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FATTY ACID COMPOSITION OF HUMAN ERYTHROCYTE MEMBRANES BY CAPILLARY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

LOUIS R. ALEXANDER* AND JOSEPH B. JUSTICE, Jr.

Emory University, Chemistry Department, Atlanta, GA 30322 (U S A.)

and

JOHN MADDEN

Emory University School of Medicine, Department of Psychiatry and Biochemistry, and Georgia Mental Health Institute, Human and Behavioral Genetics Laboratory, Atlanta, GA 30306 (U.S.A.)

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SUMMARY

Capillary gas chromatographic and gas chromatographic—mass spectrometric methods were employed for profiling total fatty acid content of human erythrocyte membranes. The protocol was designed to efficiently separate, identify, and accurately quantify the fatty acid composition in human erythrocyte membranes. Washed erythrocyte "ghosts" were saponified in aqueous methanolic sodium hydroxide solution and methylated with boron trichloride and acid catalysis. Extracted total fatty acid methyl esters (FAMEs) were analyzed using a highly polar cyanopropylsiloxane SP 2560 fused-silica capillary column. Total run time was 55 min, and 45 FAMEs were tentatively identified by relative retention times compared to those of known FAMEs. Confirmation of identities by mass spectral structure elucidation revealed saturated, mono- and polyunsaturated, and branched-chain FAMEs. The presence of four fatty aldehydes was also confirmed as dimethyl acetal derivatives. Identification of *cis/trans* isomers was based on relative retentin times and characteristic profile of the *cis/trans* FAME standard. Quantification of FAMEs for normal subjects showed some variation in relative amounts, consistent with expectations based on literature reports on total or phospholipid FAMEs from human erythrocytes. Separation of individual components of fatty acid families (n-3), (n-6), and (n-9) is demonstrated. Losses in relative amounts of polyunsaturated fatty acids upon storing samples were also detectable by this rapid method.

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INTRODUCTION

The human erythrocyte membrane is essentially a fluid phospholipid bilayer and protein [1]. Fatty acid components of the phospholipid may be saturated or unsaturated and have different lengths of carbon chains, giving a distinctive chromatographic profile upon analysis. Definition of the normal profile by a clinically practical method, the results of which are characterized by mass spectrometry (MS) to confirm gas chromatographic (GC) identification based on standard relative retention times (RRTs), is reported herein.

Highly polar cyanopropylsiloxane-coated SP 2560 capillary columns have recently been produced (Supelco, Bellefonte, PA, U.S.A.) expressly for *cis/trans* fatty acid determinations. Analysis of complex biological matrices, including erythrocyte membranes, requires these more effective columns for separation and quantification of total fatty acid content. We selected the 100-m fused-silica SP 2560 capillary column for its improved capacity and overall efficiency in an attempt to separate all fatty acid moieties on a single column using a straightforward linear temperature program.

EXPERIMENTAL

Materials

Organic solvents, HPLC grade, were purchased from Fisher Scientific or Burdick & Jackson Labs. The fatty acid methyl ester (FAME) standards GLC 68A and U-83-M were obtained from Nu-Chek Prep (Elysian, MN, U.S.A.); 4-5556 (FAME KIT 14), 4-7015 (PUFA No. 2 Animal Source), and 4-7048 (GLC 110 Branch Chain Mix) were obtained from Supelco. Eicosapentaenoic acid methyl ester (E-5506) was purchased from Sigma (St. Louis, MO, U.S.A.). Boron trichloride—methanol (10%) was purchased from Applied Science Labs. The fused-silica column (SP 2560, 100 m × 0.25 mm I.D., 0.2 μm film thickness non-bonded) was obtained from Supelco. Activated silicic acid, Unisil, 200—325 mesh, was obtained from Clarksville (Williamsport, PA, U.S.A.), and Hanks Balanced Salt Solution (HBSS) from Gibco Labs. (Grand Islands, NY, U.S.A.).

Blood collection

Washed erythrocyte "ghosts" were provided by Georgia Mental Health Institute, Human Genetics Laboratory. Each blood sample was drawn by filling a 10-ml EDTA(K_3) vacutainer (Becton and Dickinson, Rutherford, NJ, U.S.A.) which was immediately cooled in ice. Red blood cells were promptly separated from the plasma by centrifugation at 500 *g* for 30 min in a refrigerated unit at 4°C. Plasma and buffy coat were removed before proceeding with wash procedure. Packed red blood cells were resuspended in HBSS and transferred to 30-ml centrifuge tubes. Red cells were washed three times with centrifugation of each suspension at 400 *g* for 30 min at 4°C.

