

Lipid Composition of Lymphocyte Plasma Membrane from Pig Mesenteric Lymph Node

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1. The lipid fraction of the plasma membrane of pig mesenteric lymph-node lymphocytes contained primarily (94%) neutral lipids and phospholipids in about equal weights. The remaining lipid comprised glycosphingolipids (1.8%), gangliosides (0.27%) and probably ceramides (1.3%). The major phospholipid was phosphatidylcholine (46% of the total), and mono- and tri-hexosylceramides accounted for 72% of the glycosphingolipids. Haematoside was distributed between the glycosphingolipid and ganglioside fractions. The major ganglioside was monosialoganglioside. About 90% of the sialic acid was *N*-glycolylated. 2. A comparison of the lipid composition of the plasma-membrane fraction with that of the initial lymph-node homogenate showed that the purified membrane contained increased proportions of phospholipids, especially sphingomyelin, glycosphingolipids and gangliosides. 3. Fatty acid analyses showed that the membrane phosphatidylcholine was rich in palmitic acid, that the sphingomyelin and phosphatidylethanolamine were high in myristic acid and that the glycosphingolipids were rich in oleic acid.

Comprehensive investigations of the lipid compositions of the plasma membrane of eukaryotic cells have, in general, been restricted to erythrocytes (Maddy, 1966), liver and hepatoma cells (see, e.g., van Hoesen & Emmelot, 1972) and certain fibroblast cell lines (Weinstein *et al.*, 1969; Renkonen *et al.*, 1972). Also, few precise analyses of the nature and distribution of plasma-membrane glycosphingolipids have been reported, apart from some recent studies of fibroblast (L cells and BHK21 cells) and intestine plasma membranes (Weinstein *et al.*, 1970; Renkonen *et al.*, 1972; Forstner & Wherrett, 1973), and little information is available on the fatty acid moieties of the glycolipids. This lack of knowledge is somewhat surprising in view of the important roles in mediating cell behaviour that have been ascribed to the plasma-membrane lipids, in general, and to the glycosphingolipids (Hakomori, 1973), phospholipids (Emmelot & van Hoesen, 1975; Resch & Ferber, 1975) and the hydrocarbon chains of the fatty acid residues (de Kruyff *et al.*, 1973; McElhaney *et al.*, 1973), in particular.

The present paper describes the glycolipid and phospholipid compositions of a purified preparation of pig lymphocyte plasma membrane, and the distribution of fatty acid residues among the individual lipids. This information should provide a suitable

basis for analysing the significance of the differences in plasma-membrane lipid composition that are apparently induced during lymphocyte activation (Resch & Ferber, 1975) and that probably occur between different lymphocyte subpopulations (e.g. peripheral blood lymphocytes have been reported to have a higher content of glycosphingolipids than the cells of the lymphoid tissues; Levis & Kesse-Elias, 1974).

Materials and Methods

Chemicals were of analytical grade and solvents were redistilled before use. Lymphocyte plasma membrane was prepared from the mesenteric lymph nodes of young pigs (average age about 7 months), as previously described (Allan & Crumpton, 1970). Lymph node, which had been disrupted by pressing through a perforated plate, was diluted with an equal volume of 10mM-Tris/HCl buffer/0.15M-NaCl, pH7.4, and filtered through muslin to yield the tissue homogenate [referred to by Allan & Crumpton (1970) as 'filtrate']. Samples of the tissue homogenate and the purified plasma-membrane fraction were dried from the frozen state immediately after preparation and were subsequently resuspended in water (about 50mg/ml) before lipid extraction.

Authentic samples of fatty acids were purchased from Applied Science Laboratories, State College, PA, U.S.A. Phenyl *N*-acetyl- α -D-glucosaminide, *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K., and cytolipin H (lactosylceramide) was from Miles Laboratories, Elkhart, IN 46514, U.S.A.

