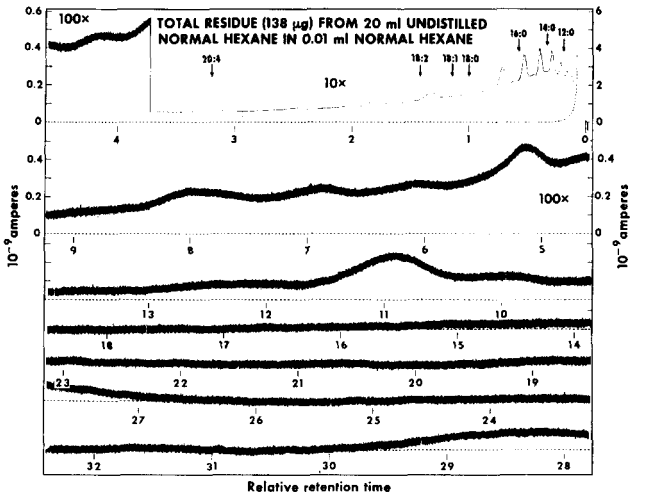

FIG. 1. Chromatogram of total residue from 20 ml of undistilled normal hexane, technical grade (95% Mol % minimum), Phillips Petroleum Company, Bartlesville, Oklahoma. The sum of the responses due to the components resolved represents approximately one-half the response expected for an equivalent mass of methyl esters of fatty acids. All gas chromatographic analyses were made in one of two apparatuses described in detail elsewhere (4, 5). Each unit employed a beta-particle ionization detector (6) and a 132-cm glass column of 6 mm inside diameter. Column packing consisted of 70% by weight 48–65 mesh chromosorb and 30% by weight polydiethyleneglycolsuccinate[1] (PDEGS). Columns were conditioned approximately 3 weeks at the operating temperature of 195° before usage. Relative retention times along the ordinates are expressed relative to that of methyl stearate (18:0 = 1).

Potential contamination in the analysis of methyl esters of fatty acids by gas-liquid chromatography*

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» In gas-liquid chromatography (GLC), as in other microanalytical techniques, it is especially important to guard against the intrusion of contaminants. At the microgram level, contaminants may be introduced at any stage of sample handling or processing and may originate from such sources as glassware, aluminum foil, filter paper, solvents, plastics (tubing or bottle cap liners), and even skin lipids of the investigator. Once introduced, they may accompany the sample all the way to the point of measurement and yield spurious and misleading peaks in the resulting gas-liquid chromatograms. Also, scrupulous care must be exercised to clean injection apparatus such as syringe barrels and micropipettes.

In our studies of the fatty acids of blood lipids and lipoproteins, we have noted that organic solvents and filter paper are indeed potential sources of contamination. While this is not suprising to trained microchemists, the widespread adoption of gas-liquid chromatography as an analytical tool finds many research workers measuring microgram and submicrogram quantities for the first time. It therefore seems pertinent to reemphasize the necessity for certain precautions.

Lipid extraction as well as subsequent lipid-separation techniques by silicic acid chromatography frequently require the use of relatively large volumes of solvents for the extraction or fractionation of relatively small amounts of lipids. Thus, it becomes very important to check all solvents used for potential contamination. For instance, it is absolutely essential to distill solvents such as hexane and pentane obtained from commercial sources. Figures 1 and 2 show the total contamination resulting from the residue obtained from 20 ml of raw and distilled normal hexane, respectively. Reagent-grade chloroform, methanol, and absolute ethanol also yield residues that introduce, on a much smaller scale, contaminations in the gas-liquid chromatogram.

In certain types of lipid extraction (1) as well as during hydrogenation procedures (2), it may be necessary or convenient to use filter paper. In our experience, it is absolutely essential to pre-extract such filter paper

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[1] Cambridge Industries Company, Inc., 101 Potter St., Cambridge 42, Mass.

