Ginoza, W., Atkinson, D., and Wildman, S. (1954), *Science 119*, 269.
Jaenicke, R., and Lauffer, M. A. (1969), *Biochemistry 8*, 3083.
Khalil, M. T., and Lauffer, M. A. (1967), *Biochemistry 6*, 2474.
Lauffer, M. A. (1944), *J. Am. Chem. Soc. 66*, 1195.
Lauffer, M. A. (1962), *in* Molecular Basis of Neoplasia, Austin, Texas, The University of Texas M. D. Anderson Hospital and Tumor Institute, pp 180–206.
Lauffer, M. A. (1964), *Biochemistry 3*, 731.
Lauffer, M. A. (1966a), *Chimia Aarau 20*, 89.
Lauffer, M. A. (1966b), *Biochemistry 5*, 1952.
Lauffer, M. A. (1966c), *Biochemistry 5*, 2440.
Lauffer, M. A., Ansevin, A. T., Cartwright, T. E., and Brinton, C. C., Jr. (1958), *Nature 181*, 1338.
Lauffer, M. A., Shalaby, R. A., and Khalil, M. T. M. (1967), *Chimia Aarau 21*, 460.
Lauffer, M. A., and Stevens, C. L. (1968), *Advan. Virus Res. 13*, 1.
Lauffer, M. A., and Szent-Gyorgyi, A. G. (1955), *Arch. Biochem. Biophys. 56*, 542.
McLaren, A. D., and Rowen, J. W. (1951), *J. Polymer Sci. 7*, 289.
Paglini, S. (1968), *Anal. Biochem. 23*, 247.
Paglini, S., and Lauffer, M. A. (1968), *Biochemistry 7*, 1827.
Shalaby, R. A., and Lauffer, M. A. (1967), *Biochemistry 6*, 2465.
Smith, C. E., and Lauffer, M. A. (1967), *Biochemistry 6*, 2457.
Stauffer, H. P., Srinivasan, S., and Lauffer, M. A. (1969), Program and Abstracts of the Biophysical Society, Los Angeles, Calif., Abstract TPM-F1.
Stevens, C. L., and Lauffer, M. A. (1965), *Biochemistry 4*, 31.
Tremaine, J. H., and Lauffer, M. A. (1960), *J. Phys. Chem. 64*, 568.

Human Platelet Membrane Protein*

Ralph L. Nachman and Barbara Ferris

ABSTRACT: Protein derived from isolated membranes of washed human platelets was solubilized in sodium dodecyl sulfate and analyzed by acrylamide gel disc electrophoresis. The preparations obtained from intact as well as lipid extracted membranes contained a heterogeneous population of protein molecules with a predominant molecular weight range of 20,000 to 90,000. The protein residue from lipid extracted membrane retained antigenic integrity as evidenced by reactivity with antiserum to whole platelet protein. The nondelipidated membrane protein preparation reacted with an antiserum to isolated intact platelet membranes. These studies offer a biochemical approach to the future elucidation of the structure–function relationships of the membrane phenomena involved in primary hemostasis.

Cell membrane structure is probably an important determinant of the physiologic activities of a given cell. The participation of platelets in the complex events of the hemostatic process is an example of specific biologic activity. In contrast to erythrocytes and leukocytes, platelets adhere to collagen in the connective tissue of blood vessel walls and undergo marked structural and biochemical alterations during hemostasis (Spaet and Zucker, 1964; Lusher, 1967). There is good evidence that these reactions are membrane-associated phenomena (Marcus *et al.*, 1967). The primary role of the platelet membrane as a surface catalyst has been previously mentioned (Nachman and Marcus, 1968). In this study the protein constituents of isolated "intact" as well as lipid-extracted human platelet membrane preparations have been analyzed. The results indicate that the platelet membrane protein (as solubilized in sodium dodecyl sulfate) consists of a heterogeneous population of molecules of relatively low molecular weight. In addition, one of the membrane subunit proteins appeared to retain antigenic specificity in both delipidated and nonlipid extracted membrane preparations.

