## Chapter 46: Erythrocyte Membrane Disorders

# INTRODUCTION


### SUMMARY

The human erythrocyte membrane consists of a lipid bilayer containing transmembrane proteins and an underlying membrane skeleton, which is attached to the bilayer by linker protein complexes. The membrane is critical in maintaining the unique biconcave disk shape of the erythrocyte and enabling it to withstand the circulatory shear stress. The integrity of the membrane is ensured by vertical interactions between the skeleton and the transmembrane proteins, as well as by horizontal interactions between skeletal proteins. Inherited defects of membrane proteins compromise these interactions and alter the shape and deformability of the cells, which ultimately results in their premature destruction and hemolytic anemia. The disorders are typically autosomal dominant and exhibit significant clinical, laboratory, biochemical, and genetic heterogeneity.

*Hereditary spherocytosis* is a common condition characterized by spherically shaped erythrocytes on the blood film, reticulocytosis, and splenomegaly. The underlying defect is a deficiency of one of the membrane proteins, including ankyrin, band 3, $\alpha$-spectrin, $\beta$-spectrin, or protein 4.2. This weakens the vertical membrane interactions, resulting in loss of membrane and surface area. Spherocytes have diminished deformability, which predisposes them to entrapment and destruction in the spleen. *Hereditary elliptocytosis* is characterized by the presence of elliptical erythrocytes on the blood film. The principal abnormality affects horizontal membrane protein interactions and typically involves $\alpha$-spectrin, $\beta$-spectrin, protein 4.1R, or glycophorin C. The membrane skeleton is destabilized and unable to maintain the biconcave disk shape, which manifests as an elliptical distortion of the cells in the circulation. *Hereditary pyropoikilocytosis* is a rare, severe hemolytic anemia characterized by markedly abnormal erythrocyte morphology caused by defective spectrin. *Southeast Asian ovalocytosis* is largely asymptomatic and is caused by a defect in band 3. The blood film shows large oval red cells with a transverse ridge across the central area. *Acanthocytosis* is typified by contracted, dense erythrocytes with irregular projections, which may be seen in patients with severe liver disease, abetalipoproteinemia, various neurologic disorders, certain aberrant red cell antigens, and postsplenectomy. *Stomatocytosis* is a rare group of inherited disorders associated with abnormal membrane permeability and red cell cation content, which either cause overhydration or dehydration of the cells.


### Acronyms and Abbreviations:

AE1, anion exchanger-1; $\alpha^{LELY}$, $\alpha$-spectrin low-expression Lyon; $\alpha^{LEPRA}$, $\alpha$-spectrin low-expression Prague; AGLT, acidified glycerol lysis test; ANK, ankyrin; AQP1, aquaporin-1; BCSH, British Committee for Standards in Haematology; BPG, 2,3-bisphosphoglycerate; CDAII, congenital dyserythropoietic anemia type II; EMA, eosin 5′-maleimide; 4.1R, erythrocyte isoform of protein 4.1; GLT, glycerol lysis test; GLUT-1, glucose transporter-1; GP, glycophorin; GP-A, -B, -C, -D, -E, various members of glycophorin family; GSSG, oxidized glutathione; HARP, hypobetalipoproteinemia, acanthocytosis, retinitis pigmentosa, and pallidal degeneration syndrome; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; HS, hereditary spherocytosis; HSt, hereditary stomatocytosis; MAGUK, membrane-associated guanylate kinase; MARCKS, myristoylated alanine-rich C kinase substrate; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; OF, osmotic fragility; PKAN, pantothenate kinase-associated neurodegeneration; RhAG, Rh-associated glycoprotein; SAO, southeast Asian ovalocytosis; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; UGT1, uridine diphosphate glucuronosyltransferase 1.

The erythrocyte membrane plays a critical role in the function and structure of the red cell. It is a key determinant of the unique biconcave disk shape and provides the cell with a finely tuned combination of flexibility and durability. These properties enable the erythrocyte to withstand the circulatory shear pressure and allow it to undergo extensive and repeated distortion while negotiating the microvasculature and the spleen, thus ensuring survival during its average 120-day life span. The red cell membrane maintains a nonreactive surface so that erythrocytes do not adhere to the endothelium or aggregate and occlude capillaries. It provides a barrier with selective permeability, which retains vital components inside the cell and permits the efflux of metabolic waste. To facilitate the transfer

of carbon dioxide and to maintain pH homeostasis, the membrane exchanges chloride and bicarbonate anions, and it also actively controls the cation and water content of the erythrocyte. The membrane sequesters reducing agents required to prevent oxidative damage to hemoglobin and other cellular components, and it plays a role in regulating metabolism by reversibly binding and inactivating selected glycolytic enzymes.

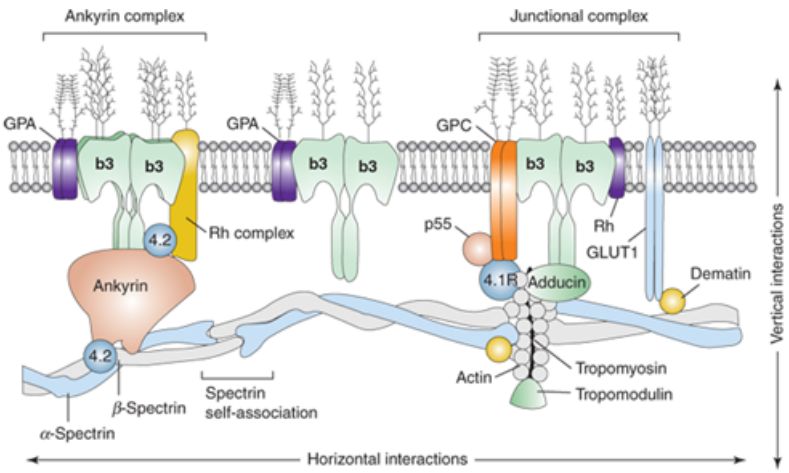
Abnormalities of the erythrocyte membrane alter the shape of the cell and compromise its integrity and ability to survive the rigors of circulation, which leads to premature destruction and hemolysis. Erythrocyte membrane disorders comprise an important group of hereditary hemolytic anemias, which are classified according to the altered red cell morphology and include hereditary spherocytosis (HS), hereditary elliptocytosis (HE) and related disorders, and the hereditary stomatocytosis (HSt) syndromes. This chapter summarizes our current understanding of the erythrocyte membrane in normal cells followed by a discussion of the underlying molecular defects and their role in the pathophysiology and clinical manifestations of these disorders. The main emphasis is on spherocytosis and elliptocytosis, the two most common and best characterized diseases.

## OVERVIEW OF THE ERYTHROCYTE MEMBRANE

The erythrocyte membrane is the most studied plasma membrane and serves as a paradigm for all cellular membranes. Mature erythrocytes are readily accessible; they contain no intracellular organelles, which facilitates the isolation of pure erythrocyte membranes; and “experiments of nature” resulting in abnormal erythrocyte morphology have provided unique opportunities to investigate the function of membrane components. These studies have revealed the primary structure and several important functions of the red cell membrane. Ongoing research, using the latest molecular technologies, continues to yield important insights into our understanding of membrane structure–function relationships, as well as genotype–phenotype correlations.

The erythrocyte membrane is a complex structure consisting of a relatively fluid lipid bilayer stabilized by an underlying two-dimensional membrane skeleton, which maintains the integrity of the biconcave disk shape of the erythrocyte (Fig. 46–1). The skeleton provides the cell with the strength and flexibility to deform rapidly and repeatedly and thus endure the shear stress encountered in the tiny capillaries of the microcirculation and in the spleen. The lipid bilayer separates the erythrocyte cytoplasm from the external plasma environment and contains phospholipids and cholesterol, as well as integral transmembrane proteins, which are tethered to the skeleton by interactions with linker proteins.

Figure 46–1.
Schematic model of the human erythrocyte membrane. The molecular assembly of the major proteins is indicated. *Vertical* interactions are perpendicular to the plane of the membrane and are represented by the ankyrin and junctional protein complexes that connect the membrane spectrin skeleton to the integral proteins embedded in the lipid bilayer. *Horizontal* interactions occur parallel to the plane of the membrane and involve spectrin tetramers and protein 4.1R. The proteins and lipids are not drawn to scale. b3, Band 3; GPA/GPC, glycophorin A/C; GLUT-1, glucose transporter-1.






## COMPONENTS OF THE ERYTHROCYTE MEMBRANE

## MEMBRANE LIPIDS

The lipid bilayer comprises approximately 50 percent of the membrane mass and contains unesterified cholesterol and phospholipids in approximately equal amounts, with small amounts of glycolipids and phosphoinositides (Chap. 31).[1,2] Mature erythrocytes are unable to synthesize fatty acids, phospholipids, or cholesterol *de novo,* and they depend on lipid exchange and limited phospholipid repair.[3]

Cholesterol regulates the fluidity of the membrane and is present in both leaflets, whereas the phospholipids are asymmetrically distributed. The choline phospholipids, phosphatidylcholine and sphingomyelin, are predominantly located in the outer leaflet and play a role in plasma lipid exchange and renewal of membrane phospholipids. Glycolipids carry several important red cell antigens, including A, B, H, and P, and are only found in the external leaflet with their carbohydrate moieties extending into the plasma. The aminophospholipids, phosphatidylserine and phosphatidylethanolamine, as well as phosphatidylinositol are located in the inner leaflet of the lipid bilayer.

This asymmetric distribution of phospholipids is maintained by a dynamic process involving flippase and floppase enzymes, which translocate the aminophospholipids to the inner and outer leaflets, respectively.[4,5] A scramblase mediates bidirectional movement of phospholipids down their concentration gradient.[6] Asymmetry of the phospholipids is important for the survival of the erythrocyte since exposure of phosphatidylserine on the outside surface of the cell, as found in sickle cell disease and thalassemia, has several deleterious consequences. It activates the coagulation cascade and may contribute to thromboses[4]; it facilitates adhesion to the vascular endothelium; it provides a recognition signal for macrophages to phagocytose these cells; and it decreases the interaction of skeletal proteins with the bilayer, which destabilizes the membrane.

Lipid rafts have been identified in erythrocytes.[7] They form detergent-resistant membrane microdomains, enriched in cholesterol and sphingolipids, and are associated with several proteins, including stomatin and flotillin-1 and -2. These rafts play a role in signaling and invasion of malaria parasites.[8]

## MEMBRANE PROTEINS

Pioneering studies resolved the major proteins of the red cell membrane by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and numbers from 1 to 8 were assigned to each protein starting with the largest protein, which migrated the slowest (Chap. 31).[9] Subsequent research revealed minor bands between the major proteins and these were designated with decimals. Analysis of the individual proteins led to the renaming of some of them, such as band 1 and 2, which are now known as $\alpha$- and $\beta$-spectrin, respectively. Technologic advances have enabled an in-depth analysis of the erythrocyte proteome by mass spectrometry, revealing a total of 340 membrane proteins.[10] Table 46–1 summarizes the properties of the major components.

Table 46–1.

**Major Red Cell Membrane Proteins**

| Band | Protein | Mr (gel) | Mr (calc) | Copies per Cell ($\times 10^3$) | Percentage of Total[a] | Gene Symbol | Chromosomal Localization | Amino Acids | Gene Size (kb) | No. of Exons | Involvement in Hemolytic Anemias |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $\alpha$-Spectrin | 240 | 280 | 240 | 16 | *SPTA1* | 1q22–q23 | 2429 | 80 | 52 | HE, HS, HPP |
| 2 | $\beta$-Spectrin | 220 | 246 | 240 | 14 | *SPTB* | 14q23–q24.2 | 2137 | >100 | 32 | HE, HS, HPP |
| 2.1 | Ankyrin[b] | 210 | 206 | 120 | 4.5 | *ANK1* | 8p11.2 | 1881 | >100 | 40 | HS |
| 2.9 | $\alpha$-Adducin[c] | 103 | 81 | 30 | 2 | *ADDA* | 4p16.3 | 737 | 85 | 16 | N |
| 2.9 | $\beta$-Adducin[c] | 97 | 80 | 30 | 2 | *ADDB* | 2p13–2p14 | 726 | ~100 | 17 | N |
| 3 | Anion exchanger-1 | 90–100 | 102 | 1200 | 27 | *EPB3* | 17q21–qter | 911 | 17 | 20 | HS, SAO, HAc |
| 4.1 | Protein 4.1 | 80 | 66 | 200 | 5 | *EL11* | 1p33–p34.2 | 588[d] | >100 | 23 | HE |
| 4.2 | Protein 4.2 | 72 | 77 | 200 | 5 | *EB42* | 15q15–q21 | 691 | 20 | 13 | HS |
| 4.9 | Dematin[e] | 48 + 52 | 43 | 40[f] | 1 | *EPB49* | 8p21.1 | 383 | – | – | N |
| 4.9 | p55[e] | 55 | 53 | 80 | – | *MPP1* | Xq28 | 466 | – | – | N |
| 5 | $\beta$-Actin | 43 | 42 | 400–500 | 5.5 | *ACTB* | 7pter–q22 | 375 | >4 | 6 | N |
| 5 | Tropomodulin | 43 | 41 | 30 | – | *TMOD* | 9q22 | 359 | – | – | N |
| 6 | G3PD[g] | 35 | 37 | 500 | 3.5[g] | *GAPD* | 12p13.31–p13.1 | 335 | 5 | 9 | N |
| 7 | Stomatin | 31 | 32 | – | 2.5 | *EPB72* | 9q33–q34 | 288 | 12 | 7 | HSt |
| 7 | Tropomyosin | 27 + 29 | 28 | 80 | 1 | *TPM3* | 1q31 | 239 | – | – | N |
| PAS-1 | Glycophorin A[h] | 36 | – | 500–1000 | 85 | *GYPA* | 4q28–q31 | 131 | >40 | 7 | HE |
| PAS-2 | Glycophorin C[h] | 32 | 14 | 50–100 | 4 | *GYPC* | 2q14–q21 | 128 | 14 | 4 | HE |

| Band | Protein | Mr (gel) | Mr (calc) | Copies per Cell ($\times 10^3$) | Percentage of Total[a] | Gene Symbol | Chromosomal Localization | Amino Acids | Gene Size (kb) | No. of Exons | Involvement in Hemolytic Anemias |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PAS-3 | Glycophorin B[h] | 20 | – | 100–300 | 10 | *GYPB* | 4q28–q31 | 72 | >30 | 5 | N |
| | Glycophorin D[h] | 23 | – | 20 | 1 | *GYPD* | 2q14–q21 | 107 | 14 | 4 | N |
| | Glycophorin E | – | – | – | – | *GYPE* | 4q28–q31 | 59 | >30 | 4 | N |

–, Information not available; G3PD, glyceraldehyde 3-phosphate dehydrogenase; HAc, hereditary acanthocytosis; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; HS, hereditary spherocytosis; HSt, hereditary stomatocytosis; N, no hematologic abnormalities reported; SAO, southeast Asian ovalocytosis.

[a]Quantitation based on scanning of sodium dodecylsulfate polyacrylamide gel electrophoresis of red cell membranes prepared from healthy blood donors. For glycophorins, values indicate the fraction of periodic acid-Schiff–positive material.

[b]Bands 2.1, 2.2, 2.3, and 2.6 are protein isoforms of erythroid ankyrin, at least some of which are produced by alternative splicing of ankyrin messenger RNA.

[c]Because adducin comigrates with band 3, no numerical band designation is available.

[d]Numerous erythroid and nonerythroid isoforms of protein 4.1 produced by alternative splicing have been described. Values correspond to the major erythroid protein 4.1 isoform.

[e]Both dematin and p55 migrate within the 4.9 band.

[f]Forty thousand dematin trimers are present in one red cell.

[g]Variable amounts of band 6 are detected in red cell membranes.

[h]Detectable on periodic acid-Schiff–stained gels only.

The membrane proteins are classified as either integral or peripheral based on the ease with which they can be removed from whole red cell membrane preparations in the laboratory. Integral or transmembrane proteins are embedded in the lipid bilayer by hydrophobic interactions and require detergents to extract them. They often protrude from the bilayer and extend into the plasma and/or the interior of the erythrocyte and these structural features correlate with their functions as transport proteins, receptors, signaling molecules, and carriers of red cell antigens.

Peripheral proteins constitute the membrane skeleton and are loosely attached to the cytoplasmic face of the lipid bilayer and can be extracted by high or low salt concentrations or by high pH. Attachment is mediated indirectly by covalent or noncovalent interactions with the cytoplasmic domains of the transmembrane proteins, as well as by direct interactions with the inner leaflet of the lipid bilayer. These associations are dynamic and the affinity of binding is regulated by post-translational modifications of the proteins, including phosphorylation, methylation, glycosylation, or lipid modification (myristoylation, palmitoylation, or farnesylation). Peripheral proteins typically function either as structural proteins and form part of the membrane skeleton or they serve as linker proteins attaching the skeleton to the bilayer.

Many erythrocyte proteins belong to superfamilies and have homologues in nonerythroid cells that are structurally related but are encoded by different genes. This genetic diversity explains why the clinical expression of most (but not all) red cell membrane protein

mutations is confined to the erythroid lineage. Several proteins exist in different isoforms, created by tissue- and developmental stage-specific alternative splicing or by the use of alternative initiation codons or promoters. Many of the membrane proteins are large, multifunctional proteins and therefore the position of a mutation determines the functional abnormality and clinical phenotype.

### Integral Membrane Proteins

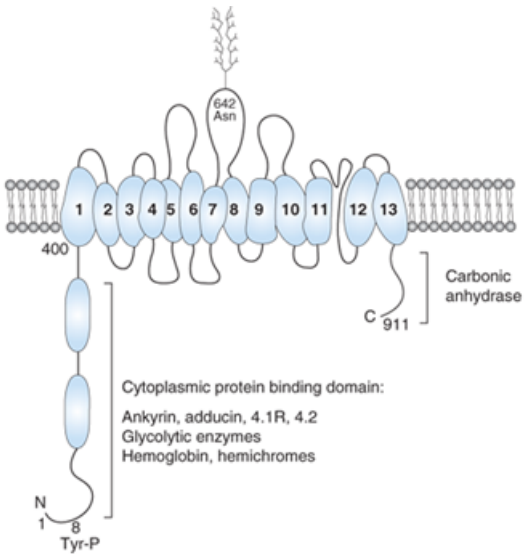
The most abundant and important transmembrane proteins are band 3, which is the anion exchanger (AE1) of the erythrocyte, and the glycophorins (GPs).

