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# CHARACTERIZATION OF THE MEMBRANE MATRIX DERIVED FROM THE MICROSOMAL FRACTION OF RAT HEPATOCYTES

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## SUMMARY

A highly purified membrane preparation derived from the microsomal fraction of rat hepatocytes has been chemically characterized and fractionated by means of gel filtration. The preparation has been freed of ribosomes and intravesicular protein and has a composition on a w/w basis of 52.1 % protein, 45.0 % phospholipid, 2.9 % carbohydrate and no RNA. 97±2 % of the total membrane phosphorus is accounted for as phospholipid phosphorus.

Determination of the molecular weight distribution of the constituent polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis gave values ranging from 171 000 to 16 000 for the major classes of proteins. Although several membrane glycoproteins have been identified, the most prominent species has an apparent molecular weight of 171 000, 40% of the total microsomal protein is present in the 49 000–60 000 molecular weight region. Examination of the intrinsic polypeptide composition of membranes obtained from smooth and degranulated rough endoplasmic reticulum revealed no detectable qualitative differences.

Sodium dodecyl sulfate-solubilized microsomal membrane proteins were separated by gel filtration into much simplified molecular weight classes, some of which showed predominantly a single electrophoretic component. Amino acid analysis of individual fractions showed a noticeable trend toward a decreasing ratio of acidic to basic residues with decreasing molecular weight.

Membrane phosphorus was distributed between two chromatographic fractions: one containing the membrane phospholipid (97 % of the total) as well as essentially all the cholesterol, the other, at the inclusion volume of the gel filtration system, containing small molecular weight species (3 % of the total phosphorus). The absence of a ribonuclease-resistant RNA component eluting near the void volume clearly distinguishes the microsomal membrane from the nuclear envelope.

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Abbreviation: TKM buffer, 0.05 M Tris · HCl, pH 7.55, 25 mM KCl, 5 mM $MgCl_2$.

## INTRODUCTION

Microsomal membrane from rat liver offers several advantages for the study of membrane biochemistry. First, since it is derived from the endoplasmic reticulum, it is representative of a highly specialized structural entity within the cell, serving in the anchoring of the major part of the cell's protein-synthesizing system, as well as assisting in the transport of the protein products of this system to the exterior of the cell. Second, microsomal membrane possesses a particularly important membrane-integrated enzyme system responsible for the metabolism of drugs, carcinogens, and certain naturally occurring compounds. While the latter enzymatic properties have been enthusiastically studied [1–10], the isolation and chemical characterization of the proteins primarily responsible for the structural organization of the membrane have received scant attention.

For the most part, separations of these proteins have been achieved by analytical polyacrylamide gel electrophoresis, a technique which can provide only a limited amount of information concerning molecular properties of complex mixtures of proteins. Kiehn and Holland [11], using sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated the multiplicity of proteins present in microsomal membrane as well as in other cellular membranes. By comparing the gel patterns of a variety of membranes, they concluded that no two were exactly alike in protein composition, although they suggested that some compositional overlap probably does occur. In a later study, Schnaitman [12] reached much the same conclusions. In addition, from his gel comparisons of the protein compositions of smooth and rough microsomal membranes, he concluded that these membranes showed significant differences in polypeptide composition. Hinman and Phillips [13] have disputed the latter conclusion on the grounds that the observed differences can be attributed to contamination by non-membrane proteins. By thorough washing, they succeeded in preparing smooth and rough membrane which yield essentially identical electrophoretic patterns. Morin et al. [14], using a different preparatory procedure, have reached a similar conclusion.

In this paper, we describe the characterization of the microsomal membrane matrix in terms of chemical composition, molecular weight distribution of constituent polypeptides, separation into molecular weight classes by Sephadex gel filtration, and the amino acid composition of individual fractions. As a part of our continuing studies on the physicochemical and biochemical interrelationships between the nuclear envelope and the endoplasmic reticulum [15–20], this work forms the basis for the detailed protein fractionation, purification, and characterization studies in progress in our laboratory.

## MATERIALS AND METHODS

*Chemicals.* All chemicals used were reagent grade.

