Studies on Structural Units of Human Erythrocyte Membrane. I. Separation, Isolation, and Partial Characterization*

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ABSTRACT: The objectives of this research on erythrocyte membranes were to characterize membrane preparations chemically, examine their solubility characteristics, and separate and partially characterize the solubilized components. Mature erythrocyte membranes were studied because erythrocytes are devoid of subcellular organelles and their membranes may serve as prototypes for parenchymal cell membranes. The hemoglobin-free membrane showed barely detectable adenosine triphosphatase (ATPase) activity; amino acid analyses were reproducible and the membrane material was found to be a lipoglycoprotein with 55% protein, 35% lipid, and 10% carbohydrate. Quantitative solubility studies were done in detergents, bile salt, organic amides, acetic acid, sodium hydroxide (pH 13), and sodium sulfide. Almost complete solubilization was obtained in anionic and nonionic detergents and in urea. Column chromatography on polyacrylamide gels (Bio-Rad laboratories) was done. Studies were performed when membrane was dissolved in sodium dodecyl sulfate (SDS) using P-30 through P-300. All material was excluded on P-30. As the exclusion limit of the polyacrylamide gels increased from P-100 through P-300, the amount of material retarded increased until, with P-300, 98% of SDS-solubilized membrane was retarded. On P-100, there was a retarded and one excluded molecular weight class when membrane was dissolved in SDS, in the nonionic detergent, and in the bile salt; only an excluded molecular weight class was obtained with urea and sodium hydroxide. The columns separated SDS-solubilized membrane into two principle molecular weight classes, having reproducibly different amino acid distributions and different equilibrium characteristics. The weight-average molecular weight as determined by approach to sedimentation equilibrium experiments of the smaller molecular weight class isolated on gel filtration columns was 40,400; the molecular weight of its protein was 22,200. The different molecular weight classes are believed to represent different repeating units of erythrocyte structural membrane.

It has been stated that "structure within the living membrane is a treacherous problem for study; but no problem is more intriguing, and none in biophysics more important" (Casey, 1962). Despite the recognized importance of biological membranes, there is still disagreement about their over-all structure. As recently as 1961, Ponder concluded on reviewing the state of knowledge of the molecular structure of the red cell membrane, "If the reader of this chapter has concluded that the author is not convinced about the structure, or even the necessary existence of the cell membrane as it is generally described, he will not be far wrong." The author continues in his summary in a similar vein.

Advances have been achieved by high-resolution electron microscopy which has allowed direct visualization of cell membranes. From these studies, concepts of the dynamic molecular organization of the cell membrane have been obtained by Robertson (1959), Sjöstrand (1963), and Fernandez-Moran *et al.* (1964). It has been emphasized by Robertson (1959), Fernandez-Moran (1962), and Korn (1966) that quantitative information about the detailed chemical nature and interrelations of cell membranes is necessary to assign definitive structure. Useful information has been abstracted from work on synthetic membranes; this information and the theories synthesized therefrom have not been sufficiently tested in natural biological systems. In biological systems, pioneering work has been done on mitochondrial membranes whereby correlations of structure and function on an electron microscopic and a biochemical level have been done on a repeating unit.

A structural protein from mitochondrial membrane has been isolated, solubilized, and characterized by Criddle *et al.* (1962). In addition, lipoprotein structures have been isolated from myelin and have been subjected to limited biophysical and biochemical studies (Hulcher, 1963; Gent *et al.*, 1964). Membranes of the pleuropneumonia-like organism *Mycoplasma laidlawii* were dissolved by Razin *et al.* (1965) in sodium lauryl sulfate; the dissolved material consisted of ultracentrifugally

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"homogeneous" subunits which could be reaggregated to form membrane-like structures. Membranes obtained from *Micrococcus lysodeikticus* and *Sarcina lutea* were dissolved by detergents and two major components were seen in the analytical ultracentrifuge; when *M. lysodeikticus* membrane was dissociated by ultrasonic vibrations, a single peak was observed in the analytical ultracentrifuge (Salton and Netschey, 1965). The evidence for repeating units of membranes has been reviewed recently by Green and Perdue (1966).

In early studies on erythrocyte membrane, Moskowitz and Calvin (1952) isolated from human erythrocyte ghosts a large component, elinin, with a molecular weight of 4×10^7. This component was found to contain protein, carbohydrate, and lipid. Azen *et al.* (1965) separated protein components from human erythrocyte membrane using starch gel electrophoresis. Aggregates of membrane protein having a molecular weight of around 300,000 were solubilized with butanol from ox erythrocytes (Maddy, 1966). Mitchell and Hanahan (1966), using hypertonic NaCl solutions, effected partial solubilization of protein and lipoprotein fractions of human erythrocyte stroma. Morgan and Hanahan (1966), using ultrasonic vibration, isolated and partially characterized a soluble lipoprotein from erythrocyte stroma which contained 94% lipid and 6% protein and had an average molecular weight of 163,000. This fraction contained 68–80% of the original stromal lipid but only 9–20% of the original stromal protein.

We have studied the solubility characteristics of human erythrocyte membranes using different solvents. The solubilized components have been separated by gel filtration and the physical–chemical properties of one of the separated components have been investigated. This approach has been useful in comparing the results on human erythrocyte membrane to the results of parallel studies on human liver cell membranes conducted in this laboratory (R. T. Cook, W. E. Neeley, and S. Bakerman). Interpretations relating to the structure of cell membranes based on the physical–chemical data are presented.

Experimental Procedure

Preparation. Red cell membrane was prepared from recently outdated whole human blood obtained through the courtesy of the Community Blood Bank of Kansas City. Only group O, Rh-negative blood was used to eliminate possible "contamination" of the final preparation by A, B, and Rh-positive factors. Three pints of blood were usually combined and processed for each preparation. The cells were packed by centrifugation at 1000*g* for 30 min and the plasma and buffy coat were removed by suction; this and all subsequent procedures were carried out with temperatures maintained between 2 and 6°.

The main objective, which was to obtain cell membranes free of hemoglobin, was achieved by washing the erythrocytes repetitively with very large amounts of a solvent that has been shown to free the hemoglobin from the membrane. In a general way, the method of preparation consisted of diluting the erythrocytes with buffer, homogenizing to help release attached hemoglobin, and then collecting the residue by centrifugation. This process was continued until white membrane was obtained.

The details of the procedure, step by step, were as follows. The packed erythrocytes were diluted to 1500 ml with phosphate buffer, which was made up to pH 8 (Dodge *et al.*, 1963). The cells were homogenized in a Waring blender for 3 min and centrifuged for 4 hr (11,700*g*, 8500 rpm, Servall preparative centrifuge, GSA rotor), and the supernatant was decanted and discarded. The packed membranes were diluted to 900 ml, homogenized with a Waring blender for 3 min, then with several loose strokes with a Dounce homogenizer, and centrifuged for 1 hr (30,000*g*, 17,500 rpm, Spinco Model L preparative centrifuge, rotor no. 21). The supernatant was decanted and discarded, and the membranes were diluted, homogenized, and collected by centrifugation. These steps were repeated until the supernatant was clear. After about the seventh centrifugation, a light-colored layer was obtained in each of the centrifuge tubes on top of a darker pigmented layer. The light-colored layers were decanted off and combined; the dark layers were combined and reprocessed. Homogenization, centrifugation with elution, and combination of the light-colored layers were continued until white membrane was obtained. The white layer was collected and dialyzed against at least 1000 times its volume of distilled water. Just prior to use, the membrane, dispersed in water, was removed from the dialysis bags, combined, made up to a known volume in a volumetric flask, stirred constantly with a magnetic stirrer, and used as stock solution.