Preparation of lipids

Total lipids were extracted by the method of Folch *et al.* (1957) with 19ml of chloroform/methanol (2:1, v/v) per ml of suspension. The organic phase was washed once with 0.1M-KCl and once with the aqueous upper phase as described by Folch *et al.* (1957), evaporated to constant weight under N₂ and the residue weighed. The lipid extract (50mg) was fractionated by adding it to a column (0.7cm \times 5cm) of silicic acid (Bio-Rad Laboratories, St. Albans, Herts., U.K.; special for lipid chromatography) in chloroform. Neutral lipids were separated by washing the column with chloroform, and ceramides, glycosphingolipids and phospholipids were successively eluted with ethyl acetate, acetone/methanol (9:1, v/v) and chloroform/methanol (1:1, v/v) respectively (Vance & Sweeley, 1967; Levis & Kesse-Elias, 1974). The ceramide fraction was further purified after mild alkaline hydrolysis (Miras *et al.*, 1966) by rechromatography under the same conditions. Individual glycosphingolipids were isolated from the glycosphingolipid fraction after mild alkaline hydrolysis and silicic acid column chromatography (Karli & Levis, 1974) by preparative t.l.c. on plates of washed silica gel H (E. Merck, Darmstadt, Germany) as described by Gray (1967), with chloroform/methanol/water (65:25:4, by vol.) as solvent. The glycosphingolipids were revealed by spraying with water, scraped from the plate and extracted four times with 3 ml of chloroform/methanol (2:1, v/v). In some cases, neutral glycosphingolipids were separated from acidic glycosphingolipids by DEAE-cellulose chromatography (Miras *et al.*, 1966). Qualitative t.l.c. was performed on silica-gel G plates prepared by E. Merck and developed with chloroform/methanol/water (65:25:4, by vol.). The glycosphingolipids were revealed by spraying with 50% (v/v) H₂SO₄ and heating at 160°C for 10min.

Gangliosides were recovered from the combined aqueous upper phases of the Folch extraction (see above) after drying from the frozen state. The residue was hydrolysed with sodium methoxide as described by Kanfer (1969), taken up with an equal volume of 0.025M-EDTA (tetrasodium salt; pH12), dialysed for 2 days against deionized water in the cold and freeze-dried. Significant losses of gangliosides during dialysis were avoided

by keeping their concentration as high as possible (Kanfer & Spielvogel, 1973). The dried residue was dissolved in a small volume of chloroform/methanol (2:1, v/v) at about 50°C and added to a column of silicic acid. Fatty acids and their derivatives that had been released during alkaline hydrolysis were eluted with chloroform, and the gangliosides were subsequently obtained by washing with acetone/methanol (3:2, v/v); the column was standardized by using a sample of rat brain gangliosides.

Lipid analysis

The identity and purity of all lipid fractions, sugars and fatty acids were assessed relative to authentic samples by t.l.c. and/or g.l.c. Individual lipids were also analysed by the following procedures.

Glycosphingolipids. Glycosphingolipids were characterized in terms of their sugar and fatty acid composition after treatment with 0.8M-HCl in dry methanol at 80°C for 24h (Sweeley & Walker, 1964). Methyl glycosides were converted into their trimethylsilyl derivatives (Carter & Gaver, 1967) and were subsequently determined by using a 3% SE-30 column (152cm \times 0.4cm; support, 100–200 mesh Diatomite CQ) at 175°C in a Pye–Unicam aerograph. Amounts of sugars were measured by reference to an internal standard of mannitol, a known amount of which was added to each glycolipid sample after methanolysis (Sweeley & Walker, 1964). To test this procedure mannitol was added to a known sample of cytolipin H before methanolysis. More than 90% of the sugars of the glycosphingolipid were recovered. Carbohydrate compositions are expressed as molar ratios of sugars relative to glucose. Methyl esters of the fatty acids were resolved on a 12% diethylene glycol succinate column (260cm \times 0.30cm; support, 80-mesh Chromosorb W) in a Varian aerograph equipped with a Hewlett–Packard Integrator 3370B. As 2-hydroxy fatty acid methyl esters were not eluted under the above conditions, these were analysed on an SE-30 column at 215°C before and after separation from other fatty acid methyl esters by t.l.c. (Levis & Kesse-Elias, 1974).

Ceramides. These were identified by comparing their positions on t.l.c. in chloroform/methanol/water (65:20:3, by vol.) with those given by authentic ceramides prepared from brain cerebroside (Carter *et al.*, 1961). Further identification was based on t.l.c. of the lipid base released by alkaline hydrolysis (Miras *et al.*, 1966). For quantitative measurement ceramides were methanolysed as described above for glycosphingolipids. Fatty acid esters were determined in duplicate by using a SE-30 column at 215°C; a known amount of methyl tricosanoate was included in one run as an internal standard and used for their quantitative measurement.

