A Comparative Study of the Molecular Structures of the Plasma Membranes and the Smooth and the Rough Endoplasmic-Reticulum Membranes from Rat Liver

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Plasma-membrane as well as smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions were isolated from the microsomal pellet of rat liver. The purity of these fractions, as determined by marker-enzyme activities, electron microscopy, cholesterol content and RNA content, was found to be adequate for a comparative structural study. Major differences in lipid and protein composition were found to exist between the plasma membrane and the endoplasmic reticulum, but not between the smooth and the rough fractions of the endoplasmic reticulum. Differences in the location of membrane protein thiol groups and the mobility of the membrane phospholipids were observed between the plasma membranes and the endoplasmic reticulum, and these could be explained by differences in protein and lipid composition. However, by employing fluorescence and spin-labelling techniques structural changes were also observed between the smooth and the rough endoplasmic-reticulum fractions. These results suggest that the structural heterogeneity existing between the two latter membrane fractions occurs near or on their membrane surfaces and is not due to the greater number of ribosomes bound to the rough endoplasmic-reticulum fraction.

The use of Ficoll barriers by Kamat & Wallach (1965) to prepare plasma-membrane vesicles from the microsomal pellet of Ehrlich ascites-carcinoma cells, and a report by Graham *et al.* (1968) that this procedure can be employed successfully with the liver, raised the possibility of isolating plasma membranes and the smooth and the rough endoplasmic-reticulum membranes from the same liver microsomal pellet. Thus a comparative structural study of the plasma-membrane and the smooth- and the rough-endoplasmic-reticulum-membrane fractions could be performed without the possibility of contamination of the endoplasmic-reticulum-membrane fractions with plasma-membrane vesicles. It is assumed that contamination by vesicles derived from the Golgi apparatus will be negligible, owing to the relatively small amount of this material found in the microsomal pellet (Fleischer & Fleischer, 1970).

It was found that employment of Ficoll gradients including a low concentration of Ca^{2+} (0.5 mM) resulted in an adequate preparation of the plasma-membrane vesicles. The resulting endoplasmic reticulum was then separated into rough, smooth and degranulated fractions by published procedures (Dallner *et al.*, 1963; Williams *et al.*, 1968).

The protein and lipid compositions of the four fractions were compared by gel electrophoresis and t.l.c. The structure of the membranes of the different fractions was monitored by the binding of the fluorescent dye 8-anilidonaphthalene-1-sulphonic acid and

by e.s.r.* spin-labelling techniques employing protein and lipid spin-labels (McConnell & McFarland, 1970).

Materials and Methods

Preparation of membrane fractions

The livers of four male Sprague-Dawley rats (175–200 g) were removed, minced and homogenized in a Teflon-glass homogenizer (0.17 mm clearance) with 250 ml of 0.25 M-sucrose–0.2 mM-MgSO₄–5 mM-tris-HCl buffer, pH 7.5. The homogenate was centrifuged for 10 min at 600 g to remove the nuclei and cell debris. Two centrifugations at 6000 and 10000 g were performed to remove the mitochondria and lysosomes. The mitochondria-free supernatant was then centrifuged for 1 h at 200000 g; the pellet was homogenized in 10 mM-tris-HCl buffer, pH 7.5, and centrifuged at the same speed for 45 min. A third wash was performed in 0.5 mM-CaCl₂–1 mM-tris-HCl buffer, pH 7.5. The pellet was then homogenized in 20 ml of 0.5 mM-CaCl₂–1 mM-tris-HCl buffer, pH 7.5, layered on a 10–20% (w/v) Ficoll gradient (prepared with 0.5 mM-CaCl₂–1 mM-tris-HCl buffer, pH 7.5), and centrifuged overnight at 205000 g in an SW40 rotor. A white layer appeared at the top of the gradient, and this was taken as the plasma-membrane fraction. The fraction from the 51–20% region of the gradient, yellow in colour, was collected as the endoplasmic reticulum. Both fractions were then washed with 1 mM-tris-HCl buffer, pH 7.5, to remove the Ficoll.

