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Direct gas chromatographic examination of total lipid extracts

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ABSTRACT The conditions for gas–liquid chromatography described in a previous publication for the improved separation of natural triglycerides have been further modified to permit a direct examination of total lipid extracts. For this purpose the range of temperature programming has been extended to 100–350°C. Under optimum conditions, complete separations and quantitative estimates are obtained for free fatty acids, free sterols, diglycerides, steryl esters, and triglycerides. The separations are based on differences in molecular weights. Glycerophosphatides present in the sample seem to be pyrolyzed to compounds with retention times similar to those of diglycerides.

The accuracy of the method is examined for standard mixtures of neutral lipids and fatty acids, and applications are illustrated with samples of the total plasma lipids of man. The lipid compositions obtained are compared with those data derived by conventional analyses. Although the method is rapid, its successful application to quantitative routine analyses requires extreme care.

KEY WORDS gas–liquid chromatography · high-temperature programming · on-column injector · dual column operation · total lipid extracts · pyrolysis of lecithins · plasma lipids

During the last 10 yr GLC has successfully been applied to the resolution of most homologues or isomers within a given class of lipids. The initial separations of fatty acids (1) led to the development of GLC techniques for the determination of steroids and bile acids (2). The improvements in column technology and instrumentation required for steroid work paved the way for the elaboration of methods (3) of GLC fractionation of natural triglycerides and steryl esters. Further advances made in the preparation of column packings and in the operation of the instruments have permitted the introduction of GLC systems of wide application. Thus, the improved analytical methods employed for the separation of the high molecular weight triglycerides (4) can also effectively resolve many of the low molecular weight fatty acid esters as well as the free fatty acids themselves and the sterols commonly occurring in natural lipid extracts.

The present report shows that properly selected GLC columns in combination with effective instrumental controls can provide both qualitative and quantitative information about the composition of relatively crude extracts of complex lipid mixtures.

MATERIALS

The synthetic neutral lipid and free fatty acid mixtures examined in this study were prepared in the laboratory from purified materials supplied by various companies and investigators. Free fatty acids and cholesteryl esters were obtained from Applied Science Laboratories Inc., State College, Pa., and were claimed to be at least 99% pure. Cholesterol (Amerchol) was donated by American Cholesterol Products, Inc., Edison, N.J. The plant sterols were prepared in the laboratory from commercial concentrates of soybean sterols. The 1,2-dimyristin, 1,2-dipalmitin, and 1,2-distearin were gifts from Dr. E. Baer, who also supplied the synthetic dimyristoyl and distearoyl lecithins used in the investigation of the fate of the phospholipids during GLC. The 1-stearoyl 2-palmitoyl diglyceride was a gift from Dr. D. Buchnea. The fatty acid esters were donated by the National Heart Institute (Mixtures D, E, and F) and the Hormel Institute (Mixtures No. 1, 2, and 8). Stripped lard was given by Distillation Products Industries, Rochester, N.Y.

The human plasma samples were obtained from young adults on controlled experimental diets (5). The samples of the lymph lipids were from dogs with continuously draining thoracic duct fistulas (6). Samples of organ

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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lipids were obtained from rats killed in the laboratory during the course of other experiments.

METHODS

Preparation of Lipid Extracts

Plasma samples were obtained by centrifugation of whole blood in the usual way (7). 1–2 ml of the plasma was quantitatively transferred to a heavy-walled centrifuge tube and lyophilized. To the dry residues, 10 ml of methanol was added, and the tubes were stoppered with corks covered with aluminum foil. The contents of each tube were thoroughly mixed and left standing for 4–8 hr in the dark at room temperature. Other samples of plasma were treated with methanol in the same ratio without prior freeze-drying.

Then 20 ml of chloroform was added to each tube and the contents were again mixed and allowed to stand at room temperature. After about 2 hr, the suspension was filtered through a defatted Whatman No. 1 filter paper and the filtrate and chloroform washings were evaporated to dryness in a rotary evaporator at 37°C. The evaporator flask (100 ml capacity) was modified by pushing out the bottom to form a small cone of about 2 ml volume. After evaporation the lipid residue could be conveniently collected into this cone by washing the flask with small amounts of solvent. The total lipid extracts were collected in screw cap vials (9 ml capacity) and sealed with polyethylene liners. Prior to use both liners and caps were extracted with hot chloroform–methanol 2:1. Samples of lymph and tissue homogenates were treated in an identical manner.