Concentrations. Stock solutions of water-dispersed membrane were used for subsequent studies. These were prepared by combining in a single dialysis bag several preparations which had been previously dialyzed exhaustively against water. The dispersion in the dialysis bag was thoroughly mixed and measured aliquots in triplicate were introduced with a pipet into boats shaped from heavy-duty aluminum foil. These boats had been dried at 70° for 24 hr, allowed to cool in a desiccator, and weighed. The pipets were rinsed with distilled water and the washings were also introduced into the aluminum boats. The dispersion in the boats was taken to near dryness by evaporation over nitrogen and finally to dryness in a vacuum oven at 70° for 24 hr and then weighed on an analytical balance (sensitivity ± 0.02 mg). The drying process was repeated until a constant weight was obtained (± 0.05 mg). These stock solutions of known concentration were then used as a source of material for quantitative lipid, carbohydrate and amino acid analyses, and solubility studies.

Some of the solvents used to solubilize the membrane in later procedures could not be completely removed by dialysis against water. In such instances, concentration was determined in terms of protein by quantitative amino acid analyses on aliquots of the solubilized material.

Hemoglobin. Hemoglobin was determined with a Cary recording spectrometer, Model 11, between 400 and 600 mμ on ten different white membrane preparations dispersed in water at concentrations of 2–6 mg/ml using matched cells with quartz end plates and a 5-cm path length. It should be noted that hemoglobin has a very strong absorption at 420 mμ (Soret band).

Adenosine Triphosphatase (ATPase)[1] *Activity.* A water dispersion of the membrane was assayed for ATPase activity. The incubation mixture consisted of 0.1 ml of each of the following: 0.05 M ATP, 0.0035 M DPNH, 0.01 M phosphoenolpyruvate, 0.004 M $MgCl_2$, and a total of one international unit of pyruvate phosphokinase and lactic dehydrogenase, all made up to 3-ml final volume in 0.033 M KH_2PO_4 (pH 7). Since ATPase from erythrocyte membrane is dependent on the Na^+:K^+ ratio, the ratio was adjusted to 5:1. Although sodium ion shows about a 30% inhibition of K^+ activation of pyruvate phosphokinase at the molarity of the ions (Kachmar and Boyer, 1953), essentially there would be no effect on the activity of the assay system since the concentration of pyruvate phosphokinase was present to a very large excess. Then 0.1 and 0.4 ml of the membrane preparation containing 1.5 mg/ml was used. The activity of ATPase was measured spectrophotometrically at 340 mμ to reflect the extent of the consumption of DPNH (Kornberg, 1955).

Electron Microscopy. Samples for the electron microscope were prepared by centrifuging a water suspension of the membrane preparation at 190,000*g* for 1 hr. The pellet was fixed with 2% OsO_4 and embedded in epoxy resin. The electron microscope used was an RCA Model EMU 3 G at 5 kv at a magnification of approximately 72,000.

Amino Acid Analyses. Amino acid analyses were done on the water-dispersed membrane, lipid-extracted protein, solubilized material, and the components separated by gel filtration. Aliquots of the solutions were hydrolyzed in evacuated tubes in 6 N HCl at 110° for 20 hr. When complete assignment of the amino acids was required, 40- and 80-hr hydrolyses were done. After hydrolysis, the HCl was evaporated to dryness in a stream of nitrogen and the dried samples were dissolved in 0.2 N citrate buffer of pH 2.2. Tryptophan was analyzed on separate aliquots hydrolyzed under vacuum with 3.7 M NaOH for 16–24 hr at 110°. The excess NaOH was neutralized with HCl. Separate aliquots were oxidized with performic acid and, after acid hydrolysis, used for the estimation of cysteic acid (Moore, 1963). The analyses were performed on a Spinco Model 120 amino acid analyzer (Spackman *et al.*, 1958) with accelerated system.

Carbohydrate. Total neutral sugars were estimated by the anthrone reaction using the method of analysis developed by Goa (1955), an equimolar mixture of mannose and galactose was used as standard. The different neutral sugars are now being examined in this laboratory by S. Bakerman, W. Gourley, S. Kobeck, D. McKillop, D. Pence, and G. Wasemiller (unpublished data). Until this is done, the neutral sugar content cannot be precisely assigned. Sialic acids were determined by the thiobarbituric acid assay of Warren (1958). The hexosamines were measured with a Spinco-Beckman amino acid analyzer, Model 120, following the procedures of Spackman *et al.* (1958). Glucosamine and galactosamine eluted at 51 and 77 ml, respectively, after phenylalanine.

Lipid Extraction and Analyses. Lipid extraction was done on aliquots of the stock solutions using the solvents recommended by Ways and Hanahan (1964). The objectives were different in as much as we also attempted to quantitate and analyze the lipid-extracted residue. Extractions were done on Teflon filter paper in a Soxhlet extractor, or repetitively in the same glass tubes. The amount of extracted lipid was determined by gravimetric methods and by phosphorus determination on the residue and lipid extract. The phosphorus was determined following combustion by the method of King (1932) and analyzed by the method of Bartlett (1959). Cholesterol was determined by the method of Rosenthal *et al.* (1957).

Solubility. In preliminary experiments, a broad spectrum of solvents was used to test solubility of membrane dispersed in water. It was readily apparent that the more conventional solvents, such as simple salts and buffers, did not dissolve membrane. Elevated pH was employed, in conjunction with some of the solvents listed below, because it tended to increase the solubility.

Solubility was tested in the following solvents: detergents (anionic, SDS, 0.5%, pH 11; nonionic, alkylphenol–ethylene oxide condensate type, Kyro EO, 0.5–1.0%, pH 9, Proctor and Gamble); bile salt (sodium deoxycholate, 0.5%, pH 11); organic amides (urea, 6 M, pH 11; guanidine-HCl, 1.5 M, pH 11); acid (acetic acid, 33%); base (NaOH, pH 13); and reducing agent (Na_2S, 1 M). A sufficient quantity of each solvent was added to the water-dispersed membrane to give the final concentration of solvent as listed above. The concentration of the membrane in the different solvents varied from 2 to 5 mg/ml. The pH was adjusted with 0.2 N NaOH to the respective pH; during the 5 days that the membrane was in contact with solvent, the pH decreased apparently due to ion binding by the membrane and/or solvent. The pH was readjusted to the value given above. The solutions were kept at 4° and then centrifuged for 1 hr (78,410*g*, 30,000 rpm, Spinco Model L preparatory centrifuge, rotor no. 30). The supernatants were carefully removed with a syringe without disturbing the residues and stored at 4°.

Membrane was solubilized also with 1-butanol, following the procedure of Maddy (1964). Aliquots of the aqueous phase were centrifuged as above.

The solutions were prepared for hydrolysis for

[1] Abbreviations used: ATP, adenosine 5′-triphosphate; SDS, sodium dodecyl sulfate; DPNH, reduced diphosphopyridine nucleotide; ESU, erythrocyte structural unit; ESP, erythrocyte structural protein; MSP, mitochondrial structural protein.

amino acid analyses in different ways. The solutions of the anionic and nonionic detergents and of the NaOH were hydrolyzed directly following addition of concentrated hydrochloric acid to make a final concentration of 6 N. The acetic acid and 1-butanol were evaporated to dryness and 6 N HCl was added. Aliquots of the solutions of bile salt, organic amides, and the Na_2S were dialyzed against large volumes of water and the precipitates were quantitatively removed from the dialysis bag and then hydrolyzed; this was done because these solvents caused spurious values to occur in the analyses of the amino acids of the membrane if acid hydrolysis was performed in their presence. The solubility of membrane components in the different solvents was calculated from the results of amino acid analyses before and after centrifugation.

Gel Filtration. The membrane preparations, which had been solubilized with different solvents and centrifuged for 1 hr (78,410*g*, 30,000 rpm, Spinco Model L preparatory centrifuge, rotor no. 30) to remove insoluble residue, were chromatographed by gel filtration. Polyacrylamides, Bio-Gels P-30 through P-300 (Bio-Rad Laboratories, Richmond, Calif.), were usually employed to isolate the solubilized components of the membrane. In early studies, Sephadex (Pharmacia Fine Chemicals, Inc., New Market, N. J.) had been used for the same purpose. There was no difference in the capacity of these different gels (Bio-Gels *vs.* Sephadex) to resolve the components.

The polyacrylamide gels were boiled with the solvent for 30 min to ensure hydration. P-100 through P-300 gels were wet sieved many times through a 115-mesh screen and only particles larger than 115 mesh were utilized.

