This monograph is focused on anemias. Among the most common disorders in the world, anemias are conditions that cause the number of red blood cells (erythrocytes) in the circulation to fall. In humans, red cells provide the sole means for efficient oxygen acquisition (in the lungs), transport (in the circulation), and delivery (via capillaries perfusing vital tissues). They are thus essential for survival. In the most severe cases, anemias can lead to major organ dysfunction or non-function due to oxygen deprivation, producing heart failure, coma, or even death. In more moderate situations, anemia can produce protean symptoms and physical signs of inadequate oxygen transport, such as pallor, exercise intolerance, malaise, cognitive changes, congestive heart failure, and weakness. In many cases, however, anemias tend to be milder and can be entirely asymptomatic, detectable only as deviations from the patient’s normal red cell values in laboratory tests.

As discussed in detail in many chapters of this book, literally hundreds of factors govern the production, destruction, or loss, via bleeding, of red cells. Reductions in the red cell mass can thus have many hundreds of possible causes. While a number of these arise from intrinsic abnormalities in the production (erythropoiesis), structure, or function of erythrocytes themselves, in most patients with anemia, the fall in red cell mass is due to extrinsic factors that impair erythropoiesis, cause hemorrhage, or lead to accelerated destruction of red cells. Thus, a very mild anemia can be a sign of a potentially fatal condition, such as colon cancer or a myelodysplastic syndrome, while some of the most severe anemias, though requiring emergent lifesaving interventions, can be due to relatively readily managed conditions such as severe folic acid or vitamin B12 deficiency. Discerning the reason for an individual’s anemia, however mild, is thus an imperative of good clinical practice.

The body’s need for large quantities of red cells is intimately related to the need for large quantities of hemoglobin, the body’s major oxygen transport protein. Since oxygen is minimally soluble in plasma, a higher-efficiency means of transport from the lungs to the tissues is required. In most metazoan species, this bulk transport depends on “heme” or “heme-like” pigments. These consist of a transition metal (e.g., iron or cobalt) coordinately bound to a planar porphyrin molecule. In mammals, this compound is invariably heme, consisting of reduced iron (Fe^{2+}) encased in protoporphyrin IX. Unfortunately, heme is minimally soluble in plasma. It would, in any event, be rapidly catabolized and excreted by the liver and kidneys. In order to achieve a sufficient capacity for oxygen transport, heme is packaged in a highly soluble, tetrameric group of proteins called hemoglobins. In addition to allowing enough heme to be soluble, hemoglobins also modulate the interaction between heme and oxygen so as to ensure reversible binding and release of oxygen over the physiologic range of oxygen tensions encountered in the lungs and the vascular beds perfusing the interior tissues of the body.

Enormous quantities of hemoglobin are needed to ensure life-sustaining oxygen transport. Unfortunately, hemoglobins themselves cannot persist for more than a few minutes in the circulation, where they dissociate into dimers and are rapidly cleared by the kidneys. The amount of energy and nutrients needed to replenish literally pounds of hemoglobin lost every day would overwhelm the entire resources of the body. Nature has addressed this problem by packaging hemoglobin into erythrocytes. These highly durable cells are capable of lasting 4 months in the blood. All of the vital cells, tissues, organs, and physiologic functions of the body thus depend on the continued production and prolonged survival of red cells to provide them with life-sustaining amounts of oxygen.

The abundance of red cells in the circulation is assessed clinically by a series of laboratory tests. They measure the “concentration” of red cells or hemoglobin in whole blood, or the volume of red cells as a percentage of the volume of whole blood. These are summarized in Table 1.1, along with a number of other basic laboratory tests useful for characterizing anemias and assessing possible underlying causes. The red cell count (RBC) is measured by automated particulate counters and tabulated as the number of cells per cubic millimeter of whole blood; normal values range from 4 to 5.5 cells/cu.mm. The hemoglobin value (Hb or Hgb) measures the concentration of hemoglobin, calculated as grams of hemoglobin per 100 cubic centimeters of whole blood. Normal values range from 12.5 to 15.5 mg/100 cc. Finally, the hematocrit measures the total volume of red cells as a percentage of the volume of whole blood; normal values range from 38 to 45%. As noted in the table, “normal” values vary somewhat by age and gender.
These measurements of the amount of red blood cells are invariably included as part of the routine complete blood count (CBC), which also provides information about the numbers and types of white blood cells and platelets, and some additional information useful for further assessment of anemias. These include the means corpuscular volume (MCV), which is expressed as the average volume, in femtoliters, of the red cells in the circulation. Certain forms of anemia in femtoliters, are associated with smaller- or larger-than-normal-sized red cells in the circulation. Recognizing the change in size can be a very useful first step in narrowing down the possible causes of anemia. The mean corpuscular hemoglobin or MCH measures the total amount of hemoglobin in picograms per microliter of red cells, while the mean corpuscular hemoglobin concentration (MCHC) measures the concentration of hemoglobin within red cell cytoplasm and is expressed as picograms of hemoglobin per picoliter of cytoplasm. The reticulocyte count is a measure of the percentage of total red cells that are newly released into the circulation (“reticulocytes”). These cells retain traces of their bone marrow progenitors that alter their staining properties on peripheral blood smears, as discussed later in this chapter, but only for about one day out of the roughly 100- to 120-day lifespan of normal red cells. The normal value for the reticulocyte count in the setting of a normal hemoglobin and hematocrit is thus about 0.8–1%. Given the relative persistence of reticulocytes and mature erythrocytes in the circulation, this percentage represents the physiological replacement needs. Levels above this indicate increased production of red cells, usually in response to red cell loss or destruction, while levels at or below normal values despite the presence of anemia indicate inadequate marrow response to the need for more red cells. This value is thus very helpful in assessing whether anemias are due to failure to produce sufficient numbers of red cells or to excess red cell loss or destruction, or both.