* Abbreviations: e.s.r., electron spin resonance; ATPase, adenosine triphosphatase.

The smooth and the rough endoplasmic reticulum were prepared by employing a 1.3M-sucrose barrier, by the method of Dallner *et al.* (1963). Finally the degranulation of the rough fraction was performed by dialysis against EDTA followed by a sucrose barrier as described by Williams *et al.* (1968). The microsomal pellet appeared to be composed of 60–70% rough membranes, 10–15% smooth membranes and 20–25% plasma membranes.

Enzyme assays

5'-Nucleotidase. This assay was performed as described by Emmelot *et al.* (1964). Membrane protein concentrations of 100–200 µg/ml and an incubation time at 37°C of 10 min were employed.

Na⁺+K⁺-transport ATPase. This assay was performed as described by Emmelot *et al.* (1964) except that the buffer employed to measure total ATPase activity was 66 mM-NaCl–33 mM-KCl–5 mM-MgCl₂–10 mM-ATP–25 mM-tris–HCl buffer, pH 7.4, and that for the Mg²⁺-activated ATPase was 5 mM-MgCl₂–10 mM-ATP–25 mM-tris–HCl buffer, pH 7.4.

NADPH-cytochrome *c* reductase, succinate-cytochrome *c* reductase and *p*-nitrophenyl phosphatase. The cytochrome *c* reductase assays were performed as described by Sottocosa *et al.* (1967) and the phosphatase assay as described by Ray (1970).

Electron microscopy

Samples were fixed in glutaraldehyde, postfixed with OsO₄, dehydrated by an ethanol series, embedded in Epon, and the grids stained with uranyl acetate and lead citrate as described by Reynolds (1963). Micrographs were obtained with a Hitachi 12D electron microscope.

Determination of lipid, RNA and protein

Protein concentrations were assayed by the method of Lowry *et al.* (1951) and cholesterol concentrations by the method of Abell *et al.* (1952). The RNA content was determined by employing the orcinol reagent (Schneider, 1957). Phosphorus was determined by the method of Bartlett (1959).

The phospholipids were extracted by the method of Folch *et al.* (1957) and separated by one-dimensional t.l.c. on silica gel G as described by Skipski *et al.* (1962). The quantitative measurement of the spots was performed as described by Kahovcava & Odavic (1969).

The membrane proteins were analysed on 1% sodium dodecyl sulphate–polyacrylamide gels as described by Lenard (1970) except that 150–200 µg of membrane protein was applied/gel, the run was at 6 mA/gel for 3–4 h and the gel staining and destaining system of Hinman & Phillips (1970) was employed.

Fluorescence studies

A Turner Fluorometer III equipped with a 460 nm emission filter and a 360 nm excitation filter was employed to determine the binding of 8-anilino-naphthalene-1-sulphonate to the different membrane fractions in 0.5 mM-CaCl₂–1 mM-tris–HCl buffer, pH 7.5. The dye concentration was calculated from an absorption coefficient of $4.95 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 350 nm (Weber & Young, 1964).

Spin-labelling studies

The *N*-ethylmaleimide spin-label was bought from Syva Associates (Palo Alto, Calif., U.S.A.). The fatty acid labels employed were based on stearic acid:

and were the 7-nitroxide ($n = 10$, $m = 5$) and the 5-nitroxide ($n = 12$, $m = 3$). The preparation of these labels has already been described (Simpkins *et al.*, 1971b) except that the 5-oxo acid was prepared as described by Jost *et al.* (1971). The labelling of the membranes with the protein and fatty acid labels was performed as described by Simpkins *et al.* (1971b) except that the buffer employed was 0.5 mM-CaCl₂–1 mM-tris–HCl buffer, pH 7.5, and the washing was performed by centrifugation of the membrane fractions at 150 000g.