Preparation of erythrocyte ghosts

Essentially the method of Hanahan and Ekholm [2] was followed to form the erythrocyte ghosts. Red blood cells were lysed with distilled water at room

temperature for 1 h. Erythrocyte ghosts were washed three times with distilled water and recovered via ultracentrifugation at 20 000 *g* for 1 h at 4°C. Fatty acids were promptly extracted for chromatographic analysis.

Total fatty acid extraction

A rapid procedure reported by Metcalfe and Schmitz [3] and Moss et al. [4] was modified for extraction of erythrocyte fatty acids. Washed erythrocyte ghosts were saponified with 3 ml of 5% sodium hydroxide in 50% aqueous methanol in a 20 × 150 mm PTFE-lined screw-capped test tube. Equivalent reagent blanks and known fatty acid standards were processed identically. Tubes were flushed with nitrogen and placed in a boiling water bath for 30 min. To each of the cooled tubes was added 1 ml of 6 *M* hydrochloric acid. Contents were mixed, the pH was adjusted to 2—3, and 4 ml of 10% boron trichloride—methanol were added. Tubes were again flushed with nitrogen and heated in a water bath at 85°C for 15 min. To the cooled tubes were added 2 ml of saturated sodium chloride and 10 ml of hexane—diethyl ether (1:1) (the diethyl ether contained 1 ppm butylated hydroxy toluene (BHT) as a preservative). Tubes were inverted slowly thirty times; then phases were allowed to separate. The organic (top) layer was removed and transferred to a 20-ml beaker. Extraction was repeated with two additional 10-ml volumes of hexane—diethyl ether. The extracts were combined and evaporated at room temperature to approximately 200 μl under a nitrogen blanket. Anhydrous sodium sulfate was added to beakers, and the contents of each beaker were quantitatively transferred to a Unisil chromatography column. These columns were prepared by placing 3.5—4.0 g of Unisil in a Pasteur pipette containing a silanized glass wool plug. Beakers were rinsed with 1 ml of hexane and the extract was transferred to Unisil columns. Each column was eluted with 10 ml of 3% diethyl ether—hexane into a 10-ml beaker to remove the FAME fraction. Final extracts were evaporated to 200 μl under nitrogen. The FAME extracts were quantitatively transferred to 500-μl PTFE-lined screw-cap vials, evaporated to dryness, and reconstituted in 50 μl of hexane for analysis.

Gas chromatographic analysis

A Perkin-Elmer 3920 B gas chromatograph with dual flame ionization detectors and split capillary injector was used for all analyses. A Hewlett-Packard 3390A integrator was employed to collect peak height data. The capillary column was a highly polar fused-silica SP 2560 column (100 m × 0.25 mm I.D., 0.2 μm film thickness) which was coated with a specially deactivated cyanopropylsiloxane. The column oven temperature was linearly programmed from 80°C to 220°C at 8°C/min. Initial and final times were 2 and 32 min, respectively, requiring a total run time of 55 min. The split ratio at 80°C was 1:30 with helium carrier gas at a flow-rate of 0.67 ml/min and injector and interface temperatures were 210°C and 225°C, respectively. The sample injections were 0.5 μl (in hexane) and peaks were tentatively identified by comparison with retention behavior of known standards of FAME mixtures. Identifications were confirmed by GC—MS structure elucidation.

Gas chromatographic—mass spectrometric analysis

A Finnigan 4000 quadrupole mass spectrometer with a 6115 data system was used in the electron-impact (EI) mode to verify identification and elution order of FAMEs on the SP 2560 capillary column. In addition, solid probe analysis of selected individual FAME standards was performed for the poly-unsaturated moieties that produce very weak molecular ions in order to verify fragmentation patterns in the absence of any column bleed background. FAME samples were cold-trapped into the gas chromatograph at 50°C in the splitless mode for 15 sec. After an initial time of 2 min at 80°C, the GC oven was programmed to 220°C at 8°C/min. The final hold time was 32 min. Helium carrier gas was employed at a flow-rate of 0.6 ml/min. Injector temperature was 230°C and ionizer temperature was maintained at 250°C. Scan-rate was set at 1 sec per scan over a mass range of 50—450 a.m.u. and the electron energy was 70 eV.

