The Molecular Basis and Physiological Implications of Red Cell Deformability

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INTRODUCTION

The biorheological properties and behaviors of cells and tissues have a biochemical and molecular basis (Chien & Sung, 1990). Recent advances in concepts and techniques in molecular biology have been utilized to elucidate the molecular basis of red blood cell (RBC) membrane deformability, which is an important factor in determining blood rheology and hence blood flow.

The RBC membrane is composed mainly of proteins and lipids. The membrane proteins, which contribute to approximately one-half of the membrane weight, play a significant role in the shape maintenance and the deformability of RBCs (Lux, 1988). In recent years, molecular biological techniques have been used to establish the molecular composition of nearly all of these proteins (Becker & Benz, 1990; Chien & Sung, 1990). The DNAs encoding these proteins have been cloned and sequenced. From the nucleotide and amino acid sequences, information has been deduced on the secondary structure of the proteins and their interaction sites. Studies on genomic DNAs have contributed to the un-.

derstanding of the mechanisms of regulation of these genes. The molecular basis of the rheological behavior of the RBC membrane is beginning to emerge, and this will eventually lead to an elucidation of the molecular basis of physiological regulation of blood flow in health and disease (Chien, 1990). Studies on RBC membrane rheology represent an excellent example of how molecular biology can be applied to the study of physiological functions at organ-system levels.

ERYTHROCYTE MEMBRANE PROTEINS AND DEFORMABILITY

RBC membrane proteins are generally identified by treating the RBC ghost membrane with sodium dodecyl sulfate (SDS) and applying it onto polyacrylamide gel for electrophoresis. The gel can be stained to show the proteins which have been separated electrophoretically according to size (Fig. 1). The protein bands are numbered starting from the top of the gel, where the high molecular weight species are located, giving rise to protein bands 1, 2, 2.1, 3, 4.1, 4.2, 4.9, 5, etc.

RBC membrane proteins may be classified as (a) integral proteins that are inserted into the lipid bilayer perpendicular to the plane of the membrane, (b) skeletal proteins that form a network lining the endoface parallel to the membrane, and (c) linking proteins
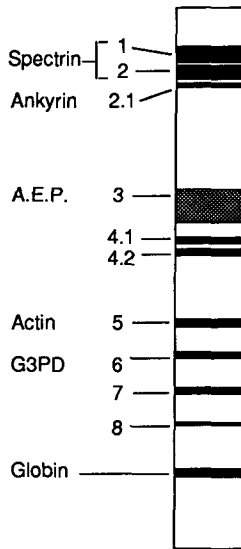

Fig. 1. *Schematic illustration of the SDS-PAGE patterns of RBC membrane proteins stained with Coomasie blue dye. A. E. P. denotes anion exchange protein, and G3PD represents glyceraldehyde-3-phophate dehydrogenase. From Chien & Sung (1990).*

that connect the skeletal proteins to the integral, transmembrane proteins.

Most of the integral proteins of the RBC membrane are transmembrane glycoproteins that span the entire bilaryer, e.g. glycophorins and protein band 3, but some of the integral proteins penetrate only partially into the lipid bilayer from the endofacial or exofacial side of the RBC membrane. The transmembrane glycoproteins typically have an extracellular domain with their carbohydrate groups protruding into the surrounding medium, an intramembranous domain consisting of peptide segments with a high content of hydrophobic amino acids, and a cytoplasmic domain that protrude into the intracellular cytoplasm and interact with the membrane skeletal network.

The skeletal proteins form a network lining the endoface of the membrane; they are also referred to as peripheral proteins. Some of the skeletal proteins are isoforms of the con-

tractile proteins found in muscle, e.g. actin, myosin and tropomyosin (Becker & Benz, 1990). Other membrane proteins were first thought to be specific for RBCs, e.g. spectrin, but immunological and molecular biological studies have shown that isoforms of these proteins are present in most nonerythroid cells and tissues.

Linking proteins connect the skeletal proteins, which form a network parallel to the membrane, to the transmembrane integral proteins, which form posts in the lipid bilayer perpendicular to the plane of the membrane. They function as molecular brokers (Lux et al, 1990) interacting with the skeletal and integral proteins and provide the hinges for the formation of a three-dimensional membrane protein network. The main linking proteins are protein bands 2.1 (ankyrin), 4.1 and 4.2. Because these linking proteins remain together with the skeletal proteins in the triton shell prepared from RBC ghosts, they are often considered together with the skeletal proteins.

