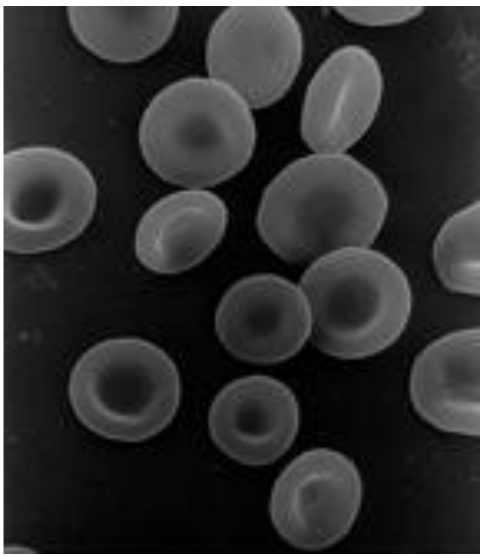
Haematology

Red Blood Cell

Red blood cells (**RBCs**), also called **erythrocytes**, are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues--via blood flow through the circulatory system. RBCs take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries. The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network. In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second in human adults. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells. Red blood cells are also known as **RBCs**, **red cells**, **red blood corpuscles** (an archaic term), **haematids**, **erythroid cells** or **erythrocytes** (from Greek *erythros* for "red" and *kytos* for "hollow vessel", with *-cyte* translated as "cell" in modern usage). Packed red blood cells (pRBC) are red blood cells that have been donated, processed, and stored in a blood bank for blood transfusion.

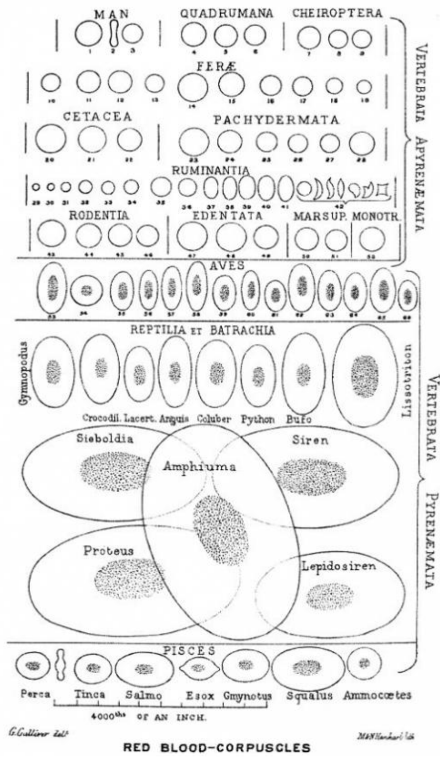


Erythrocytes consist mainly of hemoglobin, a complex metalloprotein containing heme groups whose iron atoms temporarily bind to oxygen molecules (O_2) in the lungs or gills and release them throughout the body. Oxygen can easily diffuse through the red blood cell's cell membrane.

Hemoglobin in the erythrocytes also carries some of the waste product carbon dioxide back from the tissues; most waste carbon dioxide, however, is transported back to the pulmonary capillaries of the lungs as bicarbonate (HCO_3^-) dissolved in the blood plasma. Myoglobin, a compound related to hemoglobin, acts to store oxygen in muscle cells.

The color of erythrocytes is due to the heme group of hemoglobin. The blood plasma alone is straw-colored, but the red blood cells change color depending on the state of the hemoglobin: when combined with oxygen the resulting oxyhemoglobin is scarlet, and when oxygen has been released the resulting deoxyhemoglobin is of a dark red burgundy color, appearing bluish through the vessel wall and skin. Pulse oximetry takes advantage of this color change to directly measure the arterial blood oxygen saturation using colorimetric techniques. Hemoglobin also has a very high affinity for carbon monoxide, forming carboxyhaemoglobin which is a very bright red in color. Flushed, confused patients with a saturation reading of 100% on pulse oximetry are sometimes found to be suffering from carbon monoxide poisoning.

The sequestration of oxygen-carrying proteins inside specialized cells (as opposed to oxygen carriers being dissolved in body fluid) was an important step in the evolution of vertebrates as it allows for less viscous blood, higher concentrations of oxygen, and better diffusion of oxygen from the blood to the tissues. The size of erythrocytes varies widely among vertebrate species; erythrocyte width is on average about 25% larger than capillary diameter, and it has been hypothesized that this improves the oxygen transfer from erythrocytes to tissues.



RED BLOOD-CORPUSCLES

Nucleus

Erythrocytes in mammals are *anucleate* when mature, meaning that they lack a cell nucleus. In comparison, the erythrocytes of other vertebrates have nuclei; the only known exceptions are salamanders of the *Batrachoseps* genus and fish of the *Maurolicus* genus with closely related species.

The elimination of the nucleus in vertebrate erythrocytes has been offered as an explanation for the subsequent accumulation of non-coding DNA in the genome. The argument runs as follows: Efficient gas transport requires erythrocytes to pass through very narrow capillaries, and this constrains their size. In the absence of nuclear elimination, the accumulation of repeat sequences is constrained by the volume occupied by the nucleus, which increases with genome size.