Results

The yield of plasma membranes (approx. 0.5 mg of membrane protein/g of liver tissue) is comparable with that reported for membranes prepared by Dounce homogenization (Emmelot *et al.*, 1964). Ca²⁺ was included in the last wash of the microsomal pellet and in the Ficoll gradient, as it produced a better membrane separation and thus a correspondingly greater yield of membranes. This is also true with membranes prepared by Dounce homogenization (Ray, 1970).

The plasma-membrane fraction has high specific activities of Na⁺+K⁺-transport ATPase, 5'-nucleotidase and *p*-nitrophenyl phosphatase, but a low NADPH-cytochrome *c* reductase activity (see Table 1). The Na⁺+K⁺-transport ATPase and 5'-nucleotidase activities compare well with those obtained with other plasma-membrane fractions obtained from rat liver by Dounce homogenization (Dod & Gray, 1968; Emmelot *et al.*, 1964; Graham *et al.*, 1968; Evans, 1970a). The microsomal NADPH-cytochrome *c* reductase activity was low in the plasma-membrane fraction but high in the smooth- and the rough-endoplasmic-reticulum-membrane

EXPLANATION OF PLATE 1

Electron micrographs of the plasma membranes (a), smooth endoplasmic reticulum (b) and rough endoplasmic reticulum (c) from rat liver

The samples were fixed and stained as described in the text. The final magnification was $\times 45000$.

EXPLANATION OF PLATE 2

Photographs of the polyacrylamide gels of the plasma-membrane (a), smooth-endoplasmic-reticulum-membrane (b) and rough-endoplasmic-reticulum-membrane (c) fractions from rat liver

Experimental details are given in the text.

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Table 1. *Enzyme activities of the plasma-membranes and the smooth- and the rough-endoplasmic-reticulum membranes from rat liver*

Experimental details are given in the Materials and Methods section. The specific activities are expressed as μmol of substrate hydrolysed/h per mg of membrane protein at 37°C , and are given as means $\pm$ s.d. with the numbers of determinations in parentheses. The values in brackets refer to the increase in specific activity relative to that found in the homogenate.

Membrane fraction	5'-Nucleotidase	$\text{K}^+ + \text{Na}^+ + \text{Mg}^{2+}$ -stimulated ATPase	$\text{K}^+ + \text{Na}^+$ -transport ATPase	NADPH-cytochrome <i>c</i> reductase	<i>p</i> -Nitrophenyl phosphatase	Succinate-cytochrome <i>c</i> reductase
Liver homogenate	1.5 ± 0.5 (3)	8.0 ± 0.5 (4)	0.7 ± 0.5 (4)	0.6 ± 0.1 (4)	0.05 ± 0.01 (4)	0.600 ± 0.050 (4)
Plasma membrane	28.0 ± 3.0 (3) [18]	35.0 ± 5.0 (4) [4]	12.0 ± 1.0 (4) [16]	0.6 ± 0.2 (6)	0.50 ± 0.10 (6) [10]	0.010 ± 0.005 (3) [0.02]
Smooth endoplasmic reticulum	9.0 ± 2.0 (3) [6]	6.0 ± 2.0 (4) [0.8]	0.0 ± 0.5 (4)	3.0 ± 1.0 (6) [5]	0.05 ± 0.01 (4)	0.005 ± 0.005 (3) [0.01]
Rough endoplasmic reticulum	3.0 ± 0.5 (3) [2]	5.0 ± 2.0 (4) [0.6]	0.0 ± 0.5 (4)	5.0 ± 1.0 (6) [8]	0.11 ± 0.05 (4) [2]	0.005 ± 0.005 (3) [0.01]

fractions, and its activity in the latter two fractions was comparable with the values reported by Dallman *et al.* (1969) and Van Tol (1970). All three membrane fractions possessed negligible mitochondrial contamination, as shown by very low succinate-cytochrome *c* reductase activities.