A schematic drawing of the RBC membrane proteins is shown in Fig. 2. In the last few years, molecular biological techniques have been successfully employed to establish the molecular composition of nearly all of the RBC membrane proetins, including spectrins, glycophorins, protein 3, protein 4.1, protein 4.2, and ankyrin. Combination of biophysical measurements with molecular information has facilitated the understanding of the roles of these proteins in affecting membrane rheology.

RBC membrane rheology can be determined by several techniques. One of the most commonly employed method is micropipette aspiration (Chien et al, 1978). Micropipettes with internal radii of less than 1 μm are prepared with the use of a micropipette puller and filled with a prefiltered buffer solution. The filled micropipette is mounted on a hydraulic micromanipulator and its wide end connected to a pressure regulation and recording system. The RBC suspension is loaded into a small round chamber located on the
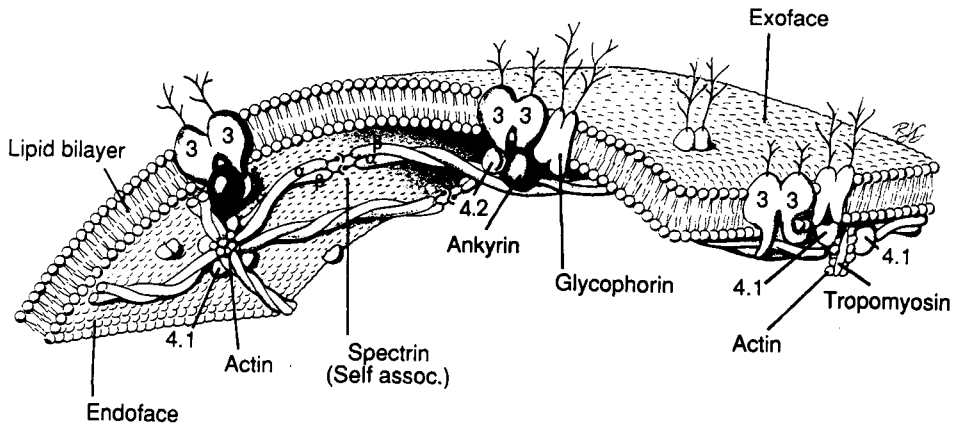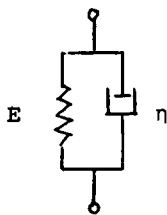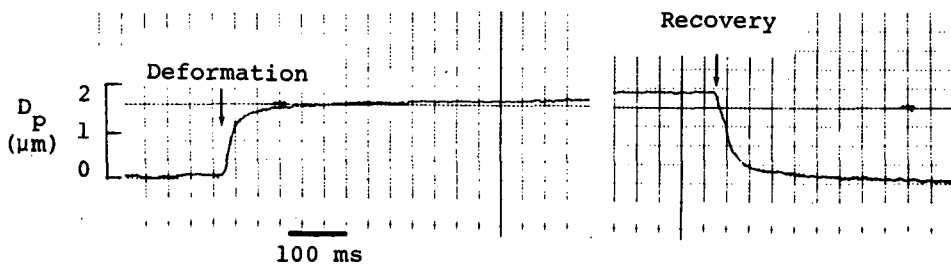
Fig. 2. Schematic drawing showing the organization of RBC membrane proteins. From Chien & Sung (1990).

E: Elastic modulus $= \dfrac{\text{Stress}}{\text{Strain}} = 3 \times 10^{-3}$ dyn/cm

η: Viscosity $= \dfrac{\text{Stress}}{\text{Rate of strain}} =$ E x Time constant

η in deformation $= 1\text{-}4 \times 10^{-4}$ dyn-s/cm

η during recovery $= 4 \times 10^{-4}$ dyn-s/cm

Fig. 3. Recorder tracing of video dimension analyzer output tracking the deformation and recovery of RBC membrane subjected to micropipette aspiration. A Kelvin model consisting of a viscous element (η) in parallel to an elastic element (E) is used to analyze the data. The normal values for the membrane elastic modulus and the viscosity during deformmation and recovery are given.

stage of an inverted microscope. The optical image is monitored and recorded with the use of a video camera and tape recorder system. The micropipette tip is positioned at the surface of a RBC. A desired level of negative pressure is set and transmitted to the micropipette to cause a time-dependent aspiration of a portion of the cell. The susequent release of the pressure leads to a time-dependent recession of the aspirate segment out of the micropipettes and recovery of the cell shape.

