Little Red Wonders
A Short Biography of the Red Blood Cell

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Human red blood cells exist to facilitate exchange of respiratory gases between the lungs and the tissues of the body. The article is a ‘biographical sketch’ of red blood cells, describing how their structure, chemistry and activities are geared to serve this purpose. In the process, the cause of some red blood cell disorders has been explained in a simplified manner.

Introduction

Of the many cellular elements in blood, the ones which are the most abundant are the red blood cells (RBCs). They are also called erythrocytes. Their red color, and hence the red color of blood, is due to the pigment hemoglobin. Red cells occur in huge numbers – about 4 to 5 million per microlitre of blood. This implies that at any given moment some 20,000 to 25,000 billion of them are circulating in the average adult, making up around 45% of the total blood volume. These cells help to transport the respiratory gases, oxygen and carbon dioxide, between lungs and tissues. Nature has equipped them to discharge this function continuously for a period of 4 months during which each of them makes a 300 mile journey around the microcirculation.

Genesis

In the fetus, RBCs are produced by the liver and spleen but after birth they are ‘born’ exclusively in the bone marrow. Till about the age of 5 years the marrow of essentially all bones take part in erythropoiesis, the process of RBC production. Thereafter, the marrow of long bones increasingly fill up with fat and suspend erythropoiesis. Beyond age 20, most RBCs are produced by the
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marrow in the vertebrae, ribs, sternum and iliac bones. Nevertheless, all marrow retain the capacity to generate RBCs and may be stimulated into erythropoiesis at times of crisis.

Inside the marrow there is a rich cellular environment with a recognizable hierarchy. The most primitive cells in the marrow can give rise to all types of cellular elements of blood. They are called pluripotent hemopoietic stem cells. These cells cannot be identified under the microscope but their existence can be inferred from specialized cell culture techniques. Stem cells have a singular capacity for self-renewal. A lifetime supply of incalculable numbers of mature blood cells relies on only a few thousand stem cells present at birth.

The earliest cells in the bone marrow that can be recognized through microscopic staining techniques as being committed to the red cell lineage are called proerythroblasts. The proerythroblast undergoes successive cell divisions with synthesis of more and more hemoglobin and corresponding alteration of staining characteristics. As hemoglobin fills up the intermediate cells (called normoblasts), the endoplasmic reticulum on which it is synthesized is gradually absorbed. The other organelles are also lost progressively and eventually the nucleus too is extruded from the cell. Shedding of the nucleus reduces weight and allows transformation to a more flexible biconcave shape than the spheroidal precursor. At this stage the cell is called a reticulocyte because the remainder of the cellular organelles appear as a small amount of basophilic material which, upon staining, gives the appearance of a network or reticulum. The reticulocytes squeeze into the blood capillaries from the marrow space and within a day or two lose the residual basophilic material. The mature RBC is now essentially a flexible bag packed with hemoglobin and the bare minimum of enzymes to carry out the metabolic processes essential to survival and function. Each proerythroblast gives rise to 8 to 16 mature RBCs. At any given moment, up to 2% of red cells in circulation can be reticulocytes. This percentage increases substantially when red cells are being rapidly released into circulation to compensate
for blood loss or premature destruction.

The total mass of circulating red cells is regulated in such a manner that the numbers are sufficient for adequate tissue oxygenation but not large enough to impede blood flow through small vessels. Various factors can influence the rate of red cell production but the one that is most important is probably the state of tissue oxygenation. Reduced oxygen saturation in the tissues (tissue hypoxia) is a very powerful stimulus for accelerated erythropoiesis. The response is mediated by erythropoietin, a glycoprotein hormone synthesized by specialized cells in the kidneys and released into circulation within minutes of sensing reduced oxygen supply. The erythropoietin stimulus is so important that in its absence, as can happen in severe and chronic kidney disease, few red cells are produced by the marrow and severe anemia results. Conversely, when erythropoietin concentration is elevated, the rate of red cell production can climb to 10 times the normal or even more. People residing at high altitudes have a greater mass of RBCs in the bloodstream because the rarefied atmosphere at such altitudes tends to produce tissue hypoxia which is offset by increased erythropoietin secretion. The hormone can now be produced through recombinant DNA technology. This has come as a great boon to individuals who suffer from severe anemia due to erythropoietin deficiency from renal disease or otherwise. On the flip side, there are reports that unscrupulous athletes are, in recent times, abusing erythropoietin to increase their red cell mass to gain a clandestine advantage in endurance sports.