The definitions and utility of the other tests listed in Table 1.1 are addressed later in this chapter or in the subsequent relevant parts of this textbook. For our present purpose, it is sufficient to note that anemias can be detected and at least operational definitions of “anemia” need not necessarily require a reduction in red cell mass sufficient to compromise normal...
physiologic function, such as cardiovascular status, exercise tolerance, and the like. Indeed, some of the most important reasons for a decline in red cell mass from the patient’s “norm” might not cause any symptoms or even cause the blood counts to fall outside of the “normal” range. The underlying reason for an altered red cell mass often is more important than the actual extent of the change in the amounts of red cells.

Chapter 2 describes the process by which red cells are produced in the bone marrow and the mechanisms by which accelerated red cell production is stimulated in response to excessive red cell loss or destruction. Chapter 3 describes the mechanisms by which senescent or infected red cells are removed from the circulation, thereby potentially shortening red cell survival. This chapter outlines the essential components of the red cell that allow for its prolonged survival in the circulation. An understanding of these components is required for understanding anemia, because derangements in the structure, function, or physical status of these components represent major etiologies of anemia.

**Essential Components of the Red Cell**

The mammalian erythrocyte develops over 2–3 weeks through a highly concerted cellular differentiation program called erythropoiesis. Erythropoiesis begins with the pluripotent hematopoietic stem cell. After a complex series of differentiation events and maturation processes, each red cell circulates as a biconcave disk-shaped cell exquisitely adapted to provide oxygen transport for nearly 120 days. Along the way, it loses its nucleus and all of its cytoplasmic organelles. It enters the blood stream as a reticulocyte, distinguished from its more mature erythrocyte descendants by the retention of a few polyribosomes that support a limited repertoire of protein synthesis, 90% of which is devoted to producing hemoglobin. Within 24 hours, even that capacity is lost. The mature erythrocyte thus has no ability to reproduce or to generate new proteins, lipids, or nucleic acids. It retains only a rudimentary system for generating energy (ATP) from glucose and a modest ability to rebalance redox status in the presence of oxidative stresses.

Despite these limitations, the circulating human erythrocyte is remarkably resilient. In normal individuals, it traverses the circulation nearly 300,000 times during its 4-month lifespan. During this journey, it encounters enormous mechanical, osmotic, and biochemical stresses. The diameters of capillaries in many capillary beds are only 2–3 μm, whereas the erythrocyte has a normal diameter of 7.5 μm. Because of the geometric redundancy of its disk shape and the pliability and tensile strength of its membrane, the red cell is able to withstand the considerable shear stress and distortion of being “squeezed” through these narrow passages. Indeed, it recovers its normal biconcave disk shape as it enters the more capacious venous circulation. Erythrocytes also encounter striking and rapid changes in osmolarity when passing through the collecting system of the kidney, enormous changes in pH in the renal pelvis, spleen, and other “stagnant” vascular beds, and massive shifts in oxygen tension while traversing the pulmonary arteries, capillaries, and veins. During each passage through the hepatic and splenic circulation, the red cell is brought into proximity with the (RE) system of macrophages, which detect flaws on its surfaces. Cells deemed too damaged are ingested and catabolized. Given these enormous stresses and the limited repertoire it possesses for repair and replacement of essential components, the red cell’s endurance is indeed remarkable.

**Hemoglobin: The Predominant Component of the Circulating Erythrocyte**

Red cells can support the oxygen demands of the organism only because they are able to carry enough hemoglobin for a sufficient period of time to support oxidative metabolism and, therefore, life. Hemoglobin is the overwhelmingly predominant component of the erythrocyte. Red cells do have other important functions, such as contributing to the regulation of blood pH and modulating vascular tone via uptake and release of nitrous oxide. However, these other, “minor” physiologic impacts of erythrocytes are also largely mediated by hemoglobin. It is thus important to review first the structure, function, and regulated biosynthesis of hemoglobin.

**Hemoglobin Structure and Function**

As shown in Figure 1.1, the predominant hemoglobin produced during fetal and adult life are “heterotetramers” comprised of two “α” globin polypeptide subunits and two “non-α” or “β-like” subunits. Each of these globin chains is a helical protein that enfolds a single heme moiety. Heme, in turn, consists of protoporphyrin IX coordinately complexed to a single reduced (Fe“2+”) iron ion. In humans, α chains are 141 amino acids long, and non-α chains are 146 amino acids long. The chains are folded into seven (α) or eight (β) helical segments in such a way as to create a highly hydrophobic core. This interior pocket holds the planar heme moiety at an angle appropriate to interact reversibly with oxygen over the physiologic range of partial pressures of oxygen. The helical segments present mostly hydrophilic (charged) amino acid side chains to the aqueous environment of the erythrocyte cytoplasm while maintaining an interlocking set of neutral and hydrophobic amino acids in the interior of the molecule. In addition to promoting the proper tight binding of heme, these surfaces also support a series of hydrogen bonds, electrostatic, weak-force, and hydrophobic interactions that hold the tetramer together.

Two functional parameters are most critical to understanding the derangements of hemoglobin structure or function that can lead to abnormalities of red cell number. The first is the extraordinarily high intracytoplasmic solubility of the hemoglobin tetramer, required because hemoglobin must...
accumulate in very high concentrations. Red cells are inherently viscous. If red cells accumulate in excessive numbers (hematocrits greater than 50–55), their viscosity creates significant resistance to blood flow, increasing cardiac afterload. This puts a "cap" on how many red cells can safely circulate. Within that number, there must be sufficient numbers of hemoglobin molecules to carry adequate oxygen to tissues. Thus, if the red cell mass is maintained at the rheological optimal hematocrit level of 35–50, then each red cell must carry roughly 30–35 grams of hemoglobin per 100 cc of red cell cytoplasm. This is a very high level of protein to be maintained in a soluble state. The circulatory system, the regulation of red cell mass, and the cytoplasmic hemoglobin concentration are exquisitely evolved to balance these two parameters.