*Animals.* Male Sprague-Dawley rats weighing $190 \pm 10$ g were used in all studies. Animals were fasted for at least 16 h and killed by decapitation.

*Preparation of microsomal membrane.* For the preparation of total microsomal membrane, minced livers were added in 12-g portions to a Potter-Elvehjem homogenizer (0.006–0.009 inch clearance), suspended in 25 ml of 0.25 M sucrose, 0.05 M

Tris · HCl buffer, pH 7.55, containing 25 mM KCl and 5 mM $MgCl_2$ (TKM buffer) and homogenized with approx. 15 up and down strokes of a teflon pestle. The homogenate was diluted with an additional 25 ml of 0.25 M sucrose/Tris buffer and filtered through four layers of cheesecloth. The combined filtrates were centrifuged at 20 000 × *g* (max.) for 10 min in a Sorvall RC-2 centrifuge equipped with a SS-34 head and the supernatant carefully removed without disturbing the loosely packed upper portion of the pellet. Crude microsomal pellets were obtained by centrifugation of the supernatant in a Spinco T-42 rotor at 142 800 × *g* (max.) for 1 h. The membrane fraction was then sonicated in a small volume of 10 % (w/v) potassium citrate/TKM buffer at 0 °C to yield a homogeneous suspension and 2 M sucrose/10 % (w/v) potassium citrate/TKM buffer was added to adjust the density to greater than 1.20 g/cm$^3$. The membrane suspension was placed in either Spinco SW25.2 or SW27 tubes and successively overlayered in a stepwise manner with solutions of 10 % citrate/TKM buffer containing sufficient sucrose to yield densities of 1.20, 1.18, and 1.16 g/cm$^3$. Volumes of each zone were adjusted to the amount of membrane processed, but no less than 5 ml in any zone was used and the 1.16 g/cm$^3$ zone was greater than 15 ml. After centrifugation at approx. 100 000 × *g* (max.) for 15–18 h, the 1.16 g/cm$^3$ layer containing the membrane was removed, diluted at least 3-fold with TKM buffer and centrifuged at 142 800 × *g* (max.) for 1 h in the T-42 rotor. The red-brown microsomal membrane pellets were suspended in 10 % potassium citrate/TKM buffer by sonication and centrifuged again in the T-42 rotor at 142 800 × *g* for 1 h. This sonication and washing procedure was repeated one more time and was then followed by the same treatment in distilled water.

Smooth and rough microsomal membranes were prepared by method b (i) of Murray et al. [21] and purified using the discontinuous sucrose/citrate gradient and subsequent washing procedures described above.

*Solubilization of membrane.* Washed microsomal membrane was sonicated into water suspension at a protein concentration of approx. 35 mg/ml. To this homogeneous suspension were added the appropriate quantities of 1 M Tris · HCl, pH 7.5, crystalline sodium dodecyl sulfate, β-mercaptoethanol, and disodium EDTA to give a solution of the composition: 0.1 M Tris · HCl, pH 7.5, 1 % (v/v) β-mercaptoethanol, 0.5 mM EDTA, and approx. 7 % (w/v) sodium dodecyl sulfate.

*Disc gel electrophoresis.* Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Bornens and Kasper [17]. Samples of total microsomal membrane or rough or smooth membrane were dissolved in 0.1 M Tris · HCl, pH 7.5, 0.5 % (w/v) sodium dodecyl sulfate, 1 % (v/v) β-mercaptoethanol and were allowed to stand at room temperature for at least 4 h prior to electrophoresis. Heating of the samples had no effect on the gel patterns and was therefore not routinely used. Protein concentrations were approx. 0.2 % (w/v).

Attempts to detect possible proteolysis either during solubilization or in the interim between solubilization and fractionation showed no evidence of protein degradation. The experiments were performed in the following way: identical membrane samples were dissolved (a) as described above, (b) as described above, but with immediate heating at 100 °C for 3 min, (c) in a solution of composition 0.1 M Tris · HCl, pH 7.5, 5 % (w/v) sodium dodecyl sulfate, 5 % (v/v) β-mercaptoethanol, (d) as in (c) but with heating at 100 °C for 3 min, (e) in 0.1 M Tris · HCl, pH 7.5, 1 M urea, 0.1 % (v/v) β-mercaptoethanol, and (f) as in (e) but with heating at 100 °C

for 3 min. All samples were then allowed to stand at room temperature for 24 h, dialyzed into the conventional sodium dodecyl sulfate polyacrylamide gel buffer, and electrophoresed as usual.

