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THE LIPIDS OF THE PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM FROM CULTURED BABY HAMSTER KIDNEY CELLS (BHK21)

OSSI RENKONEN, CARL G. GAHMBERG, KAI SIMONS AND LEEVI KÄÄRIÄINEN
Department of Biochemistry, Department of Serology and Bacteriology, and Department of Virology, University of Helsinki, Helsinki (Finland)

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SUMMARY

Plasma membranes and endoplasmic reticulum of two clones of cultured baby hamster kidney cells (BHK21) were prepared by the method of WALLACH AND KAMAT (D. F. H. WALLACH AND V. B. KAMAT, *Methods Enzymol.*, 8 (1966) 164). The two membranes differed significantly from each other in their neutral, phospho- and sphingoglycolipid compositions.

INTRODUCTION

We are stydying the envelope of the Semliki Forest virus as a membrane model for structural and assembly studies[1,2]. This virus acquires its envelope as it leaves the host cell by budding through the plasma membrane[3] and possibly also through vacuoles of the endoplasmic reticulum[4]. How the proteins and lipids are incorporated into the virus envelope is not known.

We use BHK21 cells, fibroblasts derived from hamster kidney. The plasma membrane lipids of these cells have previously been studied by KLENK AND CHOPPIN[5,6], who used the fluorescein mercuric acetate method of WARREN *et al.*[7] to isolate the plasma membrane. This method suffers from the drawback that proteins are denatured. Endoplasmic reticulum lipids of these cells have not been analyzed previously.

We have used the method of WALLACH AND KAMAT[8] to isolate the plasma membrane and endoplasmic reticulum of BHK21 cells. The purity of the membranes has been assessed by enzymatic and immunological analysis[9]. Their protein and glycoprotein compositions[10] have been studied in this laboratory and have been found to be significantly different.

The present report describes the lipid class composition analyzed from small samples of plasma membrane and endoplasmic reticulum of BHK21 cells. A report describing the lipid class composition of Semliki Forest virus grown in these cells will be published elsewhere[11].

MATERIALS AND METHODS

Cells

Monolayer cultures of two BHK21 cell lines, Wi-2 (ref. 12) and C-13 (ref. 13) were grown at 37° in 32-ounce prescription bottles in 40 ml BHK21 tissue culture

Abbreviations: BHT, butylated hydroxytoluene.

medium[14] containing 10 % calf serum and 10 % tryptose phosphate broth. The doubling time was 16–18 h. The cultures were usually harvested before they reached the saturation density.

Plasma membranes and endoplasmic reticulum of BHK21 cells

They were obtained essentially as described by WALLACH AND KAMAT[8]. The two membranes were characterized by analysis of marker enzymes, surface antigens and gangliosides as previously described[9,15]. The specific activity of (Na^+–K^+)-activated ATPase was 10–20 times higher in the plasma membrane fraction than in the original homogenates. Surface antigens were concentrated likewise 10–20 times in the plasma membrane fraction. The specific activity of NADH dehydrogenase (EC 1.6.99.3) was 2–4 times higher in the endoplasmic reticulum fraction than in the original homogenate[9].

Extraction of lipids

The isolated membranes were lyophilized, and samples containing 1–10 mg protein were extracted essentially as described by PURO[16]. The completeness of the extractions was evaluated as described by WEINSTEIN *et al.*[17]. Less than 1 % of total fatty acids were found in the protein residue of the whole cells, and the lipid extraction is believed to be 99 % complete even with the isolated membranes.

Fractionation of the lipid extracts

This is summarized on the flow sheet shown in Fig. 1.

Preparation and assay of ganglioside extracts

Chloroform and water were added to the lipid extracts to form the Folch system, chloroform–methanol–water (8:4:3, by vol.)[18]. The two liquid layers were separated, the upper layer removed, and the lower layer washed twice with fresh upper layer. The original upper layer, together with the two wash phases, was dialyzed for 3 days against water and lyophilized. The dialysis causes no loss of lipid-bound neuraminic acid[16]. The dry residue was extracted three times with chloroform–methanol (2:1, by vol.) giving the ganglioside extracts. The gangliosides were quantitated as described by WEINSTEIN *et al.*[17]. The quantities of neuraminic acid were multiplied by 1.78 × 4.0 to obtain the weight of the gangliosides[19].