Hemoglobin tetramers are sufficiently soluble to be present at these concentrations. However, each of the individual components (Fe ions, heme, protoporphyins, and individual globin chains) is minimally soluble in physiologic aqueous solutions. It follows that the enormous amount of hemoglobin production needed during the relatively short (5–7 day) period of terminal erythroblast maturation (see below) must be accomplished in such a way that significant excess amounts of free globin, free heme, or Fe are not permitted to accumulate. Conversely, the structural integrity of the intact tetramer and its enfolded heme moieties must be preserved during the circulating life of the erythrocyte. Finally, hemoglobins must be protected from noxious biochemical alterations, most prominently oxidation of either their amino acid residues or their heme moieties. When oxidized, hemoglobins lose the
exquisite spatial relationships among their components that maintain the tetramer’s state; they then dissociate into their insoluble components and precipitate.

Precipitated hemoglobins, as well as the insoluble inclusions formed by dissociated heme-bearing globin subunits, are rapidly catabolized into highly reactive, toxic aggregates known as hemipyrroles. They oxidize the delicate lipids and proteins of the red cell membrane and cytoskeleton, deranging their structure and function. These in turn render the red cell rigid and create surface abnormalities that are detected by the RE system as a damaged or infected cell destined to be removed from the circulation.

Hemoglobin tetramers can also associate with one another in more ordered ways to form polymers rather than frank precipitated aggregates (Heinz bodies). Hemoglobin tends to polymerize when the hemoglobin molecules carry mutated amino acids that increase the affinity of one intact tetramer for others. These “tactoids” of hemoglobin form fibrous and viscous structures within the cytoplasm, thereby altering the fluid dynamics of the cells in the circulation. Red cells bearing such polymers tend to be viscous and rigid. In addition, polymers often adhere to, and alter, the membrane and its cytoskeleton, causing a variety of surface abnormalities that can produce a hemolytic anemia. The altered membrane exteriors can also make the cells more adherent to the vascular wall itself (“sticky” red cells). The most prominent and clinically important example is HbS, or sickle hemoglobin. As described in Chapter 10, HbS arises from a single amino acid mutation that favors polymer formation, but only when hemoglobin is deoxygenated. These polymers alter the red cell as described earlier, producing hemolytic anemia and impaired blood flow in the microvasculature, causing a proten set of clinical manifestations.

The solubility of hemoglobin in erythrocyte cytoplasm is also highly dependent on a system of metabolic enzymes that generate reducing compounds, notably reduced glutathione. Reduced glutathione reverses oxidative damage to hemoglobin and membrane components. In the circulation, erythrocytes are constantly exposed to oxidant molecules generated by infection, inflammation, and the byproducts of energy metabolism, rendering hemoglobin susceptible to being oxidized and precipitated. Indeed, a significant cause of hemolytic anemia (see Chapters 13 and 17) is impairment of one or more of the enzymes in these reducing pathways, especially when the reduced antioxidant capacity is overly stressed by exposure to oxidative stress, notably by certain drugs, toxins, and dietary components.

The other clinically relevant hemoglobin structure-function relationships are those affecting reversible oxygen binding. The hemoglobin tetramer has evolved to possess an oxygen affinity that actually increases as the concentration of oxygen in its environment increases. This creates the so-called “sigmoidal” oxygen binding curve shown in Figure 1.2. A detailed description of the physical chemistry of this phenomenon is beyond the scope of this discussion. Nonetheless, it is important to note that, at low oxygen tensions, hemoglobin has a low affinity for oxygen until the oxygen concentration is sufficient to break so-called salt bridges that would otherwise bar access of oxygen to the iron moiety within heme. This binding shifts the configuration of heme molecules within the remaining chains of the tetramer to make the breakage of those salt bridges easier. Thus, as oxygen tension rises, the hemoglobin becomes more avid for oxygen, and the amount of binding increases steeply over a relatively narrow range of oxygen tension. In other words, “oxygen binding begets more oxygen binding.” At about 60–65 mm Hg PaO₂, all four of the heme groups become fully oxygen saturated. Further increases in oxygen concentration have little effect on the oxygen-carrying capacity of the blood because the additional amount of nonhemoglobin oxygen that can be dissolved in the blood is virtually negligible.

Figure 1.2 reveals one very important aspect of the oxygen-binding properties of hemoglobin, namely that it is virtually completely saturated with oxygen at an arterial PaO₂ of 60–65 mm Hg. This is far below the PaO₂ (90–100 mm Hg) in the normal pulmonary capillary circulation. Thus, the ability of red cells to acquire their maximum payload of oxygen in the lungs is protected by the oxygen binding properties of normal human hemoglobins. Figure 1.2 also reveals that, at
the normal PaO₂ of mixed capillary blood (roughly 40 mm Hg), hemoglobin rapidly loses much of its oxygen over a relatively narrow range of PaO₂. Changes in the inherent oxygen affinity of hemoglobin thus tend to have a much greater impact on the delivery of oxygen to the tissues than they do on the ability to acquire oxygen in normal lungs. Increased oxygen affinity offers virtually no advantage in terms of acquiring oxygen unless the ambient oxygen is extremely low or pulmonary function is seriously compromised. However, “high-affinity” hemoglobins are disadvantageous at the capillary level, where much less oxygen is released (see Figure 1.2). Tissue hypoxia triggers erythropoietin release because the kidney perceives the reduced oxygen delivery as reflecting reduced red cell mass. This leads to erythrocytosis; when the red cell mass increases beyond hematocrits of 50–55, blood viscosity increases and hemodynamics are compromised.

Conversely, the oxygen affinity of hemoglobin can be reduced significantly without substantially reducing the amount of oxygen acquired in the lung. As shown once again by Figure 1.2, the wide range of PaO₂s over which hemoglobins will be fully saturated allows for this change. However, at the PaO₂ of capillary beds, low-affinity hemoglobins deliver far more oxygen to the tissues than “normal” hemoglobins. These individuals thus have little or no erythropoietin drive and can function physiologically quite well at lower red cell masses, which, when measured in routine blood labs, appear to be “anemias.”

The clinical aspects of inheriting high-affinity and low-affinity hemoglobins are discussed in Chapter 11. Even though these are not major causes of anemia, they are instructive because they demonstrate in vivo the impact of tissue oxygen delivery on the regulation of red cell mass.