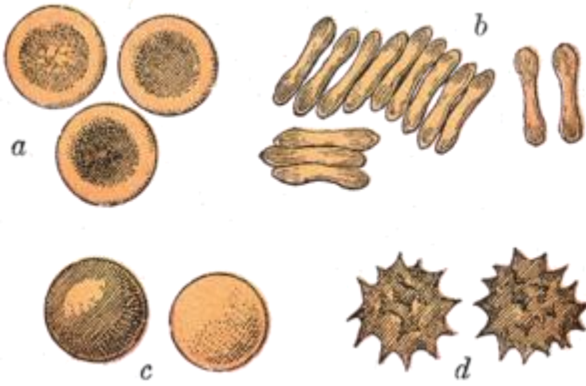
Secondary functions

When erythrocytes undergo shear stress in constricted vessels, they release ATP, which causes the vessel walls to relax and dilate so as to promote normal blood flow.
When their hemoglobin molecules are deoxygenated, erythrocytes release S-nitrosothiols, which also act to dilate blood vessels, thus directing more blood to areas of the body depleted of oxygen.
Erythrocytes can also synthesize nitric oxide enzymatically, using L-arginine as substrate, as do endothelial cells. Exposure of erythrocytes to physiological levels of shear stress activates nitric oxide synthase and export of nitric oxide, which may contribute to the regulation of vascular tonus.
Erythrocytes can also produce hydrogen sulfide, a signalling gas that acts to relax vessel walls. It is believed that the cardioprotective effects of garlic are due to erythrocytes converting its sulfur compounds into hydrogen sulfide.
Erythrocytes also play a part in the body's immune response: when lysed by pathogens such as bacteria, their hemoglobin releases free radicals, which break down the pathogen's cell wall and membrane, killing it.

Mammalian erythrocytes

Mammalian erythrocytes are unique among the vertebrates as they are non-nucleated cells in their mature form. These cells have nuclei during early phases of erythropoiesis, but extrude them during development as they mature in order to provide more space for hemoglobin. In mammals, erythrocytes also lose all other cellular organelles such as their mitochondria, Golgi apparatus and endoplasmic reticulum.
As a result of not containing mitochondria, these cells use none of the oxygen they transport; instead they produce the energy carrier ATP by the glycolysis of glucose and lactic acid fermentation on the resulting pyruvate.
Because of the lack of nuclei and organelles, mature red blood cells do not contain DNA and cannot synthesize any RNA, and consequently cannot divide and have limited repair capabilities. This also ensures that no virus can evolve to target mammalian red blood cells.
Mammalian erythrocytes are typically shaped as biconcave disks: flattened and depressed in the center, with a dumbbell-shaped cross section, and a torus-shaped rim on the edge of the disk. This distinctive biconcave shape optimises the flow properties of blood in the large vessels, such

as maximization of laminar flow and minimization of platelet scatter, which suppresses their atherogenic activity in those large vessels. However, there are some exceptions concerning shape in the artiodactyl order (even-toed ungulates including cattle, deer, and their relatives), which displays a wide variety of bizarre erythrocyte morphologies: small and highly ovaloid cells in llamas and camels (family Camelidae), tiny spherical cells in mouse deer (family Tragulidae), and cells which assume fusiform, lanceolate, crescentic, and irregularly polygonal and other angular forms in red deer and wapiti (family Cervidae). Members of this order have clearly evolved a mode of red blood cell development substantially different from the mammalian norm. Overall, mammalian erythrocytes are remarkably flexible and deformable so as to squeeze through tiny capillaries, as well as to maximize their apposing surface by assuming a cigar shape, where they efficiently release their oxygen load.
In large blood vessels, red blood cells sometimes occur as a stack, flat side next to flat side. This is known as *rouleaux formation*, and it occurs more often if the levels of certain serum proteins are elevated, as for instance during inflammation.
The spleen acts as a reservoir of red blood cells, but this effect is somewhat limited in humans. In some other mammals such as dogs and horses, the spleen sequesters large numbers of red blood cells which are dumped into the blood during times of exertion stress, yielding a higher oxygen transport capacity.



Typical mammalian erythrocytes: (a) seen from surface; (b) in profile, forming rouleaux; (c) rendered spherical by water; (d) rendered crenate by salt. (c) and (d) do not normally occur in the body.

Human erythrocytes

A typical human erythrocyte has a disk diameter of approximately 6.2–8.2 µm and a thickness at the thickest point of 2–2.5 µm and a minimum thickness in the centre of 0.8–1 µm, being much smaller than most other human cells. These cells have an average volume of about 90 fL with a surface of about 136 μm^2, and can swell up to a sphere shape containing 150 fL, without membrane distension.

Adult humans have roughly $20–30 \times 10^{12}$ (20–30 trillion) red blood cells at any given time, comprising approximately one quarter of the total human body cell number (women have about 4

to 5 million erythrocytes per microliter (cubic millimeter) of blood and men about 5 to 6 million; people living at high altitudes with low oxygen tension will have more). Red blood cells are thus much more common than the other blood particles: there are about 4,000–11,000 white blood cells and about 150,000–400,000 platelets in each microliter of human blood.

Human red blood cells take on average 20 seconds to complete one cycle of circulation.

As red blood cells contain no nucleus, protein biosynthesis is currently assumed to be absent in these cells, although a recent study indicates the presence of all the necessary biomachinery in the cells to do so.

The blood's red color is due to the spectral properties of the hemic iron ions in hemoglobin. Each human red blood cell contains approximately 270 million of these hemoglobin biomolecules, each carrying four heme groups; hemoglobin comprises about a third of the total cell volume. This protein is responsible for the transport of more than 98% of the oxygen (the remaining oxygen is carried dissolved in the blood plasma). The red blood cells of an average adult human male store collectively about 2.5 grams of iron, representing about 65% of the total iron contained in the body.

Life cycle

Human erythrocytes are produced through a process named erythropoiesis, developing from committed stem cells to mature erythrocytes in about 7 days. When matured, in a healthy individual these cells live in blood circulation for about 100 to 120 days (and 80 to 90 days in a full term infant). At the end of their lifespan, they become senescent, and are removed from circulation. In many chronic diseases, the lifespan of the erythrocytes is markedly reduced (e.g. patients requiring haemodialysis).

Erythropoiesis

Erythropoiesis is the development process by which new erythrocytes are produced; it lasts about 7 days. Through this process erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second in a healthy adult. (In the embryo, the liver is the main site of red blood cell production.) The production can be stimulated by the hormone the developing cells are known as reticulocytes; these comprise about 1% of circulating red blood cells.