#### Band 3

The red cell contains approximately 1.2 million copies of AE1, a multifunctional and major integral membrane protein (see Table 46–1). It has a molecular mass of 102 kDa, but migrates as a diffuse band on sodium dodecylsulfate (SDS) gels as a result of heterogeneous N-glycosylation. The 911-amino-acid protein consists of two functional domains; an N-terminal 43-kDa cytoplasmic domain and a 52-kDa transmembrane channel, including a short 33-amino-acid C-terminal cytoplasmic tail[11] (Fig. 46–2). The anion exchange domain encompasses 13 $\alpha$ helical transmembrane segments and one nonhelical segment all connected by hydrophilic loops.[12,13] The short cytoplasmic tail binds carbonic anhydrase II to form a metabolon with the transmembrane domain, enabling the exchange of $HCO_3^-$ and $Cl^-$ anions, which is a critical function of the red cell.[14] The extracellular surface of the transmembrane domain of band 3 carries several antigens, including Diego, I/i, and Wright blood groups.

Figure 46–2.

Schematic model of human erythrocyte band 3. The N and C terminal regions of the protein extend into the cytoplasm and provide binding sites for several red cell proteins and enzymes. The transmembrane domain forms an anion exchange channel and consists of 13 $\alpha$ helical segments embedded in the lipid bilayer and one nonhelical segment. Asparagine 642 is linked to complex carbohydrates, which protrude on the exterior of the red cell. Tyrosine 8 is phosphorylated. The domains are not drawn to scale.






The N-terminal phosphorylated cytoplasmic domain serves as a major hub for protein-protein interactions, which perform key functions (see Figs. 46–1 and 46–2).[15] It regulates metabolic pathways by sequestering key glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and aldolase, which are inactive when bound. Phosphorylation at tyrosine 8 prevents binding, which liberates the active enzymes.[16] The cytoplasmic domain interacts with hemoglobin and hemichromes and plays a role in red cell aging[17]; it associates with several peripheral membrane proteins, including the erythrocyte isoform of protein (4.1R),[18,19] protein 4.2,[20] and adducin,[21] as well as phosphatases and kinases. This domain also serves as the major attachment site of the membrane to the underlying skeleton through its interaction with ankyrin, which binds to spectrin (see Figs. 46–1 and 46–2).[22,23]

Band 3 associates with other transmembrane proteins to form macromolecular complexes (see Fig. 46–1).[24] This includes the major GP, GPA,[25] and the Rh protein complex, consisting of Rh-associated glycoprotein (RhAG), Rh, CD47, LW, and GPB (Chap. 136).[24] In addition,

band 3 participates in the protein 4.1-based junctional complex of proteins.[19]

Band 3 is encoded by the *SLC4A1* gene, which produces different tissue-specific isoforms.[11,26] The erythroid isoform is controlled by a promoter upstream of exon 1, whereas transcription of the kidney isoform is initiated from a promoter in intron 3, resulting in a protein lacking the first N-terminal 65 amino acids.

#### Glycophorins

GPs are integral membrane glycoproteins composed of an extracellular hydrophilic N-terminal domain, a single $\alpha$-helical membrane-spanning domain, and a C-terminal cytoplasmic tail. GPA, GPB, and GPE are homologous and are encoded by closely linked genes that arose by duplication of the ancestral GPA gene.[27] GPC and GPD are encoded by the same gene but make use of alternate initiation codons.[28]

GPs have very high sialic acid content and are responsible for most of the external negative charge of red cells, which prevents the adherence of cells to each other and the vascular endothelium. The GPs carry a large number of blood group antigens, including MN, SsU, Miltenberger, En(a–), $M^K$, and Gerbich (Chap. 136). They also function as receptors for *Plasmodium falciparum*, the most virulent malaria parasite. Within the lipid bilayer of the membrane, GPA interacts with band 3 as part of a macromolecular complex, and may serve as a chaperone for band 3 targeting to the membrane.[25] GPC associates with protein 4.1R and p55, thereby providing an additional contact site between the membrane and the skeleton (see Fig. 46–1).[19] These interactions play a role in stabilizing the membrane.

#### Other Integral Membrane Proteins

The Rh-RhAG group of proteins is part of a macromolecular band 3 complex, which stabilizes the membrane. RhAG belongs to the ammonium transporter family of proteins, but its function is controversial. Numerous other proteins are embedded in the lipid bilayer, many of which are implicated in clinical immunohematology and membrane disorders, such as the XK, Kell, Kidd, Duffy, and Lutheran glycoproteins (see Fig. 46–1).[11,19] Additional integral membrane proteins include ion pumps and channels, such as stomatin, aquaporin, glucose transporter (GLUT-1), and various cation and anion transporters.

### Peripheral Membrane Proteins

Underlying the lipid bilayer is the peripheral membrane skeleton, an interlocking network of structural proteins, which plays a critical role in maintaining the shape and integrity of the red cell. The major proteins of the erythrocyte membrane skeleton are spectrin, actin, proteins 4.1R, 4.2, 4.9, p55, and the adducins, which interact in a horizontal plane. Linker proteins mediate the vertical attachment of the skeleton to integral membrane proteins in the lipid bilayer (see Fig. 46–1). The primary connecting protein is ankyrin, which links spectrin to the cytoplasmic domain of band 3, as well as to the Rh–RhAG complex. Protein 4.1R provides an additional link with GPC and band 3.

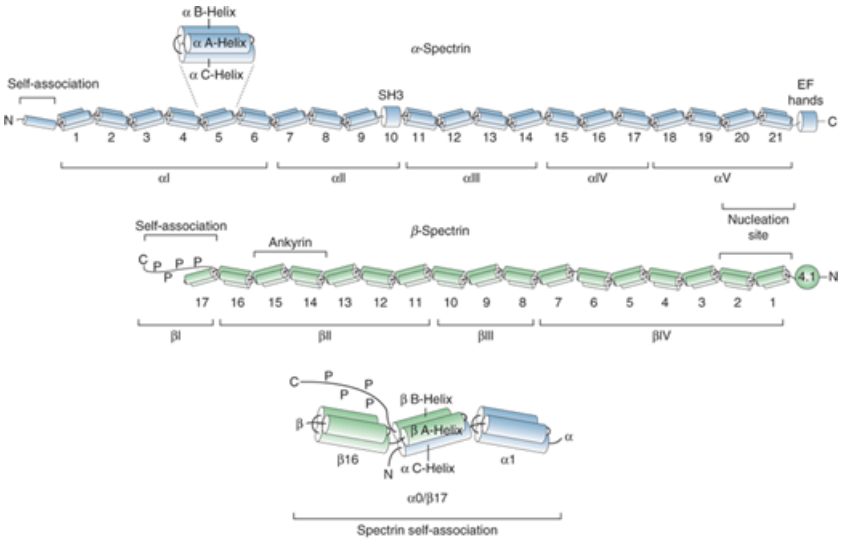
#### Spectrin

Spectrin is the major constituent of the erythrocyte membrane skeleton and is present at approximately 240,000 molecules per cell.[29] It is a multifunctional protein composed of two homologous but structurally distinct subunits, $\alpha$ and $\beta$, encoded by separate genes, which may have evolved from duplication of a single ancestral gene[30] (see Table 46–1 and Fig. 46–3). Both $\alpha$- and $\beta$-spectrin contain tandem homologous spectrin repeats that are approximately 106 amino acids long and are folded into three antiparallel helices, A, B, and C. Each repeat is connected to the adjacent repeat by short ordered $\alpha$-helical linkers (Fig. 46–3).[31,32] Erythrocyte $\alpha$-spectrin is a 280-kDa protein comprising 20 complete repeats, an N-terminal partial repeat, a central SH3 domain, and a C-terminal calcium-binding EF hand. The $\beta$-spectrin subunit is a 246-kDa polypeptide consisting of 16 complete repeats, an N-terminal actin binding domain, a partial repeat near the C-terminus, and a nonhomologous phosphorylated C-terminus. Mild trypsin treatment of spectrin cleaves the two subunits into distinct structural domains: $\alpha$I-V and $\beta$I-IV. The triple helical structure of the spectrin repeats renders the molecule highly flexible and enables it to extend and condense reversibly, which provides the red cell with elasticity and durability to withstand the shear stress encountered in the circulation.

Figure 46–3.

Schematic model of human erythrocyte $\alpha$- and $\beta$-spectrin. The proteins consist of multiple homologous spectrin repeats of approximately 106 amino acids numbered from the N-terminal. Each repeat is composed of three $\alpha$ helices. Nonhomologous regions include an SH3 domain and calcium-binding EF hands in $\alpha$-spectrin, a protein 4.1R binding domain, and a C-terminal phosphorylated tail in $\beta$-spectrin. The nucleation site indicates the initial region of interaction between $\alpha$ and $\beta$ monomers to form an antiparallel heterodimer. Spectrin heterodimer self association into tetramers involves helix C of the $\alpha$0 partial repeat of $\alpha$-spectrin and helices A and

B of the partial $\beta$17 repeat of $\beta$-spectrin to form a complete triple helical repeat. Ankyrin binds to repeats 14 and 15 of $\beta$-spectrin. Limited tryptic digestion of spectrin cleaves the proteins into discrete $\alpha$I-V and $\beta$I-IV domains.



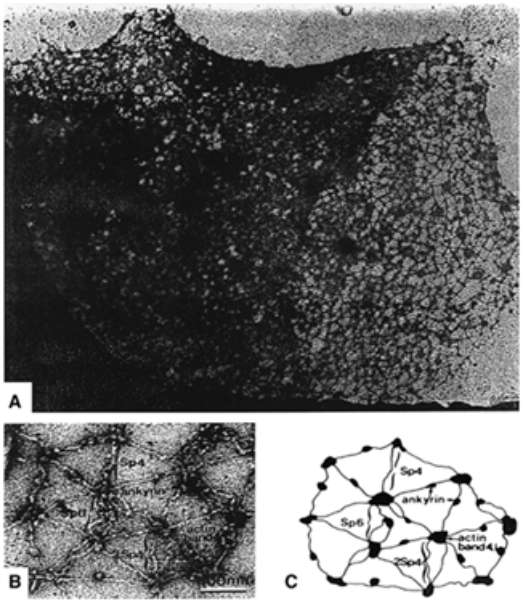



The core structure of the erythrocyte skeleton consists of spectrin heterotetramers, which are strong but flexible filaments. Tetramers are assembled from the monomers in a series of events. For initial heterodimer formation, the $\alpha$- and $\beta$-spectrin chains align in an antiparallel fashion and interact with high affinity through long-range electrostatic interactions at a nucleation site, comprising repeats $\alpha$20–21 and $\beta$1–2.[33] This triggers the association of the remaining repeats in the two subunits in a zipper-like fashion. Repeats at the N-terminus of $\alpha$-spectrin ($\alpha$I domain) and the C-terminus of $\beta$-spectrin ($\beta$I domain) are the regions involved in heterodimer self-association to form tetramers. Partial repeat $\beta$17 consists of two helices (A and B) which interact with the single helix C of partial repeat $\alpha$0 to form a complete triple helical repeat (see Fig. 46–3). The interface of this tetramerization site is dominated by hydrophobic contacts supplemented by electrostatic interactions.[34] Phosphorylation of the C-terminal region of $\beta$-spectrin beyond the self-association site decreases the mechanical stability of the membrane.

At the opposite tail end of the spectrin tetramers, the N terminus of $\beta$-spectrin binds to short F-actin filaments, which is potentiated by 4.1R, to form the core of a junctional complex,[35] which links six tetramers together into a hexagonal skeletal network (Fig. 46–4).[36] The C-terminal EF hand of $\alpha$-spectrin enhances this spectrin-actin-4.1R interaction.[37] Numerous other proteins participate in the junctional complex, including adducin, protein 4.9, p55, tropomodulin and tropomyosin (see Fig. 46–1).[19] Protein 4.1R binds to GPC and band 3, which serves as a secondary attachment site of the skeleton to integral membrane proteins. The main interaction tethering the skeleton to the lipid bilayer is accomplished by ankyrin, which links $\beta$-spectrin to band 3 (see Fig. 46–1). The ankyrin binding site is a flexible pocket formed by repeats 14 and 15 of $\beta$-spectrin near the C-terminal end of the molecule.[38,39] Spectrin also interacts with phosphatidylserine on the inner leaflet of the lipid bilayer.

**Figure 46–4.**

Electron micrograph of the human erythrocyte membrane skeleton. Membrane lipids and transmembrane proteins have been removed and the skeletons were extended during preparation and negative staining to reveal the structure. **A.** Low-magnification image reveals an ordered network of proteins. **B** and **C.** High-magnification image and schematic of the hexagonal lattice showing spectrin tetramers (Sp4) and hexamers (Sp6) or double tetramers (2Sp4). Junctional complexes contain actin filaments and protein 4.1R. Globular ankyrin molecules are bound to spectrin tetramers. *(Reproduced with permission from Liu SC et al: Visualisation of the hexagonal lattice in the erythrocyte membrane skeleton.* J Cell Biol *1987 Mar;104(3):527-536.)*





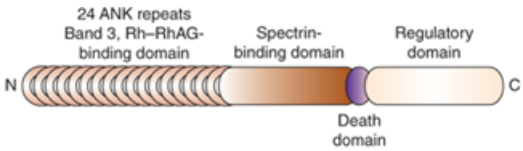

Nonrepeat sequences in spectrin provide the recognition sites for binding to modifiers, including kinases and calmodulin. The functions of spectrin are to maintain the biconcave disk shape of the red cell, regulate the lateral mobility of integral membrane proteins, and provide structural support for the lipid bilayer.

#### Ankyrin

Erythrocyte ankyrin is encoded by the *ANK1* gene, which contains three separate tissue-specific promoters and first exons that are spliced to a common exon 2.[40] The 206-kDa protein is a versatile binding partner and has three functional domains: an N-terminal 89-kDa membrane-binding domain, that contains sites for band 3 and other ligands; a central 62-kDa spectrin-binding domain, and a C-terminal 55-kDa regulatory domain that is responsible for the different isoforms of the protein, which influence ankyrin–protein interactions (Fig. 46–5).[29]

#### Figure 46–5.

Schematic of human erythrocyte ankyrin. The N-terminal domain consists of 24 ANK repeats, which bind to band 3 and the Rh–RhAg complex. The central domain attaches to spectrin. The C-terminal domain varies in different isoforms of ankyrin, which are produced by alternative splicing of the gene. This domain also contains a conserved death domain of unknown function.






The membrane-binding domain contains 24 tandem ankyrin (ANK) repeats, which are stacked into a superhelical array that is coiled into a solenoid. This structure behaves like a reversible spring, which may contribute to the elasticity of the membrane.[22] Each 33-amino-acid ANK repeat is highly conserved and forms an L-shaped structure composed of two antiparallel $\alpha$ helices separated by a $\beta$ hairpin.[41] The ANK repeats are connected by unstructured loops and provide an interface for numerous protein–protein interactions. Erythrocyte ANK repeats specifically bind to band 3 and the Rh–RhAG macromolecular complex.[19,29]

The spectrin-binding domain contains a small unique subdomain termed *ZU5-ANK*, which has a $\beta$-strand core with several surface loops and binds to $\beta$-spectrin through hydrophobic and electrostatic interactions.[42] The regulatory domain contains a highly conserved death domain of unknown function in the red cell. The C-terminal section of the regulatory domain varies in the different isoforms of ankyrin, proteins 2.1 to 2.6, which are created by alternative splicing[43] and which exhibit different binding affinities for band 3 and spectrin. Phosphorylation of ankyrin reduces binding to band 3 and spectrin tetramers.

#### Protein 4.1R

The gene encoding protein 4.1 produces diverse isoforms in different tissues and different developmental stages. This diversity is accomplished by the use of alternate first exons under the control of different promoters, and alternate initiation codons. This

transcriptional regulation is coupled to complex pre-mRNA splicing events.[44,45] The erythrocyte isoform, 4.1R, is produced from the downstream initiation codon and contains exon 16, which encodes an essential part of the spectrin-actin binding domain.

Protein 4.1R is a globular phosphoprotein that contains four structural and functional domains of 30 kDa, 16 kDa, 10 kDa, and 22 to 24 kDa (Fig. 46–6). The N-terminal 30-kDa domain is responsible for binding to the cytoplasmic domains of band 3 and GPC, as well as to p55, thereby linking the skeleton to the lipid bilayer.[19] The 10-kDa domain enhances the interaction between spectrin and actin in the junctional complex, which connects spectrin tetramers to each other. The functions of the other two domains are not characterized. Phosphorylation of 4.1R inhibits spectrin–actin–4.1R complex formation and also decreases binding to band 3. Protein 4.1R binds weakly to phosphatidylserine in the lipid bilayer.

Figure 46–6.
Schematic of human erythrocyte protein 4.1R. The protein consists of four domains, with the 30-kDa and the 10-kDa domains involved in binding to other red cell membrane proteins. The C-terminal domain has an asparagine residue at position 502 in isoform 4.1a, which is deamidated in older red cells to form aspartic acid and isoform 4.1b.