Electron micrographs of the three membrane preparations are presented in Plate 1. The plasma-membrane fraction is vesicular in appearance and appears similar to the light subfraction of plasma membranes prepared from Dounce homogenization (Evans, 1970a). The presence of ribosomes can be seen in the rough fraction as opposed to the smooth fraction of the endoplasmic reticulum (cf. Plate 1b and 1c). The latter two fractions are vesicular and appear similar to those presented by Dallman *et al.* (1969).

The amount of cholesterol present in the plasma-membrane fraction is very much higher than that found in the endoplasmic reticulum (Table 2). The absolute values for the plasma membranes are somewhat higher than those reported by Dod & Gray (1968) and a little lower than those reported by Emmelot *et al.* (1964). The RNA content of the plasma membranes was low when compared with that of the endoplasmic reticulum. The smooth fraction of the endoplasmic reticulum possessed less than did the rough fraction, but the RNA content of the latter fraction is low compared with those presented by other workers (Glaumann & Dallner, 1968; Evans & Gurd, 1971). This is probably due to the low-ionic-strength buffers that have to be employed to prepare the plasma-membrane vesicles.

The phospholipid composition of the three fractions (Table 3) appears not to differ greatly, although the endoplasmic reticulum possesses less sphingomyelin and more phosphatidylcholine or phosphatidylinositol than the plasma membrane. These results are in agreement with the values presented by Dod & Gray (1968) for plasma membranes and by Glaumann & Dallner (1968) for the endoplasmic reticulum.

The protein composition of the three fractions as shown by gel electrophoresis shows striking differences between the plasma membranes and the membrane fractions from the endoplasmic reticulum (Plate 2 and Fig. 1). However, there is little difference between the smooth and rough fractions of the endoplasmic reticulum. Similar patterns for these latter two fractions have been presented by Hinman & Phillips (1970). Schimke & Dehlinger (1971) and Evans (1970b) have also shown differences between the plasma and the endoplasmic-reticulum-membrane protein compositions, although their plasma-membrane specimens were prepared by Dounce homogenization.

The binding of the fluorochrome 8-anilinonaphthalene-1-sulphonate to the membrane shows some

Table 2. *Cholesterol, RNA and phosphorus contents of the plasma-membrane and the smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions from rat liver*

Experimental details are given in the Materials and Methods section. The values are expressed in terms of $\mu\text{g}/\text{mg}$ of membrane protein. Errors are given as means $\pm$ S.D., with the numbers of determinations in parentheses.

	Plasma membrane	Smooth endoplasmic reticulum	Rough endoplasmic reticulum	Degranulated endoplasmic reticulum
Cholesterol	100 $\pm$ 5 (4)	42 $\pm$ 6 (4)	23 $\pm$ 3 (4)	32 $\pm$ 4 (4)
RNA	20 $\pm$ 5 (8)	90 $\pm$ 15 (8)	170 $\pm$ 20 (8)	50 $\pm$ 5 (8)
Total phosphorus	26 $\pm$ 3 (6)	35 $\pm$ 5 (6)	35 $\pm$ 5 (6)	30 $\pm$ 5 (6)

Table 3. *Phospholipid compositions of the plasma-membrane and the smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions from rat liver*

Experimental details are given in the Materials and Methods section. The values are expressed as percentages of total phospholipid present in the extract, and are given as means (S.D. $\pm$ 3 %) of six determinations in each case.

Phospholipid	Plasma membrane	Smooth endoplasmic reticulum	Rough endoplasmic reticulum	Degranulated endoplasmic reticulum
Phosphatidylcholine	5	5	3	2
Sphingomyelin	20	11	4	7
Phosphatidylcholine + phosphatidylinositol	45	59	61	62
Phosphatidylserine	8	5	4	6
Phosphatidylethanolamine	22	20	28	23

Fig. 1. *Densitometer traces of the polyacrylamide gels presented in Plate 2*

Experimental details are given in the text. The apparent molecular weights were obtained by running gels with haemoglobin, bovine serum albumin and γ -globulin as marker proteins.

startling differences between the membrane fractions (Fig. 2). The plasma membrane and the smooth endoplasmic reticulum both appear to have a similar number of binding sites/mg of membrane protein (Fig. 2b), this being smaller than that observed with the rough endoplasmic reticulum. This difference is not due to the presence of ribosomes on the rough endoplasmic reticulum as their removal by EDTA results in a membrane possessing a similar number of binding sites (cf. rough endoplasmic reticulum and degranulated membranes; Figs. 2a and 2b).