During the playback of the recorded video

image, the tip of the aspirated segment is tracked with the use of a video dimension analyzer. The time-dependent deformation in response to a step aspiration pressure is analyzed by using a Kelvin model in which the RBC membrane is simulated as a viscous element (η) in parallel to an elastic element (E) (Fig. 3). The membrane elastic modulus and viscosity of normal RBCs are used to compare with the results obtained from patients with RBC membrane abnormalities.

ERYTHROCYTE SKELETAL PROTEINS

Spectrin

Spectrin constitutes approximately 3/4 of the RBC membrane skeleton by weight, and it plays a significant role in determining the rheological properties of the RBC membrane. Spectrin is a heterodimer composed of two intertwined long-chain subunits (α-subunit: 240 KD and β subunit: 220 KD) aligned in antiparallel directions. Human and murine erythrocyte α-spectrins and β-spectrins have been cloned (Becker & Benz, 1990). There is considerable homology between the human and mouse amino acid sequences. Nonerythroid spectrins have been cloned from other tissues, e.g. fodrin in the brain (Wasenius et al, 1989). Nonerythroid α-spectrins are extremely well conserved among different species (Leto et al, 1988), more so than the erythroid α-spectrins and nonerythroid β-spectrins. It appears that the functional requirements placed on nonerythroid spectrins, which are found in relatively stationary cells, are relatively uniform in different species, e.g. for structural support and other cellular functions. On the other hand, the needs for RBCs to constantly undergo dynamic shape changes during its life-time and to adapt to hemodynamic and other conditions in different species may lead to evolutionary alterations of erythroid spectrins to accommodate the changing functional needs.
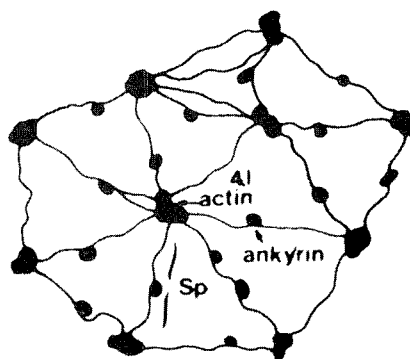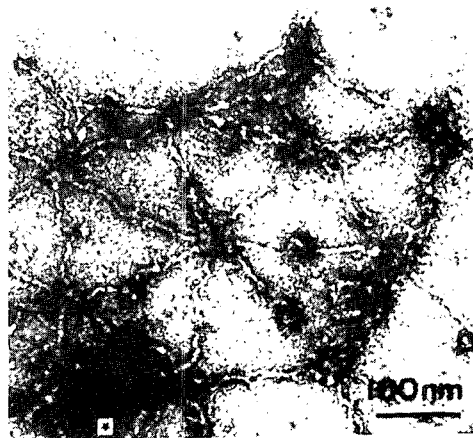
The organization of the erythroid spectrin

Fig. 4. *Top: Spread membrane skeleton derived from Triton-treated human RBC ghosts examined by negative-staining electron microscopy. Bottom: A schematic drawing of the electron micrograph. Shown are a septagonal subunit in the lower left and a pentagonal subunit on the upper right. The asterisk at the lower left corner of the electron micrograph indicates a dense region of residual membrane material. Modified from Liu et al (1987).*

molecules confers to them mechanical flexibility which probably plays a significant role in RBC membrane deformability. Both α and β subunits in all spectrins contain many homologous 106-amino acid repeats (20 and 18 repeats, respectively). Each of these repeats is arranged in triple α-helices (Speicher & Marchesi, 1984).

Electron microscopy has demonstrated the polygonal structure of the RBC skeletal net-

work (Liu et al, 1987) (Fig. 4). In this network, the "threads" consist mainly of the spectrin molecules and the "joints" consist of the linking proteins interacting with spectrin and other membrane proteins. Such a network structure would favor uniaxial deformation at constant area (Evans, 1973; Skalak et al, 1973). The rheological behavior of the membrane depends on the mechanical properties of the skeletal protein molecules, especially spectrin, and the binding characteristics between the linking proteins and their adjoining molecules (Lux, 1988).

In hereditary spherocytosis a decrease in RBC membrane spectrin content as a result of defects in the β-spectrin, has been correlated with an increase in osmotic fragility of RBCs (Speicher et al, 1984; Leto et al, 1988), and there is a decrease in RBC membrane viscoelastic coefficients which may be related to the decrease in membrane spectrin content (Waugh & Agre, 1988).