Structure

The human red blood cell appears like a biconcave disc with a dumb-bell shape in cross-section (Figure 1). In this unique shape it measures approximately 7.8 μm across and 1.7 μm thick at the periphery, but is only about 1 μm or less in the central part. The average volume is about $90 \times 10^{-15}$ l (90 femtolitres). In a stained blood film under the light microscope, the thinner central part appears a paler red than the surrounding region and
there is very little variation in shape and size between individual cells. Clues to various disorders may be obtained from size or shape alterations. For instance, in the condition known as hereditary spherocytosis, the RBCs are spheroidal in shape (hence 'spherocytes'), smaller in diameter than normal and stain more intensely than usual erythrocytes. These abnormal cells are prone to premature destruction.

The biconcave shape enhances surface area for gas exchange and also confers deformability to the cell. Thus the cell can adopt an umbrella shape while traversing small blood capillaries which are only about 5 μm in caliber or even less. Maintenance of the biconcave shape depends partly upon the pliable structure of the red cell membrane which is depicted schematically in Figure 2. The membrane consists of a collapsible lattice of specialized proteins anchored to a lipid bilayer containing cholesterol and phospholipids. The protein scaffolding maintains cell shape while the lipid bilayer provides a water-repellent skin. A particular membrane protein called spectrin is most abundant and it is this protein which is deficient in hereditary spherocytosis, leading to loss of the biconcave shape and instability of the lipid layer. The cholesterol in the lipid bilayer is inserted inbetween the phospholipid moieties in a manner that stiffens the membrane but still permits optimum flexibility. The red cell needs this flexibility to withstand the stresses of turbulent circulation.

**Figure 2. A schematic representation of the red cell membrane showing the various protein components.**
Chemistry

Biochemically, RBCs are not very active cells. The absence of mitochondria precludes aerobic energy production and the lack of other cellular organelles prevents synthesis of new proteins. However, they do need an energy source to maintain their structure and also the metabolic machinery to defend themselves against oxidative damage (Figure 3). As in other cells, adenosine triphosphate (ATP) serves as the energy source. It is generated through glycolysis (Embden–Meyerhof pathway). This is a sequence of enzyme-catalyzed biochemical reactions in which glucose is metabolized to lactate without the help of
G6PD deficiency depletes GSH stores in the red cell making it vulnerable to oxidative damage.

molecular oxygen. Two ATP molecules are generated in the process. ATP drives the sodium and calcium pumps in the cell membrane, maintaining osmotic pressure inside the cell, and also provides energy required for recovery of cell shape. Pyruvate kinase is a key enzyme in the glycolytic pathway.

A small amount of oxidative glucose breakdown occurs via the hexose monophosphate shunt, in which glucose-6-phosphate is shunted down an alternative pathway for conversion to fructose-6-phosphate by the enzyme glucose-6-phosphate dehydrogenase (G6PD). Reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is a by-product of the G6PD catalyzed reaction plays a vital role in detoxification of oxidants via another molecule called reduced glutathione (GSH). Accumulation of toxic oxidants in the cell greatly enhances activity in the shunt pathway.

Deficiency of the G6PD enzyme can occur as an X-linked recessive genetic disorder. Males are affected while female carriers show half-normal enzyme levels. G6PD deficiency depletes GSH stores in the red cell making it vulnerable to oxidative damage. Things are normal till a stressor precipitates premature destruction of circulating RBCs in large numbers leading to an episode of 'hemolytic' anemia. Common triggers include oxidant chemicals such as the anti-malaria drug, primaquine or the anti-leprosy drug, dapsone. This is why doctors are careful while prescribing such drugs and may order G6PD estimation beforehand. Treatment is to stop the offending drug and support the patient through the crisis. Blood transfusion may be necessary in severe cases.

