BBA 73131

Comparative studies of erythrocyte membranes by gel electrophoresis

Current concepts of membrane structure are based upon interacting systems of proteins and lipids[1–4]. An understanding of the molecular nature of membrane structure(s), therefore, requires a detailed knowledge of the number and types of membrane proteins. In the present study, a method for the solubilization and electrophoresis of whole erythrocyte membranes has been developed and used to fractionate the protein components from five different mammalian species in the hope that this information will further our knowledge concerning the types of membrane structures which can occur.

Erythrocyte membranes were prepared from fresh blood according to the procedure of DODGE *et al.*[5] and lyophilized before use in electrophoresis experiments. Membrane samples were dissolved at a concentration of 10 mg/ml in 2.4% (w/v) sodium dodecyl sulfate–0.1 M phosphate buffer (pH 7.8) containing 0.14 M mercaptoethanol and 20% (v/v) glycerol and allowed to stand 18–20 h at room temperature under a nitrogen atmosphere. Acrylamide gels (6%) were prepared and run according to the procedure of SHAPIRO *et al.*[6] on samples containing 100 μg of membrane protein. Gels were stained for protein using 0.025% Coomassie Blue and destained with 7% acetic acid. Carbohydrate bands were visualized by the periodate–Schiff method of ZACHARIAS *et al.*[7].

Fig. 1 shows the protein patterns obtained by sodium dodecyl sulfate acrylamide gel electrophoresis of solubilized (left to right) human, dog, cat, cow and horse erythrocyte membranes. A number of features of these patterns are worth noting. (1) Considerable heterogeneity of the erythrocyte membrane proteins is evidenced by the large number of separate bands. Visual inspection indicates at least 12 major bands for the human membranes and 15 for the dog. A densitometer tracing of the human membrane gel pattern indicated approx. 25 bands. (2) There is no apparent common pattern of proteins among the various species to suggest a common membrane structure based on similar proteins. The slow-running doublet which appears at the top of all the gels appears to be the only significant common feature. This material can be extracted from the membranes by EDTA and appears to be identical to the protein isolated by TILLACK *et al.*[8] from a number of different erythrocyte membranes. (3) The molecular weights of the components vary from about 20000 to 190000 when compared to a series of protein standards. However, the acceptance of the absolute values of the molecular weights should be viewed with caution since deviations from linearity in the molecular weight plots are likely near the ends of the gels, and highly hydrophobic membrane proteins may show altered behavior in sodium dodecyl sulfate solutions when compared to the common standard proteins. (4) The protein patterns from human erythrocyte membranes are virtually identical to those reported recently by LENARD[9] in a description of a similar sodium dodecyl sulfate solubilization and electrophoresis technique. This is particularly important since a number of fractionation techniques for erythrocyte membrane proteins have not proven to be readily reproducible.

