Chemical Characterization of the Proteins and Glycoproteins of Mouse Liver Plasma Membranes Solubilized by Sequential Extraction with Aqueous and Organic Solvents

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(Received 13 August 1971)

1. A procedure for the stepwise fractionation of the proteins of mouse liver plasma membranes is described. 2. Of the membrane protein 20–25% was soluble in 50mM-sodium carbonate–bicarbonate buffer (pH 9.7). This fraction contained a large number of proteins but only 1 major glycoprotein. It was low in sialic acid, amino sugars and phospholipid. 3. Extraction of the alkali-insoluble residue with aq. 33% pyridine solubilized an additional 30–35% of the membrane protein. The pyridine-soluble membrane components were enriched in sialic acid and glucosamine and it was shown that this procedure resulted in the selective extraction of glycoproteins. 4. Gel filtration in sodium dodecyl sulphate resolved the pyridine-soluble proteins into five fractions of decreasing molecular weight and an inverse relationship between molecular weight and sialic acid content was indicated.

The surface (plasma) membrane of mammalian cells is composed of proteins and lipids with a small amount of carbohydrate, in the form of glycoproteins and glycolipids. Detailed chemical analysis of these membrane components has, with the exception of the erythrocyte, been largely prevented by the unavailability of sufficient quantities of purified membrane fractions. However, the recent development of a variety of methods for the isolation and purification of liver plasma membranes (Neville, 1960; Emmelot *et al.*, 1964; Touster *et al.*, 1970) and particularly the introduction of zonal-centrifugation techniques which facilitate large-scale preparations (Hinton *et al.*, 1970; Anderson *et al.*, 1968; Weaver & Boyle, 1969; Evans, 1970a) has enabled fractionation and analysis of this membrane to be undertaken. Lipids of the liver plasma membrane have been extensively studied and several differences between the lipid composition of cell surface and intracellular membranes demonstrated (Patton, 1970; Dod & Gray, 1968; Ray *et al.*, 1969; Keenan & Morré, 1970). Membrane proteins are largely insoluble under conventional aqueous conditions and this continues to hinder the isolation and characterization of these membrane components. Polyacrylamide-gel electrophoresis, however, has demonstrated the heterogeneity of membrane proteins (Lenard, 1970; Nachman & Ferris, 1970; Schnaitman, 1969; Kiehn & Holland, 1968) and has revealed differences between the protein composition of liver surface and intracellular membranes (Zahler *et al.*, 1970; Evans, 1970a,b). Elucidation of the significance of these structural differences to the function of the various membranes is dependent on the development of

methods for the resolution of membranes into simplified fractions so that the chemical, physical and biological properties of individual components can be studied.

The present paper describes a procedure for the stepwise fractionation of mouse liver plasma membranes by extraction with aqueous pyridine (Blumenfeld, 1968) after preliminary extraction in EDTA–carbonate buffer at pH 9.7. Because of the implication of carbohydrates in a variety of membrane functions (Curtis, 1967; Cook, 1968) particular attention has been paid to the distribution of glycoproteins in the various fractions obtained. A preliminary report of this work has appeared (Gurd *et al.*, 1971).

Experimental

Preparation and fractionation of plasma membranes

Parkes white mice weighing 25–30g were used for the preparation of liver plasma membranes by rate zonal centrifugation as described by Evans (1970a). Membrane fractions obtained from the zonal rotor were pooled, diluted with water and centrifuged at 100000g for 30min. The membrane pellet was re-suspended in 8% sucrose–5mM-tris–HCl buffer, pH 7.6, with 20–25 strokes of a tight-fitting Dounce homogenizer (stated radial clearance 0.003in), layered over a cushion of 47% (w/v) sucrose and centrifuged in a Beckman SW 27 rotor at 100000g for 30min. The membranes, which formed a sharp band at the 47%–8% sucrose interface, were removed with a Pasteur pipette. A pellet which formed at

the bottom of the tube was discarded. The plasma membranes (which represented a 25–30-fold purification of 5'-nucleotidase) were then washed five times with ice-cold 0.9% NaCl – 5 mM-tris – HCl buffer, pH 7.6, by centrifugation at 100000g for 30 min.

