

BLOOD

Blood functions and properties

Blood is a fluid tissue, vital vehicle of communication between the tissues and it has very important functions. It transports oxygen from the lungs to the body tissues and carbon dioxide from the tissues to the lungs. It transports nutritive substances and metabolites to the tissues and removes waste products to the kidneys and other organs of excretion. It has an essential role in the maintenance of fluid balance. The blood distributes hormones from the endocrine glands to the organs they influence. It also helps regulate body temperature by carrying excess heat from the interior of the body to the surface layers of the skin, where the heat is dissipated to the surrounding air. Other functions are: immunological role because of intervention of leucocytes; intervention in stopping hemorrhage by intervention of platelets.

Blood consists of a fluid part called plasma in which is suspended formed elements: red cells or erythrocytes, white cells or leukocytes and platelets or thrombocytes. Plasma accounts for about 55 per cent of the volume and the formed elements account for about 45 per cent. The plasma exchanges substances continuously with the interstitial fluid through the pores of the capillary membranes. These pores are highly permeable to almost all solutes in the extracellular fluid except the proteins. Therefore, the extracellular fluids are constantly mixing, so that the plasma and interstitial fluids have about the same composition except for proteins, which have a higher concentration in the plasma.

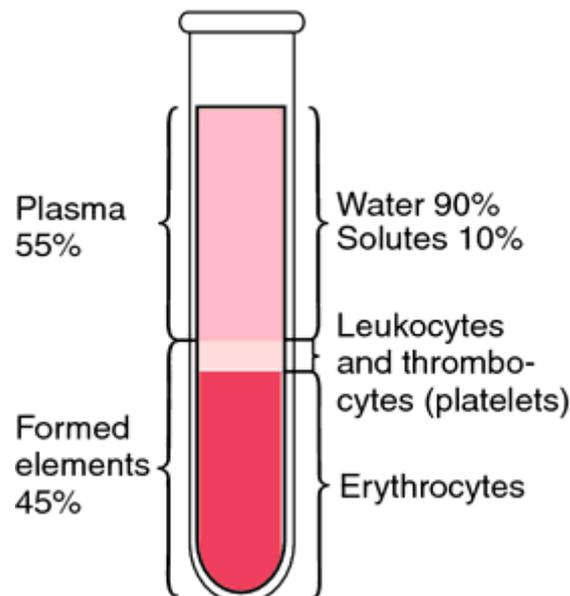


Figure 1 Separation of blood into cells and plasma

The properties of the blood

1. **The blood volume** is especially important in the control of cardiovascular dynamics.

The normal total circulating blood volume of adults is about 8 per cent of body weight (about 5 liters in a 70 kg man). At any one time, assuming a blood volume of 5 liters, about 1 liter will be in the lungs, about 3 liters in the systemic venous circulation and the remaining liter in the heart, systemic arteries, arterioles and capillaries.

The measurement of blood volume can be done by the dilution of specific markers. For a marker to permit the accurate measurement of the volume of a particular compartment it must

be evenly distributed throughout that compartment and it should not be metabolized or alter any physiological variable.

To measure plasma volume, a substance must be used that does not readily penetrate capillary membranes but remains in the vascular system after injection. One of the most commonly used substances for measuring plasma volume is serum albumin labeled with radioactive iodine (^{125}I -albumin) or it can use dyes that avidly bind to the plasma proteins, such as Evans blue dye (also called T-1824). As the amount of a marker that was injected is known, it is a simple matter to calculate the volume in which it has been diluted.

Example: to a patient with a body weight of 70 kg was injected with 100 mg solution of the dye. Further assume that sample of blood was taken after 10 min and the plasma was found to contain 0,037 mg/ml of dye. The plasma volume is:

$$\text{Concentration} = \text{amount of dye/volume} \rightarrow \text{volume} = \text{amount of dye/concentration}$$

$$\text{Plasma volume} = 100/0,037 = 2702 \text{ ml}$$

In practice some dye is lost from the circulation and the corrections for the lost dye need to be applied to improve the accuracy of the estimate. After allowing sufficient time for equilibration, the volume of a fluid compartment to a first approximation is given by:

$$\text{Volume} = \frac{\text{Amount of marker infused} - \text{Amount excreted}}{\text{Concentration in plasma}}$$

If one measures plasma volume using the methods described earlier, blood volume can also be calculated if one knows the hematocrit using the following equation:

$$\text{Total blood volume} = \frac{\text{Plasma volume}}{1 - \text{Hematocrit}}$$

Another way to measure blood volume is to inject into the circulation red blood cells that have been labeled with radioactive material. After these mix in the circulation, the radioactivity of a mixed blood sample can be measured, and the total blood volume can be calculated using the dilution principle. A substance frequently used to label the red blood cells is radioactive chromium (^{51}Cr), which binds tightly with the red blood cells.

Physiological and pathological variations

A. Physiological:

- Increased in males, in pregnancy by 20-30% due to plasma volume, trained personnel, altitude, massive fluid ingestion (transient), heat exposure,
- Falls in the elderly, dehydration, the transition from supine to standing, obese people

B. Pathology:

- Increase in polycythemia
- Decreases after hemorrhage and shock.

Regulation of blood volume

Despite varying amounts of liquid ingestion, blood volume remains constant. Adjustment is done differently for globular volume or plasma volume. Plasma volume adjustment is done by reflex and humoral mechanisms.

1 reflex mechanism. There are volume receptors in the left atria, sensitive for changes in blood volume; when blood volume increased → atria distension → impulses to nc. solitary tract (brainstem) by n. vagi → anterior hypothalamus reflex reduction in ADH secretion → eliminates excess water through urine restoring blood volume. Plasma volume expansion influences cardioaortici sinocarotidieni baroreceptors: increased cardiac output and blood pressure excites baroreceptors triggering a reflex similar to the one of stimulation of volume receptors. Blood volume reduction → decreased blood pressure → reflex vasoconstrictor of renal vessels with glomerular filtration decreased → fluid retention in the body. Reflexes initiated by volume receptors bring the blood volume to normal in about 1 h, but they adapt completely within 1-3 days.

2 Humoral regulation involve: ADH

Aldosterone

Atrial natriuretic factor

Plasma proteins

ADH is a hormone secreted by the anterior hipotalamus and stored in posterior pituitary. Secretion is controlled by reflex mechanisms and by the value of osmotic pressure of blood: increase of osmotic pressure → stimulates ADH secretion → retains water in the body.

Aldosterone is a hormone secreted by adrenal glands. It stimulates reabsorption of Na^+ and Cl^- followed by osmotic water reabsorption in renal tubules leading to reduced urine volume.

Atrial natriuretic factor is a hormone secreted in the walls of the two atria (especially right atria). When blood volume increased → atria distension → FNA release → increases Na^+ excretion by the kidneys → reduced blood volume.

Plasma proteins retain water in tree circulator because of their colloid osmotic pressure. When protein levels are below 5.5 g% water passes in the interstitial space causing edema.

Adjustment of the corpuscular volume is made according to the degree of tissue oxygenation: hypoxia (low concentration of O_2 at tissue level) stimulates erythropoiesis leading to increased globular volume. Reduction of globular volume occurs when tissues needs in O_2 decrease, eg hypothyroidism.

2. The colour is red in arteries and darker red in veins, the explanation being the oxidation or reduction of haemoglobin.

3. ph 7,35-7,45

4. Normal density of the blood is between 1,050 and 1,060; the blood density is given by the blood cells, the organic substances, especially the proteins, the mineral substances dissolved in plasma. As the mineral substances present in plasma are maintained at strictly constant level, the variations of the plasma density are dependent mainly on: the number of the blood cells (hematocrit); the concentration of the proteins (proteinemia); the concentration of the hemoglobin. Plasma density = 1.024 - 1.028 (average 1.027); deproteinated plasma density = 1.006

Physiological variations of density of blood

1. Increased blood density: perspiration (loss of water); during exercise (the spleen contracts); altitude (increased nr. of erythrocytes)
2. Decreased blood density occurs in the period of pregnancy (the plasma volume increases with 20-30%), after massive ingestion of liquids.

Pathological variations

1. Increased values: diarrhea, vomiting, polycythemia (Vera and secondary polycythemia) -shock (hemoconcentration)
2. Decreased values: anemia, hemorrhage (bleeding).
4. **Viscosity of plasma** depend on these factors: number and size of RBCs, their load in HGB; number and size of WBC; number and size of PLT.
5. **Clotting** occurs when blood is taken out of the vessels that circulate blood normal. Coagulation represents the transformation from liquid to solid, gelatinous dish in the form in which it is. After 2-3 hours early clotting table blood is divided into two parts: a solid red and called clot and some liquid, transparent and slightly yellowish serum called serum. Serum has essentially the same composition as plasma except that its fibrinogen and clotting factors II, V, and VIII have been removed and it has a higher serotonin content because of the breakdown of platelets during clotting.
6. **Osmotic pressure** is the pressure which needs to be applied to a solution to prevent the inward flow of water across a semipermeable membrane or the minimum pressure needed to nullify osmosis. Depends on the number of Brownian particles moving. When the osmotic pressure of the solution outside the blood cells is higher than the osmotic pressure inside the red blood cells, the solution is hypertonic. The water inside the blood cells exits the cells in an attempt to equalize the osmotic pressure, causing the cells to shrink. When the osmotic pressure outside the red blood cells is the same as the pressure inside the cells, the solution is isotonic with respect to the cytoplasm and this is the usual condition of red blood cells in plasma. When the solution outside of the red blood cells has a lower osmotic pressure than the cytoplasm of the red blood cells, the solution is hypotonic with respect to the cells. The cells take in water in an attempt to equalize the osmotic pressure, causing them to swell and potentially burst. Normal values 290-300 mOsm/l; solution NaCl 0.9 g% is isotonic.
7. **Oncotic pressure**, or **colloid osmotic pressure**, is a form of osmotic pressure exerted by proteins in blood plasma that usually tends to pull water into the circulatory system. Because large plasma proteins cannot easily cross through the capillary walls, their effect on the osmotic pressure of the capillary interiors will, to some extent, balance out the tendency for fluid to leak out of the capillaries. In other words, the oncotic pressure tends to pull fluid into the capillaries. In conditions where plasma proteins are reduced, e.g. from being lost in the urine (proteinuria) or from malnutrition, there will be a reduction in oncotic pressure and an increase in filtration across the capillary, resulting in excess fluid buildup in the tissues (edema). The large majority of oncotic pressure in capillaries is generated by the presence of high quantities of albumin which constitute approximately 80% of the total oncotic pressure exerted by blood plasma on interstitial fluid. The total oncotic pressure of an average capillary is about 25 mmHg with albumin contributing approximately 22 mmHg of this oncotic pressure. Because blood proteins cannot escape through capillary endothelium, oncotic pressure of capillary beds tends to draw water into the vessels. It plays an important role in capillary exchange processes: the hydrostatic pressure (35 mmHg) in the capillary pushes water and micro molecules in interstitial spaces by a value of 10 mmHg (the difference between hydrostatic pressure and colloid osmotic pressure) and in capillary venous hydrostatic pressure is only 15 mmHg, so the water goes from interstitial spaces into vessels (resorption).