For gas chromatography, the extracts were diluted with a known amount (about 400 μg) of tridecanoin used as internal standard, and after evaporation to dryness, the final volume was adjusted to about 0.25 ml with chloroform. Approximately 0.5–1 μl was injected into the gas chromatograph.

Diazomethylation

In order to convert the free fatty acids into the methyl esters, we dissolved the total lipid extracts in absolute methanol (0.25 ml) and added an excess of freshly distilled diazomethane in diethyl ether (8) until the yellow color persisted. The solutions were allowed to stand for 10–15 min in the fume hood before the excess reagents and solvents were evaporated under nitrogen. For injection into the gas chromatograph, the dry residue was taken up in 0.25 ml of chloroform.

Acetylation

To convert any compounds with free hydroxyl groups into the acetates, we dissolved the total lipid extract in the minimum amount (0.1 ml) of dry pyridine and added an excess of acetic anhydride (0.5 ml). The mixture was kept overnight (12 hr) at room temperature before the excess reagents and solvents were removed under nitrogen. The dry residue was dissolved in 0.25 ml of chloroform for injection into the gas chromatograph.

Thin-Layer Chromatography

The origin and the identity of the various peaks detected on GLC was confirmed by TLC, which also provided an independent indication of the proportions of the lipid classes. For this purpose the total lipid extract, or a suitable aliquot of it, was applied as a band to Silica Gel H (20 × 20 cm, 0.25 mm thick layer) and developed in hexane–diethyl ether 9:1 (9). Under these conditions separate bands were obtained for total phospholipids (retained at the origin), monoglycerides, free sterols and diglycerides, free fatty acids, triglycerides, fatty acid methyl esters, and steryl esters. The proportions of the chemical classes in the total lipid extract were determined by GLC after tridecanoin had been added to the eluate of each band as internal standard.

Gas Chromatography

GLC of the mixtures of standards and of the total lipid extracts was performed on a Beckman GC-4 Gas Chromatograph with a specially modified on-column injector heater, a diagram of which is shown in Fig. 1. The gas chromatograph was equipped with a differential electrometer, dual hydrogen flame ionization detectors, and dual columns. The columns were stainless steel tubes ($^1/_8$ inch O.D. × 2 ft.) and were filled with 3% JXR (a methyl silicone) or 1% OV-17 (a phenyl silicone) packings on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa.). Before

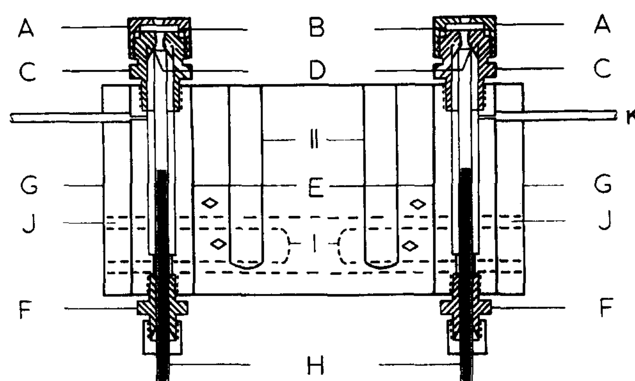

FIG. 1. Cross-sectional view of modified injector heater and upper end of column. *A*, cap; *B*, septum; *C*, removable top of injector barrel; *D*, top of column; *E*, injector barrel; *F*, column inlet fitting; *G*, position of new heating block; *H*, column packing; *I*, positions of old cartridge heaters; *II*, positions of new cartridge heaters; *J*, location of old heating block; *K*, carrier gas inlet. Lower pair of diamonds, old location of heat sensors; upper pair, new location of heat sensors.

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use the columns were conditioned and equilibrated as previously described (4).

The carrier (N_2) flow through the working column was usually set at 150 ml/min at 200°C. The instrument was equipped with automatic flow controllers, which were able to overcome minor changes in the resistance of the column during temperature programming. The air and hydrogen flows were adjusted to give maximum detector response. The detector temperature was set at 340°C and was maintained within 0.5°C of the setting by means of a special temperature controller (supplied with the instrument). The temperatures of the on-column injector (300°C) and the heater (320°C) of the detector line (a stainless steel tube, $^1/_{16}$ inch O.D. × 6 inches, between end of column and detector) were maintained by simple Powerstats, and they consequently varied by as much as 10°C from the settings. The oven temperature was linearly programmed at 4–6°C/min over the range of 100–325°C by means of a Mylar programming sheet on which the temperature profile is traced with silver plastic tape. Temperature spans were controlled by a step switch and a 10-turn potentiometer that allowed settings accurate to 0.1°C.