Materials and Methods

Platelet Membranes. Human platelets were separated from whole blood and platelet rich concentrates[1] as previously described (Nachman *et al.*, 1967a) and washed six times in Alsever's solution (Sigueria and Nelson, 1961) followed by four washes in Gaintner buffer (Gaintner *et al.*, 1962). Phase microscopy of multiple representative preparations revealed no red blood cells and less than one leukocyte per 10,000 platelets. Sucrose density gradient ultracentrifugation of platelet homogenates was performed as previously described (Marcus *et al.*, 1966). The platelet membrane consisting of plasma membranes as well as intracellular granule membranes was washed two times in 20–30 volumes of buffered saline (pH 7.4) (NaCl 0.15 M, phosphate 0.0175 M) at 114,000*g*. Representative batches of washed platelet membrane pellets

* Department of Medicine, New York Hospital–Cornell Medical Center, New York, New York 10021. *Received July 7, 1969.* This work was supported by grants from the American Cancer Society, T-483, U. S. Public Health Service, AM05615, and by a grant from the Kreizel Foundation.

[1] Kindly supplied by the New York Blood Center.

were delipidated by a modified Folch technique (Marcus and Zucker-Franklin, 1965). The membranes were mixed vigorously with a 20-fold volume of chloroform–methanol (2:1) at 4°. After 2 hr the precipitate was collected by centrifugation for 30 min at 10,000*g* at 4°. The precipitate was resuspended in chloroform–methanol as above and the extract repeated two more times. The residue was collected by filtration through Whatman No. 52 filter paper following the final extraction and dried in a desiccator. In subsequent determinations the absence of lipid phosphorus was assumed to indicate the absence of neutral lipid. Lipid extracted and untreated membrane preparations were dialyzed 18 hr against deionized water and lyophilized. Lyophilized membranes were stored at −20°. Delipidated platelet membranes utilized in early experiments were kindly provided by Dr. Aaron Marcus.

Polyacrylamide Gel Electrophoresis. Gel systems containing 10% acrylamide with 0.1% sodium dodecyl sulfate were prepared as previously described (Vinuela *et al.*, 1967). Gels containing 4% and 7.5% acrylamide with 0.1% sodium dodecyl sulfate were also used in several experiments. The effect of changing the concentration of the sodium dodecyl sulfate in the buffer gels was studied by comparing the observed electrophoretic patterns in 10% gels containing 0.1%, 0.5%, 1%, and 2% sodium dodecyl sulfate. The gels were stained with 1% Amido-Schwartz in 7.5% acetic acid for 90 min. Destaining was performed with the use of an electrolytic destainer (Shandon Scientific Co., Inc., Sewickley, Pa.).

Gradient Centrifugation. Sucrose density gradient centrifugation of platelet membrane solutions was performed at 16° in 5-ml cellulose nitrate tubes in a swinging bucket SW65LTi rotor with a Spinco Model L ultracentrifuge at 40,000 rpm for 18 hr. Gradients were 1% sodium dodecyl sulfate in 5–20% sucrose. Protein markers included purified immunoglobulin G (IgG) and purified type K Bence-Jones protein.

Column Chromatography. Membrane protein in 1% sodium dodecyl sulfate was chromatographed on Sepharose 4 B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The columns (2.5 × 98 cm) were packed and equilibrated with several column volumes of 1% sodium dodecyl sulfate. Pure molecular weight markers including human IgG (mol wt 160,000), bovine serum albumin (mol wt 67,000), and horse cytochrome (mol wt 12,400) were chromatographed under identical conditions using the same columns following extensive washing with buffer.

Molecular Weight Estimations in Sodium Dodecyl Sulfate Acrylamide Gels. This was performed as previously described using 1% sodium dodecyl sulfate gels (Shapiro *et al.*, 1967). The molecular weight markers included purified bovine serum albumin, egg ovalbumin (mol wt 45,000), beef pancreas chymotrypsinogen A (mol wt 25,000), and sperm whale myoglobin (mol wt 17,800). R_F values were determined by comparison with 1% bromophenol blue marker.