Gangliosides. Qualitative analyses were performed by t.l.c. on plates (8.5cm×8.5cm) of silica gel G (E. Merck), which were developed three times in chloroform/methanol/water (65:35:8, by vol.), the plates being dried between runs. Gangliosides were revealed by spraying with the resorcinol reagent of Svennerholm (1957) and heating the plates covered with a glass plate at 100°C for 10min. They were tentatively identified by comparing their positions with those given by a sample of rat brain gangliosides run at the same time. Quantitative analyses were based on the amount of lipid-bound sialic acid. This was determined by the method of Yu & Ledeen (1970) by using g.l.c. on a column of 3% OV-1 (support, 100–200 mesh Diatomite CQ) at 210°C; phenyl *N*-acetyl- α -D-glucosaminide was included as an internal standard.

Phospholipids. Phospholipids were separated by t.l.c. in two dimensions on 10cm×10cm silica-gel G plates (E. Merck) by using chloroform/methanol/NH₃ (sp.gr. 0.895)/water (24:14:1:1, by vol.) in the first dimension and chloroform/methanol/NH₃/water (24:32:1:1, by vol.) in the second dimension (Kwiterovich *et al.*, 1970). Detection of spots and identification of phospholipids were performed as described previously (Levis *et al.*, 1972). For quantitative determination, spots and blanks were scraped into test tubes and phosphorus was determined by using triplicate samples as described previously (Levis *et al.*, 1972). Amounts of phosphatidylserine and phosphatidylinositol are presented together, since in certain t.l.c. runs these two phospholipids were not sharply separated. For fatty acid analysis phospholipids were separated on washed silica-gel H plates (20cm×20cm) by using the solvent systems of Kwiterovich *et al.* (1970). Spots were detected by spraying with water, and the phospholipids were extracted from the silica gel

with several washings of chloroform/methanol (2:1, v/v). Methanolysis of phospholipids was performed with 0.8M-HCl in dry methanol for 2h at 75°C and the recovered methyl esters of the fatty acids and dimethylacetals from plasmalogens were resolved on a 10% polyethylene glycol adipate column (152cm×0.4cm; support, 100–200 mesh Diatomite CQ) at 195°C in a Pye–Unicam aerograph as described previously (Levis *et al.*, 1972).

Other methods

Phosphorus was determined as described by Bartlett (1959) and protein was measured as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Lipid composition

The lipid compositions of three separate samples of pig lymphocyte plasma membrane are shown in Table 1 together with the compositions of the homogenates of mesenteric lymph node from which the membrane samples were prepared. The lipid fraction of the purified plasma membrane comprised about 94% by weight of neutral lipids and phospholipids, in a ratio of about 1:1 (by wt.). The glycosphingolipids and gangliosides accounted for about 1.8 and 0.27% of the total lipid respectively. One sample of plasma membrane contained 1.3% (by wt. of total lipid) of ceramides, but another sample contained a trace amount only. The reason for this variation is not known. Table 1 shows that samples of plasma membrane differing in weight by a factor of 26 gave similar results, and that as little as 5mg (dry wt.) of membrane (equivalent to about

Table 1. Lipid compositions of pig lymphocyte plasma membrane and of the homogenate of pig mesenteric lymph node

Results are given for three separate experiments. The dry weights of plasma-membrane preparations nos. 1, 2 and 3 used for analysis were 5, 100 and 130mg respectively. N.D., Not determined. Experimental details are given in the text. 'Total lipids' are the fraction soluble in the lower organic phase of Folch *et al.* (1957); results are expressed as % of dry wt. of sample. Neutral lipids, phospholipids, ceramides and glycosphingolipids are expressed as % dry wt. of lipid fraction added to the silicic acid column (see the Materials and Methods section). Weights of ceramides and glycosphingolipids were calculated by using mol.wts. of 600 and 1000 respectively, whereas the weight of phospholipid was calculated by multiplying the amount of lipid P by 25. Gangliosides were recovered from the upper aqueous phase of Folch *et al.* (1957), and expressed as μ mol of sialic acids/g of total lipid. Weights of gangliosides were calculated by using a mol.wt. of 1600.