In the experiments with P-30 through P-100, columns were 50 cm long and 1.9 cm in diameter. In the experiments with P-150 through P-300, columns were poured up to 350 cm; column heights of at least 200 cm were required to resolve components. Reasonable flow rates in columns of these lengths were obtained only following multiple sieving of the gel prior to use. Chromatography was carried out at room temperature. The gels were poured into the glass columns with constant stirring and finally equilibrated for at least 18 hr with solvent. The solvents used to elute the membrane preparations were the same as those used to solubilize the membrane, except the pH was 9 for those solvents where higher pH was used. The gels were supported by a coarse grid overlaid with 400-mesh nylon screen cloth (Tabor, Ernst, and Traber Co., N. Y.). The void volume was determined using high molecular weight dextrans.

The effluent from the columns was continuously monitored at 280 mμ and then collected using a Gilson Medical Electronics Model VL fraction collector (Gilson Medical Electronics, Middleton, Wis.). When necessary, the fractions were monitored at 215 mμ with a Cary recording spectrometer, Model 11. At 215 mμ, greater sensitivity was attained in detecting material in the eluent.

Physical Characteristics of Material Retarded on P-100 Columns. Physical constants, including partial specific volume, viscosity, sedimentation coefficient, and molecular weight, were obtained for that component of the cell membrane which was retarded on G-100; this material was identical in amino acid analyses with the material retarded on P-100. The membrane was solubilized with SDS and the retarded component was isolated on G-100 which had been equilibrated with the detergent. This component was collected, dialyzed exhaustively against at least 1000 times its volume of water, and then dialyzed with 0.1% SDS, 0.1 M NaCl, and 0.001 M Na_2HPO_4 (pH 9).

Partial Specific Volume. Density measurements were made using the density gradient column of Linderstrøm-Lang and Lanz (1938). Gradient columns made up of bromobenzene–kerosene solutions were prepared in an insulated water bath having an electronic temperature control, monitored by a Beckman thermometer to $\pm 0.003°$. In addition, density was also measured using a pair of similar 10-ml pycnometers. The densities of the water-dispersed membrane and of the retarded peak from molecular sieve chromatography were obtained.

Viscosity. Viscosity was measured in a calibrated semimicrocapillary viscometer in the same water bath as used in the density measurements. The kinetic energy correction, determined by measuring the flow of water at two different temperatures, was insignificant. The volume of the sample was 0.45 ml, and the flow time for water was 195 sec at 20°.

Analytical Ultracentrifugation. All experiments were carried out using a Spinco Model E ultracentrifuge equipped with phase-plate schlieren optics and RTIC unit. Kel-F centerpieces were used except for the synthetic boundary runs. When experiments were carried out near 30°, the rotors were heated to around this temperature just prior to the runs. All of the sedimentation velocity experiments were performed at 50,740 rpm using a 30-mm centerpiece and an An-E rotor. By using a longer path-length cell than the more conventional 12-mm cell, lower concentrations were employed and more accurate sedimentation coefficients were obtainable following extrapolation to infinite dilution. Sedimentation coefficients were corrected to $s_{20,w}$ values by the usual procedures. All of the molecular weight determinations were done by Archibald's (1947) approach to sedimentation equilibrium method at 17,980 rpm using the schlieren optical system and a four-place An-J rotor and employing both the meniscus and bottom of the cell. A schlieren diaphragm angle of 70° was used in all molecular weight calculations; at this angle, and with sufficiently long runs, the concentration gradient close to the meniscus was gradual enough to allow smooth-line extrapolation between meniscus and curvature. Photographic plates were measured with a two-dimensional microcomparator (Nikon) and also with a planimeter following photographic enlargement at a magnification of tenfold. The results by these two methods were almost identical. The initial concentrations were obtained from a separate ultracentrifuge run using a standard 12-mm, 4°

TABLE I: Amino Acid Content of Protein from the Human Erythrocyte Membrane.

Amino Acid	Residues/1000 Total Residues						
	Hr of Hydrolysis						
	16.5[a]	24[b]	42[b]	42[a]	78.5[b]	79[a]	Cor Values[f]
Lysine	49.8	50.0	50.6	48.2	50.4	49.4	47.7
Histidine	25.1	25.5	25.6	24.3	25.2	24.4	24.0
Arginine	50.5	49.3	51.7	48.8	50.3	50.9	48.2
Tryptophan	3.8	4.5	3.4	3.6	3.7	3.7	3.7
Aspartic acid	87.2	82.9	83.4	90.2	84.1	83.3	81.7
Threonine[c]	56.6	58.0	53.4	51.1	48.8	50.6	57.2
Serine[c]	71.1	62.7	63.0	57.8	49.4	50.8	70.4
Glutamic acid	129.2	125.8	124.5	123.8	128.0	126.3	121.2
Proline	50.8	51.1	49.2	48.5	50.4	49.4	47.8
Glycine	70.0	67.1	69.3	67.0	68.3	69.2	65.7
Alanine[d]	83.2	82.3	83.6	80.0	83.2	84.2	80.7
Cystine (half)	15.1	14.8	14.7	14.6	15.1	15.0	14.3
Valine[d]	56.6	57.7	68.0	71.9	69.0	73.1	68.6
Methionine[c]	24.3	24.3	22.5	24.3	22.1	19.3	24.3
Isoleucine[d]	39.1	47.7	46.5	49.2	53.5	51.4	50.6
Leucine[d]	118.6	123.2	121.0	120.9	128.1	128.3	123.5
Tyrosine[c]	25.5	26.0	24.0	26.2	22.5	23.6	25.4
Phenylalanine	43.3	46.6	45.7	49.3	47.9	47.0	44.8
Total residues	999.8	999.5	1000.1	99.97	1000.0	999.9	999.8
Per cent nitrogen[e]							16.5

[a] Represents one kind of preparation. [b] Represents a preparation different from *a*. [c] Extrapolated to zero time of hydrolysis. [d] Average of 78.5- and 79-hr hydrolysates. [e] Per cent of nitrogen in the amino acid residues. [f] Following the extrapolations as in *c* and *d* above, the sum of the residues was 1057; therefore, the corrected values were redistributed to represent 1000 residues.

sector, valve-type, synthetic boundary centerpiece. The molecular weight of this component was corrected for the weight of the bound detergent. This was done by dialyzing the solution exhaustively against distilled water to remove unbound SDS and then weighing the complex. The amount of protein in the complex was determined from the amino acid analyses; the amount of lipid was approximated from the amount of phosphorus and from the amount of cholesterol, and the amount of carbohydrate was approximated from the anthrone reaction. The procedure and methods of calculation were essentially those of Schachman (1957).

Experimental Results

Electron Microscopy. The electron micrographs of the membrane following dialysis against water showed double, closely parallel, irregular lines which resembled what one would expect to see in the plasma membranes of intact parenchymal cells similarly treated. Although no components other than membranes were recognized, electron micrographs do not serve as an optimal method for the recognition of "contamination" in this system.

Chemical Composition

Hemoglobin. There were no absorptions for the water-dispersed membrane between 400 and 600 mμ. In a 5-cm cell, it is possible to detect at 420 mμ 2×10^{-4} mg/ml of hemoglobin. Since the concentration of the membrane was at least 1000 times the concentration of hemoglobin in the subsequent measurements, then hemoglobin did not contribute to the chemical or physical analyses.

ATPase Activity. No ATPase activity was detected in 0.15 mg of the water-dispersed membrane. With 0.6 mg, there was a slight increase in absorption at 340 mμ. Increased amounts of membrane caused too much scattering of light at this wavelength, prohibiting accurate measurement of enzyme activity. One of the main purposes of the multiple washings was to reduce the concentration of ATPase below the level of detection by amino acid analyses. This was obviously achieved.

Amino Acid Analyses. The amino acid analyses of two different preparations of lipid-extracted red cell membrane protein are shown in Table I. The analyses for each timed hydrolysis are almost identical. Twenty

additional analyses were done on 20-hr hydrolysates, and there was no significant difference from these results. The significance of these data is that membrane can be prepared which has a reproducible protein content.