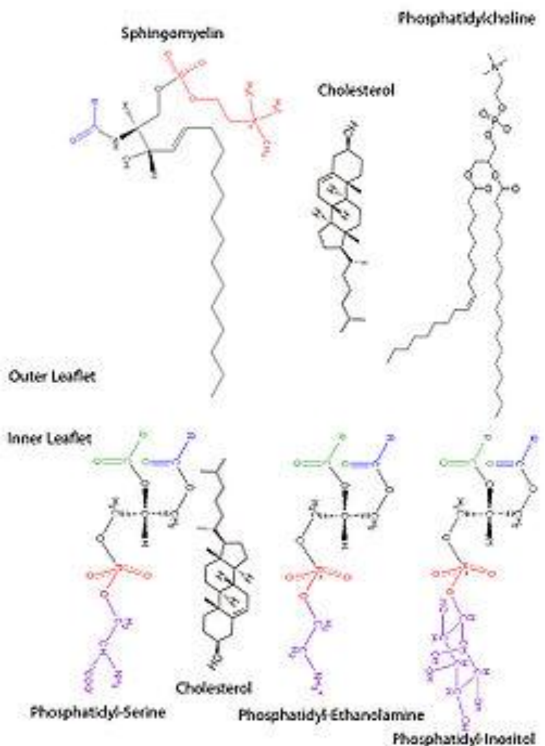
Functional lifetime

The functional lifetime of an erythrocyte is about 100–120 days, during which time the erythrocytes are continually moved by the blood flow push (in arteries), pull (in veins) and a combination of the two as they squeeze through microvessels such as capillaries.

Senescence

The aging erythrocyte undergoes changes in its plasma membrane, making it susceptible to selective recognition by macrophages and subsequent phagocytosis in the mononuclear phagocyte system (spleen, liver and lymph nodes), thus removing old and defective cells and continually purging the blood. This process is termed eryptosis, erythrocyte programmed cell death. This process normally occurs at the same rate of production by erythropoiesis, balancing the total circulating red blood cell count. Eryptosis is increased in a wide variety of diseases including sepsis, haemolytic uremic syndrome, malaria, sickle cell anemia, beta-thalassemia, glucose-6-phosphate dehydrogenase deficiency, phosphate depletion, iron deficiency and Wilson's disease. Eryptosis can be elicited by osmotic shock, oxidative stress, energy depletion as well as a wide variety of endogenous mediators and xenobiotics. Excessive eryptosis is observed in erythrocytes lacking the cGMP-dependent protein kinase type I or the AMP-activated protein kinase AMPK. Inhibitors of eryptosis include erythropoietin, nitric oxide, catecholamines and high concentrations of urea.

Much of the resulting breakdown products are recirculated in the body. The heme constituent of hemoglobin are broken down into Fe^{3+} and biliverdin. The biliverdin is reduced to bilirubin, which is released into the plasma and recirculated to the liver bound to albumin. The iron is released into the plasma to be recirculated by a carrier protein called transferrin. Almost all erythrocytes are removed in this manner from the circulation before they are old enough to hemolyze. Hemolyzed hemoglobin is bound to a protein in plasma called haptoglobin, which is not excreted by the kidney.



composition

The membrane of the red blood cell plays many roles that aid in regulating their surface deformability, flexibility, adhesion to other cells and immune recognition. These functions are highly dependent on its composition, which defines its properties. The red blood cell membrane is composed of 3 layers: the glycocalyx on the exterior, which is rich in carbohydrates; the lipid bilayer which contains many transmembrane proteins, besides its lipidic main constituents; and the membrane skeleton, a structural network of proteins located on the inner surface of the lipid bilayer. Half of the membrane mass in human and most mammalian erythrocytes are proteins. The other half are lipids, namely phospholipids and cholesterol.

Membrane lipids

The most common erythrocyte cell membrane lipids, schematically disposed as they are distributed on the bilayer. Relative abundances are not at scale.

The erythrocyte cell membrane comprises a typical lipid bilayer, similar to what can be found in virtually all human cells. Simply put, this lipid bilayer is composed of cholesterol and phospholipids in equal proportions by weight. The lipid composition is important as it defines many physical properties such as membrane permeability and fluidity. Additionally, the activity of many membrane proteins is regulated by interactions with lipids in the bilayer.

Unlike cholesterol, which is evenly distributed between the inner and outer leaflets, the 5 major phospholipids are asymmetrically disposed, as shown below:

Outer monolayer

- Phosphatidylcholine (PC);
- Sphingomyelin (SM).

Inner monolayer

- Phosphatidylethanolamine (PE);
- Phosphoinositol (PI) (small amounts).
- Phosphatidylserine (PS);

This asymmetric phospholipid distribution among the bilayer is the result of the function of several energy-dependent and energy-independent phospholipid transport proteins. Proteins called “Flippases” move phospholipids from the outer to the inner monolayer, while others called “floppases” do the opposite operation, against a concentration gradient in an energy dependent manner. Additionally, there are also “scramblase” proteins that move phospholipids in both directions at the same time, down their concentration gradients in an energy independent manner. There is still considerable debate ongoing regarding the identity of these membrane maintenance proteins in the red cell membrane.

The maintenance of an asymmetric phospholipid distribution in the bilayer (such as an exclusive localization of PS and PIs in the inner monolayer) is critical for the cell integrity and function due to several reasons:

- Macrophages recognize and phagocytose red cells that expose PS at their outer surface. Thus the confinement of PS in the inner monolayer is essential if the cell is to survive its frequent encounters with macrophages of the reticuloendothelial system, especially in the spleen.