Split sodium dodecyl sulfate polyacrylamide gels were prepared by casting gels of the usual composition in 1 cm diameter tubes. After polymerization, two 5-mm diameter transparent plastic cylinders approx. 1 cm in length (sections of plastic drinking straws work well) were inserted side by side into the top of the tube until they were imbedded into the gel surface approx. 1 mm. Samples to be compared were then applied by underlayering one into each of the compartments.

Electrophoretic analysis of chromatographic fractions was achieved by direct utilization of the protein-containing fractions eluted from the columns, or, if necessary, aliquots were first concentrated by acetone precipitation and resolubilized into a smaller volume of sample buffer.

*Gel filtration in sodium dodecyl sulfate.* The technique of Jones and Kennedy [22] as modified by Bornens and Kasper [17] for fractionation of rat hepatocyte nuclear envelope was applied to microsomal membrane. Three $2.5 \times 100$ cm serially connected Pharmacia up-flow columns of Sephadex G-150 were used. Initial flow rates were approx. 10 ml/h at 40 cm pressure head, but diminished to unsuitable levels after two or three fractionations and could be restored only by repouring the columns.

*Chemical analysis.* Protein was determined by the method of Lowry et al. [23] using Worthington twice crystallized ovalbumin as a standard.

Total phosphorus was determined by the method of Bartlett [24] and lipid phosphorus by the same method after extracting membrane lipid into chloroform/methanol (2 : 1, v/v), and washing with 0.88 % (w/v) KCl according to Folch et al. [25].

Total carbohydrate was determined on samples delipidated by treatment with 100 % acetone. The method of DuBois et al. [26] was used with D-galactose as a standard.

Sialic acid was determined on samples prepared as described in the previous paragraph. Hydrolysis was carried out by the procedure of Warren [27], and the liberated sialic acid analyzed by the method of Jourdian et al. [28].

*Amino acid analysis.* Protein for amino acid analysis was hydrolyzed for 24 h at 110 °C in 3 ml of 6 M glass-distilled HCl containing 50 µl of 5 % (v/v) phenol and 2 µl of mercaptoacetic acid [29]. Chromatography was performed on the Beckman 120 C amino acid analyzer according to the method of Spackman et al. [30]. Quantitation of cysteine and cystine was performed by the performic acid oxidation method [31] and also by reduction and S-carboxymethylation [32].

## RESULTS

### *Chemical composition of microsomal membrane*

If biochemical studies are to enhance our understanding of the molecular structure of membranes, then the membrane preparation investigated must be a well defined, reproducibly obtainable cellular subfraction which accurately reflects the in vivo composition of the membrane from which it is derived. For this reason, the microsomal membrane used in this study is not only operationally defined by the procedures involved in its preparation, but also chemically defined by thorough

characterization of the final product. The general chemical composition (w/w) of microsomal membrane prepared as described in Materials and Methods was protein, 52.1 %; phospholipid, 45.0%; carbohydrate, 2.9%; 0 % RNA. It should be emphasized that the membrane preparation used in this study is a mixture of degranulated rough and smooth surfaced membranes. Detailed preliminary studies have shown that the polypeptide composition of the membrane matrix derived from smooth endoplasmic reticulum is qualitatively indistinguishable from that of the rough endoplasmic reticulum.

Several items are noteworthy, some of them because they reflect compositional similarities to other cellular membranes, particularly the nuclear envelope to which the endoplasmic reticulum forms a morphological continuum with the outer leaflet, and some because they diverge from values reported for microsomal membrane prepared by other methods. With regard to the former comparison, the carbohydrate composition of microsomal membrane (2.9 %, w/w) is quantitatively similar to that of rat hepatocyte nuclear envelope [15], but quite different from those of the more immune-adapted [33–38] plasma membrane of erythrocyte (8 %, w/w) [39] or rat hepatocyte (5–10 %, w/w) [39].