A summary of the analytical results is presented in the right-hand column of Table I. By grouping the amino acids, the following distributions are obtained: polar side chains, 476 (arginine, aspartic acid, glutamic acid, histidine, lysine, serine, threonine, and tyrosine); and nonpolar side chains, 524 (all others). Other groupings are as follows: basic groups, 120 (lysine, arginine, and histidine); acidic groups, 121 (glutamic and aspartic acids minus 82 amides); hydroxylic side chains, 153 (serine, threonine, and tyrosine); sulfur-containing amino acids, 39 (half-cystine and methionine); and heterocyclic-containing amino acids, 76 (tryptophan, proline, and histidine).

Protein Content. The protein content was calculated from quantitative amino acid analyses of 20-hr hydrolysates of different membrane preparations in which the concentration had been measured from the dry weight. An average value of 55% protein (range 48–68%) was obtained based on 12 different preparations.

Carbohydrate. The carbohydrate content was 10% of the dry weight of the membrane. Since sugar acids and alcohols were not assayed, the total carbohydrate content of the membrane may not have been determined. The distributions of the carbohydrates were: neutral sugars, 6%; sialic acids, 1.5; and hexosamines. 2.5%. Ludewig (1960) reported a value of 4.4% for the carbohydrate content of human red cell stroma. Maddy (1966) solubilized 90–95% of the protein of ox erythrocytes which contained 8% by weight of carbohydrate.

Lipid Content. Following exhaustive extraction with chloroform–methanol (2:1, v/v) about 15% of the phosphorus and about 20% of the cholesterol remained behind in the lipid-extracted residue. In addition, 75% of the neutral sugars, about 80% of the sialic acids, and 0.5–3% of the protein was soluble in chloroform–methanol. This solvent contained 33% of the dry weight of the membrane. The most reliable value for lipids was thought to be obtained by the difference between the weight of the preparations and the assignment of the protein and carbohydrate content. In this manner, the lipid content of the membrane preparations was 35%; the intact ghost contains 35% lipid (Maddy, 1964).

Summary of Chemical Composition. The chemical composition of the water-dispersed human erythrocyte membrane is given in Table II.

TABLE II: Chemical Composition of Human Erythrocyte Membrane.

Component	%
Protein	55[a]
Carbohydrate	10[b]
Lipid	35[c]

[a] Average of 12 different membrane preparations. [b] Total of values of neutral sugars, sialic acids, and hexosamines. [c] See text.

TABLE III: Solubility of Human Erythrocyte Membrane.

Solvent	Solubility (%)
SDS, 0.5%, pH 11	91.8[a] (range 82.2–100)
Kyro EO (Proctor and Gamble), 0.1%, pH 9	94.0[b] (range 92.8–95.9)
Sodium deoxycholate, 0.5%, pH 11	54.6
Urea, 6 M, pH 11	89.4[c] (89.2 and 89.6)
Guanidine, 1.5 M, pH 11	47.1
Sodium sulfide, 1 M	62.7
Acetic acid (66%)	82.5
Sodium hydroxide (pH 13)	76.8
Butanol[d]	15.3

[a] Average of ten different preparations. [b] Average of three different preparations. [c] Average of two different preparations. [d] Prepared by the method of Maddy (1964).

Solubility

The solubility of the membrane in a number of different solvents is shown in Table III. Almost complete solubilization of the membrane could be attained with the anionic detergent (SDS) and with the nonionic detergent (Kyro EO); cationic detergents were ineffective in solubilizing the membrane. The bile salt (deoxycholate) solubilized a little more than one-half of the membrane. The membrane was almost as soluble in 6 M urea as in the detergents. Elevated pH, in combination with these solvents, was important in effecting solubilization; elevated pH alone dissolved about 75% of the membrane. The values in Table III are not maximum values since increased concentration of solvent, elevated pH, and increased time of contact with solvent increase the per cent of the membrane dissolved.

Solubility studies were also done on the residue following lipid extraction. The maximum amount of the protein residue that could be dissolved was about 30%, and this was done with the detergents and urea.

Gel Filtration

Gel filtration, with both polyacrylamide and dextran, was employed to separate the components of the dissolved membrane into different molecular weight classes. In most of the experiments, the Bio-Gels (P-30 through P-300) were used; Sephadex G-100 was utilized in many of the early experiments. Identical elution patterns

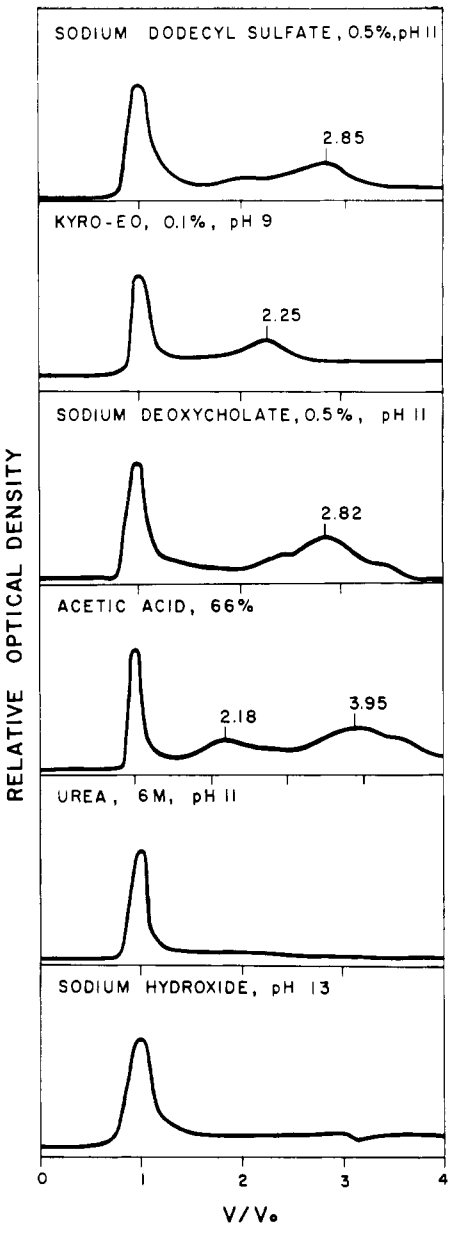

FIGURE 1: Gel filtration patterns on P-100 of human erythrocyte membrane solubilized with different solvents. For each solvent, material was obtained in the void volume, $V/V_0 = 1$; with urea and sodium hydroxide, no material was retarded; with the detergents and the bile salt, retarded material eluted as a single peak; with acetic acid, retarded material eluted as two peaks.

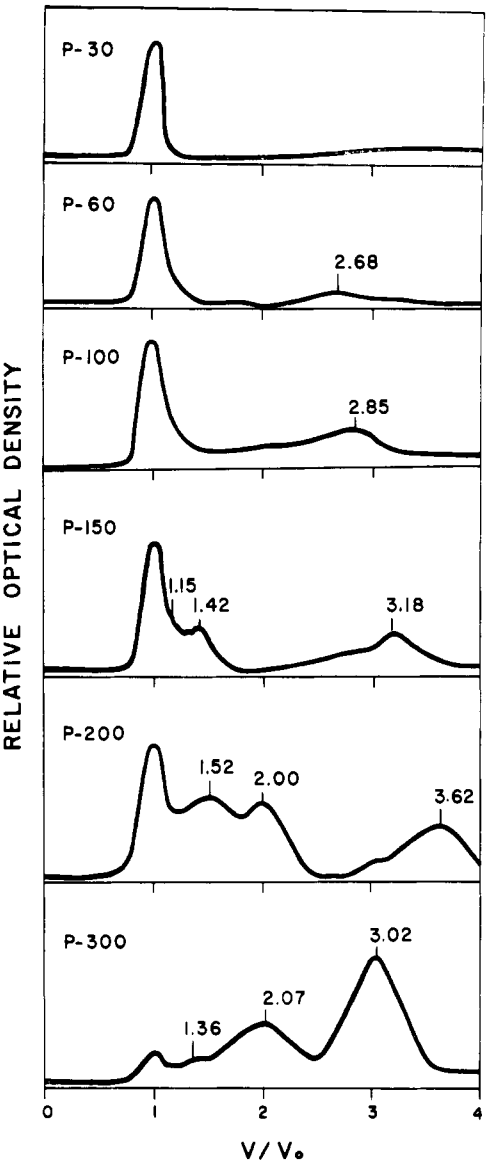

FIGURE 2: Gel filtration on P-30 through P-300 of human erythrocyte membrane solubilized with 0.5% SDS (pH 11). The columns were eluted with 0.3% SDS (pH 9). All material was excluded, $V/V_0 = 1$ on P-30. As the exclusion limit for the polyacrylamides increased, the amount of material retarded increased until with P-300, 98% was retarded.

were obtained regardless of whether the polyacrylamide or the dextran gels were used.