Spectrin dimers undergo head-to-head self association to form tetramers, as well as higher oligomers. Studies on the distribution of the spectrin molecular size by using non-denaturing gels have shown that greater than 90% of the spectrin in normal human RBC membrane exist in tetrameric form (Morrow & Marchesi, 1981). Defective spectrin self-association as a result of molecular alterations in the N-terminus of α-spectrin or the C-terminus of β-spectrin is found in approximately 30% of hereditary elliptocytosis patients and all hereditary pyropoikilocytosis patients (Liu et al, 1982; Knowles et al, 1983). Micropipette studies on RBCs from such hereditary elliptocytosis patients have shown increases in RBC membrane viscosity and elastic modulus which can be correleated with an increase in spectrin dimer percentage (Chabanel et al, 1989)

In addition to self association, the other important interactions for spectrin are spectrin-ankyrin-protein 3 and spectrin-actin-protein 4.1 interactions, as well as the interactions with glycophorins and protein 3 via protein

4.1. Abnormalities in ankyrin (see below) can reduce the stability of spectrin attachment to the membrane, thus causing a decrease in membrane spectrin and an increase in membrane fragility (Agre et al, 1986). A deficiency in protein 4.1 (see below), through a defective spectrin-actin binding, can also lead to hereditary elliptocytosis (Allosisio et al, 1981; Conboy et al, 1986).

Actin, Tropomyosin and Myosin

Actin constitutes a major component of the cytoskeleton of all cells and comprise an essential part of the contractile apparatus of all muscles. Mammalian cells contain at least six actin isoforms, including four muscle actins and two nonmuscle actins. These actin isoforms, especially the four muscle actins, have closely similar primary structures. Actin is well conserved across animal species. The erythrocyte actin is organized into double helical filaments (F-actin), in close association with tropomyosin (Fowler & Bennett, 1984). The role of actin in RBC membrane is primarily to interact with protein 4.1 and spectrin to form a ternary complex (Cohen & Korsgren, 1980) which allows the connection of spectrin tetramers with each other to form an extended skeletal network.

In RBC membrane, the nonmuscle tropomyosin forms a coiled-coil dimer lying in the major groove of the F-actin thin filament to induce actin polymerization (Folwer & Bennett, 1984). This relation is similar to that found between actin and tropomyosin in muscles. Tropomyosin has been identified in human RBC membranes as a dimer with two polypeptides of ~29 kD and 27 kD in molecular weights (Folwer & Bennett, 1984). In the RBC membrane skeleton, two such tropomyosin molecules associate with a short actin filament 12-17 monomers long. The RBC tropomyosin probably functions to mechanically stabilize the actin filaments and regulate the interaction between spectrin and actin.

RBC membrane contains tropomodulin, a 43 kD protein, which binds to tropomodulin

and inhibits its binding to actin (Fowler, 1990). Human RBC tropomodulin has recently been cloned and sequenced.

Myosin, which is an important contractile protein in muscles, is also found in RBCs (Fowler et al, 1985). The amount of myosin in RBCs, however, is very small and its role is less well understood, It is possible that the RBC myosin functions together with tropomyosin in an actomyosin contractile apparatus responsible for ATP-dependent changes in RBC shape (Fowler et al, 1985).

ERYTHROCYTE LINKING
PROTEINS

Protein 4.1

Protein 4.1 is a phosphoprotein with a molecular weight of approximately 80 kD. Its central domain binds to the spectrin tail (Correas et al, 1988), resulting in a marked increase of the binding affinity of spectrin to actin in RBC membrane (Goodman et al, 1982). The importance of protein 4.1 as a linking protein in the membrane skeleton is enhanced by its binding to several transmembrane glycoproteins, thus coupling the horizontal network to the vertical posts in the bilayer.

Human erythroid protein 4.1 has been cloned and sequenced (Conboy et al, 1986), allowing the idenitification of the amino acid sequences of the various domains, including the hydrophobic domain for the binding of transmembrane proteins and the highly charged domain for the binding of spectrin and actin. Protein 4.1 is present in several tissues, e.g., brain, platelets, endothelial cells, lens cells, fibroblasts, and lymphoid cells, in various isoforms (Conboy, 1988). cDNA cloning has established the existence of several nucleotide sequence "motifs", which are inserted or deleted by alternative splicing in a tissue-spectific manner to produce the isoforms (Conboy et al, 1988; Tang et al, 1988). Comparison of Protein 4.1 sequences of human

lymphoid (Tang et al, 1988) and erythroid cells (Conboy et al, 1986; Conboy et al, 1988) has revealed complete homology except for five "motifs". The resulting variations in amino acid sequences of these isoforms constitute the molecular basis for the functional differences between erythroid and lymphoid 4.1 proteins.