The phospholipid to protein ratio (0.86, w/w) is considerably higher than reported by other investigators [40–47]. This increase probably reflects the thorough removal of exogenous, non-membrane proteins from the final membrane fraction. Although it might well be argued that the rigorous washing procedures required to eliminate such contamination might incur losses of intrinsic membrane proteins, the assurance that the purified proteins to be characterized are of genuine membrane origin is deemed worth the somewhat diminished yield of membrane.

Another property of the microsomal membrane which further establishes its chemical nature is the ratio of phosphorus extractable into chloroform/methanol (2 : 1, v/v), i.e. phospholipid phosphorus, to total membrane phosphorus. The mean of five determinations was 0.97 with a standard deviation of 0.02, indicating that essentially all ribosomal and non-ribosomal RNA is removed from the membrane by the preparatory procedures and furthermore that phosphoproteins, if present, are below a detectable level. These results contrast to those of Kashnig and Kasper [15] for nuclear envelope, wherein approx. 9 % by weight of the total membrane phosphorus persisted as RNA resistant to RNAase digestion. Presumably, this RNA is associated with the nuclear pore complex and is not ribosomal in origin.

The amino acid composition of total microsomal membrane (Table I) is indistinguishable from the composition of the membrane matrix derived from either the smooth or rough endoplasmic reticulum. Furthermore, amino acid analysis of microsomal membranes prepared from various Morris hepatomas failed to reveal significant differences in composition when compared to membrane from normal liver (Blackburn, G. R. and Kasper, C. B., unpublished results). Considering the fact that each membrane is a complex mixture of many proteins, it is not surprising that alterations, evident from gel electrophoresis patterns (Blackburn, G. and Kasper, C. B., manuscript in preparation), are not reflected as changes in gross amino acid content. Using the assignments of Dickerson and Geis [48], the distribution of non-polar, neutral polar, acidic, and basic side chains for the microsomal membrane is calculated from Table I to be 52, 15, 19, and 14 %, respectively. Most notable is the high level of aspartic and glutamic acids ($\approx$ 21 %) and leucine ($\approx$ 11 %). This

TABLE I

AMINO ACID COMPOSITIONS OF SELECTED FRACTIONS FROM THE CHROMATOGRAM OF FIG. 2

Results are expressed in mol per 100 mol of amino acid; cysteine, cystine, and tryptophan were not determined.