Protein determinations were performed by the Folin method (Kabat and Mayer, 1961). Total neutral sugars were estimated by the anthrone reaction using galactose as standard (Williams and Chase, 1968). Sialic acids were determined by a thiobarbituric acid assay method (Warren, 1959).

Residual phosphorus in the delipidated membrane preparation was measured as previously described (Bartlett, 1959) and expressed as phospholipid. The total lipid in the nondelipidated membrane material was calculated as 0.58 mg of total lipid/mg of protein based on the studies of Marcus *et al.* (1969).

TABLE I: Solubility of Lipid Extracted Platelet Membrane.

Solvent	Per Cent Protein Solubilized
1% sodium dodecyl sulfate	98
88% formic acid	86
Urea (6 M, pH 11)	44
Phosphate saline (pH 7.4)	4

Immunodiffusion studies were performed as previously described (Korngold *et al.*, 1962). Antihuman platelet protein and antimembrane serum were prepared as described (Nachman, 1965; Nachman *et al.*, 1967b). Antifibrinogen was obtained commercially (Lloyd Bros., Inc., Cincinnati, Ohio). Antiserum to erythrocyte stromal protein was kindly supplied by Dr. Hartwig Cleve, Cornell University Medical College.

Results

Solubility of the Delipidated Membrane Protein. The membrane protein powder was solubilized in a wide variety of solvents by mixing 4 mg with 1 ml of the solvent and vigorously stirring for 6 hr at room temperature. Protein that did not sediment at 114,000*g* for 1 hr was considered soluble. The relative solubility of the protein in different buffer systems is shown in Table I. More than 98% of the membrane powder was soluble in 1% sodium dodecyl sulfate. This was true using multiple specimens from different platelet batches. Accordingly, for the subsequent studies, platelet membrane protein was solubilized at 4 mg/ml in 1% sodium dodecyl sulfate.

Composition of the Membrane Preparation. For these studies the isolated membranes from multiple platelet fractionations were analyzed. The results are seen in Table II.

Characterization of the Proteins in the Membrane. Polyacrylamide gel electrophoresis of the sodium dodecyl sulfate treated lipid extracted membrane preparations using the standard 10% gels with 0.1% sodium dodecyl sulfate revealed multiple protein bands (Figure 1). Ten to fifteen separate bands were repeatedly seen following multiple gel analyses. Polyacrylamide gel electrophoresis of the sodium dodecyl sulfate treated intact nonlipid extracted membrane proteins revealed a similar degree of molecular heterogeneity but with greater resolution of the faster moving bands (Figure 3A). The electrophoretic patterns of the membrane proteins were

TABLE II: Composition of Platelet Membranes.

Component	Intact (%)	Defatted (%)
Protein	57	90
Lipid	33	1
Carbohydrate[a]	8	8

[a] Hexosamine not determined.

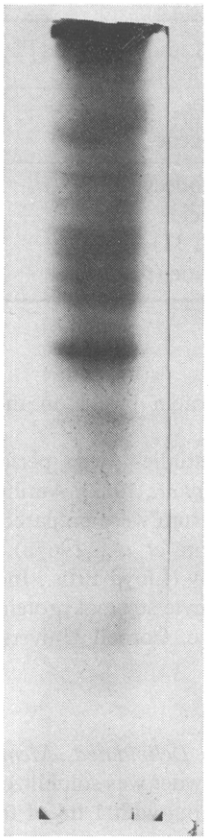

FIGURE 1: Heterogeneity of platelet membrane protein. Acrylamide gel electrophoresis of protein derived from isolated delipidated membranes. The protein was dissolved in 1% sodium dodecyl sulfate at a concentration of 4 mg/ml. Anode is at the bottom. Sample was applied at the cathodal end.

not significantly altered by using 7.5% acrylamide gels with 0.1% sodium dodecyl sulfate. Acrylamide gel patterns (4%) were consistently smeared and generally unsatisfactory. Increasing the sodium dodecyl sulfate concentration in the gel buffers to 0.5%, 1%, or 2% similarly did not significantly alter the electrophoretic pattern of the membrane protein