The recorder, Beckman Model 1005, was a linear 10 inch potentiometric instrument with less than 0.1% dead band, 0.035% resolution, and 0.5 sec full scale pen response. It had a high degree of common mode and ripple rejection. The peak areas were computed by means of a disc integrator.

The following other GLC instruments were subjected to a similar evaluation using the same column packings and comparable column lengths and diameters: Aerograph Model 204-1 (Varian Aerograph, Walnut Creek, Calif.) with dual stainless steel columns, dual hydrogen flame detectors, differential electrometer, and a linear temperature programmer; Aerograph Model 600 D (Varian Aerograph) with a single stainless steel column, a hydrogen flame detector and an F & M Model 240 Linear Temperature Programmer (F & M Scientific Corp., Avondale, Pa.); F & M Model 402 High Efficiency Chromatograph with dual glass columns, dual hydrogen flame detectors, differential electrometer, and a linear temperature programmer. The Aerograph models were equipped with Electronik 15 and the F & M model with Electronik 16 (Honeywell, Inc., Philadelphia, Pa.) 1 mv strip chart recorders.

Conventional Analyses

Total free fatty acids in plasma were determined by the method of Dole and Meinertz (10). Total plasma triglycerides were estimated as described by Jagannathan (11). Total plasma cholesterol was measured by using the procedure of Abell, Levy, Brodie, and Kendall (12). The concentration of free cholesterol was obtained as outlined by Cook and Rattray (13).

RESULTS AND DISCUSSION

Previous studies (3, 14, 15) have shown that the short (1–2 ft of packed length), narrow-bore ($^1/_{16}$ inch I.D., $^1/_8$ inch O.D.) columns, which are successfully employed in triglyceride separations, are capable of resolving much more complex lipid mixtures. The rather simple and frequently inadequate instrument controls employed in the past, however, have prevented a full exploitation of the potentialities of this resolution. In an attempt to use the resolving power of these columns to the full, advanced instrumentation was sought, which could be adapted to high temperature operation with a minimum of modification. Of the commercial instruments evaluated, the most promising results were obtained with the Beckman GC-4 Chromatograph when the operating conditions specified under Methods were used. These working parameters have been adjusted for use with short columns and high molecular weight compounds, and differ considerably from those recommended by the company for conventional columns at relatively low temperatures.

Special attention is called to the change in the design of the injector heater, which was found to be essential for obtaining any success at all for our purposes. As shown in Fig. 1, the original heating block, which had been installed in a horizontal position and which provided a hot collar at a point about 3 inches below the top of the column, is replaced with a custom-made upright heater of about the same mass as the old block. The new heater surrounds the inlet end of the column completely and extends from the top of the column to the oven. With the block's position changed, the cartridge-type heating elements can also be placed parallel with the column. As a result, the inlet end of the column is maintained at a uniform temperature, and both hot and cold spots are avoided. The relocated heat sensors indicate temperature near the injection site when a 10 μl Hamilton syringe with a 2 inch needle is used.

Fig. 2 shows a representative gas chromatogram of a mixture of standard nonphospholipids of the type commonly encountered in plasma, lymph, and various animal tissues. The components are eluted in the order of increasing molecular weight. All peaks, except those of the free fatty acids, are symmetrical normal curves. The apparent tailing of the fatty acids is due not to adsorption on the column, but to a lack of equilibration of the more volatile and more rapidly migrating components between the mobile and the stationary phases, for longer columns and slower flow rates yield more symmetrical curves. Furthermore, the appearance of the fatty acid peaks on the short columns is not materially improved by

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TABLE 1 Recoveries of Free Fatty Acids and Neutral Lipids from Two Pairs of GLC Columns

