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Erythrocyte Membrane Proteins in Sickle Cell Anemia (38107)

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Evidence is accumulating that erythrocytes from patients with sickle cell anemia have membrane abnormalities. Sickled erythrocytes have increased potassium fluxes (1). The phospholipids of Hb SS erythrocytes differ from normal erythrocytes in their reactivity with 1-fluoro 2,4-dinitrobenzene, suggesting structural differences affecting the availability of phosphatidyl ethanolamine and phosphatidyl serine amino groups (2).

The property of reversibility from the sickle shape to a discocyte with oxygenation may be partially a membrane phenomenon because ghosts made from irreversibly sickled cells (ISC) remain sickled (3), and when ISC are oxygenated, the fibrillar fine structure of deoxygenated Hb SS is no longer seen on electron microscopy, although the cell remains distorted (4). Depletion of ATP while the Hb SS erythrocyte is sickled under nitrogen prevents the cell's return to the discocyte when reoxygenated (5). ISC have decreased membrane deformability (6). An alteration in membrane proteins has been suggested (7). In this paper, we describe disc polyacrylamide gel electrophoretic analysis of the membrane proteins of normal erythrocytes, SS erythrocytes, and ISC formed *in vitro*.

Methods. Blood was collected in heparin from healthy laboratory personnel with AA hemoglobin (black and white) and four patients with SS disease who had not been transfused within 4 mo. The blood was processed at 4° within 1 hr after collection. Ghosts were prepared from 25–50 ml of blood by washing the erythrocytes thrice with 5 m*M* sodium phosphate–0.15 *M* NaCl (pH 8.0), and aspirating the buffy coat (8). The cells were lysed with 5 m*M* sodium phosphate (pH 8) and the resultant ghosts washed thrice. The protein concentration of the ghost suspension was adjusted to 5 mg/ml and the sample frozen at −70° until used.

ISC were formed *in vitro* by incubation with NaF under nitrogen (5). SS erythrocytes were suspended in phosphate-buffered salt solution or serum to which NaF was added to a final concentration of 20 m*M* and incubated under nitrogen for 3 hr at 37°. ATP levels were determined as described by Kornberg (9). The cells were judged to be ISC when greater than 75% remained distorted upon oxygenation. Ghosts from the ISC were then prepared.

Polyacrylamide gel electrophoresis was performed as described by Neville (10). The ghosts were dissolved in 1% Na_2CO_3, 4% SDS, and 10% 2-mercaptoethanol. Forty-five micrograms of membrane protein were layered on the discontinuous sulfate–borate polyacrylamide gel electrophoresis system (pH 9.5). The initial current was 0.5 mA/tube until the sample had entered the stacking gel and then increased to 1.5 mA/tube. Running time was approximately 2½ hr at 25°. The gels were fixed and stained with Coomassie blue (8). Gels were scanned with a Photovolt Densitometer. Photographs were taken using a yellow filter.

Results. No differences were observed in the polyacrylamide gel patterns of normal human erythrocytes and those of Hb SS erythrocytes (Fig. 1), either visually or on scanning with the densitometer. At least 25 bands were clearly seen. The numbering system employed is that of Steck (11). The reticulocyte counts of the Hb SS blood samples ranged from 11 to 22%. Production of *in vitro* ISC failed to modify the protein patterns. ATP levels decreased during incubation from 1.75 ± 0.22