| Amino acid | Microsomal membrane | Fraction | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L |
| Lysine | 6.47 | 6.77 | 6.04 | 6.70 | 6.67 | 6.61 | 7.03 | 6.91 | 6.70 | 7.50 | 5.73 | 6.30 | 6.25 |
| Histidine | 2.10 | 2.26 | 2.45 | 2.31 | 2.19 | 2.57 | 2.81 | 2.74 | 2.27 | 2.50 | 1.91 | 2.24 | 2.34 |
| Arginine | 4.72 | 5.22 | 5.66 | 4.96 | 4.91 | 4.77 | 5.04 | 4.88 | 5.90 | 6.56 | 5.61 | 5.89 | 5.08 |
| Aspartic acid | 9.62 | 12.3 | 9.44 | 9.70 | 10.0 | 9.55 | 9.61 | 8.94 | 8.74 | 7.50 | 8.83 | 8.54 | 8.59 |
| Threonine | 5.33 | 5.50 | 5.57 | 5.43 | 5.53 | 5.38 | 5.04 | 5.18 | 5.67 | 5.00 | 5.25 | 5.49 | 5.08 |
| Serine | 6.47 | 6.35 | 6.33 | 6.24 | 5.88 | 6.24 | 5.86 | 6.50 | 6.58 | 6.25 | 6.20 | 7.93 | 8.98 |
| Glutamic acid | 10.8 | 11.4 | 11.3 | 11.3 | 11.0 | 10.8 | 10.1 | 10.7 | 9.88 | 8.75 | 9.07 | 8.94 | 10.2 |
| Proline | 6.12 | 5.78 | 5.85 | 5.77 | 5.79 | 6.00 | 6.92 | 6.00 | 4.99 | 4.69 | 5.25 | 5.28 | 5.47 |
| Glycine | 7.17 | 7.33 | 7.27 | 7.27 | 7.19 | 6.98 | 6.45 | 6.52 | 7.26 | 6.88 | 8.00 | 7.93 | 8.20 |
| Alanine | 7.08 | 6.77 | 6.80 | 6.93 | 7.19 | 6.49 | 5.86 | 6.20 | 7.26 | 7.19 | 8.11 | 7.93 | 7.81 |
| Valine | 6.73 | 7.19 | 7.27 | 6.70 | 7.10 | 6.85 | 6.68 | 6.30 | 7.26 | 6.56 | 6.44 | 6.10 | 6.25 |
| Methionine | 2.53 | 2.12 | 2.27 | 2.31 | 2.02 | 2.45 | 2.70 | 2.44 | 2.50 | 2.50 | 2.15 | 2.03 | 2.34 |
| Isoleucine | 5.07 | 4.94 | 5.10 | 5.08 | 4.82 | 5.14 | 5.39 | 5.08 | 4.65 | 5.31 | 4.53 | 4.47 | 4.69 |
| Leucine | 11.1 | 8.46 | 11.3 | 11.1 | 10.8 | 11.3 | 11.2 | 11.0 | 12.9 | 12.8 | 13.4 | 11.8 | 10.5 |
| Tyrosine | 3.50 | 3.10 | 3.21 | 3.35 | 3.33 | 3.43 | 3.40 | 4.06 | 2.64 | 4.06 | 3.82 | 3.66 | 3.12 |
| Phenylalanine | 5.16 | 4.51 | 4.91 | 4.85 | 5.09 | 5.51 | 5.86 | 5.59 | 4.88 | 5.94 | 5.73 | 5.28 | 5.08 |

observation also holds for the nuclear envelope [17], liver plasma membrane [49], and erythrocyte membrane [50].

*Polypeptide composition of microsomal membrane and comparison to the smooth and rough subfractions*

Fig. 1 shows a split sodium dodecyl sulfate polyacrylamide gel comparison of the molecular weight classes of proteins derived from the total, rough, and smooth fractions of microsomal membrane, after removal of ribosomes and intravesicular protein. Based on this electrophoretic system, the polypeptide patterns of the three membrane preparations appear to be qualitatively indistinguishable. This does not imply, however, that significant differences may not exist within a specific molecular weight class. Because of the strong similarities among the three fractions and because