Component	Weight	Pair A Run 1	Pair A Run 2	Pair A Average	Pair A Correction Factor*	Pair A Corrected Area†	Pair B Run 1	Pair B Run 2	Pair B Average	Pair B Correction Factor*	Pair B Corrected Area†
	%	per cent of total									
Myristic acid	4.4	4.3	4.2	4.3	1.00	4.3	5.3	5.1	5.2	1.00	4.6
Palmitic acid	4.6	4.4	4.3	4.4	1.00	4.4	5.3	5.0	5.2	1.00	4.6
Stearic acid	7.6	7.3	7.8	7.5	1.00	7.4	9.5	9.2	9.3	1.00	8.1
Arachidic acid	4.8	4.4	4.6	4.5	1.00	4.5	5.3	5.1	5.2	1.00	4.6
Behenic acid	2.4	2.6	2.4	2.5	1.00	2.4	2.7	2.5	2.6	1.00	2.3
Cholesterol	11.5	12.2	11.8	12.0	1.00	11.8	14.4	13.6	14.0	1.00	12.3
Tridecanoin	11.1	11.4	11.4	11.4	1.00	11.3	11.5	11.3	11.4	1.05	10.6
Cholesteryl myristate	9.4	9.5	9.7	9.6	1.00	9.5	9.3	9.0	9.1	1.10	8.8
Cholesteryl palmitate	8.8	9.2	8.8	9.0	1.00	8.9	8.8	9.0	8.9	1.15	9.0
Cholesteryl oleate	10.9	11.2	11.2	11.2	1.00	11.0	9.7	9.9	9.8	1.20	10.4
Stripped lard	24.5	23.5	23.8	23.6	1.05	24.5	18.2	20.3	19.3	1.45	24.7

Column pairs A and B were made in identical fashion. Results for pair B are shown to illustrate the finding that some columns give incomplete recovery of higher molecular weight substances. Column conditions were as given in Fig. 2.

* Correction factors were computed from GLC analyses of binary mixtures of internal standard and test lipid by means of the expression: correction factor = weight %/area %. The internal standard, tridecanoin, was assumed to be completely recovered from all columns.

† "Corrected Area" = (observed area × correction factor × 100)/total corrected area.

methylation, although the recovery may improve over that of free fatty acids from the columns of poorer quality.

Table 1 compares the recorded proportions of the peak areas with the weight proportions of the standards in the mixture. Data are given for two different pairs of columns. For pair A, the area percentages correspond closely to those of the original weights. A further adjustment of the peak areas for slight differences in the response shown by the different compounds in the flame detector or for incompleteness in recoveries, results in a nearly perfect match. Pair B, on the other hand, gives a rather poor correlation, as the recoveries of cholesteryl oleate and of triglycerides are low. Ideally, columns of this quality would not be chosen for quantitative separations, but they are adequate to illustrate the methods used in adjusting the recoveries of the various compounds from the less perfect columns. The correction factors, therefore, are empirical and do not apply from one laboratory to another, or even from one pair of columns to the next. Furthermore, the correction factors tend to vary from day to day, and frequent checks are necessary for quantitative work even with high-quality columns.

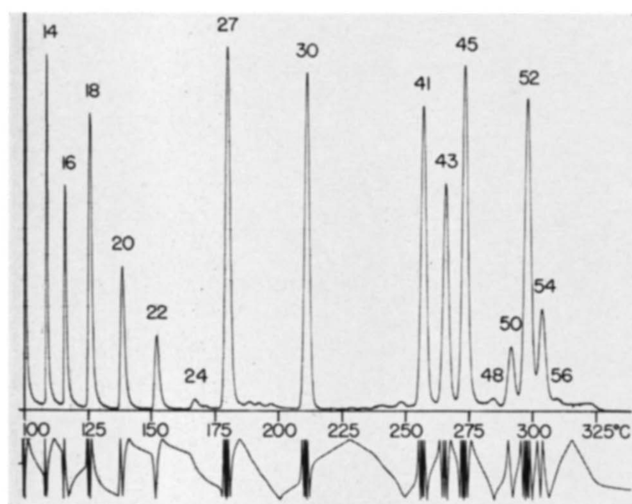

Fig. 2. GLC of standard mixture of free fatty acids and neutral lipids. Peaks 14–24 represent saturated straight-chain fatty acids of 14–24 carbon atoms per molecule; peak 27, cholesterol; peak 30, tridecanoin; peaks 41, 43, and 45 represent cholesteryl myristate, palmitate, and oleate; peaks 48–56 identify triglycerides of stripped lard by the total number of carbon atoms in the fatty acid moieties. Beckman GC-4 Gas Chromatograph; columns, 3% (w/w) JXR (a methyl silicone) on Gas-Chrom Q (100–120 mesh); temperature program as shown. Chart speed, 1 inch/2 min. Attenuation, 5 × 10^3. Sample, 1 μl of 0.1% (w/v) total lipid solution in chloroform.