P-100 and G-100 Different Solvents. The membrane preparations which had been dissolved in different solvents (Table III) were eluted on P-100. The results are shown in Figure 1. The elution pattern showed a peak at the excluded volume, $V/V_0 = 1$, for all solvents. The elution pattern for SDS, Kyro EO, and deoxycholate showed one retarded component while the elution pattern of the acetic acid solution showed two retarded components. No components were retarded on the columns from the urea or sodium hydroxide solutions. Following elution from the columns the peaks were collected and dialyzed exhaustively against water, and aliquots were obtained for amino acid analyses. The concentration of the dissolved components in the absorption peaks was calculated from the amino acid analyses and is given in Table IV. The greatest per cent of retarded membrane, measured as protein, as compared to the amount of membrane in

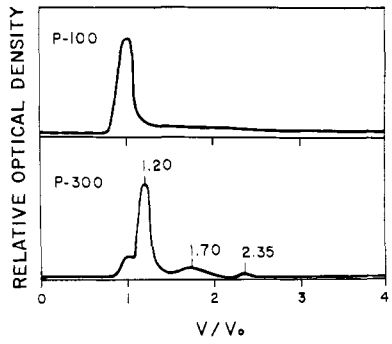

FIGURE 3: Gel filtration on P-100 and P-300 of human erythrocyte membrane solubilized with 6 M urea (pH 11). The columns were eluted with 4 M urea (pH 9). All material was excluded on P-100. The material (82%) was retarded with P-300.

TABLE IV: Relative Distribution of the Components of Human Erythrocyte Membrane Solubilized with Different Solvents and Separated on Column Chromatography Using Molecular Sieves P- or G-100.[a]

Solvent	Distrbtn of Components (%)	
	Excluded	Retarded
SDS, 0.5% pH 11	50	50[b]
Kyro EO, 0.1%, pH 9	70	30
Sodium deoxycholate, 0.5%, pH 11	25	75
Acetic acid, 66%	45	55

[a] Refer to Figure 1 for description of chromatographs. [b] Represents sum of retarded material in the original chromatograph plus the additional retarded component obtained following resolubilization of excluded component.

TABLE V: Relative Distribution of the Components of Human Erythrocyte Membrane Solubilized with SDS and Separated on Column Chromatography Using Molecular Sieves P-30 through P-300.[a]

Polyacrylamides (Bio-Gels)	Distrbtn of Components (%)	
	Excluded	Retarded
P-30	100	0
P-60	56	44
P-100	50	50
P-200	15	85
P-300	2	98

[a] Refer to Figure 2 for description of chromatographs.

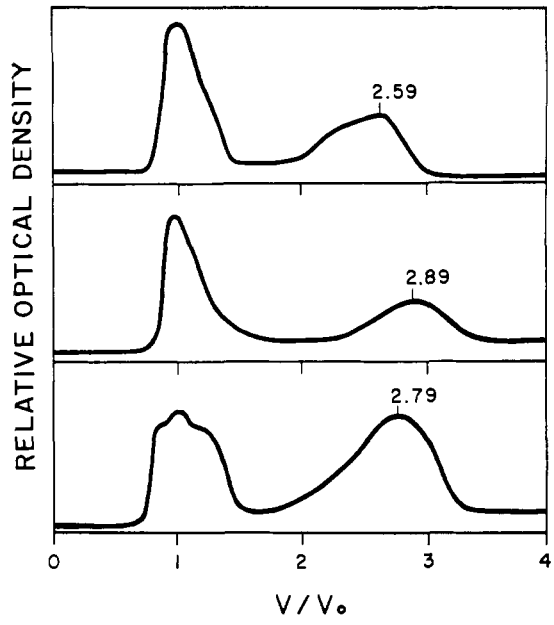

FIGURE 4: Equilibrium studies on P-100 of human erythrocyte membrane solubilized with 0.5% SDS, pH 11. (upper pattern) Gel filtration pattern following solubilization of membrane for 2 days. There was an excluded peak, $V/V_0 = 1$, and a single retarded component, $V/V_0 = 2.59$. (middle pattern) The material from the void volume (upper pattern) was collected and allowed to remain in contact with the detergent for an additional week. The elution pattern shows both excluded and retarded material. (lower pattern) The material from the retarded component (upper pattern) was collected and dialyzed against water. The precipitate was dried, resolubilized with SDS, and rechromatographed. The elution pattern again shows two peaks, excluded and retarded.

the excluded volume, was obtained in solutions of deoxycholate. The amount of material in the excluded volume and in the retarded peak could be shifted by varying the concentration of the detergent (see equilibrium studies with SDS below).

P-30 through P-300, SDS. The characteristics of the membrane dissolved in SDS were studied with all of the available polyacrylamide gels, *i.e.*, P-30 through P-300. This was done in order to ascertain the upper and lower exclusion limits of the membrane preparations. Figure 2 shows the results of these experiments. With the exception of P-30, all of the gels caused retardation of some of the membrane protein. There was only one retarded peak on the P-60 and P-100 columns. As the exclusive limit for the polyacrylamide gels increased, the amount of material retarded increased until with P-300, 98% of the protein was retarded. A comparison of the per cent membrane in the excluded volume to the per cent of membrane retarded, in terms of protein, is shown in Table V.

P-300. Four molecular weight classes of the SDS-

TABLE VI: Amino Acid Distribution[a] on Fractions of Equilibrium Experiments on Bio-Gel P-100 Columns.

	Residues/1000 Total Residues					
			Equilibrium Studies on P-100			
	Original Chromatograph		Fractions Derived from Excluded Material		Fractions Derived from Retarded Material	
Amino Acid	Excluded	Regarded	Excluded	Retarded	Excluded	Retarded
Lysine	51.6	61.6	46.5	63.8	58.0	62.3
Histidine	20.8	17.7	18.7	16.8	18.5	20.8
Arginine	46.7	44.2	47.5	40.4	51.2	42.1
Aspartic acid	78.8	100.2	81.4	107.1	91.3	95.3
Threonine	59.5	52.7	57.1	53.7	50.9	55.0
Serine	68.8	50.4	67.8	60.1	53.1	48.5
Glutamic acid	105.2	106.9	103.3	105.0	115.3	110.0
Proline	52.8	46.0	54.7	38.3	46.0	42.2
Glycine	86.3	123.0	84.9	126.9	110.1	129.0
Alanine	84.0	117.4	87.7	117.1	110.9	116.3
Valine	72.0	68.1	71.7	63.5	70.2	67.7
Methionine	21.0	21.4	22.0	17.3	20.2	20.6
Isoleucine	53.4	46.4	55.5	46.4	48.6	46.8
Leucine	118.9	86.4	123.3	81.6	92.2	79.8
Tyrosine	28.1	24.5	24.5	27.3	24.9	24.4
Phenylalanine	52.2	32.9	53.5	34.6	33.2	39.2

[a] Refer to Figure 4 and text for explanation of chromatographs; 22-hr hydrolysates.

dissolved membrane were eluted on P-300. The peaks were located at V/V_0 = 1, 1.36, 2.07, and 3.02. The membrane protein (98%) was retarded and 96% was equally distributed between two peaks, V/V_0 = 2.07 and 3.02. The recovery of membrane, in terms of protein, from the column was 97%. Further column studies were done with solutions of urea because this solvent, as opposed to the detergents, could be completely removed from the membrane by dialysis against water. On P-100 (Figure 3) there were no retarded components for the urea-dissolved material. On P-300 (Figure 3) the elution pattern of urea solutions does not show a molecular weight class as small as that observed with the detergents and the bile salt. The distribution of protein in the different peaks was: V/V_0 = 1, 18%; V/V_0 = 1.20, 42%; V/V_0 = 1.70, 29%; and V/V_0 = 2.35, 11%.