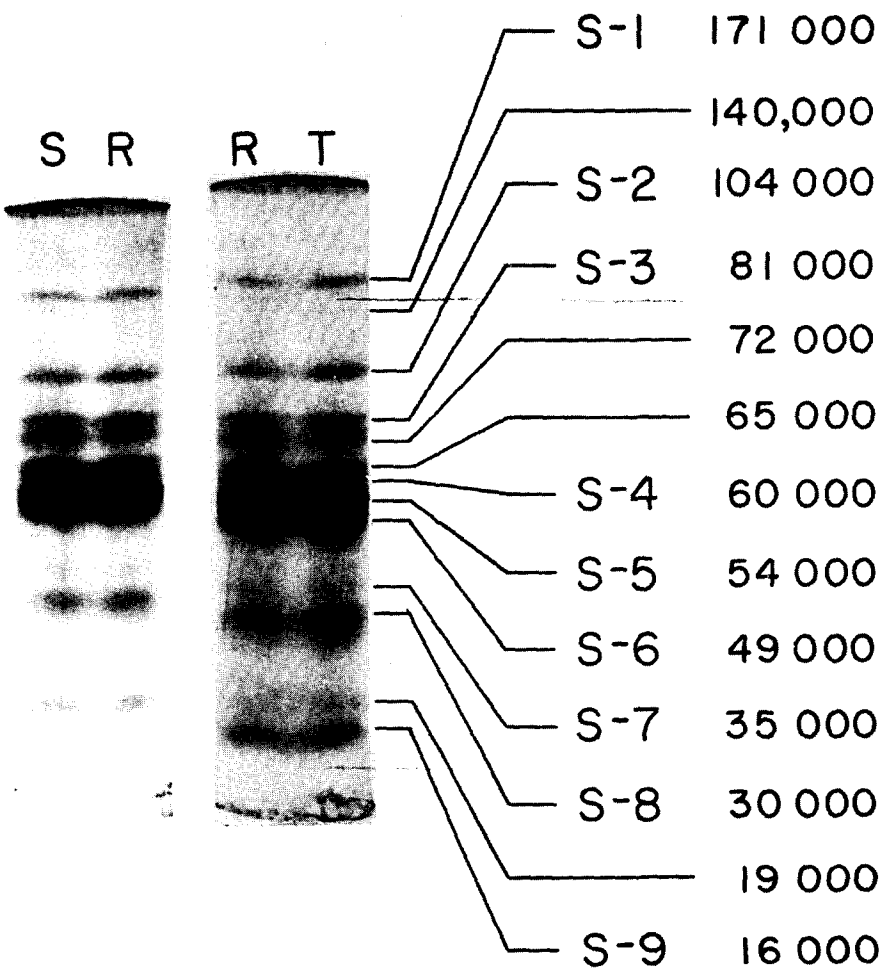


Fig. 1. Comparison of the polypeptide composition of rough (R), smooth (S), and total (T) microsomal membranes by split sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The molecular weight of the major proteins is also given.

the yield of total membrane (3–4 mg/g of liver) is much greater than that of either of the two subfractions prepared separately (approx. 0.5 mg/g for rough and 1.5 mg/g for smooth), all further studies reported in this series utilized the total membrane fraction.

The molecular weight assignments for the major polypeptide chains are also indicated in Fig. 1. Among the most noticeable features of the pattern is the intensity of the bands located in the 49 000–60 000 molecular weight region of the gel. Protein analysis of this material (see below) revealed that approx. 40 % (w/w) of the total membrane protein is present in this region. The remaining 60 % is accounted for by polypeptides having a maximum molecular weight of 171 000 and a minimum of 16 000. The major glycoprotein of the microsomal membrane has an apparent molecular weight of 171 000.

Of crucial importance in the interpretation of polyacrylamide gel patterns and the data which they provide is the assurance that the patterns obtained reflect the in vivo situation and that they have not been altered by interim proteolysis. An attempt to detect proteolytic degradation was carried out as described in Materials and Methods, and under the conditions examined, no evidence of proteolysis was observed.

*Column fractionation studies*

Fractionation of sodium dodecyl sulfate-solubilized microsomal membrane on the three column Sephadex G-150 system gave the result shown in Fig. 2; in Fig. 3 are shown the results of sodium dodecyl sulfate polyacrylamide gel analysis of protein fractions from selected regions of the chromatogram (A–L). It can be seen that in some regions of the chromatogram, separations are of sufficient resolution to allow isolation of single components (e.g. Fractions F and H). Protein analysis revealed that 40 % by weight of the total membrane protein is found between tubes 180 and 210 (molecular weights from approx. 40 000 to 60 000). It should be noted that the peak in