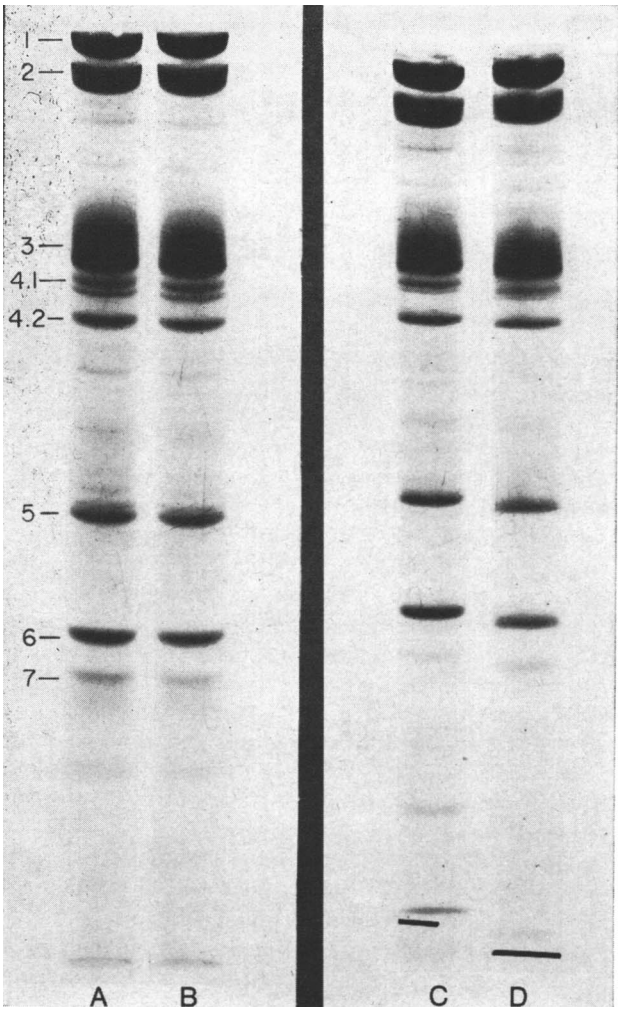

FIG. 1. Polyacrylamide gels of membrane proteins from normal and SS erythrocytes. A. Normal erythrocyte; B. SS erythrocyte; C. NaF-incubated normal erythrocyte; D. NaF-incubated SS erythrocyte (ISC); Numbering of bands according to Steck (11).

to 0.37 ± 0.15 μM/ml ± 1 SE erythrocytes. The ghosts of these cells were distorted under phase microscopy.

Discussion. The presence of a membrane lesion in the Hb SS erythrocytes has been postulated. Reversibility of the sickled shape upon reoxygenation appears to be a membrane property. In patients with sickle cell disease, the percentage of ISC is often low and the amount of blood required to study membrane proteins of ISC formed *in vivo* is prohibitive. ISC can be formed *in vitro* by incubation under nitrogen in the presence of NaF to deplete ATP. After incubation, restoration of ATP levels does not affect the distorted cell shape (5).

Neville's discontinuous polyacrylamide gel system allowed consistent reproducibility and excellent band separation of membrane proteins. Our major protein bands agree with those in the literature (11). We were unable to demonstrate a difference in the protein pattern of either freshly collected Hb SS erythrocytes or ISC from those of normal subjects. The absence of a discernible protein abnormality does not negate its presence. The membrane protein composition of ISC formed *in vitro* may differ from those formed *in vivo*, since the lipid content differs between *in vivo* and *in vitro* formed ISC (5). Membrane proteins are difficult to solubilize unless harsh

techniques are used. Partial dissolution by mild techniques (e.g., butanol, low ionic strength, or acetic acid) solubilizes varying amounts of protein and may be affected by lipid–protein interaction. Therefore, changes in protein patterns after milder solubilization procedures may reflect only changes in extraction and not real differences in protein composition. SDS and sulfhydryl-reactive agents produce total membrane protein solubilization. However, the strongly anionic SDS obviates charge difference and allows protein separation by molecular weight only. Therefore, changes in surface charge or minor changes in molecular weight of membrane proteins may be missed. Although there were no differences in bands between normal and Hb SS erythrocyte membrane proteins, physical changes may still be present. Possibilities include charge differences, minor molecular weight changes, interaction with inorganic cations, or noncovalent binding between proteins. These possibilities require further study.

Summary. Erythrocyte membrane proteins from normal subjects and four patients with sickle cell anemia were studied. The proteins were solubilized with SDS and 2-mercaptoethanol and separated on a discontinuous disc polyacrylamide gel system. No variations in major protein groups were found between normal and hemoglobin SS-containing erythrocytes. ISC formed *in vitro* were also studied and showed no deviation from the normal pattern. The significance of these studies has been discussed.

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