# Lipid Class and Fatty Acid Composition of Rat Liver Plasma Membranes Isolated by Zonal Centrifugation*

Raymond C. Pfleger,† Norman G. Anderson, and Fred Snyder

ABSTRACT: Plasma membranes of rat liver were isolated by zonal centrifugation for analysis of lipid classes and fatty acid composition. The yield of plasma membranes by this technique was 880 μg of protein and 390 μg of total lipid per g of liver. Neutral lipids represented 27% of the total extractable lipids, and this was mainly cholesterol. Phosphatidylcholine accounted for almost 40% of the phospholipids in the plasma membranes of rat liver. Phosphatidylethanolamine and sphingomyelin amounted to approximately 20 and 18%, respectively, of the total phospholipids. Phosphatidylserine and phosphatidylinositol were not resolved from each other; this mixed fraction contained approximately 13% of the total lipid phosphorus. Lysophosphatidylcholine and polyglycerolphosphatides plus phosphatidic acid were present as minor components of the membrane lipids. An unidentified polar lipid containing no phosphorus was resolved from other phospholipids and accounted for approximately 12% of the total lipids. The alkyl and alk-1-enyl ethers of glycerol liberated from the total lipids of liver plasma membranes were 0.97 and 0.24%, respectively. The fatty acid composition of all phospholipids, triglycerides, and free fatty acids was determined by gas–liquid partition chromatography. The fatty acid composition of phosphatidylcholine and phosphatidylethanolamine was similar. About twice as much palmitate (28%) and stearate (31%) as linoleate (14%) and arachidonate (14%) was found in these fractions. The fatty acid composition of the mixture of phosphatidic acid and polyglycerolphosphatides was similar to that of phosphatidylcholine and phosphatidylethanolamine.

The sphingomyelin fraction of the liver cell membranes contained most of the polyunsaturated acids ($C_{20}$ and above), whereas lysophosphatidylcholine contained the highest percentage of saturated fatty acids. The unidentified polar lipid containing no phosphorus had a fatty acid composition of 16:0 (32%), 18:0 (48%), 18:1 (10%), and 18:2 (10%). Triglycerides were comprised mainly of 16:0, 18:1, and 18:2 acyl moieties. The plasma membranes of rat liver isolated by zonal centrifugation appear to have many features of lipid composition similar to those reported by others for pure membranes of rat erythrocytes.

Precise knowledge of the composition of mammalian cell membranes has been limited because of the difficulty in preparing pure material. Until recently our understanding of the composition of cell membranes was limited to data concerning the erythrocyte (Ponder, 1961; De Gier and Van Deenen, 1961), which might be a poor model for mammalian cells. But in 1960, Neville described a procedure for isolating rat liver cell membranes, and several other investigators (Emmelot *et al.*, 1964; Takeuchi and Terayama, 1965; Ashworth and Green, 1966) partially characterized the lipids found in such membrane preparations. Recently, Skipski and coworkers (1965), using the isolation procedure of Neville, presented a detailed analysis of lipid classes in plasma membranes of rat liver. However, the plasma membranes isolated by the technique of Neville (1960) and the modifications of Emmelot *et al.* (1964) and Finean and coworkers (1966) were shown by Finean *et al.* to yield a preparation contaminated (10–15%) with mitochondria and endoplasmic reticulum. A later report by Skipski's group (Barclay *et al.*, 1967) also indicated that the membrane fraction contained swollen mitochondria with fragmented cristae, amorphous material, and small vesicles of unknown origin.

Liver cell plasma membranes essentially free of other particulate constituents were recently sedimented (El-Aaser *et al.*, 1966) in the A-XII low-speed zonal centrifuge rotor (Anderson *et al.*, 1966b) as a band that occupied an intermediate position between nuclei and mitochondria, confirming the view that cell-membrane fragments tend to sediment faster than mitochondria. Anderson *et al.* (1967, 1968a) have extended the zonal technique to achieve sequentially a high-resolution rate-zonal separation and an isopycnic-zonal separation of pure liver cell membranes in the A-XII rotor. This paper describes the lipid classes and fatty acid composition of each lipid class found in the plasma membranes purified by zonal centrifugation. It also indicates the value of zonal rotor systems which can be partially unloaded

* From the Lipid Research Laboratories, Medical Division of the Oak Ridge Associated Universities, Oak Ridge, Tennessee, under contract with the U. S. Atomic Energy Commission and with the Joint National Institutes of Health, Atomic Energy Commission Molecular Anatomy Program (the Molecular Anatomy Program is sponsored by the U. S. Atomic Energy Commission, the National Cancer Institute, the National Institute of General Medical Sciences, and the National Institute for Allergy and Infectious Diseases), Oak Ridge National Laboratory (operated for the U. S. Atomic Energy Commission by the Nuclear Division of Union Carbide Corp.), Oak Ridge, Tennessee 37830. *Received April 4, 1968.*

† Present address: Lovelace Foundation for Medical Education and Research, Albuquerque, N. M. 87108.

during a rate separation and therefore allow one fraction to be banded isopycnically in the same rotor. The advantage of unloading from the rotor edge is apparent; new experimental rotors (B-XXIII and B-XXIX) have been designed which may be unloaded from either the center or the edge.

## Experimental Procedures

*Isolation of Rat Liver Plasma Membranes by Zonal Centrifugation.* Livers from a total of 10 male Sprague–Dawley rats weighing approximately 300 g and maintained on a Purina Laboratory diet were used to prepare 13 different liver-membrane samples. Fresh unperfused rat liver was diced with scissors in chilled 1 mM $NaHCO_3$ (pH adjusted to 7.6 with NaOH) and homogenized with 15 even strokes of a loose-fitting Dounce homogenizer. The homogenate was filtered through eight layers of cheesecloth and diluted to a final volume of 30 ml/g of liver. The suspension was centrifuged for 15 min at 260 rpm (1720*g*) at 0° in an International PR-2 centrifuge using 250-ml tubes in the no. 259 head. The supernatant was discarded and the loose pellet was rehomogenized (five strokes) and resuspended in the bicarbonate buffer (12 ml, 1 mM $NaHCO_3$/g of liver). This suspension was used for zonal centrifugation.

