Lipid composition of subcellular particles of human blood platelets

AARON J. MARCUS,* HARRIS L. ULLMAN, and LENORE B. SAFIER

Hematology Section, New York Veterans Administration Hospital, New York 10010; and the Department of Medicine, Cornell University Medical College, New York 10021

ABSTRACT Human platelets can be fractionated into three main subcellular components: granules, membranes, and a soluble fraction. In this study we determined the phospholipid and neutral lipid content of the granules and membranes. Quantitative relationships between lipids and protein were examined. The fatty acid and aldehyde composition of individual phospholipids and neutral lipids was also determined.

Whole platelets had a lower lipid to protein ratio than did the subcellular particles, but the basic lipid composition of the granules, membranes, and platelets was similar. The phospholipid composition of platelets and subcellular fractions was found to differ only in that granules had a lower percentage of lecithin. Each of the phospholipid classes displayed a distinctive fatty acid pattern which was the same in all fractions and in whole platelets.

The major neutral lipid was free cholesterol. Cholesteryl esters, triglycerides, and free fatty acids were minor components. The molar ratio of cholesterol to phospholipid in the platelet membranes was lower than that of brain myelin and erythrocyte ghosts. Some differences in fatty acid composition of the neutral lipids of platelet fractions were found.

A special lipid composition or constituent that would correlate with platelet function has not been found.

SUPPLEMENTARY KEY WORDS cell membranes · free cholesterol · proteolipids · cardiolipin · cerebrosides

PLATELETS play an important role in primary hemostasis, blood coagulation, and possibly occlusive vascular disease (1, 2). The rapidity with which platelet aggregation and adhesion occur in these processes suggests that they take place at the plasma membrane. Platelets contain intracellular granules, each of which is also enclosed by a membrane. In previous investigations, subcellular platelet components were isolated and some of their coagulation and biochemical properties were studied (3, 4). Three main subcellular components were defined. One consisted of membranes, including both the plasma membrane and membranes derived from intracellular structures. The second consisted of intracellular granules, at least some of which were identified as lysosomes, and others as mitochondria. The third component consisted of soluble material that remained at the top of the isopycnic sucrose density gradient in which the particles were separated.

In the studies to be reported, the lipid composition of whole platelets and of subcellular platelet particles was determined. The ratios of lipid to protein and of cholesterol to phospholipid were established and compared to similar ratios in other tissues.

MATERIALS AND METHODS

Human platelets were collected and processed, and subcellular fractions were prepared, as previously reported (3). Lipids were extracted by a modification of the Folch technique (5).

Thin-Layer and Paper Chromatography

Phospholipids were studied by quantitative thin-layer chromatography based on the methods of Skipski and associates (6, 7). The most useful solvent system consisted of chloroform–methanol–acetic acid–water 50:28:

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Abbreviations: TLC, thin-layer chromatography; PI, inositol phosphoglycerides; PS, serine phosphoglycerides; PE, ethanolamine phosphoglycerides; GLC, gas–liquid chromatography; BHT, 2,6-di-*tert*-butyl-*p*-cresol; DMA, dimethyl acetal(s); EGA, ethylene glycol adipate; ADP, adenosine diphosphate. Fatty acids designated by chain length : number of double bonds.

* Address requests for reprints to: Dr. Aaron J. Marcus, Hematology Section-13 West, New York Veterans Administration Hospital, 408 First Avenue, New York 10010.

10:5 (7). Separations were more complete and reproducible if solvent evaporation was minimized by placing the chromatography tank in a sealed plastic bag during the separation procedure. The finished chromatograms were examined by various detection methods, such as exposure to iodine vapor, spraying with ammonium molybdate–perchloric acid, or charring with 50% sulfuric acid at 180°C for 10 min. The identified components, as well as corresponding blank areas, were scraped into Nash combination tubes (11-381-50, Fisher Scientific Company, Springfield, N.J.) and the phospholipids were digested by the sulfuric acid–peroxide method of Parker and Peterson (8), with the following modifications: the temperature of the heating block was held at 190° C for the entire digestion period, and three drops of 30% hydrogen peroxide were added instead of two. Phosphorus was determined as described by these authors (8). Recoveries of lipid phosphorus averaged 96.8 ± 6.5%.

