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THE LIPID COMPOSITION OF RAT-LIVER PLASMA MEMBRANES

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SUMMARY

1. Plasma membranes were prepared from rat-liver cells by the method of NEVILLE[3].

2. The lipid composition of the membranes was compared with that of the mitochondria. Sphingomyelin was a major component (33 % of the total phospholipid) of the plasma membranes but was not detected in the mitochondria, whereas cardiolipin, which accounted for 10 % of the mitochondrial phospholipids, was not found in the plasma membranes.

3. The molar ratio of cholesterol to phospholipid in the plasma membrane (0.6) was much higher than that in the mitochondria (0.1).

4. Glycosphingolipids (ceramide monohexoside, dihexoside, trihexoside and gangliosides) were identified as minor components of the plasma membrane (approx. 7 % of the total lipid) but were not detected in the mitochondria.

INTRODUCTION

The boundaries of mammalian cells and cell organelles consist mainly of lipids and proteins which are intimately associated in such a way that they form a physical lipoprotein membrane. One of the most important factors in the relationship of a mammalian cell to its surrounding environment is the stability and structural integrity of this plasma membrane.

At the present time interpretations of the ultrastructure of biological membrane systems are in a state of flux[1] and it seems that electron-microscopic evidence of structure that is often obtained[2] may have been misinterpreted. It is beyond doubt however that lipids and especially phospholipids and cholesterol are essential components in the molecular organisation of mammalian cell membranes. A knowledge of the lipid compositions of different plasma membranes and of possible ways in which a particular composition can affect the stability, molecular organisation and normal function of the membrane is of considerable value in the understanding of membrane behaviour.

Rat-liver plasma membranes have been prepared by several groups of workers[3,4–7] and SKIPSKI *et al.*[6] and TAKEUCHI AND TERAYAMA[7] have made detailed studies of the lipid composition. In conjunction with studies being carried out on the action of phospholipases on the plasma membrane of rat-liver cells[8] a quantitative study was made of the lipid composition of the plasma membranes. The results of this work

which include a comparison with the lipid composition of the mitochondrial membranes of the rat liver are presented in this paper.

METHODS AND RESULTS

Phosphorus was estimated by the methods of BARTLETT[9] and GRAY AND MACFARLANE[10] and protein by the method of LOWRY *et al.*[11].

Free cholesterol was estimated by the following procedure. A sample of the total lipids (2–3 μg P) was applied to a micro glass plate (8.2 cm × 3.5 cm) coated with a thin layer (0.3 mm) of silica gel H. The neutral lipids were separated with light petroleum (b.p. 40–60°)–diethyl ether–acetic acid (70:30:1, v/v/v) solvent and made visible by spraying the plate with 2,4-dichlorofluorescein (0.1% in ethanol). A cholesterol standard was chromatographed at the same time. The area of silicic acid containing the cholesterol in the lipid sample was removed from the plate and transferred to a small glass column. The cholesterol was eluted with chloroform–methanol (2:1, v/v), the eluate was evaporated to dryness and redissolved in a known volume of chloroform. Samples of the solution were analysed by gas chromatography on a column of 1% QF1 on Gas Chrom P (80–100 mesh) at 245°. A quantitative estimation was obtained by including a known amount of cholesterol acetate–cholesterol mixture (2:1, by wt.) in the samples as an internal standard and measuring the areas of the two peaks obtained on the chromatogram chart. Detector (flame ionisation) response to various ratios of cholesterol acetate and cholesterol was checked for linearity. The method was suitable to estimate 1–5 μg of cholesterol.

The phospholipids and glycolipids in the lipid samples were separated by thin-layer chromatography as described by GRAY[12,13]. Individual lipids were identified by comparison with standard lipid markers. Additional evidence of identity of the various phospholipids was obtained by chromatography at 2° on silicic acid-impregnated paper with a solvent of diisobutyl ketone–acetic acid–water (40:20:3, v/v/v)[14]. A quantitative estimation of individual phospholipids in a sample was obtained (a) by measuring, after chromatographic separation[15], the amounts of water-soluble phosphate esters obtained by deacylation of the lipids with mild alkali[16] and (b) by measuring the phosphorus content of individual lipids separated by thin-layer chromatography.