About 60 percent of the blood is plasma and 40 per cent is red blood cells, but these percentages can vary considerably in different people, depending on gender, weight, and other factors. The hematocrit ratio (or the packed cell volume) describes the proportion of the total blood volume occupied by the erythrocytes. If we centrifuge a sample of whole blood in a test tube for a short time at low speed, the heavier red cells are packed at the bottom of the tube while the plasma can be seen as a clear pale yellow fluid above them. A thin layer of white cells and platelets separates the packed red cells from the plasma. It is impossible to completely pack the red cells together; therefore, about 3 to 4 per cent of the plasma remains entrapped among the cells, and the true hematocrit is only about 96 per cent of the measured hematocrit.

In men, the measured hematocrit is normally about 40 percent and in women, it is about 36. In severe anemia, the hematocrit may fall as low as 10% a value that is barely sufficient to sustain life. Conversely, there are some conditions in which there is excessive production of red blood cells, resulting in polycythemia. In these conditions, the hematocrit can rise to 65%.

Plasma and its balances: hydro-electrolytic, acid base. Composition of plasma. Physiology of plasma proteins

The total blood volume and the plasma volume may be measured using the dilution techniques. Normal adults have 35-45 ml of plasma per kilogram body weight (or 4% of body weight); plasma volume is 2,8-3 liters in men and 2,4 liters in women.

Because the plasma and interstitial fluid are separated only by highly permeable capillary membranes, their ionic composition is similar. The most important difference between these two compartments is the higher concentration of protein in the plasma; because the capillaries have a low permeability to the plasma proteins, only small amounts of proteins are leaked into the interstitial spaces in most tissues. Because of the Donnan effect, the concentration of positively charged ions (cations) is slightly greater (about 2 per cent) in the plasma than in the interstitial fluid. The plasma proteins have a net negative charge and, therefore, tend to bind cations, such as sodium and potassium ions, thus holding extra amounts of these cations in the plasma along with the plasma proteins. Conversely, negatively charged ions (anions) tend to have a slightly higher concentration in the interstitial fluid compared with the plasma, because the negative charges of the plasma proteins repel the negatively charged anions. However, the concentration of ions in the interstitial fluid and in the plasma is considered to be about equal. The plasma and the interstitial fluid, contains large amounts of sodium and chloride ions, reasonably large amounts of bicarbonate ions, but only small quantities of potassium, calcium, magnesium, phosphate, and organic acid ions. The composition of extracellular fluid is carefully regulated by various mechanisms, but especially by the kidneys. This allows the cells to remain continually bathed in a fluid that contains the proper concentration of electrolytes and nutrients for optimal cell function.

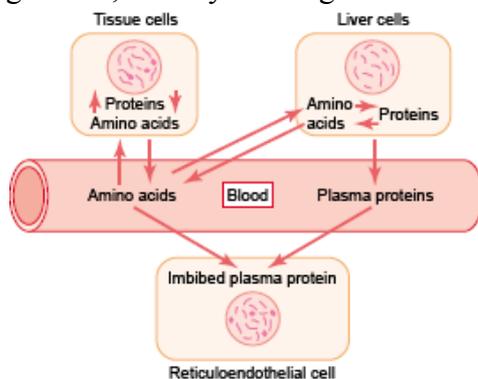
The plasma consists of 95% water and the remaining 5% being made up by a variety of substances in solution and suspension. These include mineral ions (e.g. sodium, potassium, calcium, chloride), small organic molecules (glucose, aminoacids, fatty acids) and plasma proteins. The major constituents of the plasma, proteins and inorganic ions are normally present at roughly constant levels. The chief inorganic cation of plasma is sodium and chloride is the principal anion of the plasma. Sodium has a concentration of 140-145 mmoles/l and chloride around to 100 mmoles/l; electroneutrality is achieved by the presence of other anions like bicarbonate, phosphate, sulphate, proteins. The ionic components of the plasma maintain both its osmolality (280-300 mOsm per kg water) and its pH(7,35-7,45) within physiological limits.

Table 1

Constituent	Concentration	Observation
Water	94,5 g/l	
Ions:		
Bicarbonate	25 mmol/l	Carriage of CO ₂ and H ⁺ buffering
Chloride	105 mmol/l	Principal extracellular anion
Inorganic phosphate	1,1 mmol/l	
Calcium total	2,5 mmol/l	Ionized calcium is about 1,5 mmol/l
Magnesium	0,8 mmol/l	
Potassium	4 mmol/l	
Sodium	144 mmol/l	Principal extracellular cation
Hydrogen ions	40 mmol/l	Correspond to a pH 7,4
Organic molecules:		
Glucose	4,5 mmol/l	
Cholesterol	2 g/l	
Fatty acids(total)	3 g/l	
Protein (total)	65-85 g/l	

Plasma proteins

The plasma proteins consist of albumin, globulin, and fibrinogen fractions and the globulin fraction is subdivided into numerous components. One classification divides it into $\alpha_1, \alpha_2, \beta$ and γ globulins and fibrinogen. A major function of albumin is to provide colloid osmotic pressure in the plasma, which prevents plasma loss from the capillaries. The capillary walls are relatively impermeable to the proteins in plasma, and the proteins exert an osmotic force of about 25 mm Hg across the capillary wall (oncotic pressure) into the blood. The plasma proteins are responsible for 15% of the buffering capacity of the blood, because of the weak ionization of their substituent COOH and NH₂ groups. At the normal pH of 7.40, the proteins are mostly anionic form and constitute a significant part of the anionic complement of plasma. The globulins perform a number of enzymatic functions in the plasma, but equally important, they are principally responsible for the body's both natural and acquired immunity against invading organisms. Fibrinogen polymerizes into long fibrin threads during blood coagulation, thereby forming blood clots that help repair leaks in the circulatory system.



Most of the plasma proteins are synthesized in the liver; other are produced in the plasma cells (leukocytes). These principal functions are listed in Table 2. There is a constant state of equilibrium, as shown in Figure 2, among the plasma proteins, the amino acids of the plasma, and the tissue proteins. It has been estimated that normally about 400 grams of body protein are synthesized and degraded each day as part of the continual state of flux of amino acids.

Figure 2 Equilibrium among tissue and plasma proteins (after Guyton)

This demonstrates the general principle of reversible exchange of amino acids among the different proteins of the body. Even during starvation or severe debilitating diseases, the

ratio of total tissue proteins to total plasma proteins in the body remains relatively constant at about 33:1.

Table 2

Fraction	Principal function	Serum/plasma concentration
Albumin	Binding and carrier protein; osmotic regulation	4500-5000 mg/dl
Orosomuroid	inflammation	
Alfa 1 antiprotease	General protease inhibitor in serum and tissue secretions	1,3-1,4 mg/dl
Alfa fetoprotein	Osmotic regulation	Normally in fetal blood
Alfa2 macroglobulin	Inhibitor of serum endoproteases	150-420 mg/dl
Antithrombin III	Protease inhibitor of intrinsic coagulation system	17-30 mg/dl
Ceruloplasmin	Transport of copper	15-60 mg/dl
C-reactive protein	inflammation	< 1 mg/dl
Fibrinogen	Precursor to fibrin	200-450 mg/dl
Haptoglobin	Binding and transport of cell-free hemoglobin	40-180 mg/dl
Hemopexin	Binding of porphyrins	50-100 mg/dl
Transferrin	Transport of iron	3-6,5 mg/dl
Angiotensinogen	Precursor of angiotensin II	
Apolipoprotein B	Lipoprotein particles	
Proteins coagulation factors: II, VII, IX, X	Blood clotting	20 mg/dl
Antithrombin C, protein C	Inhibition of blood clotting	
Insulin-like growth factor I	Mediator of some hormonal effects	
Steroid hormone binding globulin	Carrier for steroids	3,3 mg/dl
Thyroid binding prealbumin	Carrier for thyroid hormone	25 mg/dl
Thyroid binding globulin	Carrier for thyroid hormone	1,5 mg/dl

The total amount of protein in plasma is called proteinemia whose normal values are between 6.5 -8 g%. Proteinemia that is more than 8 g% is called hyperproteinemia and when it is less than 6 g% is called hypoproteinaemia. Plasma protein levels are maintained during starvation until body protein stores are markedly depleted. In prolonged starvation, malabsorption syndrome due to intestinal diseases, liver disease, nephrosis plasma protein levels are low. The consequence is decrease in the plasma oncotic pressure and edema tends to develop.

Acid-Base Balance

Precise H^+ regulation is essential because the activities of almost all enzyme systems in the body are influenced by H^+ concentration. Therefore, changes in hydrogen concentration alter virtually all cell and body functions. Compared with other ions, the H^+ concentration of the body fluids normally is kept at a low level. To achieve homeostasis, there must be a balance between the intake or production of H^+ and the net removal of H^+ from the body.

Molecules containing hydrogen atoms that can release hydrogen ions in solutions are referred to as acids. An example is hydrochloric acid (HCl), which ionizes in water to form hydrogen ions (H^+) and chloride ions (Cl^-). Likewise, carbonic acid (H_2CO_3) ionizes in water to form H^+ and bicarbonate ions (HCO_3^-). A base is an ion or a molecule that can accept an H^+ . For example,



The proteins in the body function as bases, because some of the amino acids that make up proteins have net negative charges that readily accept H^+ . The protein hemoglobin (dissociation of the imidazole groups of the histidine residues) in the red blood cells and proteins in the other cells of the body are among the most important of the body's bases.

A strong acid is one that rapidly dissociates and releases especially large amounts of H^+ in solution, like HCl. Weak acids have less tendency to dissociate their ions and, therefore, release H^+ with less vigor. An example is H_2CO_3 . A strong base is one that reacts rapidly and strongly with H^+ and, therefore, quickly removes these from a solution. A typical example is OH^- , which reacts with H^+ to form water. A typical weak base is HCO_3^- because it binds with H^+ much more weakly than does OH^- . Most of the acids and bases in the extracellular fluid that are involved in normal acid-base regulation are weak acids and bases. The most important ones are H_2CO_3 and bicarbonate base.