The phospholipids were also studied by one- and two-dimensional chromatography on filter paper impregnated with silica gel (9). The one-dimensional solvent system was a mixture of two parts of diisobutyl ketone (2,6-dimethyl-4-heptanone)–acetic acid–water 40:25:5 to one part of chloroform–methanol 1:1 (G. V. Marinetti, personal communication). The dried chromatograms were sprayed with Rhodamine 6G (0.005%) and viewed under UV light. The stained areas were circled, cut out, and further subdivided into smaller pieces (10). They were then transferred to Nash combination tubes and digested according to the method of Letters (10), modified by the use of a heating block at 190°C for 30 min. Control blanks were prepared as follows: the area of the identified spots was delineated by tracing through onion skin paper and the corresponding area of silica gel-impregnated paper, which had been subjected to an identical chromatographic run, was cut out. Recoveries of lipid phosphorus averaged 93.5 ± 7.8%.

Column Chromatography

Silicic acid column chromatography was carried out by methods previously utilized in this laboratory (11). Prior to phospholipid separation, the "neutral lipid" fraction (arbitrarily defined as the lipid eluted from silicic acid with nonpolar solvents) was eluted with chloroform and stored at −85°C. In instances where column fractions overlapped—for example, PI–PS and lecithin–sphingomyelin—the separations were completed by TLC. Aliquots of column fractions were weighed on a Cahn microbalance. Recoveries averaged 99.7 ± 2.3%.

Neutral lipids were separated by column chromatography on silicic acid, as described by Hirsch and Ahrens (12). Since the platelet neutral lipids differed quantitatively from those of plasma, the elution scheme was modified accordingly. Column eluates were monitored by application of aliquots to glass fiber filter paper and subsequent charring (13), and identified by TLC (14). Recoveries, as determined by dried weight, were 104.7 ± 4.9%. Platelet cholesteryl esters, free fatty acids, and triglycerides were present in small quantities; they were collected from the column as a single fraction and separated by TLC. The thin-layer plates were coated with Camag silica gel (Type D-O, Arthur H. Thomas Co., Philadelphia, Pa.) (14) and developed with petroleum ether (bp 30–60°C)–diethyl ether–glacial acetic acid 82:18:1 (15). The separated lipids were then prepared for GLC.

Gas–Liquid Chromatography

Fatty acid methyl esters and dimethyl acetals (DMA) were prepared from either column eluates or spots scraped from thin-layer plates. The lipids were treated with boron trifluoride–methanol reagent (Applied Science Laboratories Inc., State College, Pa.) as described by Morrison and Smith (16), and as modified by Dodge and Phillips (17). When lipid spots from thin-layer plates were esterified, corresponding blank areas of silica gel were subjected to the same procedure and the product was also gas chromatographed. Areas of the peaks, which did appear, were subtracted from those of the sample under study.

The methyl esters and DMA were chromatographed on polar [ethylene glycol adipate (EGA) polyester] and nonpolar (Apiezon L) columns by techniques previously described (11). Methyl ester mixture L-207 (Applied Science Lab.) was used as a reference standard for the long-chain fatty acids occurring in the sphingomyelins. Linearity of detector response to these acids was validated by the use of Applied Science mixture "KF."

GLC was also employed to determine fatty acid composition and to quantify cholesteryl esters, free fatty acids, and triglycerides. Internal standards (Applied Science Labs.) were added to the methyl esters under study as follows: methyl eicosanoate (20:0) to the cholesteryl esters and methyl heneicosanoate (21:0) to the free fatty acids and triglycerides. These were selected because their retention times differed from those of the methyl esters in the compounds under study. Weighed samples of the internal standards were added to the methylated compounds prior to the extraction step of the esterification procedure.