Two forms of 4.1R, a and b, are present in red cells, with protein 4.1b predominating in young erythrocytes. The difference between the two isoforms relates to the gradual deamidation of asparagine 502 to aspartic acid in a nonenzymatic, age-dependent manner, which influences the mobility of the protein on SDS gels.[46]

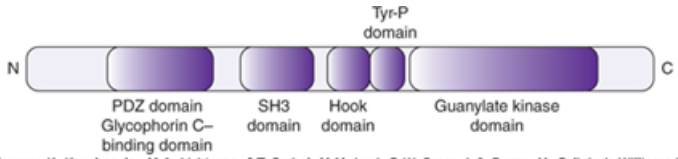
Protein 4.2

Protein 4.2 is a member of the transglutaminase family of proteins,[47] but it has no enzyme activity as it lacks the critical triad of residues that form the active transglutaminase site. The exact role of protein 4.2 has not been elucidated, but it stabilizes the link between the skeleton and the lipid bilayer. Protein 4.2 interacts with several proteins, including the cytoplasmic domain of band 3, and this binding site has been identified as a hairpin region toward the center of the protein 4.2 molecule.[11,47] Interactions with the ANK repeats in the membrane-binding domain of ankyrin[47] and CD47, a component of the Rh complex, have been documented.[19,47] *In vitro* binding studies have revealed an association of protein 4.2 with 4.1R and spectrin. Protein 4.2 binds calcium adjacent to the spectrin-binding loop suggesting that calcium may regulate this interaction. The protein undergoes posttranslational palmitoylation and myristoylation, which suggests an interaction with the lipid bilayer.[47]

p55

This molecule is a phosphoprotein member of the membrane-associated guanylate kinase (MAGUK) family of proteins.[48] In the red cell it is found as part of a ternary complex with GPC and 4.1R and it strengthens the link between the skeleton and the bilayer.[19] p55 contains five domains, including an N-terminal PDZ domain, which binds to GPC; an SH3 domain; a central HOOK domain interacting with the 30-kDa domain of 4.1R; a region with tyrosine phosphorylation sites; and a C-terminal guanylate kinase domain (Fig. 46–7).[48] The protein is extensively palmitoylated, reflecting an interaction with the membrane bilayer.

Figure 46–7.
Schematic of human erythrocyte p55. The protein is part of the membrane-associated guanylate kinase family and the kinase domain is close to the C terminus. Adjacent is a tyrosine phosphorylation zone. The central HOOK domain binds protein 4.1R.



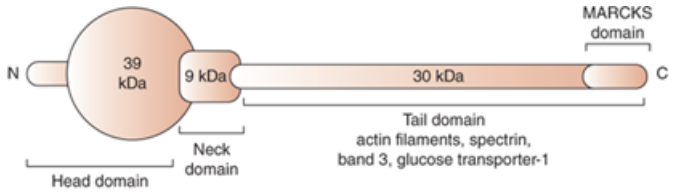



Adducin

Adducin, a calcium/calmodulin-binding phosphoprotein located at the spectrin–actin junctional complex, is composed of $\alpha\beta$ adducin heterodimers, which are structurally similar proteins encoded by separate genes. Adducins contain a 39-kDa globular head region, a

small neck region of 9 kDa implicated in oligomerization to form $\alpha_2\beta_2$ heterotetramers, and a 30-kDa cytoplasmic tail with a myristoylated alanine-rich C kinase substrate (MARCKS) phosphorylation domain at the C terminus (Fig. 46–8). The adducin tails cap actin filaments and promote interaction of spectrin and actin.[49] They also bind band 3 and the GLUT-1, and thus form part of the macromolecular junctional complex linking the spectrin skeleton to the lipid bilayer (see Fig. 46–1).[21,50] The function of adducin is regulated by calcium-dependent calmodulin binding and differential phosphorylation. A primary deficiency of adducin in human disease has not been described; however, mice with targeted inactivation of $\alpha$- or $\beta$-adducin suffer from compensated spherocytic anemia, suggesting that the adducin mutations may be candidates for recessively inherited hemolytic anemia.[51]

Figure 46–8.
Schematic of human erythrocyte adducin. The domain structure for $\alpha$ and $\beta$ adducin is similar. The neck domain is responsible for oligomerization and the tail represents the major binding site for other red cell membrane proteins. MARCKS, myristoylated alanine-rich C kinase substrate.





Actin and Actin-Binding Proteins

The erythrocyte contains $\beta$-type actin assembled into short F-actin protofilaments of 14 to 16 monomers. The length of the filaments is regulated by a "molecular ruler" of two rod-shaped tropomyosin molecules, which are bound along the filament, as well as by two tropomodulin molecules, which cap the filaments at the pointed ends.[52] At the barbed end actin is capped by an adducin heterodimer. Dematin or protein 4.9 is a trimeric phosphoprotein, which bundles the actin filaments,[53] but also acts as a linker molecule by binding to the transmembrane GLUT-1.[21,50]

## MEMBRANE ORGANIZATION

The structure of the erythrocyte membrane is determined by multiple protein–protein interactions between (1) integral membrane proteins within the lipid bilayer, (2) peripheral proteins in the skeleton, and (3) linker proteins, which tether the skeleton to the transmembrane proteins (see Fig. 46–1). Protein–lipid interactions within the bilayer or between the anionic phospholipids and the underlying membrane skeleton also play a role in cohesion of the membrane components. By using the cytoplasmic domains of embedded proteins as attachment points, the membrane skeleton not only affixes itself to the lipid bilayer but also influences the topology of the transmembrane proteins and constrains their lateral and rotational mobility.

The membrane skeleton resembles a lattice-like network, with approximately 60 percent of the lipid bilayer directly laminated to the underlying skeleton.[36] Electron microscopy of stretched membrane skeletons indicate that the individual proteins can be visualized as a highly ordered meshwork of hexagons (see Fig. 46–4).[36] The corners of each hexagon consist of a globular macromolecular junctional complex of proteins, including 4.1R and actin, which interact with spectrin tetramers, as well as tropomyosin, tropomodulin, adducin, dematin, and p55.[19,21,50] Spectrin tetramers form the arms of the hexagons, cross-bridging individual junctional complexes. These *horizontal* protein interactions are important in the maintenance of the structural integrity of the cell, accounting for the high tensile strength of the erythrocyte (see Fig. 46–1).

The spectrin/actin skeleton is anchored to the phospholipid bilayer by two major membrane protein complexes: (1) an ankyrin complex that contains transmembrane proteins, band 3, GPA, Rh, and RhAG complex proteins, as well as peripheral proteins ankyrin, protein 4.2, and several glycolytic enzymes, and (2) a distal junctional complex that contains the membrane-spanning proteins band 3, GPC, GLUT-1, Rh, Kell, and XK proteins, in addition to peripheral proteins 4.1R, actin, tropomyosin, tropomodulin, adducin, dematin, and p55. These *vertical* protein–protein interactions are critical in the stabilization of the lipid bilayer, preventing loss of microvesicles from the cells (see Fig. 46–1).

The avidity of these horizontal and vertical interactions is modulated by posttranslational modifications of the participating proteins, especially phosphorylation. The erythrocyte contains multiple protein kinases and phosphatases that constantly phosphorylate and dephosphorylate specific serine, threonine, and tyrosine residues on band 3, $\beta$-spectrin, ankyrin, 4.1R, adducin, and dematin, in a dynamic manner, thereby tightly regulating the structural properties of the membrane. Additionally, membrane protein associations are also influenced by a variety of intracellular factors, including calcium, calmodulin, phosphoinositides, and polyanions such as 2,3-bisphosphoglycerate (BPG). Red cell membrane proteins are also subject to a variety of other posttranslational modifications, including myristoylation, palmitoylation, glycosylation, methylation, deamidation, oxidation, and limited proteolytic cleavage, but the functional effects of these alterations are generally not known.

## CELLULAR DEFORMABILITY AND MEMBRANE STABILITY

In performing its primary function of oxygen delivery to the tissues, the erythrocyte has to repeatedly negotiate tiny capillaries in the microvasculature, as well as narrow slits in the spleen, which are much smaller than the diameter of the cell. Consequently, it has to undergo extensive distortion and deformation without fragmentation or loss of integrity, and this property of deformability is critical for survival during its average 120-day life span. The structure of the red cell membrane endows the cell with unique material properties, which makes it highly flexible, yet incredibly resilient, and enables a very rapid response to circulatory shear stress.

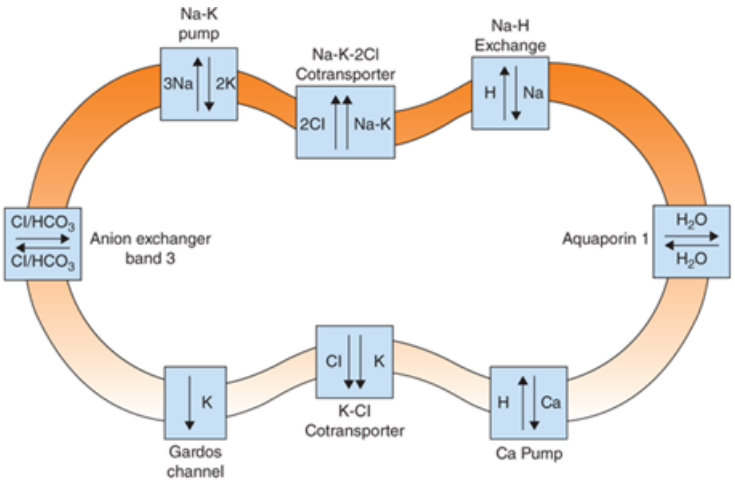
Elegant biophysical studies have identified three features that regulate the deformability of the cell: (1) the biconcave disk shape, which reflects the cell surface-area-to-volume ratio; (2) the viscoelastic properties of the membrane, which depend on the structural and functional integrity of the membrane skeleton; and (3) the cytoplasmic viscosity, which is determined primarily by intracellular haemoglobin.[54]

The unique biconcave disk shape of the erythrocyte provides a high ratio of surface area to cellular volume and this excess of membrane is critical for survival of the cell. It enables the red cell to stretch and distort when it passes through the microcirculation and protects it from destruction. To maintain the shape of the cell and to prevent loss of membrane microvesicles, the lipid bilayer and the skeleton have to be in direct contact with each other. The cohesion between the two sections of the membrane depends on protein–protein interactions between transmembrane proteins and peripheral proteins in the vertical plane of the membrane. These contacts are represented by the two macromolecular complexes (ankyrin–band 3 complex and the junctional complex) anchoring the skeleton to the integral proteins. To prevent fragmentation of the membrane and loss of the biconcave disk shape, the structural integrity of the membrane skeleton is critical. In this regard, the horizontal interactions of the peripheral proteins of the junctional complex, mainly 4.1R and actin, which link the tail ends of the spectrin tetramers together, is a major determinant of membrane stability. Spectrin heterodimer self-association, which links the head regions of the spectrin tetramers, is also of paramount importance.

The viscoelastic properties of the membrane are intrinsic features of the spectrin skeleton. The enormous distortion imposed on the cell during passage through the microvasculature is accommodated by the dynamic dissociation of spectrin tetramers into dimers, and subsequent reassociation to restore the original shape once the shear stress is removed.[55] The lattice structure of the skeleton facilitates this flexibility, as the individual hexagons are either in a compact configuration, with the junctional complexes close to each other and the spectrin tetramers coiled between them, or in an extended configuration, which allows large unidirectional deformation without disruption of the skeleton (see Fig. 46–4). The structure of the spectrin repeats also play a major role in the elasticity of the skeleton. Each triple helical repeat behaves partly as an independently folding unit and has a different thermal stability.[56] Cysteine labeling studies indicated that shear stress forced the unfolding of the least stable repeats.[57] These studies highlight the flexibility of the spectrin repeats and support the concept that their unfolding and refolding contributes to the deformability of the membrane. In addition, the elasticity of the ANK repeats may also facilitate the dynamic changes in the membrane during circulatory shear stress.[22]

Red cell viscosity is largely determined by the concentration of intracellular hemoglobin, which is tightly regulated to minimize cytoplasmic viscous dissipation during cellular deformation. As the mean cell hemoglobin concentration rises above 37 g/dL, the viscosity increases exponentially, and this compromises the deformability of the cell under increased circulatory shear stress.[54] The hemoglobin concentration is critically dependent on red cell volume, which is primarily determined by the total cation content of the cell. Numerous membrane pumps and ion channels regulate the transport of sodium and potassium across the membrane (Fig. 46–9).

Figure 46–9.
Principal ion transport and ion exchange channels and passive permeability pathways of the human erythrocyte.






## MEMBRANE PERMEABILITY

The red cell membrane displays selective permeability to cations and anions and it maintains a high potassium, low sodium, and very low calcium content within the cell.[58] Ion transport pathways in the red cell membrane (see Fig. 46–9) include energy-driven membrane pumps, gradient-driven systems, and various channels. Several transport mechanisms exist for cations, including two energy-driven pumps.[58] The sodium pump is a $Na^+K^+$ adenosine triphosphatase (ATPase) that extrudes three sodium ions in exchange for two potassium ions entering the red cell. Calcium is pumped out of the cell by a calmodulin-activated $Ca^{2+}$ ATPase, which protects the cell from deleterious effects of calcium, such as echinocytosis (Chap. 31), membrane vesiculation, calpain activation, membrane proteolysis, and cellular dehydration.[58] The $Ca^{2+}$-activated $K^+$ channel, also called the Gardos channel, causes selective loss of $K^+$ in response to increased intracellular $Ca^{2+}$. The $Na^+K^+$ gradient established by the sodium pump is used by several passive, gradient-driven systems to move ions across the red cell membrane.[58] The systems include the $K^+Cl^-$ cotransporter, the $Na^+K^+2Cl^-$ cotransporter, and the $Na^+H^+$ exchanger.

Chloride and bicarbonate anions are readily exchanged through band 3. The red cell is highly permeable to water, which is transported by aquaporin-1 (AQP1),[59] and glucose is taken up by the glucose transporter.[60] The membrane also contains an ATP-driven oxidized glutathione (GSSG) transporter and amino acid transport systems.[58] Larger charged molecules, such as ATP, do not cross the membrane.

## RED CELL MEMBRANE DISORDERS

Hemolytic anemias resulting from defects in the erythrocyte membrane comprise an important group of hereditary anemias. The disorders are characterized by altered red cell morphology, which is reflected in the nomenclature of HS, HE, hereditary pyropoikilocytosis (HPP) and southeast Asian ovalocytosis (SAO), which are the most common disorders in this group. Protein studies have identified the underlying membrane abnormalities and advances in molecular biology have enabled further characterization of these disorders and, in many cases, identification of the causative mutations. These molecular analyses have provided additional information on the pathogenesis of these disorders and important insights into the structure–function relationships of erythrocyte membrane proteins.

As predicted in 1984 by Jiri Palek[61] and confirmed by subsequent studies, protein defects that compromise *vertical* interactions between the membrane skeleton and the lipid bilayer result in destabilization of the bilayer, loss of membrane microvesicles and spherocyte formation; whereas mutations affecting *horizontal* protein interactions within the membrane skeletal network disrupt the skeleton resulting in defective shape recovery and elliptocytes (Table 46–2). Red cell membrane disorders exhibit significant heterogeneity in their clinical, morphologic, laboratory and molecular characteristics.

Table 46–2.

**Erythrocyte Membrane Protein Defects in Inherited Disorders of Red Cell Shape**

| Protein | Disorder | Comment |
|---|---|---|
| Ankyrin | HS | Most common cause of typical dominant HS |
| Band 3 | HS, SAO, NIHF, HAc | “Pincered” HS spherocytes seen on blood film presplenectomy; SAO results from 9-amino-acid deletion |
| $\beta$-Spectrin | HS, HE, HPP, NIHF | “Acanthocytic” spherocytes seen on blood film presplenectomy; location of mutation in $\beta$-spectrin determines clinical phenotype |
| $\alpha$-Spectrin | HS, HE, HPP, NIHF | Location of mutation in $\alpha$-spectrin determines clinical phenotype; $\alpha$-spectrin mutations most common cause of typical HE |
| Protein 4.2 | HS | Primarily found in Japanese patients |
| Protein 4.1 | HE | Found in certain European and Arab populations |
| GPC | HE | Concomitant protein 4.1 deficiency is basis of HE in GPC defects |

GPC, glycophorin C; HAc, hereditary acanthocytosis; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; HS, hereditary spherocytosis; NIHF, nonimmune hydrops fetalis; SAO, southeast Asian ovalocytosis.

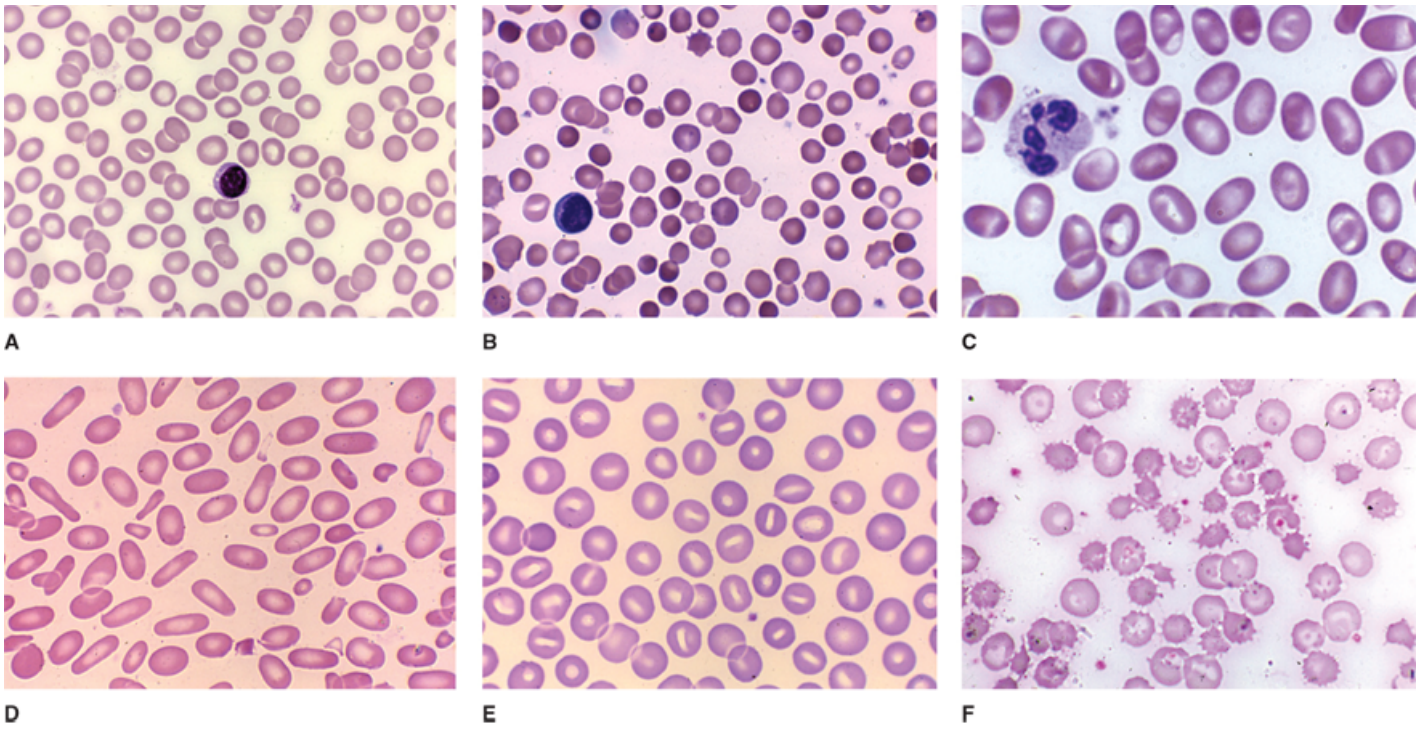
## HEREDITARY SPHEROCYTOSIS

### Definition and History

Hereditary spherocytosis is characterized by the presence of osmotically fragile spherical red blood cells on the blood film (Fig. 46–10B). The disorder was first described in 1871 as microcythemia in a case history by two Belgian physicians.[62]

**Figure 46–10.**

Blood films from patients with erythrocyte membrane disorders. **A.** Normal blood film. **B.** HS with dense spherocytes. **C.** SAO with large ovalocytes exhibiting a transverse ridge. **D.** HE with elongated elliptocytes and some poikilocytes. **E.** HSt with cup-shaped stomatocytes. **F.** Hereditary abetalipoproteinemia with acanthocytes. *(Reproduced with permission from Lichtman's Atlas of Hematology, www.accessmedicine.com.)*






### Epidemiology

HS occurs in all racial and ethnic groups. It is the most common inherited hemolytic anemia in individuals of northern European ancestry, affecting approximately 1 in 2000 individuals in North America and Europe.[63] It is also common in Japan and in Africans from southern Africa. Males and females are affected equally.