The corrected areas (as percentages) in Table 1 are obtained by multiplying each observed area by the appropriate correction factor (1.00 to 1.45 for pair B, for example), dividing the product by the sum of all the corrected areas, and adjusting to 100, if necessary.

Fig. 3 shows a gas chromatogram recorded for the total lipid extract of a normal sample of fasting human plasma. The peaks of the free fatty acids are relatively small, while those of the steryl esters and triglycerides are quite prominent. The unsaturated fatty acids have been eluted together and just ahead of the corresponding saturated acids, giving two peaks for each carbon number. Saturated and unsaturated steryl esters and triglycerides of the same carbon number are not resolved with this nonpolar phase unless they differ by at least four double bonds per molecule (e.g. cholesteryl arachidonate and cholesteryl arachidate, tristearin, and trilinolein).

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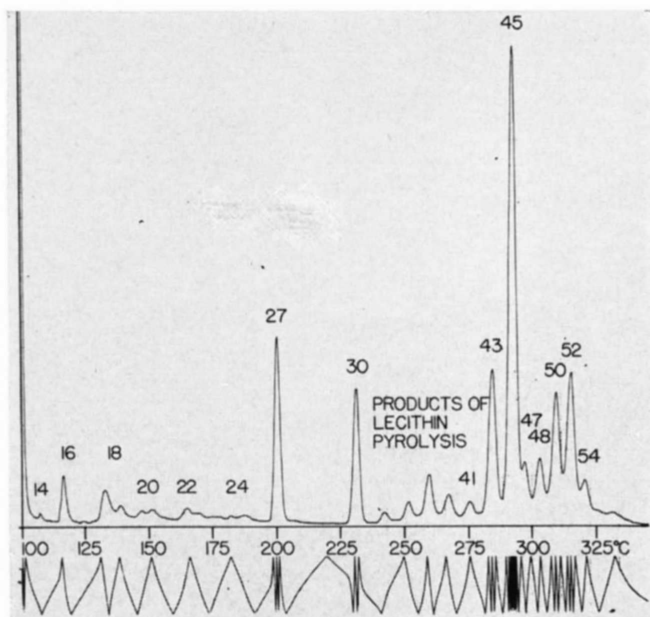

Fig. 3. GLC of a total lipid extract of human plasma (fasting subject). Peaks 14–24, free fatty acids; peak 27, cholesterol; peak 30, tridecanoin; peaks 41, 43, 45, and 47, cholesteryl esters of fatty acids with 14, 16, 18, and 20 fatty acid carbon atoms; peaks 48–54, triglycerides designated by the total number of carbon atoms in the fatty acid moieties. The series of four peaks between peaks 30 and 41 (carbon-numbers 32, 34, 36, 38) probably represent three overlapping pairs of fatty acid diesters resulting from pyrolysis of lecithins. Operating conditions as given in Fig. 2.

The series of peaks appearing in the chromatogram (Fig. 3) between peaks 30 and 43 are due to the pyrolysis products of the plasma lecithins (A. Kuksis and L. Marai, manuscript in preparation). These peaks represent what are believed to be the fatty acid diesters of propenediol. Two isomeric peaks of about equal concentration are produced per molecule of lecithin. From synthetic lecithins these materials are produced in 60–70% yield. When they are eluted the retention times are similar to those of the diglycerides of corresponding carbon number. The mixture of plasma lecithins yields a rather complex picture because of overlapping of the various peak pairs. However, it is obvious that most of the lecithins are made up of combinations of C_{16}, C_{18}, and C_{20} acids. Little or no free fatty acid is released during the pyrolysis of the lecithins under the described conditions. The fate of phosphatidyl serines and ethanolamines seems to be similar to that of lecithins; that of sphingomyelin has not been examined. On exposure to diazomethane the phosphatidyl serines and ethanolamines are converted to the dimethyl esters of phosphatidic acids (16), which are also pyrolyzed.

The origin and the identity of all the intact lipids detected on the gas chromatogram of the total lipid extract was confirmed by GLC of the individual lipid classes recovered from TLC. Each chemical class gave all and only those peaks expected for it on the basis of carbon-number analysis.