Additional Materials and Methods

Column separations were carried out under nitrogen with redistilled, deoxygenated solvents. All lipids were applied to thin-layer plates in a closed chamber (25 0976, Brinkmann Instruments Inc., Westbury, N.Y.)

under nitrogen. Upon completion of the chromatographic run, plates were dried under a stream of nitrogen in a vacuum desiccator. It was found necessary to employ the antioxidant BHT (50 mg/100 ml of solvent) (17) in TLC separations of compounds containing large amounts of highly unsaturated fatty acids, such as PI and PS. BHT was also included whenever the TLC separation procedure took more than 30 min. Since the BHT produced artifactual peaks during GLC analysis, especially on the polar column, it was separated from the methyl esters by rapid TLC on Silica Gel HR (Brinkmann) in hexane–diethyl ether–acetic acid 90:10:1 (18). This solvent system was also used to purify the methyl esters formed from the free fatty acids and triglycerides, as well as to remove free cholesterol after methylation of cholesteryl esters. Methyl esters were eluted from TLC plates by the method of Dodge and Phillips (17).

Proteolipid protein was measured in extracted lipids by the method of Hess and Lewin (19), as modified by Lees (20).

Cholesterol was determined by the method of Zak and associates (21). The color was allowed to develop in the dark for 1 hr at 4°C. When necessary, the saponification procedure of Abell and coworkers was used (22), with a single modification: saponification was carried out in a 60°C water bath for 60 min.

TLC to detect cerebroside was carried out by the method of Honegger (23). The method of Suzuki and coworkers was used for TLC of cerebroside sulfate (24).

RESULTS

The crude lipid composition of whole platelets and subcellular platelet fractions is shown in Table 1. The analyses were carried out on material pooled from 60 subjects with no evidence of a platelet disorder. Each determination was carried out in triplicate and is reported as the average of such analyses. The ratio of phospholipid to total lipid was arrived at as follows. Neutral lipid fractions obtained by column chromatography were weighed directly. The phospholipid weight was calculated by multiplying the phosphorus value by a factor derived from the fatty acid composition of each phospholipid and from the relative proportion of each phospholipid present in the fraction. It was of interest that the factor so derived was approximately 25, which corresponds to the figure in general use. "Total lipid" was the sum of "neutral lipid" and phospholipid.

TABLE 1 HUMAN PLATELET LIPIDS

	Lipid / Protein	Phospholipid / Total Lipid
	w/w	%
Whole platelets	0.28	79*
Platelet membranes	0.58	78*
Platelet granules	0.56	75*
Soluble fraction	0.11	—

* Pooled material from 60 subjects. No. of determinations at least 3. Total lipid = phospholipid (P × 25) plus neutral lipid (determined gravimetrically).

The lipid to protein ratio was higher in the membranes and granules than in whole platelets and the soluble fraction, but the relative concentration of phospholipid in granules, membranes, and whole platelets was similar.

Phospholipid composition is shown in Table 2. The granules contained proportionately less lecithin than whole platelets or platelet membranes and somewhat more sphingomyelin, cardiolipin, and lysolecithin. Cardiolipin could not be clearly identified by TLC since it migrated to the solvent front, but it was discernible in the paper system.

Fatty acid and aldehyde analysis of the phospholipids is shown in Table 3. Results obtained with the polar and nonpolar GLC columns were nearly identical. The maximal difference for any acid was 3%. Analyses of the DMA are reported from the Apiezon column, since the less acidic nature of this column has been found to give greater accuracy and reproducibility (11). The sphingomyelin results were taken from the EGA column, since the retention times of its constituent methyl esters were too long on Apiezon. The PS, PI, and lecithin figures represent averages of Apiezon and EGA results. The methyl ester of 22:4($n-6$) was identified by the use of types I and II separation factors, as described by Ackman and Burgher (25), and as employed by Dodge and Phillips (17).

These compositions were quite similar in whole platelets, granules, and membranes. The PE was characterized by a rather large number of long-chain unsaturated fatty acids and, as previously reported, more than 60% of this phosphatide was in the plasmalogen form (11).

When calculated on a molar basis, more than 40% of the fatty acid chains in the inositol phosphatides consisted of arachidonic acid, in amount exceeding that found in any of the other platelet phospholipids. Only traces of plasmalogen were found in the PI fractions. The sphingomyelins contained a large proportion of long-chain saturated fatty acids.

