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Glass fiber paper strip charring: A rapid and simple method for monitoring column chromatography of lipids*

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» When eluates from the column chromatography of lipids are collected fractionally, it is desirable to have

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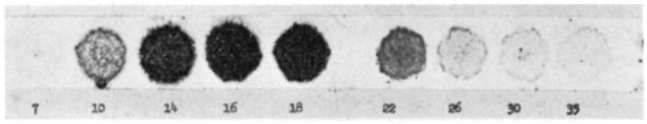

FIG. 1. The charring strip from a series of tubes containing neutral lipid that had been separated from phospholipid on a silicic acid column by eluting with chloroform. The blank area between tubes 18 and 22 was used to obtain a blank value for the strip when it was read densitometrically.

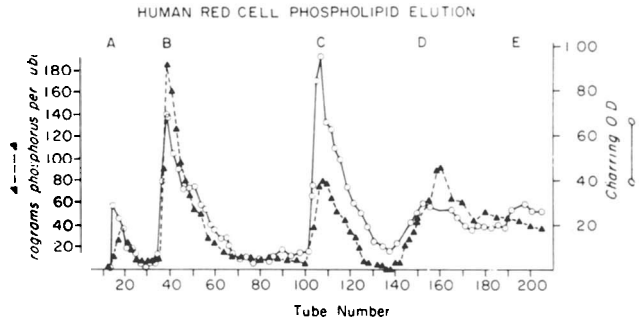

FIG. 2. Column chromatogram of human red cell phospholipids illustrating the close correlation between the peaks determined by phosphorus assays and by charring. Tube volume, 2.9 ml; charring aliquots, 0.05 ml. Peaks eluted with the following solvents (v/v): A, chloroform–methanol 9:1; B, chloroform–methanol 5:1; C, ethyl acetate–methanol 3:2; D + E, ethyl acetate–methanol 1:1.

a record of the elution pattern. When phospholipids are chromatographed, phosphorus assays may be performed for this purpose. Similarly, total weights, cholesterol assays, determinations of fatty acid esters, or a combination of these provide good records for neutral lipid elutions. All of these assays are time consuming, and none of them can be performed rapidly enough to eliminate a time lag between the elution of a given fraction and its assay. Recently, Hirsch has reported the use of a refractometer to monitor column effluents, but this instrument is expensive and the method is, to some extent, still in its developmental stages (1). Lands and Dean have reported a method for detecting small amounts of lipid by spotting samples of the effluent on a ferrotype plate (2). As reported, this procedure certainly has the virtue of being simple and semi-quantitative.

Muldrey et al. have shown that measurement of the optical density of charred spots of phospholipids separated by chromatography on glass fiber paper can be used for quantitative estimation (3). This suggested that column effluents could be monitored by charring measured aliquots spotted on glass fiber paper. Accordingly, the following method was developed and has been applied successfully to both neutral and phospholipid separations. Aliquots of effluent containing 2–100 μg of lipid can be used and a useful semi-quantitative elution pattern can be obtained.

Materials. Reeve and Angel (Clifton, N. J.) #101 glass fiber filter paper and Gelman Instrument Company (Chelsea, Mich.) Type A glass fiber filter paper, were found to be satisfactory. The former was preferred because it was smoother and gave more homogeneous charring. If handled carefully and protected from dust and dirt, the paper can be used as delivered. Heating at 500° for $^1/_2$ hr prior to use insure complete cleanliness. Concentrated reagent grade sulfuric acid was used throughout. To char the paper strips, a muffle furnace or oven capable of heating to 250–300° can be used, but an ordinary hot plate, the surface of which will attain temperatures of the same magnitude, is also satisfactory. The glass fiber paper is cut into strips that vary in width depending on the nature of the densitometer to be employed, or 1.5–3 cm if the method is to be used only visually. In these studies, a Photovolt Corporation Model 52-C densitometer was used without filters. Care was taken to keep the strips clean by avoiding contact with hands and bench tops. Handling and cutting the glass fiber paper inside ordinary paper towels proved very satisfactory. Aliquots of column effluent were pipetted from consecutive (or appropriately selected) fractions and discharged onto the smooth side of the strip in succession, leaving a blank area of 3–5 cm in the center of the strip (Fig. 1). The aliquots can be 0.05–0.10 ml, again depending on the type of densitometer to be used. All aliquots can be pipetted with the same pipette (a 0.10 ml serological pipette has been found most satisfactory) if excess solvent is blown out between each sample and the pipette is rinsed twice with each new fraction before pipetting it. After a strip is completed and the solvents have dried, the strip is sprayed with concentrated sulfuric acid and then heated in the oven or on a hot plate covered with clean tin foil. Ten to fifteen seconds at 250–300° is usually sufficient to give maximal charring. A representative neutral lipid peak as visualized after charring is shown in Fig. 1. After charring, the spots can be evaluated visually and rated on some arbitrary scale. If a semi-quantitative evaluation is desired, the strips can be impregnated in white oil (mineral oil), blotted free of excess oil (but not so that any of the areas appear dry), and read in the densitometer using the blank center area to zero each separate strip. If consecutive strips are used to construct an elution diagram, it is advisable to spot the last fraction on each strip as the first on the next strip, in order to indicate variations in the paper strips themselves.