The ganglioside extracts prepared from whole BHK21 cells contained all of the lipid-bound neuraminic acid, but in addition they also contained 4–7 % of the total phosphorus extracted from the cells. However, much of this phosphorus (33–67 %) remained stationary during phospholipid thin-layer chromatography and probably did not represent phospholipids. We estimate that only 2–3 % of the whole cell or membrane phospholipids were lost from the lower Folch layer to the ganglioside fraction.

Separation of neutral lipids, neutral glycolipids and phospholipids from each other

The washed lower Folch phases contained all lipids except the gangliosides. They were filtered through a bed of Na^+-charged Chelex resin essentially as described by CARTER AND WEBER[20]. This treatment, which converts the anionic lipids into sodium salts, is necessary for successful separation of glycolipids and phospholipids

in the next phase of the analysis (K. TYÖRINOJA AND O. RENKONEN, unpublished observations). The phospholipids were recovered quantitatively from the Chelex bed.

The eluates from the Chelex beds were fractionated by silicic acid chromatography into "neutral lipids", neutral glycolipids and phospholipids. The chromatography was performed with samples of 3–4 mg lipid on 1.00-g columns of Unisil (Clarkson Chemical Co., N. J.). The "neutral lipids" were eluted with 10 ml chloroform, the neutral glycolipids with 50 ml acetone, and the phospholipids with 50 ml chloroform–methanol (1:1, by vol.) *plus* 50 ml of methanol[21]. Thin-layer chromatography showed that essentially all "neutral lipids" were eluted with the chloroform, neutral glycolipids with the acetone, and phospholipids with the last two solvents.

Quantitation of the principal lipid fractions

"Neutral lipids" were quantitated by evaporating aliquots containing 100–300 μg lipid on aluminium planchets and weighing them with a Mettler M5-microbalance:

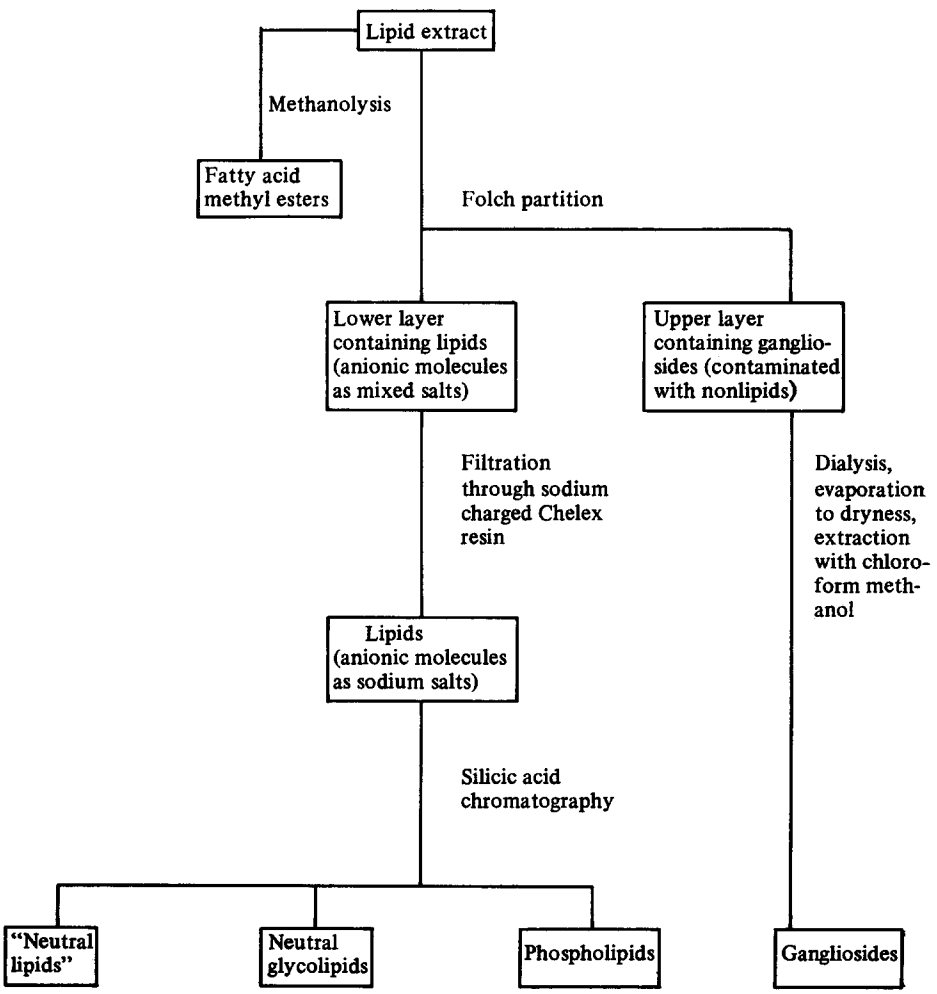

Fig. 1. Flow sheet of the lipid fractionation.

Most of the butylated hydroxytoluene (BHT) could be evaporated at 20 torr at 20° without loss of lipids. The lipid samples subjected to Unisil chromatography contained 1000–1500 μg "neutral lipids"; the blanks carried through Chelex filtration and Unisil chromatography contained less than 100 μg of apparent "neutral lipids".

Neutral glycolipids were quantitated by converting their carbohydrates, glucose and galactose, into methyl glycosides and subjecting these to quantitative gas-liquid chromatography as trimethylsilyl ethers[22]. The quantity of hexoses was multiplied by 3 to obtain the weight of neutral glycolipids. The actual lipid samples subjected to Unisil chromatography contained 25 μg or more of neutral glycolipids. Blanks carried through Chelex filtration and Unisil chromatography generally contained about 4 μg of apparent glycolipids. The high blank values appeared to be caused by glucose contamination, so the glucose values are probably less reliable than the galactose values.

Phospholipids were quantitated by phosphate analysis[23]. The weight of phosphorus was multiplied by 25 to obtain the quantity of phospholipids. The phosphorus in the blanks was negligible.

Separation and quantitation of individual lipid classes

"Neutral lipids" were fractionated by thin-layer chromatography on Silica gel G plates which were developed first with hexane–ether (19:1, by vol.), dried for 20 min and redeveloped with hexane–diethyl ether–acetic acid (50:50:1, by vol.). In the first run the solvent front moved 15 cm, in the second only 10 cm. The different lipid classes were detected with iodine vapour and quantitated by the method of MARSH AND WEINSTEIN[24].

Neutral glycolipid classes were separated with thin-layer chromatography using Silica gel G plates and chloroform–methanol–water (60:20:3, by vol.) as solvent. The lipids were detected with iodine vapor or by the aniline–diphenylamine–phosphoric acid stain of BAILEY AND BOURNE[25]. The quantities of the neutral glycolipid classes were calculated from the gas–liquid chromatography-results for the original glycolipid fraction.

Phospholipid classes were separated by two-dimensional thin-layer chromatography on ready-made plates of Silica gel F254 (Merck, Darmstadt, Germany). The first dimension was run twice in chloroform–methanol–7 M aqueous ammonia (65:20:4, by vol.). The second dimension was developed with chloroform–acetone–methanol–acetic acid–water (50:20:10:10:5, by vol.). Between the different runs the plates were dried for 20 min at 20° with an air blower. The different phospholipid classes were detected with iodine vapor and quantitated by measuring phosphorus essentially as described by ROUSER *et al.*[26]. Phosphorus recovery from all analyses was 86 ± 11 % (S.D.). The low and variable yield probably reflects the fact that the area near the origin was not analyzed: this area contained non-lipid phosphorus compounds.

Ganglioside classes were separated by thin-layer chromatography using Silica gel G plates. The developing solvent was propanol–water (7:3, by vol)[27] or propanol–conc. ammonia–water (6:2:1, by vol.)[28]. The former solvent did not separate *N*-acetyl- and *N*-glycolylneuraminyl-lactosylceramides from each other, but did separate these two hematosides from other gangliosides when referenc lipids were chromatographed. The second solvent was used to separate the two hematosides from each other. The

plates were stained with the resorcinol reagent of SVENNERHOLM[29]. The ganglioside classes were quantitated by use of the method of SUZUKI[30].