The major component of the neutral lipid fractions was free cholesterol, which represented 85, 90, and 88% of the whole platelet, membrane, and granule neutral lipid. It comprised 18, 20, and 23% of the total lipid of these respective preparations. Ratios of cholesterol to phospholipid for platelet membranes are compared with those in other cell membranes in Table 4.

TABLE 2 PHOSPHOLIPID COMPOSITION OF PLATELETS AND SUBCELLULAR FRACTIONS*

	No. of Analyses	PE	PS	PI	Lec	Sph	Lysolec	Origin	SF
		Thin-Layer Chromatography							
		mean ± SD							
Whole platelets	7	27.0 ± 1.4	10.1 ± 0.7	5.0 ± 0.6	38.4 ± 1.5	17.0 ± 0.9	0.7 ± 0.4	0.7 ± 0.6	1.2 ± 0.6
Granules	5	28.7 ± 1.2	11.7 ± 0.5	3.9 ± 0.5	31.8 ± 1.1	20.2 ± 0.8	1.9 ± 0.8	0.3 ± 0.3	1.5 ± 0.5
Membranes	5	27.2 ± 0.6	9.9 ± 1.0	6.1 ± 0.6	38.3 ± 1.2	16.9 ± 0.6	0.4 ± 0.3	1.0 ± 0.4	0.2 ± 0.3

	No. of Analyses	PE	PS	PI	Lec	Sph	Lysolec	Cardio	?
		Silica Gel Paper Chromatography							
		mean ± SD							
Whole platelets	5	25.4 ± 0.8	10.1 ± 1.7	4.3 ± 1.5	39.8 ± 1.6	18.3 ± 1.1	0.8 ± 0.7	0.5 ± 0.4	0.6 ± 0.3
Granules	4	27.1 ± 0.9	11.4 ± 2.2	3.8 ± 0.2	33.4 ± 0.8	20.0 ± 0.2	1.9 ± 0.2	1.3 ± 0.6	0.8 ± 0.4
Membranes	6	26.5 ± 1.3	8.2 ± 3.1	4.2 ± 0.5	41.7 ± 2.0	17.2 ± 1.1	0.3 ± 0.5	0.3 ± 0.2	1.8 ± 1.1

Lec, lecithin; Sph, sphingomyelin; Lysolec, lysolecithin; SF, solvent front; Cardio, cardiolipin.
* Percentage of lipid phosphorus.

TABLE 3 PHOSPHOLIPID FATTY ACIDS AND ALDEHYDES OF WHOLE PLATELETS, PLATELET MEMBRANES, AND GRANULES

	PE			PS			PI			Lecithin			Sphingomyelin		
	W.P.*	Gran.*	Memb.*	W.P.	Gran.	Memb.	W.P.	Gran.	Memb.	W.P.	Gran.	Memb.	W.P.	Gran.	Memb.
	weight %†														
16:0 DMA	9.2	8.7	8.7	0.60			0.92		2.0	0.80	0.47	0.36			
16:0	3.4	3.0	3.5	0.97		0.36	1.6	0.94	1.4	34.1	31.0	31.0	24.6	22.5	21.9
16:1	0.26	0.24	0.51	0.67	0.20	0.51	0.46	0.24	0.55	1.6	1.8	1.4	1.2	0.26	0.15
18:0 DMA	17.9	17.8	18.2	0.19	tr.	0.15	0.12	tr.		tr.					
18:0	13.7	13.8	14.1	44.7	44.2	46.5	44.7	44.3	44.4	14.1	15.6	15.7	5.8	5.7	5.6
18:1 DMA	2.7	4.7	3.1												
18:1	5.9	5.8	5.5	26.7	26.0	25.0	8.7	9.0	9.2	27.0	27.5	27.4	1.3	0.83	0.72
18:2	2.3	2.1	2.2		tr.	tr.	0.05	0.44	0.33	6.9	7.2	7.5		0.16	0.12
18:3	1.6	1.4	1.5												
20:0	0.64	0.41	0.52	1.6	1.5	1.7	0.84	0.48	0.47	0.38	0.94	0.51	9.4	9.6	9.8
20:4	31.8	28.9	31.6	22.6	25.2	24.7	41.8	44.1	41.2	12.0	11.8	12.5	tr.	tr.	
22:0													29.3	29.2	31.5
22:1													2.0	4.0	5.8
22:4 (*n*-6)	4.4	5.6	3.8												
22:5	1.8	2.0	1.1	tr.	tr.	tr.				tr.		0.81			
22:6	1.2	1.6	1.2	tr.	tr.	tr.						tr.			
23:0													1.2	2.9	2.1
24:0													10.9	7.3	7.7
24:1													12.8	15.4	12.0