Preparation no. ...	Plasma membrane			Homogenate		
	1	2	3	1	2	3
Total lipids	46	49.5	45.0	N.D.	N.D.	N.D.
Neutral lipids	N.D.	49.8	51.6	N.D.	78.0	76.3
Phospholipids	53.9	44.6	42.6	25.5	16.4	18.4
Ceramides	N.D.	1.3	(trace)	N.D.	1.2	0.99
Glycosphingolipids	1.6	1.7	2.1	0.49	0.45	0.31
Gangliosides (brain type)	N.D.	1.50	1.87	N.D.	0.35	0.42

2.5×10^9 lymphocytes; Allan *et al.*, 1971) gave a reliable analysis for the total amount of glycosphingolipid. The latter amount of plasma membrane also yielded sufficient glycosphingolipids for qualitative analysis by t.l.c. A comparison of the lipid compositions of the purified plasma membrane with those of the initial homogenate showed that the membrane contained more phospholipids (2–3-fold), glycosphingolipids (3–4-fold) and gangliosides (about 5-fold).

The phospholipid compositions of the purified plasma membrane and the initial lymph-node homogenate are shown in Table 2. The most notable

Table 2. *Phospholipid compositions of pig lymphocyte plasma membrane and of the initial lymph-node homogenate*

Phospholipids were separated by t.l.c. in two dimensions, detected by charring with 50% (v/v) H_2SO_4 , and measured by removing spots and determining their P contents; the weight of phospholipid was calculated by multiplying the weight of lipid P by 25. Experimental details are given in the text. The results represent the means of triplicate analyses (\pm s.d. are given for preparation no. 3). The values for phosphatidylcholine and phosphatidylethanolamine include the respective lyso compounds.

Preparation no. ...	Phospholipids (% of total)			
	Plasma membrane		Homogenate	
	2	3	2	3
Phosphatidylcholine	47.8	44.5 \pm 1.5	64.8	48.1 \pm 4.3
Phosphatidylethanolamine	20.1	20.9 \pm 4.8	18.1	36.4 \pm 2.8
Phosphatidylserine + phosphatidylinositol	14.5	16.1 \pm 2.5	9.2	9.3 \pm 5.1
Sphingomyelin	17.5	18.5 \pm 5.6	8.1	6.2 \pm 4.8

feature of the results is that the plasma-membrane phospholipid contained a relatively high proportion of sphingomyelin and, to a lesser extent, of phosphatidylserine plus phosphatidylinositol compared with the initial tissue homogenate. The reason for the marked variation in the amounts of phosphatidylcholine and phosphatidylethanolamine in the two samples of homogenate is not known.

The glycosphingolipids of the lymphocyte plasma membrane and of the lymph-node homogenate were qualitatively analysed by t.l.c. after purification as described in the Materials and Methods section. In preparation no. 3 the glycosphingolipids were separated into neutral and acidic fractions before analysis. The different samples of plasma membrane and homogenate gave similar results with spots in the positions corresponding (in order of increasing mobility) to those of haematoside, globoside (aminoglycolipid), trihexosylceramide, dihexosylceramide and monohexosylceramide. Haematoside was the only acidic component detected in the lower chloroform phase after Folch extraction. Quantitative analysis of the glycosphingolipids was carried out by g.l.c. after fractionation by t.l.c. and subsequent methanolysis. The results (Table 3) indicate that the mono- and tri-hexosylceramides represent the predominant glycolipid constituents of the plasma membrane.

Analysis by t.l.c. of the ganglioside fractions of the purified membrane and the initial homogenate revealed similar patterns. Comparison of these patterns with that given by rat brain gangliosides showed that monosialogangliosides were present to the largest extent and that trisialogangliosides were barely detectable. The mobilities of the mono- and di-sialogangliosides were noticeably lower than those of the corresponding components of rat brain. These differences in polarity most probably reflect differences in the fatty acid and sialic acid com-

Table 3. *Glycosphingolipid compositions of pig lymphocyte plasma membrane and of the lymph-node homogenate*

The glycosphingolipids were isolated from the lower chloroform phase after Folch extraction. Amounts of glycosphingolipids were calculated from the amount of glucose, assuming 1 mol of glucose/mol of glycolipid, and represent the means of duplicate analyses. Individual analyses differed by less than 10%. Glucose/galactose molar ratios represent the means of the analyses for preparations nos. 2 and 3. Experimental details are given in the text.