Equilibrium Studies, P-100 (G-100), SDS. In early studies on G-100, it was noted that when aliquots were obtained from the same preparations of SDS-dissolved membrane on successive days, the relative amount of the retarded material increased in proportion to a decrease in the excluded material. Therefore, it was thought that at least some of the material in the void volume contained higher molecular forms of the retarded material. This hypothesis was tested in the following manner. P-100 runs were made with the SDS-soluble material (Figure 4, upper pattern). The material from the void volume was isolated and allowed to remain in contact with SDS for an additional week. Chromatography was repeated and the elution pattern corresponded to the original chromatograph (Figure 4, middle pattern). Likewise, it was thought that it should be possible to polymerize the retarded material to the material in the void volume. This hypothesis was tested by collecting the material which was retarded (Figure 4, upper pattern) and dialyzing this material against water. The precipitate was dried, redissolved in SDS, and immediately rechromatographed. The chromatograph again showed a peak at the void volume followed by the material which was retarded (Figure 4, lower pattern). These equilibrium studies were done 20 times on five different preparations.

In summary, gel filtration on P-100 of SDS-dissolved membrane showed a peak at the void volume and a peak which was retarded; some of the material eluting in the void volume could be broken down to the material which was retarded; the material which was retarded could be aggregated to material eluting in the void volume.

The amino acid distributions of the lipoprotein fractions obtained from the equilibrium runs (Figure 4) are shown in Table VI. There is a difference in amino acid distribution in the original chromatographs (Figure 4, upper pattern) between the excluded and retarded components (Table VI, columns 1 and 2). When the fractions derived from the material which was *retarded* on P-100 were rechromatographed on P-100 (Figure 4, lower pattern) the distributions of

the amino acids in the excluded and retarded fractions were more nearly similar (Table VI, columns 5 and 6) not only to each other, but to the amino acid analyses of the retarded material from the other chromatographs. These results indicated that higher molecular weight aggregates of retarded material were present in the excluded volume. When the fractions derived from the material which was *excluded* on P-100 were combined and rechromatographed on P-100 (Figure 4, middle pattern), the distribution of the amino acids in the excluded and retarded fractions were again different; however, they were similar to the distribution of the amino acids in the excluded and retarded fractions of the original chromatographs (Table VI, columns 3 and 4). These results showed that there were higher molecular weight aggregates of the protein of the retarded class in the excluded volume and that there was different protein in the excluded volume that did not tend to dissociate into a smaller molecular weight class.

Equilibrium Studies, P-300 through P-100, SDS. As already noted, gel filtration on P-300 of the SDS-dissolved membrane separated the preparation into four molecular weight classes (Figure 2, lower pattern). Two of the four molecular weight classes, $V/V_0 = 2.07$ and 3.02, accounted for 96% of the protein introduced onto the column. The amino acid distribution of the material eluting at $V/V_0 = 2.07$ on the P-300 column was similar to the distribution of the material excluded on the P-100 columns; the amino acid distribution of the material eluting at $V/V_0 = 3.02$ on the P-300 column was similar to the distribution of the material retarded on the P-100 columns. Equilibrium studies on P-100 were done on these retarded components obtained from the P-300 columns. The material, eluating at $V/V_0 = 2.07$, was introduced onto a P-100 and showed the same pattern obtained in the previous equilibrium runs. The material, $V/V_0 = 3.12$, was introduced onto a P-100 and the usual pattern, as already described, was obtained. The amino acid distributions of the different peaks were similar to that shown in Table VI. These results indicate that the material eluting at $V/V_0 = 3.02$ on the P-300 column was similar to the material retarded on the P-100 columns, and the material eluting at $V/V_0 = 2.07$ on the P-300 column was similar to the material excluded on the P-100 columns.

Chemical and Physical Characteristics of Material Retarded on the P-100 Columns

Electron Microscopy. The material, which was retarded on the P-100 column, was dialyzed exhaustively against water, and the precipitate was collected by centrifugation. The electron micrographs of the reconstituted material (Figure 5) showed a pattern similar to that of the original membrane preparations. It should be noted that not all of the detergent could be removed from the preparation, and the contribution of detergent to the electron microscopic pattern cannot be determined.

Chemical Composition. A comparison of the results obtained for phosphorus, cholesterol, and anthrone on the membrane and on the excluded and the retarded components is given in Table VII. The results are reported in terms of micrograms per milligram of protein instead of dry weight because the SDS could not be completely removed following dialysis against water. The values were almost identical in the membrane preparation and in the excluded component. Phosphorus, cholesterol, and anthrone were increased in the retarded component about 20, 25, and 15%, respectively; whether these represent real differences or are within the errors of the methods of assay has not been determined. However, it can be concluded that the retarded and excluded components are lipoglycoproteins and that the per cent of lipid, carbohydrate, and protein is not markedly different than that of the original membrane preparation.

Partial Specific Volume, $\bar{V}$. The $\bar{V}$ was measured on both the water-dispersed membrane and on the retarded material. The $\bar{V}$ of the water-dispersed membrane was 0.86 and that of the retarded material, determined in the same solvent as used in the physical

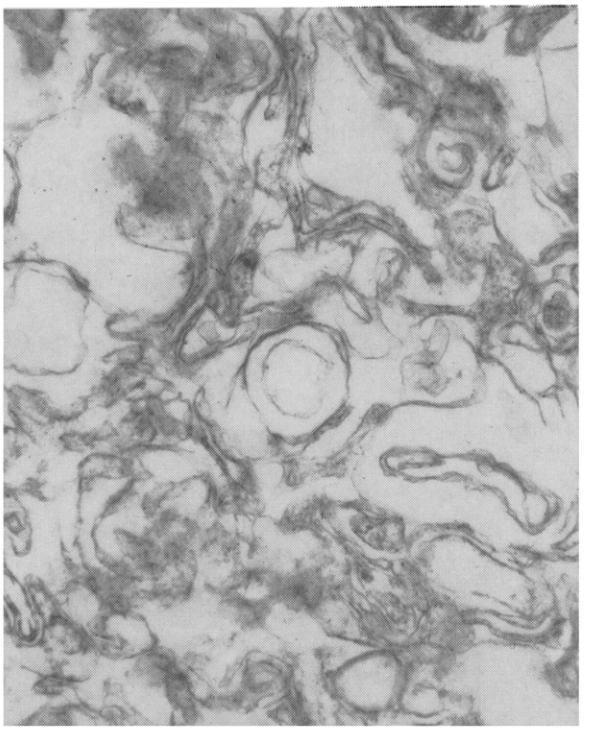

FIGURE 5: Electron micrograph of material retarded on P-100 columns. Membrane had been treated with 0.1% SDS for 2 days and chromatographed on P-100. The retarded material was collected, dialyzed exhaustively against water, and centrifuged at 190,000*g* for 1 hr. The pellet was fixed with 2% OsO_4, buffered with 2,4,6-trimethylpyridine (pH 7.45), sectioned, and stained with uranium acetate and embedded in epoxy resin. The electron micrographs were taken at an electron optical magnification of 24,000 and enlarged photographically to a final magnification of 72,000.