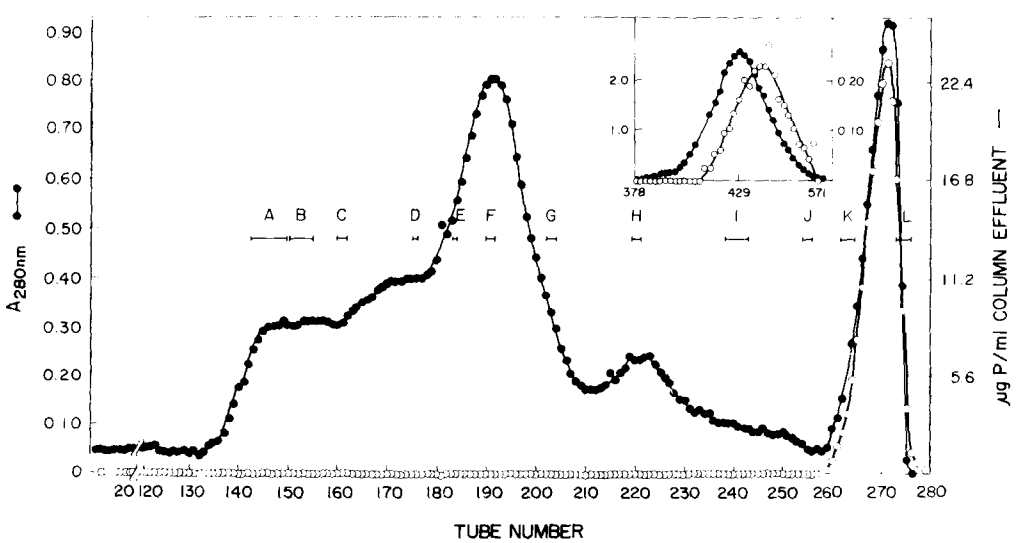


Fig. 2. Fractionation of microsomal membrane proteins by gel filtration on Sephadex G-150. See Materials and Methods for experimental details. The insert is the elution profile in the region of the column inclusion volume.

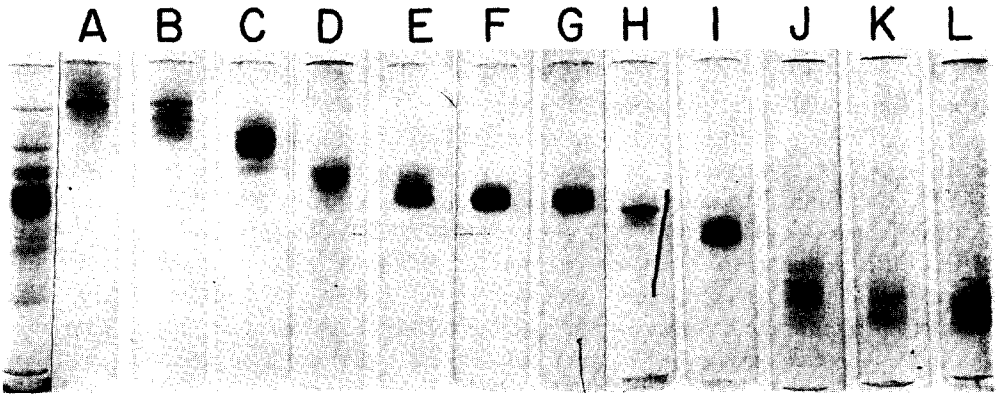


Fig. 3. Electrophoretic analysis (sodium dodecyl sulfate gels) of fractionated microsomal membrane proteins. Letters correspond to fractions in Fig. 2. The unlabeled gel at the extreme left represents the membrane proteins prior to fractionation.

the inset in Fig. 2 occurs at the inclusion volume of the system and is presumably due to low molecular weight species.

The elution profile of phosphorus-containing material is also shown in Fig. 2 97 % by weight of the total membrane phosphorus is located in the peak between tubes 260 and 280 with the remaining 3 % found in the inclusion volume peak. A phospholipid extract of the membrane has been shown to elute at the same position as the major phosphorus fraction whether chromatographed in the presence or absence of membrane protein, thereby indicating that the phospholipids are probably not bound to the proteins with which they co-elute. Essentially all membrane cholesterol is also found in this peak.

The absence of any phosphorus-containing molecules with apparent molecular weights greater than 20000 constitutes a notable difference between the microsomal and nuclear membranes. Bornens and Kasper [17] have reported the presence in nuclear envelope of an RNA species which, in the same chromatographic system used in this study, elutes at the void volume and comprises approx. 10 % by weight of the total membrane phosphorus.