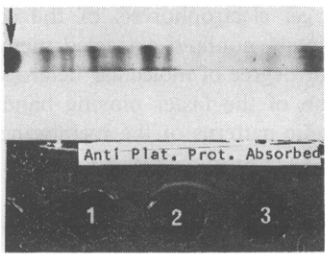

FIGURE 2: Antigenic reactivity of a slow moving component in the delipidated membrane protein preparation. Immunodiffusion of unstained 2-mm cut segments of the acrylamide gel containing the separated delipidated membrane protein. The second cut disc segment reacted with an antiserum to platelet protein absorbed with lyophilized human plasma. A simultaneously run uncut stained gel of the membrane protein is shown above the immunodiffusion study. The arrow indicates the position of the antigenically reactive protein. The top of the gel is at the left.

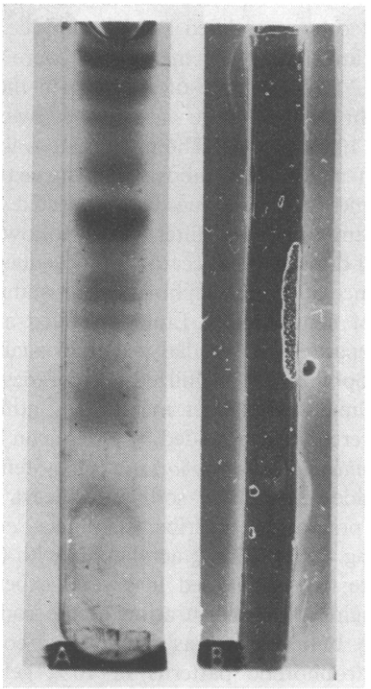

FIGURE 3: Antigenic reactivity of two separate components in the nondelipidated intact membrane protein preparation. (A) Stained half of the cut gel, anode is at the bottom. (B) Immunodiffusion analysis of the unstained half of the cut gel. The antiserum used on the left was antiplatelet protein absorbed with human plasma. The antiserum used on the right was antiplatelet membrane.

preparations. In order to more accurately determine the number of proteins in the membrane, gel electrophoretic studies were performed with membrane protein solubilized in 2%, 4%, and 6% sodium dodecyl sulfate. At these higher sodium dodecyl sulfate:protein ratios no significant differences in the gel electrophoretic patterns were observed.

Immunological Analysis of the Separated Proteins in the Platelet Membrane Solution. Despite an assumed high degree of denaturation of the individual membrane proteins, attempts were made to examine the residual antigenic integrity of the separated components by combining immunodiffusion techniques with acrylamide gel electrophoresis. The unstained acrylamide gel containing the separated defatted membrane proteins was carefully sliced into serial 2-mm disks. The gel segments were imbedded in agar plates and analyzed using various antisera. The second disk segment reacted in three separate experiments with antisera to whole platelet protein absorbed with lyophilized platelet free plasma (Figure 2). For demonstration purposes a stained gel, run at the same time, is shown. The immunologically reacting component is indicated by the arrow. No other antigenic reactivity was noted in the remaining segments of the gel. In addition, other antisera including antimembrane and antifibrinogen did not react when allowed to diffuse against the second disk segment as well as the remaining segments of the gel.

Antigenic reactivity of the nondelipidated membrane proteins was best demonstrated by a slightly different immunodiffusion technique. The unstained acrylamide gel containing the separated nonlipid extracted membrane proteins was cut in half along the longitudinal axis. One-half of the gel was

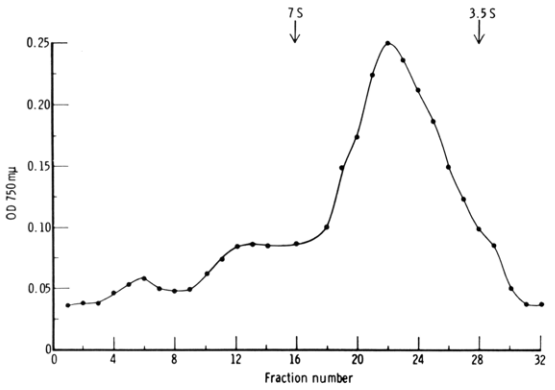

FIGURE 4: Sucrose density gradient (20–5% sucrose) of lipid extracted membrane protein in 1% sodium dodecyl sulfate. The markers used included purified human IgG and a purified type K Bence-Jones protein.