E.s.r. studies with fatty acid spin-labels (which are assumed to diffuse into the membrane lipid regions) with the nitroxide probe positioned on the fifth and seventh methylene groups show some noteworthy differences between the four fractions.

Fig. 3 shows that the spectra obtained with membranes labelled with the 5-nitroxide stearic acid shows differences existing between the smooth and the rough endoplasmic reticulum. The mobility of the nitroxide when expressed in terms of the a/b ratio or in terms of the mean angular deviation of the probe about the axis of the hydrocarbon chains of the phospholipids (see Table 4) is increased in the rough endoplasmic reticulum. This means that the lipids are more fluid near the membrane surface in the rough endoplasmic reticulum than in the smooth. This difference is not

Fig. 2. Binding of 8-anilino-1-naphthalene-sulphonate to the plasma-membrane ($\blacktriangle$) and the smooth- ($\triangle$), rough ($\circ$) and degranulated-endoplasmic-reticulum-membrane ($\bullet$) fractions from rat liver

Experimental details are given in the text. (a) Binding at increasing concentrations of dye but constant membrane protein concentration ($50\mu\text{g/ml}$); (b) binding with increasing concentration of membrane protein but constant dye concentration ($20\mu\text{M}$). The fluorescence intensity appears to be proportional to the membrane protein concentration (b).

due to the presence of ribosomes on the membrane, as their removal results in the membrane lipid regions decreasing only slightly in mobility (see Table 4).

The 7-nitroxide stearic acid, where the nitroxide label is situated 0.2–0.3 nm further away from its charged carbonyl group (and can thus be assumed to reflect the lipid structure deeper in the membrane core than the 5-nitroxide stearic acid), does not exhibit the same behaviour.

This probe (Fig. 4) shows that a difference in lipid structure becomes apparent between the plasma

Fig. 3. E.s.r. spectra obtained by labelling the plasma-membrane and the smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions from rat liver with the 5-nitroxide stearic acid spin-label

Experimental details are given in the text. (a) Spectra obtained with the plasma membrane (—) and the smooth endoplasmic reticulum (---); (b) spectra obtained with the rough (—) and the degranulated endoplasmic reticulum (---).

membrane and the smooth endoplasmic reticulum and that very little difference is seen between the smooth, rough and degranulated fractions of the endoplasmic reticulum. Removal of the ribosomes results in a small decrease in lipid fluidity similar to that shown with the 5-nitroxide label.

The *N*-ethylmaleimide spin-label, which is mainly specific for protein thiol groups (Simpkins *et al.*, 1971a) and can thus be used as a probe of membrane protein structure, shows a drastic change in the spectrum obtained with the plasma membrane as

Table 4. *Mobilities of the 7-nitroxide and the 5-nitroxide fatty acid spin-labels in the plasma-membrane and the smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions from rat liver*

Experimental details are given in the text. Values for the splitting of the outer doublet ($2T_{11}$) and the deviation between the axis of the hydrocarbon chains and the nitroxide spin hamiltonian are presented and were calculated as described by Simpkins *et al.* (1971*b*). The a/b ratio was not calculated for the 7-nitroxide stearic acid-labelled membrane owing to the difficulty in resolving the b line. The values are given as means $\pm$ s.d.