Isolation of plasma membranes from rat liver

The livers of adult hooded rats (male or female) were removed immediately after death and plasma membranes prepared by the method of NEVILLE[3]. The final stage of the isolation of the plasma membranes which entailed density-gradient centrifugation, was modified as follows: and extra sucrose layer of density 1.18 was introduced between sucrose layers of density 1.22 and density 1.16 to ensure complete separation of contaminating mitochondria[5]. A preparation, fixed in 1% osmic acid for 1 h, embedded in araldite and stained with uranyl acetate–potassium permanganate, was examined by electron microscopy which showed it to be essentially free of nuclear and mitochondrial contamination (see Fig. 1). 4–6 preparations were combined (yield average 16 mg) and freeze-dried and used for analysis of lipid composition.

Rat-liver mitochondria were prepared by the method of HOGEBOOM[17].

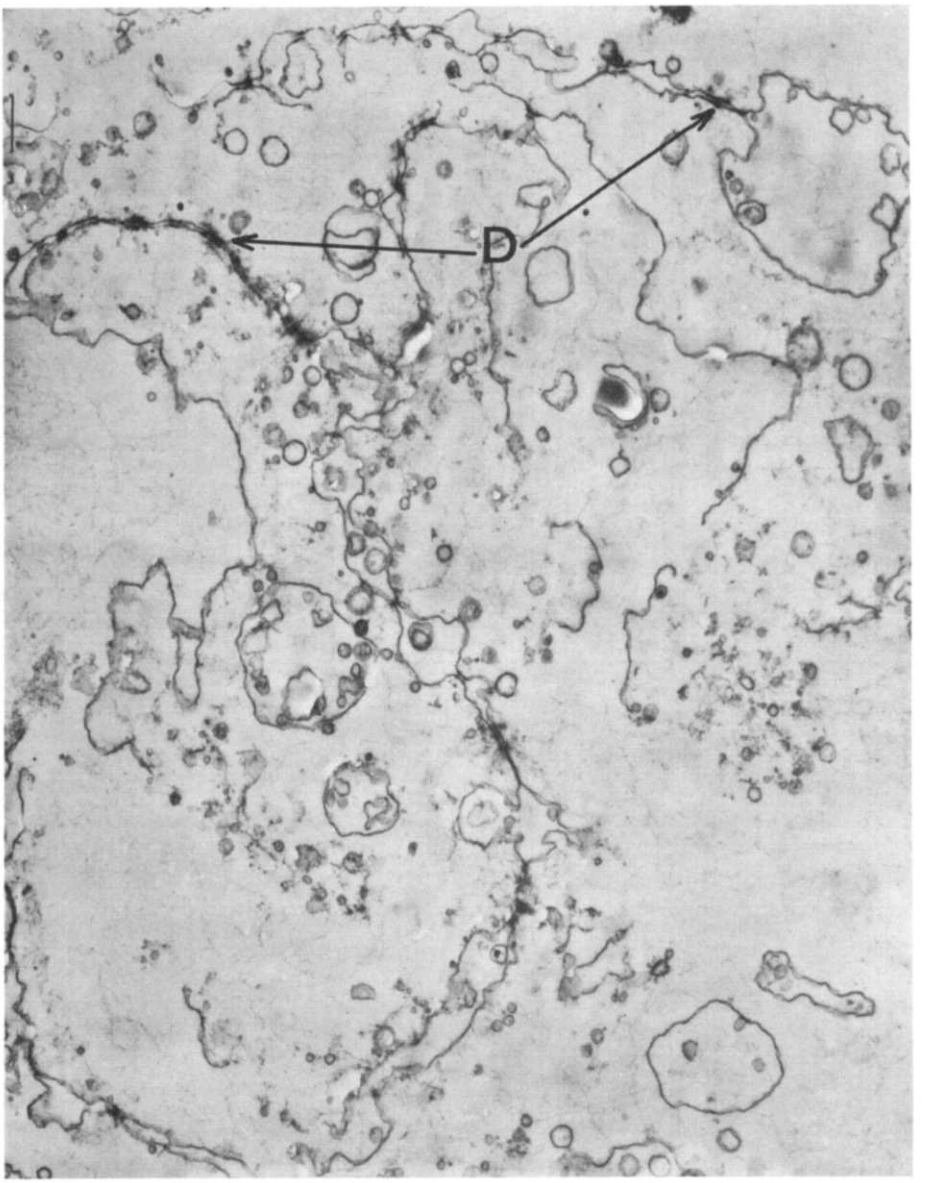

Fig. 1. Electron micrograph of isolated rat-liver plasma membrane (× 11000). Field contains several desmosomes (D) and small and large vesicles.