Preparation and analysis of fatty acid methyl esters

Fatty acids of all lipid samples were converted to methyl esters and analyzed on a packed polyester column essentially as described previously[31].

Protein

Protein was determined with the method of LOWRY *et al.*[32] using bovine serum albumin as standard. The reaction mixture contained 0.1 % sodium dodecyl sulphate.

RESULTS

The lipids of whole BHK21 cells, their plasma membrane and their endoplasmic reticulum were analyzed as shown in Fig. 1. The yields of plasma membrane and endoplasmic reticulum protein calculated from the homogenate were 0.6 and 3.0 %, respectively. The yield of plasma membrane lipids was about 4 %, and the yield of endoplasmic reticulum lipids about 8.5 % of the homogenate lipids. These values are minimum estimates for the cellular content of these membranes since the procedure used does not allow quantitative recovery.

Fatty acid composition

The Wi-2 cells as well as the isolated membranes had similar fatty acid compositions with only small quantities of polyenoic fatty acids (Table I).

The principal lipid fractions

Phospholipids formed the largest fraction in the whole cells as well as in the plasma membrane and the endoplasmic reticulum (Table II). These lipids form about two thirds of the endoplasmic reticulum lipids. The neutral lipids were enriched in the plasma membrane. So were the sphingoglycolipids. This confirms our earlier findings[15] as well as those of KLENK AND CHOPPIN[6].

TABLE I

FATTY ACID COMPOSITION OF THE TOTAL LIPIDS OF BHK21-Wi-2 CELLS, THEIR PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM

Mean values of two independent analyses are shown.

Major fatty acid	*Fatty acid (% of total fatty acid)*		
	Whole cells	*Plasma membranes*	*Endoplasmic reticulum*
16:0	21	26	21
18:0	8	12	9
18:1	48	46	51
18:2	12	9	10
20:1	2	2	2
Polyenes and long-chain acids	Trace	Trace	Trace

TABLE II

LIPID TO PROTEIN RATIOS OF THE PRINCIPAL LIPID FRACTIONS IN BHK21 CELLS, PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM

Mean values and standard deviations of 2–6 independent analyses. Values expressed in μg/mg protein.

	Whole cells		*Plasma membranes*		*Endoplasmic reticulum*	
	BHK21-Wi-2	*BHK21-C-13*	*BHK21-Wi-2*	*BHK21-C-13*	*BHK21-Wi-2*	*BHK21-C-13*
Total lipids*	291	210	1457	1599	668	660
"Neutral lipids"	93	62	587 ± 144	493	185 ± 68	189
Neutral glycolipids	nd	1.7	25 ± 6	28	3.4 ± 1.2	2.7
Phospholipids	187	138	766 ± 75	1000	466 ± 126	455
Gangliosides	10	7.5	79 ± 18	78	14 ± 5	13
Molar ratio of sphingoglycolipids** to phospholipids	0.034***	0.045	0.097	0.076	0.026	0.023

* Sum of the four main fractions.
** Neutral glycolipids *plus* gangliosides.
*** Gangliosides only.

TABLE III

FREE CHOLESTEROL AND FREE FATTY ACID CONTENT IN BHK21 CELLS, THEIR PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM MEMBRANES

Mean values and standard deviations of 2–6 independent analyses are shown.

Ratio	*Whole cells*		*Plasma membranes*		*Endoplasmic reticulum*	
	BHK21-Wi-2	*BHK21-C-13*	*BHK21-Wi-2*	*BHK21-C-13*	*BHK21-Wi-2*	*BHK21-C-13*
Free cholesterol/protein (w/w)	0.025	0.027	0.26 ± 0.03	0.28	0.067 ± 0.026	0.047
Free cholesterol/phospholipid (mole/mole)	0.28	0.36	0.70 ± 0.08	0.56	0.29 ± 0.07	0.20
Free fatty acid/protein (w/w)	0.010	0.015	0.093 ± 0.028	0.081	0.062 ± 0.026	0.060
Free fatty acid/phospholipid (mole/mole)	0.22	0.31	0.35 ± 0.12	0.23	0.41 ± 0.19	0.37