Genetic deficiency of pyruvate kinase, the key enzyme in glycolysis is also known to occur as an autosomal recessive disorder. ATP generation is reduced and the red cells become stiff and susceptible to breakdown in the minute blood channels of the spleen. Periodic blood transfusion may be required. Surgical removal of the spleen may also have to be considered to reduce transfusion requirements.
The life of the red blood cell is committed to transport of respiratory gases and it is for this reason that it is endowed with hemoglobin. Mature cells can pack hemoglobin up to a concentration of 34 g/dl which means that there is about $30 \times 10^{-12}$ g (30 picograms) of hemoglobin per cell. In the normal state, the quantity of hemoglobin is near this maximum. In anemia, the hemoglobin concentration in blood falls from the normal of about 14.5 g/dl. This can occur if red cells are produced in small numbers, or are destroyed or lost from circulation in large numbers, or are packed with less than the normal quantity of hemoglobin. In our country the commonest cause of anemia is iron deficiency, in which there is insufficient iron in the body to permit synthesis of adequate hemoglobin.

Hemoglobin transports oxygen from the lungs to the tissues. Free hemoglobin in the bloodstream cannot discharge this function effectively, for it is apt to leak out of capillaries into the tissues or is filtered out by the kidneys into urine. Inside the packaging of the red cell, however, hemoglobin can transport about 20 ml of oxygen per decilitre of blood. Researchers have tried hard to come up with artificial blood substitutes based upon chemically modified hemoglobins that will not be lost from circulation quickly, or by packaging hemoglobin inside artificial membrane envelopes. Unfortunately, these efforts are yet to pay rich dividends.

The hemoglobin molecule is complex and interesting. It has a 'globin' (protein) part comprising four polypeptide chains, each of which carries a molecule of the porphyrin pigment 'heme' (Figure 4). Each heme molecule contains a ferrous ($\text{Fe}^{2+}$) ion that can reversibly associate with one molecule of oxygen. ATP generated by red cell metabolism helps to keep the iron in the ferrous state. If the iron gets oxidized to ferric state, the hemoglobin molecule is converted to methemoglobin, which will not carry oxygen. Although heme forms the oxygen carrier part of the hemoglobin molecule, the nature of the polypeptide chain is crucial for the function of the hemoglobin molecule.
Unbalanced chain synthesis leads to reduced hemoglobinization of the red cells, reducing their life span, restricting their oxygen transport capacity and leading to anemia.

chains determines the binding affinity for oxygen. In the normal adult variety of hemoglobin, called HbA, there are 2 alpha and 2 beta chains i.e. HbA is $\alpha_2\beta_2$. Fetal hemoglobin, HbF, is $\alpha_2\gamma_2$. The gamma chains impart a higher oxygen carrying capacity to fetal blood. This is essential since fetal tissues are more vulnerable to inadequate oxygen supply. The switch-over from fetal to adult hemoglobin occurs in the first 3 to 6 months of life. The individual polypeptide chains in hemoglobin interact with each other to facilitate off-loading of the oxygen (that has associated with hemoglobin in the pulmonary circulation) at the lower oxygen concentration of the tissues. The metabolite 2,3-diphosphoglycerate (2,3-DPG), generated in an offshoot (Rapoport–Luebering shunt) of the glycolysis pathway, has an important facilitator role in this oxygen release process.

Numerous abnormalities of the globin chains, giving rise to different ‘hemoglobinopathies’, have been described. In the thalassemias, a common group of disorders in India, synthesis of the $\alpha$ or $\beta$ globin chains is genetically defective. Unbalanced chain synthesis leads to reduced hemoglobinization of the red cells, reducing their life span, restricting their oxygen transport capacity and leading to anemia. In $\beta$-thalassemia major, the $\beta$ chain is not synthesized, leading to a severe anemia that becomes apparent at around the time when the level of HbF in blood declines. Regular blood transfusions are necessary to sustain life. This brings its own problems such as that of iron overloading. In sickle-cell anemia, which is uncommon in Asians, an abnormal type of hemoglobin, HbS, is produced due to mutated $\beta$-chain synthesis. HbS tends to polymerize at low oxygen saturation, distorting the affected red cells into a rigid elongated sickle shape that makes passage through small blood capillaries difficult and increases the likelihood of rupture. Clinical manifestations of anemia and blocked capillaries follow.