Preparation no. ...	Plasma membrane			Homogenate		
	Glycolipid (μ mol/g of total lipid)		Glc/Gal (molar ratio)	Glycolipid (μ mol/g of total lipid)		Glc/Gal (molar ratio)
	2	3		2	3	
Glycosphingolipid						
Monohexosylceramide	4.80	4.03	10.3:1.0	1.14	0.60	8.0:1.0
Dihexosylceramide	0.80	1.20	1.0:1.1	0.24	0.16	1.0:1.1
Trihexosylceramide	7.10	5.80	1.0:1.7	0.95	1.13	1.0:1.9
Globoside	3.0	1.70	1.0:1.9	0.84	1.16	1.0:2.1
Haematoside	1.10	0.56	1.0:1.2	0.34	0.08	1.0:1.2

Table 4. Fatty acid compositions of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin from pig lymphocyte plasma membrane and the initial lymph-node homogenate

Fatty acid methyl esters from preparation no. 3 were prepared and analysed as described in the Materials and Methods section. The results represent the means of duplicate analyses; individual analyses did not differ by more than 6% for those fatty acids which comprised more than 5% of the total. Tr, Trace (<0.2%); PM, plasma membrane; Hom, lymph-node homogenate.

Fatty acid	Composition (% of total)					
	Phosphatidylcholine		Phosphatidylethanolamine		Sphingomyelin	
	PM	Hom	PM	Hom	PM	Hom
C _{14:0}	0.5	Tr	10.8	5.4	4.9	0.7
C _{15:0}	—	—	—	—	1.8	0.4
C _{16:0} (dimethylacetals)	—	—	4.0	2.3	—	—
C _{16:0}	49.8	27.2	18.5	30.5	19.6	32.8
C _{16:1}	Tr	Tr	4.6	1.2	Tr	Tr
C _{18:0} (dimethylacetals)	—	—	4.2	3.6	—	—
C _{18:0}	15.2	20.9	15.3	22.2	12.0	15.1
C _{18:1}	17.3	26.5	19.8	17.4	4.5	7.1
C _{18:2}	8.5	4.1	4.5	4.9	—	—
Unknown	2.2	2.4	6.6	1.8	—	—
C _{20:0}	—	—	—	—	10.3	13.3
C _{20:3}	1.0	2.7	—	—	—	—
C _{20:4}	4.8	9.2	8.3	9.0	—	—
C _{22:0}	—	—	—	—	7.9	6.3
C _{22:unsat.}	0.7	7.0	3.4	1.7	—	—
C _{23:0}	—	—	—	—	5.3	4.2
C _{24:0}	—	—	—	—	15.4	9.2
C _{24:1}	—	—	—	—	9.8	8.5
C _{25:0}	—	—	—	—	8.5	2.4
Unsaturated (%)	32.3	49.5	40.6	34.2	14.3	15.6

positions. Thus the pig gangliosides contained 90% *N*-glycolylated and 10% *N*-acetylated neuraminic acid residues, compared with the opposite distribution in the rat (see also Renkonen *et al.*, 1972). The haematoside detected in the pig ganglioside fractions is probably the *N*-acetyl derivative that originated from partition during the extraction procedure (Tao & Sweeley, 1970).

Fatty acid composition

The methyl esters of fatty acids formed by methanolysis of the various lipid fractions were analysed by g.l.c. The fatty acid compositions of the phosphatidylcholine, phosphatidylethanolamine and sphingomyelin of purified plasma membrane are shown in Table 4; the composition of the initial lymph-node homogenate is included for comparison. The results show that the plasma-membrane phosphatidylcholine was rich in palmitic acid (C_{16:0}), whereas the membrane phosphatidylethanolamine and sphingomyelin contained less palmitic acid but were much richer in myristic acid (C_{14:0}).

Table 5 shows the fatty acid compositions of the individual glycosphingolipids isolated from the plasma membrane and the lymph-node homogenate. The results indicate that especially oleic acid and

fatty acids with a chain length shorter than C₂₀ were major constituents of the plasma membrane. 2-Hydroxy fatty acid analyses revealed primarily C_{24:0} and C_{24:1} acids; these fatty acids generally accounted for about 10% of the total fatty acids of the plasma-membrane glycosphingolipids.

The fatty acid composition of the ganglioside fraction resembled that reported previously for extraneural brain-type gangliosides (Puro & Keranen, 1969; Yu & Ledeen, 1972) with the C_{16:0}, C_{18:0}, C_{18:1}, C_{20:0}, C_{22:0} and C_{24:1} acids predominating. A characteristic feature of the plasma-membrane gangliosides was the high content of C_{23:0} (tricosanoic) acid (22% of the total fatty acids), and the presence of 2-hydroxy fatty acids (also reported by Puro & Keranen, 1969).