### Genetics, Ontogeny, and Biosynthesis of Hemoglobin

Humans produce different hemoglobins at different stages of embryonic, fetal, and adult development. The primary sites of erythropoiesis also change at these different stages of development, from the yolk sac in embryonic life to the liver during fetal life, and then, permanently, to the bone marrow in the latter stages of gestation. The production of hemoglobin is tightly coupled to the process of erythropoiesis, which is described in more detail in Chapter 2. Briefly, pluripotent hematopoietic stem cells become committed to erythropoiesis at early stages of development under the influence of a complex array of growth factors. These activate the production or repression of an equally complex network of transcription factors and cofactors. Under their direction, the progenitors progress through a series of differentiation steps until they emerge as the earliest definable fully committed erythroid progenitors, the so-called burst forming units – erythroid (BFU-E). BFU-E give rise to so-called colony forming units – erythroid (CFU-E) that then differentiate to the earliest morphologic state of erythropoiesis, the proerythroblast. This cellular progression is accompanied by an increasing sensitivity to erythropoietin, which serves to promote both survival and proliferation. BFU-E appear to be more flexible than CFU-E as to which globin genes they will ultimately express.

Although completely committed to produce only erythrocytes, progenitors at the early proerythroblast stage express globin at minimal levels. As the proerythroblast matures, the globin genes, which have been poised for high levels of expression via their chromatin configuration, unleash a remarkably high level of transcriptional activity. During the next 5–7 days, this burst gives rise to the abundant amounts of hemoglobin needed for oxygen transport.

At about the same time, iron uptake increases. The increased intracellular levels of iron then trigger a complex series of gene expression events, largely at the posttranscriptional level, which greatly increase the amount and activities of the heme biosynthetic enzymes. In this manner, rapid increases in the amounts of iron, heme, and globin are coordinated. Maturation beyond the proerythroblast stage also marks the beginning of a progressive decline in the expression of most other genes except those required for producing the red cell membrane cytoskeleton and the few metabolic enzymes that will persist in the circulation. The production of hemoglobin thus ultimately constitutes nearly 95% of the total gene expression activity of late erythroblasts and reticulocytes. This progression is evident by observing the progressive morphologic stages of erythroblast maturation. As these cells mature from proerythroblasts to metachromatophilic erythroblasts to orthochromatophilic erythroblasts, there is progressive hemoglobinization (“pinking”) of the cytoplasm, reduced basophilia (due to declining amounts of ribosomes and nucleic acids), and condensation of active nuclear chromatin (euchromatin) into heterochromatin-producing nuclear pyknosis. In the very terminal stages of maturation, the nucleus is ejected, mitochondria and other organelles disappear, and the enucleate reticulocyte is released into the circulation. Hemoglobin synthesis persists for about 24 hours in the circulation before the reticulocyte matures into fully developed erythrocytes.

A highly coordinated production of α globin, non-α globins, the protoporphyrin IX component of heme, and the incorporation of iron cause each to accumulate in roughly equal amounts. Since each component is rapidly incorporated with the others to form hemoglobin, excesses of none of them occur. This ensures that only highly soluble hemoglobin tetramers and none of their insoluble components accumulate in the cytoplasm.

### The Globin Genes

The globin genes map to two compact clusters on chromosome 16 (the ζ globin and α globin genes) and chromosome 11 (the β-like globin genes, β, γ, δ, and ε). As shown in Figure 1.3, the embryonic, fetal, and adult hemoglobins are defined by their globin chain composition. Embryonic hemoglobins are
Chapter 1: Essential Elements of Red Cell Homeostasis

produced in the yolk sack during embryonic life, beginning at about 5–6 weeks of gestation: Hb Portland I (ζγ2γ2), Hb Portland II (ζ2βγ2), Hb Gower-1 (ζ2ε2), and Hb Gower-2 (α2ε2). For our purposes, no further discussion of these hemoglobins is necessary. By the gestational age of 10–11 weeks, red cell production has largely switched to the fetal liver, where the predominant hemoglobin is HbF (α2γ2), i.e., fetal hemoglobin. β globin genes are expressed, but at very low levels, throughout gestation. Production of hemoglobin F predominates until approximately 34–37 weeks’ gestation, when a largely irreversible switch to the production of adult hemoglobins (Hb A: α2β2 and Hb A2: α2δ2) begins (the Hb F > Hb A switch). At birth, virtually all new hemoglobins being produced are adult hemoglobins. Hb A is the most abundant adult hemoglobin, comprising more than 95% of total adult red cell hemoglobin. Hb A2 accounts for 2–3.5% and residual HbF about 1%, in the normal adult array of red cells. HbF is confined after birth to a small subpopulation of red cells (“F cells”), within which the HbF content is very high.

Even though the switch to biosynthesis of adult hemoglobin is virtually complete at the time of birth, the composition of hemoglobins in the peripheral blood changes more slowly. This reflects the long life span of the erythrocytes. HbF persists for a long time after the switch in biosynthesis in circulating red cells that were launched into the circulation before the switch occurred. The transition to the adult pattern of hemoglobin content in the peripheral blood is usually not complete until the latter half of the first year of life.

As discussed in Chapter 8, “hemoglobin switching” has clinical relevance for the understanding and potential therapy of anemias due to certain hemoglobinopathies. Reference to Figure 1.3A shows that individuals inheriting anemia due to α or γ globin gene defects will be affected in utero as well as in adult life, provided that the severity of the anemia is compatible with survival through gestation. However, individuals inheriting abnormalities of the β chain will be asymptomatic in utero, since dependence on β globin becomes physiologically important only several months after birth. Sickle cell anemia, a β chain hemoglobinopathy, and β thalassemia comprise major causes of morbidity and mortality among hemoglobinopathies. These patients generally do not present with symptoms until infancy, childhood, or even, in milder situations, adult life. It follows that prevention or reversal of the fetal to adult hemoglobin switch could potentially eliminate the clinical consequences of β globin chain hemoglobinopathies.

Reference to Figure 1.3A also shows that the α globin loci are duplicated, whereas the β globin locus exists as a single copy. Thus, individuals inheriting a mutation in one of the α globin alleles will tend to have a smaller percentage of their total hemoglobin affected by that mutation than individuals inheriting a comparably “severe” abnormality of the single β globin locus. The latter will impair half of all the hemoglobin produced, the former only about 25%. This feature may account for the fact that fetuses are often able to survive gestation despite the phenotypic impact of abnormal α globin production during fetal life.