Composition of the "neutral lipids"

The presence of cholesterol esters, triglycerides, free fatty acids, diglycerides and free cholesterol in BHK21-Wi-2 cells was established with thin-layer chromatography (Fig. 2). In addition an apolar lipid was observed, but it remained unidentified; it may have been a hydrocarbon fraction[33]. *O*-Alkyl diglyceride (Component B in Fig. 2) was isolated and then identified by the formation of fatty acid methyl esters and *O*-alkyl glycerols upon mild alkaline methanolysis; this lipid was stable under mild acid methanolysis which shows that *O*-alkenyl diglycerides were absent[34,35]. Thin-layer chromatography showed that BHK21-C-13 cells contained the same neutral lipid classes as the Wi-2 cells. The plasma membrane and the endoplasmic reticulum contained the same lipid classes as the whole cells.

Free cholesterol and free fatty acids were the largest neutral lipid components in whole BHK21 cells and the isolated membranes; in most samples these lipids amounted to 60–70 % of the whole neutral lipid fraction. The protein based cholesterol content of the plasma membrane was 10 times higher than that of whole BHK21 cells (Table III), whereas the molar ratio of free cholesterol to total phospholipids was 2 times higher.

The free fatty acids amounted to 5 % of total lipids in the whole cells and the plasma membrane, and to 9 % in the endoplasmic reticulum. Lipolysis was not optimally controlled in our experiments, and the high fatty acid concentrations may be artifacts. However, free fatty acids amount to 4.7–6.1 % of total lipids even in other tissue cultured cells[36,37]. Furthermore, we have found that they comprise 4–12 %

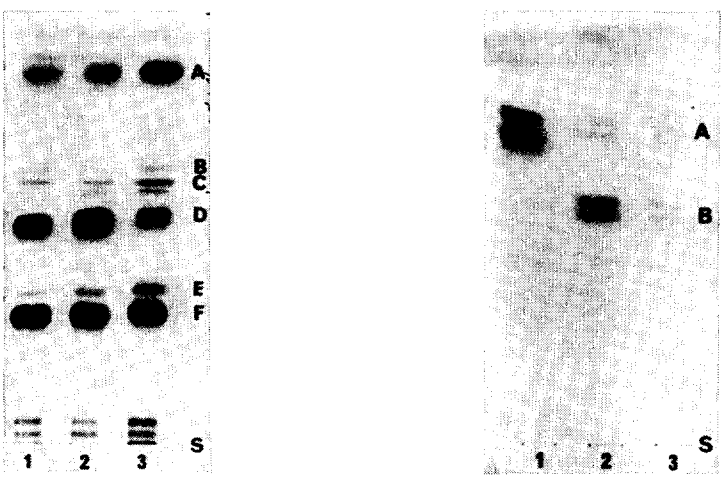

Fig. 2. Thin-layer chromatogram of "neutral lipids". 1, "neutral lipids" of plasma membrane (BHK21-Wi-2); 2, "neutral lipids" of endoplasmic reticulum (BHK21-Wi-2); 3, "neutral lipids" of whole BHK21-Wi-2 cells. A, cholesterol esters; B, *O*-alkyl diglycerides; C, triglycerides; D, free fatty acids; E, diglycerides; F, cholesterol; S, start. The plate was developed as described in the text, and stained with iodine vapor.

Fig. 3. Thin-layer chromatogram of neutral glycolipids. 1, ceramide monosaccharides from ox-brain; 2, neutral glycolipids from plasma membrane (BHK21-Wi-2); 3, neutral glycolipids from endoplasmic reticulum (BHK21-Wi-2). A, ceramide monosaccharides; B, ceramide disaccharides; S, start. The plate was developed as described in the text, and stained with the diphenylamine stain of Bailey and Bourne.

of total lipids in plasma membrane isolated from BHK21-C-13 cells by the fluorescein mercuric acetate method (O. RENKONEN, unpublished observations).