The periodate–Schiff staining patterns for electrophoretic gel separations of

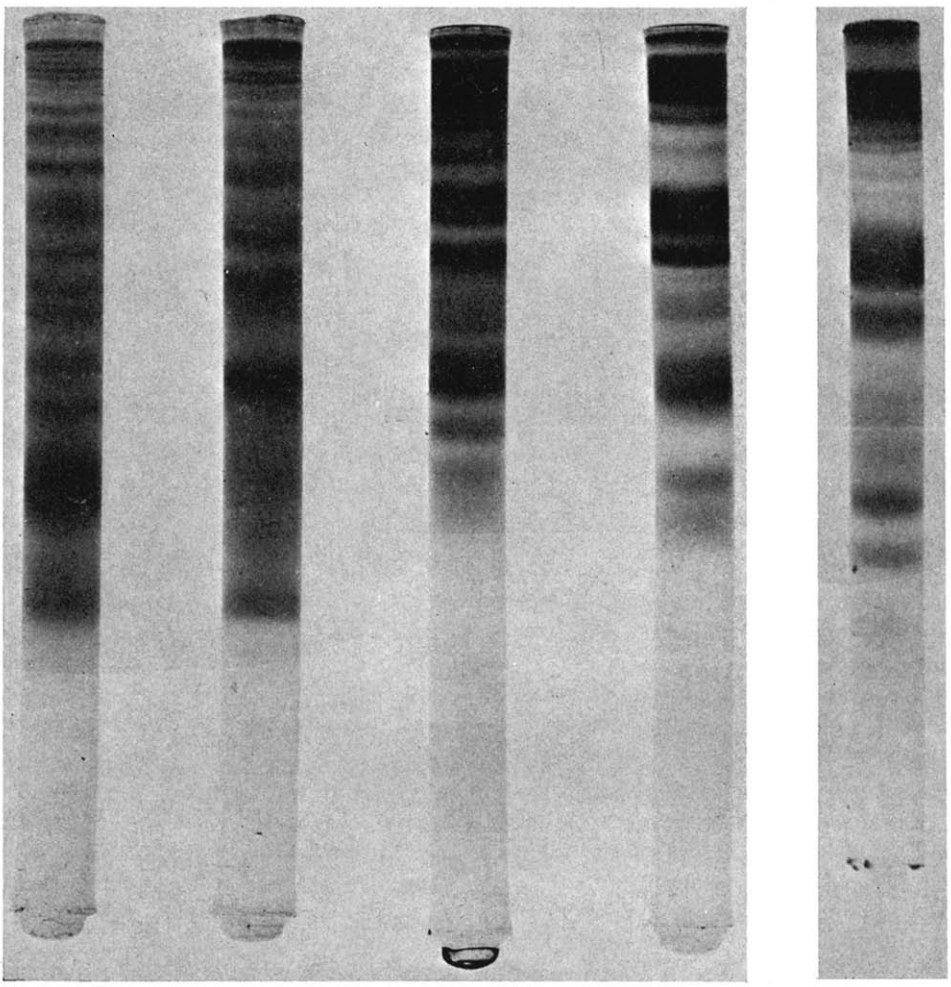

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis protein patterns of sodium dodecyl sulfate solubilized erythrocyte membranes. Left to right: human, dog, cat, cow, horse. Gels were run in 0.1 % sodium dodecyl sulfate, stained with Coomassie Blue and destained with 7 % acetic acid.

membrane proteins from the same species are shown in Fig. 2. The major fast-running band on these gels does not correspond to any protein band. This was identified as a lipid band by an exchange experiment in which [^{14}C]cholesterol was incorporated into human red cell membranes[10]. The radioactivity in the gel after electrophoresis of the membranes was shown to be coincident with the fast-running carbohydrate band by slicing and counting[11] duplicates of gels stained for carbohydrate. LENARD[9] has made a similar identification using lipid extraction before electrophoresis. The nonlipid bands which stain for carbohydrate are assumed to be glycoproteins. These bands also show considerable diversity among the different species. Again, there is no apparent evidence for a common membrane structure based on similar proteins common to each of the different species.