A great deal of research has been devoted to understanding how the globin genes first become poised to express themselves and then execute their programs of abundant yet tightly regulated fetal and adult globin gene expression. Globin genes are completely silent in all other tissues, and in other
hematopoietic progenitors, except for the 5- to 7-day period between the early erythroblast and reticulocyte stages of terminal maturation. It has become clear that the two tightly clustered arrays of globin genes are under the control of a series of promoters and enhancers that regulate their individual function but are also subservient to “super enhancers” or “master switches” called the LCR, for locus control region (Figure 1.3). Evidence suggests that the LCRs, when bound to the transcription factors and cofactors mentioned earlier, form “loops” of chromatin that connect with the promoters and enhancers flanking a particular globin gene. These looped-out regions provide access for additional factors and cofactors that form the transcriptional complex needed to produce globin mRNA. Many of these key proteins have been identified and characterized. Manipulation of LCR interactions is being attempted experimentally to modulate hemoglobin switching as a potential therapeutic modality.

Although the process of hemoglobin biosynthesis is exquisitely and tightly regulated, there is little or no direct “cross talk” between the α-like and β-like gene clusters. The fact that equal amounts of α and β globin are produced is the result of intrinsic structural features of the genes and their mRNA products. The production of globin mRNA is regulated exclusively at the transcriptional level. Globin pre-mRNAs do not undergo alternative pre-mRNA splicing. The rate of production of α globin mRNA exceeds that of β globin mRNA by about 50%, particularly in the earlier stages of erythroblast maturation. However, β globin mRNA is translated into β protein more efficiently than α globin mRNA by nearly the same relative percentages. These differences in translational efficiency are due to differences in the structure of the 5’ untranslated regions of the mRNAs. β globin mRNA has a 5’ untranslated conformation more amenable to formation of the initiation complex for binding to polyribosomes. β globin mRNA is also slightly more stable than α globin mRNA, having a cytoplasmic survival time of about 54 hours, in contrast to about 38 hours for α globin mRNA. These differences produce a net result that α and β chain production is very nearly equal. Only a very slight excess of α globin is produced, much of it in the earlier stages of maturation. The small pools of excess α globin chains are catabolized by an ubiquitin-mediated pathway, thereby preempting the formation of precipitated inclusions. However, as discussed in Chapter 8, this catabolizing capacity can be easily overcome if the α globin burden increases as the result of impairments in β globin production.

Assembly of hemoglobin from newly synthesized globin chains requires the presence of chaperone proteins. To date, there are no well-described forms of anemia due to the failure of these assembly mechanisms. However, some mutations in the globin chains themselves appear to alter globin structure sufficiently that posttranslational formation of tetramers is impaired, producing a thalassemia-like syndrome.

Hemoglobins are susceptible to many posttranslational modifications. Of note is the affinity of HbA for (2,3-BPG, biphosphoglycerate, 2,3-BPG), a metabolite of the glycolytic pathway present in the circulating red cell. 2,3-BPG decreases the oxygen affinity of hemoglobin (Figure 1.2). HbF has very low affinity for 2,3-BPG. Even though fully purified (“stripped”) preparations of HbF and HbA have nearly identical oxygen affinity, HbF-rich red cells tend to have a higher oxygen affinity in vivo and thus can “steal” oxygen from HbA. It also delivers oxygen less efficiently, which accounts at least partially for the high hematocrits of fetuses and neonates.

By virtue of its prolonged exposure to a variety of substances in the circulation, hemoglobin can also be nonenzymatically modified. These modified hemoglobins accumulate as the red cell ages. The most important modifications include the binding of hemoglobin to carbon monoxide in the environment, which can produce carbon monoxide poisoning, the creation of sulfated hemoglobins from exposure to sulfuric compounds, and the glycosylation of the hemoglobin by exposure to glucose. Indeed, "HbAIC", the best known of the glycosylated hemoglobins, tends to increase or decrease as a percentage of the total hemoglobin as a reflection of the red cell’s exposure to the amounts of glucose in the blood. HbAIC has thus become an important and useful biomarker for the chronic control of blood sugar in diabetic patients.

While interesting physiologically and in other clinical contexts, most posttranslational modifications of hemoglobin are not especially relevant for the purposes of assessing the causes of anemia or developing strategies for their management. A few exceptions are discussed in Chapter 11. However, it is worth noting that anemia can modify the utility of HbAIC measurements in diabetic patients who also have shortened red cell survivals. Since HbAIC tends to accumulate in red cells over time, HbAIC levels can be artefactually low in patients with severe hemolytic anemia.

The Red Cell Membrane and Its Cytoskeleton

The circulating erythrocyte faces daunting challenges in the circulation. These include the physical stresses of circulating under high hydrostatic pressures through sometimes turbulent vascular beds. In the microcirculation, many of the capillaries are 2–3-fold narrower than the normal red cell diameter. To return to the venous circulation in the spleen, RBCs must also slither through interendothelial cell slits only a few microns in width. The high concentration of hemoglobin exerts a tremendous internal oncotic pressure, which causes swelling and contraction of the red cell as it passes through environments that are either hypotonic or extraordinarily hypertonic, for example, in the collecting system of the kidney. Moreover, circulating erythrocytes are subjected to enormous biochemical challenges including redox fluxes in sluggish vascular beds and wide swings in oxygen tension as the red cell passes from the venous circulation into the lungs, through the arteries, and back into the capillary beds. Erythrocytes must therefore possess not only the high tensile strength but also the pliability and flexibility needed to tolerate extraordinary changes in cell }
volume, shape, and biochemistry. Finally, red cells must be able to resist adherence to the walls of the vascular tree and to one another despite their exposure to adherence molecules when passing in intimate contact with small capillaries and venules. If red cells adhered to one another, they would form aggregates that would tend to block small vessels as well. An essential adaptation for these challenges is the unique structure and function of the erythrocyte membrane and its underlying cortical cytoskeleton. Derangements of these elements that can lead to anemia are considered in considerable detail in Chapter 14. For our present purposes, it is sufficient to note the special features of this integrated membrane/cytoskeleton structure and the ways in which they provide for the longevity of the circulating red cell.