We calculated from Fig. 3 that, per 100 ml, the fasting plasma contained 19 mg of free fatty acid, 35 mg of free cholesterol, 140 mg of cholesteryl ester, 105 mg of lecithin, and 80 mg of triglyceride. These estimates are in the range of the normal values reported for plasma free fatty acid (10), cholesterol (5, 12, 13), lecithin (7), and triglyceride (5, 11).

Table 2 gives the composition of a pooled sample of fasting human plasma as determined by direct GLC of the total lipid extract, by quantitative GLC of the methyl esters derived from the various lipid classes first resolved by TLC ("combined TLC–GLC analysis"), and by conventional analysis. The various estimates are in reasonable agreement. The greatest difference is seen in the values for free fatty acids. Direct titrations give the highest estimates (20.4 mg/100 ml). Titration of the fatty acids after a preliminary isolation by TLC gives a value (18.0 mg/100 ml) close to that derived from combined TLC–GLC analysis (18.5 mg/100 ml). The lowest estimate (15.5 mg/100 ml) is derived from the direct GLC analysis. In general, the latter values were 20–30% lower than those obtained by the method of Dole and Meinertz (10).

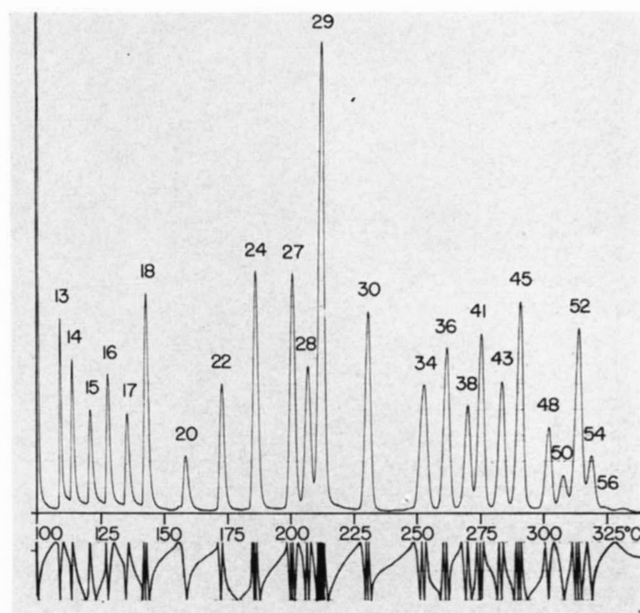

Fig. 4. GLC of standard mixture of fatty acid methyl esters and neutral lipids. Peaks 13–24, methyl esters of fatty acids designated by the number of acyl carbon atoms; peak 27, cholesterol; peak 28, campesterol; peak 29, β-sitosterol; peak 30, tridecanoin; peaks 34, 36, and 38, the acetates of 1,2-dipalmitin, 1-monostearo-2-monopalmitin, and 1,2-distearin, respectively; peaks 41–56, as in Fig. 2. Instrument and operating conditions as given in Fig. 2. Columns, 1% (w/w) OV-17 (a phenyl silicone) on Gas-Chrom Q (100–200 mesh). Sample, 1 μl of 0.5% (w/v) total lipid solution in chloroform.

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TABLE 2 DETERMINATION OF THE LIPID COMPOSITION OF A POOLED SAMPLE OF FASTING HUMAN PLASMA*

Lipid Component	Direct GLC Analysis Run 1	Direct GLC Analysis Run 2	Combined TLC–GLC Analysis (17)	Conventional Analyses Values	Conventional Analyses Reference
		mg/100 ml			
Free fatty acids	15.5	17.9	18.5	20.4†	(10)
				18.0†	(10) after TLC
C_{14}	0.1	0.2			
C_{16}	5.5	6.3			
C_{18}	9.0	10.4			
C_{20}	0.9	1.0			
Total cholesterol	128.0	135.5	130.5	125.8	(12)
Free cholesterol	45.5	44.0	40.5	45.0	(13)
Cholesteryl esters	148.5	150.5	155.5	141.5‡	(13)
C_{14}	2	3			
C_{16}	44	42			
C_{18}	95	90.5			
C_{20}	7.5	15.0			
Triglycerides	80.0	83.5	85.0	89.5§	(11)
C_{48}	12	10			
C_{50}	28	30			
C_{52}	32	33.5			
C_{54}	8	10			
Lecithins	130	125	110.5		
C_{34}	20	30			
C_{36}	40	40			
C_{38}	40	30			

* The values are averages of two determinations, except for the direct GLC analyses where the original data of two representative runs are given. C_{14}, C_{16}, etc. refer to total number of *fatty acid* carbon atoms per molecule.