Composition of neutral glycolipids and gangliosides

Thin-layer chromatography of the whole cell neutral glycolipids revealed ceramide mono- and dihexosides (Fig. 3). The double spots on thin-layer chromatography suggest that both these glycolipids contained fatty acids of different chain lengths, or alternatively unsubstituted as well as hydroxy acids. Thin-layer chromatography on borate containing plates[38] and gas–liquid chromatography of the component sugars showed that the monohexoside contained glucose and the dihexoside equimolar amounts of glucose and galactose. Our cells did not reveal ceramide tri- or tetrasaccharides on thin-layer chromatography nor did gas–liquid chromatography reveal hexosamines or fucose. HAKOMORI and co-workers[39,40] have described a ceramide trisaccharide from BHK21 cells in one line of low passage level, but not in another.

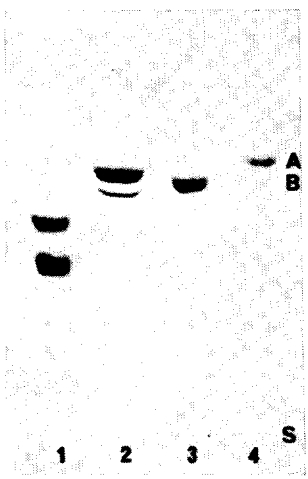

Fig. 4. Thin-layer chromatogram of gangliosides. 1, brain gangliosides; 2, gangliosides of whole BHK21-Wi-2 cells; 3, *N*-glycolylneuraminyllactosylceramide; 4, *N*-acetylneuraminyllactosylceramide. A, *N*-acetylneuraminyllactosylceramide; B, *N*-glycolylneuraminyllactosylceramide; S, start. The plate was developed with propanol–conc. ammonia–water (6:2:1, by vol.) and stained with the resorcinol stain of Svennerholm.

Thin-layer chromatography and gas–liquid chromatography revealed the same neutral glycolipids in the plasma membrane and in the endoplasmic reticulum as in the whole cells. The two components were present in about equimolar amounts in the isolated membranes.

Two gangliosides were found in our BHK21 cell clones (Fig. 4). On thin-layer chromatography they were identical with *N*-acetyl-, and *N*-glycolylneuraminyllactosylceramides. More polar gangliosides were observed on thin-layer chromatography only occasionally and in trace quantities.

Partial hydrolysis of the two gangliosides gave results compatible with the thin-layer chromatography analyses[15]. Quantitation revealed 90 % *N*-acetyl derivative and 10 % *N*-glycolyl derivative in whole BHK21-Wi-2 cells[15].

The plasma membrane of BHK21 cells revealed only *N*-acetyl-, and *N*-glycolyl-

TABLE IV

COMPOSITION OF PHOSPHOLIPIDS IN BHK21 CELLS, THEIR PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM

Mean values and standard deviations of 2–5 independent analyses are shown.

	Phospholipid (% of total fatty acid)					
	Whole cells		*Plasma membranes*		*Endoplasmic reticulum*	
	BHK21-Wi-2	*BHK21-C-13*	*BHK21-Wi-2*	*BHK21-C-13*	*BHK21-Wi-2*	*BHK21-C-13*
Phosphatidic acid	1.8	2.1	3.8 ± 2.6	5.7	1.3 ± 1.0	2.6
Phosphatidylethanolamine	21	23	18 ± 3	21	14 ± 4	15
Phosphatidylcholine	48	55	38 ± 9	40	55 ± 6	55
Phosphatidylserine	5.2	2.6	8.9 ± 0.8	7.1	3.6 ± 0.9	2.5
Phosphatidylinositol		1.1	2.3 ± 0.5	3.2	4.3 ± 0.7	5.0
Sphingomyelin *	14 **	11	18 ± 7	17	10 ± 4	9.0
Lysophosphatidylcholine	2.9	1.3	6.4 ± 1.1	3.4	6.9 ± 4.8	5.7

* Includes lysophosphatidylethanolamine.