The red cell membrane consists of a phospholipid and cholesterol bilayer punctuated with transmembrane proteins (Figure 1.4). These support ion and nutrient transport. The vast majority of these molecules are glycoproteins or lipoproteins whose attached residues tend to be negatively charged, thus creating a repellant electrostatic "cloud" around the outer surface of the cell. Most abundant among these is glycophorin, which is heavily modified by sialic acid on its outer surface. In addition to their functional role, these exterior-facing moieties are polymorphic both at the amino acid sequence level and in the composition of their carbohydrate and lipid modifications. These polymorphisms are recognized clinically as the major and minor blood group antigens. As discussed in Chapters 12, 29, and 30, the blood group antigens are important in the characterization and management of immune hemolytic anemias, in bone marrow transplantation, and in transfusion medicine.

The lipid components that make up the phospholipid bilayer of the membrane are also critical both structurally and functionally. The major lipid component of the erythrocyte membrane is cholesterol, but a wide variety of lipids and phospholipids, including sphingomyelin, also contribute to the stability of the red cell membrane and its interaction with its external environment. It is important to note that three major phospholipid classes — phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine — are particularly germane to the pathophysiology of a number of the anemias discussed in this text. Phosphatidylserine can act as a cofactor in the activation of prothrombin; thus, its presence on the outer leaflet, facing into plasma, could cause the red cell to have procoagulant activity. Given the mass of red cells in the circulation, this could create a thrombophilic state. In normal erythrocytes, phosphatidylserine is maintained almost exclusively on the inner leaflet of the lipid bilayer. Phosphatidylcholine and phosphatidylethanolamine predominate on the outer surfaces of the lipid bilayer.

Lipid asymmetry is maintained by a system of enzymes called "liphases," "flopases," and "scramblases." As suggested by their names, these enzyme systems have the effect of "flipping" the polar heads of specific phospholipids in one direction (inward), in the other direction (outward), or in both directions, respectively. In erythrocytes, as in other cells, influx of calcium into the cytoplasm causes evasion of phosphatidylserine to the outer leaflet. As discussed in other chapters, this is relevant in hemolytic anemias such as sickle cell anemia, in which oxidation of membrane structures by precipitation or polymerization leads to altered ion transport, calcium influx, and alterations of the phospholipid translocating systems.

The fragile nature of this bilayer is not only determined by its lipid composition but also by the manner in which the lipids are arranged. As suggested by the name, phospholipids form bilayers because the charged heads of their polar groups (the glycerophosphocholine) and the fatty acid chains (the lipids) are repelled by each other, the outer head groups are facing into the cell and the fatty acid tails are facing outward, thus creating a hydrophobic core that cannot be penetrated by water. Phospholipids make up the bulk of the erythrocyte membrane and consist of two kinds of structural classes: the plasma and cell membranes, which are also critical both structurally and functionally. The major lipid component of the erythrocyte membrane is cholesterol, but a wide variety of lipids and phospholipids, including sphingomyelin, also contribute to the stability of the red cell membrane and its interaction with its external environment. It is important to note that three major phospholipid classes — phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine — are particularly germane to the pathophysiology of a number of the anemias discussed in this text. Phosphatidylserine can act as a cofactor in the activation of prothrombin; thus, its presence on the outer leaflet, facing into plasma, could cause the red cell to have procoagulant activity. Given the mass of red cells in the circulation, this could create a thrombophilic state. In normal erythrocytes, phosphatidylserine is maintained almost exclusively on the outer surface of the lipid bilayer. Phosphatidylcholine and phosphatidylethanolamine predominate on the outer surfaces of the lipid bilayer.

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resulting in eversion of phosphatidylserine. While the pathophysiologic importance of this phenomenon is unknown, it is clear that sickle red cells are more adherent to the vascular wall, and appear to be somewhat prothrombotic. Failure of an impaired RE system to remove red cells with everted phosphatidylserine in patients who have been splenectomized might also contribute to the hypercoagulable state seen post-splenectomy.

The cytoplasmic domains of the key transmembrane proteins are important for the physical strength and flexibility of the red cell, because these are sites at which the phospholipid bilayer becomes attached to the underlying protein cytoskeleton (Figure 1.4). Indeed, without anchorage to an underlying protein meshwork, the red cell membrane has the physical properties of a soap bubble. In the circulation, red cells would quickly be emulsified into small vesicles as they are pushed through small capillaries under high hydrostatic pressure. Fortunately, the erythrocyte membrane cytoskeleton is uniquely adapted to circulate in that vasculature. The underlying protein scaffold consists primarily of spectrin. Spectrins are large (ca250 kD) helical proteins. In erythrocytes, the functional unit of spectrin is a dimer of α spectrin and β spectrin. These chains bind to one another in a head-to-tail fashion in such a way as to become entwined along a series of more than 20 helices. This structure allows the protein to be highly extensible and compressible, roughly like a spring.

Spectrins form a hexagonal latticework underneath the lipid bilayer and are attached at the junctions of one dimer with another to the cytoplasmic domains of several transmembrane proteins, the most critical of which quantitatively and functionally are the glycoporphins and the anion exchange transporter (“Band 3”). These two proteins are the most abundant in the red cell membrane. They provide multiple attachment points for the spectrin cytoskeleton. Additional flexibility is provided by binding to actin at these critical junctions.

Binding of the spectrin lattice to the cytoplasmic domains of the transmembrane proteins is mediated primarily by two multifunctional proteins, protein 4.1R and Ankyrin (Figure 1.4). Each of these molecules has separate binding regions for the cytoplasmic domains of transmembrane proteins and for the spectrin–actin cytoskeleton. They thus provide strong yet flexible attachment, much like “molecular swivels” or “molecular hinges,” conferring the freedom of motion needed for the twisting or sliding of the cytoskeleton across the inner surfaces of the phospholipid bilayer when the cells are distorted by shear stress and stretched or shriveled by osmotic changes. An added effect of these two proteins is to stabilize spectrin–spectrin and spectrin–actin interactions, thus stabilizing the entire complex. A number of other molecules, mentioned in more detail in Chapter 14, participate in this complex set of linkages and contribute to stability. Figure 1.4 illustrates these structural and functional features.