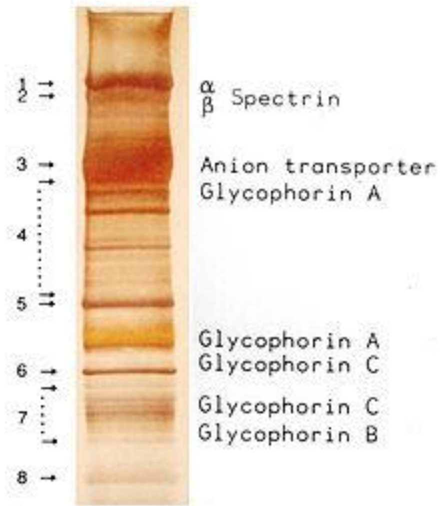
- Premature destruction of thallassemic and sickle red cells has been linked to disruptions of lipid asymmetry leading to exposure of PS on the outer monolayer.

- An exposure of PS can potentiate adhesion of red cells to vascular endothelial cells, effectively preventing normal transit through the microvasculature. Thus it is important that PS is maintained only in the inner leaflet of the bilayer to ensure normal blood flow in microcirculation.

- Both PS and phosphatidylinositol-4,5-bisphosphate (PIP2) can regulate membrane mechanical function, due to their interactions with skeletal proteins such as spectrin and protein 4.1R. Recent studies have shown that binding of spectrin to PS promotes membrane mechanical stability. PIP2 enhances the binding of protein band 4.1R to glycophorin C but decreases its interaction with protein band 3, and thereby may modulate the linkage of the bilayer to the membrane skeleton.

The presence of specialized structures named "lipid rafts" in the erythrocyte membrane have been described by recent studies. These are structures enriched in cholesterol and sphingolipids associated with specific membrane proteins, namely flotillins, stomatins (band 7), G-proteins, and β-adrenergic receptors. Lipid rafts that have been implicated in cell signaling events in nonerythroid cells have been shown in erythroid cells to mediate β2-adregenic receptor signaling and increase cAMP levels, and thus regulating entry of malarial parasites into normal red cells.[38][39]

__Membrane proteins__

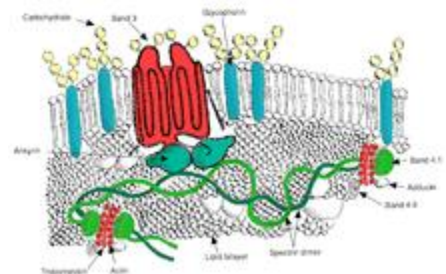


Red blood cell membrane proteins separated by SDS-Page and silverstained

The proteins of the membrane skeleton are responsible for the deformability, flexibility and durability of the red blood cell, enabling it to squeeze through capillaries less than half the diameter of the erythrocyte (7–8 μm) and recovering the discoid shape as soon as these cells stop receiving compressive forces, in a similar fashion to an object made of rubber.

There are currently more than 50 known membrane proteins, which can exist in a few hundred up to a million copies per erythrocyte. Approximately 25 of these membrane proteins carry the various blood group antigens, such as the A, B and Rh antigens, among many others. These membrane proteins can perform a wide diversity of functions, such as transporting ions and molecules across the red cell membrane, adhesion and interaction with other cells such as endothelial cells, as signaling receptors, as well as other currently unknown functions. The blood types of humans are due to variations in surface glycoproteins of erythrocytes. Disorders of the proteins in these membranes are associated with many disorders, such as hereditary spherocytosis, hereditary elliptocytosis, hereditary stomatocytosis, and paroxysmal nocturnal hemoglobinuria.

The red blood cell membrane proteins organized according to their function:



Red Blood Cell membrane major proteins

Transport

- Band 3 – Anion transporter, also an important structural component of the erythrocyte cell membrane, makes up to 25% of the cell membrane surface, each red cell contains approximately one million copies. Defines the Diego Blood Group
- Aquaporin 1 – water transporter, defines the Colton Blood Group;
- Glut1 – glucose and L-dehydroascorbic acid transporter;
- Kidd antigen protein – urea transporter;
- RhAG – gas transporter, probably of carbon dioxide, defines Rh Blood Group and the associated unusual blood group phenotype Rh_{null};
- Na^+/K^+ – ATPase;
- Ca^{2+} – ATPase;
- Na^+ K^+ $2Cl^-$ – cotransporter;
- Na^+-Cl^- – cotransporter;
- Na-H exchanger;
- K-Cl – cotransporter;
- Gardos Channel.

Cell adhesion

- ICAM-4 – interacts with integrins;
- BCAM – a glycoprotein that defines the Lutheran blood group and also known as Lu or laminin-binding protein.

Structural role – The following membrane proteins establish linkages with skeletal proteins and may play an important role in regulating cohesion between the lipid bilayer and membrane skeleton, likely enabling the red cell to maintain its favorable membrane surface area by preventing the membrane from collapsing (vesiculating).

- Ankyrin-based macromolecular complex – proteins linking the bilayer to the membrane skeleton through the interaction of their cytoplasmic domains with Ankyrin.
  - Band 3 – also assembles various glycolytic enzymes, the presumptive CO_2 transporter, and carbonic anhydrase into a macromolecular complex termed a "metabolon," which may play a key role in regulating red cell metabolism and ion and gas transport function);
  - RhAG – also involved in transport, defines associated unusual blood group phenotype Rh_{mod}.

- Protein 4.1R-based macromolecular complex – proteins interacting with Protein 4.1R.
  - Protein 4.1R – weak expression of Gerbich antigens;
  - Glycophorin C and D – glycoprotein, defines Gerbich Blood Group;
  - XK – defines the Kell Blood Group and the Mcleod unusual phenotype (lack of Kx antigen and greatly reduced expression of Kell antigens);

- RhD/RhCE – defines Rh Blood Group and the associated unusual blood group phenotype Rh_{null};
- Duffy protein – has been proposed to be associated with chemokine clearance;[42]
- Adducin – interaction with band 3;
- Dematin- interaction with the Glut1 glucose transporter.