** Includes phosphatidylinositol. Recent analyses have revealed 7% phosphatidylinositol and 7% sphingomyelin.

TABLE V

LIPID CLASS COMPOSITION OF PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM FROM BHK21 CELLS AND RAT LIVER

	Molar ratio of individual lipid classes to total phospholipids *							*Reference*
	Cholesterol	*Sphingo-glycolipids*	*Phosphatidyl-ethanolamine*	*Phosphatidyl-choline*	*Phosphatidyl-serine*	*Phosphatidyl-inositol*	*Sphingo-myelin*	
BHK21								
Plasma membrane **	0.63	0.09	0.20	0.39	0.08	0.03	0.18	Present work
Endoplasmic reticulum **	0.24	0.02	0.15	0.55	0.03	0.05	0.10	Present work
Whole cell **	0.32	0.04	0.22	0.52	0.04	0.01?	0.12	Present work
Rat liver								
Plasma membrane	0.66	0.007	0.18	0.35	0.09	0.07	0.18	33, 57, 58
Endoplasmic reticulum ***	0.09	0.001	0.18	0.47	0.08	0.11	0.06	57, 58, 42
Whole cell	0.10	0.001	0.22	0.49	0.03	0.10	0.05	33, 57, 58, 42, 59

* The sum of all phospholipid components including those not listed here, equals 1.00 in each membrane fraction.

** Average composition of Wi-2 and C-13 cells. The plasma membranes of the two clones had 8%, their endoplasmic reticulum 7%, and the whole cells themselves 9% dissimilar lipids.

*** Mean of "Rough" and "Smooth I" values.

neuraminyllactosylceramides on thin-layer chromatography in similar proportions as in the cells. The gangliosides in the endoplasmic reticulum of Wi-2 cells were studied, but only with the neutral thin-layer chromatography solvent, which revealed the unresolved spot of *N*-acetyl-, and *N*-glycolylneuraminyllactosylceramides.

Composition of phospholipids

Whole BHK21 cells contained all the common animal phospholipid classes. The plasma membrane and the endoplasmic reticulum contained the same major phospholipid classes as the whole cells.

The quantitative relationships between the major phospholipids are shown in Table IV. The plasma membrane phospholipids of both cell clones contained less phosphatidylcholine but more phosphatidic acid, phosphatidylserine, sphingomyelin, and lysophosphatidylcholine than the whole BHK21 cells. The cardiolipin content was also smaller in the plasma membrane than in the whole cell, but our small samples did not allow quite reliable analyses of this minor lipid.

High lysophosphatidylcholine is not generally found in the plasma membrane and we cannot exclude the possibility that the high value in our experiments is an artifact caused by membrane-bound phospholipase A (EC 3.1.1.4)[41].

The endoplasmic reticulum phospholipids were clearly different from those of the plasma membrane. The most characteristic feature of the endoplasmic reticulum phospholipids was the very high phosphatidylcholine content. On the whole the phospholipids of BHK21 endoplasmic reticulum resembled rather much those of rat liver endoplasmic reticulum[42].

The phospholipids of whole BHK21-Wi-2 cells contained plasmalogens as evidenced by the release of dimethyl acetals upon mild methanolysis. *O*-Alkyl phospholipids were also present since $LiAlH_4$ reduction[43] gave both *O*-alk-1-enyl- and *O*-alkylglycerols. Measurement of alkali-stable phospholipid suggested that these ether phospholipids were also present in the plasma membrance and in the endoplasmic reticulum of BHK21-Wi-2 cells.

DISCUSSION

It appears that plasma membranes of identical lipid/protein ratios are difficult to obtain when different methods are applied to a given cell type. This has been widely experienced in the early work on erythrocyte ghosts[44], and it is hardly surprising that work with the more complex eucaryotic cells meets with the same difficulty. In our experiments the plasma membrane were isolated by the method of WALLACH AND KAMAT[8], and the samples derived from the two clones of BHK 21 cells both had a lipid/protein ratio of 1.5 (w/w). KLENK AND CHOPPIN[5] on the other hand, isolated plasma membrane from BHK21 cells using the fluorescein mercuric acetate method of WARREN *et al.*[7], and found that the lipid content was only 31 % of the dry weight of the membrane. Plasma membrane preparations of different lipid/protein ratios have also been obtained from HeLa cells when different methods were used: MCLAREN *et al.*[45] recorded a very high ratio of over 1.6 (w/w) with a modification of the WALLACH–KAMAT method, whereas BOSMANN, *et al.*[46] reported a ratio of only 0.67 (w/w) when using Dounce homogenization and sucrose density-gradient centrifugation. The WALLACH–KAMAT procedure may give high lipid/protein values rather generally, MOLNAR *et al.*[47] found a value of 0.91 in Ehrlich ascites