### Etiology and Pathogenesis

The hallmark of HS erythrocytes is loss of membrane surface area relative to intracellular volume, which accounts for the spherical shape and loss of central pallor of the cell (Figs 46-10B and 46-11C). Spherocytes exhibit decreased deformability and are thus selectively retained, damaged and ultimately destroyed in the spleen, which causes the hemolysis experienced by HS patients. The HS red cell membrane is destabilized by a deficiency of critical membrane proteins, including spectrin, ankyrin, band 3 and protein 4.2, which decreases the vertical interactions between the skeleton and the bilayer, resulting in the release of microvesicles and loss of surface area (Fig. 46–12). It is hypothesized that two mechanisms underlie the membrane loss: (1) in cells with spectrin/ankyrin deficiency, sections of the lipid bilayer and band 3 are not in contact with the skeleton, which will increase the lateral and rotational mobility of band 3, allowing lipid microvesicles containing band 3 to be generated, and (2) in cells with decreased amounts of band 3/protein 4.2, the stabilizing effect of the transmembrane section of band 3 on the lipid bilayer is lost, facilitating the formation of band 3-free microvesicles.[13]

Figure 46–11.
Scanning electron micrographs of erythrocytes with abnormal morphology due to membrane defects. **A.** Normal discocyte. **B.** Echinocyte. **C.** Spherocyte. **D.** Stomatocytes. **E.** Ovalocytes. **F.** Elliptocytes. **G.** Acanthocytes. *(Reproduced with permission from* Lichtman's Atlas of Hematology, *www.accessmedicine.com.)*

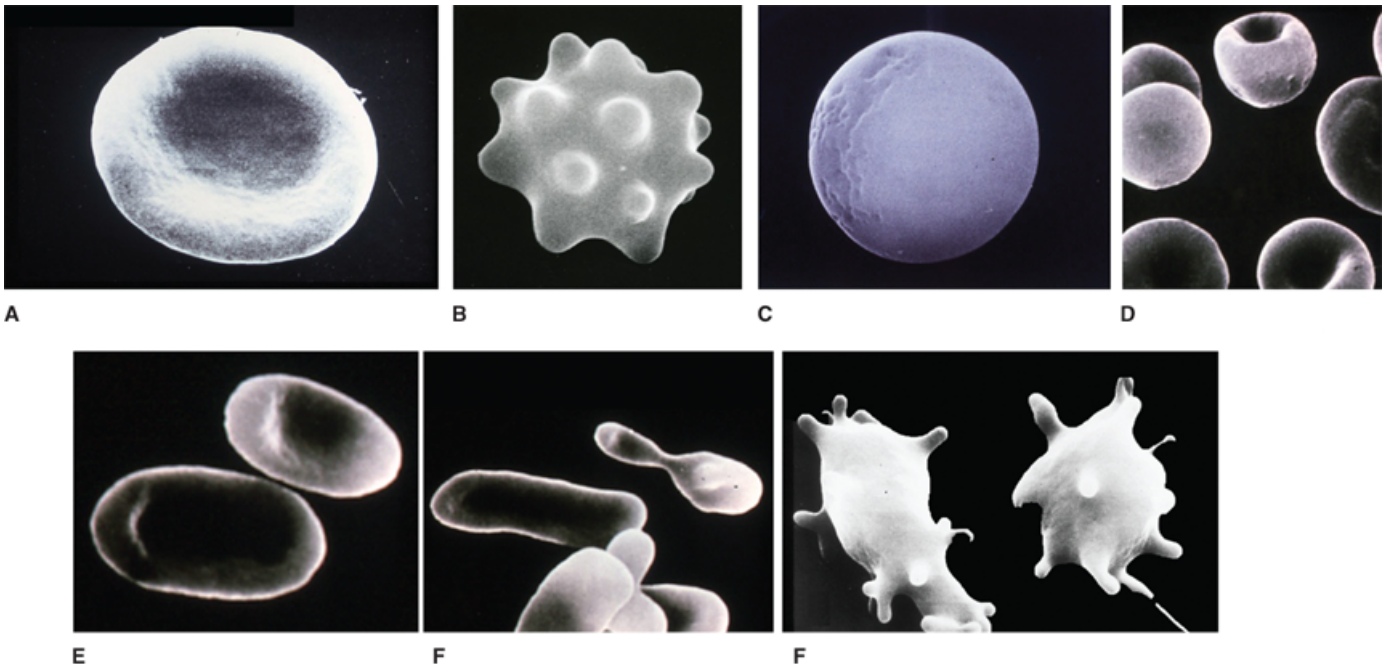
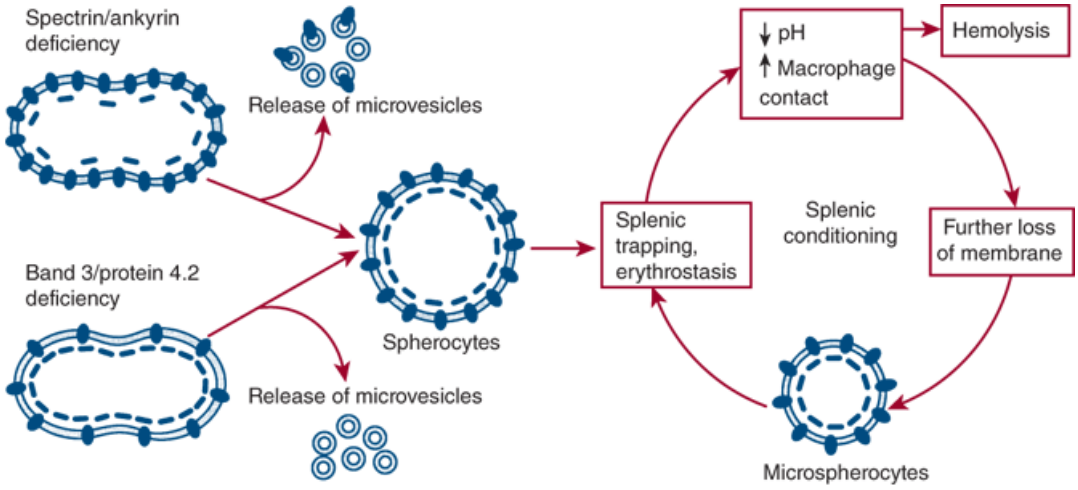





Figure 46–12.

Pathobiology of hereditary spherocytosis (HS). The primary defect in HS is a deficiency of one of the membrane proteins, which destabilizes the lipid bilayer and leads to a loss of membrane in the form of microvesicles. This reduces the surface area of the cell and leads to spherocyte formation. Red cells with a deficiency of spectrin or ankyrin produce microvesicles containing band 3, whereas a reduced amount of band 3 or protein 4.1R gives rise to band 3–free microvesicles. Spherocytes have decreased deformability and are trapped in the spleen where the membrane is further damaged by splenic conditioning, which ultimately results in hemolysis.






### Red Cell Membrane Protein Defects

Analysis of HS red cell membrane proteins by several research groups has revealed quantitative abnormalities of spectrin, ankyrin, band 3, and protein 4.2 in 70 to 90 percent of the cases.[13,63,64] This spectrum of defects is found worldwide in all the HS cohorts that have been studied; however, the relative frequency of each defect varies with the geographical area and ethnic group. In the United States, parts of Europe, and in Korea, the most common defect is ankyrin deficiency (30 to 60 percent),[63,65,66] whereas it is relatively uncommon elsewhere in the world (<15 percent). In other parts of Europe[64,67] and in South Africa (unpublished), band 3 deficiency is the main defect. In Japan, almost half of the HS cases are caused by a decreased amount of protein 4.2, and in Korea and South Africa, this defect is the second most common, but in other populations it is rare (<6 percent).[63,64,65] The underlying gene mutations have not been

investigated in all HS subjects, but the limited research that has been conducted on the defective genes has identified more than 140 different mutations, which are often unique to a family.

#### Ankyrin

Concomitant ankyrin and spectrin deficiency was first described in two patients with severe atypical HS and the primary defect was identified as an ankyrin abnormality.[68] Subsequent DNA analysis of the *ANK1* gene in patients with typical HS identified several mutations,[69] and numerous other studies have shown that ankyrin/spectrin deficiency is a common cause of HS. Ankyrin binds to spectrin with high affinity and attaches it to the membrane, which stabilizes the molecule. Because ankyrin is present in limiting amounts, a deficiency of ankyrin causes an equivalent loss of spectrin.

Different types of ankyrin mutations have been identified throughout the gene, indicating that there are several mechanisms that ultimately result in a decreased amount of ankyrin in the membrane. Interestingly, the majority of these mutations are frameshift and nonsense mutations that either result in unstable transcripts that are destroyed by nonsense-mediated mRNA decay or else produce a truncated defective ankyrin molecule.[66] More than 50 mutations have been documented and they are typically family-specific, although a few recurrent mutations have been described[69,70] and 15 to 20 percent of mutations are *de novo*.[63] Missense mutations have been documented in all the ankyrin domains and are thought to disrupt normal ankyrin–protein interactions. A few splicing mutations have been identified, including a mutation in intron 16, which created a new splice acceptor site and a complex pattern of aberrant splicing.[71] Both parents were heterozygous for this mutation and the proband was homozygous, indicating that homozygosity for an ankyrin mutation is compatible with life.

Mutations in the erythroid-specific promoter of the *ANK1* gene are common in recessive HS. A dinucleotide deletion impairs the binding of a transcription factor complex, which leads to a reduced number of ankyrin transcripts.[72] Point mutations in a barrier insulator element of the promoter also decrease transcription of the gene.[73]

Cytogenetic studies have identified a few ankyrin-deficient HS patients with a contiguous gene syndrome that includes deletion of the ankyrin gene locus at 8p11.2. These patients additionally suffer from dysmorphic features, psychomotor retardation, and hypogonadism.[74]

#### Band 3

A subset of HS patients present with a band 3 deficiency, typically accompanied by a secondary decrease in protein 4.2, a result of the reduction in protein 4.2 binding sites in the cytoplasmic domain of band 3. The extent of band 3 deficiency in heterozygous patients ranges between 20 and 50 percent, depending on the severity of the mutation, and the compensatory effect of the *in trans* normal allele. Mushroom-shaped “pincered” cells are commonly seen on the blood film of HS patients with a band 3 abnormality.

More than 55 underlying mutations have been described; they are variable and occur throughout the band 3 gene.[13,66] Null mutations are typically family-specific and are caused by frameshift or nonsense mutations, or, in a few cases, by abnormal splicing, all of which result in truncated nonfunctional proteins or unstable transcripts that are not translated into protein. Missense mutations are common and often occur in several kindred. Highly conserved arginine residues at the internal boundaries of the transmembrane segments of the protein (see Fig. 46–2) are frequently mutated, including residues 490, 518, 760, 808, and 870.[66,75] The mutations probably interfere with the cotranslational insertion of band 3 into the endoplasmic reticulum and ultimately into the red cell membrane. Short in-frame insertions or deletions have been documented and presumably also impair insertion of the mutant protein into the lipid bilayer.

Mutations in the cytoplasmic domain of band 3 impact on the interaction of band 3 with proteins in the membrane skeleton, or may alter the conformation of the protein rendering it unstable and prone to degradation prior to insertion into the membrane. Some cytoplasmic mutations, such as band 3 Cape Town and band 3 Mondega, are silent in the heterozygous state, but exacerbate the clinical presentation when inherited *in trans* to another mutation.[76,77]

#### Spectrin

Erythrocytes from HS patients with defects in spectrin or ankyrin are deficient in spectrin. The degree of deficiency correlates with the severity of hemolysis, the response to splenectomy and the ability to withstand mechanical shear stress.[78,79] Visualization of the membrane skeleton of these red cells revealed a decreased density of the spectrin filaments connecting the junctional complexes.[80] The causative mutations occur in either $\alpha$- or $\beta$-spectrin genes.

α-Spectrin

Defects in $\alpha$-spectrin are rare and are associated with severe recessive HS. During erythropoiesis $\alpha$-spectrin is synthesized in a two- to fourfold excess over $\beta$-spectrin and heterozygotes thus still produce sufficient $\alpha$-spectrin to form heterodimers with all the $\beta$-spectrin molecules, which will not result in spectrin deficiency. The defect will only be manifested in individuals who are homozygous or doubly heterozygous for mutations in $\alpha$-spectrin. The mechanism underlying spectrin deficiency has not been fully elucidated, but a low-expression allele or a polymorphism inherited *in trans* to a causative null mutation plays a role. An example of a low-expression allele is $\alpha^{LEPRA}$ (low-expression Prague), which produces less than 20 percent of the normal amount of $\alpha$-spectrin transcripts as a result of a splicing and mRNA processing defect, but does not cause any symptoms even in the homozygous state. However, in combination with another mutation on the other $\alpha$-spectrin allele, which produces a nonfunctional truncated protein, it causes severe spectrin deficiency and anemia.[81] A polymorphic missense mutation in the $\alpha$I domain in spectrin Bug Hill has been identified in several patients with spectrin-deficient, recessive HS who carry another uncharacterized $\alpha$-spectrin gene defect that causes the disease.[82] Extensive analysis of the $\alpha$-spectrin gene in a proband with severe nondominant HS revealed a partial maternal isodisomy of chromosome 1, resulting in homozygosity of the 1q23 region containing the maternal *SPTA1* gene, which carried an R891X nonsense mutation.[83] Uniparental disomy therefore unmasked a recessive mutation in the mother, which caused severe clinical symptoms in the child.

β-Spectrin

The production of $\beta$-spectrin polypeptides is the limiting factor in spectrin heterodimer formation and one mutant allele is sufficient to cause spectrin deficiency in autosomal dominant HS. The blood films of these patients typically show a subpopulation of spiculated cells (acanthocytes and echinocytes) in addition to spherocytes.[63] Mutations in $\beta$-spectrin are found throughout the gene and are mainly null mutations caused by frameshift, nonsense, splicing, and initiator codon defects, which silence the mutant allele.[84] With a few exceptions the mutations are all kindred-specific. Truncated $\beta$-spectrin chains have also been described and are caused by frameshift mutations, in-frame deletions, or exon skipping. These mutations lead to, for example, reduced synthesis of an unstable protein,[85] or they impair the interaction with ankyrin and thereby the insertion of spectrin into the membrane.[86] A few missense mutations have been identified, including $\beta$-spectrin$^{Kissimmee}$, which is caused by a mutation in the 4.1R/actin–binding domain of the protein.[87] The mutant protein is unstable and does not bind to 4.1R, and thus it only interacts weakly with actin, which may explain why these red cells are deficient in spectrin.[87]

#### Protein 4.2

Protein 4.2 deficiency is common in Japanese patients with recessively inherited HS who exhibit almost a complete absence of the protein.[63] Defects in this protein also occur in whites and other population groups, and 13 mutations have been described in the 4.2 gene of individual kindred, including missense mutations and in-frame deletion and insertion of nucleotides. Nonsense, frameshift, and splicing defects result in premature termination of translation and these mutant truncated proteins are not detected on the membrane, indicating that they are unstable and presumably degraded.[47] Amino acids 306 to 320 are highly conserved and five of the known mutations (three missense and two nonsense) occur in this region, which is adjacent to the hairpin that binds to band 3 in the predicted tertiary structure of protein 4.2.[88] The only recurrent and most common mutation, protein 4.2 Nippon, is caused by a point mutation that affects mRNA processing.[89] Patients are either homozygous for this mutation or heterozygous for a second mutation on the other allele.[47] Mutations have also been identified in individual patients with recessive HS from Europe, Tunisia, and Pakistan. South African kindred with autosomal dominant HS from a deficiency of protein 4.2 have been noted, but the underlying mutations have not been investigated.

### Secondary Membrane Defects

The decreased membrane surface area in hereditary spherocytes involves a symmetrical loss of each species of membrane lipid. The relative proportions of cholesterol and phospholipids are therefore normal and the asymmetrical distribution of phospholipids is maintained.

HS red cells exhibit increased cation permeability, presumably secondary to the underlying membrane defect.[90] The excessive sodium influx activates the $Na^+$-$K^+$ ATPase cation pump, which increases ATP turnover and glycolysis. Spherocytes are dehydrated, especially cells obtained from the splenic pulp, but the underlying mechanism has not been clearly defined. The acidic environment of the spleen and oxidative damage by splenic macrophages increase the activity of the $K^+Cl^-$ cotransporter, which may play a role in dehydration. The

hyperactive $Na^+$-$K^+$ ATPase pump may also contribute as three sodium ions are extruded in exchange for two potassium ions, and this loss of monovalent cations is accompanied by the loss of water. Dehydration may also be related to loss of surface area.