Membrane fraction	5-Nitroxide stearate spin-label			7-Nitroxide stearate spin-label	
	a/b ratio	$2T_{11}$ (G)	Mean angular deviation between v and u	$2T_{11}$ (G)	Mean angular deviation between v and u
Plasma membrane	0.75 ± 0.05	27.9 ± 0.1	21.0 ± 0.5	26.9 ± 0.1	24.5 ± 0.5
Smooth endoplasmic reticulum	0.65 ± 0.05	27.7 ± 0.1	22.0 ± 0.5	25.7 ± 0.1	28.0 ± 0.5
Rough endoplasmic reticulum	0.55 ± 0.05	26.1 ± 0.1	26.5 ± 0.5	25.2 ± 0.1	29.5 ± 0.5
Degranulated endoplasmic reticulum	0.55 ± 0.05	26.7 ± 0.1	25.0 ± 0.5	25.5 ± 0.1	28.5 ± 0.5

compared with those obtained with membrane fractions of the endoplasmic reticulum (Fig. 5). The change in the a/b ratio (see Fig. 5) can be explained by the existence of more exposed thiol residues on the plasma membrane than on the endoplasmic reticulum. The spectrum obtained with these plasma membranes agrees well with that observed with other plasma membranes such as the erythrocyte 'ghost', and those obtained with the endoplasmic-reticulum-membrane fractions (which show an apparently smaller percentage of mobile thiol groups) are similar to that obtained with rat liver mitochondrial membrane fragments (H. Simpkins, unpublished work).

Discussion

It appears reasonable to assume from the marker-enzyme activities, electron micrographs and the RNA and cholesterol compositional data that plasma-membrane and smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions of adequate purity to perform a comparative structural study were prepared from the microsomal pellet of the rat liver. It must, however, be remembered that the so-called smooth fraction has been shown to contain elements of the Golgi apparatus (Fleischer & Fleischer, 1970), although the amount appeared to be relatively small (~10%). A preparation of the rough endoplasmic reticulum containing a greater number of attached ribosomes can be obtained by employing buffers of higher osmotic and ionic strength.

The compositional and enzymic data generally compare well with results obtained by other workers.

Thus the plasma-membrane fraction contained a greater amount of sphingomyelin, a decreased amount of phosphatidylinositol and phosphatidylcholine and a relatively large amount of cholesterol. The endoplasmic reticulum and the plasma membrane also differed markedly in protein composition. Thus the change observed in the number of exposed thiol residues between the smooth endoplasmic reticulum and the plasma membrane can be explained by their respective protein compositions. Similarly the decrease in mobility observed between the smooth-endoplasmic-reticulum and the plasma-membrane lipid regions as shown by the 7-nitroxide spin-label spectrum may be explained by the condensing effect of cholesterol, which is known to decrease the mobility of the hydrocarbon chains of phospholipid films (Hsia *et al.*, 1971).

The results that are difficult to explain and that to some extent complement each other are those obtained by studying the binding of the dye 8-anilino-naphthalene-1-sulphonate to the membrane and those obtained by spin-labelling the membrane with the 5-nitroxide stearic acid label. Both techniques show a large difference between the smooth and the rough endoplasmic reticulum and very little difference between the smooth endoplasmic reticulum and the plasma membrane.

The binding of 8-anilino-naphthalene-1-sulphonate is difficult to interpret; Chance (1970) has suggested that the fluorochrome binds at the solvent/membrane interface and that changes observed in the fluorescence of this dye reflect an alteration near or on the surface of the membrane. This idea is to some extent compatible with our results, which show similar fluorescence intensities produced by both the smooth-

Fig. 4. *E.s.r.* spectra obtained by labelling the plasma-membrane and the smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions from rat liver with the 7-nitroxide stearic acid spin-label

Experimental details are given in the text. (a) Spectra obtained with the plasma membrane (—) and the smooth endoplasmic reticulum (----); (b) spectra obtained with rough (—) and the degranulated endoplasmic reticulum (----).