cell plasma membrane isolated with this method. It is possible that certain membrane components are lost during some isolation procedures, and exogenous material carried with the membranes in others: in addition morphologically homogenous membranes may be split into different fractions[48]. Despite the different lipid/protein ratios our plasma membrane samples and those of KLENK AND CHOPPIN[5] were remarkably similar in lipid class composition. In this respect the methods of WARREN *et al.*[7] and of WALLACH AND KAMAT[8] appear to give similar results.

Comparison of all reliably analyzed lipids of the whole cells with those of the plasma membrane and the endoplasmic reticulum is presented in Table V. These values show that: (1) The plasma membrane and the whole cell had 22 % dissimilar lipids. (2) The endoplasmic reticulum and the whole cell had only 11 % dissimilar lipids. (3) The plasma membrane and the endoplasmic reticulum had 30 % dissimilar lipids*. In other words, the plasma membrane lipids differed more from the whole cell lipids than did the endoplasmic reticulum lipids. It is interesting that the lipids of Semliki Forest virus membrane resemble those of the host cell plasma membrane and contained only 13 % dissimilar lipids[11].

Five features appeared typical of our plasma membrane when their lipid composition was compared with that of the whole cell (Tables I and V). These were: (1) High molar ratio of cholesterol to phospholipid. (2) High molar ratio of sphingomyelin to phospholipid. (3) High molar ratio of sphingoglycolipid to phospholipid. (4) High molar ratio of phosphatidylserine to phospholipid. (5) High content of palmitic and stearic acid. The cellular distribution of the sphingoglycolipids, cholesterol, sphingomyelin and phosphatidyl serine was so uneven that a sizeable fraction of the cellular content of these lipids must have been confined to the plasma membrane. These features appear typical to plasma membrane of many animal cells prepared with a variety of methods[5,6,17,49].

The endoplasmic reticulum lipids resembled those of whole BHK21 cells so closely that only slightly higher phosphatidylcholine (*plus* lysophosphatidylcholine) and lower phosphatidylethanolamine seem to distinguish this organelle from the whole cells. In general the lipids of the BHK21 endoplasmic reticulum resemble those of the rat liver endoplasmic reticulum (20 % dissimilar) (Table V). This organelle may therefore have specific features of lipid composition which are more or less the same in many animal cells. Since the whole cell lipids and the endoplasmic reticulum lipids are so similar both in BHK21 and in rat liver (Table V), the endoplasmic reticulum either contains a large fraction of the cellular lipids, or it resembles other cellular organelles in its lipid composition. In rat liver the nuclear membranes[50], the outer mitochondrial membranes[51,52], the rough[42], and the smooth endoplasmic reticulum[42] do indeed resemble each other in the lipid composition. Also the Golgi complex is fairly similar[53]. By contrast the inner mitochondrial membrane has a lipid composition which is different from both the plasma membrane and the endoplasmic reticulum type of membranes[51-55].

There is then evidence which indicates the presence of at least three different

* The fraction of dissimilar lipids in two membrane preparations, both containing the same number of phospholipid molecules, is obtained as follows: The smaller number of cholesterol molecules is subtracted from the larger one, and the smaller number of phosphatidylcholine molecules from the larger one, *etc.* The sum of these differences is divided by the sum of all molecules in the two sets of lipids.

membrane classes in animal cells. We can think of three possible causes for the differences in lipid composition between these membranes: First, differences in the composition of membrane proteins with selective lipid binding properties; secondly, differences in lipid metabolizing enzymes, and/or availability of lipid precursors; and thirdly, differences in lipid transfer between the organelle and other parts of the cell and the external medium. It is clear that further study of these three possibilities will be rewarding to membranology in general, and possibly, to neoplasma studies in particular since BERGELSON *et al.*[56] have reported that in hepatoma cells the differences in the lipid composition of plasma membrane, endoplasmic reticulum and mitochondria found in normal liver cells no longer pertain.

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