#### Molecular Determinants of Clinical Severity

Affected individuals of the same kindred typically experience similar degrees of hemolysis. However, in some families the clinical expression is variable and this may be influenced by several factors. Low-expression alleles decrease transcription of the gene or influence the expression or incorporation of the protein into the membrane, but there is no phenotypic effect in the heterozygous state because the normal allele compensates for the deleterious effect. However, when inherited with a mutant allele that causes HS, it exacerbates the clinical expression of the disease. Examples of low-expression alleles that influence HS include band 3 Genas, band 3 Mondego, and two $\alpha$-spectrin alleles, $\alpha^{LELY}$ and $\alpha^{LEPRA}$.[77,81,91,92,93,94]

Variable penetrance of the defective gene, a *de novo* mutation or a mild form of recessively inherited HS may also influence the clinical severity. Double heterozygosity for two mild band 3 mutations can have an additive effect[76] and rare cases caused by homozygous defects in band 3 result in severe transfusion-dependent hemolytic anemia or fetal death.[91,95,96] Coinheritance of other hematologic disorders or Gilbert syndrome, caused by homozygosity for a polymorphism in the promoter of the uridine diphosphate-glucuronosyltransferase (UGT1) gene, can also alter the clinical symptoms.[63,97,98]

#### Role of the Spleen

The spleen plays a secondary but important role in the pathophysiology of HS. Spherocytes are retained and ultimately destroyed in the spleen and this is the primary cause of the chronic hemolysis experienced by HS patients (see Fig. 46–12). The reduced deformability of spherocytes impedes their passage through the interendothelial slits separating the splenic cords of the red pulp from the splenic sinuses. The decrease in red cell deformability is primarily related to a loss of surface area and, to a lesser extent, to an increase in internal viscosity as a result of mild cellular dehydration. *Ex vivo* experiments using perfused human spleens and red cells treated with lysophosphatidylcholine to induce spherocytosis revealed that the degree of splenic retention correlated with the reduction in the surface-area-to-volume ratio.[99]

The spleen is a metabolically hostile environment with a decreased pH, low concentrations of glucose and ATP, and increased oxidants, all of which are detrimental to the red cell. Spherocytes are “conditioned” during erythrostasis in the spleen and become more osmotically fragile and increasingly spherocytic.[100] Exposure to macrophages in the spleen eventually leads to erythrophagocytosis and destruction.

#### Inheritance

In approximately 75 percent of HS patients, inheritance is autosomal dominant. In the remaining patients, the disorder may be autosomal recessive or result from *de novo* mutations, which is relatively common.[101,102] Mutations in $\alpha$-spectrin or protein 4.2 are often associated with recessive HS.

#### Clinical Features

The clinical manifestations of HS vary widely. The typical clinical picture combines evidence of hemolysis (anemia, jaundice, reticulocytosis, gallstones, splenomegaly) with spherocytosis (spherocytes on the blood film and increased osmotic fragility) and a positive family history. Mild, moderate, and severe forms of HS have been defined according to differences in hemoglobin, bilirubin, and reticulocyte counts (Table 46–3), which can be correlated with the degree of compensation for hemolysis. Initial assessment of a patient with suspected HS should include a family history and questions about history of anemia, jaundice, gallstones, and splenectomy. Physical examination should seek signs such as scleral icterus, jaundice, and splenomegaly.

Table 46–3.

**Classification of Hereditary Spherocytosis**

| Laboratory Findings | HS Trait or Carrier | Mild Spherocytosis | Moderate Spherocytosis | Moderately Severe Spherocytosis* | Severe Spherocytosis† |
|---|---|---|---|---|---|
| Hemoglobin (g/dL) | Normal | 11–15 | 8–12 | 6–8 | <6 |
| Reticulocytes (%) | 1–2 | 3–8 | ± 8 | ≥10 | ≥10 |
| Bilirubin (mg/dL) | 0–1 | 1–2 | ± 2 | 2–3 | ≥3 |
| Spectrin content (% of normal)‡ | 100 | 80–100 | 50–80 | 40–80§ | 20–50 |
| Blood film | Normal | Mild spherocytosis | Spherocytosis | Spherocytosis | Spherocytosis and poikilocytosis |
| Osmotic fragility | | | | | |
| Fresh blood | Normal | Normal or slightly increased | Distinctly increased | Distinctly increased | Distinctly increased |
| Incubated blood | Slightly increased | Distinctly increased | Distinctly increased | Distinctly increased | Markedly increased |

*Values in untransfused patients.

†By definition, patients with severe spherocytosis are transfusion-dependent. Values were obtained immediately prior to transfusion.

‡Normal: $245 \pm 27 \times 10^3$ spectrin dimers per erythrocyte.

§Spectrin content is variable in this group of patients, presumably reflecting heterogeneity of the underlying pathophysiology.

Adapted with permission from Eber SW, Armbrust R, Schröter W: Variable clinical severity of hereditary spherocytosis: Relation to erythrocytic spectrin concentration, osmotic fragility, and autohemolysis. *J Pediatr* 1990 Sep;117(3):409-416.

#### Typical Hereditary Spherocytosis

Approximately 60 to 70 percent of HS patients have moderate disease, which typically presents in infancy or childhood but may present at any age. In children, anemia is the most frequent finding (50 percent of cases), followed by splenomegaly, jaundice, or a positive family history.[13,63] No comparable data exist for adults. Hemolysis may be incompletely compensated with mild to moderate anemia (see Table 46–3). The moderate anemia may often be asymptomatic; however, fatigue and mild pallor or both may be present. Jaundice may be intermittent and is seen in about half of patients, usually in association with viral infections. When present, jaundice is acholuric, characterized by unconjugated hyperbilirubinemia without detectable bilirubinuria. Palpable splenomegaly is evident in most (>75 percent) older children and adults. Typically the spleen is modestly enlarged (2 to 6 cm below the costal margin), but it may be massive. No proven correlation exists between the spleen size and the severity of HS. However, given the pathophysiology and response of the disease to splenectomy, such a correlation probably exists.

#### Mild Hereditary Spherocytosis

Approximately 20 to 30 percent of HS patients have mild disease with “compensated hemolysis,” that is, red blood cell production and destruction are balanced, and the hemoglobin concentration of the blood is normal (see Table 46–3).[63,103] The life span of spherocytes is decreased, but patients adequately compensate for hemolysis with increased marrow erythropoiesis. These patients are usually

asymptomatic. Splenomegaly is mild, reticulocyte counts are generally less than 6 percent, and spherocytes on the blood film may be minimal, which complicates the diagnosis. Many of these individuals escape detection until adulthood when they are being evaluated for unrelated disorders or when complications related to anemia or chronic hemolysis occur. Hemolysis may become severe with illnesses that further increase splenomegaly, such as infectious mononucleosis, or may be exacerbated by other factors, such as pregnancy or sustained, vigorous exercise. Because of the asymptomatic course of HS in these patients, diagnosis of HS should be considered during evaluation of incidentally noted splenomegaly, gallstones at a young age, or anemia resulting from parvovirus B19 infection or other viral infections.

#### Moderately Severe and Severe Hereditary Spherocytosis

Approximately 5 to 10 percent of HS patients have moderately severe disease, as evidenced by indicators of anemia that are more pronounced than in typical moderate HS, and an intermittent requirement for transfusions (see Table 46–3). This category includes patients with dominant and recessive HS. A small number (<5 percent) of patients have severe disease with life-threatening anemia and are transfusion-dependent. They almost always have recessive HS. Most have severe spectrin deficiency, which is thought to result from a defect in $\alpha$-spectrin,[78,79] but defects in ankyrin or band 3 have also been identified.[91,96] Patients with severe HS often have irregularly contoured or budding spherocytes or bizarre poikilocytes in addition to typical spherocytes and microspherocytes on the blood film. Added to the risks of recurrent transfusions, patients often suffer from hemolytic and aplastic crises and may develop complications of severe uncompensated anemia, including growth retardation, delayed sexual maturation, and aspects of thalassemic facies.

#### Asymptomatic Carriers

Parents of patients with recessive HS are clinically asymptomatic and do not have anemia, splenomegaly, hyperbilirubinemia, or spherocytosis on the blood films. However, most have subtle laboratory signs of HS (see Table 46–3), including slight reticulocytosis, diminished haptoglobin levels, and slightly elevated incubated osmotic fragility, particularly the 100 percent red cell lysis point, which occurs at a higher sodium chloride concentration in carriers compared to normal subjects.[103] The acidified glycerol lysis test may also be useful to detect carriers. In North America and parts of Europe, approximately 1 percent of the population is estimated to be silent carriers.[63]

### Pregnancy and Hereditary Spherocytosis

Most patients do well during pregnancy[104] although anemia may be exacerbated by plasma volume expansion and increased hemolysis. A few patients are symptomatic only during pregnancy. Transfusions are rarely required.

### Hereditary Spherocytosis in the Neonate

Jaundice is the most common finding in neonates with HS, present in approximately 90 percent of cases. It may be accentuated by coinheritance of Gilbert syndrome, caused by homozygosity for a polymorphism in the promoter of the *UGT1* gene (Chaps. 33 and 47).[63,97,98] Less than half of infants are anemic and severe anemia is rare. A few cases of hydrops fetalis from homozygosity or compound heterozygosity for band 3 or spectrin defects have been reported.[91,105,106]

### Complications

#### Gallbladder Disease

Chronic hemolysis leads to formation of bilirubinate gallstones, the most frequently reported complication in up to half of HS patients. Coinheritance of Gilbert syndrome markedly increases the risk of gallstone formation. Although gallstones have been detected in children, they mainly occur in adolescents and young adults.[13,63] Routine management should include interval ultrasonography to detect gallstones because many patients with cholelithiasis and HS are asymptomatic. Interval ultrasonography allows prompt diagnosis and treatment and prevents complications of symptomatic biliary tract disease, including biliary obstruction, cholecystitis, and cholangitis.

#### Hemolytic, Aplastic and Megaloblastic Crises

Hemolytic crises are the most common and are usually associated with viral illnesses and typically occur in childhood.[13,63] They are generally mild and characterized by jaundice, splenomegaly, anemia and reticulocytosis. Medical intervention is seldom necessary. During rare severe hemolytic crises, red cell transfusion may be required.

Aplastic crises following virally induced marrow suppression are uncommon but may result in severe anemia requiring hospitalization and transfusion with serious complications, including congestive heart failure or even death.[13,63] The most common etiologic agent in these cases is parvovirus B19 (Chap. 36). The virus selectively infects erythropoietic progenitor cells and inhibits their growth leading to the characteristic finding of a low number of reticulocytes despite severe anemia. Aplastic crises usually last for 10 to 14 days and may bring asymptomatic, undiagnosed HS patients with compensated hemolysis to medical attention.[63]

Megaloblastic crises may occur in HS patients with increased folate demands, such as pregnant patients, growing children, or patients recovering from an aplastic crisis. This complication can be prevented with appropriate folate supplementation.

#### Other Complications

Leg ulcers, chronic dermatitis on the legs and gout are rare manifestations of HS, which usually heal rapidly after splenectomy. In severe cases, skeletal abnormalities resulting from expansion of the marrow can occur. Extramedullary hematopoiesis can lead to tumors, particularly along the thoracic and lumbar spine or in the kidney hila, in nonsplenectomized patients with mild to moderate HS.[13,63] Postsplenectomy, the masses involute and undergo fatty metamorphosis.

HS has been suggested to predispose patients to hematologic malignancies, including myeloproliferative disorders, particularly myeloma, but cause and effect have not been proven. Thrombosis has been reported in several HS patients, usually postsplenectomy. Untreated HS may aggravate other underlying diseases, such as congestive heart disease and hemochromatosis.[13,63]

### Nonerythroid Manifestations

Clinical manifestations are confined to the erythroid lineage in the majority of patients with HS, but a few exceptions have been observed. Several HS kindred have been reported with cosegregating nonerythroid manifestations, particularly neuromuscular abnormalities including cardiomyopathy, slowly progressive spinocerebellar degenerative disease, spinal cord dysfunction, and movement disorders. Erythrocyte ankyrin and $\beta$-spectrin are also expressed in muscle, brain, and spinal cord, which raises the possibility that these HS patients suffer from defects of one of these proteins.[13]

An isoform of band 3 is expressed in the kidney and heterozygous defects of band 3 have been described in patients with inherited distal renal tubular acidosis and normal erythrocytes. This finding is in contrast to most patients with heterozygous mutations of band 3, who have normal renal acidification and abnormal erythrocytes. Kindred with HS *and* renal acidification defects resulting from band 3 mRNA processing mutations, band $3^{Pribram}$ and band $3^{Campinas}$, have been described.[107,108] Homozygosity for band $3^{Coimbra}$, a V488M missense mutation, resulted in the absence of band 3 and renal tubular acidosis in a severely affected HS infant.[91]

### Laboratory Features

Laboratory findings in HS are variable, which correlate with the heterogeneous clinical presentation.

#### Blood Film

Erythrocyte morphology in HS is not uniform. Typical HS patients have blood films with easily identifiable spherocytes lacking central pallor (see Fig. 46–10B and 46-11C). Patients with mild HS may present with only a few spherocytes, and at the other end of the spectrum, severely affected patients exhibit numerous dense microspherocytes and bizarre erythrocyte morphology with anisocytosis and poikilocytosis. Blood films from patients with band 3 defects often exhibit “pincered” or mushroom-shaped red cells, whereas spherocytic acanthocytes are associated with $\beta$-spectrin mutations. When examining blood from a patient with suspected spherocytosis, a high-quality film with the erythrocytes properly separated and some cells with central pallor in the field of examination are important because spherocytes can be an artifact.

#### Erythrocyte Indices

Most patients have mild to moderate anemia with hemoglobin in the 9 to 12 g/dL range (see Table 46–3). Mean corpuscular hemoglobin concentration (MCHC) is increased (>36 g/dL) because of relative cellular dehydration in approximately half of patients, but all HS patients have some dehydrated cells. Some automated hematology analyzers measure the hemoglobin concentration of individual red cells and a demonstration of a population of hyperdense erythrocytes can be useful as a screening test for HS, especially when combined with an increased red cell distribution width. Mean corpuscular volume (MCV) is usually normal except in cases of severe HS, when MCV is slightly decreased.

#### Markers of Hemolysis

Other laboratory features of HS are markers of ongoing hemolysis. Reticulocytosis, variably increased lactate dehydrogenase, increased urinary and fecal urobilinogen, unconjugated hyperbilirubinemia, and decreased serum haptoglobin reflect hemolysis and increased erythropoiesis (Chaps. 32 and 33). The reticulocyte count may appear to be elevated disproportionately relative to the degree of anemia.

#### Erythrocyte Fragility Tests

Spherocytes have a decreased surface area relative to cell volume and this renders them osmotically fragile. Several laboratory tests exploit this characteristic and are used to diagnose HS. The most common osmotic fragility (OF) test measures lysis of red cells, either from freshly drawn blood or after incubation of the sample at 37°C for 24 hours, in a range of hypotonic concentrations of sodium chloride. Spherocytes typically swell and burst much more readily than normal biconcave disk-shaped red cells. Other tests based on the same principle measure the rate and extent of cell lysis in buffered glycerol solutions and include the glycerol lysis test (GLT) and the acidified glycerol lysis test (AGLT). These tests, however, have relatively poor sensitivity and do not detect all cases of mild HS or those with small numbers of spherocytes, including patients who had recent blood transfusions.[63,67,109] These tests may also be unreliable and give normal results in the presence of iron deficiency, obstructive jaundice, or during the recovery phase of an aplastic crisis.[63] In addition, these tests do not differentiate HS from other disorders with secondary spherocytosis, such as the autoimmune hemolytic anemias (Chap. 54).

Other fragility tests include the cryohemolysis test, based on the sensitivity of HS red cells to cooling at 0°C in hypertonic conditions, and the autohemolysis test, but these tests also do not detect all cases of HS.[13] The reduced surface area of spherocytes can be measured by osmotic gradient ektacytometry, but the highly specialized equipment required for this procedure is only available in a few research-oriented laboratories.

#### Eosin 5′-Maleimide Flow Cytometry Test

Eosin 5′-maleimide (EMA) is a fluorescent dye that binds to the transmembrane proteins, band 3, Rh protein, Rh glycoprotein, and CD47.[110] Patients with HS exhibit decreased fluorescence compared to controls, irrespective of the underlying defective membrane protein, although not all patients with HS are detected. In addition, lower fluorescence values are also observed in patients with HE, HPP, some red cell enzymopathies, and other abnormalities of band 3, such as congenital dyserythropoietic anemia type II (CDAII; Chap. 39). The sensitivity and specificity of the test vary, depending on the cutoff value of the fluorescence, which differs between laboratories.[109,111,112,113]

#### Molecular Diagnostics

Because HS can be caused by mutations in several different genes and because there are very few common mutations, a simple DNA test to diagnose HS is not feasible. Initial analysis of the red cell membrane proteins by quantitative SDS-PAGE is required to identify the underlying defective protein. The sensitivity of this test varies between laboratories and different patient populations, but typically an abnormality is defined in 75 to 93 percent of cases.[63,64,67] Patients with clinically identified HS and normal SDS-PAGE results may have a slight decrease of 10 to 15 percent in one of the membrane proteins, which may be missed by the densitometric analysis, or they may have an abnormality in a protein that is currently not quantified and not linked to HS, for example, adducin.

Knowledge of the defective protein facilitates subsequent DNA/RNA investigations to characterize the gene defect, although this approach is challenging as the genes causing HS are large and contain many exons. Polymorphisms may be used to identify reduced expression from one allele or loss of heterozygosity because of a null mutation. In families with variable clinical expression of HS, a molecular investigation into low-expression alleles and other modifying genes is useful. A molecular diagnosis is informative in patients with atypical features; severe disease; unclear or recessive inheritance; *de novo* mutations; or undiagnosed hemolytic anemia. Identification of silent carriers and prenatal diagnosis also require molecular testing.