The blood H^+ concentration is normally maintained within tight limits around a normal value of about 40 nEq/L. Normal variations are only about 3 to 5 nEq/L, but under extreme conditions, the H^+ concentration can vary from as low as 10 nEq/L to as high as 160 nEq/L without causing death. Because H^+ concentration normally is low, and because these small numbers are cumbersome, it is customary to express H^+ concentration on a logarithm scale, using pH units. pH is related to the actual H^+ concentration by the following formula

$$pH = -\log[H^+] \quad [H^+] \text{ is } H^+ \text{ concentration expressed in equivalents per liter}$$

From this formula, one can see that pH is inversely related to the H^+ concentration; therefore, a low pH corresponds to a high H^+ concentration, and a high pH corresponds to a low H^+ concentration. The normal pH of arterial blood is 7.4, whereas the pH of venous blood and interstitial fluids is about 7.35 because of the extra amounts of carbon dioxide (CO_2) released from the tissues to form H_2CO_3 in these fluids (Table 3).

Table 3

	pH
Extracellular fluid	
Arterial blood	7.40
Venous blood	7.35
Interstitial fluid	7.35
Intracellular fluid	6-7.4
Urine	4.5-8
Gastric HCl	0.8

Because the normal pH of arterial blood is 7.4, a person is considered to have acidosis when the pH falls below this value and to have alkalosis when the pH rises above 7.4. The lower limit of pH at which a person can live more than a few hours is about 6.8, and the upper limit is about 8.0. Intracellular pH usually is slightly lower than plasma pH because the metabolism of the cells produces acid, especially H_2CO_3 . Depending on the type of cells, the pH of intracellular fluid has been estimated to range between 6.0 and 7.4. Hypoxia of the tissues and poor blood flow to the tissues can cause acid accumulation and decreased intracellular pH.

There are two primary systems that regulate the H^+ concentration in the body fluids:

(1) the chemical acid-base buffer systems of the body fluids, which immediately combine with acid or base to prevent excessive changes in H^+ concentration;

(2) the biological systems: respiratory center, which regulates the removal of CO_2 (and, therefore, H_2CO_3) from the extracellular fluid; acts within a few minutes to eliminate CO_2 and, therefore, H_2CO_3 from the body; the kidneys, which can excrete either acid or alkaline urine, thereby readjusting the extracellular fluid H^+ concentration toward normal during acidosis or alkalosis. Although the kidneys are relatively slow to respond compared with the other defenses, over a period of hours to several days, they are by far the most powerful of the acid-base regulatory systems.

Buffer systems

A buffer is any substance that can reversibly bind H^+ . The general form of the buffering reaction is:



A free H^+ combines with the buffer to form a weak acid (H buffer) that can either remain as an unassociated molecule or dissociate back to buffer and H^+ . When the H^+ concentration increases, the reaction is forced to the right, and more H^+ binds to the buffer, as long as buffer is available. Conversely, when the H^+ concentration decreases, the reaction shifts toward the left, and H^+ is released from the buffer. In this way, changes in H^+ concentration are minimized. The action of acid-base buffers can perhaps best be explained by considering the buffer system that is quantitatively the most important in the extracellular fluid—the bicarbonate buffer system.

The bicarbonate buffer system consists of a water solution that contains two ingredients: a weak acid, H_2CO_3 , and a bicarbonate salt, such as NaHCO_3 . H_2CO_3 is formed in the body by the reaction of CO_2 with H_2O .



This reaction is slow, and exceedingly small amounts of H_2CO_3 are formed unless the enzyme carbonic anhydrase is present. There is no carbonic anhydrase in plasma, but there is an abundant supply in red blood cells. It is also found in high concentration in gastric acid-secreting cells, abundant in the walls of the lung alveoli and in renal tubular cells. Carbonic anhydrase is a protein with a molecular weight of 30,000 that contains an atom of zinc in each molecule. The sulfonamides also inhibit this enzyme, and sulfonamide derivatives have been used clinically as diuretics because of their inhibitory effects on carbonic anhydrase in the kidney.

The second component of the system, bicarbonate salt, occurs predominantly as sodium bicarbonate (NaHCO_3) in the extracellular fluid. NaHCO_3 ionizes almost completely to form HCO_3^- and Na^+ , as follows:

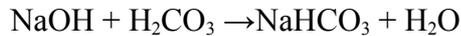


When a strong acid such as HCl is added to the bicarbonate buffer solution, the increased H^+ released from the acid ($\text{HCl} \rightarrow \text{H}^+ + \text{Cl}^-$) is buffered by HCO_3^-



As a result, more H_2CO_3 is formed, causing increased CO_2 and H_2O production. The excess CO_2 greatly stimulates respiration, which eliminates the CO_2 from the extracellular fluid.

When a strong base, such as sodium hydroxide (NaOH), is added to the bicarbonate buffer solution:



The weak base NaHCO_3 replaces the strong base NaOH. At the same time, the concentration of H_2CO_3 decreases causing more CO_2 to combine with H_2O to replace the H_2CO_3 . The net result, therefore, is a tendency for the CO_2 levels in the blood to decrease, but the decreased CO_2 in the blood inhibits respiration and decreases the rate of CO_2 expiration. The rise in blood HCO_3^- that occurs is compensated for by increased renal excretion of HCO_3^- .

All acids, including H_2CO_3 , are ionized to some extent. For any acid, the concentration of the acid relative to its dissociated ions is defined by the dissociation constant K.

$$K = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \rightarrow [\text{H}^+] = K \times \frac{[\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-]}$$

The concentration of undissociated H_2CO_3 cannot be measured in solution because it rapidly dissociates into CO_2 and H_2O or to H^+ and HCO_3^- . Most clinical laboratories measure the blood CO_2 pressure (P_{CO_2}) rather than the actual amount of CO_2 . The amount of CO_2 in the blood is a linear function of P_{CO_2} times the solubility coefficient for CO_2 ; under physiologic conditions, the solubility coefficient for CO_2 is 0.03 mmol/mm Hg at body temperature. This means that 0.03 millimole of H_2CO_3 is present in the blood for each millimeter of mercury P_{CO_2} measured. However, the CO_2 dissolved in the blood is directly proportional to the amount of undissociated H_2CO_3 .

$$[\text{H}_2\text{CO}_3] = 0.03 \times P_{\text{CO}_2}$$

The dissolved carbon dioxide in the blood reacts with water to form carbonic acid. This reaction would occur much too slowly to be of importance were it not for the fact that inside the red blood cells is a protein enzyme called carbonic anhydrase, which catalyzes the reaction between carbon dioxide and water and accelerates its reaction rate about 5000-fold. The reaction occurs so rapidly in the red blood cells that it reaches almost complete equilibrium within a very small fraction of a second and this allows tremendous amounts of carbon dioxide to react with the red blood cell water even before the blood leaves the tissue capillaries. In another fraction of a second, the carbonic acid formed in the red cells (H_2CO_3) dissociates into hydrogen and bicarbonate ions (H^+ and HCO_3^-). Most of the hydrogen ions then combine with the hemoglobin in the red blood cells, because the hemoglobin protein is a powerful acid-base buffer. In turn, many of the bicarbonate ions diffuse from the red cells into the plasma, while chloride ions diffuse into the red cells to take their place. This is made possible by the presence of a special bicarbonate-chloride carrier protein in the red cell membrane that shuttles these two ions in opposite directions at rapid velocities. Thus, the chloride content of venous red blood cells is greater than that of arterial red cells, a phenomenon called the chloride shift. The reversible combination of carbon dioxide with water in the red blood cells under the influence of carbonic anhydrase accounts for about 70 per cent of the carbon dioxide transported from the tissues to the lungs.

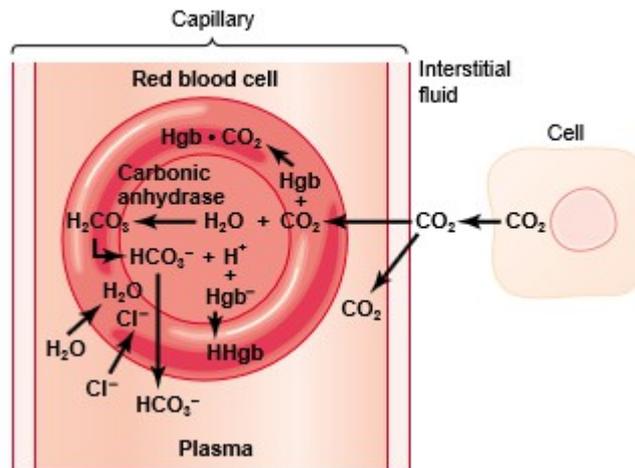


Figure 3 Membrane phenomenon Hamburger

The bicarbonate concentration is regulated mainly by the kidneys, whereas the P_{CO_2} in extracellular fluid is controlled by the rate of respiration. By increasing the rate of respiration, the lungs remove CO_2 from the plasma, and by decreasing respiration, the lungs elevate P_{CO_2} . Normal physiologic acid-base homeostasis results from the coordinated efforts of both of these organs, the lungs and the kidneys, and acid-base disorders occur when one or both of these control mechanisms are impaired.

Henderson-Hasselbalch Equation

The Henderson-Hasselbalch equation, in addition to defining the determinants of normal pH regulation and acid-base balance in the extracellular fluid, provides insight into the physiologic control of acid and base composition of the extracellular fluid.

$$pH = pK + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

From the Henderson-Hasselbalch equation, it is apparent that an increase in HCO_3^- concentration causes the pH to rise, shifting the acid-base balance toward alkalosis. An increase in P_{CO_2} causes the pH to decrease, shifting the acid-base balance toward acidosis.

When disturbances of acid-base balance result from a primary change in extracellular fluid bicarbonate concentration, they are referred to as metabolic acid-base disorders. Therefore, acidosis caused by a primary decrease in bicarbonate concentration is termed metabolic acidosis, whereas alkalosis caused by a primary increase in bicarbonate concentration is called metabolic alkalosis. Acidosis caused by an increase in P_{CO_2} is called respiratory acidosis, whereas alkalosis caused by a decrease in P_{CO_2} is termed respiratory alkalosis.

When the two components of the buffer system are equal, the pH of the solution is the same as the pK (6.1) of the bicarbonate buffer system. When base is added to the system, part of the dissolved CO_2 is converted into HCO_3^- , causing an increase in the ratio of HCO_3^- to CO_2 and increasing the pH, as is evident from the Henderson-Hasselbalch equation. When acid is added, it is buffered by HCO_3^- , which is then converted into dissolved CO_2 , decreasing the ratio of HCO_3^- to CO_2 and decreasing the pH of the extracellular fluid.