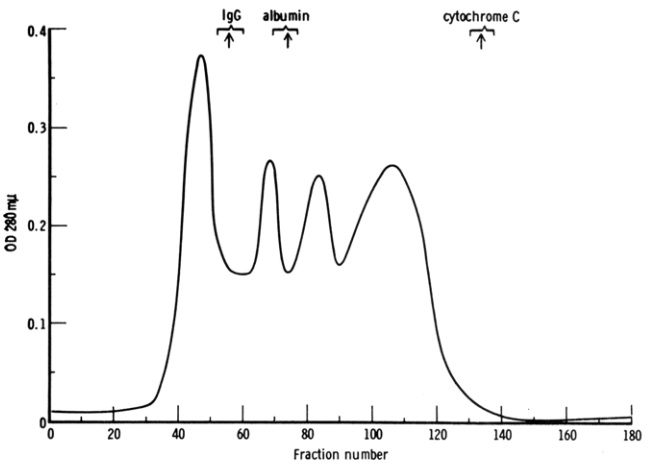

FIGURE 5: Gel filtration chromatography of lipid extracted membrane protein on Sepharose 4B in 1% sodium dodecyl sulfate. The elution volumes of the separate molecular weight markers are shown.

stained in the usual fashion (Figure 3A) while one-half was imbedded in agar (Figure 3B). Antibody troughs were cut adjacent to each edge of the gel and filled separately with platelet protein antiserum absorbed with plasma (left) and with antiplatelet membrane serum (right). The antiplatelet protein reacted with a slow moving component near the origin similar to the results obtained with the delipidated membrane protein. Antimembrane reacted with a relatively fast moving minor component. No reactivity was observed with antifibrinogen serum. None of the membrane protein preparations, either intact or lipid extracted, reacted with antierythrocyte stromal protein serum on immunodiffusion.

Molecular Weight Approximations of the Proteins in the Delipidated Membrane Preparation. Sedimentation velocity studies of the membrane proteins were performed in 5–20% sucrose containing 1% sodium dodecyl sulfate. The major protein peak appeared approximately midway between 3.5 S and 7 S markers (Figure 4). Gel filtration chromatography of the membrane protein is shown in Figure 5. Four major protein peaks were observed. The majority of the proteins separated in the molecular weight range between 67,000 (bovine serum albumin) and 12,400 (cytochrome *c*). A closer estimation of the molecular weights of the membrane protein population was obtained by comparison of the relative migrations in the sodium dodecyl sulfate gel of the individual proteins with known molecular weight markers (Figure 6). The majority of the proteins migrated with R_F values indicating a molecular weight from 20,000 to 90,000. Those components migrating only small distances into the gel were not accurately measured by this technique.

In order to assess more accurately the nature of the heterogeneity of the membrane protein, the high and low molecular weight peaks obtained from the Sepharose column were isolated, dialyzed extensively *vs.* water, and lyophilized. The protein fractions were reconstituted in 1% sodium dodecyl sulfate and analyzed by polyacrylamide gel electrophoresis (10% gels, 0.1% sodium dodecyl sulfate). The material from the high molecular weight peak scarcely entered the gel remaining essentially at the top. The material from the low molecular weight peak entered the gel and seven to eight bands were detected in the 20,000 to 70,000 molecular weight range of the gel.