Enzyme estimations

The activities of the following enzymes were measured: 5′-nucleotidase (EC 3.1.3.5) (EMMELOT *et al.*[5]); succinate dehydrogenase (EC 1.3.99.1) (DE DUVE *et al.*[18]); glucose-6-phosphatase (EC 3.1.3.9) (SWANSEN[19]); leucyl-β-naphthylamidase (EC 3.4.1.1) (GOLDBERG AND RUTENBERG[20]).

Extraction of membrane lipids

The freeze-dried plasma membranes (34.2 mg containing 57 % protein) were extracted with 10 volumes of chloroform–methanol (1:1, v/v) and twice with 10 volumes of chloroform–methanol (2:1, v/v). The combined extracts were washed with 0.2 volumes of 0.1 M KCl (ref. 21). The chloroform solution was evaporated to dryness under vacuum at room temperature and the lipid residue weighed. The lipid accounted for 34 % of the dry weight of the plasma membrane. The lipids of rat-liver mitochondria were extracted by the same procedure (59 mg dry weight of mitochondria contained 22.5 % lipid).

The total lipid extract (9.23 mg, 242 μg P) from a sample of plasma membrane was loaded on a small column of silica gel H (1 g) in chloroform. Chromatography was carried out as described by GRAY[12]. The neutral lipids accounted for 34 % of the total lipid by weight (3.14 mg). The acidic phospholipid fraction (phosphatidylserine, phosphatidylinositol, cardiolipin, *etc.*) (24.0 μg P), phosphatidylethanolamine (23.6 μg P) and phosphatidylcholine and sphingomyelin (179.5 μg P) accounted for 94 % of the phosphorus applied to the column. The phospholipids in a lipid extract from the mitochondrial membranes were separated by a similar procedure. The major lipid in the neutral lipid fraction from the plasma membranes was cholesterol. Free fatty

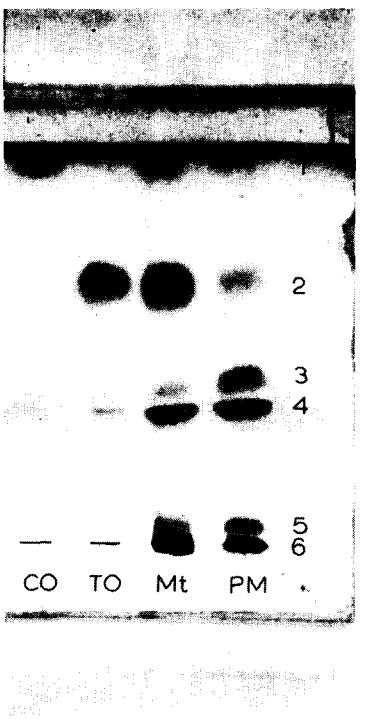

Fig. 2. The neutral lipids of rat-liver plasma membranes and rat-liver mitochondria. Thin-layer chromatography on silica gel H (0.3 mm thick); solvent, light petroleum–diethyl ether–acetic acid (70:30:1, v/v/v). Identification: CO, cholesterol oleate; TO, triolein; Mt, mitochondrial lipids; PM, plasma-membrane lipids; 1, cholesterol esters; 2, triglycerides; 3, free fatty acids; 4, cholesterol; 5, unknown lipid; 6, phospholipids.

acids, cholesterol esters, triglycerides and unidentified slow running components were present in smaller amounts (Fig. 2).