Surface electrostatic potential

The zeta potential is an electrochemical property of cell surfaces that is determined by the net electrical charge of molecules exposed at the surface of cell membranes of the cell. The normal zeta potential of the erythrocyte is −15.7 millivolts (mV). Much of this potential appears to be contributed by the exposed sialic acid residues in the membrane: their removal results in zeta potential of −6.06 mV.

Clinical notes

Separation and blood doping

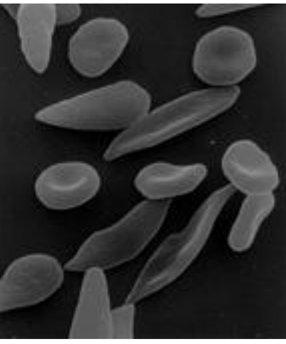
Red blood cells can be obtained from whole blood by centrifugation, which separates the cells from the blood plasma in a process known as blood fractionation. Packed red blood cells, which are made in this way from whole blood with the plasma removed, are used in transfusion medicine.[44] During plasma donation, the red blood cells are pumped back into the body right away and only the plasma is collected.

Some athletes have tried to improve their performance by blood doping: first about 1 litre of their blood is extracted, then the red blood cells are isolated, frozen and stored, to be reinjected shortly before the competition. (Red blood cells can be conserved for 5 weeks at −79 °C or −110 °F) This practice is hard to detect but may endanger the human cardiovascular system which is not equipped to deal with blood of the resulting higher viscosity. Another method of blood doping involves injection with erythropoietin in order to stimulate production of red blood cells. Both practices are banned by the World Anti-Doping Agency.

Artificially grown red blood cells

In 2008 it was reported that human embryonic stem cells had been successfully coaxed into becoming erythrocytes in the lab. The difficult step was to induce the cells to eject their nucleus; this was achieved by growing the cells on stromal cells from the bone marrow. It is hoped that these artificial erythrocytes can eventually be used for blood transfusions.[45]

Diseases and diagnostic tools

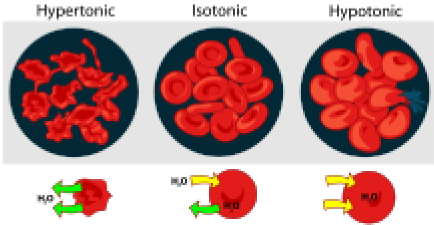


Affected by Sickle-cell disease, red blood cells alter shape and threaten to damage internal organs.

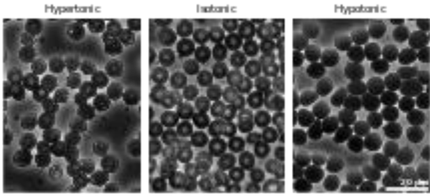
Blood diseases involving the red blood cells include:

- Anemias (or anaemias) are diseases characterized by low oxygen transport capacity of the blood, because of low red cell count or some abnormality of the red blood cells or the hemoglobin.
  - Iron deficiency anemia is the most common anemia; it occurs when the dietary intake or absorption of iron is insufficient, and hemoglobin, which contains iron, cannot be formed
  - Sickle-cell disease is a genetic disease that results in abnormal hemoglobin molecules. When these release their oxygen load in the tissues, they become insoluble, leading to mis-shaped red blood cells. These sickle shaped red cells are less deformable and viscoelastic meaning that they have become rigid and can cause blood vessel blockage, pain, strokes, and other tissue damage.
  - Thalassemia is a genetic disease that results in the production of an abnormal ratio of hemoglobin subunits.
  - Hereditary spherocytosis syndromes are a group of inherited disorders characterized by defects in the red blood cell's cell membrane, causing the cells to be small, sphere-shaped, and fragile instead of donut-shaped and flexible. These abnormal red blood cells are destroyed by the spleen. Several other hereditary disorders of the red blood cell membrane are known.
  - Pernicious anemia is an autoimmune disease wherein the body lacks intrinsic factor, required to absorb vitamin B_{12} from food. Vitamin B_{12} is needed for the production of hemoglobin.
  - Aplastic anemia is caused by the inability of the bone marrow to produce blood cells.

- Pure red cell aplasia is caused by the inability of the bone marrow to produce only red blood cells.



Effect of osmotic pressure on blood cells



Micrographs of the effects of osmotic pressure

- Hemolysis is the general term for excessive breakdown of red blood cells. It can have several causes and can result in hemolytic anemia.

    - The malaria parasite spends part of its life-cycle in red blood cells, feeds on their hemoglobin and then breaks them apart, causing fever. Both sickle-cell disease and thalassemia are more common in malaria areas, because these mutations convey some protection against the parasite.

- Polycythemias (or erythrocytoses) are diseases characterized by a surplus of red blood cells. The increased viscosity of the blood can cause a number of symptoms.

    - In polycythemia vera the increased number of red blood cells results from an abnormality in the bone marrow.

- Several microangiopathic diseases, including disseminated intravascular coagulation and thrombotic microangiopathies, present with pathognomonic (diagnostic) red blood cell fragments called schistocytes. These pathologies generate fibrin strands that sever red blood cells as they try to move past a thrombus.

- Hemolytic transfusion reaction is the destruction of donated red blood cells after a transfusion, mediated by host antibodies, often as a result of a blood type mismatch.