The B-XV titanium zonal rotor recently described (Anderson *et al.*, 1968b) has a capacity of 1666 ml. It was operated in a modified Spinco Model L centrifuge equipped with an $\int\omega^2 dt$ integrator previously described (Anderson *et al.*, 1966a) and a device for sensing and controlling temperature. A 190-ml membrane suspension was layered over a 500-ml sucrose gradient (19–35 w/w% sucrose) formed by a Spinco Model 131 gradient pump. The membrane suspension was followed by an overlay (200 ml, 1 mM $NaHCO_3$) and centrifuged at 5000 rpm until $\omega^2 t = 2 \times 10^8$. The first 800 ml (20 40-ml fractions) at the top of the gradient was unloaded by displacing the lighter portion by a 45% sucrose pushout solution, which was the same concentration as the cushion.

After removal of the centripetal 800 ml, containing the bulk of the soluble, mitochondrial, and microsomal fractions (Figure 1), a 50-ml $H_2O$ overlay was added and the rotor was accelerated to 20,000 rpm and run until $\omega^2 t = 3.5 \times 10^9$ at unloading. The membrane fragments were found to be sharply banded at an average of 41.04% (w/w) sucrose. The 260-mμ optical density profile of Figure 1 shows the sharp banding of the membrane fraction (tubes 22–26). Observations of the banded membranes in numerous phase-contrast and electron micrographs (Figure 2) confirmed the high purity of the preparation. Other criteria used to judge the purity for the plasma membranes were (1) absence of cytochrome oxidase (D. H. Brown, personal communication), (2) high concentration of alkaline ATPase (Lieberman *et al.*, 1967; Anderson *et al.*, 1968a), and (3) absence of material banding above 42% sucrose during isopycnic centrifugation (Figure 1). Each of the membrane fractions was diluted 2:1 (v/v) with bicarbonate buffer and centrifuged at 30,000 rpm for 30 min at 0° in a no. 30 rotor. The sucrose was drawn off and discarded, and the membrane samples were stored at −23° until further analysis.

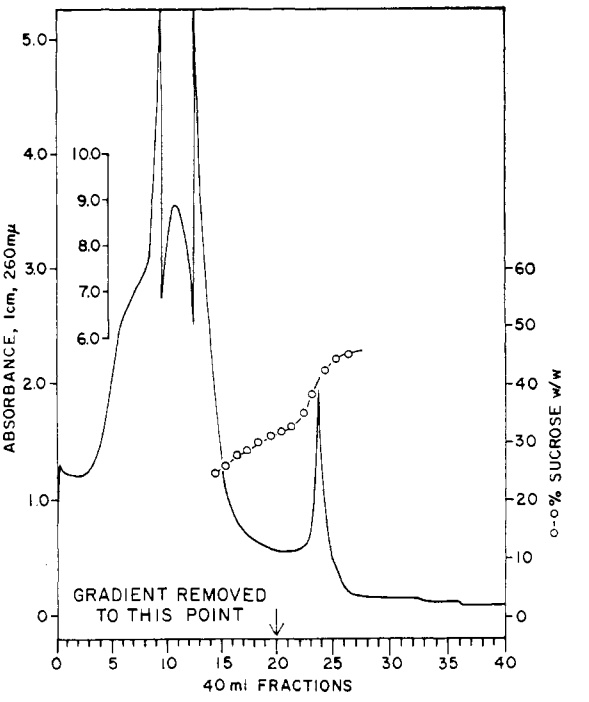


FIGURE 1: Rate-zonal and isopycnic-zonal two-stage separation of plasma membranes of rat liver in a sucrose (19–35 w/w %) gradient in the B-XV titanium rotor. The overlay, soluble protein fraction, mitochondria, and microsomes (part of gradient to the left of the arrow) were recovered after a short rate-zonal centrifugation ($\omega^2 t = 2.11 \times 10^8$). The plasma membranes banded sharply at 41.04 w/w% sucrose after centrifuging until $\omega^2 t = 3.5 \times 10^9$.

*Total Protein and Total Lipid Analyses.* The membranes were thawed and resuspended in 20–30 ml of 1 mM bicarbonate. Aliquots were taken for lipid extraction and for protein analysis using the Lowry method (Lowry *et al.*, 1951) with the Folin reagent. Total membrane lipids were extracted twice by the procedure of Bligh and Dyer (1959), first from the 1 mM bicarbonate solution in which the membranes were suspended and then from 3% $NH_4OH$ to assure complete extraction of the polyglycerolphosphatides (Rouser *et al.*, 1963). Aliquots of the total lipid extract were weighed on a Cahn electrobalance;[1] all aliquots that were dried for weighings were discarded. The following precautions were used to minimize alterations in lipids during the extraction, storage, and chromatographic procedures. (1) All tissue samples were maintained at Dry-Ice or liquid nitrogen temperatures during the period immediately after zonal centrifugation until lipid extraction. (2) All extractions were carried out in the cold. (3) All lipids were stored in solvent at −27° prior to chromatography. The measurement of polyunsaturated fatty acid standards that have been handled under these experimental conditions is quantitative. The experimental conditions for the preparation and analysis of lipid samples are based on

[1] Cahn Electrobalance, Model M-10, W. H. Curtin & Co. Memphis, Tenn.