RESULTS AND DISCUSSION

The findings indicate that the method described affords a rapid and quantitative profile of fatty acid derivatives of human erythrocyte membranes. Tentative identification of FAME constituents by GC with flame ionization detection (FID) was confirmed by GC—MS analysis and compared with reports [5—8] in the literature. The labeled chromatographic profile (Fig. 1) includes methylated isomers, odd-numbered-carbon FAMEs, double-bond positional

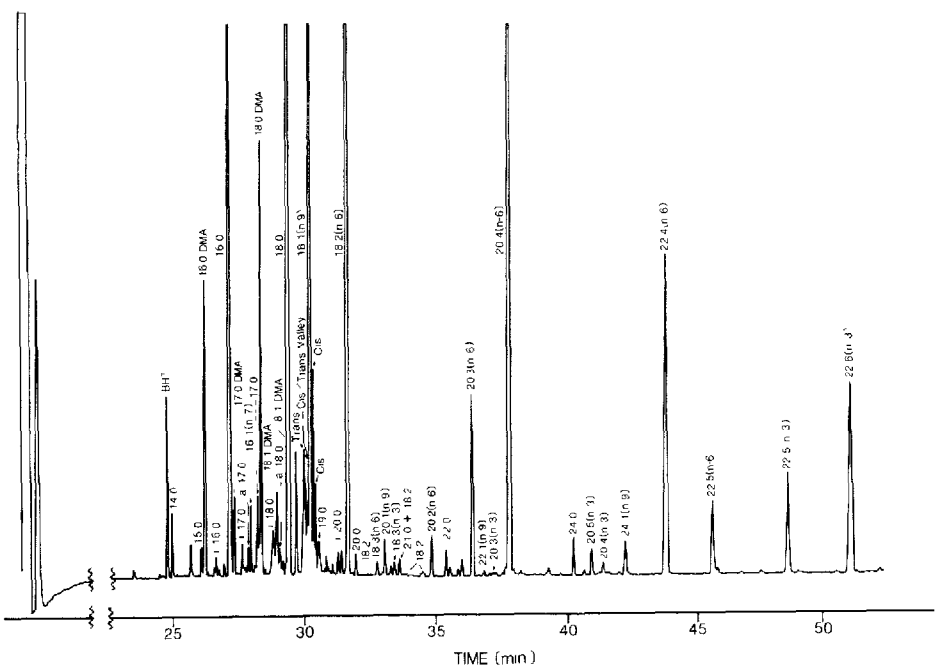

Fig. 1 GC—FID profile of human erythrocyte membrane FAMEs on an SP 2560 fused-silica capillary column (100 m × 0.25 mm I.D., 0.2 μm film thickness) Temperature program: 80°C for 2 min, 8°C/min to 220°C, hold for 32 min. BHT = antioxidant, butylated hydroxy-toluene; DMA = dimethylacetal derivatives of fatty aldehydes; i = *iso*, a = *anteiso*.

isomers, and dimethyl acetals (DMA) detected in extracts of human erythrocyte membranes. Branched-chain *iso* and *anteiso* isomers of fourteen to eighteen and twenty carbon atoms were unequivocally demonstrated at the GC retention times labeled on Fig. 1. Fatty acid families (n-3), (n-6), and (n-9), are completely resolved with the exception of 18:1(n-9). Blank samples revealed a peak for BHT and two additional very small peaks that eluted near

TABLE I

RETENTION BEHAVIOR OF FATTY ACID METHYL ESTERS ON A 100 m × 0 25 mm, 0.2 μm FILM THICKNESS SP 2560 FUSED-SILICA CAPILLARY COLUMN

Injections are 0.5 μl in hexane (n = 31)