Amino acid compositions of fractions A–L are presented in Table I. One of the most noticeable features of the data is that there is not a strong difference between the compositions of the various fractions, even those whose gel patterns suggest sufficient homogeneity for meaningful compositional analysis, e.g. fractions F and H. In general, all fractions are characterized by a rather high level of aspartic and glutamic acids (17–24 μmol %) and a relatively constant proportion of non-polar amino acids (approx. 50 μmol %). There is, however, a definite trend toward a decreasing ratio of acidic to basic residues in progressing from the high to low molecular weight range although fractions J, K, and L do not follow the trend. Such a progression has also been reported for the fractionated proteins of nuclear envelope [17].

## DISCUSSION

This study has sought to accomplish three ends: first, to describe a procedure for the preparation of microsomal membrane free of non-membrane contaminants,

second, to chemically characterize that preparation and to demonstrate that its protein composition had not been altered by proteolysis, and third, to investigate techniques whereby the intrinsic membrane proteins might be successfully separated into individual molecular weight classes.

The first of these goals was accomplished with modification of existing procedures [15]. It is interesting to note that the addition of two washing steps employing 10 % potassium citrate results in a dramatic increase of the phospholipid to protein ratio from 0.47 [43] to 0.86. Morphologically, these two membrane preparations do not differ significantly; however, the marked difference in ratio emphasizes the need for thorough washing to remove intravesicular contents if a serious study of the intrinsic membrane proteins is to be made. Recently, Kreibich et al. [51] have reported on the selective release of vesicular contents by treatment of microsomes with low concentrations of deoxycholate. Membrane preparations exhibiting low phospholipid to protein ratios should be viewed with caution since they may reflect incomplete removal of non-membrane proteins.

Another interesting aspect of the overall chemical composition is that the carbohydrate content of the microsomal membrane closely resembles that of the nuclear envelope [15, 20] but is substantially less than values reported for various plasma membrane preparations [39]. Typically intracellular membranes show a low level of sialic acid; for example, both the nuclear envelope and the microsomal membrane contain approximately one-tenth to one-fifth the content of sialic acid found in the cell membrane. In addition, glycolipids also appear to be restricted to the plasma membrane.

The absence of RNA in the final preparation recommends the citrate washing procedure for removal of non-ribosomal membrane RNA which has been reported by numerous investigators (e.g. refs. 52–56). The chemical nature, biological role, and distribution of this tightly attached RNA are obscure; however, evidence has been recently introduced suggesting that mRNA directly interacts with the microsomal membrane [57–59] and that this association survives conditions used for removing ribosomes. In contrast to the microsomal membrane described in this report, the nuclear envelope as isolated by the discontinuous sucrose-citrate method contains approx. 3 % of a non-ribosomal RNA [15, 20] which is presumably related to the pore complex, a structural entity unique to the envelope.

Characterization of the constituent membrane polypeptides revealed molecular weights ranging from 171 000 to 16 000. Three of the major components, S-4 (60 000) through S-6 (49 000), account for approx. 40 % of the total membrane protein. Of this group, cytochrome *P*-450 is known to have a molecular weight of approx. 50 000 while the subunit of UDP-glucuronosyltransferase has a molecular weight of 59 000 (Gorski, J. and Kasper, C. B., manuscript in preparation). No evidence of proteolytic alteration of the sodium dodecyl sulfate polyacrylamide gel pattern could be detected, and in agreement with observations from other laboratories [13, 14, 60] no significant differences between the proteins derived from the membrane matrix of rough and smooth microsomes were observed.

Gel filtration of the sodium dodecyl sulfate-solubilized membrane proteins yielded a series of protein fractions of varying degrees of purity. Amino acid analysis of these fractions revealed the general tendency of a decrease in the ratio of acidic to basic amino acids correlated with a decline in molecular weight.

Electrophoretic analysis using sodium dodecyl sulfate polyacrylamide gels indicated that several fractions in the range of 60 000 or less contained predominantly one or at the most two components. These results imply that these fractions are composed of one predominant molecular weight class and clearly demonstrate the efficacy of the high resolution gel filtration system in greatly reducing the complexity of the initial mixture of membrane proteins. Nonetheless, effecting a complete separation of all the major microsomal polypeptides requires the use of ancillary techniques. These techniques and the characterization of individual membrane proteins and the establishment of their relationship to the nuclear envelope are the subject of continuing investigation in our laboratory.

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