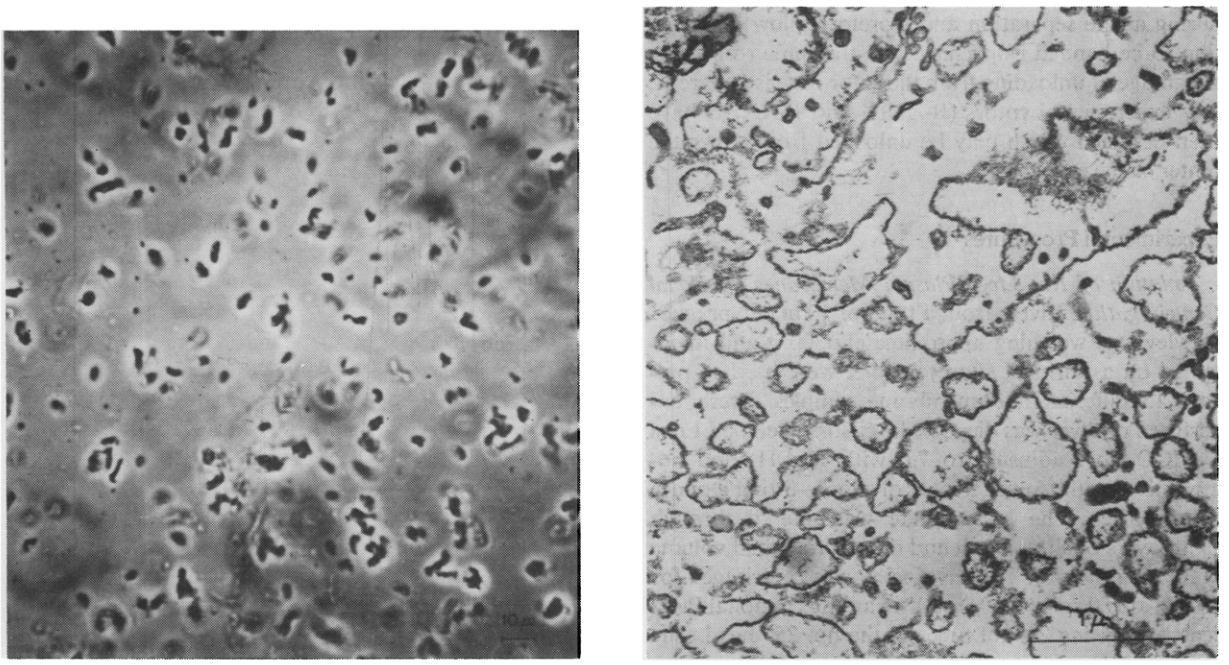

FIGURE 2: Phase-contrast (A, left) and electron micrographs (B, right) of plasma membranes of rat liver isolated in the B-XV titanium rotor by the rate- and isopycnic-zonal centrifugation technique described in the text.

the suggestions of James (1960) and Entenman (1961). In some samples the neutral and phospholipids were quantitatively separated on a silicic acid column (Bio-Sil HA,[2] 325 mesh) and the percentage of each in the total lipid extract was determined by weight (Wood and Snyder, 1968; Borgström, 1952).

*Thin-Layer Chromatography*. The neutral lipids were resolved (Figure 3) on thin layers (250 $\mu$) of silica gel G[3] in a solvent system consisting of hexane–diethyl ether–acetic acid (70:30:1, v/v). The components were measured quantitatively by a photodensitometric analysis (Privett *et al.*, 1965). The polar lipids were routinely chromatographed in two systems (Figure 3), both of which used silica gel HR[3] (250 $\mu$) made into a slurry with 1 mM $Na_2CO_3$. The solvent systems were chloroform–methanol–acetic acid–0.9% NaCl (50:25:8:4, v/v) and chloroform–methanol–2 M ammonium hydroxide (60:35:8, v/v). No elution procedures were used in the analysis of lipids separated by thin-layer chromatography, since in general such procedures are not always quantitative. We chose to make use of quantitative techniques whereby the lipid classes were measured either directly on the adsorbent layers by photodensitometry or by colorimetric procedures carried out in the presence of adsorbent. All procedures used were validated against known lipid standards. The standards used were obtained from The Hormel Foundation (cholesteryl oleate, triolein, oleic acid, and cholesterol) and Applied Science Laboratories, Inc., and Supelco, Inc. (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylserine, lysolecithin, cardiolipin, and phosphatidylinositide). The phospholipid classes were quantitated after thin-layer chromatography by measuring the phosphorus distribution on silica gel scrapings according to the method of Rouser *et al.* (1966). Recoveries of phosphorus along the entire chromatographic lane were compared to the phosphorus content of total aliquots chromatographed; recoveries were quantitative. Alkyl and alk-1-enyl glyceryl ethers were determined on aliquots of the total lipids by photodensitometric thin-layer chromatography of the $LiAlH_4$ hydrogenolysis products (Wood and Snyder, 1968).

*Gas–Liquid Partition Chromatography*. Lipid samples from membrane preparations were individually chromatographed and the percentage composition of the fatty acid methyl esters was determined from a half-height analysis of the chromatographic peaks. The lipids resolved on the thin layers were scraped into methylation tubes. Transmethylation of the acyl groupings with $H_2SO_4$–methanol (Ways *et al.*, 1963) was not affected by the presence of the silica gel, which remained with the aqueous methanol phase when the fatty acid methyl esters were extracted with petroleum ether (bp 30–60°). Free fatty acids were methylated with diazomethane (DeBoer and Backer, 1954) after they were scraped and eluted from the adsorbent with $CHCl_3$. Gas chromatography data obtained with fatty acid standards and known phospholipids (after transesterification) agreed with the known fatty acid composition.