The washed membranes were suspended (1–2 mg of protein/ml) in 50 mM- Na_2CO_3 – NaHCO_3 buffer, pH 10.2, containing 10 mM-EDTA (final pH of the membrane suspension, 9.6–9.8) and gently agitated for 60 min at 37°C. The suspension was centrifuged at 100000g for 30 min and the pellet was washed once with water (100000g, 30 min). The NaHCO_3 – Na_2CO_3 supernatant and water wash were combined and constituted the alkali-soluble fraction. The residue was resuspended in water and 0.5 vol. of ice-cold pyridine was added (final concentration of membrane, 3–4 mg of protein/ml). The membrane-pyridine mixture was homogenized with 10–15 strokes of the tight-fitting Dounce homogenizer and immediately centrifuged at 170000g for 60 min. The clear supernatant was carefully removed and constituted the pyridine-soluble fraction. The pyridine-soluble membrane components were subjected to gel chromatography on Sepharose-6B (Pharmacia, Uppsala, Sweden) as follows. Pyridine was removed by dialysis through 18/32 Visking tubing for 16–20 h against 5% (w/v) sodium dodecyl sulphate – 0.5 M-urea – 30 mM- β -mercaptoethanol. Dialysis was accompanied by a 4–5-fold decrease in the solution volume. After dialysis 40 mg of sodium dodecyl sulphate/ml was added and the solution was boiled for 4–5 min. A portion of this solution was used for gel filtration.

The fractionation procedure is summarized in Scheme 1.

Gel filtration

Sepharose-6B was suspended in the elution buffer (0.5% sodium dodecyl sulphate – 0.5 M-urea – 30 mM-tris – HCl buffer, pH 8.0) and fines were removed. Membrane samples, up to 60 mg, were placed on the column (100 cm $\times$ 0.9 cm) and were eluted at a flow rate of 6–8 ml/h and 2.5 ml fractions were collected.

Polyacrylamide-gel electrophoresis

This was performed with 7.5% (w/v) polyacrylamide gels contained in cylindrical glass tubes by the modification of the method of Summers *et al.* (1965) described by Evans (1970a). Gels contained 0.1% sodium dodecyl sulphate and 0.5 M-urea and were run at 4 mA per tube. The electrolyte (pH 8.4) contained 5 mM-tris, 40 mM-glycine and 0.01% sodium dodecyl sulphate. After electrophoresis gels were cut in half and stained either for protein with Coomassie Blue or for carbohydrate by using the periodic acid-Schiff stain as described by Zacharias *et al.* (1969). Samples obtained from gel filtration were concentrated against Aquacide II (Calbiochem) before electrophoresis. The alkali-soluble fraction was dialysed overnight at 4°C against 5 mM-tris – HCl buffer, pH 8.0, and concentrated against Aquacide II before the addition of 5% (w/v) sodium dodecyl sulphate and 0.5 M-urea. Approx. 10% of the protein

Scheme 1. Fractionation of mouse liver plasma membranes

came out of solution during dialysis. The pyridine-soluble components were analysed after dialysis against sodium dodecyl sulphate-urea-mercaptoethanol as described above.