Fatty acid	t_R (mean ± S.D., min)	RRT (mean ± S.D.)
14 0	24.650 ± 0.05413	0.839 ± 0.00062
iso-15:0	25.448 ± 0.05510	0.864 ± 0.00049
anteiso-15:0	25.597 ± 0.05221	0.872 ± 0.00058
14:1 (n-5)	25.876 ± 0.05111	0.878 ± 0.00067
15 0	25.798 ± 0.05100	0.879 ± 0 00057
iso-16:0	26 405 ± 0.06455	0.899 ± 0.00022
anteiso-16.0	26.602 ± 0.05545	0.906 ± 0.00038
16:0	26.986 ± 0.06352	0.919 ± 0 00027
iso-17:0	27.494 ± 0.05706	0.936 ± 0.00067
anteiso-17.0	27.739 ± 0.05605	0 945 ± 0.00075
16:1(n-7)	27.851 ± 0.07262	0.948 ± 0.00067
17 0	28.078 ± 0.06973	0.956 ± 0.00067
iso-18:0	28.682 ± 0.06237	0.977 ± 0.00042
anteiso-18:0	28.988 ± 0.05973	0.987 ± 0 00062
18:0	29.368 ± 0.07415	1.000 ± 0 00000
trans-18:1	30.004 ± 0.06792	1 022 ± 0.00046
trans-18:1	30.117 ± 0 06300	1.026 ± 0.00052
cis-18:1(n-9)	30.269 ± 0.07171	1.031 ± 0.00035
cis-18:1	30.371 ± 0.06831	1.034 ± 0.00049
cis-18:1	30.456 ± 0 06290	1.037 ± 0.00074
19·0	30.583 ± 0.06144	1.041 ± 0.00070
iso-20:0	31.327 ± 0.06581	1.067 ± 0.00074
cis-18:2(n-6)	31 716 ± 0.06979	1.080 ± 0.00069
20:0	32.053 ± 0.07038	1.091 ± 0.00091
18:3(n-6)	33 105 ± 0.07108	1.127 ± 0 00093
20:1(n-9)	33.212 ± 0.06908	1.131 ± 0.00095
18·3(n-3)	33.612 ± 0.07197	1 144 ± 0.00101
21:0	33.817 ± 0.07002	1.151 ± 0.00099
20 2(n-6)	35.122 ± 0.07474	1.196 ± 0.00107
22:0	35.678 ± 0.08273	1.214 ± 0.00028
20:3(n-6)	36.765 ± 0.08162	1.252 ± 0.00120
22:1(n-9)	37.251 ± 0.08352	1.268 ± 0.00116
20:3(n-3)	37.709 ± 0.08343	1.284 ± 0.00119
20·4(n-6)	38.238 ± 0.09621	1.302 ± 0.00114
24:0	40.766 ± 0.08733	1.388 ± 0.00159
20:5(n-3)	41.552 ± 0.09359	1.415 ± 0 00169
24.1(n-9)	42.971 ± 0.08667	1.463 ± 0.00154
22:4(n-6)	44.589 ± 0.11245	1.518 ± 0.00255
22·5(n-6)	46.470 ± 0.11242	1.582 ± 0.00305
22:5(n-3)	49.469 ± 0.12542	1.684 ± 0.00374
22:6(n-3)	51 976 ± 0.12954	1.770 ± 0.00402

the retention times of 16:0 and 18:1(n-9) FAMEs. Mass spectra of the two small peaks resembled those of hydrocarbons. The methylated composite standards and individual FAMEs of 20:4(n-6), 20:5(n-3), 22:4(n-6), and 22:6(n-3) were chromatographed for verification of GC retention behavior.

Injections of 1 μl or larger produced column overload as evidenced by leading edges on later eluting peaks. Increased sample volumes also produced considerable variability in retention data which was not the case with 0.5-μl injections. Table I is a tabulation of FAME retention behavior using 0.5-μl injections in hexane on the 100-m SP 2560 capillary column which demonstrates excellent precision with absolute retention time (t_R) and relative retention time (RRT) data. These data were collected over a four-month time period with special care being given to monitoring of linear velocity and split ratio.

Identities of the chromatographic peaks detected by using the FAME standards were in all cases consistent with the mass spectra of material eluting at the respective retention times. The MS pattern in Fig. 2 is characteristic of the saturated FAMEs [9—11]. Fragment ions are indicated by arrows. These ions are formed by α- and β-cleavages of methylated fatty acids. Cleavage at the α-position produces ions R^+, $R{-}C{\equiv}O^+$, $^+O{\equiv}C{-}OCH_3$, and $^+OCH_3$ [9—11]. The R^+ (M − 59) ion occurs at low intensity or may be absent [9, 10, 12]. Ions that are characteristic of the methoxy group in the methyl ester, $^+O{\equiv}C{-}CH_3$ (*m/z* 59) and $^+OCH_3$ (*m/z* 31), are low in abundance, but their presence provides diagnostic information [9—11].

Cleavage β to the carbonyl group results in a γ-hydrogen transfer and the formation of a rearranged radical ion at *m/z* 74 which is the base peak [11]. Other ion fragments are of the form $[(CH_2)_nCOOCH_3]^+$ yielding *m/z* 87, 101, etc. [9—11].