The methyl esters were analyzed on an Aerograph Model 600D Hy-Fi gas chromatograph[4] equipped with a hydrogen flame detector. An aluminum column (5 ft × 1/8 in.) packed with 15% ethylene glycol succinate methyl silicone polymer (EGSS-X) coated on

[2] Bio-Rad Laboratories, Richmond, Calif.

[3] Silica gel according to Stahl, Brinkmann Instruments, Inc., Westbury, Long Island, N. Y.

[4] Wilkens Instruments & Research, Inc., Santa Monica, Calif.

TABLE I: Lipid, Phospholipid Phosphorus, and Protein Values for Plasma Membranes of Rat Liver.

| | |
|---|---|
| mg of protein/g wet wt of liver (13)[a] | 0.88 ± 0.24 |
| mg of total lipid/g wet wt of liver (13) | 0.39 ± 0.05 |
| mg of total lipid/mg of membrane protein (13) | 0.44 ± 0.21 |
| mg of phospholipids/mg of membrane protein (10) | 0.27 ± 0.04 |
| mg of total lipid/μmoles of membrane phospholipid phosphorus (10) | 1.48 ± 0.40 |
| mg of protein/μmoles of membrane phospholipid phosphorus (10) | 2.28 ± 0.68 |
| μmoles of cholesterol/μmoles of membrane phospholipid phosphorus (7) | 0.74 ± 0.12 |
| mg of total membrane phospholipid phosphorus/mg of total membrane phosphorus (6) | 0.95 ± 0.02 |

[a] The numbers in parentheses represent the number of membrane preparations analyzed as separate determinations.

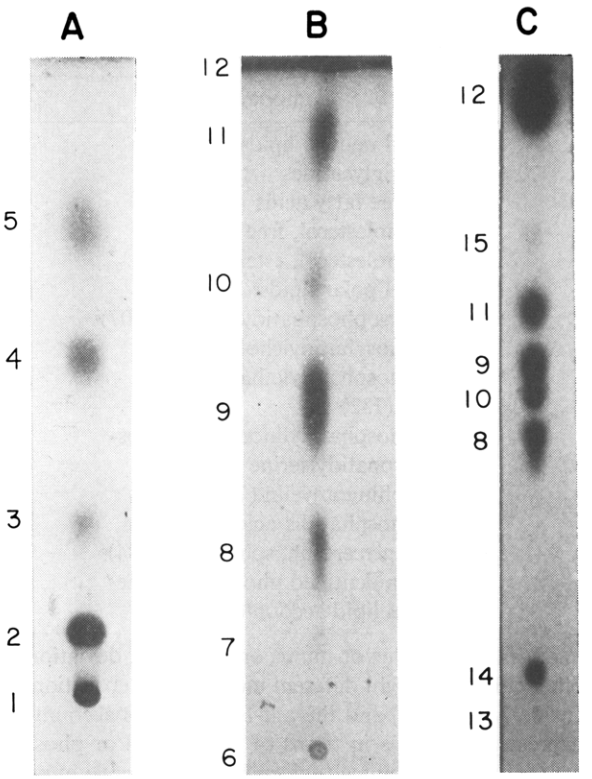


FIGURE 3: Thin-layer chromatograms of lipid material extracted from plasma membranes of rat liver. (A) Thin-layer chromatographic system using silica gel G and a solvent system of hexane–diethyl ether–acetic acid (70:30:1, v/v); (B) thin-layer chromatography system using silica gel H and a solvent system of chloroform–methanol–acetic acid–0.9% NaCl (50:25:8:4, v/v); (C) thin-layer chromatography system using silica gel H and a solvent system of chloroform–methanol–2 M ammonium hydroxide (60:35:8, v/v). The numbers 1, 6, and 13 are at the origin of the chromatograms. The other numbers designate lipid compounds based on known standards: (1) phospholipids, (2) cholesterol, (3) fatty acids, (4) triglycerides, (5) cholesterol esters, (7) lysolecithin, (8) sphingomyelin, (9) lecithin, (10) phosphatidylinositol + phosphatidylserine, (11) cephalin, (12) neutral lipids, (14) unknown phosphorus-free material, and (15) polyglycerolphosphatides.

Gas-Chrom P[5] (100–120 mesh) was prepared, conditioned, and used according to the procedure of Wood and Snyder (1966). The column and detector temperatures were maintained at 180° by a Wilkens Model 328 isothermal (±0.1°) temperature controller. The flash heater was operated at 250°. An Oscar Model 556 aquarium pump[6] supplied 300 cc of air/min to the detector. Carrier gas (helium) and hydrogen flow rates were both 25 cc/min. The signal from the chromatograph was recorded by a 1.0-mV Leeds and Northrup 6-in. strip-chart recorder at a chart speed of 0.5 in./min. Identification of chromatographic peaks was based solely on the retention time of known methyl esters of fatty acids.

## Results and Discussion

A protein profile (Figure 1) and electron and phase-contrast micrographs (Figure 2) of the plasma membranes of rat liver cells, isolated by zonal centrifugation at *d* 1.185–1.194 g/cc in the continuous gradient under isopycnic conditions, demonstrate that these membranes were not contaminated with mitochondria, endoplasmic reticulum, or other cell organelles. Previous workers using a discontinuous gradient centrifugation procedure have isolated plasma membranes of rat liver at interfaces of *d* 1.16–1.18 g/cc (Emmelot *et al.*, 1964; Finean *et al.*, 1966), *d* 1.16–1.20 g/cc (Takeuchi and Terayama, 1965), and *d* 1.16–1.22 g/cc (Neville, 1960; Skipski *et al.*, 1965). We obtained 0.88 mg of membrane protein/g of fresh liver using the zonal technique (Table I) as compared with 0.41 mg/g reported by Emmelot *et al.* (1964) and 0.52 mg/g reported by Skipski *et al.* (1965). Apparently the isolation of membrane fractions at an interface after flotation of the sample through a sucrose gradient accounts for previous low yields of membranes since the protein layer at the interface under these conditions probably could not be defined sharply.