Chemical determinations

Protein was determined by the procedure of Lowry *et al.* (1951) with bovine serum albumin as a standard. Sialic acid was measured by the procedure of Aminoff (1961) after hydrolysis in 50mM-H₂SO₄ at 80°C for 1h; a correction factor for interfering colour development was applied (Warren, 1963). For the determination of glucosamine and galactosamine, membrane samples were hydrolysed in 4M-HCl *in vacuo* for 4h at 110°C and analysed according to the method of Fanger & Smyth (1970) with a Beckman 120C amino acid analyser. Destruction of 10–15% of standard hexosamine solutions occurred when these were hydrolysed under conditions identical with those used for hydrolysis of membrane samples. The values for hexosamine presented in Table 1 were not corrected for destruction. Amino acid analysis was performed on the same apparatus after hydrolysis of samples for 24h at 100°C in 6M-HCl *in vacuo*. Corrections of 5 and 10% were made for destruction of threonine and serine respectively. For the determination of phospholipids, membrane samples were extracted 3 times with chloroform-methanol (2:1, v/v) at room temperature over 24h. Portions of the organic extract were dried and hydrolysed with 70% (w/v) HClO₄. Phosphorus was determined by the method of Bartlett (1959). The phospholipid content was calculated by assuming 25 µg of phospholipid/µg of lipid phosphorus (Kopaczyk *et al.*, 1966). Lipid was extracted from the pyridine-soluble fraction and fractions obtained from gel filtration by the addition of 3.2 vol. of chloroform-methanol (1:2, v/v) followed by 1 vol. each of chloroform and water (Bligh & Dyer, 1959).

The chloroform phase was removed and the extraction repeated. The combined chloroform phases were used for phosphorus determination as above and the aqueous phases for chemical analyses of lipid-free samples. Sialic acid and protein determinations on the fractions obtained by gel filtration were performed after dialysis against water for 16h at 4°C. For chemical analysis the alkali-soluble components were concentrated by precipitation with 5% (w/v) trichloroacetic acid at 4°C. Sialic acid, amino sugar and amino acid determinations on the pyridine-soluble fraction were performed after the removal of pyridine under vacuum at 40°C; pyridine was not removed for protein determinations.

Materials

Chemicals of AnalaR grade were obtained from British Drug Houses Ltd. (Poole, Dorset, U.K.) and biochemicals from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Glass-distilled water was used throughout.

Results

Fractionation of mouse liver plasma membranes

Of the membrane protein 10–15% was soluble in 0.9% NaCl. This is less than the soluble protein obtained from rat liver membranes under similar conditions (Emmelot *et al.*, 1964) but confirms the previous results with mouse liver membranes prepared by the present rate-zonal-centrifugation procedure (Evans, 1970a). Additional treatment of the washed membranes with 0.9% NaCl under conditions similar to those used for extraction with alkaline EDTA did not solubilize any more protein. Solubilization of 20–25% of the membrane protein occurred when the washed membranes were incubated for 60min at 37°C in 50mM-NaHCO₃-Na₂CO₃ buffer, pH 10.2, containing 10mM-EDTA.

Table 1. *Chemical properties of mouse liver plasma-membrane fractions*

Values are given $\pm$ s.d. and represent the average of three separate preparations. Protein is expressed as a percentage of starting protein, phospholipid as mg/g of protein and sialic acid and amino sugars as μ mol/g of protein. Hexosamine values are not corrected for destruction which occurred under the conditions of acid hydrolysis used.

Fraction	Protein (%)	Phospholipid (mg/g of protein)	Sialic acid (μ mol/g of protein)	Glucosamine (μ mol/g of protein)	Galactosamine (μ mol/g of protein)
Plasma membranes	100	450 $\pm$ 60	47 $\pm$ 8	85 $\pm$ 19	34 $\pm$ 12
Alkali-soluble	22 $\pm$ 3	150 $\pm$ 40	17 $\pm$ 3	54 $\pm$ 9	<5
Pyridine-insoluble	36 $\pm$ 3	730 $\pm$ 110	50 $\pm$ 5	73 $\pm$ 16	37 $\pm$ 24
Pyridine-soluble: total	33 $\pm$ 1	480 $\pm$ 50	70 $\pm$ 3	138 $\pm$ 10	41 $\pm$ 4
Pyridine-soluble: lipid-depleted			60 $\pm$ 5	139, 126*	26, 21*

* Results of two separate preparations.

