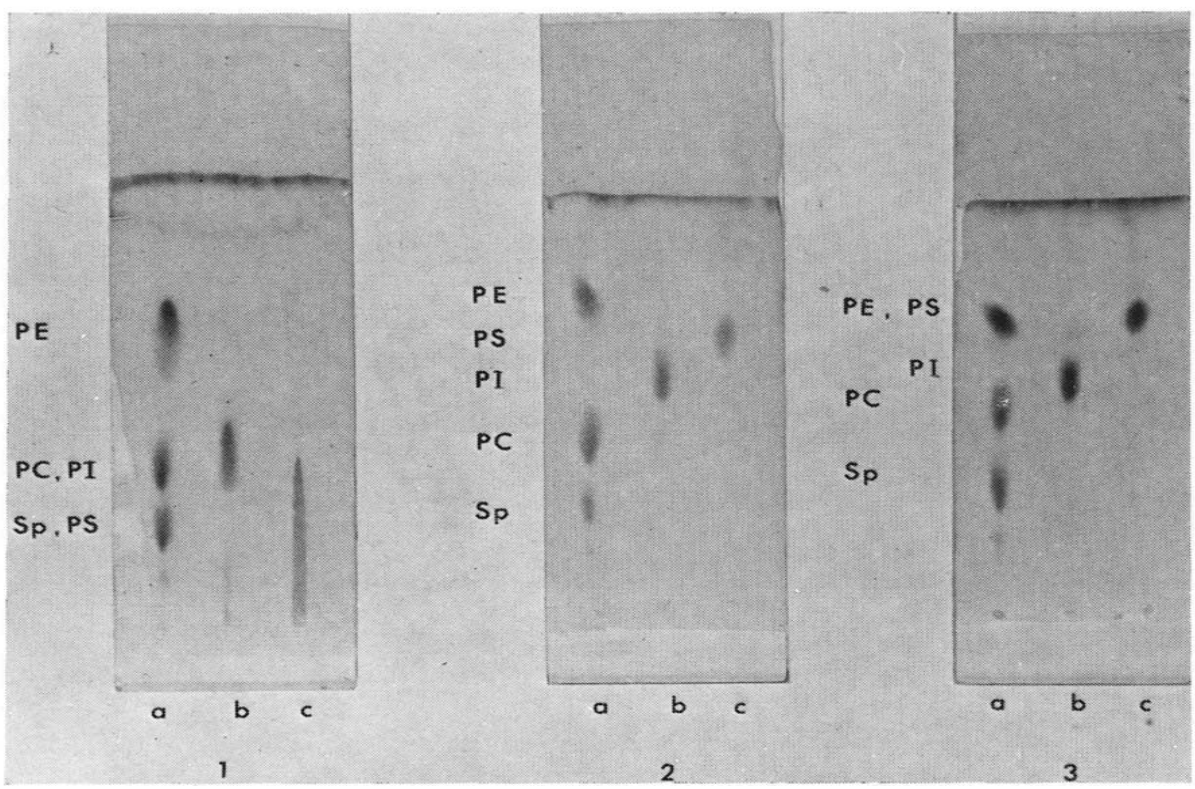

Fig. 2. Thin-layer chromatography of polar lipids. Plates: 1, Silica Gel G–water; 2, Silica Gel G–1% $(NH_4)_2SO_4$; 3, Silica Gel G–10% $(NH_4)_2SO_4$. Lipids: PE, p osphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; Sp, sphingomyelin. Solvent: chloroform–methanol–water (65:25:3.5).

phenomenon has been reported previously[2] and in this respect, impregnation of Silica Gel G with ammonium sulfate had a similar effect to impregnation with oxalic acid, potassium hydroxide[2] or sodium carbonate[3]. The mobility of phosphatidylserine on the impregnated plate was independent of the amount applied to the plate in the range of 5 to 50 μg (*cf.* SKIPSKI *et al.*[3]). The presence of ammonium sulfate in the Silica Gel G modified the mobility of the inositol and serine phosphatides. This was particularly evident when 1% $(NH_4)_2SO_4$ solution was used in the preparation of the plate (Fig. 2, plate 2). Partial separations of the serine (lane c) and ethanolamine phosphatides (lane a) and of the phosphatidylcholine (lane a) and phosphatidylinositol were evident on this plate.

Preparation of Silica Gel G plates with solutions of ammonium sulfate provided a simple means for the detection of lipids by charring without recourse to spraying with corrosive reagents. The separation of the non-polar lipids was not adversely affected by the presence of $(NH_4)_2SO_4$ in the gel and there was an improvement in the fractionation of polar lipids on such plates, the streaking problem normally encountered with phosphatidylserine being eliminated. Since this technique provides a means for the uniform dispersion of the charring agent throughout the gel layer, its application to thin-layer densitometry is currently being investigated.

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