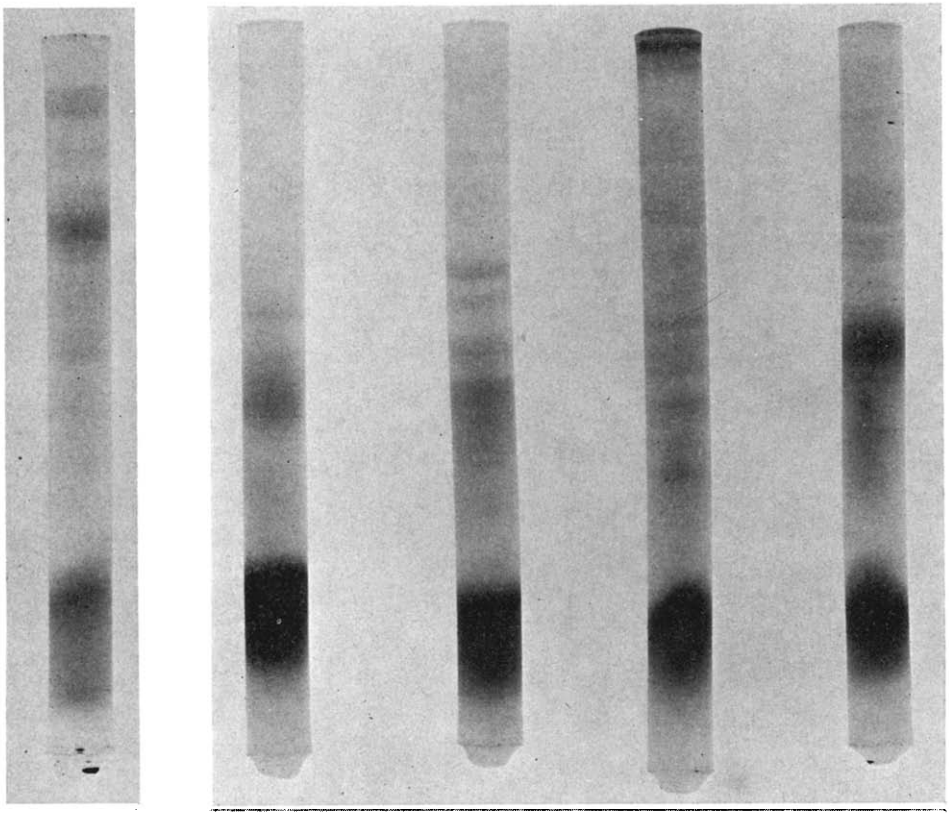

Fig. 2. Carbohydrate patterns of sodium dodecyl sulfate solubilized erythrocyte membranes after polyacrylamide gel electrophoresis in 0.1 % sodium dodecyl sulfate. Left to right: human, dog, cat, cow, horse. Gels were stained by the periodate–Schiff procedure of ZACHARIAS *et al.*[7].

Comparative studies of erythrocyte membranes are essential to an understanding of their structure if one is to avoid oversimplification of structural interpretations. The sodium dodecyl sulfate gel electrophoresis technique offers a simple, fast and reproducible method for analyzing membrane protein components. One problem which must be considered, however, is the possibility that the solubilization technique does not result in complete disaggregation of the membrane proteins to their individual polypeptide chains. In this case, some of the bands could represent aggregates of lower molecular weight chains, resulting in a more complex pattern with more protein appearing at higher molecular weight. If such aggregates are formed, they must be extremely stable for the human membranes, since they have resisted such diverse treatments as urea, hydroxylamine (pH 8.0), guanidinium thiocyanate, 1.0 M salt, piperidine[9] and heat[9].

The present study confirms the heterogeneity of the proteins of erythrocyte membranes suggested by earlier work[9,12–14] and offers further promise of the possibility of relating specific membrane proteins to particular structural or functional aspects of the membrane. It will be of particular interest to try to relate the various species differences in functional properties of red cells to changes in the content and organization of their membrane proteins.

We are grateful to the personnel of the College of Veterinary Medicine, Oklahoma State University, for collecting and supplying samples of animal bloods. The technical assistance of Joan Summers, Joyce Sternberg and Ellen Nichter is gratefully acknowledged. This work was supported by the Oklahoma Agricultural Experiment Station, National Institutes of Health Grant GM 16,870, American Cancer Society Grant P-563 and American Cancer Society Institutional Grant IN-91A.

Department of Biochemistry,
Oklahoma Agricultural Experiment Station,
Oklahoma State University,
Stillwater, Okla. 74074 (U.S.A.)

KERMIT L. CARRAWAY
DAVID KOBYLKA

1 J. D. ROBERTSON, *Symp. Biochem. Soc.*, 16 (1959) 3.
2 A. A. BENSON, *J. Am. Oil Chemists' Soc.*, 43 (1966) 265.
3 D. F. H. WALLACH AND A. GORDON, *Federation Proc.*, 27 (1968) 1263.
4 J. LENARD AND S. J. SINGER, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 1828.
5 J. T. DODGE, C. MITCHELL AND D. J. HANAHAN, *Arch. Biochem. Biophys.*, 100 (1963) 119.
6 A. L. SHAPIRO, E. VINUELA AND J. V. MAIZEL, JR., *Biochem. Biophys. Res. Commun.*, 28 (1967) 815.
7 R. M. ZACHARIAS, T. E. ZELL, J. H. MORRISON AND J. J. WOODLOCK, *Anal. Biochem.*, 30 (1969) 148.
8 T. W. TILLACK, S. L. MARCHESI, V. T. MARCHESI AND E. STEERS, JR., *Biochim. Biophys. Acta*, 200 (1970) 125.
9 J. LENARD, *Biochemistry*, 9 (1970) 1129.
10 K. R. BRUCKDORFER, P. A. EDWARDS AND C. GREEN, *European J. Biochem.*, 4 (1968) 506.
11 R. S. BASCH, *Anal. Biochem.*, 26 (1968) 184.
12 S. ROSENBERG AND G. GUIDOTTI, *J. Biol. Chem.*, 244 (1969) 5118.
13 R. F. A. ZWAAL AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 163 (1968) 44.
14 M. D. POULIK AND P. K. LAUF, *Nature*, 208 (1965) 874.

Received May 22nd, 1970