Although similar amounts of protein were solubilized in its absence, the addition of EDTA at this stage was required to ensure the maximum extraction of protein and sialic acid by pyridine. Thus in a typical experiment 27% of the protein and 41% of the sialic acid were extracted by pyridine if EDTA was added to the NaHCO_3 – Na_2CO_3 buffer whereas the corresponding values after alkaline extraction in the absence of EDTA were 12% and 25%.

Chemical properties and gel electrophoresis of plasma-membrane fractions

The protein, phospholipid and carbohydrate content of the total plasma membrane and the three membrane fractions are presented in Table 1. The sialic acid and amino sugar content of the total plasma membrane is somewhat higher than previously reported (Evans, 1970a; Evans & Gurd, 1971). These higher values may in part be due to the loss of non-membrane protein during the several washes with 0.9% NaCl. The sialic acid content now found is similar to that reported for rat liver plasma membranes by Ray (1970) and Touster *et al.* (1970).

The three membrane fractions differ in chemical composition. The alkali-soluble components are low in carbohydrate and phospholipid, whereas the pyridine-soluble fraction is enriched in sialic acid and glucosamine. The presence of glycolipids in liver plasma membranes is well documented (Dod & Gray, 1968; Stahl & Trams, 1968; Ray *et al.*, 1969). Therefore to determine if the pyridine-extracted carbohydrates represented glycoprotein or glycolipid, sialic acid and amino sugars were determined after lipid extraction. Of the sialic acid 15% and of the galactosamine 40% were soluble in chloroform–

methanol but none of the glucosamine was extracted. However, the lipid-depleted aqueous phase was still enriched in carbohydrate and these results confirm the selective extraction of membrane glycoproteins by pyridine. The failure of chloroform–methanol to extract glucosamine confirms our previous results, which indicated that this hexosamine is present in the plasma membrane predominantly in the form of glycoprotein (Evans & Gurd, 1971). Amino acid analysis revealed only minor differences between the three membrane fractions and the unfractionated membrane (Table 2). The values are similar to those reported for fractionated rat liver membranes (Simon *et al.*, 1970).

The protein and glycoprotein components of the alkali-soluble and pyridine-soluble fractions were analysed by gel electrophoresis in sodium dodecyl sulphate (Plate 1). This system separates proteins on the basis of molecular weight (Shapiro *et al.*, 1967; Weber & Osborn, 1969) and both fractions are seen to contain several proteins distributed over a wide molecular-weight range. A total of 13 distinct bands was detected in the pyridine-soluble fraction compared with about 20 in the intact membrane (Evans, 1970a). This represents a simplification over the water-soluble pyridine-extracted fraction of rat liver plasma membranes (Simon *et al.*, 1970). Only one major glycoprotein was present in the alkali-soluble fraction whereas the majority of the pyridine-soluble proteins contained carbohydrate. These results are in agreement with the distribution of carbohydrate in these two fractions as shown in Table 1. Comparison with proteins of known molecular weight run on polyacrylamide gels under identical conditions suggested that the major alkali-soluble glycoprotein had a molecular weight of

Table 2. *Amino acid composition of liver plasma-membrane fractions*

Values are expressed as mol/100 mol. Values for the total plasma membrane are calculated from the data given by Evans (1970a).