Branched-chain FAMEs (*iso* and *anteiso*) yield fragment ions comparable to

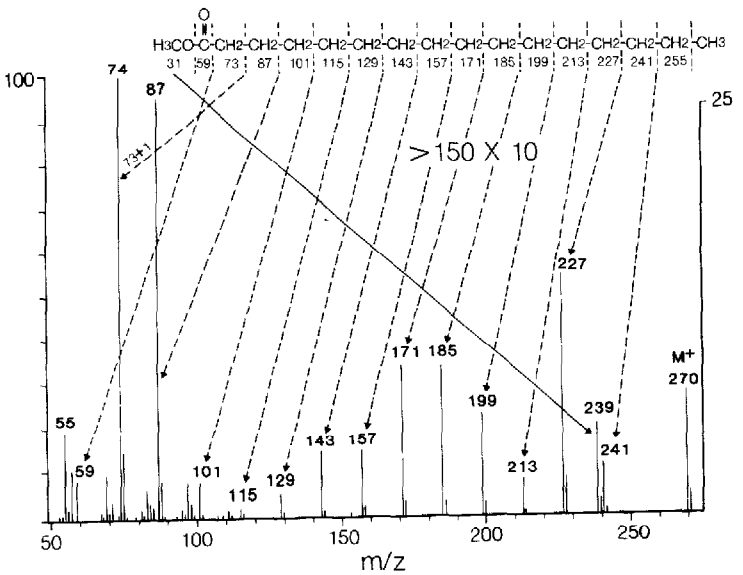

Fig. 2. Fragmentation pattern of methyl hexadecanoate (16:0 FAME). Fragment ions, of the form R^+, $R{-}C{\equiv}O^+$, $^+O{\equiv}C{-}CH_3$, $^+OCH_3$, and $[(CH_2)_nCOOCH_3]^+$, are indicated by arrows. The mass spectrometer was used in the EI mode, the electron energy was 70 eV.

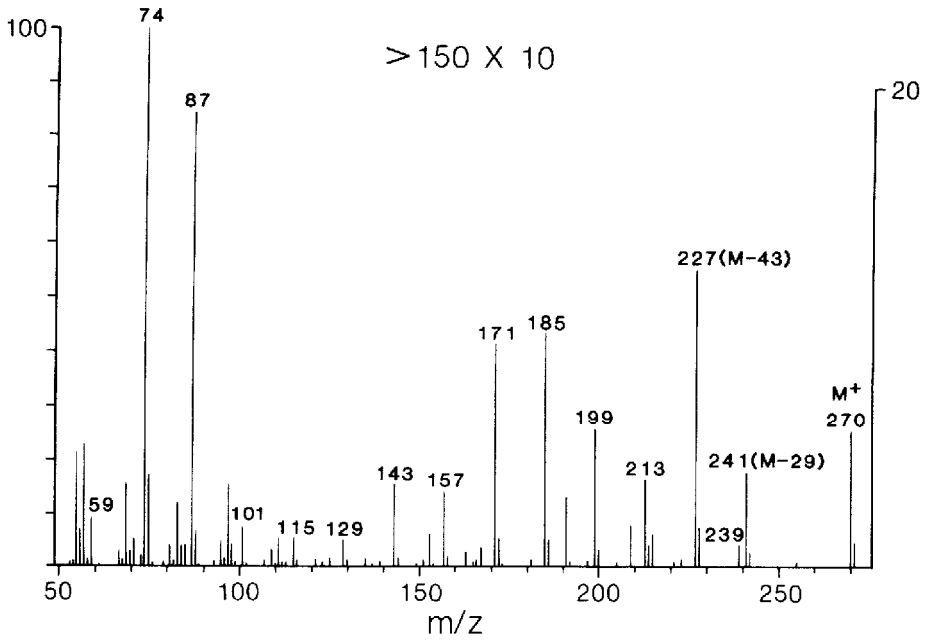

Fig. 3. Mass spectrum of *anteiso* methyl hexadecanoate (a 16:0 FAME). The mass spectrometer was used in the EI mode; the electron energy was 70 eV.

those observed in saturated FAMEs. Identification of these FAMEs is based on retention behavior (Fig. 1) and the relative abundance of the M — 29 and M — 31 ion fragments (Fig. 3). The *m/z* 241 ion is substantially larger than *m/z* 239 in the *anteiso*-16:0 FAME (Fig. 3). The earlier eluting *iso* FAME fragmentation pattern reveals more evenly distributed intensities of these ions.