RBCs discharge some other functions. They contain large quantities of carbonic anhydrase, an enzyme which tremen-
dously speeds up the reaction between carbon dioxide and water to generate carbonic acid. The latter dissociates spontaneously into $\text{H}^+$ and $\text{HCO}_3^-$ ions. The process is reversed in the capillaries of the lungs and the carbon dioxide diffuses out. Large quantities of carbon dioxide can thus be transported from the tissues to the lungs in the form of bicarbonate. Hemoglobin in red cells also has excellent buffering capacity, helping to preserve the acid-base balance of the body. Indeed, almost 50% of the buffering capacity of blood comes from hemoglobin.

Incidentally, blood groups are defined on the basis of antigens carried by RBCs. The two major blood group systems are the ABO and Rh systems. Incompatibility in these groups between blood donor and recipient can lead to destruction of donor RBCs in the bloodstream of the recipient creating several problems. Blood grouping is a simple laboratory procedure and everyone should know his or her blood group. The knowledge may come in handy during an emergency.

**Senility and Demise**

On an average RBCs circulate in blood for 120 days after leaving the bone marrow. Beyond this period, the rate of glycolysis declines, correspondingly lowering ATP levels in the cell. This leads to other signs of aging such as reduced pliability of the cell membrane, progressive disruption of transmembrane ion transport, swelling of the cell, oxidative denaturation of cellular proteins and failure to keep the hemoglobin in the Fe$^{2+}$ rather than in the Fe$^{3+}$ state. There is also reduced quantity of membrane lipids. Once the membrane becomes stiff and fragile, the cell is liable to rupture in some tight spot in the circulation. This mostly occurs in the spleen when the 8 μm cells try to squeeze through the 3 to 5 μm channels in the red pulp of the organ.

The hemoglobin released from ruptured RBCs is quickly engulfed by macrophage cells of the body, particularly in the liver, spleen and bone marrow. The globin part of the molecule is broken down to release amino acids which are recycled. The
Iron is released back into the blood and is carried by transferrin, the iron transport protein of the body, to the bone marrow where it is used up for the production of new erythrocytes, or to the liver and other tissues for storage in combination with ferritin, the iron storage protein. The heme is metabolized in stages to the yellow bile pigment, bilirubin. Bilirubin is released into the blood. A part of this bilirubin is taken up by liver cells, coupled with glucoronic acid to make it more water soluble (a process called bilirubin conjugation), and then secreted in bile. Bile is stored in the gall bladder. Upon bile release into the intestine, the bilirubin diglucoronide is converted by the action of bacteria in the gut to urobilinogen, and then to stercobilinogen and stercobilin. The latter pigments impart the normal yellow color to stools. A part of the urobilinogen is absorbed from the intestines and is later excreted by the kidneys as urobilinogen and its oxidation product urobilin. These processes are summarized in Figure 5.

**Figure 5. Bilirubin metabolism pathways.**
Increasing levels of bilirubin in blood imparts a yellow color to the eyes and later to the entire skin. This is the familiar disorder called jaundice. There are different types of jaundice depending upon whether excess RBCs are being prematurely destroyed in the circulation or bilirubin excretion is being hampered inside or outside the liver. Recognizing the type of jaundice is important as treatment must be tailored accordingly. But that’s another story.

Please Note
Resonance, Vol.5, No.5, 2000

Title: Evolution of the Atmosphere and Oceans: Evidence from Geological Records by P V Sukumaran

Page 10, line 2, 20 to 30% should be read as 20 to 30 \%\%
Page 12, line 28, (13C=+15\%) should read as (\delta^{13}C=+15\%)
Page 13, line 1, 51 \pm 10\% should read as 51 \pm 10 \%
Page 13, line 4, \sim 55\% should read as \sim 55\%\%
Page 13, Box 3 was wrongly inserted, please see the correct one below.

Box 3. Events Related to Atmospheric Evolution

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