In summary, the red cell membrane and its cytoskeleton are adapted to the cellular transport and communication needs of the circulating erythrocyte and also provide the critical tensile strength, flexibility, and metabolic adaptability needed to endure 4 months in the circulation. Unfortunately, they are also delicate structures. They are susceptible to irreversible damage because of the limited capacity of circulating red cells to regenerate or repair damaged and destroyed components. Inherited defects in or acquired damage to any of these protein components can lead to hemolytic anemias. However, it is also important to recognize that other conditions, such as enzymopathies, immune damage to red cells, polymerization of hemoglobin in sickle cell anemia, or exposure to drugs or ingested agents that injure the membrane, can also cause the membrane to be a key intermediary of premature red cell destruction.

Enzymes of Red Blood Cell Intermediary Metabolism

The systems supporting energy homeostasis and metabolic stability of the red cell were once thought to be extraordinarily simple. Recent studies have revealed that red cells possess a somewhat broader repertoire of metabolic capabilities than previously appreciated. For example, there are limited but important signal transduction pathways that persist in red cells and appear to help control interactions between the red cell, other blood cell types, and the vascular wall. Similarly, enzymes involved in nitric oxide metabolism remain in the circulation for quite a long time. However, compared to most cells or even cell remnants like the platelet, the red cell is rather simple metabolically. For the purposes of understanding anemias, it is sufficient for us to review its three major metabolic capabilities, one for generating energy (ATP) and two for maintaining the balance of oxidized and reduced molecules (redox state).

As shown in Figure 1.5, the anaerobic pathway for glycolysis in red cells is rather primitive in comparison to other cell types. Lacking mitochondria, erythrocytes do not use oxygen. Other metabolic pathways are adapted to circulate in that vasculature. The underlying protein scaffold consists primarily of spectrin. Spectrins are large (ca250 kD) helical proteins. In erythrocytes, the functional unit of spectrin is a dimer of α spectrin and β spectrin. These chains bind to one another in a head-to-tail fashion in such a way as to become entwined along a series of more than 20 helices. This structure allows the protein to be highly extensible and compressible, roughly like a spring.

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ATP within red cells is required for membrane homeostasis and to fuel the other two metabolic pathways needed to generate intracellular reducing power. ATP is needed to maintain red cell phospholipid levels, to fuel membrane pumps, and to support kinase reactions that modulate the behavior of membrane cytoskeletal proteins and signaling components. For example, reversible phosphorylation of protein 4.1R alters its tightness of binding to the spectrin actin cytoskeleton and thus modulates membrane fluidity and, thereby, red cell hemodynamics.

The hexose monophosphate shunt (HMPS) begins with the rate-limiting enzyme glucose-6-phosphate-dehydrogenase (G6PD). Its connection to glycolytic metabolism is shown in Figure 1.5. Through a series of enzyme reactions, the HMPS generates reduced glutathione molecules critical for correcting oxidative damage to hemoglobin and red cell membrane proteins. Deficiencies of these enzymes due to inherited mutations are associated with diminished reducing power and consequence oxidation and precipitation of hemoglobin. By far the most common of these conditions is G6PD deficiency. It is discussed in more detail in Chapter 13. Exposure to toxic levels of oxidizing compounds (e.g., phenylhydrazine) can also overwhelm even the normal complement of glutathione and produce very similar types of hemolysis.

The cytochrome B5 reductase ("methemoglobin reductase system") is a complex of several enzymes that are expressed in multiple isoforms during red cell development and remain intact for much of the red cell lifespan. As noted elsewhere, the iron moiety within hemoglobin is susceptible to oxidation from its reduced ferrous $\text{Fe}^{2+}$ to oxidized ferric state $\text{Fe}^{3+}$, generating methemoglobin, which is a useless respiratory pigment and can also be unstable in the red cell. The cytochrome B5 reductase system generates reduced nicotinamide adenine dinucleotide (NADH), which can reduce ferric $\text{Fe}^{3+}$ iron to ferrous $\text{Fe}^{2+}$ iron. Deficiency of this system by virtue of inherited defects or by exposure to toxins such as nitrite or nitrate compounds results in methemoglobinemia.

Even though the red cell is limited in its arsenal of metabolic capabilities, it manages, at the price of high levels of glucose consumption, to generate sufficient ATP and reducing power to preserve the normal lifespan of the red cell. The consequences, direct or indirect, of impairments in this system can cause multiple forms of anemia that are discussed in other chapters.

**Red Cell Synthesis and Destruction**

While discussed in much more detail in Chapter 3, it is worth mentioning here a few of the basic mechanisms of normal red cell synthesis and destruction. Anemias resulting from excessive red cell destruction invariably result from alterations within or on the red cell that signal systems intended normally for the removal of senescent red cells. These alterations cause the RE system to catabolize these altered red cells before their intended time. Three basic mechanisms are postulated to result in the eventual loss of red cells from the circulation.

The metabolic hypothesis derives from the inability of red cells to replace their proteins. Although launched with a completely normal array of proteins, key enzymes "wear out," and oxidative damage to membrane components and to

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**Figure 1.5** Intermediary metabolism of the red cell. The vertically organized set of metabolic reactions shows the Embden Meyerhof pathway of anaerobic glycolysis, generating a net gain of two ATP molecules and generating lactic acid as a byproduct. However, two major shunts exist. The first, hexose monophosphate shunt (HMPS), for which glucose-6-phosphate-dehydrogenase is the rate-limiting first enzyme, generates reduced nucleotides and, ultimately reduced glutathione to combat oxidative stresses in the red cell. The Rappaport Luebering Shunt (RLS) is also shown. This generates 2,3 biphosphoglycerate (2,3 BPG), which can modulate oxygen affinity in accordance with metabolic needs.
hemoglobin accumulates. It is believed that these changes eventually create sufficient damage to the red cell membrane that abnormalities are sensed on the exterior of the cell (e.g., exteriorization of phosphotidylserine), creating signals that activate macrophages in the RE system to engulf the red cell and catabolize it.