Total lipids of the membranes prepared by zonal centrifugation weighed 0.39 mg/g of fresh liver weight (Table I). This amount of lipid extracted from membranes is in good agreement with the values reported by Skipski *et al.* (1965). The ratio of total lipids to protein of our membrane preparation was 0.44, which is about equal to that reported by Bakerman and Wasemiller (1967) for human erythrocyte membranes. We found that the ratio of phospholipids to protein was 0.27 (Table I). Emmelot and coworkers (1964) demonstrated that practically all membrane phosphorus consisted of phospholipid phosphorus, which we also found to be

[5] Applied Science Laboratories, Inc., State College, Pa.

[6] The Oscar Co., Knoxville Tropical Fish and Pet Supply Co., Knoxville, Tenn.

TABLE II: Lipid Composition of Plasma Membranes of Rat Liver.

| Compounds | % of Total Lipid Extracted[a] | % of Total Phosphorus |
|---|---|---|
| Total neutral lipids | 27.0 ± 6.3 | |
| Triglycerides | 2.19 ± 1.23 | |
| Free fatty acids | 2.31 ± 0.87 | |
| Cholesterol, free | 21.2 ± 3.4 | |
| Cholesterol, esterified | 1.28 ± 0.72 | |
| Total polar lipids[b] | 62.1 ± 7.4 | |
| Lysophosphatidylcholine (507)[c] | 1.15 ± 0.98 | 4.21 ± 1.87 |
| Phosphatidylcholine (776)[c] | 19.2 ± 4.6 | 37.4 ± 5.2 |
| Phosphatidylethanolamine (732)[c] | 9.96 ± 1.34 | 21.5 ± 2.3 |
| Phosphatidylinositol + phosphatidylserine (832)[c] | 6.89 ± 1.20 | 13.0 ± 2.5 |
| Sphingomyelin (736)[c] | 8.01 ± 1.17 | 17.2 ± 4.8 |
| Phosphatidic acid + polyglycerolphosphatides (1084)[c] | 1.70 ± 0.54 | 3.23 ± 1.53 |
| Unidentified phosphorus free[d] | 11.9 ± 3.6 | |
| Total lipid accounted for | 91.1 ± 10.9 | 109.2 ± 12.4 |

[a] Mean value plus or minus one standard deviation for ten different membrane preparations. [b] The total glyceryl ether content of eight different membrane preparations was 1.26 ± 0.33% of the total lipids; the alkyl ethers accounted for 0.97 ± 0.35% and the alk-1-enyl ethers accounted for 0.24 ± 0.06%. Each value should be multiplied by 3 to express percentages in terms of their diacyl or phospholipid types. [c] Weight calculated on the basis of phosphorus content and assuming a molecular weight (value in parentheses) for each lipid class based on fatty acid composition listed in Table III. [d] Weight based on photodensitometric measurement using sphingomyelin (bovine) as a standard. Recent evidence has indicated that a portion of the unidentified acyl component is contaminated with sucrose from the gradient solution.

true. Our ratio of milligram of protein per micromole of phosphorus (Table I) also agrees quite closely with the results reported by Emmelot *et al.*

The neutral lipids comprise 27% of the total lipids present in rat liver membranes. Skipski *et al.* (1965) found that the neutral lipids of plasma membranes of rat liver were 34.6% of the total lipids, but their higher value might be attributed to the fact that Skipski and coworkers accounted for only 73.6% of the total lipids in their analyses of the lipid classes present in the membranes. Thin-layer chromatographic analysis of the neutral lipid fraction showed that it contained 80% unesterified cholesterol. Triglycerides, free fatty acids, and cholesterol esters as a group represented less than 6% of the total lipids (Table II) and these classes could be detected only when cholesterol was overloaded on the chromatograms (Figure 3). Monoglycerides, diglycerides, alkylglycerols and their esters, and fatty alcohols were not detected in chromatographic systems that can resolve these lipid classes. These solvent systems included hexane–diethyl ether–acetic acid (90:10:1, v/v) and hexane–diethyl ether–methanol–acetic acid (80:20:10:1, v/v) (Snyder and Piantadosi, 1966).

In our work, the polar lipids represented 62% of the total lipids extracted from the plasma membranes: phosphatidylcholine, an unidentified phosphorus-free polar lipid, phosphatidylethanolamine, sphingomyelin, and phosphatidylserine plus phosphatidylinositol accounted for approximately 19, 12, 10, 8, and 7%, respectively, of the total lipids (Table II). Trace amounts of lysolecithin (1.2%) and polyglycerolphosphatides plus phosphatidic acid (1.7%) also were present. The unidentified polar compound that chromatographed on thin layers of silica gel HR in the $NH_4OH$ system (Figure 3) was more polar than lysolecithin; although acyl groupings were found, no phosphorus could be detected. The relative quantities of polar lipids in the membranes isolated by zonal centrifugation are similar to those reported by Skipski *et al.* (1965). In the results of Takeuchi and Terayama (1965), only the phosphatidylcholine phosphorus value agrees with our data. The work of Emmelot and coworkers (1964) showed that the choline phosphatides (lysolecithin and lecithin) comprised 50% of the phospholipids, which does not agree with the values observed by us or by Skipski's group (1965). The phospholipid content of the yolk-sac membrane of the chick embryo was reported by Noble and Moore (1967) to be 76.8% lecithin and only 9.8 and 1.65% cephalin and sphingomyelin, respectively. These data and those of Ashworth and Green (1966), on the composition of mitochondrial, microsomal, and plasma membranes, point out that there is not necessarily a close parallel in structure between membranes from different sources and between different types of membrane components.