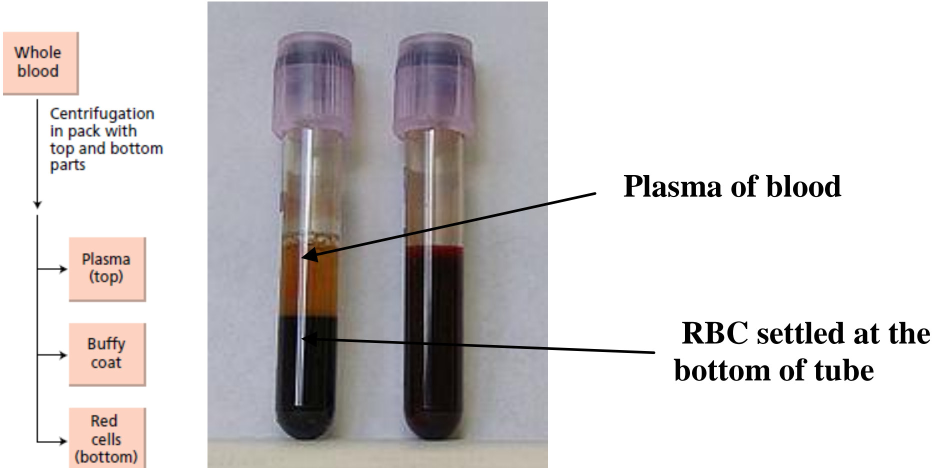
Several blood tests involve red blood cells, including the *RBC count* (the number of red blood cells per volume of blood), the hematocrit (percentage of blood volume occupied by red blood cells), and the erythrocyte sedimentation rate. Many diseases involving red blood cells are

diagnosed with a blood film (or peripheral blood smear), where a thin layer of blood is smeared on a microscope slide. The blood type needs to be determined to prepare for a blood transfusion or an organ transplantation.

Hematocrit

The **hematocrit** (**Ht** or **HCT**, British English spelling **haematocrit**), also known as **packed cell volume** (**PCV**) or **erythrocyte volume fraction** (EVF), is the volume percentage (%) of red blood cells in blood. It is normally 45% for men and 40% for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count. Anemia refers to an abnormally low hematocrit, as opposed to polycythemia, which refers to an abnormally high hematocrit. Both are potentially life-threatening disorders.

The packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes. This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Since a tube is used, this can be calculated by measuring the lengths of the layers.



With modern lab equipment, the hematocrit is calculated by an automated analyzer and not directly measured. It is determined by multiplying the red cell count by the mean cell volume. The hematocrit is slightly more accurate as the PCV includes small amounts of blood plasma trapped between the red cells. An estimated hematocrit as a percentage may be derived by tripling the hemoglobin concentration in g/dL and dropping the units.

There have been cases in which the blood for testing was inadvertently drawn proximal to an intravenous line that was infusing packed red cells or fluids. In

these situations, the hemoglobin level in the blood sample will not be the true level for the patient because the sample will contain a large amount of the infused material rather than what is diluted into the circulating whole blood. That is, if packed red cells are being supplied, the sample will contain a large amount of those cells and the hematocrit will be artificially very high. On the converse, if saline or other fluids are being supplied, the blood sample would be diluted and the hematocrit will be artificially low.

Elevated:-

In cases of dengue fever, a high hematocrit is a danger sign of an increased risk of dengue shock syndrome.

Polycythemia vera (PV), a myeloproliferative disorder in which the bone marrow produces excessive numbers of red cells, is associated with elevated hematocrit.

Chronic obstructive pulmonary disease (COPD) and other pulmonary conditions associated with hypoxia may elicit an increased production of red blood cells. This increase is mediated by the increased levels of erythropoietin by the kidneys in response to hypoxia.

Professional athletes' hematocrit levels are measured as part of tests for blood doping or erythropoietin (EPO) use; the level of hematocrit in a blood sample is compared with the long-term level for that athlete (to allow for individual variations in hematocrit level), and against an absolute permitted maximum (which is based on maximum expected levels within the population, and the hematocrit level that causes increased risk of blood clots resulting in strokes or heart attacks).

Anabolic androgenic steroid (AAS) use can also increase the amount of RBCs and, therefore, impact the hematocrit, in particular the compounds boldenone and oxymetholone.

If a patient is dehydrated, the hematocrit may be elevated.

Capillary leak syndrome also leads to abnormally high hematocrit counts, because of the episodic leakage of plasma out of the circulatory system.

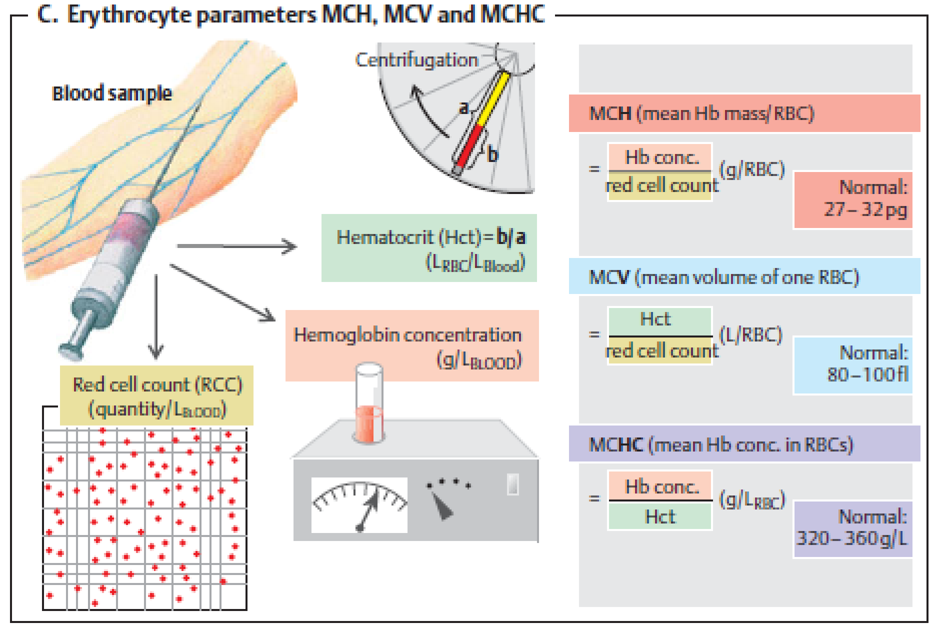
Sleep apnea has been known to cause elevated hematocrit levels.