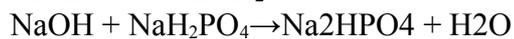
The bicarbonate buffer system is the most powerful extracellular buffer in the body. This apparent paradox is due mainly to the fact that the two elements of the buffer system, HCO_3^- and CO_2 , are regulated, respectively, by the kidneys and the lungs. As a result of this

regulation, the pH of the extracellular fluid can be precisely controlled by the relative rate of removal and addition of HCO_3^- by the kidneys and the rate of removal of CO_2 by the lungs.

Although the phosphate buffer system is not important as an extracellular fluid buffer, it plays a major role in buffering renal tubular fluid and intracellular fluids. The main elements of the phosphate buffer system are H_2PO_4^- and HPO_4^{2-} . When a strong acid such as HCl is added to a mixture of these two substances, the hydrogen is accepted by the base HPO_4^{2-} and converted to H_2PO_4^- .



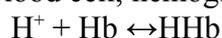
The result of this reaction is that the strong acid, HCl, is replaced by an additional amount of a weak acid, NaH_2PO_4 , and the decrease in pH is minimized. When a strong base, such as NaOH, is added to the buffer system, the OH^- is buffered by the H_2PO_4^- to form additional amounts of HPO_4^{2-} and H_2O .



In this case, a strong base, NaOH, is traded for a weak base, NaH_2PO_4 , causing only a slight increase in pH. The phosphate buffer system has a pK of 6.8, which is not far from the normal pH of 7.4 in the body fluids; this allows the system to operate near its maximum buffering power. However, its concentration in the extracellular fluid is low, only about 8 per cent of the concentration of the bicarbonate buffer. The phosphate buffer system is also important in buffering intracellular fluid because the concentration of phosphate in this fluid is many times that in the extracellular fluid. Also, the pH of intracellular fluid is lower than that of extracellular fluid and therefore is usually closer to the pK of the phosphate buffer system compared with the extracellular fluid.

Proteins are among the most plentiful buffers in the body because of their high concentrations, especially within the cells. Approximately 60 to 70 per cent of the total chemical buffering of the body fluids is inside the cells, and most of this results from the intracellular proteins. However, except for the red blood cells, the slowness with which H^+ and HCO_3^- move through the cell membranes often delays for several hours the maximum ability of the intracellular proteins to buffer extracellular acid-base abnormalities. In addition to the high concentration of proteins in the cells, another factor that contributes to their buffering power is the fact that the pKs of many of these protein systems are fairly close to 7.4.

In the red blood cell, hemoglobin (Hb) is an important buffer, as follows:



Respiratory regulation of acid-base balance

The second line of defense against acid-base disturbances is control of extracellular fluid CO_2 concentration by the lungs. An increase in ventilation eliminates CO_2 from extracellular fluid, which, by mass action, reduces the H^+ concentration. Conversely, decreased ventilation increases CO_2 , thus also increasing H^+ concentration in the extracellular fluid. CO_2 is formed continually in the body by intracellular metabolic processes. After it is formed, it diffuses from the cells into the interstitial fluids and blood, and the flowing blood transports it to the lungs, where it diffuses into the alveoli and then is transferred to the atmosphere by pulmonary ventilation.

If the metabolic formation of CO_2 remains constant, the only other factor that affects Pco_2 in extracellular fluid is the rate of alveolar ventilation. The higher the alveolar ventilation, the lower the Pco_2 ; conversely, the lower the alveolar ventilation rate, the higher the Pco_2 . Not only does the alveolar ventilation rate influence H^+ concentration by changing the Pco_2 of the body fluids, but the H^+ concentration affects the rate of alveolar ventilation. The alveolar ventilation rate increases four to five times normal as the pH decreases from the

normal value of 7.4 to the strongly acidic value of 7.0. Conversely, when plasma pH rises above 7.4, this causes a decrease in the ventilation rate.

Because increased H^+ concentration stimulates respiration, and because increased alveolar ventilation decreases the H^+ concentration, the respiratory system acts as a typical negative feedback controller of H^+ concentration. That is, whenever the H^+ concentration increases above normal, the respiratory system is stimulated, and alveolar ventilation increases. This decreases the P_{CO_2} in extracellular fluid and reduces H^+ concentration back toward normal. Conversely, if H^+ concentration falls below normal, the respiratory center becomes depressed, alveolar ventilation decreases, and H^+ concentration increases back toward normal.

Respiratory control cannot return the H^+ concentration all the way back to normal when a disturbance outside the respiratory system has altered pH. Ordinarily, the respiratory mechanism for controlling H^+ concentration has an effectiveness between 50 and 75 per cent. However, abnormalities of respiration can also cause changes in H^+ concentration. For example, an impairment of lung function, such as severe emphysema, decreases the ability of the lungs to eliminate CO_2 ; this causes a buildup of CO_2 in the extracellular fluid and a tendency toward respiratory acidosis. Also, the ability to respond to metabolic acidosis is impaired because the compensatory reductions in PCO_2 that would normally occur by means of increased ventilation are blunted. In these circumstances, the kidneys represent the sole remaining physiologic mechanism for returning pH toward normal after the initial chemical buffering in the extracellular fluid has occurred.

Renal control of acid-base balance

The kidneys control acid-base balance by excreting either an acidic or a basic urine. Excreting an acidic urine reduces the amount of acid in extracellular fluid, whereas excreting a basic urine removes base from the extracellular fluid. The overall mechanism by which the kidneys excrete acidic or basic urine is as follows. Large numbers of HCO_3^- are filtered continuously into the tubules, and if they are excreted into the urine, this removes base from the blood. Large numbers of H^+ are also secreted into the tubular lumen by the tubular epithelial cells, thus removing acid from the blood. If more H^+ is secreted than HCO_3^- is filtered, there will be a net loss of acid from the extracellular fluid. Conversely, if more HCO_3^- is filtered than H^+ is secreted, there will be a net loss of base.

Each day the body produces about 80 milliequivalents of nonvolatile acids, mainly from the metabolism of proteins. These acids are called nonvolatile because they are not H_2CO_3 and, therefore, cannot be excreted by the lungs. The primary mechanism for removal of these acids from the body is renal excretion. The kidneys must also prevent the loss of bicarbonate in the urine, a task that is quantitatively more important than the excretion of nonvolatile acids. Both the reabsorption of bicarbonate and the excretion of H^+ are accomplished through the process of H^+ secretion by the tubules. Because the HCO_3^- must react with a secreted H^+ to form H_2CO_3 before it can be reabsorbed, 4320 milliequivalents of H^+ must be secreted each day just to reabsorb the filtered bicarbonate. Then an additional 80 milliequivalents of H^+ must be secreted to rid the body of the nonvolatile acids produced each day, for a total of 4400 milliequivalents of H^+ secreted into the tubular fluid each day.

When there is a reduction in the extracellular fluid H^+ concentration (alkalosis), the kidneys fail to reabsorb all the filtered bicarbonate, thereby increasing the excretion of bicarbonate. Because HCO_3^- normally buffers hydrogen in the extracellular fluid, this loss of bicarbonate is the same as adding an H^+ to the extracellular fluid. Therefore, in alkalosis, the removal of HCO_3^- raises the extracellular fluid H^+ concentration back toward normal. In acidosis, the kidneys do not excrete bicarbonate into the urine but reabsorb all the filtered bicarbonate and produce new bicarbonate, which is added back to the extracellular fluid. This

reduces the extracellular fluid H^+ concentration back toward normal. Thus, the kidneys regulate extracellular fluid H^+ concentration through three fundamental mechanisms: (1) secretion of H^+ , (2) reabsorption of filtered HCO_3^- , and (3) production of new HCO_3^- . Thus, each time an H^+ is formed in the tubular epithelial cells, an HCO_3^- is also formed and released back into the blood. The net effect of these reactions is “reabsorption” of HCO_3^- from the tubules, although the HCO_3^- that actually enters the extracellular fluid is not the same as that filtered into the tubules. The reabsorption of filtered HCO_3^- does not result in net secretion of H^+ because the secreted H^+ combines with the filtered HCO_3^- and is therefore not excreted.

Table 4 Primary acid-base disturbances

	pH	Pco ₂	HCO ₃ ⁻	HCO ₃ ⁻ / H ₂ CO ₃
Normal	7.4	40 mmHg	24 mEq/l	18-22
Respiratory acidosis	↓	↑↑	↑	< 18
Respiratory alkalosis	↑	↓↓	↓↓	> 22
Metabolic acidosis	↓	↓	↓↓	< 18
Metabolic alkalosis	↑	↑	↑↑	> 22

Referring to Henderson- Hasselbalch equation, we can see that acidosis occurs when the ratio of HCO_3^- to CO_2 in the extracellular fluid decreases, thereby decreasing pH. If this ratio decreases because of a fall in HCO_3^- , the acidosis is referred to as metabolic acidosis. If the pH falls because of an increase in PCO_2 , the acidosis is referred to as respiratory acidosis. This increases the bicarbonate part of the bicarbonate buffer system, which, in accordance with the Henderson-Hasselbalch equation, helps raise the extracellular pH and corrects the acidosis. If the acidosis is metabolically mediated, additional compensation by the lungs causes a reduction in PCO_2 , also helping to correct the acidosis.

In metabolic acidosis, there is also a decrease in pH and a rise in extracellular fluid H^+ concentration. However, in this case, the primary abnormality is a decrease in plasma HCO_3^- . The primary compensations include increased ventilation rate, which reduces PCO_2 , and renal compensation, which, by adding new bicarbonate to the extracellular fluid, helps minimize the initial fall in extracellular HCO_3^- concentration.

The compensatory responses to alkalosis are basically opposite to those that occur in acidosis. In alkalosis, the ratio of HCO_3^- to CO_2 in the extracellular fluid increases, causing a

rise in pH (a decrease in H^+ concentration), as is evident from the Henderson- Hasselbalch equation.

In respiratory acidosis, the compensatory responses available are (1) the buffers of the body fluids and (2) the kidneys, which require several days to compensate for the disorder. Respiratory alkalosis is caused by overventilation by the lungs. Rarely does this occur because of physical pathological conditions. A physiologic type of respiratory alkalosis occurs when a person ascends to high altitude. The low oxygen content of the air stimulates respiration, which causes excess loss of CO_2 and development of mild respiratory alkalosis. Again, the major means for compensation are the chemical buffers of the body fluids and the ability of the kidneys to increase HCO_3^- excretion.

The term metabolic acidosis refers to all other types of acidosis besides those caused by excess CO_2 in the body fluids. Metabolic acidosis can result from several general causes: (1) failure of the kidneys to excrete metabolic acids normally formed in the body, (2) formation of excess quantities of metabolic acids in the body, (3) addition of metabolic acids to the body by ingestion or infusion of acids, and (4) loss of base from the body fluids, which has the same effect as adding an acid to the body fluids. Some specific conditions that cause metabolic acidosis are the following.