Our data show that ether-linked lipids are present in pure plasma membranes of rat liver. Thin-layer chro-

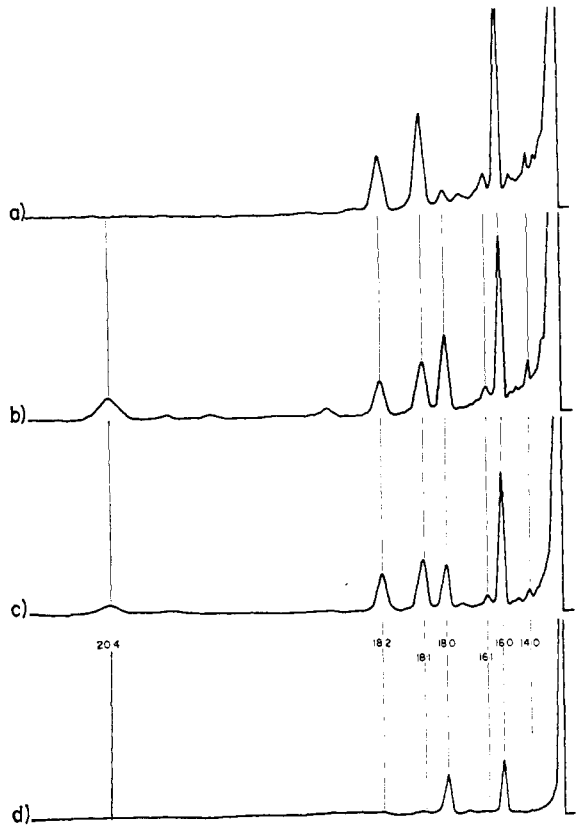


FIGURE 4: Gas–liquid partition chromatograms of the fatty acid ester components of (a) triglycerides, (b) free fatty acids, (c) polyglycerolphosphatides plus phosphatidic acid, and (d) unknown phosphorus-free lipid material, from plasma membranes of rat liver.

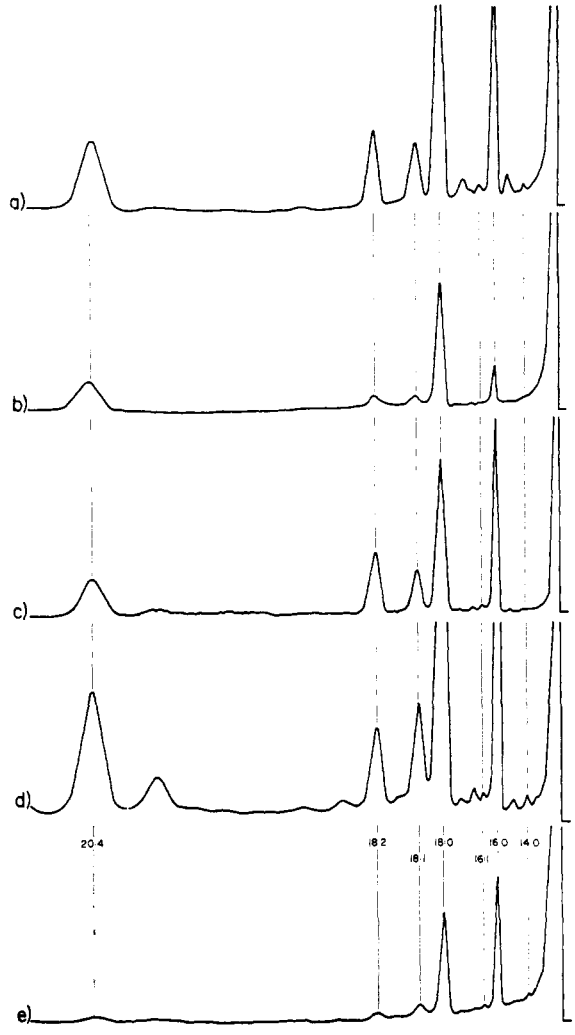


FIGURE 5: Gas–liquid partition chromatograms of the fatty acid ester components of (a) phosphatidylethanolamine, (b) phosphatidylserine plus phosphatidylinositol, (c) phosphatidylcholine, (d) sphingomyelin, and (e) lysophosphatidylcholine, from plasma membranes of rat liver.

matography, which is capable of resolving ether- and the corresponding acyl-linked lipids in the neutral lipid fraction (Snyder and Piantadosi, 1966), indicated that the ether lipids were phospholipids. An analysis of the ether lipids showed that the alk-1-enyl and alkyl ethers of glycerol amounted to 0.24 and 0.97%, respectively, of the total lipids. Since the total lipid extract had to be used for these analyses, it was not possible to determine the contribution of the alk-1-enyl and alkyl ethers to a specific phospholipid class. The biochemical significance of ether-containing lipids, which are present as minor constituents in all mammalian cells, is not yet understood. However, we can guess that their presence in membranes could provide structural stability to the orientation of lipid–protein and lipid–lipid interactions. Takeuchi and Terayama (1965) have reported the presence of plasmalogens in plasma membranes of rat liver, but their method of isolation and detection of the alk-1-enyl ethers was not specified. Glyceryl ethers have also been found in nonmammalian membranes (*Tetrahymena pyriformis* W.) by Thompson (1967); he found that a portion of the alkyl ethers was the ethanolamine-containing phosphonic acid derivative.