Lowered :-

The mean corpuscular volume (MCV) and the red cell distribution width (RDW) can be quite helpful in evaluating a lower-than-normal hematocrit, because they can help the clinician determine whether blood loss is chronic or acute, although acute blood loss typically does not manifest as a change in hematocrit, since hematocrit is simply a measure of how much of the blood volume is made up of red blood cells. The MCV is the size of the red cells and the RDW is a relative measure of the variation in size of the red cell population. A low hematocrit with a low MCV with a high RDW suggests a chronic iron-deficient anemia resulting in abnormal hemoglobin synthesis during erythropoiesis. One unit of packed red blood cells will elevate the hematocrit by about 3%.

Groups of individuals at risk for developing anemia include:

- infants without adequate iron intake
- children going through a rapid growth spurt, during which the iron available cannot keep up with the demands for a growing red cell mass
- menstruating women, who have a greater need for iron because of blood loss during menstruation
- pregnant women, in whom the growing fetus creates a high demand for iron
- patients with chronic kidney disease whose kidneys no longer secrete sufficient levels of the hormone erythropoietin that promotes RBC proliferation. Erythropoietin prevents the death of cells in the erythrocyte cell line in the bone marrow. Therefore, erythropoietin allows those cells to continue to mature, exit the bone marrow and become RBCs.



ABO Blood Group System

The **ABO blood group system** is the most important blood type system (or blood group system) in human blood transfusion. The associated anti-A and anti-B antibodies are usually IgM antibodies, which are produced in the first years of life by sensitization to environmental substances, such as food, bacteria, and viruses. ABO blood types are also present in some other animals, for example rodents and apes, such as chimpanzees, bonobos, and gorillas

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

History of discoveries of the blood types

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who identified the O, A, and B blood types in 1900. Landsteiner originally described the O blood type as type "C", and in parts of Europe it is rendered as "0" (zero), signifying the lack of A or B antigen. Landsteiner was awarded the Nobel Prize in Physiology or Medicine in 1930 for his work. Alfred von Decastello and Adriano Sturli discovered the fourth type, AB, in 1902.

Ukraine marine uniform imprint, showing the wearer's blood type as "B (III) Rh+"

Due to inadequate communication at the time, it was subsequently found that the Czech serologist Jan Janský had independently pioneered the classification of human blood into four groups, but Landsteiner's independent discovery had been accepted by the scientific world while Janský remained in relative obscurity. Janský's nomenclature is, however, still used in Russia and states of the former USSR, in which blood types O, A, B, and AB are respectively designated I, II, III, and IV.[5] The designation A and B with reference to blood groups was proposed by Ludwik Hirszfeld.

In America, W.L. Moss published his own (very similar) work in 1910.

Ludwik Hirszfeld and E. von Dungern discovered the heritability of ABO blood groups. Felix Bernstein demonstrating the correct blood group inheritance pattern of multiple alleles at one locus in 1924. Watkins and Morgan, in England, discovered that the ABO epitopes were conferred by sugars, to be specific, N-acetylgalactosamine for the A-type and galactose for the B-type. After much published literature claiming that the ABH substances were all attached to glycosphingolipids, Finne et al. (1978) found that the human erythrocyte glycoproteins contain polylactosamine chains that contains ABH substances attached and represent the majority of the antigens. The main glycoproteins carrying the ABH antigens were identified to be the Band 3 and Band 4.5 proteins and glycophorin. Later, Yamamoto's group showed the precise glycosyl

Antigens

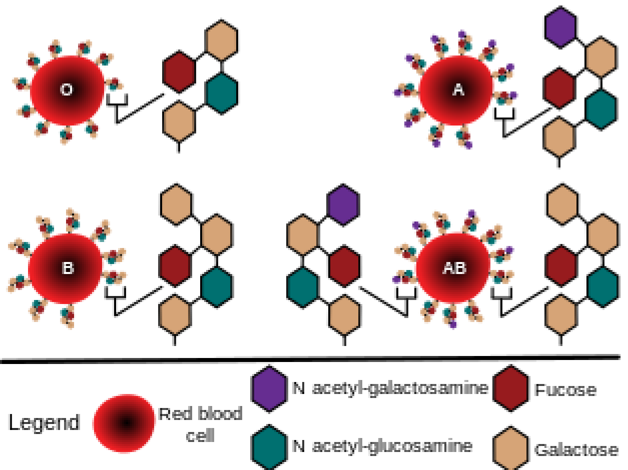
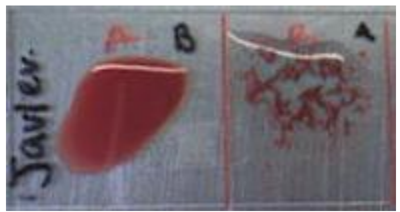


Diagram showing the carbohydrate chains that determine the ABO blood group



Student blood test. Three drops of blood are mixed with anti-B (left) and anti-A (right) serum. Agglutination on the right side indicates blood type A.

The central principle of the ABO system is that antigens – in this instance, sugars physically exposed on the exterior of red blood cells – differ between individuals, who have immunological tolerance only toward what occurs in their own bodies. As a result, many humans express isoantibodies – antibodies against isoantigens, natural components present in the bodies of other members of the same species but not themselves. Isoantibodies may be present against the A and/or B antigens in people who do not themselves have the same antigens in their own blood. These antibodies act as haemagglutinins, which cause blood cells to clump and break apart if they carry the foreign antigens. This harsh response, though an adaptive reaction useful against infection, can cause death when large amounts of such cells are encountered after a blood transfusion, a circumstance not encountered in natural selection prior to modern history. Because A and B antigens are chemically modified from a precursor form that is also present in type O individuals, people with type A and B antigens can accept blood from type O individuals.