Protein 4.1 cDNA probes have been used to study the structural organization of the protein 4.1 genes in normal individuals and in patients with hereditary elliptocytosis due to protein 4.1 deficiency (Tchernia et al, 1981; Conboy et al, 1986). The homozygous patients had complete absence of protein 4.1 in their RBC membranes, marked elliptocytosis, and severe hemolytic anemia; the heterozygous patients had 50% of the normal level of protein 4.1 in their RBC membranes, mild elliptocytosis, and no clinically important anemia. Southern blot analysis of restriction enzyme-digested genomic DNA and fragments of cDNA clones suggested that a partial gene deletion in the region preceding the initiation codon of the protein 4.1 gene is responsible for the disease (Tchernia et al, 1981; Conboy, 1988).

Ankyrin

Erythrocyte ankyrin (protein 2.1) is a 210-kD globular protein that attaches spectrin to protein 3 (Bennett & Stenbuck, 1980). Although each spectrin tetramer has two ankyrin-binding sites, the stoichiometry is one spectrin tetramer to one ankyrin. Unphosphorylated ankyrin binds preferentially to spectrin tetramers and oligomers, rather than to spectrin dimers. In vitro phosphorylation of ankyrin by the RBC membrane cyclic AMP-independent casein kinase I abolishes this preferential binding (Lu et al, 1985; Cianci et al, 1988) and reduces ankyrin-protein 3 binding (Soong et al, 1985).

Some cases of hereditary spherocytosis has been shown to be related to defects in the ankyrin gene (Soong et al, 1985; Lux et al, 1990). A deficiency or instability of ankyrin,

by reducing the stability of spectrin attachment to the membrane, leads to a decrease in membrane spectrin. This may contribute to the correlation observed between the degree of spectrin deficiency and the extent of spherocytosis or clinical severity in hereditary spherocytosis (Agre et al, 1986).

There exists a mutant mice strain (nb/nb) with hereditary hemolytic anemias involving the ankyrin gene (Bernstein, 1980). The deficiency of normal ankyrin in the nb/nb RBC membranes, probably through a weakening of the binding of skeletal network to the bilayer membrane, leads to the formation of nodules and tethers and irregular cell shapes. Micropipette tests on nb/nb RBCs have shown that the membrane rheology is normal during the deformation phase, but there is a lowering of viscosity during recovery (Sung et al, 1984). It is to be noted that the nb/nb RBC membrane also has a reduced spectrin content (Bodine et al, 1984).

Human erythrocyte ankyrin has been recently cloned and sequenced (Lux et al, 1990). The deduced amino acid sequence extends for 1880 amino acids. The 89-kD domain located on the 5' end consists of 22 successive repeats formed by successive gene duplications (Lux et al, 1990). These repeats could form the binding sites for integral and skeletal proteins. The C-terminal amino acid form a functional domain (55-kD) that regulates the binding of ankyrin to spectrin and protein 3.

Searching of databases has shown very similar repeats in several invertebrate, yeast and viral proteins which are related to the cell cycle (e.g., events involved in mitosis) and viral infectivity (Lux et al, 1990). Furthermore, ankyrin-like repeats have recently been found in *bcl-3*, a candidate proto-oncogene reated to B-cell chronic lymphocytic leukemia. These results indicate that ankyrin-like repeats are involved in cell growth and differentiation.

Protein 4.2

Protein 4.2, which represents ~5% of the protein mass of human RBC membranes, interacts with the cytoplasmic domain of protein 3, ankyrin, and protein 4.1 (Steck, 1974; Korsgren & Cohen, 1986). Protein 4.2 probably functions to stabilize ankyrin in the membrane (Korsgren & Cohen, 1988; Rybicki et al, 1988). Human RBC protein 4.2 has recently been cloned from a reticulocyte library (Korsgren et al, 1990; Sung et al, 1990). Two isoforms, differing by 30 amino acids, have been obtained (Sung et al, 1990), predicting proteins with molecular weights of ~77 and ~80 kD.

Sequence searches have shown that RBC protein 4.2 has a high degree of homology with human factor XIII subunit a and guinea pig liver transglutaminase in regions where the two latter proteins have transglutaminase activity. The critical cysteine residue required for the enzymatic crosslinking of substrates, however, is missing in protein 4.2, which has an alanine at this position instead of cysteine (Korsgren et al, 1990; Sung et al, 1990). It is possible that protein 4.2 may use this site to undergo reversible interactions with other RBC membrane proteins without forming covalent crosslinks (Hayashi et al, 1974). Protein 4.2, along with protein 4.1, has been proposed to be one of the last membrane proteins synthesized during RBC maturation. It is possible that protein 4.2 contributes to the stabilizaton of the membrane skeleton through its binding with membrane proteins and thus protecting them from being degraded (e.g. by proteases) or crosslinked (e.g., by cytoplasmic transglutaminase) (Sung et al, 1990).