While no attempts have been made to determine the exact quantitative significance of the charring response,

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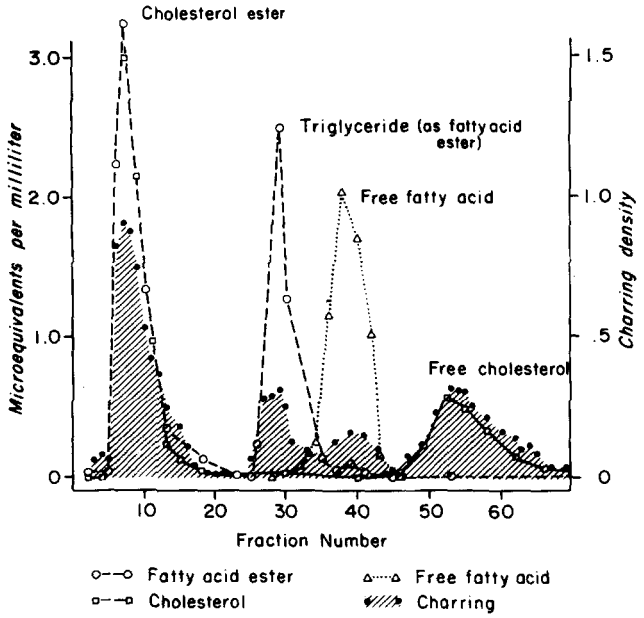

FIG. 3. Chromatogram of plasma neutral lipids eluted by the method of Barron and Hanahan (4). Tube volume, 3.4 ml; charring aliquots, 0.05 ml. The peaks were eluted with the following solvents (v/v): Cholesterol ester, hexane–benzene 85:15; triglyceride and free fatty acid, diethyl ether–hexane 5:95; free cholesterol, diethyl ether–hexane 20:80. In the cholesterol ester peak, note that the cholesterol and fatty acid ester peaks are superimposed.

two elution patterns are presented (Figs. 2 and 3) to show that, with both phospholipids and neutral lipids, the correlation with conventional methods of assay is excellent.

In Fig. 2, the peak charring density and the peak phosphorus response in Fraction B (predominantly phosphatidyl ethanolamine) have been arbitrarily set to almost the same height. In Fraction C (phosphatidyl serine, phosphatidyl inositol, glycolipid), the charring response is then much greater than the phosphorus assay. This has been observed consistently with this fraction of red cell lipids. Lipid phosphorus is usually 2.4% of total weight in Fraction C whereas it is 3.8–4.0% in B. In Fractions D and E (lecithin and sphingomyelin), it is again approximately 4% and, as shown, the two curves again nearly coincide. The irregularity of the charring in the region of lecithin and sphingomyelin has been seen frequently and is unexplained.

In the neutral lipid diagram, the charring is greater in the cholesterol ester fraction relative to the fatty acid ester content than in the triglyceride fraction, as expected from the carbon/ester ratio for these lipid classes (43:1 for cholesteryl oleate, and 19:1 for triolein). The free fatty acid peak is also nicely shown by the charring method. The hydroxamate reaction in this same peak gives low values, probably due to contamination with triglyceride.

Thus, charring of small amounts of column effluents on glass fiber paper provides a sensitive means of detecting eluted compounds. The correlation between peaks determined by charring and by more conventional methods is shown to be good in both phospholipid and neutral lipid chromatograms. There is a semi-quantitative relationship of the intensity of charring to the amount of carbon present.

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