	Plasma membranes	Alkali-soluble	Pyridine-soluble	Pyridine-insoluble
Lysine	7.9	6.4	6.5	7.5
Histidine	2.3	2.3	1.9	2.7
Arginine	5.2	5.3	5.6	5.9
Aspartic acid	7.9	8.7	8.2	9.7
Threonine	4.5	5.2	4.8	5.5
Serine	6.0	7.5	7.5	6.0
Glutamic acid	11.4	11.7	11.9	12.7
Proline	4.9	4.4	5.5	4.3
Glycine	8.7	7.9	9.5	7.9
Alanine	8.7	8.1	8.2	7.8
Valine	6.6	7.0	6.9	6.9
Isoleucine	5.5	5.9	5.2	5.6
Leucine	9.8	11.0	10.3	9.7
Tyrosine	3.1	3.6	3.2	3.4
Phenylalanine	4.7	5.2	4.8	4.3

EXPLANATION OF PLATE I

Polyacrylamide-gel electrophoresis in 0.1 % sodium dodecyl sulphate (pH 8.4) of alkali-soluble (left-hand pair) and pyridine-soluble membrane components. After electrophoresis gels were cut in half and stained either with Coomassie Blue for protein (a) or with the periodic acid-Schiff stain for carbohydrates (b). The arrow indicates the major glycoprotein present in the alkali-soluble fraction. The direction of electrophoresis was from the top to the bottom.

EXPLANATION OF PLATE 2

Polyacrylamide-gel electrophoresis in 0.1% sodium dodecyl sulphate (pH 8.4) of Sepharose-6B fractions I-V. Gels were cut in half and stained either with Coomassie Blue for protein (a) or with the periodic acid-Schiff stain for carbohydrate (b). The direction of electrophoresis was from the top to the bottom.

approximately 120000. It has recently been demonstrated that some glycoproteins behave anomalously on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (Segrest *et al.*, 1971; Bretscher, 1971) and therefore the assignment of this molecular weight must be regarded as tentative.

Fractionation of pyridine-soluble components

The enrichment in glycoproteins achieved by pyridine extraction coupled with the finding that these proteins cover a wide molecular-weight range suggested that additional fractionation and purification of the glycoproteins might be achieved by gel chromatography. The elution profile obtained when the pyridine-soluble fraction was applied to a column of Sepharose 6B equilibrated with sodium dodecyl sulphate is shown in Fig. 1. With the exception of an initial peak at the void volume the majority of the protein was retarded and eluted over the entire column volume with no distinct peaks being apparent. The presence of β -mercaptoethanol during dialysis was required to decrease aggregation and to minimize the amount of protein that was excluded from the column. Phospholipid separated from the majority of the protein (Fig. 1 and Table 3) and this is in accord with previous demonstrations of the ability of detergents to dissociate membrane proteins and lipids (Bont *et al.*, 1969; Engelman *et al.*, 1967; Philippot, 1971).

Gel electrophoresis and chemical properties of Sepharose-6B fractions

Fractions were pooled as indicated in Fig. 1 and analysed by gel electrophoresis (Plate 2). It is evident

that resolution on the basis of molecular weight takes place although the various fractions still exhibit some heterogeneity. The proteins of fractions IV and V run with the dye front and are of low molecular weight (<25000). They are retained during dialysis and hence their molecular weight should be within the range 6000–25000. The diffuse periodate–Schiff-positive–Coomassie Blue-negative area which runs in front of the proteins of fractions IV and V is indicative of the presence of glycolipids (Lenard, 1970).

The protein, phospholipid and sialic acid contents of the pooled Sepharose-6B fractions are shown in Table 3. In different preparations the recovery of protein applied to the column varied between 80 and 100%. In accord with the results of gel electrophoresis (Plates 1 and 2) each of the fractions contains glycoprotein, represented by sialic acid that is not extracted with chloroform–methanol, and glycolipid is present in fractions IV and V, as indicated by the presence of sialic acid soluble in chloroform–methanol. An inverse relationship between the molecular weight and the carbohydrate content of pyridine-soluble membrane glycoproteins is suggested by the results

Discussion

The stepwise procedure for the fractionation of mouse liver plasma membranes described in this paper achieves partial resolution of the plasma membrane into its constituent proteins. Although the heterogeneity of liver plasma-membrane proteins has been previously demonstrated by gel electrophoresis (Evans, 1970a,b; Zahler *et al.*, 1970) little pro-