-Renal Tubular Acidosis. This type of acidosis results from a defect in renal secretion of H^+ or in reabsorption of HCO_3^- , or both.

-Vomiting of intestinal contents. Vomiting of gastric contents alone would cause loss of acid and a tendency toward alkalosis because the stomach secretions are highly acidic.

-Diabetes Mellitus. Diabetes mellitus is caused by lack of insulin secretion by the pancreas (type I diabetes) or by insufficient insulin secretion to compensate for decreased sensitivity to the effects of insulin (type II diabetes). In the absence of sufficient insulin, the normal use of glucose for metabolism is prevented. Instead, some of the fats are split into acetoacetic acid, and this is metabolized by the tissues for energy in place of glucose. With severe diabetes mellitus, blood acetoacetic acid levels can rise very high, causing severe metabolic acidosis. In an attempt to compensate for this acidosis, large amounts of acid are excreted in the urine, sometimes as much as 500 mmol/day.

-Ingestion of acids. Rarely are large amounts of acids ingested in normal foods. However, severe metabolic acidosis occasionally results from the ingestion of certain acidic poisons. Some of these include acetylsalicylics (aspirin) and methyl alcohol (which forms formic acid when it is metabolized).

-Chronic renal failure. When kidney function declines markedly, there is a buildup of the anions of weak acids in the body fluids that are not being excreted by the kidneys.

Metabolic Alkalosis is caused by increased extracellular fluid bicarbonate concentration. When there is excess retention of HCO_3^- or loss of H^+ from the body, this results in metabolic alkalosis. Metabolic alkalosis is not nearly as common as metabolic acidosis, but some of the causes of metabolic alkalosis are as follows.

-Administration of diuretics (except the carbonic anhydrase inhibitors). All diuretics cause increased flow of fluid along the tubules, usually causing increased flow in the distal and collecting tubules. This leads to increased reabsorption of Na^+ from these parts of the nephrons. Because the sodium reabsorption here is coupled with H^+ secretion, the enhanced sodium reabsorption also leads to an increase in H^+ secretion and an increase in bicarbonate reabsorption. These changes lead to the development of alkalosis, characterized by increased extracellular fluid bicarbonate concentration.

-Excess aldosterone. When large amounts of aldosterone are secreted by the adrenal glands, a mild metabolic alkalosis develops. Aldosterone promotes extensive reabsorption of Na^+ from the distal and collecting tubules and at the same time stimulates the secretion of H^+ by the

intercalated cells of the collecting tubules. This increased secretion of H^+ leads to its increased excretion by the kidneys and, therefore, metabolic alkalosis.

-Vomiting of gastric contents. Vomiting of the gastric contents alone, without vomiting of the lower gastrointestinal contents, causes loss of the HCl secreted by the stomach mucosa. The net result is a loss of acid from the extracellular fluid and development of metabolic alkalosis. This type of alkalosis occurs especially in neonates who have pyloric obstruction caused by hypertrophied pyloric sphincter muscles.

-Ingestion of alkaline drugs. A common cause of metabolic alkalosis is ingestion of alkaline drugs, such as sodium bicarbonate, for the treatment of gastritis or peptic ulcer.

Red Blood Cells (Erythrocytes)

The major function of red blood cells (erythrocytes) is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues. The red blood cells have other functions besides transport of hemoglobin. For instance, they contain a large quantity of carbonic anhydrase, an enzyme that catalyzes the reversible reaction between carbon dioxide (CO_2) and water to form carbonic acid (H_2CO_3), increasing the rate of this reaction several thousandfold. The rapidity of this reaction makes it possible for the water of the blood to transport enormous quantities of CO_2 in the form of bicarbonate ion (HCO_3^-) from the tissues to the lungs, where it is reconverted to CO_2 and expelled into the atmosphere as a body waste product. The hemoglobin in the cells is an excellent acid-base buffer (as is true of most proteins), so that the red blood cells are responsible for most of the acid-base buffering power of whole blood.

Normal red blood cells are biconcave discs having a mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the center. The average volume of the red blood cell is 90 to 95 cubic micrometers. The shapes of red blood cells can change remarkably as the cells squeeze through capillaries. Actually, the red blood cell is a "bag" that can be deformed into almost any shape. Furthermore, because the normal cell has a great excess of cell membrane for the quantity of material inside, deformation does not stretch the membrane greatly and, consequently, does not rupture the cell, as would be the case with many other cells.

Normal values are between:

- 4 to 5 million / mm^3 for women
- 5 ÷ 5.5 million / mm^3 for men

Red blood cells have the ability to concentrate hemoglobin in the cell fluid up to about 34 grams in each 100 milliliters of cells. The concentration does not rise above this value, because this is the metabolic limit of the cell's hemoglobin forming mechanism. Furthermore, in normal people, the percentage of hemoglobin is almost always near the maximum in each cell. However, when hemoglobin formation is deficient, the percentage of hemoglobin in the cells may fall considerably below this value, and the volume of the red cell may also decrease because of diminished hemoglobin to fill the cell. When the hematocrit (normally 40 to 45 per cent) and the quantity of hemoglobin in each respective cell are normal, the whole blood of men contains an average of 15 grams of hemoglobin per 100 milliliters of cells; for women, it contains an average of 14 grams per 100 milliliters.

Production of Red Blood Cells

In the early weeks of embryonic life nucleated red blood cells are produced in the yolk sac. During the middle trimester of gestation, the liver is the main organ for production of red blood cells, but reasonable numbers are also produced in the spleen and lymph nodes. During the last month or so of gestation and after birth, red blood cells are produced exclusively in the bone marrow. The bone marrow of essentially all bones produces red blood cells until a person is 5 years old. The marrow of the long bones, except for the proximal portions of the humeri and tibiae, becomes quite fatty and produces no more red blood cells after about age 20 years. Beyond this age, most red cells continue to be produced in the marrow of the membranous bones, such as the vertebrae, sternum, ribs and ilia. Even in these bones, the marrow becomes less productive as age increases.

The blood cells begin their lives in the bone marrow from a single type of cell called the pluripotential hematopoietic stem cell, from which all the cells of the circulating blood are eventually derived. Figure 4 shows the successive divisions of the pluripotential cells to form the different circulating blood cells. As these cells reproduce, a small portion of them remains exactly like the original pluripotential cells and is retained in the bone marrow to maintain a supply of these, although their numbers diminish with age. The intermediate-stage cells are very much like the pluripotential stem cells, even though they have already become committed to a particular line of cells and are called committed stem cells. The different committed stem cells, when grown in culture, will produce colonies of specific types of blood cells. A committed stem cell that produces erythrocytes is called a colony-forming unit-erythrocyte, and the abbreviation CFU-E is used to designate this type of stem cell. Likewise, colony-forming units that form granulocytes and monocytes have the designation CFU-GM, and so forth.

Growth and reproduction of the different stem cells are controlled by multiple proteins called growth inducers. Four major growth inducers have been described, each having different characteristics. One of these, interleukin-3, promotes growth and reproduction of virtually all the different types of committed stem cells, whereas the others induce growth of only specific types of cells. The growth inducers promote growth but not differentiation of the cells. This is the function of another set of proteins called differentiation inducers. Each of these causes one type of committed stem cell to differentiate one or more steps toward a final adult blood cell. Formation of the growth inducers and differentiation inducers is itself controlled by factors outside the bone marrow. For instance, in the case of erythrocytes, exposure of the blood to low oxygen for a long time results in growth induction, differentiation, and production of greatly increased numbers of erythrocytes. In the case of some of the white blood cells, infectious diseases cause growth, differentiation, and eventual formation of specific types of white blood cells that are needed to combat each infection.

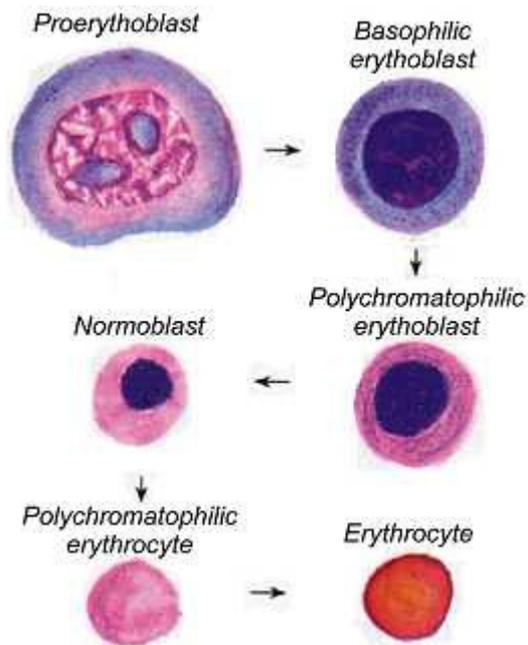


Figure 4

The first cell that can be identified as belonging to the red blood cell series is the proerythroblast, shown at the starting point in Figure. Once the proerythroblast has been formed, it divides multiple times, eventually forming many mature red blood cells. The first-generation cells are called basophil erythroblasts because they stain with basic dyes; the cell at this time has accumulated very little hemoglobin. In the succeeding generations, as shown in Figure 4, the cells become filled with hemoglobin to a concentration of about 34 per cent, the nucleus condenses to a small size, and its final remnant is absorbed or extruded from the cell. At the same time, the endoplasmic reticulum is also reabsorbed. The cell at this stage is called a reticulocyte because it still contains a small amount of basophilic material, consisting of remnants of the Golgi apparatus, mitochondria, and a few other cytoplasmic organelles. During this reticulocyte stage, the cells pass from the bone marrow into the blood capillaries by diapedesis (squeezing through the pores of the capillary membrane). The remaining basophilic material in the reticulocyte normally disappears within 1 to 2 days, and the cell is then a mature erythrocyte. Because of the short life of the reticulocytes, their concentration among all the red cells of the blood is normally slightly less than 1 per cent.

The total mass of red blood cells in the circulatory system is regulated within narrow limits. Any condition that causes the quantity of oxygen transported to the tissues to decrease ordinarily increases the rate of red blood cell production. Thus, when a person becomes extremely anemic as a result of hemorrhage or any other condition, the bone marrow immediately begins to produce large quantities of red blood cells. Also, destruction of major portions of the bone marrow by any means, especially by x-ray therapy, causes hyperplasia of the remaining bone marrow, thereby attempting to supply the demand for red blood cells in the body. At very high altitudes, where the quantity of oxygen in the air is greatly decreased, insufficient oxygen is transported to the tissues, and red cell production is greatly increased. In this case, it is not the concentration of red blood cells in the blood that controls red cell production but the amount of oxygen transported to the tissues in relation to tissue demand for oxygen. Various diseases of the circulation that cause decreased blood flow through the peripheral vessels, and particularly those that cause failure of oxygen absorption by the blood as it passes through the lungs, can also increase the rate of red cell production. This is especially apparent in prolonged cardiac failure and in many lung diseases, because the tissue hypoxia resulting from these conditions increases red cell production, with a resultant increase in hematocrit and usually total blood volume as well.