The second hypothesis, the “immunological” hypothesis, is based on the observation that at least one abundant membrane protein, the anion exchange transporter (Band 3), forms, over time, tetramers that are antigenic and are recognized by isoantibodies present in nearly every normal human. It is possible these are only examples of other preformed antibodies that recognize exteriorized antigens that accumulate over time. What is clear is that red cells contain, in their early phases of circulating life, abundant supplies of the membrane protein CD47. CD47 is known to be a “don’t eat me” signal by virtue of its interaction with a macrophage protein SHPS-1. CD47 levels decline on the surface of the red cell as it ages, making macrophages more likely to recognize and ingest red cells bearing any of these antigen–antibody complexes.

The third mechanism, the geometric hypothesis, may simply be a final common pathway of the aforementioned aging phenomena. In order to traverse the tortuous and sluggish microcirculation of the RE system in the spleen and liver, red cells must maintain their “slipperiness,” pliability, and elasticity. Even normal red cells have “bits” on their membrane removed as they come in intimate contact with RE system macrophages. These lost membrane “bits” are likely surface abnormalities that are “polished” away by the RE system to facilitate the red cell’s ability to circulate efficiently. As the red cell ages and loses more and more membrane to this polishing process, it begins to be unable to maintain its biconcave disk shape and becomes rounder (a spherocyte), because the smallest surface that can enclose the interior volume of hemoglobin and cytoplasm is a sphere. The sphere, however, is a rigid structure. It is thus less able to navigate the microcirculation of the RE system and has a higher probability of being catabolized.

It is likely that all three of these mechanisms contribute to the eventual demise of senescent red cells. It is well documented that most metabolic enzymes decline as the red cells age, albeit at different rates, reducing both energy-generating and reducing capability. It is clear that the immunological factors considered above cause erythrocytes to be more recognizable as targets for immune clearance as cells age. In addition, either of these mechanisms will result in gradual loss of membrane function, causing the cells to become more rigid spherocytes.

Regardless of how red cells get destroyed, the process of red cell death generates molecules that serve as important markers of the rate of red cell destruction and are thus critical tools for assessing whether anemia is due to premature red cell destruction (i.e., a hemolytic anemia). Once an erythrocyte is engulfed by the macrophage, its membrane, hemoglobin, and enzyme systems are efficiently catabolized to their component parts. Notably, the iron moieties of hemoglobin are recycled by being transferred to circulating transferrin, which carries the iron either to the bone marrow for use in producing hemoglobin from newly developing red cells or, if present in excess supply, to the liver and the macrophage for storage in the form of ferritin and hemosiderin. The heme moiety is catabolized to biliverdin and then to bilirubin (See Chapter 6). Bilirubin is released into the circulation and is conjugated in the liver. When red cell destruction levels are increased, the bilirubin level rises. When hemolysis is brisk, the newly released “unconjugated” or “indirect” bilirubin fraction rises disproportionately high compared to bilirubin that is hepatically conjugated. Thus, hemolytic anemia is accompanied by higher serum bilirubin levels with a preponderance of increase in the indirect bilirubin fraction.

Some enzymes that are abundant in the red cell, such as lactic dehydrogenase (LDH), also “leak” out of the macrophage, resulting in increased LDH levels in the circulation. The leakage of free heme and hemoglobin subunits, while a small percentage of the total in red cells, is sufficient to saturate the available supplies of circulating proteins, such as haptoglobin and hemopexin, designed to bind and sequester them. The levels of free haptoglobin and hemopexin thus tend to decline during periods of hemolysis. Finally, when hemolysis is brisk, there is hypertrophy of the spleen and liver due to an increase in the proliferation of macrophages attempting to destroy the abnormal red cells. In particularly prolonged or severe states of hemolysis, this can also occur in the liver so that hepatosplenomegaly is a cardinal sign of a brisk hemolytic anemia.

The normal pathway for destruction of red cells at the end of their lifespan is almost exclusively “extravascular,” meaning that it occurs in the sinusoids of the RE system, which, technically, are not part of the normal vascular tree (because they are not lined by endothelial cells). However, in some circumstances, such as extreme oxidative stress, overt mechanical destruction (e.g., due to heart valves or arteriovenous malformation), certain autoimmune conditions in which the classical pathway of complement is fully activated, acute fresh water drowning, severe thermal burns, and so forth, direct fragmentation of red cells can occur within the circulation. Under these conditions, large quantities of hemoglobin are released directly into the blood, where it dissociates into its subunits and is filtered through the kidneys. This results in the generation of red urine that looks like hematuria but is in fact hemoglobinuria. Plasma hemoglobin levels also rise; serum LDH levels can rise to extremely high levels because the enzyme is released directly into the blood. Hemoglobin was once thought to be damaging to the renal tubules. However, the primary damage to the kidneys in states of extreme intravascular hemolysis appears to be due to the trapping of other components of the destroyed red cells such as the red cell membrane and its cytoskeleton in the interstices of the renal tubules.

Conclusion
Red cells are critical to human survival. Without them, little or no oxygen can reach our vital organs. To serve their function,
each red cell must support the substantial metabolic and osmotic burdens placed on it by the high intracellular concentration of the hemoglobin molecules needed to transport adequate amounts of oxygen. These demands are met because each red cell is the product of a remarkable set of maturation and differentiation events that provide for the physical and biochemical strength and flexibility needed to traverse the circulation with its payload of hemoglobin and oxygen nearly 300,000 times during its lifespan. Many intrinsic and extrinsic factors can damage the highly evolved and adaptive yet fragile membrane and metabolic components that ensure this durability and/or impair the high rates of erythropoiesis needed to replace worn-out or lost red cells. These comprise the basic causes of anemia. They determine the clinical management challenges that are reviewed in the remainder of this text.

Further Reading