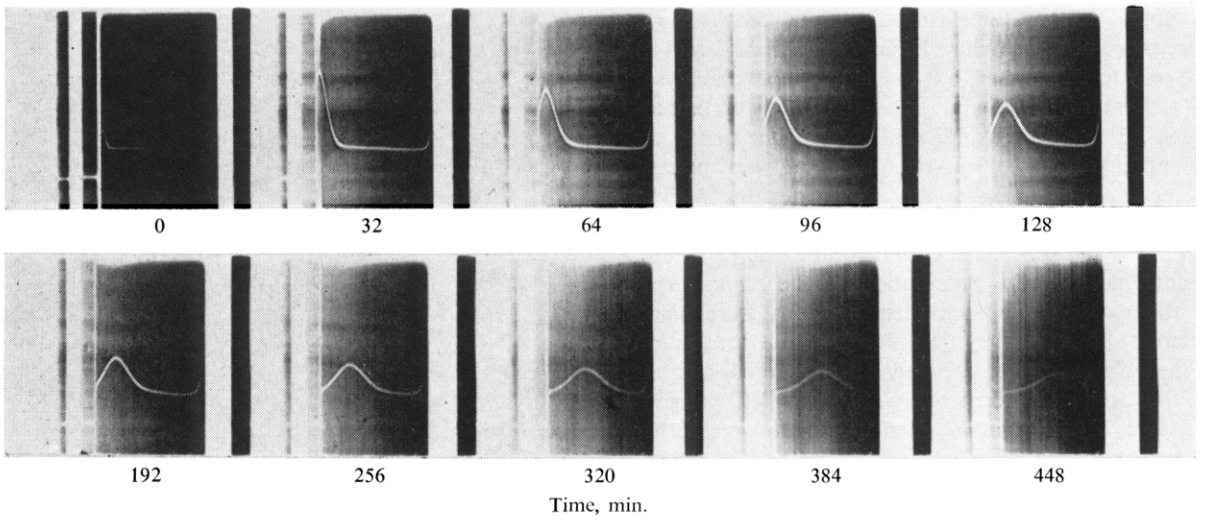

FIGURE 6: Sedimentation pattern (from left to right) of retarded material isolated from P-100 columns. The patterns were obtained at 25°, bar angle 70°, 50,980 rpm, in a cell containing a 30-mm centerpiece. Complete sedimentation required about 8 hr. The concentration of material was about 5 mg/ml and the solvent was 0.1% SDS, 0.1 M NaCl, and 0.001 M Na_2HPO_4. Following the initial photograph, the next four frames were made at 32-min intervals followed by 64-min intervals.

measurements, 0.1% SDS, 0.1 M NaCl, and 0.001 M Na_2HPO_4 (pH 9), was 0.84 and that of the solvent alone was 0.88. For proteins, the usual value for $\bar{V}$ is around 0.7; for cell membranes, the lipid content (35%) would tend to increase the $\bar{V}$, and the carbohydrate content (7%) would tend to decrease the $\bar{V}$. The justification for equating $\bar{V}$ of the insoluble membrane to that of the solubilized membrane was based on the findings of McMeekin and Marshall (1952) who found that the $\bar{V}$ of suspended insoluble protein was closely approximated to the value of soluble protein. The values for $\bar{V}$ were close to those observed in the high-density lipoproteins and to that found for the α-lipoproteins, 0.841 (Oncley *et al.*, 1947).

Viscosity. The intrinsic viscosity for the retarded material was found to be 0.147 dl/g.

Sedimentation. Sedimentation in the analytical ultracentrifuge revealed a single, slowly sedimenting peak that tended to gradually broaden during the 8 hr required for almost complete sedimentation (Figure 6). A plot of $1/s_{20,w}$ *vs.* concentration (Figure 7) was used for the extrapolation to zero concentration, and a value of 0.27 S was obtained for $s_{20,w}$ following corrections to the standard state for temperature, viscosity, and density.

Molecular Weight. Figures 8 and 9 show the analytical ultracentrifuge patterns for the boundary runs and the approach to sedimentation equilibrium runs,

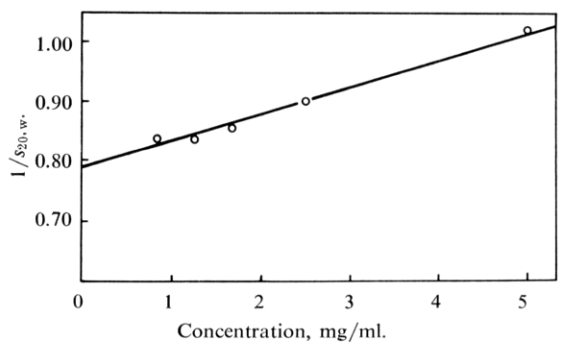

FIGURE 7: Concentration dependence of the reciprocal of the sedimentation coefficient of material retarded on P-100 columns.

TABLE VII: Lipid (Phosphorus and Cholesterol) and Carbohydrate (Anthrone) Composition of Human Erythrocyte Membrane and the Components (Retarded and Excluded), Separated on Bio-Gel P-100 Columns, Following Solubilization with SDS.

	Composition (μg/mg of protein)[a]		
		Bio-Gel P-100 Columns	
Assay	Membrane	Excluded	Retarded
Phosphorus	23	23	28
Cholesterol	197	200	249
Anthone	151	151	176

[a] The protein content was obtained by amino acid analyses. The results are reported in terms of micrograms per milligram of protein instead of dry weight because the SDS could not be completely removed following dialysis against water.

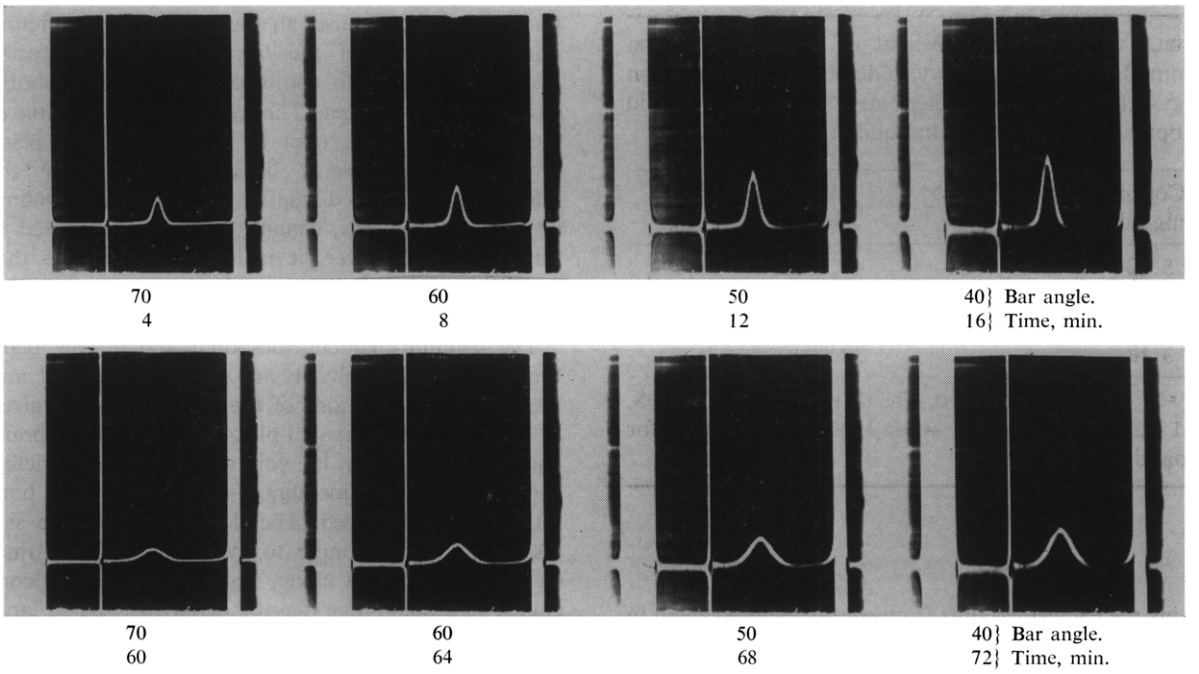

FIGURE 8: Analytical ultracentrifuge patterns from a run in the synthetic boundary cell on retarded material isolated from P-100 columns. The bar angles and time are indicated in the pictures.