The principal stimulus for red blood cell production in low oxygen states is a circulating hormone called erythropoietin, a glycoprotein with a molecular weight of about 34,000. In the absence of erythropoietin, hypoxia has little or no effect in stimulating red blood cell production. But when the erythropoietin system is functional, hypoxia causes a marked increase in erythropoietin production, and the erythropoietin in turn enhances red blood cell production until the hypoxia is relieved. In the normal person, about 90 per cent of all erythropoietin is formed in the kidneys; the remainder is formed mainly in the liver. It is not known exactly where in the kidneys the erythropoietin is formed. One likely possibility is that the renal tubular epithelial cells secrete the erythropoietin, because anemic blood is unable to deliver enough oxygen from the peritubular capillaries to the highly oxygen-consuming tubular cells, thus stimulating erythropoietin production.

At times, hypoxia in other parts of the body, but not in the kidneys, stimulates kidney erythropoietin secretion, which suggests that there might be some nonrenal sensor that sends an additional signal to the kidneys to produce this hormone. In particular, both norepinephrine and epinephrine and several of the prostaglandins stimulate erythropoietin production. It has been determined that the important effect of erythropoietin is to stimulate the production of proerythroblasts from hematopoietic stem cells in the bone marrow. In addition, once the proerythroblasts are formed, the erythropoietin causes these cells to pass more rapidly through the different erythroblastic stages than they normally do, further speeding up the production of new red blood cells. The rapid production of cells continues as long as the person remains in a low oxygen state or until enough red blood cells have been produced to carry adequate amounts of oxygen to the tissues despite the low oxygen; at this time, the rate of erythropoietin production decreases to a level that will maintain the required number of red cells but not an excess. In the absence of erythropoietin, few red blood cells are formed by the bone marrow.

Because of the continuing need to replenish red blood cells, the erythropoietic cells of the bone marrow are among the most rapidly growing and reproducing cells in the entire body. Therefore, as would be expected, their maturation and rate of production are affected greatly by a person's nutritional status. Especially important for final maturation of the red blood cells are two vitamins, vitamin B12 and folic acid. Both of these are essential for the synthesis of DNA, because each in a different way is required for the formation of thymidine triphosphate, one of the essential building blocks of DNA. Therefore, lack of either vitamin B12 or folic acid causes abnormal and diminished DNA and, consequently, failure of nuclear maturation and cell division. Furthermore, the erythroblastic cells of the bone marrow, in addition to failing to proliferate rapidly, produce mainly larger than normal red cells called macrocytes, and the cell itself has a flimsy membrane and is often irregular, large, and oval instead of the usual biconcave disc. These poorly formed cells, after entering the circulating blood, are capable of carrying oxygen normally, but their fragility causes them to have a short life, one half to one third normal. Therefore, it is said that deficiency of either vitamin B12 or folic acid causes maturation failure in the process of erythropoiesis.

A common cause of red blood cell maturation failure is failure to absorb vitamin B12 from the gastrointestinal tract. This often occurs in the disease pernicious anemia, in which the basic abnormality is an atrophic gastric mucosa that fails to produce normal gastric secretions. The parietal cells of the gastric glands secrete a glycoprotein called intrinsic factor, which combines with vitamin B12 in food and makes the B12 available for absorption by the gut. The minimum amount of vitamin B12 required each day to maintain normal red cell maturation is only 1 to 3 micrograms, and the normal storage in the liver and other body tissues is about 1000 times this amount. Therefore, 3 to 4 years of defective B12 absorption are usually required to cause maturation failure anemia.

Folic acid is a normal constituent of green vegetables, some fruits, and meats (especially liver). However, it is easily destroyed during cooking. Also, people with gastrointestinal absorption abnormalities, such as the frequently occurring small intestinal disease called sprue, often have serious difficulty absorbing both folic acid and vitamin B12. Therefore, in many instances of maturation failure, the cause is deficiency of intestinal absorption of both folic acid and vitamin B12.

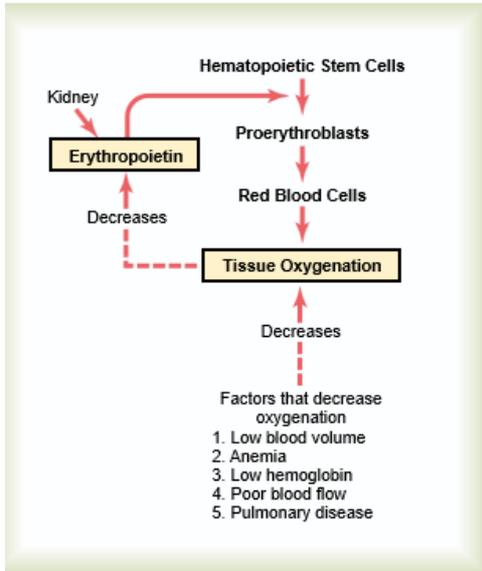


Figure 5

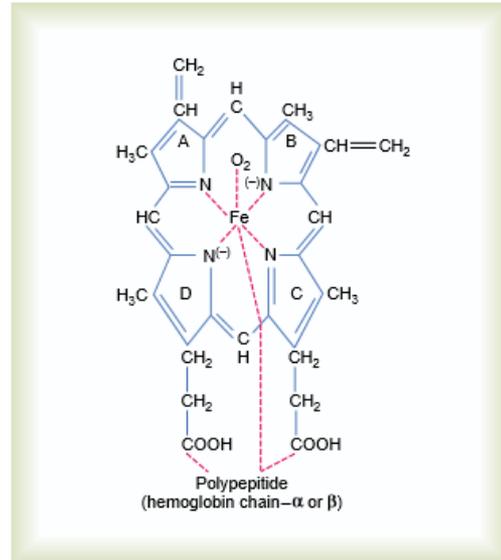


Figure 6 Basic structure of the hemoglobin molecule

Formation of Hemoglobin

Synthesis of hemoglobin begins in the proerythroblasts and continues even into the reticulocyte stage of the red blood cells. Therefore, when reticulocytes leave the bone marrow and pass into the blood stream, they continue to form minute quantities of hemoglobin for another day or so until they become mature erythrocytes. Each heme molecule combines with a long polypeptide chain, a globin synthesized by ribosomes, forming a subunit of hemoglobin called a hemoglobin chain (Figure 6). Each chain has a molecular weight of about 16,000; four of these in turn bind together loosely to form the whole hemoglobin molecule. There are several slight variations in the different subunit hemoglobin chains, depending on the amino acid composition of the polypeptide portion. The different types of chains are designated alpha chains, beta chains, gamma chains, and delta chains. The most common form of hemoglobin in the adult human being, hemoglobin A, is a combination of two alpha chains and two beta chains.

Because each hemoglobin chain has a heme prosthetic group containing an atom of iron, and because there are four hemoglobin chains in each hemoglobin molecule, one finds four iron atoms in each hemoglobin molecule; each of these can bind loosely with one molecule of oxygen, making a total of four molecules of oxygen (or eight oxygen atoms) that can be transported by each hemoglobin molecule. The types of hemoglobin chains in the hemoglobin molecule determine the binding affinity of the hemoglobin for oxygen. Abnormalities of the chains can alter the physical characteristics of the hemoglobin molecule as well. For instance, in sickle cell anemia, the amino acid valine is substituted for glutamic acid at one point in each of the two beta chains. When this type of hemoglobin is exposed to low oxygen, it forms elongated crystals inside the red blood cells that are sometimes 15

micrometers in length. These make it almost impossible for the cells to pass through many small capillaries, and the spiked ends of the crystals are likely to rupture the cell membranes, leading to sickle cell anemia.

The most important feature of the hemoglobin molecule is its ability to combine loosely and reversibly with oxygen. Oxygen does not combine with the two positive bonds of the iron in the hemoglobin molecule. Instead, it binds loosely with one of the so-called coordination bonds of the iron atom. This is an extremely loose bond, so that the combination is easily reversible. Furthermore, the oxygen does not become ionic oxygen but is carried as molecular oxygen (composed of two oxygen atoms) to the tissues, where, because of the loose, readily reversible combination, it is released into the tissue fluids still in the form of molecular oxygen rather than ionic oxygen.

Iron Metabolism

Iron is important for the formation not only of hemoglobin but also of other essential elements in the body (e.g., myoglobin, cytochromes, cytochrome oxidase, peroxidase, catalase). The total quantity of iron in the body averages 4 to 5 grams, about 65 per cent of which is in the form of hemoglobin. About 4 per cent is in the form of myoglobin, 1 per cent is in the form of the various heme compounds that promote intracellular oxidation, 0.1 per cent is combined with the protein transferrin in the blood plasma, and 15 to 30 per cent is stored for later use, mainly in the reticuloendothelial system and liver parenchymal cells, principally in the form of ferritin.

When iron is absorbed from the small intestine, it immediately combines in the blood plasma with a beta globulin, apotransferrin, to form transferrin, which is then transported in the plasma. The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body. Excess iron in the blood is deposited especially in the liver hepatocytes and less in the reticuloendothelial cells of the bone marrow. In the cell cytoplasm, iron combines mainly with a protein, apoferritin, to form ferritin. Apoferritin has a molecular weight of about 460,000, and varying quantities of iron can combine in clusters of iron radicals with this large molecule; therefore, ferritin may contain only a small amount of iron or a large amount. This iron stored as ferritin is called storage iron. Smaller quantities of the iron in the storage pool are in an extremely insoluble form called hemosiderin. This is especially true when the total quantity of iron in the body is more than the apoferritin storage pool can accommodate. Hemosiderin collects in cells in the form of large clusters that can be observed microscopically as large particles. In contrast, ferritin particles are so small and dispersed that they usually can be seen in the cell cytoplasm only with the electron microscope. When the quantity of iron in the plasma falls low, some of the iron in the ferritin storage pool is removed easily and transported in the form of transferrin in the plasma to the areas of the body where it is needed. A unique characteristic of the transferrin molecule is that it binds strongly with receptors in the cell membranes of erythroblasts in the bone marrow. Then, along with its bound iron, it is ingested into the erythroblasts by endocytosis. There the transferrin delivers the iron directly to the mitochondria, where heme is synthesized. In people who do not have adequate quantities of transferrin in their blood, failure to transport iron to the erythroblasts in this manner can cause severe hypochromic anemia—that is, red cells that contain much less hemoglobin than normal.