Discussion

These studies were designed to characterize the types of protein which make up a significant portion of the human platelet membrane and to define the degree of heterogeneity. A major difficulty in designing studies of this type involves the marked insolubility of cell membranes in standard buffer systems. In previous investigations of red cell membranes (Bakerman and Wassmiller, 1967), *Bacillus subtilis* (Bishop *et al.*, 1967), as well as mycoplasma (Engelman *et al.*, 1967), the anionic detergent sodium dodecyl sulfate has proven to be a useful membrane solubilizing agent. Rosenberg and Guidotti (1968) reported that more than 98% of the erythrocyte membrane protein was solubilized in 1% sodium dodecyl sulfate. Our studies indicate that the platelet membrane behaves in a similar manner (Table I).

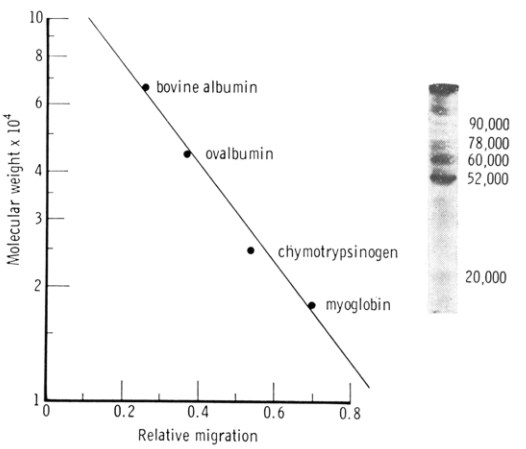

FIGURE 6: Molecular weight estimation of the separated lipid extracted membrane proteins. A semilog plot comparing the relative migrations in sodium dodecyl sulfate gels of purified markers to molecular weights is shown on the left. A stained gel of the membrane protein is shown on the right with superimposed approximate molecular weight values of the individual bands as calculated from the R_F values.

Studies on membrane proteins in other cell systems have developed along two separate lines, one involving lipid extracted membrane preparations, the other intact nondelipidated membranes. It was deemed advisable in most of our work to utilize both approaches in an attempt to relate lipid–protein interactions to potential membrane function. Practically no lipid was detected in the chloroform–methanol extracted platelet membrane preparations while approximately 33% of the intact membrane consisted of lipid (Table II).

Marcus *et al.* (1969) have recently reported an extensive analysis of the lipid composition of isolated platelet membranes. Our studies revealed that carbohydrate constituted approximately 8% of both platelet membrane preparations. It should be noted that this figure is probably slightly lower than the true value since hexosamines were not included in these determinations.

Marked heterogeneity was noted in the acrylamide gel electrophoresis of the platelet membrane proteins. Ten to fifteen separate bands were consistently seen. The possibility must be considered that some of the separate bands detected in the acrylamide gel system might represent subunit monomeric polypeptides split off larger native polymeric molecules by the action of the anionic detergent (Bakerman and Wassmiller, 1967). In order to evaluate this possibility further, an attempt was made to determine the relationship of the components of molecular weight 20,000–90,000 to the larger molecular weight protein which remained on the top of the gels following electrophoresis. Solubilization of the membrane preparations in sodium dodecyl sulfate solutions as high as 6% as well as variation in the concentration of acrylamide in the gels and increasing the sodium dodecyl sulfate concentration in the gel buffers did not significantly alter electrophoretic patterns of the membrane proteins. The heterogeneity of the membrane protein preparation was further confirmed when four major peaks were eluted by gel chromatography (Figure 5). The isolated protein from the high and low molecular weight peaks from Sepharose chromatography yielded different bands in polyacrylamide gel electrophoresis. Similar observations were noted by Rosenberg and Guidotti (1968) working with human erythrocyte membranes. The results suggest to us that human platelet membrane proteins are really proteins of various size and not specific aggregates of membrane subunits. We have previously identified three separate membrane antigens in intact membrane preparations by immunological techniques (Nachman and Marcus, 1968). The platelet contractile protein, thrombosthenin, has also been isolated from intact subcellular membrane preparations (Nachman *et al.*, 1967a). It is also likely that platelet fibrinogen is intimately associated with the platelet membrane (Nachman *et al.*, 1967b). It should be emphasized that the membrane preparation utilized in this study refers not only to plasma membranes but also to intracellular granule membranes (Marcus *et al.*, 1966). Thus one might expect an increased number of individual different proteins in comparison to a pure plasma membrane preparation such as that derived from human erythrocytes (Schneiderman and Junga, 1968).