### Differential Diagnosis

Clinical features and family history should accompany an initial laboratory investigation comprising a complete blood count with a blood film, reticulocyte count, direct antiglobulin test (Coombs test), and serum bilirubin. Other causes of anemia should be excluded, particularly autoimmune hemolytic anemia, CDAII and HSt. Further diagnostic tests (discussed in “Laboratory Features” earlier), are not standardized as reflected by a European survey of 25 centers.[114] A consistent finding was that all the laboratories used at least two tests to make a final diagnosis, as none of the currently available methods have 100 percent sensitivity. The EMA test was most commonly

used. Recent guidelines from the British Committee for Standards in Haematology (BCSH)[115] advocate the use of the EMA test or cryohemolysis, but OF is not recommended for routine use.

In neonates, ABO incompatibility should be considered, but its differentiation from HS becomes clear several months after birth. Other causes of spherocytic hemolytic anemia, such as autoimmune hemolysis, clostridial sepsis, transfusion reactions, severe burns, and bites from snakes, spiders, bees, and wasps (Chaps. 52 to 54), should be viewed in the appropriate clinical context. Occasional spherocytes are seen in patients with a large spleen (e.g., in cirrhosis or myelofibrosis) or in patients with microangiopathic anemias (Chap. 51), but differentiation of these conditions from HS does not usually present diagnostic difficulties.

HS may be obscured in disorders that increase the surface-to-volume ratio of erythrocytes, such as obstructive jaundice, iron deficiency (Chap. 43), $\beta$-thalassemia trait, or hemoglobin SC disease (Chaps. 48 and 49), and vitamin $B_{12}$ or folate deficiency (Chap. 41).

### Therapy and Prognosis

#### Splenectomy

Splenic sequestration is the primary determinant of erythrocyte survival in HS patients. Thus, splenectomy cures or alleviates the anemia in the overwhelming majority of patients, reducing or eliminating the need for red cell transfusions, which has obvious implications for future iron overload and hemochromatosis-related end-organ damage. The incidence of cholelithiasis is decreased. Postsplenectomy, spherocytosis, and altered OF persist, but the “tail” of the OF curve, created by conditioning of a subpopulation of spherocytes by the spleen, disappears. Erythrocyte life span nearly normalizes, and reticulocyte counts fall to normal or near-normal levels. Changes typical of the postsplenectomy state, including Howell-Jolly bodies, target cells, Pappenheimer bodies (siderocytes), and acanthocytes (Chaps. 2 and 31), become evident on the blood film. Postsplenectomy, patients with the most severe forms of HS still suffer from shortened erythrocyte survival and hemolysis, but their clinical improvement is striking.[79]

#### Complications of Splenectomy

Early complications of splenectomy include local infection, thrombotic complications and in particular hepatic and mesenteric thrombosis, bleeding, and pancreatitis, presumably resulting from injury to the tail of the pancreas incurred during spleen removal. In general, the morbidity of splenectomy for HS is lower than the morbidity of other hematologic disorders. Chapters 5 and 6 discuss the complications of splenectomy.

#### Indications for Splenectomy

In the past, splenectomy, which has a low operative mortality, was considered routine in HS patients. However, the risk of overwhelming postsplenectomy infection and the emergence of penicillin-resistant pneumococci have led to reevaluation of the role of splenectomy in the treatment of HS.[116] Considering the risks and benefits, a reasonable approach is to splenectomize all patients with transfusion-dependent severe spherocytosis and all patients suffering from significant signs or symptoms of anemia, including growth failure, skeletal changes, leg ulcers, and extramedullary hematopoietic tumors. Other candidates for splenectomy are older HS patients suffering from vascular compromise of vital organs.

Whether patients with moderate HS and compensated, asymptomatic anemia should undergo splenectomy is controversial. Patients with mild HS and compensated hemolysis can be followed and referred for splenectomy if clinically indicated. Treatment of patients with mild to moderate HS and gallstones is debatable, particularly because new treatments for cholelithiasis, including laparoscopic cholecystectomy, and endoscopic sphincterotomy, lower the risk of this complication. If such patients have symptomatic gallstones, a combined cholecystectomy and splenectomy can be performed, particularly if acute cholecystitis or biliary obstruction has occurred. No evidence indicates any benefit to performing cholecystectomy and splenectomy separately, as performed in the past.

Because the risk of postsplenectomy sepsis is very high during infancy and early childhood, splenectomy should be delayed until age 5 to 9 years if possible and to at least 3 years if feasible, even if chronic transfusions are required in the interim. No evidence indicates further delay is useful. In fact, further delay may be harmful because the risk of cholelithiasis increases dramatically in children older than 10 years.

When splenectomy is warranted, laparoscopic splenectomy has become the method of choice in centers with surgeons experienced in the technique.[117] If desired, the procedure can be combined with laparoscopic cholecystectomy. Laparoscopic splenectomy results in less postoperative discomfort, a quicker return to preoperative diet and activities, shorter hospitalization, decreased costs, and smaller scars. The risk of bleeding increases during the operation and approximately 10 percent of laparoscopic operations (for all causes) must

be converted to standard splenectomies. Even very large spleens (>600 g) can be removed laparoscopically because the spleen is placed in a large bag, diced, and eliminated via suction catheters.

Partial splenectomy via laparotomy has been advocated for infants and young children with significant anemia associated with erythrocyte membrane disorders.[118] The goal of this procedure is to allow for palliation of hemolysis and anemia while maintaining some residual splenic immune function. Long-term followup data for this procedure have been variable.

Prior to splenectomy, patients should be immunized with vaccines against pneumococcus, *Haemophilus influenzae* type B, and meningococcus, preferably several weeks preoperatively. Use of prophylactic antibiotics postsplenectomy for prevention of pneumococcal sepsis is controversial. Prophylactic antibiotics (penicillin V 125 mg orally twice daily for patients younger than 7 years or 250 mg orally twice daily for those older than 7 years, including adults) have been recommended for at least 5 years postsplenectomy by some and for life by others. The optimal duration of prophylactic antibiotic therapy postsplenectomy is unknown. Presplenectomy and, in severe cases, postsplenectomy, HS patients should take folic acid (1 mg/day orally) to prevent folate deficiency.

#### Splenectomy Failure

Splenectomy failure is uncommon. Failure may result from an accessory spleen missed during splenectomy, from development of splenunculi as a consequence of autotransplantation of splenic tissue during surgery, or from another intrinsic red cell defect, such as pyruvate kinase deficiency (Chap. 47). Accessory spleens occur in 15 to 40 percent of patients and must always be sought. Recurrence of hemolytic anemia years or even decades following splenectomy should raise suspicion of an accessory spleen particularly if Howell-Jolly bodies are no longer found on blood film (Chaps. 2 and 31). Definitive confirmation of ectopic splenic tissue can be achieved by a radiocolloid liver–spleen scan or a scan using $^{51}$Cr-labeled, heat-damaged red cells.

#### Genetic Counseling

After a patient is diagnosed with HS, family members should be examined for the presence of HS. A history, physical examination for splenomegaly, complete blood count, examination of the blood film for spherocytes, and a reticulocyte count should be obtained for parents, children, and siblings, if available.

### HEREDITARY ELLIPTOCYTOSIS AND PYROPOIKILOCYTOSIS

#### Definition and History

HE is characterized by the presence of elliptical or oval erythrocytes on the blood films of affected individuals (Figs 46-10D and 46-11F). In 1904, Dresbach, a physiologist at Ohio State University in Columbus, Ohio, published the first description of elliptical red blood cells in one of his students, noticed during a laboratory exercise in which the students were examining their own blood.[119] The report elicited controversy because the student died soon thereafter, leading to speculation that he had actually suffered from pernicious anemia. The demonstration of elliptocytosis in three generations of one family established the hereditary nature of this disorder.[120] A related disorder, HPP is a rare disease first described in 1975 in children with severe neonatal anemia with abnormal poikilocytic red cell morphology reminiscent of that seen in patients suffering from severe burns (Figure 46-13).[121] The erythrocytes from these patients exhibited increased thermal sensitivity.

#### Epidemiology and Inheritance

HE has a worldwide distribution but the true incidence is unknown because the disease is heterogeneous and many patients are asymptomatic. In the United States, the incidence is estimated to be 1 in 2000 to 4000 individuals.[13,122] HE occurs in all racial groups but is more prevalent in individuals of West African descent, possibly because elliptocytes may confer some resistance to malaria.[123,124] HPP is typically found in patients of African origin, but it has also been diagnosed in subjects of European and Arabic descent.[122,125,126]

#### Etiology and Pathogenesis

The primary abnormality in HE and HPP erythrocytes is defective horizontal interactions between components of the membrane skeleton, which weakens the skeleton and compromises its ability to maintain the biconcave disk shape of the red cell during circulatory shear stress. Investigations of erythrocyte membrane proteins in these disorders have identified abnormalities in $\alpha$- and $\beta$-spectrin,

protein 4.1, and GPC.[122] The most common defects occur in spectrin, the main structural protein of the erythrocyte membrane skeleton, and they impair the ability of spectrin dimers to self-associate into tetramers and oligomers, thereby disrupting the skeletal lattice.[55] Abnormalities in 4.1R diminish the interaction between the tail ends of spectrin tetramers in the junctional complex and thus destabilize the skeleton. Deficiency of GPC/GPD is associated with reduced levels of 4.1R, which presumably is responsible for the elliptocytosis.

When the integrity of the skeleton is compromised, the capacity of the erythrocyte to undergo flow-induced deformation and rearrangement of the skeleton is reduced. Disruption of the dynamic dissociation and reassociation of spectrin tetramers causes mechanical instability of the membrane, which precludes the recovery of the normal biconcave disk shape of the cell after prolonged and repeated unidirectional axial distortion in the microcirculation.[127] HE reticulocytes have a normal shape when released into the circulation but the mature red cells become progressively more elliptical as they age and ultimately the abnormal shape becomes permanent.[13,122] As the severity of the defect increases, poikilocytes are formed and the cells become prone to fragmentation. HPP patients exhibit a combination of horizontal (impaired spectrin tetramer formation) and vertical (spectrin deficiency) defects, with the latter causing microspherocytes and exacerbating the hemolytic anemia.[128,129]

#### Red Cell Membrane Protein Defects

##### Spectrin

Mutations that affect spectrin heterodimer self-association are found in the majority of HE patients and in all patients with HPP. This functional defect results in an increased percentage of spectrin dimers relative to tetramers,[130] which is reflected on a structural level by an abnormal tryptic digest pattern of the protein, whereby the normal peptide is decreased with a concomitant increase in an abnormal peptide of lower molecular weight. Most of the defects affect the 80-kDa $\alpha$I domain of $\alpha$-spectrin and of the nine structural variants the most common are Sp$\alpha^{I/74}$, Sp$\alpha^{I/65}$, and Sp$\alpha^{I/46\text{ or }50a}$.[128]

More than 50 mutations have been identified in either $\alpha$- or $\beta$-spectrin genes. The majority of the mutations are missense mutations that substitute highly conserved amino acids or those in close proximity. The abnormal amino acids typically have a different charge, or in the case of glycine or proline substitutions, they disrupt the helical structure of the spectrin repeats, which alter the interactions between $\alpha$ and $\beta$ subunits. Interestingly, mutations in $\alpha$-spectrin primarily occur in helix C of the repeats, which highlights the importance of this helix in the triple helical bundle (see Fig. 46–3). Several mechanisms have been identified by which the mutations impair spectrin tetramer formation.

Sp$\alpha^{I/74}$ mutations are mostly missense mutations found at the self-association site, which consists of helix C of the $\alpha$0 partial spectrin repeat that interacts with helices B and C of $\beta$-spectrin partial repeat 17 to form a complete triple helical bundle.[34] *In vitro* studies on missense mutations in $\alpha$0 revealed that the mutant peptides were stable folded structures, similar to wild type, but their binding affinities to $\beta$-spectrin peptides were variable. This suggested that their effect on tetramer formation was exerted through defective molecular recognition and disruption of protein-protein interactions at the contact site, rather than an altered structure.[131] These findings contrasted with mutations in the $\beta$17 repeat of $\beta$-spectrin, which perturbed the structural conformation of this partial repeat and the adjacent $\beta$16 repeat.[132] Codon 28 in helix C of $\alpha$0 has been identified as a mutation "hotspot" since four different point mutations occur in this position, resulting in different amino acid substitutions, and the mutations have also been found in several unrelated kindred.[133] Arginine 28 is a highly conserved amino acid and any changes in this position are typically associated with severe HE or HPP.[133,134] An interesting case of HE Sp$\alpha^{I/74}$ involving an intragenic crossover in the $\alpha$-spectrin gene and uniparental disomy, together with an underlying R34P mutation, was recently described in a Utah family.[126]

Sp$\alpha^{I/74}$ defects are also caused by mutations in $\beta$-spectrin, which presumably expose the $\alpha$I domain of spectrin to increased tryptic digestion. These abnormalities are all located in partial repeat 17. Missense mutations are found in both helices A and B of the $\beta$17 repeat, but some in helix A are particularly severe, including spectrin$^{Providence}$, spectrin$^{Cagliari}$, and spectrin$^{Buffalo}$, which cause severe fetal or neonatal anemia and nonimmune hydrops fetalis when inherited in the homozygous state.[105,106,135] Frame-shift mutations and splicing defects predominate in helix B, resulting in truncated spectrin molecules lacking the self-association site.[13,122,136]

Sp$\alpha^{I/65}$ is a mild defect, even in the homozygous state, because of a duplication of leucine 154 in helix C of the $\alpha$1 repeat.[137] It is very common in blacks from West and Central Africa, as well as Arabs in North Africa, suggesting genetic selection, possibly by protecting

carriers against *P. falciparum* malaria.[13,122,123]

Sp$\alpha^{I/46\text{ or }50a}$ mutations are distal from the self-association site and usually occur close to the helical linker regions between individual repeats and often involve the substitution of an amino acid with a proline residue, which is a helix breaker.[13,122] *In vitro* studies on Q471P between repeats 4 and 5 of $\alpha$-spectrin showed that the mutation uncoupled the repeats and caused cooperative unfolding, which abolished the stabilizing influence of the helical linker on adjacent repeats.[138] Because $\beta$-spectrin has fewer repeats than $\alpha$-spectrin, the alignment of the heterodimers places $\alpha$4 and $\alpha$5 in contact with $\beta$16 and $\beta$17, suggesting that unfolding of the mutant spectrin repeats interferes with the self-association site and prevents tetramer formation.[139] The L260P mutation is in a similar position to Q471P, but is between repeats $\alpha$2 and $\alpha$3 of spectrin. When heterodimers are aligned, repeats $\alpha$03 are not in contact with $\beta$-spectrin and they represent an open dimer configuration, which facilitates tetramer formation. Open dimers are in equilibrium with closed dimers whereby $\alpha$0 to $\alpha$3 are folded onto $\beta$16 and $\beta$17 of the same dimer, thus preventing bivalent tetramer formation.[139] *In vitro* experiments on the L260P mutation revealed a conformational change, which stabilized the mutant spectrin in the closed dimer configuration and reduced tetramer assembly.[140]

Mutations in the $\alpha$I domain of spectrin implicated in HE are rare. Spectrin$^{St\ Claude}$ is caused by a single point mutation in intron 19 of $\alpha$-spectrin,[141,142] which creates complex splicing events that ultimately impair the function of both $\alpha$- and $\beta$-spectrin, resulting in decreased binding to ankyrin, defective spectrin self-association and spectrin deficiency.[141] These membrane abnormalities have profound effects on red blood cell morphology and survival, manifesting as severe HE.

#### Protein 4.1R

Defects in the erythrocyte isoform of protein 4.1 associated with HE are relatively common in some Arab and European populations.[13] Heterozygotes exhibit partial deficiency of 4.1R, manifesting as mild or asymptomatic HE, whereas homozygotes lack 4.1R and p55, have a reduced content of GPC, and present with severe HE. These red blood cells are mechanically unstable and fragment at moderate shear stress, but the stability can be restored by reconstituting the deficient red cells with 4.1R or the 4.1R spectrin–actin binding domain.[143] The 4.1R null erythrocytes demonstrate decreased invasion and growth of *P. falciparum* parasites *in vitro*.[144]

Mutations in the 4.1R gene often affect the erythroid-specific initiation codon, which abolishes transcription, or else they tend to cluster in the spectrin-actin binding domain where exon deletions or duplications result in mutant proteins that are smaller or larger than normal.[122]

#### Glycophorin C

GPC and GPD carry the Gerbich antigens and rare patients with the Leach phenotype are Gerbich-negative and lack both GPs. The underlying mutations are either a 7-kb deletion of genomic DNA or a frameshift mutation.[145] Heterozygous carriers are asymptomatic, with normal red blood cell morphology, whereas homozygous subjects exhibit elliptocytes on the blood film and present with mild HE, presumably as a result of the concomitant partial deficiency of 4.1R.[13,145]

### Molecular Determinants of Clinical Severity

HE patients exhibit marked clinical heterogeneity ranging from asymptomatic carrier to severe, transfusion-dependent anemia. In patients with spectrin heterodimer self-association defects, the resultant increase in spectrin dimers and concomitant decrease in spectrin tetramers, weakens the membrane skeleton and facilitates the formation of elliptocytes under circulatory shear stress. The most important determinants of the severity of hemolysis in these patients are the percentage of spectrin dimers and the spectrin content of the membrane skeleton. These parameters are influenced by the degree of dysfunction of the mutant spectrin, and the gene dose (heterozygote versus homozygote or compound heterozygote).[128] Genotype–phenotype correlations indicate that the order of clinical severity of $\alpha$I domain defects is Sp$\alpha^{I/74}$ > Sp$\alpha^{I/46–50a}$ > Sp$\alpha^{I/65}$ and it depends on the position of the mutations within the proteins, as well as the type of mutation. Defects in the spectrin dimer self-association contact site leading to Sp$\alpha^{I/74}$ mutants are the most severe[128] and, for example, codon 28 mutations, which affect a highly conserved and critical arginine residue, are generally associated with phenotypically severe HE or HPP.[133] A more distal mutation such as the duplication of leucine 154, which causes Sp$\alpha^{I/65}$, is phenotypically very mild, even in the homozygous state.[137] Proline or glycine helix-breaking mutations resulting in Sp$\alpha^{I/46\text{ or }50a}$ are more severe even though they are further away from the self-association site.[138]

The clinical expression of HE often varies within the same kindred, despite all the affected individuals carrying the same causative mutation. This heterogeneity is a result of the inheritance of modifier alleles or additional defects. The low-expression $\alpha^{LELY}$ is the most common polymorphism affecting spectrin content and clinical severity. The allele is characterized by an L1857V amino acid substitution, and partial skipping of exon 46 in 50 percent of the $\alpha$-spectrin mRNA.[94] The six amino acids encoded by exon 46 are essential for spectrin heterodimer assembly and therefore Sp$\alpha^{LELY}$ results in a reduced amount of spectrin, as monomers are rapidly degraded.[146] The Sp$\alpha^{LELY}$ allele is clinically silent, even when homozygous, because $\alpha$-spectrin is normally synthesized in three- to fourfold excess.[147] Inheritance of Sp$\alpha^{LELY}$ *in cis* to an elliptocytogenic $\alpha$-spectrin mutation ameliorates symptoms,[148] whereas inheritance *in trans* causes a relative increase in the mutant spectrin and therefore exacerbates the disease.[94]

Coinheritance of other molecular defects also plays a role in modifying the clinical expression. HPP patients are very severely affected because they are homozygous or doubly heterozygous for spectrin self-association mutations and are also deficient in spectrin.[129] Several molecular mechanisms have been identified that underlie the spectrin deficiency, including an RNA processing defect[149]; reduced $\alpha$-spectrin mRNA and protein synthesis[150]; abnormal splicing resulting in a premature stop codon[151]; and degradation of $\alpha$-spectrin.[150] A recent study revealed the complexity of genotype–phenotype interactions in two large Utah families of northern European descent in whom a novel R34P mutation in $\alpha$-spectrin was associated with three morphologic phenotypes.[126] This heterogeneity was caused by an intricate interplay and coinheritance of other factors, including Sp$\alpha^{LELY}$ *in trans,* reduced transcription from the $\alpha$-spectrin gene and intragenic crossover.[126]

In neonates the clinical severity of HE can be affected by the weak binding of BPG to fetal hemoglobin leading to an increase in free BPG, which, in turn, destabilizes the spectrin–actin–protein 4.1 interaction.[152] Finally, hemolytic anemia can be exacerbated by several acquired conditions, including those that alter microcirculatory stress to the cells.