The acidic phospholipid fraction was evaporated to dryness and redissolved in 1 ml of chloroform. The phospholipids present in a sample of the fraction were separated and estimated by quantitative two-dimensional thin-layer chromatography[13]. The phospholipids in the remainder of the fraction were deacylated with mild alkali and the alkali-stable lipids were recovered from the reaction mixture. A sample of the lipid was analysed by thin-layer chromatography on silica gel H in chloroform–methanol–water (65:25:4, v/v/v) solvent and several components were identified as glycolipids by comparison with authentic standards (Fig. 3). Ceramide monohexoside (the major glycolipid component), dihexoside, trihexoside, a trace of aminoglycolipid and at least one ganglioside component were identified. No glycolipids were detected in a total lipid extract from mitochondria.

The composition of the lipid extracts from both the plasma membranes and the mitochondrial membranes were compiled from results obtained on several different membrane preparations (Table I).

EMMELOT *et al.*[5] and COLEMAN AND FINEAN[22] measured the activities of certain enzymes in their membrane preparations to assess the degree of contamination by

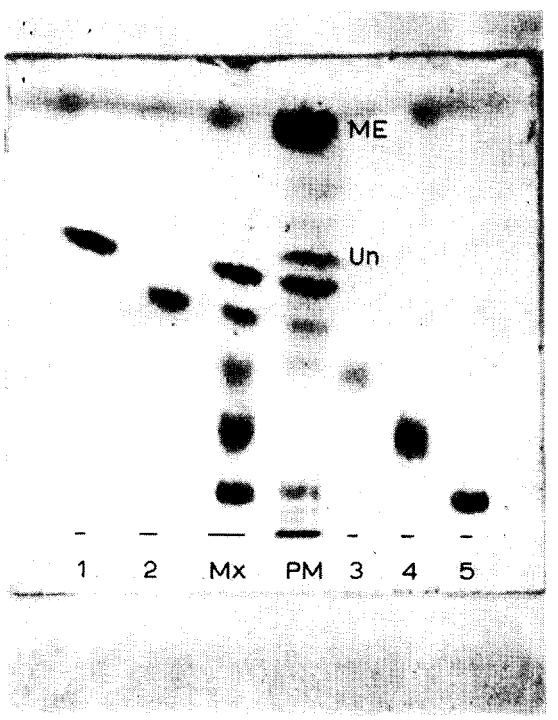

Fig. 3. Glycosphingolipids present in rat-liver plasma membranes. Thin-layer chromatography on silica gel H (0.3 mm thick); solvent, chloroform–methanol–water (65:25:4, v/v/v). Identification, reference markers: 1, ceramide monohexoside; 2, ceramide dihexoside; 3, ceramide trihexoside; 4, aminoglycolipid; 5, ganglioside (ceramide–glucose–galactose–sialic acid, 1:1:2:1, from horse spleen); Mx, mixture of 1–5; PM, alkali-stable lipid fraction from plasma membranes; ME, methyl esters of fatty acids from phospholipids; Un, unknown lipid.

other cellular fragments. This procedure was followed in the present study (Table II) The results indicated that the preparations were free from mitochondria but were contaminated to some extent with microsomal fragments.

TABLE I

THE LIPID COMPOSITION OF RAT-LIVER PLASMA MEMBRANES AND RAT-LIVER MITOCHONDRIA

Lipids	*Plasma membranes*		*Mitochondria*	
	% of total lipids	*Phospholipids as % of total phospholipids*	*% of total lipids*	*Phospholipids as % of total phospholipids*
Cholesterol	17		4	
Free fatty acids Cholesterol esters Triglycerides Unidentified	17		15	
Phosphatidylethanolamine		11		23
Phosphatidylserine		6		10
Phosphatidylinositol		6		9
Phosphatidylcholine	59	41	80	49
Cardiolipin				10
Sphingomyelin		33		—
Ceramide trihexoside Ceramide dihexoside Ceramide monohexoside Gangliosides	7*		—	

* Glycolipid = (total lipid − neutral lipid) − phospholipid (phosphorus value × 25).

TABLE II

SPECIFIC ACTIVITIES OF ENZYMES IN PLASMA MEMBRANE PREPARATIONS AS COMPARED WITH THE LIVER HOMOGENATES

Specific activity is expressed as μmoles/mg protein per hour at 38° (ref. 22).