Fig. 1. Gel filtration of pyridine-soluble membrane proteins

Fractionation was carried out in 0.5% sodium dodecyl sulphate–0.5 M-urea–30 mM-tris–HCl buffer, pH 8.0, on a column (100 cm $\times$ 0.9 cm) of Sepharose 6B. The flow rate was 6–8 ml/h and 2.5 ml fractions were collected. Tubes were monitored for extinction at 280 nm (—). Horizontal divisions represent fractions that were pooled. The histogram represents total phospholipid in the respectively pooled fractions. No phospholipid was detected in fractions I and II.

Table 3. *Chemical properties of Sepharose-6B fractions*

The pyridine-soluble fraction of mouse liver plasma membranes was fractionated on a column of Sepharose-6B as described in the Experimental section. The results of two separate experiments are presented. Protein is expressed as mg/fraction and the numbers in parentheses indicate the percentage of the total protein recovered in each fraction. Phospholipid is expressed as mg/fraction and sialic acid as $\mu\text{mol/g}$ of protein. In Expt. 1 41 mg of protein containing $78 \mu\text{mol}$ of sialic acid/g of protein was added to the column. The corresponding values for Expt. 2 were 20 and 68.

Expt. no. ... Fraction no.	Protein (mg/fraction)		Phospholipid (mg/fraction)		Sialic acid ($\mu\text{mol/g}$ of protein)			
					Total fraction		Lipid-depleted fraction	
	1	2	1	2	1	2	1	2
I	6.1 (15)	3.3 (16)	<0.1	<0.1	46	38	42	35
II	12.8 (31)	6.3 (31)	<0.1	<0.1	80	62	78	61
III	9.2 (22)	4.9 (24)	3.9	0.1	114	57	97	57
IV	4.2 (10)	4.0 (20)	9.7	5.4	230	112	121	106
V	2.2 (5)	2.0 (10)	8.2	4.5	229	191	148	140

gress has been made in the fractionation and analysis of these proteins. The two soluble membrane fractions now described account for 50–60% of the membrane protein. Although similar in overall amino acid composition, the alkali- and pyridine-soluble fractions contain different amounts of carbohydrate and lipid and differ in their complements of protein and glycoprotein.

Aqueous media of low ionic strength have been used to solubilize erythrocyte 'ghost' proteins (Marchesi *et al.*, 1969; Harris, 1969; Burger *et al.*, 1968; Mazia & Ruby, 1968) and 50–70% of the protein of rat liver plasma membranes has previously been found to be soluble in 50mM- K_2CO_3 (Neville, 1967; Simon *et al.*, 1970). The smaller amount (20%) of mouse liver plasma-membrane proteins now found to be extracted under alkaline conditions may in part be due to the more rigorous removal of non-membrane proteins as noted above and in part to the use of a lower pH value in the present procedure. The low phospholipid and sialic acid content of the alkali-soluble fraction is similar to the findings of Simon *et al.* (1970). Preliminary experiments have shown that this fraction gives a positive precipitin reaction when analysed by immunodiffusion against antisera to unfractionated plasma membranes indicating that some of the protein is exposed on the membrane surface (inside or outside). Glycoproteins and glycolipids have been implicated in the antigenic properties of the cell surface (Cook, 1968; Curtiss, 1967). Recently the H-2 alloantigenic specificities of mouse spleen cells have been shown to be associated with glycoproteins released from the cell surface by proteolytic digestion (Shimada & Nathenson, 1969; Yamane & Nathenson, 1970), and Davies (1971) has indicated that the

transplantation antigens extracted from the membranes of a variety of mammalian cells are glycoproteins. These results suggest that the antigen solubilized by alkali in the present study may be the major glycoprotein which is present in this fraction (Plate 1). Neville (1968) described the purification of a surface membrane antigen, having an apparent molecular weight of 70000, that was extracted from rat liver plasma membranes with 50mM- K_2CO_3 or 1mM-EDTA. The absence of any chemical characterization, however, prevents further comparison with the present results.