Monounsaturated FAME peaks produce spectra consistent with Fig. 4. The diagnostic molecular ion, M^+, and ion fragments resulting from M — 32, M — 74, and M — 116 coupled with a change in the base peak from *m/z* 74 to *m/z* 55 in the mass range 50—450 a.m.u., affords identification. Other ion fragments are identical to those found in the saturated FAMEs but usually exist in lower relative abundance [9—11].

Polyunsaturated FAMEs yield mass spectra that differ in fragmentation patterns among positional isomers [9—11]. A weak but discernable M^+ is present for FAMEs with as many as six double bonds and the base peak changes from *m/z* 55 in the monounsaturated to *m/z* 67 in the di- and triunsaturated. The peak *m/z* 91 (C_7H_7), which is very small in the monounsaturate, increases in height relative to the base peak with the number of double bonds [9]. With four or more double bonds, the M^+ becomes less apparent, except for 22:4(n-6), and the base peak is *m/z* 79 (Fig. 5). Longer integration times are required to detect these low-intensity M^+ ions, and the isotope peak (M + 1) may be too weak to be observed.

The results tabulated in Table II represent average relative amounts of FAMEs from 24 erythrocyte ghost samples, as quantified by GC—FID peak height data. Quantification using peak height measurements was selected due to wide variation in area percent calculations of closely eluting FAME peaks using the HP 3390A reporting integrator. Weight percent calculations were

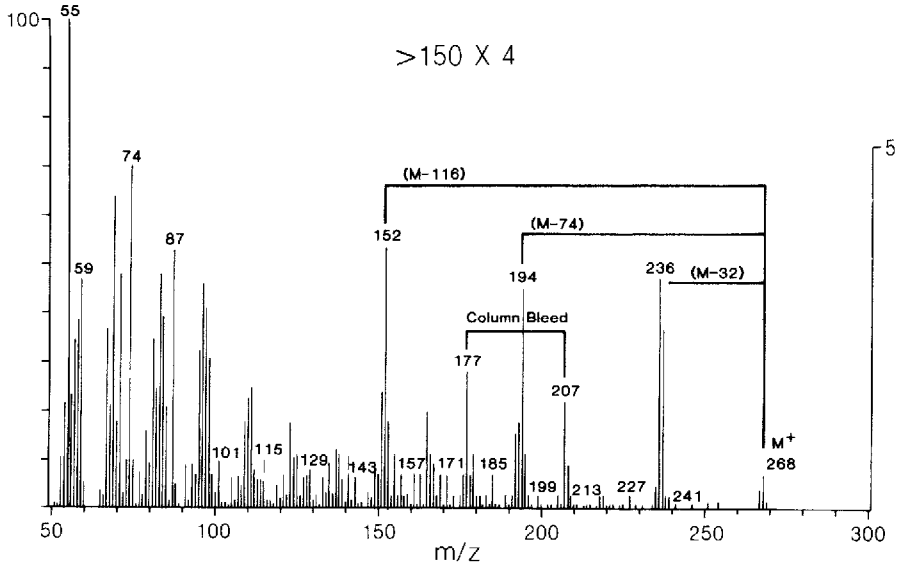

Fig. 4. Mass spectrum of methyl hexadecanoate (16 1 FAME). Monounsaturated FAMEs display intense ion fragments M — 32, M — 74, and M — 116. The mass spectrometer was used in the EI mode; the electron energy was 70 eV.