Discussion

Various arguments suggest that the initial homogenate of pig mesenteric lymph node and the purified plasma-membrane fraction were derived primarily from small lymphocytes. Thus histological examination of sections of lymph node showed the small lymphocyte as the predominant morphological cell type together with large lymphocytes, plasma

Table 5. Fatty acid compositions of glycosphingolipids from pig lymphocyte plasma membrane and the initial tissue homogenate

Fatty acid methyl esters were prepared and analysed as described in the Materials and Methods section. The results given by preparation no. 2 are presented in detail and a summary of those obtained with preparation no. 3 is included for comparison. The quoted values present the means of duplicate analyses; individual analyses did not differ by more than 10% for those fatty acids which comprised more than 5% of the total. Tr, Trace (<0.2%). PM, plasma membrane; Hom, lymph-node homogenate.

Preparation no.	Fatty acid	Composition (% of total)									
		Monohexosyl-ceramide		Dihexosyl-ceramide		Trihexosyl-ceramide		Globoside		Haematoside	
		PM	Hom	PM	Hom	PM	Hom	PM	Hom	PM	Hom
2	C _{12:0}	Tr	Tr	4.5	1.9	Tr	Tr	0.5	0.3	0.8	0.6
	C _{14:0}	2.0	2.7	6.5	8.8	7.1	3.8	4.1	2.1	9.0	6.1
	C _{15:0}	3.4	Tr	4.0	2.0	8.3	0.6	0.2	0.3	0.2	0.3
	C _{16:0}	27.2	27.2	34.6	26.2	19.5	17.5	14.9	8.9	9.8	25.7
	C _{16:1}	1.9	2.6	5.6	5.6	1.0	2.2	4.1	1.5	2.0	3.0
	C _{17:0}	2.8	0.8	Tr	0.6	0.8	0.2	2.1	0.8	0.6	0.7
	C _{18:0}	10.0	7.8	10.0	13.1	7.8	6.1	13.6	11.6	24.6	22.9
	C _{18:1}	18.3	7.8	17.1	8.2	15.2	5.6	17.6	7.0	21.8	11.3
	C _{19:0}	2.8	0.2	0.7	Tr	0.2	0.1	1.0	Tr	Tr	Tr
	Unknown	7.2	1.5	2.5	3.7	6.8	2.3	—	—	—	—
	C _{20:0}	4.9	5.3	3.6	3.4	2.9	7.6	5.5	5.6	11.1	13.5
	C _{20:1}	1.1	0.8	0.3	0.2	0.6	1.0	3.1	0.7	Tr	0.4
	C _{21:0}	1.0	2.2	Tr	2.4	1.9	0.4	3.3	0.6	6.3	1.2
	C _{22:0}	6.3	9.5	1.7	6.8	6.3	15.1	12.4	11.6	2.7	4.2
	C _{22:1}	0.1	Tr	Tr	0.6	2.1	0.2	Tr	3.8	Tr	0.6
	C _{23:0}	1.5	3.3	0.6	7.8	4.8	3.7	8.1	5.3	7.1	1.8
	C _{24:0}	6.5	17.6	3.5	3.3	11.7	15.3	4.1	17.2	1.0	4.3
	C _{24:1}	3.0	10.7	4.8	5.4	3.0	18.3	5.4	22.7	3.0	3.4
2	<C ₂₀ (%)	74.7	53.8	87.4	70.8	49.2	55.6	63.0	49.1	65.4	69.2
3		75.6	50.6	85.5	70.1	66.7	38.4	58.1	32.5	68.8	70.6
2	μmol of oleic acid/g of total lipid	0.62	0.104	0.12	0.014	0.69	0.056	0.48	0.047	0.24	0.036
3		0.74	0.047	0.20	0.013	0.88	0.063	0.30	0.081	0.12	0.009

cells, macrophages and connective tissue. Much of the connective tissue was, however, apparently discarded during the disruption of the node, and the subsequent filtration of the disrupted tissue through muslin (Allan & Crumpton, 1970). Further, the polypeptide composition of the purified plasma membrane, as assessed by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate, resembled closely that of the plasma membrane isolated from single cell suspensions comprising more than 90% small lymphocytes that were prepared by mechanically agitating the nodes (B. Barber, F. Walsh & M. J. Crumpton, unpublished work).