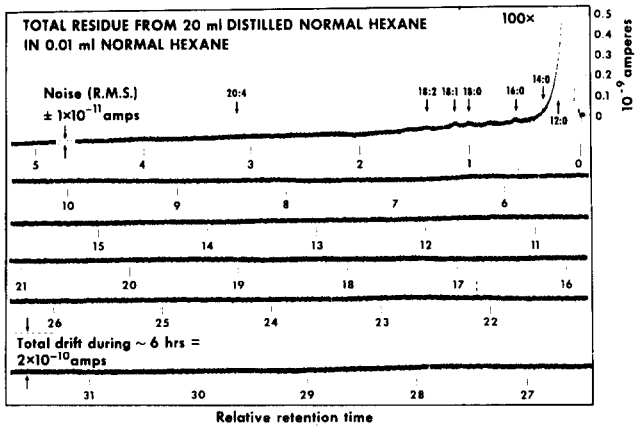

FIG. 2. Chromatogram of the total residue from 20 ml of distilled normal hexane. Components resolved are equivalent to 0.05 μg of methyl esters of fatty acids and may represent contamination, in part, from the syringe barrel of the injection apparatus. The mass of residue itself was too low (less than 10 μg) to permit an accurate determination. Injection of 0.01 ml distilled hexane (alone) gave similar patterns with resolved components, if present at all, below 0.05 μg. Relative retention times as in Fig. 1.

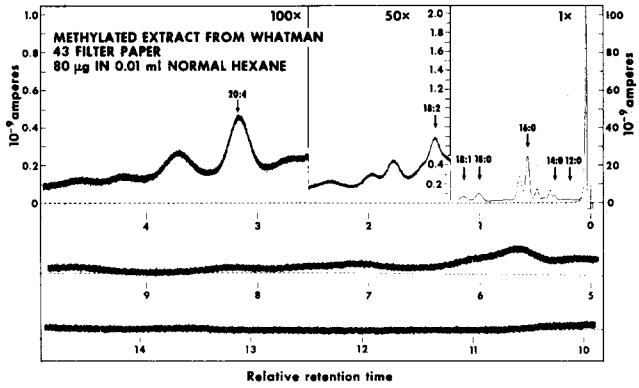

FIG. 3. Chromatogram of methylated extract from Whatman No. 43 (fat-free) filter paper (0.12 mg of residue were obtained from a disc 11 cm in diam. and 0.02 cm thick). Total components resolved are equivalent to 30 μg of methyl esters of fatty acids. Relative retention times as in Fig. 1.

if used in connection with GLC work. This is also true when using No. 43 Whatman filter paper, although it is described as "recommended . . . where fat-free paper is desirable" (3).

Several samples of 41H and 43 filter paper (Whatman) were extracted with 2:1 (v/v) mixture of chloroform and methanol, and the extracts were weighed on a microbalance. The weights of extracted material (not necessarily lipid) per filter-paper sheet ranged from 90 to 250 μg.

An example of a gas-liquid chromatogram of a methylated extract of filter paper is shown in Figure 3. The relative retention times of the principal methyl esters of fatty acids are indicated, as determined with a 55 μg calibration mixture just before or after the filter paper run. (Relative retention times, especially for the longer-chain methyl esters, may vary by as much as 10% over the useful lifetime of a particular column.) It is readily seen from the chromatogram that a methylated filter-paper extract yields a large number of GLC peaks, many of which could be erroneously identified as serum methyl esters of methyl esters of serum fatty acids. Methylated residues from other grades of filter paper give quite different chromatograms. An unmethylated extract showed several early components, but the very low total yield suggests that nearly all of the unmethylated material was held up on the column. Hydrogenation of the methylated extract from Whatman No. 43 paper produced very little change in the chromatographic pattern, except for the apparent conversion of a small amount of methyl oleate to methyl stearate. It may be inferred that most of the remaining peaks do not represent methyl esters of the common fatty acids.

Further attempts to identify these substances seem unwarranted, since the aim is to eliminate them. However, one of the extracts has infrared spectral characteristics, which are indicative of a phthalate ester. This class of compounds is commonly used as a plasticizer and has previously been encountered as a contaminant in a variety of situations in lipid chemistry.

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