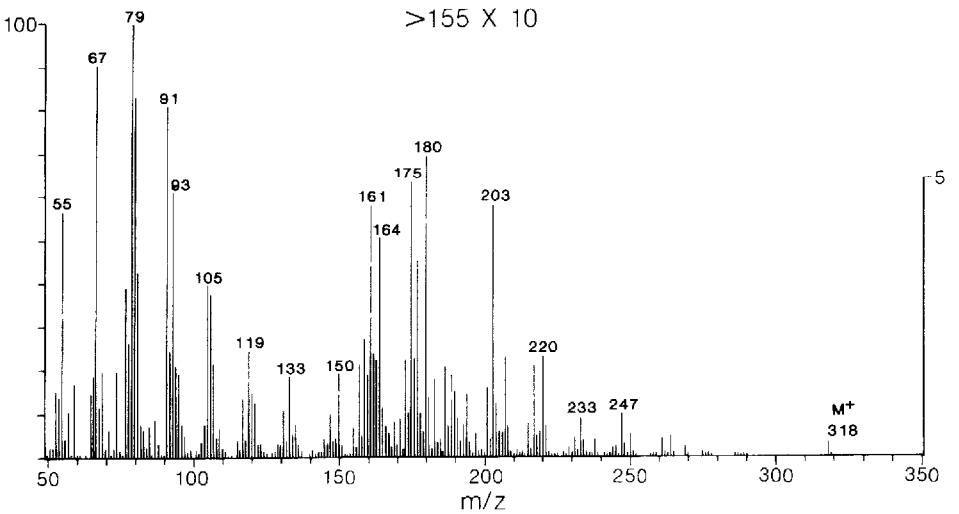

Fig. 5. Mass spectrum of methyl arachidonate, 20:4(n-6). A Finnigan 4000 quadrupole mass spectrometer with a 6115 data system was used in the EI mode. The electron energy was 70 eV.

performed using response factors obtained from FAME standards of specific concentrations shown in Table III. In cases where FAME standards were not available, interpolation based on known response factors for the similar series of compounds was used to obtain the response factors. Free fatty acids were also procured and analyzed using the same methylation procedure to assess derivatization efficiency and recovery. Quantitative recoveries of FAME standards and fatty acids were > 90% with essentially non-detectable losses due

TABLE II

RELATIVE AMOUNTS OF FAMEs FROM 24 HUMAN ERYTHROCYTE GHOST SAMPLES

FAME	Height % (mean ± S.D.)	Weight % (mean ± S.D.)
14:0	0.35 ± 0.12	0.25 ± 0.09
iso-15:0	<0.10	<0.10
anteiso-15:0	<0.10	<0.10
14:1(n-5)	<0.10	<0.10
15:0	0.19 ± 0.09	0.14 ± 0.07
iso-16:0	0 13 ± 0.07	0.10 ± 0.05
anteiso-16:0	<0.10	<0.10
16:0	22.97 ± 1.22	16 95 ± 0.09
iso-17:0	0.23 ± 0.09	0 18 ± 0.07
anteiso-17:0	0.18 ± 0.07	0.14 ± 0.05
16:1(n-7)	0.42 ± 0.16	0.31 ± 0.12
17:0	0.58 ± 0.14	0.44 ± 0.11
iso-18:0	0 28 ± 0.09	0.21 ± 0.07
anteiso-18:0	0.21 ± 0.10	0.16 ± 0.08
18:0	16.77 ± 1.68	12.82 ± 1.28
trans-18:1	1.02 ± 0.33	0.81 ± 0.26
trans-18·1	0.57 ± 0.34	0.45 ± 0.27
cis-18:1(n-9)	15.13 ± 1.08	12.06 ± 0.86
cis-18:1	1.91 ± 0.36	1.52 ± 0.29
cis-18:1	1.00 ± 0.45	0.79 ± 0.36
19:0	0.34 ± 0 11	0.28 ± 0.09
iso-20:0	0.18 ± 0.07	0.16 ± 0.06
cis-18:2(n-6)	14.36 ± 1.51	12.74 ± 1.34
20:0	0.17 ± 0.04	0.15 ± 0 04
18:2	<0 10	<0.10
18:3(n-6)	0.14 ± 0.06	0.15 ± 0.06
20·1(n-9)	0.31 ± 0.07	0.29 ± 0.07
18 3(n-3)	0.11 ± 0.05	0.11 ± 0.05
21:0+18:2	0 14 ± 0.07	0.14 ± 0 07
18:2	<0.10	<0.10
18.2	<0.10	<0.10
20:2(n-6)	0.33 ± 0.06	0.34 ± 0.06
22:0	0.19 ± 0.09	0.21 ± 0.10
20 3(n-6)	1.79 ± 0.42	2.24 ± 0.53
22:1(n-9)	<0.10	<0.10
20.3(n-3)	<0 10	<0.10
20·4(n-6)	13.36 ± 1.26	19.27 ± 1.81
24:0	0.29 ± 0.13	0.48 ± 0.22
20·5(n-3)	0.25 ± 0.10	0.67 ± 0.27
20 4(n-3)	0.13 ± 0.04	0.19 ± 0.06
24:1(n-9)	0.22 ± 0.12	0.39 ± 0.21
22 4(n-6)	2.69 ± 0.45	5.94 ± 0.99
22 5(n-6)	0.54 ± 0.16	1.41 ± 0.42
22 5(n-3)	1.04 ± 0.21	2.71 ± 0.55
22:6(n-3)	1.51 ± 0.46	4.81 ± 1 47

to the Unisil chromatography columns. We have included the relative weight percent data for identified FAME chromatographic peaks only and omitted preservative (BHT), DMA, and other spurious peaks. Amounts detected for FAMEs with less than fourteen carbon atoms were so small that peak height data sufficient for integration were not obtained.