Protein 4.2-deficient patients have RBCs with elliptocytotic and spherocytotic shapes and an increased osmotic fragility, and they suffer from anemia (Hayashi et al, 1974; Rybicki et al, 1988). These abnormalities probably occur as a result of a weakened associa-

tion between the skeletal network and the membrane bilayer in the absence of protein 4.2.

Immunoreactive analogs of protein 4.2 are also present in nonerythroid cells and tissues, including platelets, brain and kidney (Rybicki et al, 1988; Griedrichs et al, 1989). These results raise the possibility that protein 4.2 like other RBC membrane proteins such as protein 4.1 may be a ubiquitous component of cell membranes, although its function in other cells may differ from that in RBCs.

ERYTHROCYTE TRANSMEMBRANE PROTEINS

Protein 3

Protein band 3 is transmembrane glycoprotein with a molecular weight of approximately 100 kD. Its heterogeneous glycosylation results in the diffuse band on SDS-PAGE (Fig. 1). The cDNA and the gene for murine protein 3 have been cloned and sequenced (Kopito et al, 1987). Hydropathy analysis has revealed that protein 3 has many hydrophobic segments which represent membrane-spanning domains. The N-terminal 43-kD cytoplasmic peptide of protein 3 provides the binding site for ankyrin, as well as proteins 4.1 and 4.2, hemoglobin and a few glycolytic enzymes (Bennett & Stenbuck, 1980). Protein 3 is associated noncovalently into tetramers (Nigg & Cherry, 1980), and each tetramer binds one ankyrin (Becker & Benz, 1990). The C-terminal 52-kD peptide of protein 3 serves as the anion transport channel for $Cl^- - HCO_3^-$ exchange (Drickamer, 1977).

Nonerythroid isoforms of protein 3 are found in many tissues and cells, e.g. in the kidney (Alper et al, 1987), where anion transport is an important function of the renal tubules.

Glycophorins

Glycophorins are transmembrane sialogly-

coproteins which consist of an extracellular glycosylated segment that may have a receptor function, an intramembranous hydrophobic segment that traverses the lipid bilayer, and a cytoplasmic segment that interact with other membrane or cellular proteins (Lux, 1988; Cartron et al, 1991). Staining of RBC ghost membrane gels with periodic acid-Schiff (PAS) reagent shows four bands which are referred to as glycophorins A, B, C and D.

SUMMARY OF RBC MEMBRANE PROTEINS AND RHEOLOGY

The molecular structure of the individual RBC membrane proteins has been established in recent years, and knowlege is being gathered on the molecular basis of their interactions. Additional research is required to elucidate the molecular interactions involved in the formation of the membrane skeletal network and in the linking of the network to the transmembrane proteins and lipids. Such studies will provided the information needed to formulate a molecular model of membrane rheology.

The micromechanical method of assessing membrane rheology has been applied to several disease states in which there are molecular abnormalities in the RBC membrane. Further studies are needed, however, to relate rheological changes to specific alterations in membrane proteins and their interactions. With the advancement of genetic engineering technology, molecular manipulations should be attempted to induce specific rheological defects, thus making possible the structure-function correlation at the molecular level.

EFFECTS OF REDUCED RBC DEFORMABILITY ON BLOOD FLOW

In order to assess the physiological consequence of reduced red cell deformability in

RELATIVE DISTRIBUTION of CELLS
Ratio (Normal RBC₂/Normal RBC₁)

RELATIVE RESISTANCE
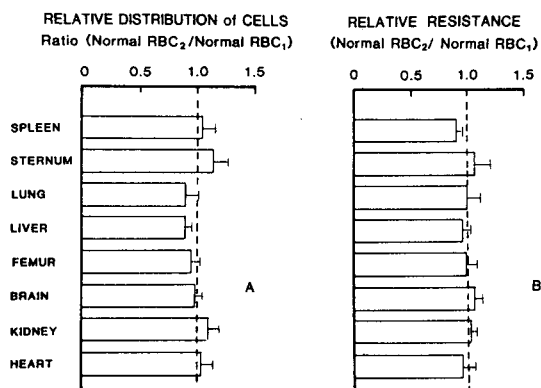(Normal RBC₂/ Normal RBC₁)

Fig. 5. A: Relative tissue distribution of normal red blood cells (RBCs) in various organs in control group. B: Relative blood flow resistance in various organs in control group. Data on lung represent values for bronchial circulation. From Simchon et al (1987).