#### Inheritance

HE is typically inherited as an autosomal dominant disorder. *De novo* mutations are rare.[134] The severity of clinical symptoms is highly variable reflecting heterogeneous molecular abnormalities, as well as the coinheritance of other genetic defects or polymorphisms that modify disease expression. A strong genetic relationship exists between HE and HPP, and parents or siblings of patients with HPP often have typical HE.

#### Clinical Features

The clinical presentation of HE is heterogeneous, ranging from asymptomatic carriers to patients with severe, life-threatening anemia. The overwhelming majority of patients with HE are asymptomatic and are diagnosed incidentally during testing for unrelated conditions. HPP patients present in infancy or early childhood with a very severe hemolytic anemia.

Asymptomatic carriers who possess the same molecular defect as an affected HE relative but who have normal or near-normal blood films have been identified. The erythrocyte life span is normal, and the patients are not anemic. Asymptomatic HE patients may experience hemolysis in association with infections, hypersplenism, vitamin $B_{12}$ deficiency, or microangiopathic hemolysis, such as disseminated intravascular coagulation or thrombotic thrombocytopenic purpura. In the latter two conditions, increased hemolysis may result from microcirculatory damage superimposed on the underlying mechanical instability of red cells.

HE patients with chronic hemolysis experience moderate to severe hemolytic anemia with elliptocytes and poikilocytes on the blood film. Red cell life span is decreased and patients may develop complications of chronic hemolysis, such as gallbladder disease. In some kindreds, the hemolytic HE has been transmitted through several generations. In other kindreds, not all HE subjects have chronic hemolysis; some have only mild hemolysis, presumably because another genetic factor modifies disease expression. The blood films of the most severe HE patients with chronic hemolysis exhibit elliptocytes, poikilocytes, fragments and small microspherocytes, reminiscent of HPP.

HPP represents a subtype of common HE, as evidenced by the coexistence of HE and HPP in the same family and the presence of the same molecular defects of spectrin.[130] HE relatives are heterozygous for an elliptocytogenic spectrin mutation, whereas HPP patients are

homozygous or doubly heterozygous and are also partially deficient in spectrin.[128,129]

#### Hereditary Elliptocytosis and Pyropoikilocytosis in Infancy

Clinical symptoms of elliptocytosis are uncommon in the neonatal period. Typically, elliptocytes do not appear on the blood film until the patient is 4 to 6 months old. Occasionally, severe forms of HE present in the neonatal period with severe, hemolytic anemia with marked poikilocytosis and jaundice. These patients may require red cell transfusion, phototherapy, or exchange transfusion. Usually, even in severely affected patients, the hemolysis abates between 9 and 12 months of age, and the patient progresses to typical HE with mild anemia. Infrequently, patients remain transfusion dependent beyond the first year of life and require early splenectomy. In cases of suspected neonatal HE or HPP, review of family history and analysis of blood films from the parents usually are of greater diagnostic benefit than other available studies.

A few cases of hydrops fetalis accompanied by fetal or early neonatal death as a result of unusually severe forms of HE have been described.[105] A severely affected hydropic infant salvaged by intrauterine transfusions (Chap. 55) and early exchange transfusion has remained transfusion dependent for more than 2 years.

### Laboratory Features

The hallmark of HE is the presence of cigar-shaped elliptocytes on blood films Figs. 46-10D and 46-11F. These normochromic, normocytic elliptocytes may number from a few to 100 percent. The degree of hemolysis does not correlate with the number of elliptocytes present. Spherocytes, stomatocytes, and fragmented cells may be seen. Osmotic fragility is abnormal in severe HE and in HPP. The reticulocyte count generally is less than 5 percent but may be higher when hemolysis is severe. Other laboratory findings in HE are similar to those of other hemolytic anemias and are nonspecific markers of increased erythrocyte production and destruction. For example, increased serum bilirubin, increased urinary urobilinogen, and decreased serum haptoglobin reflect increased erythrocyte destruction.

HPP blood films exhibit similar features to severe HE, but in addition, they reveal extreme poikilocytosis, some bizarre-shaped cells with fragmentation or budding and often only very few or no elliptocytes Fig. 46-13. Microspherocytosis is common and MCV is usually low, ranging between 50 to 70 fL. Pyknocytes are prominent on blood films of neonates with HPP. The thermal instability of erythrocytes, originally reported as diagnostic of HPP, is not unique to this disorder because it is also commonly found in HE erythrocytes.

Specialized testing has been used in difficult cases or cases requiring a molecular diagnosis. Tests on isolated membrane proteins include analysis and quantitation of the proteins by SDS-PAGE; extraction of spectrin from the membranes to evaluate the spectrin-dimer-to-tetramer ratio on nondenaturing gels, as well as limited tryptic digestion of spectrin followed by SDS-PAGE or two-dimensional gel electrophoresis to identify the defective domain. Ektacytometry may be used to measure membrane stability and deformability. Genomic DNA and/or complementary DNA analyses are used to determine the underlying mutation.

### Differential Diagnosis

Elliptocytes may be seen in association with several disorders, including megaloblastic anemias, hypochromic microcytic anemias (iron-deficiency anemia and thalassemia), myelodysplastic syndromes, and myelofibrosis. In these conditions, elliptocytosis is acquired and generally represents less than one-quarter of red cells seen on the blood film. History and additional laboratory testing usually clarify the diagnosis of these disorders. Pseudoelliptocytosis is an artifact of blood film preparation and these cells are found only in certain areas of the film, usually near its tail. The long axes of pseudoelliptocytes are parallel, whereas the axes of true elliptocytes are distributed randomly.

### Therapy and Prognosis

Therapy is rarely needed in patients with HE. In rare cases, occasional red blood cell transfusions may be required. In cases of severe HE and HPP, splenectomy has been palliative, as the spleen is the site of erythrocyte sequestration and destruction. The same indications for splenectomy in HS can be applied to patients with symptomatic HE or HPP. Postsplenectomy, patients with HE or HPP exhibit increased hematocrit, decreased reticulocyte counts, and improved clinical symptoms.

Patients should be followed for signs of decompensation during acute illnesses, characterized by acute decrease of hematocrit from nonspecific suppression of erythropoiesis by a concurrent acute event. HE and particularly HPP patients are at increased risk for

parvovirus infection generally requiring short-lasting transfusion support (Chap. 36).[153] Interval ultrasonography to detect gallstones should be performed. Patients with significant hemolysis should receive daily folate supplementation.

## SOUTHEAST ASIAN OVALOCYTOSIS

SAO, also known as Melanesian elliptocytosis or stomatocytic elliptocytosis, is widespread in certain ethnic groups of Malaysia, Papua New Guinea, the Philippines, and Indonesia,[123] but is also common in the Cape Coloured population in South Africa.[154] It is characterized by the presence of large oval red cells, many of which contain one or two transverse ridges or a longitudinal slit Figs 46-10C and 46-11E.

SAO erythrocytes are rigid and hyperstable because of a structurally and functionally abnormal band 3. SAO band 3 binds tightly to ankyrin, forms oligomers, exhibits restricted lateral and rotational mobility[155,156] and is unable to transport anions.[157] The underlying molecular abnormality is an in-frame deletion of 27 bp in the band 3 gene resulting in the loss of amino acids 400 to 408 located at the boundary of the cytoplasmic and membrane domains of band 3.[158] The defective SLC4A1 allele also carries a linked band $3^{Memphis}$ polymorphism, L56E.

SAO is a dominantly inherited trait and homozygosity is postulated to be lethal during embryonic development.[159] A recent case of homozygous SAO has been described where the fetus was kept alive by two intrauterine transfusions and since birth he has been on a monthly transfusion program.[160] Distal renal tubular acidosis was diagnosed at 3 months as a result of the inability of the SAO band 3 to transport anions.

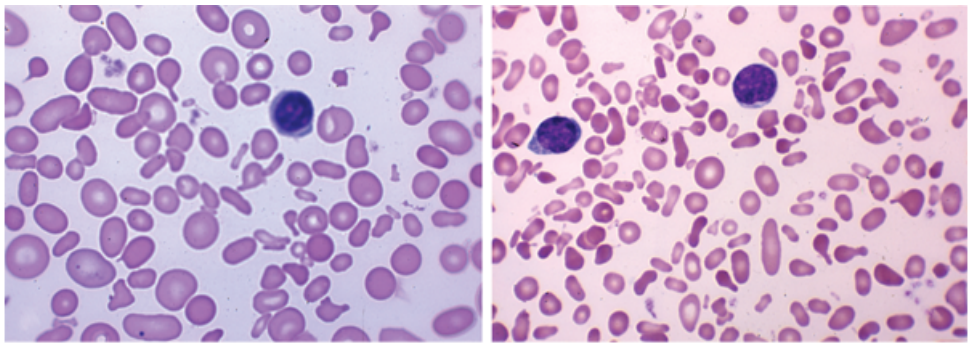
A remarkable feature of SAO erythrocytes is their resistance to infection by several species of malaria parasites. This has been demonstrated by numerous *in vitro* studies, as well as *in vivo* evidence indicating that SAO provides protection against severe malaria and cerebral malaria.[123,161] Epidemiologic data and the increased prevalence of SAO in populations challenged by malaria suggest a selective advantage of the gene.[123] Numerous factors have been implicated in the protective effect, but the precise mechanism of malaria resistance of SAO red cells has not been fully elucidated.

Clinically, the presence on the blood film of at least 20 percent ovalocytic red cells, some containing a central slit or a transverse ridge, and the notable absence of clinical and laboratory evidence of hemolysis are highly suggestive of SAO. Rapid genetic diagnosis can be made by amplifying the defective region of the band 3 gene and demonstrating heterozygosity for the SAO allele containing the 27 bp deletion.

## ACANTHOCYTOSIS

Spiculated red cells are classified into two types: acanthocytes and echinocytes. *Acanthocytes* are contracted, dense cells with irregular projections from the red cell surface that vary in width and length (Figs. 46-10F and 46-11G). *Echinocytes* have small, uniform projections spread evenly over the circumference of the red cell Fig. 46-11B. Diagnostically, the distinction is not critical, and disorders of spiculated red cells are generally classified together. Normal adults may have up to 3 percent spiculated erythrocytes, but care should be taken when preparing and examining the blood film, because spiculated cells, particularly echinocytes, are common artifacts of blood film preparation and blood storage.

Figure 46–13.
Blood films from a patient with HPP. **A.** Pre-splenectomy. **B.** Post-splenectomy. Note the prominent micropoikilocytosis, microspherocytosis, and fragmentation especially after splenectomy. *(Reproduced with permission from* Lichtman's Atlas of Hematology, *www.accessmedicine.com.)*



A B



Acanthocytes/echinocytes are found in various inherited disorders and acquired conditions. Spiculated cells can occur transiently in several instances, such as after transfusion with stored blood, ingestion of alcohol and certain drugs, exposure to ionizing radiation or certain venoms, and during hemodialysis.[13] Spiculated cells are commonly seen on the blood films of patients with functional or actual splenectomy, severe liver disease, severe uremia, abetalipoproteinemia, certain inherited neurologic disorders and abnormalities of the Kell blood group. Occasionally acanthocytes and/or echinocytes may be present in patients with glycolytic enzyme defects, myelodysplasia, hypothyroidism, anorexia nervosa, vitamin E deficiency, and in premature infants.[13] Individuals with suppressed expression of $Lu^a$ and $Lu^b$, the major antigens of the Lutheran blood group system, may also exhibit acanthocytes.[13]

The molecular mechanisms whereby acanthocytes are generated have not been fully elucidated. However, alterations in band 3 have emerged as a pivotal causative factor. The abnormal red cell membrane lipid composition and altered lipid distribution between the inner and outer leaflets of the bilayer are only found in some, but not all, of these disorders, implying that they may play a secondary role.[162]

## ACANTHOCYTOSIS IN SEVERE LIVER DISEASE

### Definition

The anemia in patients with liver disease is often called “spur cell anemia” because of the projections on the red cells. Although only a small number of patients with end-stage liver disease acquire spur cell anemia, these individuals typically account for the majority of cases of acanthocytosis seen in clinical practice.

### Etiology and Pathogenesis

The anemia in patients with liver disease is of complex etiology. Common causes include blood loss, iron or folate deficiency, hypersplenism, and marrow suppression from alcohol, malnutrition, hepatitis infection, or other factors. Acquired abnormalities of the red cell membrane may contribute to the anemia in some patients.[163]

*In vivo* acanthocyte formation in spur cell anemia is a two-step process involving accumulation of free (nonesterified) cholesterol in the red cell membrane and remodeling of abnormally shaped red cells by the spleen.[13,163] The diseased liver of the patient produces abnormal lipoproteins with excess cholesterol, which is acquired by circulating erythrocytes, increasing their cholesterol content. The cholesterol preferentially partitions into the outer leaflet, increasing the surface area to volume ratio and forming scalloped edges. In the spleen, membrane fragments are lost and the cells develop the characteristic projections of acanthocytes (see Fig. 46–13). Cholesterol interacts with band 3 and changes its conformation, which may affect the membrane skeleton and reduce the deformability of the cell,[162] causing it to be trapped and eventually destroyed in the narrow sinusoids of the spleen.

### Clinical Features

Spur cell anemia is characterized by rapidly progressive hemolytic anemia with large numbers of acanthocytes on the blood film. Splenomegaly and jaundice become more prominent and are accompanied by severe ascites, bleeding diatheses, and hepatic encephalopathy. Spur cell anemia is most common in patients with alcoholic liver disease, but similar clinical syndromes have been

described in association with advanced metastatic liver disease, cardiac cirrhosis, Wilson disease, fulminant hepatitis, and infantile cholestatic liver disease.[13]

#### Laboratory Features

Most patients have moderate anemia with a hematocrit of 20 to 30 percent, marked indirect hyperbilirubinemia, and laboratory evidence of severe hepatocellular disease. Blood films reveal significant acanthocytosis and in some patients, echinocytes, target cells and microspherocytes, many with very fine spicules, are visible (see Fig. 46–13).

#### Differential Diagnosis

Spur cell hemolytic anemia should be distinguished from other hemolytic syndromes associated with liver disease, including congestive splenomegaly, in which patients exhibit chronic, mild hemolysis and occasional spherocytes, and patients with transient hemolytic episodes.

#### Therapy, Course, and Prognosis

The anemia of spur cell anemia usually is not a significant clinical problem, but it can aggravate pre-existing anemia resulting from, for example, gastrointestinal bleeding, to the point that erythrocyte transfusion is required. The life span of spur cells is markedly decreased because of splenic sequestration, and, as expected, hemolysis abates after splenectomy. However, splenectomy is a dangerous and potentially fatal procedure in these critically ill patients and is generally not recommended.

### NEUROACANTHOCYTOSIS

The term *neuroacanthocytosis* describes a heterogeneous group of rare disorders with variable clinical phenotypes and inheritance. The common features are a degeneration of neurons and abnormal acanthocytic erythrocyte morphology. These syndromes may be divided into: (1) lipoprotein abnormalities, which cause peripheral neuropathy, such as abetalipoproteinemia and hypobetalipoproteinemia, (2) neural degeneration of the basal ganglia resulting in movement disorders with normal lipoproteins, such as chorea-acanthocytosis and McLeod syndrome, and (3) movement abnormalities in which acanthocytes are occasionally seen, such as Huntington disease-like 2 (HDL2) and pantothenate kinase-associated neurodegeneration (PKAN).