TABLE III

RESPONSE FACTORS (RF) IN HEIGHT COUNTS/ng FAME (n = 12)

Capillary column SP 2560 100 m × 0 25 mm, 0.2 μm film thickness

FAME	RF	$RRF_{18.0}$*
14:0	987.58	1.064
iso-15 0	970.32	1.045
anteiso-15:0	970 32	1.045
14:1(n-5)	932.02	1.004
15:0	970.32	1.045
iso-16·0	961.97	1 036
anteiso-16:0	961.97	1.036
16:0	961.91	1.036
iso-17·0	939 82	1.012
anteiso-17:0	939.82	1.012
16:1(n-7)	959.26	1.033
17:0	939.82	1.012
iso-18 0	928.54	1.000
anteiso-18.0	928.54	1.000
18:0	928.54	1.000
trans-18 1	890.47**	0.959**
trans-18 1	890.47**	0.959**
cis-18·1(n-9)	890.22	0.959
cis-18.1	890.47**	0.959**
cis-18.1	890.47**	0.959**
19 0	854.26	0.920
iso-20:0	781.30**	0.841**
cis-18 2(n-6)	800.00	0 862
20:0	781.30	0.841
18:2	800.40**	0.862**
18:3(n-6)	678.76	0.731
20 1(n-9)	761.06	0.820
18:3(n-3)	678.32	0.731
21:0+18·2	714.98	0.770
18:2	800.40**	0.862**
18·2	800.40**	0.862**
20 2(n-6)	694.14	0.748
22:0	621.20	0.699
20.3(n-6)	565.96	0.610
22 1(n-9)	533.08	0.574
20:3(n-3)	525.96	0.566
20:4(n-6)	492.30	0.530
24:0	426.52	0.459
20:5(n-3)	268.06	0.289
20:4(n-3)	492 13**	0.530**
24 1(n-9)	397 14	0.428
22 4(n-6)	321 09	0.346
22·5(n-6)	271.13**	0.293**
22:5(n-3)	271.13**	0.293**
22.6(n-3)	223.18	0.240

*Response factor relative to 18:0.

**Interpolated; based on known response factors for the similar series of FAMEs Response factors shown in order of elution of FAMEs.

Variation in relative amounts of particular FAMEs was as high as > 100% and as low as 6% among samples tested. The FAMEs showing the least variation, as indicated by the S.D., among samples were 16:0, 18:0, 18:2(n-6), and 20:4(n-6) which are expected to be influenced somewhat with diet and time of sample collection. This is in agreement with previous reports [7, 8] and can be followed throughout the (n-6) and (n-3) families of essential fatty acids [6, 7]. Variation in kinds of FAMEs among samples was not observed.

There are reports in the literature of mean values of erythrocyte FAMEs of selected populations, and these were compared with our results. Heckers and co-workers [5, 13] collected FAME data on erythrocyte ghosts from 25 disease-free subjects in each of two age groups and our mean values are in close agreement with these results. These investigators found more variation among erythrocyte membrane phospholipid FAME relative quantities in young individuals than among a population of older subjects. As expected, our observations fell intermediate to these findings since the population used in our study had a wide age range, < 15 years to > 70 years. Other published works presented similar results [7, 8, 14] but the total erythrocyte FAME profiles were abbreviated compared to our findings.

Losses of the unsaturated FAME during storage at −20°C were observed by comparison with freshly prepared samples. These losses were apparently due in part to the residual hemoglobin content of the washed erythrocyte ghosts [12, 15]. Since it is difficult to remove all residual hemoglobin, and in view of the reports [12, 15] on autoxidation processes of fatty acids, erythrocyte membrane fatty acid studies demand a rapid sample analysis time to obtain the best results.

MS data herein confirm the GC results obtained using this rapid extraction procedure. Literature reports [5—8] on fatty acid analysis of human erythrocyte membranes are in agreement with our results, but the procedure reported here allows a more complete analysis of minor membrane components. Therefore, we suggest that the GC method described offers utility in clinical laboratories charged with determination of altered or abnormal fatty acid profiles from erythrocyte membranes.

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