Fatty acids are designated by chain length:number of double bonds. Besides those shown, the following acids were detected in small (<1.5) percentages: 12:0, 14:0, 15:0, 17:0 DMA, 17:0, 20:1, 20:2, 20:3, and one with a probable carbon number of 22 on Apiezon.
* W.P., whole platelets; Gran., granules; Memb., membranes; tr. trace.
† Determined by GLC on EGA and (or) Apiezon.

The composition of neutral lipids in platelet membranes and granules is shown in Table 5. The presence of several minor unidentified components, in addition to the technical difficulties of quantifying the small amounts of cholesteryl esters, triglycerides, and free fatty acids, may account for the rather low total recoveries seen in this table.

The fatty acid composition of the platelet neutral lipids is compiled in Table 6. The cholesteryl ester results were derived from the EGA columns only because of the limited amount of material available. The data on triglycerides and free fatty acids represent an average of results obtained from both columns. In the cholesteryl esters there were three main fatty acids: palmitic (16:0), palmitoleic (16:1), and oleic (18:1). The principal fatty acids of the triglycerides were palmitic, stearic (18:0), and oleic. Similarly, the free fatty acid fractions contained palmitic, stearic, and oleic as major components.

Since lipid extracts of other tissues have been shown to contain small amounts of proteolipids, the platelet lipids were examined for the presence of these compounds (19, 20). Whole platelet lipid and membrane

TABLE 4 MOLAR RATIOS OF CHOLESTEROL TO PHOSPHOLIPID IN CELL MEMBRANES

Tissue	Ratio
Brain myelin*	1.32
Erythrocyte ghosts*	0.89
Platelet membranes	0.53
Intestinal microvilli*	0.46
Liver plasma membranes*	0.26

* Data from Ashworth and Green (29).

TABLE 5 COMPOSITION OF NEUTRAL LIPIDS

Component	Membranes	Granules
	% of neutral lipids	
Cholesteryl esters	0.4	0.1
Triglycerides	2.3	0.6
Free fatty acids	2.1	1.7
Free cholesterol	90.2	88.4
Diglycerides	tr.	tr.

tr., trace.

and granule lipid contained 1.8, 2.3, and 3.4% proteolipid protein, respectively.

TLC of whole platelet and particle lipids was carried out in a search for cerebroside and cerebroside sulfate (23, 24). Authentic standards and human brain lipids were simultaneously chromatographed. With this procedure no cerebroside or cerebroside sulfate was detected.

DISCUSSION

Platelets have ultrastructural, physiological, and biochemical features that distinguish them from other cells. They have no nuclei, little or no rough endoplasmic reticulum, no Golgi complex, and no centrioles (26). When blood vessels are damaged or blocked, a complex series of events is initiated. Platelets that have come in contact with collagen in the vessel wall rapidly aggregate. ADP from these platelets causes further aggregation, and eventually a hemostatic platelet plug forms (1). At this time, membrane lipoprotein of the aggregated platelets serves as a catalytic surface for the interaction of coagulation factors (27). In addition, a lipoprotein from surrounding tissues (Tissue Factor) also reacts with certain coagulation factors. This results in the formation of thrombin, which produces fibrin from fibrinogen, causes further platelet aggregation, and "consolidates" the hemostatic platelet plug (2). It is reasonable to assume that the plasma membrane of the platelets plays an important role in these reactions. Indeed, it was previously found that isolated platelet membranes were highly efficient as platelet substitutes in coagulation systems in vitro (3). One of the main purposes of this study was to determine the lipid composition of platelet membranes and compare it with that of granules and whole platelets. The finding of a specific lipid or fatty acid in the membranes might have had functional implications.