When red blood cells have lived their life span and are destroyed, the hemoglobin released from the cells is ingested by monocyte-macrophage cells. There, iron is liberated and is stored mainly in the ferritin pool to be used as needed for the formation of new hemoglobin. A man excretes about 0.6 milligram of iron each day, mainly into the feces. Additional

quantities of iron are lost when bleeding occurs. For a woman, additional menstrual loss of blood brings long-term iron loss to an average of about 1.3 mg/day.

Iron is absorbed from all parts of the small intestine, mostly by the following mechanism. The liver secretes moderate amounts of apotransferrin into the bile, which flows through the bile duct into the duodenum. Here, the apotransferrin binds with free iron and also with certain iron compounds, such as hemoglobin and myoglobin from meat, two of the most important sources of iron in the diet. This combination is called transferrin. It, in turn, is attracted to and binds with receptors in the membranes of the intestinal epithelial cells. Then, by pinocytosis, the transferrin molecule, carrying its iron store, is absorbed into the epithelial cells and later released into the blood capillaries beneath these cells in the form of plasma transferrin. Iron absorption from the intestines is extremely slow, at a maximum rate of only a few milligrams per day. This means that even when tremendous quantities of iron are present in the food, only small proportions can be absorbed. When the body has become saturated with iron so that essentially all apoferritin in the iron storage areas is already combined with iron, the rate of additional iron absorption from the intestinal tract becomes greatly decreased. Conversely, when the iron stores have become depleted, the rate of absorption can accelerate probably five or more times normal. Thus, total body iron is regulated mainly by altering the rate of absorption.

Destruction of Red Blood Cells

When red blood cells are delivered from the bone marrow into the circulatory system, they normally circulate an average of 120 days before being destroyed. Even though mature red cells do not have a nucleus, mitochondria, or endoplasmic reticulum, they do have cytoplasmic enzymes that are capable of metabolizing glucose and forming small amounts of adenosine triphosphate. These enzymes also (1) maintain pliability of the cell membrane, (2) maintain membrane transport of ions, (3) keep the iron of the cells' hemoglobin in the ferrous form rather than ferric form, and (4) prevent oxidation of the proteins in the red cells. Even so, the metabolic systems of old red cells become progressively less active, and the cells become more and more fragile, presumably because their life processes wear out. Once the red cell membrane becomes fragile, the cell ruptures during passage through some tight spot of the circulation. Many of the red cells self-destruct in the spleen, where they squeeze through the red pulp of the spleen. There, the spaces between the structural trabeculae of the red pulp, through which most of the cells must pass, are only 3 micrometers wide, in comparison with the 8-micrometer diameter of the red cell. When the spleen is removed, the number of old abnormal red cells circulating in the blood increases considerably.

When red blood cells burst and release their hemoglobin, the hemoglobin is phagocytized almost immediately by macrophages in many parts of the body, but especially by the Kupffer cells of the liver and macrophages of the spleen and bone marrow. During the next few hours to days, the macrophages release iron from the hemoglobin and pass it back into the blood, to be carried by transferrin either to the bone marrow for the production of new red blood cells or to the liver and other tissues for storage in the form of ferritin. The porphyrin portion of the hemoglobin molecule is converted by the macrophages, through a series of stages, into the bile pigment bilirubin, which is released into the blood and later removed from the body by secretion through the liver into the bile.

Anemias

Anemia means deficiency of hemoglobin in the blood, which can be caused by either too few red blood cells or too little hemoglobin in the cells. Some types of anemia and their physiologic causes are the following.

-Blood Loss Anemia. After rapid hemorrhage, the body replaces the fluid portion of the plasma in 1 to 3 days, but this leaves a low concentration of red blood cells.

-Aplastic Anemia. Bone marrow aplasia means lack of functioning bone marrow. For instance, a person exposed to gamma ray radiation from a nuclear bomb blast can sustain complete destruction of bone marrow, followed in a few weeks by lethal anemia. Likewise, excessive x-ray treatment, certain industrial chemicals, and even drugs to which the person might be sensitive can cause the same effect.

-Megaloblastic Anemia. Based on the earlier discussions of vitamin B12, folic acid, and intrinsic factor from the stomach mucosa, one can readily understand that loss of any one of these can lead to slow reproduction of erythroblasts in the bone marrow. As a result, the red cells grow too large, with odd shapes, and are called megaloblasts. Thus, atrophy of the stomach mucosa, as occurs in pernicious anemia, or loss of the entire stomach after surgical total gastrectomy can lead to megaloblastic anemia.

-Hemolytic Anemia. Different abnormalities of the red blood cells, many of which are hereditarily acquired, make the cells fragile, so that they rupture easily as they go through the capillaries, especially through the spleen. Even though the number of red blood cells formed may be normal, or even much greater than normal in some hemolytic diseases, the life span of the fragile red cell is so short that the cells are destroyed faster than they can be formed, and serious anemia results. Some of these types of anemia are the following. In hereditary spherocytosis, the red cells are very small and spherical rather than being biconcave discs. These cells cannot withstand compression forces because they do not have the normal loose, baglike cell membrane structure of the biconcave discs. On passing through the splenic pulp and some other tight vascular beds, they are easily ruptured by even slight compression. In sickle cell anemia, which is present in 0.3 to 1.0 per cent of West African and American blacks, the cells have an abnormal type of hemoglobin called hemoglobin S, containing faulty beta chains in the hemoglobin molecule. When this hemoglobin is exposed to low concentrations of oxygen, it precipitates into long crystals inside the red blood cell. These crystals elongate the cell and give it the appearance of a sickle rather than a biconcave disc. The precipitated hemoglobin also damages the cell membrane, so that the cells become highly fragile, leading to serious anemia. Such patients frequently experience a vicious circle of events called a sickle cell disease "crisis," in which low oxygen tension in the tissues causes sickling, which leads to ruptured red cells, which causes a further decrease in oxygen tension and still more sickling and red cell destruction. Once the process starts, it progresses rapidly, eventuating in a serious decrease in red blood cells within a few hours and, often, death. In erythroblastosis fetalis, Rh-positive red blood cells in the fetus are attacked by antibodies from an Rh-negative mother. These antibodies make the Rh-positive cells fragile, leading to rapid rupture and causing the child to be born with serious anemia. The extremely rapid formation of new red cells to make up for the destroyed cells in erythroblastosis fetalis causes a large number of early blast forms of red cells to be released from the bone marrow into the blood.

Polycythemia

Secondary Polycythemia. Whenever the tissues become hypoxic because of too little oxygen in the breathed air, such as at high altitudes, or because of failure of oxygen delivery to the tissues, such as in cardiac failure, the blood-forming organs automatically produce large quantities of extra red blood cells. This condition is called secondary polycythemia, and the red cell count commonly rises to 6 to 7 million/mm³, about 30 per cent above normal. A common type of secondary polycythemia, called physiologic polycythemia, occurs in natives who live at altitudes of 14,000 to 17,000 feet, where the atmospheric oxygen is very low. The blood count is generally 6 to 7 million/mm³; this allows these people to perform reasonably high levels of continuous work even in a rarefied atmosphere.

Polycythemia Vera (Erythremia). In addition to those people who have physiologic polycythemia, others have a pathological condition known as polycythemia vera, in which the red blood cell count may be 7 to 8 million/mm³ and the hematocrit may be 60 to 70 per cent instead of the normal 40 to 45 per cent. Polycythemia vera is caused by a genetic aberration in the hemocytoblastic cells that produce the blood cells. The blast cells no longer stop producing red cells when too many cells are already present. This causes excess production of red blood cells in the same manner that a breast tumor causes excess production of a specific type of breast cell. It usually causes excess production of white blood cells and platelets as well. In polycythemia vera, not only does the hematocrit increase, but the total blood volume also increases, on some occasions to almost twice normal. As a result, the entire vascular system becomes intensely engorged. In addition, many blood capillaries become plugged by the viscous blood; the viscosity of the blood in polycythemia vera sometimes increases from the normal of 3 times the viscosity of water to 10 times that of water.

Blood Types

The bloods of different people have different antigenic and immune properties, so that antibodies in the plasma of one blood will react with antigens on the surfaces of the red cells of another blood type. If proper precautions are taken, one can determine ahead of time whether the antibodies and antigens present in the donor and recipient bloods will cause a transfusion reaction. At least 30 commonly occurring antigens and hundreds of other rare antigens, each of which can at times cause antigen-antibody reactions, have been found in human blood cells, especially on the surfaces of the cell membranes. Two particular types of antigens are much more likely than the others to cause blood transfusion reactions. They are the O-A-B system of antigens and the Rh system.

O-A-B Blood Types

Two antigens—type A and type B (also called agglutinogens because they often cause blood cell agglutination)—occur on the surfaces of the red blood cells in a large proportion of human beings. It is these antigens that cause most blood transfusion reactions. Because of the way these agglutinogens are inherited, people may have neither of them on their cells, they may have one, or they may have both simultaneously. The bloods of donors and recipients are normally classified into four major O-A-B blood types, as shown in Table , depending on the

presence or absence of the two agglutinogens, the A and B agglutinogens. When neither A nor B agglutinin is present, the blood is type O. When only type A agglutinin is present, the blood is type A. When only type B agglutinin is present, the blood is type B. When both A and B agglutinogens are present, the blood is type AB.

Table 5 OAB blood group system

Blood group	Genotype	Agglutigen	Agglutinins	Frequency in population
O I	OO	----	alpha, beta	47 %
A II	OA or AA	A	beta	41 %
B III	OB or BB	B	alpha	9 %
AB IV	AB	A,B	----	3 %

Two genes, one on each of two paired chromosomes, determine the O-A-B blood type. These genes can be any one of three types but only one type on each of the two chromosomes: type O, type A, or type B. The type O gene is either functionless or almost functionless, so that it causes no significant type O agglutinin on the cells. Conversely, the type A and type B genes do cause strong agglutinogens on the cells. The six possible combinations of genes, are OO, OA, OB, AA, BB, and AB. These combinations of genes are known as the genotypes, and each person is one of the six genotypes. One can also observe from Table that a person with genotype OO produces no agglutinogens, and therefore the blood type is O. A person with genotype OA or AA produces type A agglutinogens and therefore has blood type A. Genotypes OB and BB give type B blood, and genotype AB gives type AB blood.