The lipid fraction of the purified plasma membrane accounted for about 47% of the membrane's dry weight. It was mainly composed (about 94% by weight) of roughly equal parts of neutral lipids and phospholipids (Table 1), and in this respect resembled the surface membrane of other cell types (e.g. mouse fibroblasts; Weinstein *et al.*, 1969). The remaining lipid was made up of ceramides, glycosphingolipids and gangliosides. The phospho-

lipid composition (Table 2) resembled closely that reported for the plasma membrane of pig mesenteric lymph-node lymphocytes (Chavin *et al.*, 1975). The glycosphingolipid content (20 μg/mg of total lipid) was much lower than that of the plasma membranes of other cells/tissues [e.g. BHK 21 cells, 49 μg/mg (Renkonen *et al.*, 1972), and rat intestine, 191 μg/mg (Forstner & Wherrett, 1973)], but the pattern of the individual glycosphingolipids (Table 3) conformed to that commonly encountered in mammalian extraneural tissues. The gangliosides of the lymphocyte plasma membrane contained primarily (90%) *N*-glycolylneuraminic acid rather than the *N*-acetyl derivative and in this context resembled the brain-type gangliosides isolated from other non-human extraneural tissues (Ledeen *et al.*, 1968; Wiegandt & Bucking, 1970; Yu & Ledeen, 1972; Forstner & Wherrett, 1973).

A comparison of the lipid composition of the plasma-membrane fraction with that of the initial tissue homogenate showed that the purified plasma membrane contained increased proportions of phos-

pholipids, glycosphingolipids and gangliosides. These results agree with those of other studies, which demonstrate that the sphingomyelin, glycosphingolipids and gangliosides of whole cells are preferentially located in the surface membrane to a greater extent than other lipids (e.g. mouse fibroblasts, Weinstein *et al.*, 1969; rat liver, Ray *et al.*, 1969; rat intestine, Forstner & Wherrett, 1973). As reported previously for other cell types (Renkonen *et al.*, 1972; Forstner & Wherrett, 1973) the relative proportions of the individual glycosphingolipids in the purified membrane resembled those in the initial homogenate, apart from a higher relative amount of globoside in the homogenate (Table 3).

The role of fatty acid residues in determining membrane fluidity and permeability has been stressed previously. In view of their probable importance to lymphocyte physiology the fatty acid compositions of the plasma-membrane phospholipids and glycosphingolipids were determined. Phosphatidylcholine was unique in having a fairly simple fatty acid composition compared with the more complex patterns of phosphatidylethanolamine and sphingomyelin (Table 4). Thus palmitic acid accounted for 50% of its fatty acid residues, and palmitic acid, stearic acid and oleic acid together represented 80%. Since phosphatidylcholine accounted for 22% of the total lipid, the above distribution suggests that dipalmitoyl phosphatidylcholine may be a significant lipid species of the membrane. In contrast with the other phospholipids, phosphatidylcholine contained little myristic acid, which was present in especially large amounts in phosphatidylethanolamine. Compared with the initial tissue homogenate the plasma-membrane phosphatidylcholine showed a marked tendency to accumulate palmitic acid, whereas the phosphatidylethanolamine and sphingomyelin contained higher amounts of myristic acid. An examination of the fatty acid compositions of the plasma-membrane glycosphingolipids (Table 5) revealed that they are rich in fatty acids with a chain length of less than C₂₀, especially oleic acid and to a lesser extent palmitic acid and stearic acid. Compared with the initial homogenate, all of the membrane glycosphingolipids contained more oleic acid. This accumulation was particularly striking for the major glycosphingolipids of the membrane (i.e. mono- and tri-hexosylceramide, comprising 72% of the glycosphingolipid fraction; Table 5).

The relevance of the distributions of fatty acid residues among the plasma-membrane phospholipids and glycosphingolipids is not known. It seems possible, however, that the preferential association of oleic acid with the glycosphingolipids may be related to the presence in the membrane of glycosyltransferases that are specific for acceptor glycosphingolipids containing oleic acid (Roth, 1973).

Further, if the glycosphingolipids and phospholipids are localized within particular areas of the membrane, then it is conceivable that owing to the local enrichment in oleic acid residues these areas may represent more expanded (less ordered) regions of the lipid bilayer and may confer selective permeability on the membrane.

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