The phospholipid content of the lipid of whole platelets (Table 1) was similar to that in the subcellular particles: about 80%, somewhat more than in erythrocytes (28). A consideration of lipid to protein ratios in membranes from various tissues is of interest. Ashworth and Green have indicated (29) that highly specialized plasma membranes have a high content of protein and a low

TABLE 6 NEUTRAL LIPID FATTY ACIDS OF WHOLE PLATELETS, PLATELET MEMBRANES, AND GRANULES

	Cholesteryl Esters			Triglycerides			Free Fatty Acids		
	W.P.*	Gran.*	Memb.*	W.P.	Gran.	Memb.	W.P.	Gran.	Memb.
					weight %				
12:0	2.7		0.21	0.82	0.80	0.60	1.2	0.30	0.71
14:0	6.4	1.3	1.6	7.0	4.7	4.0	4.8	1.8	3.7
14:0 br	1.4	1.3	4.7	1.5	1.6	1.6	0.54	0.25	0.51
15:0	0.78	1.3	2.9	2.7	2.2	1.6	1.6	0.68	1.4
15:0 br	4.8	2.5	1.2	0.71	0.85	0.57	0.16	0.08	0.29
16:0	16.6	15.5	17.4	29.8	24.1	22.7	38.6	19.4	26.4
16:1	34.5	44.0	37.3	11.6	8.2	7.5	4.1	3.0	4.4
17:0	2.9	7.9	4.4	0.91	1.5	0.81	0.97	0.89	1.2
17:0 anteiso	tr.	tr.	tr.	1.8	1.7	1.5	0.36	0.96	1.2
?	tr.		tr.	1.3	1.7	0.96	0.45	0.36	1.4
18:0	2.3	7.5	4.4	10.1	11.9	13.6	20.2	34.1	22.6
18:1	27.6	18.7	26.0	23.1	28.1	30.1	23.2	21.1	27.8
18:2	tr.	tr.		8.5	8.7	7.8	1.8	6.2	3.5
18:3					0.79	0.45	tr.	0.13	0.30
20:0					1.0	2.2	0.86	1.2	1.6
20:1				0.21	0.37	1.4	0.50	0.46	1.1
20:3					tr.	0.58	0.59	0.34	1.7
20:4					1.8	2.2		8.8	0.20

Fatty acids designated by chain length:number of double bonds. br, branched chain.
*W.P., whole platelets; Gran., granules; Memb., membranes; tr., trace.

phospholipid to protein ratio. For example, in intestinal microvilli the ratio of phospholipid to protein is 0.11 mg/mg of protein, but a membrane such as brain myelin, which is presumably less active metabolically, has a ratio of 0.92. Platelet membranes appear to be intermediate in this regard, with a ratio of 0.39.

We used two different chromatographic techniques for analysis of the platelet phospholipids, in order to increase the accuracy of the determinations and to insure detection of minor components. For example, cardiolipin, a lipid characteristic of mitochondria (30), was detected only on the silica gel-impregnated paper (Table 2). The granules were found to contain proportionately less lecithin than the membranes or whole platelets. The reciprocal increase in granule sphingomyelin paralleled findings in other tissues, where the sum of the choline-containing phospholipids tends to remain constant (31). As in the human red cell and brain, lecithin is the predominant phospholipid (31). The percentage of PE plus PS (the "cephalin" fraction) in whole platelets and platelet membranes is roughly equal to that of lecithin, whereas the PE and PS of the granules exceeds the amount of lecithin present.

