Simple charring method for determination of lipids

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SUMMARY A rapid method is described for charring 5–300 μg of lipids (with concentrated sulfuric acid in a test tube) and estimating them with a reproducibility of ±1%.

KEY WORDS nonspecific lipid determination · charring reaction · sulfuric acid

IN THIS COMMUNICATION we report the development of a quantitative charring assay which is rapid enough for monitoring the column chromatography of lipids and which has a sensitivity greater than many standard colorimetric assays for lipids. The main advantages of this procedure are its rapidity, sensitivity, reproducibility, and the employment of a single stable reagent (concentrated sulfuric acid).

Materials and Methods. Reagent-grade concentrated sulfuric acid, tested for the presence of organic residues by heating at 200°C for 5 min, was used. The lipids used as standards were purified by preparative thin-layer chromatography on Silica Gel G plates which had been washed in the solvents used for the lipid separations. The standard solutions were prepared in chloroform at a concentration of 30 μg/ml and aliquots of 1–5 ml were generally employed in the assay.

Solvents were removed from the lipid samples under a flow of nitrogen in test tubes placed in an aluminum heating block at 80–100°C. Solvent blanks were run routinely. After the tubes had been cooled, 2 ml of concentrated sulfuric acid was added to each tube. At 15 sec intervals the tubes were placed in an aluminum heating block at 200°C for 15 min. The temperature within the tubes in the heating block should be controlled to within ±2°C during the charring period. The tubes were placed in water at room temperature for 15 sec and then were transferred to an ice bath for 5 min; 3 ml of water was added to each tube, the contents were mixed thoroughly, and the tubes were replaced in the ice. When cool, the tubes were removed from the ice and left standing for 10 min or until all bubbles had disappeared. The optical density was measured with a Spectronic 20 spectrophotometer (Bausch & Lomb Incorporated, Rochester, N.Y.) at 375 mμ. This wavelength was

A preliminary report of this method has been published (Marsh, J. B. *Biol. Bull.* **117**: 435, 1959).

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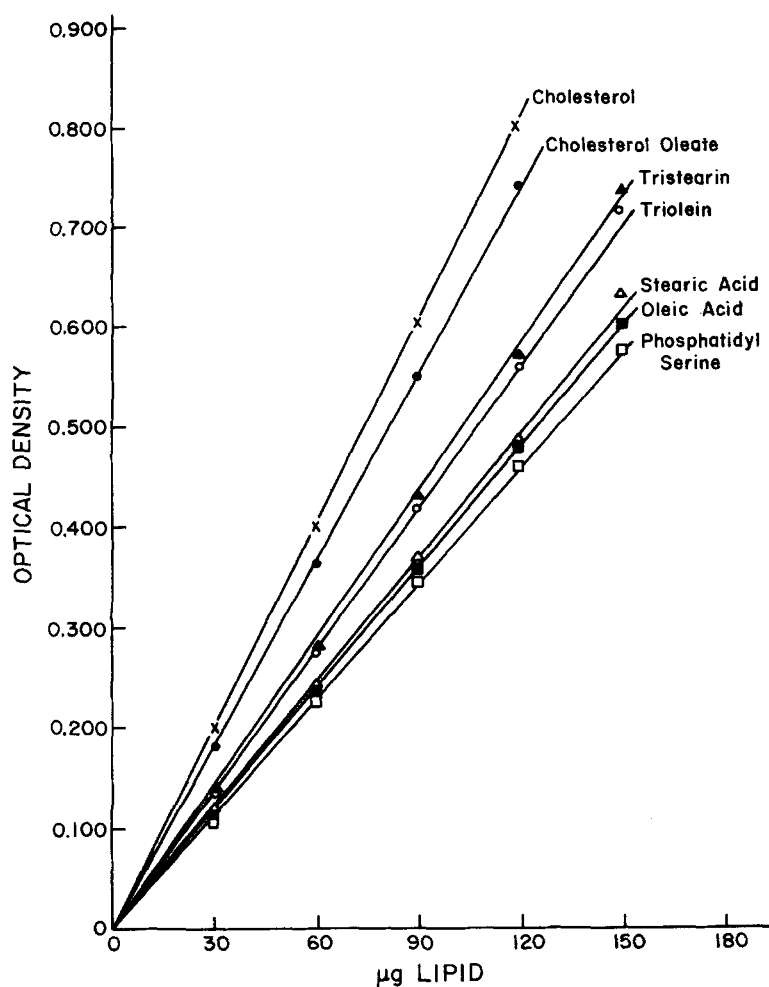