endoplasmic-reticulum and the plasma-membrane fractions. It is possible therefore that, although the plasma-membrane and smooth-endoplasmic-reticulum-membrane fractions consist of different proteins (and thus possess different numbers of exposed thiol groups) and the plasma membrane contains far more cholesterol than does the smooth membrane, little change may occur across the overall membrane surface. The absence and the presence of cholesterol may not affect the binding of the fluorochrome, because cholesterol is generally assumed to be situated in the phospholipid core (Chapman & Wallach, 1968). Thus it appears that an alteration in the structure of the membrane surface occurs between the smooth and the rough membrane fractions and that this difference is not due to a change in ribosome content, as degranulation of the rough

Fig. 5. *E.s.r.* spectra obtained by labelling the plasma-membrane and the smooth-, rough- and degranulated-endoplasmic-reticulum-membrane fractions from rat liver with a spin-label based on *N*-ethylmaleide

Experimental details are given in the text. (a) Spectra obtained with the plasma membrane (—) and the smooth endoplasmic reticulum (----); (b) spectra obtained with the rough (—) and the degranulated endoplasmic reticulum (----).

endoplasmic reticulum results in little change in the amount of 8-anilinonaphthalene-1-sulphonate binding to the membrane. The results appear contrary to those of Dallner & Azzi (1972), who reported an increased fluorescence of this dye in the smooth membrane. However, these authors did not employ buffers containing Ca^{2+} ions to isolate their membranes and to perform their fluorescence measurements. They also found that the fluorescence intensity of their smooth-membrane preparations decreased with increased bivalent cation concentration. We have found, by employing high-ionic-strength buffers (0.25 M-sucrose–25 mM-KCl–10 mM-MgCl₂–50 mM-tris–HCl buffer, pH 7.5), that the fluorescence of the two membrane fractions became virtually identical and sometimes was greater in the case of the smooth-membrane fraction. We have also found that ethidium bromide cannot be used as a probe of membrane

structure as its fluorescence is greatly affected by the presence of ribosomes on the membrane surface (H. Simpkins, unpublished work). This is expected from the results of LePecq & Paoletti (1967), who showed that the dye's fluorescence is strongly enhanced by the presence of RNA helices.

The idea of structural heterogeneity existing between the rough- and the smooth-endoplasmic-reticulum-membrane fractions is further strengthened by e.s.r. spin-labelling data employing the 5-nitroxide stearic acid probe. This is not apparent with the 7-nitroxide where the nitroxide label is located further in the membrane core.

In conclusion, the results show that the smooth and the rough endoplasmic reticulum, although possessing very similar protein and lipid constituents, possess different lipid structures near or at the membrane/solvent interface. These results may explain why Dallner and co-workers find the addition of Cs⁺ aids the separation of the smooth and the rough endoplasmic reticulum by aggregating solely the rough fraction (Glaumann & Dallner, 1968). It also has been shown (Shires *et al.*, 1971) that ribosomes require different conditions to reattach to the smooth and to the rough endoplasmic reticulum. Both observations suggest that the surface characteristics of the two membrane fractions are different. It should also be emphasized that the smooth- and the rough-endoplasmic-reticulum-membrane fractions described in the present paper are prepared by arbitrary fractionation procedures. It has been demonstrated that microsomal preparations consist of a continuous series of entities ranging from those that are ribosome-free to those vesicles heavily coated with ribosomes (Wibo *et al.*, 1971).

Our results also show that those structural differences seen between the lipid regions of the plasma membrane and the endoplasmic reticulum can be explained by the absence or the presence of cholesterol, and that the difference in the number of exposed thiol groups may solely reflect the obvious difference in protein composition as shown by the polyacrylamide-gel-electrophoresis patterns. The removal of ribosomes from the rough endoplasmic reticulum appears to result in the membrane phospholipid structure becoming somewhat less fluid and in little change in the number of exposed membrane thiol groups. However, it must also be remembered that removal of ribosomes by EDTA treatment may change the membrane structure in an ill-defined manner.

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