Enzyme	*Plasma membrane fraction (A)*	*Liver homogenate (B)*	*A/B*
5′-Nucleotidase	13.5	0.48	28.2
	20	1.16	17
	44.5	1.55	28.8
Leucyl-β-naphthylamidase	9.75	0.93	10.2
	10.3	0.94	11
Glucose-6-phosphatase	0.72	0.78	0.93
	0.81	0.63	1.28
	0.75	0.75	1
Succinate dehydrogenase	0	272	0
	10.1	500	0.02
	0	268	0

DISCUSSION

There were considerable differences in the lipid compositions of rat-liver plasma membranes and mitochondrial membranes. Phospholipids accounted for 59 % of the total lipid in the plasma membrane and 80 % of the total lipid in the mitochondria. Cardiolipin, which accounted for 10 % of the phospholipids in mitochondria, and phosphatidylglycerol, present in small amounts in mitochondria[15] were not detected in the plasma membrane lipids. Phosphatidylcholine was the major phospholipid in both the plasma and the mitochondrial membranes but phosphatidylethanolamine and phosphatidylserine occurred to a lesser extent in the plasma membrane. Sphingomyelin was not detected in the mitochondria but was a major component (33 %) of the plasma membrane phospholipids. The mitochondrial membrane prepared by the method of HOGEBOOM is a mixture of internal and external membranes whose lipid compositions are different[23] and the values in Table I represent a 'mean' of the two membranes. Even so these values are representative of both membranes with respect to the major differences in the lipid compositions of the mitochondrial and plasma membranes.

The cholesterol content of the plasma membrane was much higher than that of the mitochondria (Table I). The molar ratios of cholesterol to phospholipid for the plasma membrane was 0.6 and for the mitochondria 0.1. COLEMAN AND FINEAN[22] calculated that the cholesterol to phospholipid ratio of guinea-pig liver plasma membranes should be between 0.6 and 0.7. Values obtained from the published data on rat-liver plasma membranes vary through 0.26 (ref. 24), 0.4 (refs. 4, 7), 0.7 (ref. 6) to 0.8 (ref. 5). The plasma membranes of other tissues [24,25] also show higher ratios of cholesterol to phospholipid than do the corresponding mitochondria. The liver-cell plasma membranes contained proportionally larger amounts of free fatty acids than the mitochondria but far small amounts of triglyceride and cholesterol esters (Fig. 2).

Ceramide monohexoside, dihexoside, trihexoside and a small ganglioside were present as minor components of the plasma membranes but none were found in the mitochondria. We believe that this is the first direct evidence of the location of glycosphingolipids in the mammalian cell. They are lipid haptens[26] and they may contribute to the surface properties of the cell. It is reasonable to suppose that they are exclusive to the plasma membrane but this cannot be confirmed until pure membrane fractions of the cell endoplasmic reticulum have been examined.

Electron micrographs of the plasma membrane fraction indicated that the preparation was mainly "membranous" and was not contaminated by mitochondria and nuclei. Desmosomal regions indicated the presence of actual plasma membranes. A proportion of the vesicles and membrane fragments could nevertheless arise from other subcellular organelles. The purity of plasma membrane fractions has been indirectly assessed by the assay of various enzymes known to be associated with particular cell fractions[5,22]. Our results suggest that lipid composition may also be a reasonable guide to purity. For instance, the absence of cardiolipin indicated that the preparation was not contaminated with significant amounts of mitochondrial membrane. The high cholesterol value and the high value (33 %) for sphingomyelin compared with those in mitochondria (0 %) and whole liver cells (7 %) are further indications of a high proportion of plasma membranes. The amount of glycosphingolipid in the preparation may prove to be an even better measurement of membrane

purity and the value of about 7 % of the total lipid found in the present study represents at least a 7-fold enrichment of the content (less than 1 %, unpublished results) of the lipids of whole liver.

Most of our results are in general agreement with those of SKIPSKI *et al.*[6] who, however, reported that they were unable to account for a significant amount (26 %) of the total membrane lipids and they suggest that it was probably glycosphingolipid. Most of the lipids in our preparations, including the glycosphingolipids, were consistently accounted for and we cannot offer an explanation for the findings of SKIPSKI *et al.* The results of TAKEUCHI AND TERAYAMA[7] on the lipid composition of rat-liver plasma membranes are so different from our results and from other published data that a comparison is valueless. Their results suggest that their preparation was considerably contaminated by other cell components.

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