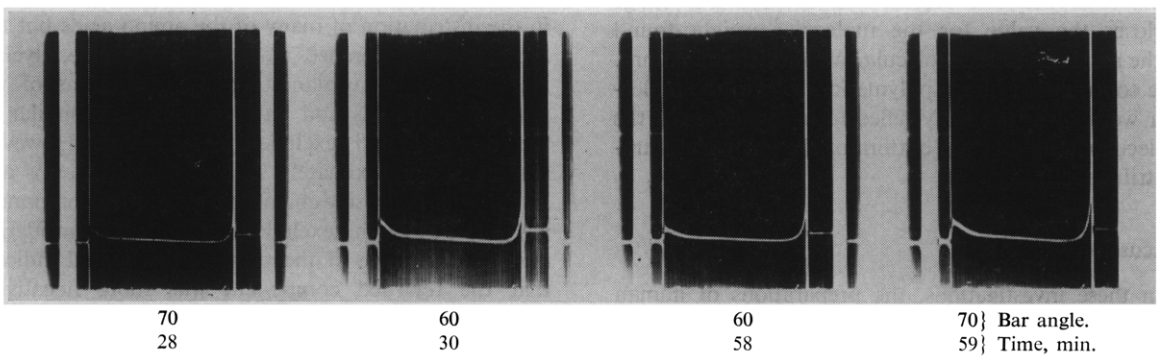

FIGURE 9: Analytical ultracentrifuge patterns during the approach to equilibrium on retarded material isolated from P-100 columns. The bar angles and time are indicated in the pictures.

respectively. Since the retarded component was complexed with SDS, the calculation of the molecular weight from the concentration gradient and from the concentration at the meniscus and bottom of the cell was corrected for the bound detergent using the equation (Hersh and Schachman, 1958) $M_c(1 - \bar{V}_c\rho) = M_r[1 - \bar{V}_r\rho + x(1 - \bar{V}_{SDS}\rho)]$, where M_c and $\bar{V}_c$ are the molecular weight and partial specific volume, respectively, of the complex of SDS with the retarded component; ρ is the density of the solution; M_r and $\bar{V}_r$ are the molecular weight and partial specific volume, respectively, of the retarded component; $\bar{V}_{SDS}$ is the partial specific volume of the SDS solution, and x is the number of grams of bound SDS per gram of retarded component. The values of $\bar{V}$, used in the calculations, were $\bar{V}_c = 0.84$, $\bar{V}_r = 0.86$, and $\bar{V}_{SDS} = 0.88$; the density of the solvent, ρ, was 1.002. The amount of bound detergent, x, was obtained from the difference between the dry weight following exhaustive dialysis against water and the chemical analyses for protein, lipid, and carbohydrate (Table VII). The amount of bound detergent of the sedimenting component, $x = 0.59$, was relatively high as compared to SDS–protein complexes; this can probably be explained by the fact that much of the SDS might be bound to the lipid of this component. The average of the molecular weights, calculated from the meniscus, was 40,400 and that from the bottom of the cell was 69,400 (Table VIII). It is interesting to compare these results on molecular weight with those obtained by

TABLE VIII: Molecular Weight of Retarded Fraction from Human Erythrocyte Membrane Isolated on Bio-Gel P-100 and Determined by the Archibald Approach to Sedimentation Equilibrium Method.[a]

Concn (mg/ml)	Speed (rpm × 10^{-3})	Molecular Weight[b] M_m	M_b
5.00	17,980	46,800	69,400
4.75		39,700	
3.75		36,100	
3.12		38,900	

[a] A $\bar{V}$ of 0.85 was used. The solvent was 0.1% SDS, 0.1 M NaCl, and 0.001 M Na_2HPO_4. [b] Corrected for bound SDS.

using the exclusion limits from gel filtration, keeping in mind the approximate nature of these values. For the SDS-dissolved material, no protein was retarded on the P-30 column (exclusion limit, 30,000) (Figure 2). However, the material used for molecular weight determinations was retarded on the P-60 column. This would place the molecular weight of the retarded component between 30,000 and 60,000; this range encloses the value for the molecular weight found at the meniscus in the molecular weight determinations. The contribution of the polymeric forms to the molecular weight was probably reflected in the values of the molecular weight at the bottom of the analytical ultracentrifuge cell.

Discussion

In these investigations, the preparations of human erythrocyte membrane have been characterized chemically. The solubility characteristics in different solvents have been studied and the solubilized components have been separated using gel filtration. There were two principle components isolated from these columns, and the smaller of these was physically and chemically characterized.

The chemical composition of the membrane is given in Table II. The most surprising value to us was the carbohydrate content. Considering the value, it would seem more appropriate to consider these membranes as lipoglycoproteins instead of the usual designation as lipoproteins.

The solubility characteristics of the membrane are shown in Table III. The erythrocyte membrane was 75% soluble at elevated pH. When the pH was lowered, the particles of membrane precipitated; the precipitate could be redissolved by increasing the pH. At elevated pH, the membrane fragments are probably negatively charged; increased solubility was most likely due to repulsion between the negatively charged particles. Elevated pH enhanced the solubility of membrane in both SDS and in the nonionic detergent. It may be that increased pH causes an alteration in the configuration of portions of the membrane, thus exposing hydrophobic areas to combine with the hydrophobic chain of the detergent. The mechanisms of action of the organic amides, urea and guanidine, are being investigated in several laboratories, whether the interaction involves disruption of hydrogen bonding, or other interactions, cannot, as yet, be predicted in this system. However, it is important to note that urea does not cause the membrane to depolymerize to a unit that is as small as obtained with detergents.

The column characteristics on P-100 of the membrane in different solvents are shown in Figure 1 and the relative distributions of the components are given in Table IV. With elevated pH and urea, the membrane components eluted in the void volume; with the detergents and bile salt, membrane components were both retarded and excluded. The detergents and bile salt disrupted the membrane to even smaller units than elevated pH or urea alone. From the data, it is concluded that membrane may be depolymerized to large aggregates at elevated pH and further depolymerized to two different subunits in the presence of detergent and bile salt.

The retarded and excluded components on P-100 columns, which have been equated to the two different components on P-300 columns, differed in their amino acid analyses (Table VI). Differences were observed in the distribution of many of the amino acids but the most striking differences were noted in leucine, glycine, alanine, and phenylalanine. The distributions of the polar amino acids and basic groups were similar in the retarded and excluded components. However, there were approximately 23 more acidic groups and 29 less hydroxylic side chains in the retarded component as compared to the excluded component. Considering only the influence of the protein, this would indicate that the retarded component was more negatively charged than the excluded component if amide groups were the same in both proteins and if these groups were not buried.

The retarded component was in partial equilibrium with its higher molecular weight forms on P-300 and P-100 columns. The conditions that tended to depolymerize this component were elevated pH, increased concentration, and time of contact with detergent. The total protein in this component following gel filtration of SDS-solubilized membrane plus gel filtration of the excluded component accounted for about 50% of the protein of the membrane. There was 20% more phosphorus in this component than in the original membrane. There also was 25% more cholesterol in this component than in the original membrane. Urea did not dissociate membrane into a molecular class that was retarded on P-100; if the retarded material was simple adsorbed protein, one would expect that the urea-solubilized subunits would be retarded on P-100. The collective data indicate that the lipid:protein ratio in this component was similar to that in the original membrane and that this component is a representative segment of the original

membrane. This segment sedimented as a single peak in the analytical ultracentrifuge and had reproducible amino acid analyses. The molecular weight was 40,400 and this component most likely represents one of the erythrocyte structural units (ESU). The molecular weight of the protein of the ESU, based on the approximate per cent protein in this segment, was 22,200. The molecular weight of the erythrocyte structural protein (ESP) is in close agreement with the molecular weight of the mitochondrial structural protein (MSP), 22,500 (Criddle *et al.*, 1966). Although the molecular weight of the ESP and MSP are of the same order of magnitude, there are differences in amino acid composition (Woodward and Munkres, 1966).

Results that tended to persuade us that the the ESU may be a fundamental structural component of other membranes were obtained with parallel studies on liver cell membranes (R. T. Cook, W. E. Neeley, and S. Bakerman, manuscript in preparation). Similarities, between the human erythrocyte membrane and human liver cell membranes were established for the following measurements: amino acid analyses of the original preparation, solubility properties, essentially identical equilibrium properties and elution patterns on the different gel filtration columns, and amino acid analyses of the different components.

Acknowledgments

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