Immunologic analysis of the separated proteins following acrylamide gel electrophoresis revealed residual antigenic activity in both delipidated and nonlipid extracted membrane preparations. Antiplatelet protein absorbed with human plasma reacted with a slow migrating component in both systems. The exact nature of this protein reactant remains to be determined. An additional antigenically reacting protein was detected in the intact membrane solution with the use of an antiserum against isolated whole membranes. Greater degrees of immunologic reactivity might have been expected when the nondelipidated membrane protein was analyzed in view of the residual lipid which theoretically could contribute to the steric integrity of the antigens. It should be noted that these immunologic experiments depend on the diffusion of sodium dodecyl sulfate soluble proteins into agar containing no sodium dodecyl sulfate. Thus the lack of antigenicity of some of the subunit membrane proteins might reflect an insolubility in the agar medium.

The denaturing effect of sodium dodecyl sulfate solubilization may vary with respect to individual proteins. Thus NADH dehydrogenase solubilized from bacterial membrane by sodium dodecyl sulfate was activated by detergent while solubilized succinic dehydrogenase was irreversibly inhibited by the detergent (Bishop *et al.*, 1967). The exact mechanism of sodium dodecyl sulfate solubilization of membrane protein remains unclear but data in some cell systems suggest that the solubilization may involve selective disaggregation of membrane components (Bishop *et al.* 1967). The recent work of Rottem *et al.* (1968) and Engelman *et al.* (1967) suggests that sodium dodecyl sulfate solubilized membrane material does not consist of homogeneous lipoprotein subunits but of separate sodium dodecyl sulfate–lipid and sodium dodecyl sulfate–protein complexes. It is clear that sodium dodecyl sulfate leads to dissociative effects on membrane components. The marked protein heterogeneity observed in our studies following sodium dodecyl sulfate solubilization of the platelet membrane would favor the concept that the solubilized membrane consists of multiple sodium dodecyl sulfate–protein complexes. Bont *et al.* (1969) similarly demonstrated heterogeneous material when isolated rat liver plasma membranes were solubilized by sodium dodecyl sulfate. Different results, however, have been observed with sodium dodecyl sulfate solubilized mycoplasma membranes (Razin *et al.*, 1965). The possibility should be considered that membranes from different cell systems may respond differently to sodium dodecyl sulfate solubilization.

The molecular weight studies on the lipid extracted membrane preparations indicated that the major population of proteins was composed primarily of small molecular weight species ranging from 20,000 to 90,000 (Figure 6). Larger molecular weight components were also detected; however, these were not well clarified in the acrylamide gel systems due to lesser degrees of gel penetration. The major protein peak in sedimentation velocity studies separated between 3.5 S and 7 S markers. It is possible that this relatively high sedimentation velocity separation represents aggregation of small subunits which were subsequently separated in the more discriminating gel electrophoresis system. Previous studies on erythrocyte membrane protein have suggested that most of the material is in the molecular weight range of 50,000 (Rosenberg and Guidotti, 1968) although smaller values closer to 20,000 have also been reported (Bakerman and Wassmiller, 1967). The structural protein of mitochondria has been reported to have a molecular weight of 22,500 (Criddle *et al.*, 1962). It would thus appear that the membrane proteins in diverse biologic systems are of relatively small molecular weight.

Previous studies on Neurospora mitochondria and human red cell membranes have revealed considerable homology as determined by two-dimensional peptide mapping (Schneider-

man and Junga, 1968). Repeated attempts to analyze the platelet membrane protein by an identical technique yielded technically unsatisfactory peptide patterns. The lack of cross-reactivity of platelet membrane protein with antierythrocyte stromal protein is suggestive evidence for the relative structural individuality of the platelet membrane. Recent electron microscopic evidence has also supported the concept of a relatively unique membrane surface specialization of platelets (Nakao and August, 1968).