RELATIVE DISTRIBUTION OF HARDENED CELLS (ratio to normal RBCs)

40% Exchange (0.025% GA)
* P ‹ 0.01

Fig. 6. Relative tissue distribution of red blood cells (RBCs) with reduced deformability (labeled as hardened cells) in experimental group. Values greater than 1 indicate a preferential trapping of RBCs with reduced deformability. GA, glutaraldehyde. From Simchon et al (1987).

terms of its hemodynamic effects on regional circulations, in vivo experiments were performed on pentobarbitalized rats (Simchon et al, 1987). RBCs were obtained from normal donor rats and labeled with ^{51}Cr or ^{111}In. One type of the labeled RBCs were partially hardened by treatment with 0.025% glutaraldehyde, which caused a decrease in RBC deformability just detectable by rheological measurements of the RBC. The other type of the labeled RBCs were untreated and served as control. The two types of isotopes were alternatively used for the partial hardening and normal controls.

The study was carried out by a two-step isovolumetric exchange transfusion, with each step involving 40% of the circulating RBCs. The first exchange was performed with normal RBCs labeled with one type of isotope, and the second exchange was performed with either normal RBCs for a second time (control group) or the partially hardened RBCs (experimental group).

The regional tissue distribution of RBCs was calulated from the ratio of tissue contents of labeled RBCs from the second injection (RBC₂) to that of the labeled normal RBCs
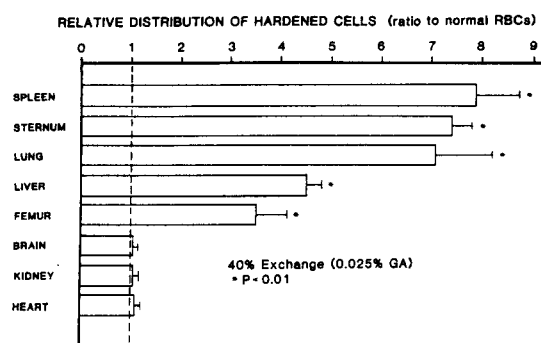
from the first injection (RBC₁). In the control group, the relative tissue distribution of RBCs from the two exchanges (RBC₂/RBC₁) was not significantly different from 1.0 in any of the organs studied (Fig. 5A), indicating that the normal RBCs given during the second exchange was not distributed differently from those given during the first exchange. In the experimental group, this relative tissue distribution was significantly > 1.0 in several organs (Fig. 6), showing that the RBCs with reduced deformability were preferentially trapped in these regions over the normal RBCs. Among the regions studied, the spleen, sternum, and lung had the greatest trapping of partially hardened RBCs, liver and femur showed the next highest trapping, and there was no preferential trapping of these cells in the brain, kidney, and myocardium.

In the control group, the relative blood flow and the relative resistance (values following the second exchange divided by values before the second exchange) were not significantly different from 1.0 (Fig. 5B). In the experimental group, the administration of RBCs with reduced deformability caused the relative blood flow to fall significantly below 1.0 in several regions; as a corollary, the relative resitance was significantly > 1.0 in these re-
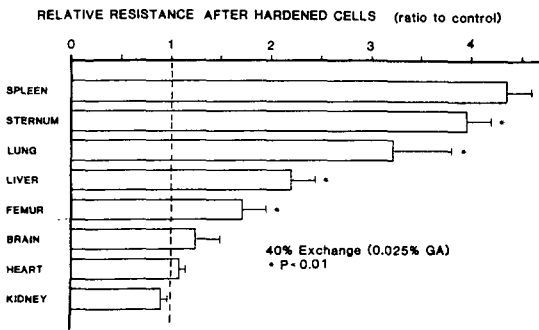
RELATIVE RESISTANCE AFTER HARDENED CELLS (ratio to control)

Fig. 7. *Relative resistance after exchange of RBCs with reduced deformability (labeled as hardened cells) to control resistance in various organs. Data on lung represent values for bronchial circulation. GA, glutaraldehyde. From Simchon et al (1987).*

Fig. 8. *Correlation of degree of increase in regional resistance with extent of trapping of red blood cells (RBCs). Data on lung represent values for bronchial circulation. C_N and C_H are concentrations of normal and hardened cells, respectively. From Simchon et al (1987).*

gions. The spleen, sternum, and lung (bronchial circulation) showed the largest decrease in flow and the greatest increase in resistance (Fig. 7) after the exchange of RBCs with reduced deformability ($p < 0.005$). There was no significant change in blood flow or resistance in the brain, kidney, and myocardium after the exchange of RBCs with reduced deformability ($p < 0.5$). The extent of the increase in regional resistance after the exchange of RBCs with reduced deformability showed a positive correlation with the degree of RBC trapping (Fig. 8), i.e., organs with preferential trapping of these cells have large flow reductions and resistance elevations. The regional variations in the trapping of RBCs with reduced deformability may reflect differences in microvascular geometry these circulatory beds and the attendant variations in predilection for plugging by these cells.