The fatty acid composition of the triglycerides isolated from the plasma membranes of rat liver is similar to that of triglycerides found in adipose tissue (Table III and Figure 4). Palmitate (36%), oleate (32%), and linoleate (22%) were the principal acyl groupings present in the triglyceride fraction; stearate and palmitoleate were present only in small amounts (3.64 and 3.50%, respectively). The free fatty acids from plasma membranes were palmitic (≃32%), stearic (≃22%), oleic (≃19%), linoleic (≃13%), arachidonic (≃6%), palmitoleic (≃3%), and myristic (≃3%) acids (Table III and Figure 4). No odd-chain saturated acids ($C_{15:0}$ or $C_{17:0}$) were detected in the free fatty acid fraction whereas all other lipid classes contained trace amounts. Phosphatidylcholine, phosphatidylethanolamine, and phosphatidic acid plus polyglycerolphosphatides contained similar percentages of palmitate (24–30%), linoleate (12–21%), and arachidonate (9–15%). Conversely, phosphatidylcholine and phosphatidylethanolamine contained less than half as much oleate as phosphatidic acid plus polyglycerolphosphatides (Table III and Figure 5).

Our work is the first to have fully examined the fatty acid composition of the lipid classes found in plasma membranes. The fatty acid composition of the phos-

TABLE III: Fatty Acid Composition of the Major Lipid Classes of Rat Liver Plasma Membranes.

| Fatty Acids[a] | LPC | SPHI | PC | PS + PI | PE | PA + PGP | Unknown | TG | FFA |
|---|---|---|---|---|---|---|---|---|---|
| <14:0 | tr | tr | 0.1 | tr | tr | tr | | tr | tr |
| 14:0 | 0.2 | 0.2 | 0.5 | tr | 0.2 | 1.7 | tr | 1.66 | 2.93 |
| 15:0 | 1.0 | 0.2 | 0.5 | tr | 0.6 | 0.4 | tr | tr | |
| 16:0 | 33.4 | 18.7 | 30.2 | 12.1 | 26.0 | 28.2 | 32 | 35.7 | 31.6 |
| 16:1 | 0.1 | 0.1 | 0.8 | 0.5 | 0.3 | 2.1 | tr | 3.50 | 3.07 |
| 17:0 | 3.4 | 0.1 | tr | tr | 1.5 | 0.9 | tr | 1.43 | |
| 18:0 | 45.7 | 39.2 | 29.8 | 49.2 | 31.9 | 15.1 | 48 | 3.64 | 21.9 |
| 18:1 | 4.0 | 3.7 | 9.6 | 4.6 | 6.5 | 22.1 | 10 | 32.3 | 19.0 |
| 18:2 | 3.7 | 4.2 | 15.3 | 5.2 | 12.4 | 20.6 | 10 | 21.8 | 13.2 |
| *A* | | 3.5 | 1.7 | 2.0 | 4.1 | tr | | tr | tr |
| 20:4 | 5.9 | 12.8 | 11.6 | 26.4 | 15.4 | 8.8 | tr | | 6.26 |
| *B* | | 17.2 | tr | tr | tr | | | | tr |

[a] The number before the colon represents the number of carbon atoms, and the number after the colon represents the number of double bonds. *A* = total percentage of minor peaks occurring between 18:2 and 20:4. *B* = total percentage of minor peaks occurring after 20:4. All values expressed as percentage of total fatty acids. LPC, lysophosphatidylcholine; SPHI, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid; PGP, polyglycerolphosphatides; TG, triglyceride; FFA, free fatty acids; tr, trace quantity.

pholipids from the chick embryo yolk-sac membrane after a 21-day incubation was shown by Noble and Moore (1967) to be quite different from our data on the acyl composition of the compounds isolated from the plasma membranes of rat liver. They showed that palmitate, stearate, oleate, and linoleate represented 31, 24, 24, and 17%, respectively, of the total acyl groupings on lysophosphatidylcholine. The amount of oleic acid in phosphatidylcholine and phosphatidylethanolamine was also much higher in the yolk-sac membranes than in the plasma membranes of rat liver.

The other polar lipid classes contained fatty acids that differed from those already discussed (Table III and Figures 4 and 5). The sphingomyelin fatty acid complement was atypical when compared with sphingolipids isolated from bovine milk, blood plasma, and brain (Morrison *et al.*, 1965; O'Brien and Rouser, 1964) and human brain (Lesch and Meier, 1964). Tissue sphingolipids generally possess a very low concentration of polyunsaturated fatty acids. Table III and Figure 5 show that in addition to the major saturated acids, palmitic (19%) and stearic (39%), the highest portion of the unsaturation in the sphingomyelin fraction was accounted for by arachidonic acid (≃13%). The combined fraction of phosphatidylserine and phosphatidylinositol also contained a relatively high concentration of arachidonate (26%). The highest levels of saturation (61–80%), represented primarily by stearate (46–49%), were found in the unidentified phosphorus-free polar lipid, the combined fraction of phosphatidylserine and phosphatidylinositol, and lysolecithin. The unidentified lipid also contained palmitate (32%), oleate (10%), and linoleate (10%). The $C_{15:0}$ and $C_{17:0}$ fatty acids were detected as minor components in all phospholipid classes.

Our detailed analyses of the lipid moieties are an essential step toward understanding the anatomical construction of membrane units at the periphery of cells. We obtained a ratio of palmitic to oleic acid in the liver membranes (2.2 in total lipids) that was strikingly similar to the ratio (2.4) observed by Kogel *et al.* (1960) in the membranes of rat erythrocytes, the only pure membranes to have been analyzed for lipid content prior to our study. The relative distribution of phospholipid classes was also similar in two plasma membranes from the same species (De Gier and Van Deenen, 1961; Van Deenen and De Gier, 1964). However, when the lipid composition of a particular plasma membrane (erythrocytes) was compared in six species (De Gier and Van Deenen, 1961), there was great variation from one species to another. Fleischer and Rouser (1965) have recently shown that membranes are similar in the different organelles within one cell but differ among cells. The similarity in the lipids of peripheral membranes from erythrocytes and hepatic cells of rats indicates that plasma membranes might have common structural and functional features. However, the ratio of calculated values of protein-to-lipid surface area (4.3) is higher in the plasma membranes of rat liver (based on the calculations and assumptions of Korn, 1966) than in erythrocyte ghosts (2.5). On the other hand, the ratio of protein-to-lipid surface areas in bacterial membranes (Korn, 1966) is similar to the ratio calculated for the plasma membranes of liver isolated by zonal centrifugation. The implication from these calculations is that different membranes must differ with respect to the orientation of lipid–protein surfaces.