A maximum titer of the anti-A and anti-B agglutinins is usually reached at 8 to 10 years of age, and this gradually declines throughout the remaining years of life. The agglutinins are gamma globulins, as are almost all antibodies, and they are produced by the same bone marrow and lymph gland cells that produce antibodies to any other antigens. Most of them are IgM and IgG immunoglobulin molecules. But why are these agglutinins produced in people who do not have the respective agglutinogens in their red blood cells? The answer to this is that small amounts of type A and B antigens enter the body in food, in bacteria, and in other ways, and these substances initiate the development of the anti-A and anti-B agglutinins. For instance, infusion of group A antigen into a recipient having a non-A blood type causes a typical immune response with formation of greater quantities of anti-A agglutinins than ever. Also, the neonate has few, if any, agglutinins, showing that agglutinin formation occurs almost entirely after birth.

Agglutinins

When type A agglutinin is not present in a person's red blood cells, antibodies known as anti-A agglutinins develop in the plasma. Also, when type B agglutinin is not present in the red blood cells, antibodies known as anti-B agglutinins develop in the plasma. Type O blood, although containing no agglutinogens, does contain both anti-A and

anti-B agglutinins; type A blood contains type A agglutinogens and anti-B agglutinins; type B blood contains type B agglutinogens and anti-A agglutinins. Finally, type AB blood contains both A and B agglutinogens but no agglutinins.

Immediately after birth, the quantity of agglutinins in the plasma is almost zero. Two to 8 months after birth, an infant begins to produce agglutinins—anti-A agglutinins cells agglutinate as a result of the agglutinins' attaching themselves to the red blood cells. Because the agglutinins have two binding sites (IgG type) or 10 binding sites (IgM type), a single agglutinin can attach to two or more red blood cells at the same time, thereby causing the cells to be bound together by the agglutinin. This causes the cells to clump, which is the process of "agglutination." Then these clumps plug small blood vessels throughout the circulatory system. During ensuing hours to days, either physical distortion of the cells or attack by phagocytic white blood cells destroys the membranes of the agglutinated cells, releasing hemoglobin into the plasma, which is called "hemolysis" of the red blood cells.

Before giving a transfusion to a person, it is necessary to determine the blood type of the recipient's blood and the blood type of the donor blood so that the bloods can be appropriately matched. This is called blood typing and blood matching, and these are performed in the following way: If the red blood cells have become clumped—that is, "agglutinated"—one knows that an antibody-antigen reaction has resulted. Type O red blood cells have no agglutinogens and therefore do not react with either the anti-A or the anti-B agglutinins. Type A blood has A agglutinogens and therefore agglutinates with anti-A agglutinins. Type B blood has B agglutinogens and agglutinates with anti-B agglutinins. Type AB blood has both A and B agglutinogens and agglutinates with both types of agglutinins.

Rh Blood Types

The Rh blood type system is also important when transfusing blood. The major difference between the O-A-B system and the Rh system is the following: In the O-A-B system, the plasma agglutinins responsible for causing transfusion reactions develop spontaneously, whereas in the Rh system, spontaneous agglutinins almost never occur. Instead, the person must first be massively exposed to an Rh antigen, such as by transfusion of blood containing the Rh antigen, before enough agglutinins to cause a significant transfusion reaction will develop. There are six common types of Rh antigens, each of which is called an Rh factor. These types are designated C, D, E, c, d, and e. A person who has a C antigen does not have the c antigen, but the person missing the C antigen always has the c antigen. The same is true for the D-d and E-e antigens. Also, because of the manner of inheritance of these factors, each person has one of each of the three pairs of antigens. The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens. Anyone who has this type of antigen is said to be Rh positive, whereas a person who does not have type D antigen is said to be Rh negative. However, it must be noted that even in Rh-negative people, some of the other Rh antigens can still cause transfusion reactions, although the reactions are usually much milder. About 85 per cent of all white people are Rh positive and 15 per cent, Rh negative. In American blacks, the percentage of Rh-positives is about 95, whereas in African blacks, it is virtually 100 per cent. When red blood cells containing Rh factor are injected into a person whose blood does not contain the Rh factor—that is, into an Rh-negative person—anti-Rh agglutinins develop slowly, reaching maximum concentration of agglutinins about 2 to 4 months later. This immune response occurs to a much greater extent in some people than in others. With multiple exposures to the Rh factor, an Rh-negative person eventually becomes strongly "sensitized" to Rh factor.

If an Rh- negative person has never before been exposed to Rh- positive blood, transfusion of Rh-positive blood into that person will likely cause no immediate reaction. However, anti-Rh antibodies can develop in sufficient quantities during the next 2 to 4 weeks to cause agglutination of those transfused cells that are still circulating in the blood. These cells are then hemolyzed by the tissue macrophage system. Thus, a delayed transfusion reaction occurs, although it is usually mild. On subsequent transfusion of Rh-positive blood into the same person, who is now already immunized against the Rh factor, the transfusion reaction is greatly enhanced and can be immediate and as severe as a transfusion reaction caused by mismatched type A or B blood.

Erythroblastosis fetalis is a disease of the fetus and newborn child characterized by agglutination and phagocytosis of the fetus's red blood cells. In most instances of erythroblastosis fetalis, the mother is Rh negative and the father Rh positive. The baby has inherited the Rh-positive antigen from the father, and the mother develops anti-Rh agglutinins from exposure to the fetus's Rh antigen. In turn, the mother's agglutinins diffuse through the placenta into the fetus and cause red blood cell agglutination. An Rh-negative mother having her first Rh-positive child usually does not develop sufficient anti-Rh agglutinins to cause any harm. However, about 3 per cent of second Rh-positive babies exhibit some signs of erythroblastosis fetalis; about 10 per cent of third babies exhibit the disease; and the incidence rises progressively with subsequent pregnancies.

After anti- Rh antibodies have formed in the mother, they diffuse slowly through the placental membrane into the fetus's blood. There they cause agglutination of the fetus's blood. The agglutinated red blood cells subsequently hemolyze, releasing hemoglobin into the blood. The fetus's macrophages then convert the hemoglobin into bilirubin, which causes the baby's skin to become yellow (jaundiced). The antibodies can also attack and damage other cells of the body.

The jaundiced, erythroblastotic newborn baby is usually anemic at birth, and the anti-Rh agglutinins from the mother usually circulate in the infant's blood for another 1 to 2 months after birth, destroying more and more red blood cells. The hematopoietic tissues of the infant attempt to replace the hemolyzed red blood cells. The liver and spleen become greatly enlarged and produce red blood cells in the same manner that they normally do during the middle of gestation. Because of the rapid production of red cells, many early forms of red blood cells, including many nucleated blastic forms, are passed from the baby's bone marrow into the circulatory system, and it is because of the presence of these nucleated blastic red blood cells that the disease is called erythroblastosis fetalis. Although the severe anemia of erythroblastosis fetalis is usually the cause of death, many children who barely survive the anemia exhibit permanent mental impairment or damage to motor areas of the brain because of precipitation of bilirubin in the neuronal cells, causing destruction of many, a condition called kernicterus.

One treatment for erythroblastosis fetalis is to replace the neonate's blood with Rh-negative blood. About 400 milliliters of Rh-negative blood is infused over a period of 1.5 or more hours while the neonate's own Rh-positive blood is being removed. This procedure may be repeated several times during the first few weeks of life, mainly to keep the bilirubin level low and thereby prevent kernicterus. By the time these transfused Rh-negative cells are replaced with the infant's own Rh-positive cells, a process that requires 6 or more weeks, the anti- Rh agglutinins that had come from the mother will have been destroyed.

In the 1970's, a dramatic reduction in the incidence of erythroblastosis fetalis was achieved with the development of Rh immunoglobulin globin, an anti-D antibody that is administered to the expectant mother starting at 28 to 30 weeks of gestation. The anti-D antibody is also administered to Rh-negative women who deliver Rh-positive babies to prevent sensitization of the mothers to the D antigen. This greatly reduces the risk of

developing large amounts of D antibodies during the second pregnancy. The mechanism by which Rh immunoglobulin globin prevents sensitization of the D antigen is not completely understood, but one effect of the anti-D antibody is to inhibit antigen-induced B lymphocyte antibody production in the expectant mother. The administered anti-D antibody also attaches to D- antigen sites on Rh-positive fetal red blood cells that may cross the placenta and enter the circulation of the expectant mother, thereby interfering with the immune response to the D antigen.

If donor blood of one blood type is transfused into a recipient who has another blood type, a transfusion reaction is likely to occur in which the red blood cells of the donor blood are agglutinated. It is rare that the transfused blood causes agglutination of the recipient's cells, for the following reason: The plasma portion of the donor blood immediately becomes diluted by all the plasma of the recipient, thereby decreasing the titer of the infused agglutinins to a level usually too low to cause agglutination. Conversely, the small amount of infused blood does not significantly dilute the agglutinins in the recipient's plasma. Therefore, the recipient's agglutinins can still agglutinate the mismatched donor cells. As explained earlier, all transfusion reactions eventually cause either immediate hemolysis resulting from hemolysins or later hemolysis resulting from phagocytosis of agglutinated cells. The hemoglobin released from the red cells is then converted by the phagocytes into bilirubin and later excreted in the bile by the liver. The concentration of bilirubin in the body fluids often rises high enough to cause jaundice—that is, the person's internal tissues and skin become colored with yellow bile pigment. But if liver function is normal, the bile pigment will be excreted into the intestines by way of the liver bile, so that jaundice usually does not appear in an adult person unless more than 400 milliliters of blood is hemolyzed in less than a day.

One of the most lethal effects of transfusion reactions is kidney failure, which can begin within a few minutes to few hours and continue until the person dies of renal failure. The kidney shutdown seems to result from three causes: First, the antigen-antibody reaction of the transfusion reaction releases toxic substances from the hemolyzing blood that cause powerful renal vasoconstriction. Second, loss of circulating red cells in the recipient, along with production of toxic substances from the hemolyzed cells and from the immune reaction, often causes circulatory shock. The arterial blood pressure falls very low, and renal blood flow and urine output decrease. Third, if the total amount of free hemoglobin released into the circulating blood is greater than the quantity that can bind with "haptoglobin" (a plasma protein that binds small amounts of hemoglobin), much of the excess leaks through the glomerular membranes into the kidney tubules. If this amount is still slight, it can be reabsorbed through the tubular epithelium into the blood and will cause no harm; if it is great, then only a small percentage is reabsorbed. Yet water continues to be reabsorbed, causing the tubular hemoglobin concentration to rise so high that the hemoglobin precipitates and blocks many of the kidney tubules. Thus, renal vasoconstriction, circulatory shock, and renal tubular blockage together cause acute renal shutdown. If the shutdown is complete and fails to resolve, the patient dies within a week to 12 days, unless treated with an artificial kidney.