FIG. 1. The relationship between optical density at 375 mμ and the quantity of lipid used in the charring assay. Lipids were charred at 200 °C for 15 min with concentrated sulfuric acid.

selected to achieve near maximum sensitivity with the spectrophotometer employed.

Results. Fig. 1 shows that a linear relationship exists between the optical density of the charred lipids and the quantity of lipid used. The relationship is linear up to 120 μg for all lipid classes tested although the slope of the line differs for the individual lipids. In 20 determinations, the average standard deviation was ±1.3%. Silicic acid, Silica Gel G, and Silica Gel H-HR (Brinkmann Instruments Inc., Westbury, N.Y.) did not interfere with the charring assay, provided that the gel was removed by centrifugation after charring.

After charring has been completed, optical density measurements may be made at any time up to 2 hr, since there is only a 2–3% decrease in the absorbancy up to that time. After 2–3 hr, tubes containing more than 120 μg of lipid often show flocculation of carbon particles. At amounts of lipid greater than 150 μg, there is a change in the slope of the line relating optical density to concentration, but the relationship then continues to be linear up to 300 μg. The sensitivity of the assay decreases by a factor of 4 from 325 to 520 mμ.

It can be seen from Fig. 1 that saturated lipids char to a slightly greater extent than unsaturated lipids of the same class. If the total volume in each tube is decreased to 2.5 ml and the tubes are read at 330 mμ (in a Beckman DU spectrophotometer) the limit of sensitivity of the assay can be lowered to 5 μg for most lipid classes and to 2–3 μg for cholesterol and cholesterol esters.

A rat liver lipid mixture and a synthetic mixture with a composition resembling that of rat liver lipid (1) were prepared and tested. These solutions also gave a linear charring response with a slope intermediate between that of triglycerides and fatty acids, reflecting the high content of phospholipid in each preparation. Although only phosphatidyl serine is shown in Fig. 1, a linear charring response was also obtained with phosphatidyl ethanolamine and lecithin.

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Discussion. With the use of multibore columns containing Florisil (2), of methylated Sephadex columns (3), and of other standard column methods the complete fractionation of lipid mixtures containing 5 μg or less is now readily accomplished. Semiquantitative column monitoring has been achieved by Muldrey, Miller, and Hamilton (4) and by Ways (5) utilizing a sulfuric acid charring technique for the detection of lipids in aliquots of column effluents spotted on glass fiber sheets. Amenta (6) has applied an acid–dichromate method to the analysis of lipids separated by chromatography. This separation of lipid classes allows the use of a single, nonspecific reaction for the quantitative determination of all lipid classes. Privett and Blank (7) and Blank, Schmit, and Privett (8) have applied an acid–dichromate charring reaction to a densitometric measurement of lipids directly on thin-layer plates. Under proper conditions, reproducible optical densities were obtained that were directly proportional to the carbon content of the substance and gave a linear response over a range of 5–25 μg of carbon.

Our results agree with the data of Blank et al. (8), which show that under constant charring conditions the extent of conversion of lipids to carbon is strongly influenced by structure and molecular weight and that the charring of compounds within the same lipid class does not vary to any considerable extent. The present method is more rapid and sensitive than many of the methods currently in use for the determination of lipids separated by column or thin-layer chromatography. In addition, the linearity of response of a natural and synthetic mixture of lipids indicates the possibility of using such mixtures as standards for the measurement of total lipid in tissue or plasma extracts, providing that the approximate lipid composition is known and that this does not change significantly under the conditions of the experiment performed.

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