#### Abetalipoproteinemia

##### Definition

Abetalipoproteinemia or Bassen-Kornzweig syndrome is a rare autosomal recessive disorder characterized by progressive ataxic neurologic disease, dietary fat malabsorption, retinitis pigmentosa, and acanthocytosis found in people of diverse ethnic backgrounds.[164]

##### Etiology and Pathogenesis

This disorder is caused by a failure to synthesize or secrete lipoproteins containing products of the apolipoprotein B (apoB) gene and this leads to changes in the plasma lipid profile.[164] The primary molecular defect is a lack of the microsomal triglyceride transfer protein, which performs an essential step in apoB-containing lipoprotein synthesis.[165] The relative distribution of erythrocyte membrane phospholipids is altered and the phosphatidylcholine content is decreased with a corresponding increase in sphingomyelin. The excess sphingomyelin is preferentially confined to the outer leaflet of the membrane bilayer, where it presumably causes an expansion of this layer and modifies the conformation of band 3, which contributes to the irregularities in cell surface contour.[162] Red cell precursors and reticulocytes have a normal shape and acanthocytosis only becomes apparent as the red cells mature in the circulation, worsening with increasing red cell age.[166]

##### Clinical Features

The disorder manifests in the first month of life by steatorrhea. Atypical retinitis pigmentosa, which often results in blindness, and progressive neurologic abnormalities characterized by ataxia and intention tremors develop between 5 and 10 years of age and progress to death in the second or third decade.[166]

##### Laboratory Features

Patients usually have mild anemia with normal red cell indices and normal or slightly increased reticulocyte counts.[166] Acanthocytosis is prominent, ranging from approximately 50 to 90 percent of red cells. Despite the red cell lipid abnormalities, the hemolysis is mild and the spleen is normal in patients with abetalipoproteinemia, in contrast to spur cell anemia. There is marked vitamin E deficiency (Chap. 44), which is thought to be a primary stimulus for the neuropathy. Coagulopathy may be observed.[164]

#### Differential Diagnosis

The related disorders hypobetalipoproteinemia, normotriglyceridemic abetalipoproteinemia, and chylomicron retention disease are associated with partial production of apoB-containing lipoproteins or with secretion of lipoproteins containing truncated forms of apoB. Patients with these disorders may experience neurologic disease and acanthocytosis, depending on the severity of the underlying defect.

#### Therapy, Course, and Prognosis

Treatment includes dietary restriction of triglycerides and supplementation with high doses of vitamins A, K, D, and E.[166] Chronic administration of vitamin E can delay or prevent the neurologic symptoms.

### Chorea-Acanthocytosis Syndrome

Chorea-acanthocytosis is a rare autosomal recessive movement disorder characterized by atrophy of the basal ganglia and progressive neurodegenerative disease with onset in adolescence or adult life.[167] In some patients, acanthocytosis may precede the onset of neurologic symptoms. The lipoproteins are normal.

Molecular studies have identified approximately 100 mutations in the *VPS13A* gene, which codes for chorein, a protein ubiquitously expressed in the brain and also found in mature red cells.[168,169,170] It is a member of a conserved protein family involved in trafficking of membrane proteins between cellular compartments, but its role in red cells and the pathogenesis of the disorder and acanthocytes is unknown. The mutations result in the absence or markedly reduced levels of chorein and founder mutations have been identified in Japanese and French-Canadian families.[167]

Patients are not anemic, and red cell survival is only slightly decreased. Plasma and erythrocyte membrane lipids, as well as membrane protein composition and content, are normal, but electron microscopy studies revealed structural abnormalities in the skeleton and an uneven distribution of intra-membrane particles. Red cell membrane fluidity is decreased. Increased serine-threonine and tyrosine phosphorylation of band 3, $\beta$-spectrin, and $\beta$-adducin has been documented.[171] In particular, abnormal activation of Lyn kinase results in increased tyrosine phosphorylation of band 3, which alters the association of band 3 with $\beta$-adducin and the junctional complex of the skeleton.[171] This may lead to localized disruption of the skeleton–membrane interaction, facilitating the formation of protrusions. In one chorea-acanthocytosis kindred a point mutation near the C terminus of band 3 has been identified, which may influence the interaction of band 3 with the skeleton.[172]

### McLeod Syndrome

The McLeod phenotype is a rare X-linked defect of the Kell blood group system, whereby cells react poorly with Kell antisera. The XK protein is an integral membrane transport channel protein that is covalently linked to the Kell antigen by disulphide bonds, and mutations in the XK gene cause a deficiency of the XK protein.[167,170] Male hemizygotes who lack XK have up to 85 percent acanthocytes on the blood film with mild, compensated hemolysis and develop late-onset multisystem myopathy or chorea known as the McLeod syndrome. Female heterozygous carriers may have occasional acanthocytes as a result of mosaicism in X chromosome inactivation. Large deletions involving not only the XK locus at Xp21.1, but also contiguous genes, result in the McLeod syndrome being associated with other diseases, such as chronic granulomatous disease of childhood, retinitis pigmentosa, Duchenne muscular dystrophy, and ornithine transcarbamylase deficiency.

Red cell membrane protein and lipid composition are normal, but the distribution of intramembrane particles is altered and increased phosphorylation of membrane proteins, notably band 3, has been noted, which again implicates band 3 as a key player in the generation of acanthocytes.

### Other Neuroacanthocytosis Syndromes

The HDL2 disorder is caused by expanded CGT/CAG trinucleotide repeat mutations in the *junctophilin-3* gene, which encodes a protein involved in junctional membrane structures and calcium regulation.[167] The disease is autosomal dominant and presents with late-onset chorea, parkinsonism, and progressive cognitive defects. Acanthocytes are present in some patients. In one unusual kindred autosomal dominant inheritance of chorea-acanthocytosis with polyglutamine neuronal inclusions was described in association with HDL2. Proteolysis of band 3 was also noted, which could contribute to the altered red cell morphology.[170,173]

Acanthocytes have been noted in some patients with PKAN (formerly known as Hallervorden-Spatz syndrome) with features of dystonia, dysarthria, and rigidity in childhood, and in HARP syndrome (hypobetalipoproteinemia, acanthocytosis, retinitis pigmentosa and pallidal degeneration). Both conditions are caused by mutations in pantothenate kinase 2, which is involved in synthesis of coenzyme A and phospholipids.[167,170,174]

#### Differential Diagnosis of Neuroacanthocytosis with Normal Lipoproteins

Chorea-acanthocytosis, McLeod syndrome, HDL2, and pantothenate kinase disorders present with overlapping neurologic symptoms and clinical phenotypes and also resemble Huntington disease, which renders the clinical diagnosis difficult. Identification of the underlying gene defects and the availability of molecular tests have markedly improved the diagnostic accuracy. This also provides insight into the underlying pathogenesis and suggests that the affected proteins, which are all linked to membrane structure, may participate in a common pathway that ultimately causes degeneration of the basal ganglia.

### HEREDITARY STOMATOCYTOSIS SYNDROMES

The intracellular concentration of the monovalent cations, $Na^+$ and $K^+$, contribute to erythrocyte volume homeostasis. A net increase in these cations causes water to enter the cells resulting in overhydrated cells or stomatocytes, whereas a net loss dehydrates the cells and forms xerocytes. Disorders of red cell cation permeability are very rare conditions that are inherited in an autosomal dominant fashion with marked clinical and biochemical heterogeneity (Table 46–4).[175]

Table 46–4.

**Heterogeneity of the Hereditary Stomatocytosis Syndromes**

| | Stomatocytosis (Hydrocytosis) | | Intermediate Syndromes | | | |
|---|---|---|---|---|---|---|
| | Severe Hemolysis | Mild Hemolysis | Cryohydrocytosis | Stomatocytic Xerocytosis | Xerocytosis with High Phosphatidylcholine | Xerocytosis |
| Hemolysis | Severe | Mild–moderate | Moderate | Mild | Moderate | Moderate |
| Anemia | Severe | Mild–moderate | Mild–moderate | None | Mild | Moderate |
| Blood film | Stomatocytes | Stomatocytes | Stomatocytes | Stomatocytes | Targets | Targets, echinocytes |
| MCV (80–100 fL)* | 110–150 | 95–130 | 90–105 | 91–98 | 84–92 | 100–110 |
| MCHC (32–36%) | 24–30 | 26–29 | 34–40 | 33–39 | 34–38 | 34–38 |
| Unincubated osmotic fragility | Markedly increased | Increased | Normal | Decreased | Markedly decreased | Markedly decreased |
| RBC $Na^{+5–12}$† | 60–100 | 30–60 | 40–50 | 10–20 | 10–15 | 10–20 |
| RBC $K^{+90–103}$ | 20–55 | 40–85 | 55–65 | 75–85 | 75–90 | 60–80 |
| RBC $Na^{+}+K^{+95–110}$ | 110–140 | 115–145 | 100–105 | 87–103 | 93–99 | 75–90 |
| Phosphatidylcholine content | Normal | ± Increased | Normal | Normal | Increased | Normal |
| Cold autohemolysis | No | No | Yes | No | No | ? |
| Effect of splenectomy‡ | Good | Good | Fair | ? | ? | ? Poor |
| Inheritance | Autosomal dominant?, autosomal recessive | Autosomal dominant | Autosomal dominant | Autosomal dominant | Autosomal dominant | Autosomal dominant |

MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell.

*Values in parentheses are the normal range.

†Values for sodium, potassium, and sodium + potassium are mEq/L RBC.

‡Splenectomy may be contraindicated in these syndromes; see text for details.

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Stomatocytes are cup-shaped red cells characterized by a central hemoglobin-free area (Figs. 46-10E and 46-11D). The molecular mechanism of stomatocyte formation has not been elucidated, but several theories have been postulated. The lipid bilayer hypothesis predicts that agents or abnormalities that expand the inner leaflet will tend to form stomatocytes.[176] Other theories relegate lipids to a secondary role and propose that membrane proteins, specifically band 3, play a major role in regulating the structure of the red cell.[162] Band 3 tetramers are attached to the spectrin skeleton and different configurations of band 3 that either face inward or outward can influence the topography of the skeleton and the shape of the cell.

## HEREDITARY XEROCYTOSIS

### Definition

Hereditary xerocytosis, also known as dehydrated HSt, is the most common form of the cation permeability defects. It is an autosomal dominant hemolytic anemia characterized by an efflux of $K^+$ and red cell dehydration. Hereditary xerocytosis is part of a pleiotropic syndrome and patients may also exhibit pseudohyperkalemia and perinatal edema.[177]

### Etiology and Pathogenesis

The underlying membrane permeability defect is complex and involves a net loss of potassium from the red cells that is not accompanied by a proportional gain of sodium. Consequently, the net intracellular cation content and cell water content are decreased. In some cases, erythrocytes exhibit an increase in phosphatidylcholine and reduced BPG content.[13]

The genetic locus for this disorder was mapped to 16q23–q24.[177] Subsequent refinement of the locus and exome sequencing of several large unrelated multigenerational kindred identified numerous missense mutations in the gene encoding the PIEZO1 protein.[178,179] Cosegregation of some of the mutations in families with multiple disease phenotypes suggested a correlation between PIEZO and perinatal edema.[178] PIEZO proteins were recently identified as mechanosensory molecules that form part of stretch-activated cation channels. The PIEZO1 protein is present in red cell membranes and two PIEZO1 mutations, R2456H and R2488Q, were demonstrated to regulate a mechanosensitive transduction channel, leading to increased cation transport in erythrocytes.[178,179]

### Clinical Features

Patients may present with symptoms of compensated hemolytic anemia, including jaundice, splenomegaly, and gallstones. Some patients may also exhibit pseudohyperkalemia and perinatal edema and even hydrops fetalis.[13,177] Variable penetrance is present in this disorder, with significant disparity in clinical symptoms between affected individuals in the same kindred. Patients display a strong tendency to iron overload (Chap. 43).[175]

### Laboratory Features

The hematologic picture is that of mild to moderate compensated hemolytic anemia (see Table 46–4) with an elevated reticulocyte count. The $K^+$ content is decreased and the $Na^+$ content is increased, but the total monovalent cation content is reduced. The MCHC is increased reflecting cellular dehydration and the MCV is frequently mildly increased.[175] Erythrocytes are resistant to osmotic lysis and the bell-shaped curve obtained by osmotic gradient ektacytometry is shifted to the left. Stomatocytes are not a prominent feature on blood films, but some target cells and spiculated cells are seen. In some of the cells, hemoglobin is concentrated (“puddled”) in discrete areas on the cell periphery.

### Therapy, Course, and Prognosis

Most patients experience only mild anemia and therapy is not required. The patients should receive folate supplementation and be monitored for complications of hemolysis. Splenectomy does not significantly improve the anemia, which suggests that xerocytes are

detected and eliminated in other areas of the reticuloendothelial system. Because of a markedly high risk of hypercoagulability and life-threatening thrombotic episodes after splenectomy, the procedure is contraindicated.[13]

## HEREDITARY STOMATOCYTOSIS/HYDROCYTOSIS

### Definition and History

Hereditary stomatocytosis, also known as hereditary hydrocytosis or overhydrated stomatocytosis, is characterized by a marked passive sodium leak, which causes red cell overhydration and macrocytosis. It is an autosomal dominantly inherited hemolytic anemia. The syndrome was first described in a girl with dominantly inherited hemolytic anemia whose blood film contained stomatocytes.[180] The hallmarks of abnormal cation transport and overhydration of the red cells were discovered subsequently.[181]

### Etiology and Pathogenesis

The red cell membrane of stomatocytes has enhanced permeability toward monovalent cations, especially sodium ions. This marked passive sodium leak into the cell represents the principal lesion in this disorder. The $Na^+$-$K^+$-ATPase pump, which normally maintains low intracellular sodium and high potassium concentrations, is stimulated but this increase in active transport, coupled to enhanced glycolysis to provide ATP, is insufficient to overcome the leak.[175,182]

The overhydrated red cells of some patients lack stomatin, a 31-kDa integral membrane protein, but no gene mutations have been found implying that the absence of the protein is a secondary phenomenon.[13,175] Stomatin interacts with GLUT-1 and converts it to a dehydroascorbic acid transporter, suggesting that it might be beneficial to inhibit this interaction in stomatocytes, because they require additional glucose for their increased ATP needs.[175]

In some stomatocytosis patients, missense mutations causing amino acid substitutions of conserved residues in the transmembrane domain of the RhAG protein, a component of the band 3–Rh–RhAG multiprotein complex in the membrane, have been described.[183] RhAG is a transport protein that may function as a gas and/or ammonium channel through pore-like structures. The mutations are thought to widen the pores allowing cations to leak through the membrane. A *de novo* missense mutation in the transmembrane domain of band 3 has been described in one patient with stomatocytosis associated with dyserythropoiesis.[184] This changed the transport function of band 3 from an anion exchanger to a cation channel. The tyrosine phosphorylation profile of the stomatocyte membranes revealed increased phosphorylation of band 3 and stomatin, as a result of enhanced activity of the Syk and Lyn tyrosine kinases, suggesting that phospho-signaling pathways involved in cell volume regulation may be perturbed.[184]

### Clinical Features

Moderate to severe anemia is present. Jaundice and splenomegaly are common, as are complications of chronic hemolysis, such as cholelithiasis. Patients exhibit a tendency for iron overload, independent of transfusion status or splenectomy. No other organ system abnormalities have been noted.[13,175] A dyserythropoietic phenotype was noted in one patient with mild anemia.[184]

### Laboratory Features

The blood film reveals striking stomatocytosis and up to 50 percent of red cells may have abnormal morphology (Figs. 46-10E and 46-11D). In addition to the anemia, red cell indices show decreased MCHC and marked macrocytosis, as reflected by an elevated MCV, which can reach 150 fL in some severely affected patients (see Table 46–4). The $K^+$ content is decreased and the $Na^+$ content is markedly increased, leading to elevated total monovalent cation content. The OF of stomatocytes is markedly increased because many of the swollen red cells approach their critical hemolytic volume, which causes a shift of the osmotic gradient ektacytometer curve to the right. Red cell deformability is decreased.

### Therapy, Course, and Prognosis

The majority of hydrocytosis patients suffer from significant lifelong anemia. They should be monitored for complications of hemolysis, such as cholelithiasis and parvovirus infection, and should receive folate supplementation. The outcome of splenectomy has been

variable, but typically it has been beneficial and improved the hemolytic anemia in severely affected patients.[13] This is expected because stomatocytes expend large amounts of ATP to pump cations in an attempt to avoid osmotic lysis and are, therefore, vulnerable in the metabolically challenging environment of the spleen. However, splenectomy should be carefully considered in patients with this disorder, since they are at high risk of developing hypercoagulability after splenectomy, leading to catastrophic thrombotic episodes.[13]

### CRYOHYDROCYTOSIS

The clinical phenotype and biochemical features of some patients with stomatocytes are intermediate between the extremes of hereditary hydrocytosis and hereditary xerocytosis. One of these disorders is cryohydrocytosis in which the mild cation leak is markedly enhanced at low temperatures. It is a very rare condition associated with mild to moderate hemolytic anemia and splenectomy appears to be beneficial.[175] Missense mutations have been found in the transmembrane section of band 3 that cluster between membrane span eight and the last two membrane-spanning domains.[184,185,186] *In vitro* studies indicated that the mutant proteins have lost their anion exchange capability and are converted to a nonselective cation channel.[175,186,187]

Two cases of cryohydrocytosis and stomatin deficiency have been described with mutations in GLUT-1, which abolish the glucose transport function of the protein and create a cation leak.[188]

### OTHER STOMATOCYTIC DISORDERS

*Rh-deficiency syndrome* designates rare individuals who either lack all Rh antigens ($Rh_{null}$) or exhibit markedly reduced ($Rh_{mod}$) Rh antigen expression. Rh antigens are carried on RhCE and RhD proteins that associate with RhAG and enable the formation of the Rh multiprotein complex in the red cell membrane. The Rh complex is either absent or markedly reduced in patients with Rh deficiency syndrome and they present with mild to moderate hemolytic anemia. Stomatocytes and occasional spherocytes are seen on the blood film and the cells have cation transport abnormalities, which cause dehydration. Hemolytic anemia is improved by splenectomy.[13,175] Chapter 136 reviews the structure, localization, and functions of the Rh antigens.

*Familial deficiency of high-density lipoproteins* is a rare condition that leads to accumulation of cholesteryl esters in many tissues, resulting in clinical findings of large orange tonsils and hepatosplenomegaly. Hematologic manifestations include moderately severe hemolytic anemia with stomatocytosis. Red cell membrane lipid analyses revealed a low cholesterol content and a relative increase in phosphatidylcholine at the expense of sphingomyelin.[13]

### ACQUIRED STOMATOCYTOSIS

Normal individuals have up to 3 percent stomatocytes on blood films. Acquired stomatocytosis is common in alcoholics particularly those with acute alcoholism. Vinca alkaloids, such as vincristine and vinblastine, may induce hemolysis with increased sodium permeability and stomatocytosis at the doses used for chemotherapy of leukemias and lymphomas.[189] Transient stomatocytosis has been observed in long distance runners immediately after a race. The molecular basis of acquired stomatocytosis is unknown.[13]

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