These studies define to a greater extent than heretofore available the nature and structure of the platelet membrane. The protein in the membrane of the human platelet thus appears to be a heterogeneous collection of molecules. It is possible that the characteristic platelet responses to external stimuli such as the presence of ADP, thrombin, and collagen may eventually be correlated with unique structural features of the specific membrane proteins. In order to characterize these processes it will be necessary to separate and analyze the individual protein components. These studies are now in progress.

References

Bakerman, S., and Wassmiller, G. (1967), *Biochemistry 6*, 1100.

Bartlett, G. R. (1959), *J. Biol. Chem. 234*, 466.

Bishop, D. G., Rutberg, L., and Samuelson, B. (1967), *Europ. J. Biochem. 2*, 454.

Bont, W. S., Emmelot, P., and Vas Dios, H. (1969), *Biochem. Biophys. Acta. 173*, 389.

Criddle, R. S., Bock, R. M., Green, D. E., and Tisdale, H. (1962), *Biochemistry 1*, 827.

Engelman, D. M., Terry, T. M., and Morowitz, H. (1967), *Biochim. Biophys. Acta 135*, 381.

Gaintner, J. R., Jackson, D. P., and Maynert, E. W. (1962), *Bull. Johns Hopkins Hosp. 111*, 185.

Kabat, E. A., and Mayer, M. M. (1961), Experimental Immuno-Chemistry, 2nd ed, Springfield, Ill., Charles C Thomas, p 557.

Korngold, L., van Leeuven, G., and Engle, R. L., Jr. (1962), *Ann. N. Y. Acad. Sci. 101*, 203.

Lusher, E. F. (1967), *Brit. J. Haematol. 13*, 1.

Marcus, A. J., Ullman, H. L., and Safier, L. B. (1969), *J. Lipid Res. 10*, 108.

Marcus, A. J., and Zucker-Franklin, D. (1965), *J. Am. Oil Chemists' Soc. 42*, 500.

Marcus, A. J., Zucker-Franklin, D., Safier, L. B., and Ullman, H. L. (1966), *J. Clin. Invest. 45*, 14.

Marcus, A. J., Zucker-Franklin, D., Ullman, H. L., and Safier, L. B. (1967), *in* Physiology of Hemostasis and Thrombosis, Johnson S. A., and Seegers, W. H., Ed., Springfield, Ill., Charles C Thomas, p 113.

Nachman, R. L. (1965), *Blood 25*, 703.

Nachman, R. L., and Marcus, A. J. (1968), *Brit. J. Haematol. 15*, 181.

Nachman, R. L., Marcus, A. J., and Safier, L. B. (1967a), *J. Clin. Invest. 46*,1380.

Nachman, R. L., Marcus, A. J., and Zucker-Franklin, D. (1967b), *J. Lab. Clin. Med. 69*, 651.

Nakao, K., and August, A. A. (1968), *Nature 217*, 960.

Razin, S., Morowitz, H. I., and Torry, T. M. (1965), *Proc. Natl. Acad. Sci. U. S. 54*, 219.

Rosenberg, S. A., and Guidotti, G. (1968), *J. Biol. Chem. 243*, 1985.

Rottem, S., Stein, O., and Razin, S. (1968), *Arch. Biochem. Biophys. 125*, 46.

Schneiderman, L. J., and Junga, I. G. (1968), *Biochemistry 7*, 2281.

Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.

Sigueria, M., and Nelson, R. A., Jr. (1961), *J. Immunol. 86*, 516.

Spaet, T. H., and Zucker, M. B. (1964), *Am. J. Physiol. 206*, 1267.

Vinuela, E., Algranati, I. D., and Ochoa, S. (1967), *Europ. J. Biochem. 1*, 3.

Warren, L. (1959), *J. Biol. Chem. 234*, 1971.

Williams, C. A., and Chase, M. W. (1968), Methods in Immunology and Immunochemistry, Vol. II, New York, N. Y., Academic, p 288.