† As palmitic acid.

‡ As cholesteryl palmitate.

§ As triolein.

The estimates for total lecithins, obtained by summation of the peak areas of the pyrolysis products and a correction for an average yield of 60%, agreed fairly well with the estimates for the total lecithins derived by combined TLC–GLC analysis. The latter values were similar to those for total cholesterol and gave, therefore, a nearly 1:1 weight ratio between total cholesterol and total phospholipid. As shown by the GLC of the individual lipid classes and of the total lipids in the absence of the phospholipids, the presence of the lecithins in the plasma lipid extract did not affect the accuracy of the estimates of the other lipid components.

Total lipid extracts of lymph and of various tissue homogenates have been shown to yield elution patterns similar to those of the plasma lipids. The ease with which differences in the qualitative appearance of the chromatogram and in quantitative composition of total lipid extracts can be observed suggests that this technique may be an excellent means for screening a large number of samples. It requires a minimum of material (5–10 μg), little time in analysis (20–30 min), and can be completed on samples of lipid obtained by simple extraction. Yet the results compare favorably in terms of accuracy with those obtained with much more laborious and time-consuming conventional determinations and are frequently more informative.

Fig. 4 shows the resolution of a more complex mixture of standard lipids. In addition to the compounds shown in Fig. 2, this mixture also contains the odd-number fatty acids C_{13}, C_{15}, and C_{17}; campesterol; β-sitosterol; the acetates of dipalmitin, monostearo-monopalmitin, and distearin; and tripalmitin. All the fatty acids in this mixture were methylated. As noted above, methylation contributed little to improved peak symmetry or resolution. In this system the free fatty acids overlap with the corresponding fatty acid methyl esters. Similarly, acetylation of the diglycerides produced little change in peak shape, recovery, or retention time of the diglyceride.

Chromatograms have also been obtained (data not given here) in which peaks of other common lipid constituents were completely resolved. Thus, coprostanol is eluted ahead of cholesterol, and cholesteryl arachidon-

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ate emerges between cholesteryl oleate and tripalmitin. The methyl ester of arachidonic acid is clearly separated from methyl arachidate. In addition to the JXR and the OV-17 columns, good peak resolution was provided also by SE-30 (methyl silicone gum, General Electric) and QF-1 (methyl fluoroalkyl silicone) columns when they had been prepared on Gas-Chrom Q. The resolution of these lipid mixtures can be further improved by using longer columns (4–6 ft packed length), but under such conditions the higher molecular weight compounds are only partially recovered and quantitative work is impaired.

Besides the scanning of total lipid extracts, short GLC columns are extremely useful for distinguishing between lipid components that are barely resolved on TLC plates. Thus, fatty acid methyl esters sometimes move only just ahead of triglycerides; occasionally, triglycerides may be confused with free fatty acids; and cholesterol and diglycerides overlap in most solvent systems used for separation of neutral lipids. The chromatographic method described can readily differentiate between all of these pairs and many more, as well as establish the degree of contamination of any of the lipid classes. Hydrocarbons, aliphatic alcohols, tocopherols, terpenes, plant steryl esters as well as simple organic and bile acids have all been recovered from similar short columns with characteristic retention times and temperatures. The GLC system is also valuable for the identification and quantitative measurement of subfractions of lipid classes separated by TLC on silver nitrate-treated adsorbents.

In conclusion, we must add that there is room for further improvement in the GLC system. The most obvious needs are those relating to more sensitive control of the inlet heater, as well as the heater of the detector line. In the instrument described, both heaters tended to either lag or superheat and required constant attention. Better automatic flow controls would maintain more regular flows of the carrier gas over the entire range of temperature programming. Finally, there is an urgent necessity for improvement in the durability of all instrumental components in order to insure reproducibility and long range performance. The presently available commercial gas chromatographs break down frequently during continuous operation at high temperature.

It is a pleasure to acknowledge the cooperation of the Toronto Office of Beckman Instruments, Inc. in the modification and testing of the inlet heaters.

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