These results indicate that modifications in RBC deformability can cause microvascular sequestration with regional specificity and that such local trapping can leads to flow reduction and resistance elevation. These findings may provide the pathophysiological link by which blood cells with abnormal deformability in disease states can lead to clinical manifestations, including infarction of
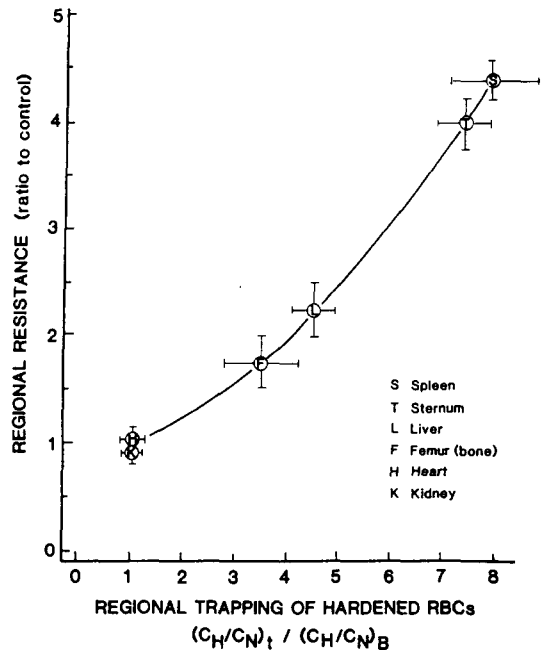
specific organs. Further studies are needed to study the regional hemodynamic effects or RBCs subjected to specific molecular modifications of membrane components.

GENERAL COMMENTS AND CONCLUSIONS

The proteins of the RBC membrane play major roles in maintaining the cell shape under resting and effecting cell deformation in response to shear stesses imparted by blood flow (Chien, 1987). The skeletal proteins from a network which is connected with the transmembrane glycoproteins through the linking proteins and interacts with lipids in the membrane bilayer (Lux, 1988). The glycoproteins serve functions such as ion transport and receptors for ligands, and their interactions with the skeletal network affect each other's

functions.

The rather ubiquitous existence of the membrane skeletal proteins, many of them first found in the RBC, as isoforms in various tissues (Becker & Benz, 1990) suggest that these proteins may also play a role in maintaining cell shape and mechanical strength in other cell types, as well as mediating other physiological functions. It has been known for some time that actin undergoes dynamic reorganization during mitosis; recent studies on protein 4.1 (Tang et al, 1991) and ankyrin (Lux et al, 1990) indicate that these and other skeletal proteins may also play a significant role in cell division and differenctiation.

Studies on hereditary hemolytic disorders have made possible the identification of the molecular domains of membrane skeletal proteins responsible for specific functions such as interactions with other proteins and allowed the assignment of the chromosomal location of the genes coding for these proteins (Becker & Benz, 1990).

In vivo hemodynamic measurements have shown that RBCs with reduced deformability are preferentially sequestrated in the spleen, lung, liver and bones, regions where clinical manifestations are seen in hematological disorders involving abnormal blood cell deformability. These regional sequestrations are closely correlated with the regional flow derangements. In order to further pursue the correlation between molecular alterations in RBC membrane and physiological changes in regional hemodynamics, transgenic mice preparations offer an approach with great potential. Experimental procedures have been develped in which the mutant gene or the corrected gen can be introduced into animals to produce transgenic mice for the investigation of the influence of such gene transfers on the induction or correction of deranged physiological functions(Jaenisch, 1988). Performance of rheological test on such RBCs with controlled molecular modifications and conduct of physiological measurements of regional hemodynamics will allow us to eluci-

date the molecular basis of the role of RBCs in flow regulation in health and disease.

In conclusion, interdisciplinary research combining molecular biology with biorheological and physiological studies will contribute to not only the elucidation of normal functions of RBSs and other cells and tissues, but also the understanding of pathophysiology of their disorders and possibly the development of new methods for diagnosis and treatment.

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