As reported by investigators studying other tissues (31–33), the phospholipid fatty acid composition of platelet organelles did not differ greatly from one organelle to another nor from that of the whole cell (Table 3). The individual platelet phospholipid classes showed distinctive fatty acid patterns. The sphingomyelins contained mainly saturated fatty acids, and in particular behenic (22:0) and palmitic. Sphingomyelin was the only phospholipid class in which lignoceric (24:0) and nervonic (24:1) acids were identified. This appears to be characteristic of sphingomyelins in other tissues (31).

The fatty acids eluted in the early portions of the lecithin peak from the silicic acid column showed (data not given) a higher degree of unsaturation than those eluted subsequently (17). Although the ratio of saturated to unsaturated fatty acids in the entire lecithin peak is close to unity, the ratios in the first and last portions of the elution indicate that a small but definite proportion of the lecithin molecules must be disaturated or diunsaturated. Such naturally occurring molecules have already been described (34).

As in plasma membranes from a large variety of tissues (29), free cholesterol predominated in the neutral lipids (Table 5). Since each platelet granule possesses a distinct membrane (1), it is not surprising that the cholesterol distribution in the granules was similar to that of membranes and whole platelets. In preliminary studies it was noted that the lipid derived from the soluble fraction contained less free cholesterol than that found in platelet membranes and granules. This is in accordance with the suggestion of Bloch, that free cholesterol in cells is mainly associated with intracellular and plasma membranes (35).

In earlier studies of myelin and erythrocytes, a cholesterol to phospholipid molar ratio of 1.0 had been established (29, 36). This was interpreted as evidence for the concept that the lipid composition of plasma membranes was similar. As more cell types were analyzed, variations in cholesterol:phospholipid ratios were found (29, 36). The ratio for platelet membranes was 0.53 (Table 4), which is considerably less than that of erythrocytes and myelin. This lower figure might be attributable to contamination of the plasma membranes with those derived from intracellular platelet organelles. However, values in this range have been found in such other plasma membranes as intestinal microvilli (29).

Quantitative neutral lipid chromatography was technically difficult because all components, with the exception of free cholesterol, were present in very small amounts (Table 5). This does not preclude the possibility that quantitatively minor constituents, such as free fatty acids and triglycerides, are important in platelet lipid metabolism (37).

In contrast to the phospholipids, neutral lipids showed some differences in fatty acid compositions between platelet membranes and granules (Table 6). For example, whole platelets contained 38.6% palmitate in the free fatty acid fraction, whereas the membranes and granules contained 26.4 and 19.4%, respectively. On the other hand, the granules contained 34.1% stearate in the free fatty acid fraction, but the membranes and whole platelets contained 22.6 and 20.2% stearate, respectively. The triglyceride fatty acids of membranes and granules resembled each other and differed slightly from those of whole platelets. The whole platelet triglycerides were similar to erythrocyte triglycerides (38). However, both differed from plasma triglycerides in their lower content of oleate and arachidonate (38). The differences between platelet cholesteryl ester fatty acids and those of serum (39) were of interest. The major fatty acid of serum cholesteryl esters is linoleate (44.5%), but only traces can be found in platelet cholesteryl esters.

Proteolipids have been defined as lipoproteins with solubility properties similar to those of lipids (40). They are mainly associated with myelin but have been found in other parts of the central nervous system and in other tissues (20). Whole platelets and platelet fractions were found to contain small amounts of proteolipid. Lees has shown that extraction of subcellular particles in the presence of sucrose results in an increase in recovery of proteolipid protein as compared to the original homogenate (20). This appears to be true for platelets also, since the granules, for example, contained 3.4% proteolipid protein whereas whole platelet lipid contained 1.8% of this component. The biological function or sig-

nificance of proteolipid remains to be elucidated.

Since certain similarities exist between the lipids of platelets and brain (11), whole platelets and subcellular fractions were examined for the presence of cerebroside and cerebroside sulfate. Although this lipid class is prominent in myelin (41), it could not be detected in platelets.

Neither platelet membranes nor granules were characterized by a specific lipid or fatty acid. Important biological activities which have been attributed to platelet membranes cannot be explained solely on the basis of lipid composition. Further understanding of these platelet functions will probably be achieved through the acquisition of more knowledge of structural relationships and interactions between lipids, proteins, and carbohydrates.

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