Of the alkali-insoluble protein 40% is soluble in 33% pyridine. The increase in pyridine-solubility that occurs when the preceding alkaline extraction is performed in the presence of EDTA is consistent with a stabilizing role for cations in membrane structure (see, e.g., Manery, 1966; or the discussion of Burger *et al.*, 1968). Similar effects of the preliminary removal of ions on membrane protein solubility have been noted for erythrocyte 'ghosts' (Rosenberg & Guidotti, 1969; Maddy, 1966) and liver plasma membranes (Neville, 1968; Simon *et al.*, 1970).

The choice of pyridine for the fractionation of mouse liver plasma membranes was based on the report of Blumenfeld (1968), which demonstrated the selective solubilization of sialylglycoproteins from erythrocyte 'ghosts' with aq. 33% pyridine. While the present work was in progress Simon *et al.* (1970) described the fractionation of rat liver plasma membranes with aqueous pyridine. Unlike the results obtained with erythrocytes (Blumenfeld, 1968), the sialic acids and neutral sugars of rat liver plasma membranes were equally distributed between the soluble and insoluble fractions. We now report the selective extraction of glycoproteins from mouse liver

plasma membranes with pyridine and in this respect the present results are similar to those obtained with erythrocyte 'ghosts' (Blumenfeld, 1968). The apparent difference in behaviour of the plasma membranes of rat (Simon *et al.*, 1970) and mouse liver on extraction with pyridine may partially be due to the variations between the present procedure and that used by Simon *et al.* (1970). Thus by using a stepwise fractionation we have obtained two soluble fractions of differing composition. The preliminary treatment with alkaline EDTA removed water-soluble membrane proteins that were low in carbohydrate content. Then the suspension of membranes in aqueous pyridine was immediately centrifuged and the pyridine-soluble proteins of the supernatant solution were studied, whereas in the procedure used by Simon *et al.* (1970) the suspension was dialysed against water before centrifugation and isolation of the water-soluble fraction of the pyridine extract. The inclusion in this fraction of proteins low in carbohydrate, which are initially removed by alkaline extraction in the present procedure, would explain why Simon *et al.* (1970) observed no enrichment of glycoproteins in their fraction. It would also account for the simpler protein composition of our pyridine-soluble fraction.

Partial resolution of the pyridine-soluble proteins was obtained by gel chromatography in sodium dodecyl sulphate. Preliminary attempts to resolve these proteins on Sephadex G-200 equilibrated with 33% pyridine (Blumenfeld *et al.*, 1970) resulted in the bulk of the protein being excluded from the column and it was necessary to replace the pyridine with sodium dodecyl sulphate to decrease aggregation and to achieve further fractionation. The role of mercaptoethanol in decreasing aggregation during dialysis would seem to be only partially related to disulphide-bond cleavage. Thus, although mercaptoethanol was not present in the eluting buffer, the results of gel electrophoresis (also performed in the absence of reducing agent) clearly demonstrated that aggregation of the proteins in the pooled fractions did not occur. Mercaptoethanol has been found to have similar effects on the behaviour of erythrocyte-'ghost' proteins (Lenard, 1970). The high amount of carbohydrate found to be associated with low-molecular-weight glycoproteins of mouse liver plasma membranes may be compared with the presence of a group of proteins (mol.wt. 20000-40000) in erythrocyte 'ghosts' that contain 4-5 times as much sialic acid as higher-molecular-weight proteins (mol.wt. greater than 75000) (Rosenberg & Guidotti, 1969). The role of these low-molecular-weight glycoproteins in membrane structure and function remains to be elucidated.

We thank Dr. D. G. Smyth for the use of the amino acid analyser. J. W. G. was supported by a post-doctoral fellowship from the Canadian Medical Research Council.

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