Acknowledgment

The authors wish to acknowledge the excellent tech-

nical assistance of C. E. Phipps, L. H. Elrod, and C. T. Rankin, Jr.

References

Anderson, N. G., Barringer, H. P., Babelay, E. F., Nunley, C. E., Bartkus, M. J., Fisher, W. D., and Rankin, C. T., Jr. (1966a), *Natl. Cancer Inst. Monograph 21*, 137.

Anderson, N. G., Barringer, H. P., Cho, N., Nunley, C. E., Babelay, E. F., Canning, R. E., and Rankin, C. T., Jr. (1966b), *Natl. Cancer Inst. Monograph 21*, 113.

Anderson, N. G., Lansing, A. I., Lieberman, I., Rankin, C. T., Jr., and Elrod, H. (1967), *Ann. Rept.* (ORNL-4171 special).

Anderson, N. G., Lansing, A. I., Lieberman, I., Rankin, C. T., Jr., and Elrod, H. (1968a), Wistar Monograph No. 8.

Anderson, N. G., Waters, D. A., Fisher, W. D., Nunley, C. E., Elrod, L. H., and Rankin, C. T., Jr. (1968b), *Anal. Biochem.* (in press).

Ashworth, L. A. E., and Green, C. (1966), *Science 151*, 210.

Bakerman, S., and Wasemiller, G. (1967), *Biochemistry 6*, 1100.

Barclay, M., Barclay, R. K., Essner, E. S., Skipski, V. P., and Terebus-Kekish, O. (1967), *Science 156*, 665.

Bligh, E. G., and Dyer, W. J. (1959), *Can. J. Biochem. Physiol. 39*, 911.

Borgström, B. (1952), *Acta Physiol. Scand. 25*, 101.

De Boer, Th. J., and Backer, H. J. (1954), *Rec. Trav. Chim. 73*, 229.

De Gier, J., and Van Deenen, L. L. M. (1961), *Biochim. Biophys. Acta 49*, 286.

El-Aaser, A. A., Fitzsimons, J. T. R., Hinton, R. H., Reid, E., Kliecis, E., and Alexander, P. (1966), *Biochim. Biophys. Acta 127*, 553.

Emmelot, P., Bos, C. J., Bendetti, E. L., and Rumke, P. (1964), *Biochim. Biophys. Acta 90*, 126.

Entenman, C. (1961), *J. Am. Oil Chemists' Soc. 38*, 534.

Finean, J. B., Coleman, R., and Green, W. A. (1966), *Ann. N. Y. Acad. Sci. 137*, 414.

Fleischer, S., and Rouser, G. (1965), *J. Am. Oil Chemists' Soc. 42*, 588.

James, A. T. (1960), *Methods Biochem. Anal. 8*, 1.

Kogl, F., De Gier, J., Mulder, I., and Van Deenen, L. L. M. (1960), *Biochim. Biophys. Acta 43*, 95.

Korn, E. D. (1966), *Science 153*, 1491.

Lesch, P., and Meier, S. (1964), *Klin. Wochnschr. 42*, 799.

Lieberman, I., Lansing, A. I., and Lynch, W. E. (1967), *J. Biol. Chem. 242*, 736.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Morrison, W. R., Jack, E. L., and Smith, L. M. (1965), *J. Am. Oil Chemists' Soc. 42*, 1142.

Neville, D. M., Jr. (1960), *J. Biophys. Biochem. Cytol. 8*, 413.

Noble, R. C., and Moore, J. H. (1967), *Can. J. Biochem. 45*, 1125.

O'Brien, J. S., and Rouser, G. (1964), *J. Lipid Res. 5*, 339.

Ponder, E. (1961), *in* The Cell, Vol. 2, Brachet, J., and Mirsky, A. E., Ed., New York, N. Y., Academic, p 1.

Privett, O. S., Blank, M. L., Codding, D. W., and Nickell, E. C. (1965), *J. Am. Oil Chemists' Soc. 42*, 381.

Rouser, G., Kritchevsky, G., Heller, D., and Lieber, E. (1963), *J. Am. Oil Chemists' Soc. 40*, 425.

Rouser, G., Siakotos, A. N., and Fleischer, S. (1966), *Lipids 1*, 85.

Skipski, V. P., Barclay, M., Archibald, F. M., Terebus-Kekish, O., Reichman, E. S., and Good, J. J. (1965), *Life Sci. 4*, 1673.

Snyder, F., and Piantadosi, C. (1966), *Advan. Lipid Res. 4*, 257.

Takeuchi, M., and Terayama, H. (1965), *Exptl. Cell Res. 40*, 32.

Thompson, G. A., Jr. (1967), *Biochemistry 6*, 2015.

Van Deenen, L. L. M., and De Gier, J. (1964), in The Red Blood Cell, Bishop, C., and Surgenor, D. M., Ed., New York, N. Y., Academic, pp 243–303.

Ways, P., Reed, C. F., and Hanahan, D. J. (1963), *J. Clin. Invest. 42*, 1248.

Wood, R., and Snyder, F. (1966), *Lipids 1*, 62.

Wood, R., and Snyder, F. (1968), *Lipids 3*, 129.