Anti-A and anti-B antibodies (called *isohaemagglutinins*), which are not present in the newborn, appear in the first years of life. Anti-A and anti-B antibodies are usually IgM type, which are not able to pass through the placenta to the fetal blood circulation. O-type individuals can produce IgG-type ABO antibodies.

The precursor to the ABO blood group antigens, present in people of all common blood types, is called the H antigen. Individuals with the rare Bombay phenotype (*hh*) do not express antigen H on their red blood cells. As the H antigen serves as a precursor for producing A and B antigens, the absence of the H antigen means that the individuals also lack A or B antigens as well (similar to O blood group). However, unlike O group, the H antigen is absent, hence the individuals produce isoantibodies to antigen H as well as to both A and B antigens. If they receive blood from someone with O blood group, the anti-H antibodies will bind to the H antigen on the red blood cells ('RBC') of the donor blood and destroy the RBCs by complement-mediated lysis. Therefore, people with Bombay phenotype can receive blood only from other *hh* donors (although they can donate as though they

were type O). Some individuals with the blood group A1 may also be able to produce anti-H antibodies due to the complete conversion of all the H antigen to A1 antigen.

Production of the H antigen, or its deficiency in the Bombay phenotype, is controlled at the H locus on chromosome 19. The H locus is not the same gene as the ABO locus, but it is epistatic to the ABO locus, providing the substrate for the A and B alleles to modify.[17] The H locus contains three exons that span more than 5 kb of genomic DNA, and encodes the fucosyltransferase that produces the H antigen on RBCs. The H antigen is a carbohydrate sequence with carbohydrates linked mainly to protein (with a minor fraction attached to ceramide moiety). It consists of a chain of β-D-galactose, β-D-N-acetylglucosamine, β-D-galactose, and 2-linked, α-L-fucose, the chain being attached to the protein or ceramide.

The ABO locus, which is located on chromosome 9, contains seven exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. The ABO locus has three main alleleic forms: A, B, and O. The A allele encodes a *glycosyltransferase* that bonds α-N-acetylgalactosamine to the D-galactose end of the H antigen, producing the A antigen. The B allele encodes a *glycosyltransferase* that bonds α-D-galactose to the D-galactose end of the H antigen, creating the B antigen.

In the case of the O allele, when compared to the A allele, exon 6 lacks one nucleotide (guanine), which results in a loss of enzymatic activity. This difference, which occurs at position 261, causes a frame shift that results in the premature termination of the translation and, thus, degradation of the mRNA. This results in the H antigen remaining unchanged in the case of O groups.

The majority of the ABO antigens are expressed on the ends of long polylactosamine chains attached mainly to band 3 protein, the anion exchange protein of the RBC membrane, and a minority of the epitopes are expressed on neutral glycosphingolipid.

Role of ABO antigens in transfusion medicine

For a blood donor and recipient to be ABO-compatible for a transfusion, the recipient must not be able to produce Anti-A or Anti-B antibodies that correspond to the A or B antigens on the surface of the donor's red blood cells (since the red blood cells are isolated from whole blood before transfusion, it is unimportant whether the donor blood has antibodies in its plasma). If the antibodies of the recipient's blood and the antigens on the donor's red blood cells do correspond, the

donor blood is rejected. On rejection, the recipient may experience Acute hemolytic transfusion reaction (AHTR).

In addition to the ABO system, the Rh blood group system can affect transfusion compatibility. An individual is either positive or negative for the Rh factor; this is denoted by a '+' or '−' after their ABO type. Blood that is Rh-negative can be transfused into a person who is Rh-positive, but an Rh-negative individual can create antibodies for Rh-positive RBCs.

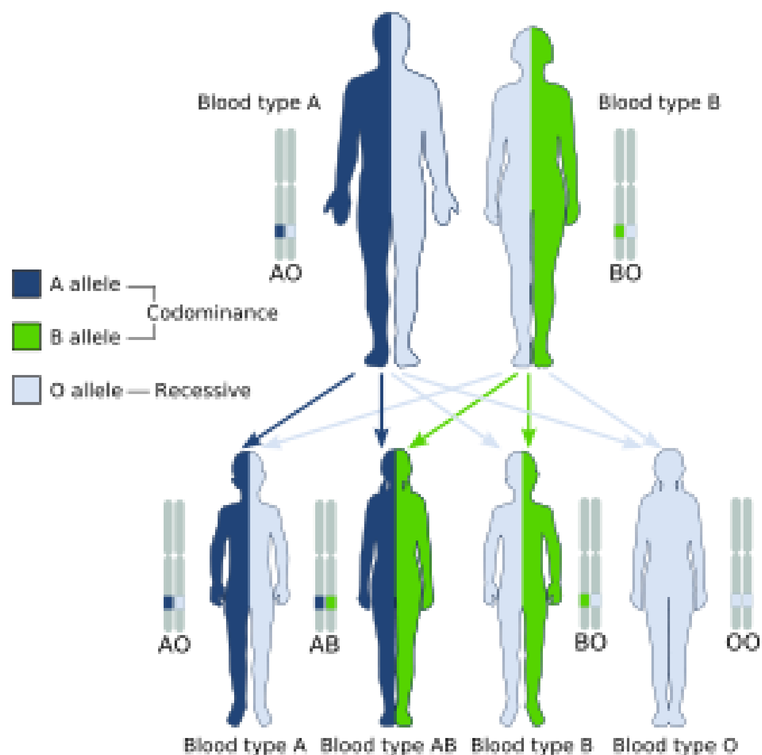
Because of this, the AB+ blood type is referred to as the "universal recipient", as it possesses neither Anti-B or Anti-A antibodies in its plasma, and can receive both Rh-positive and Rh-negative blood. Similarly, the O− blood type is called the "universal donor"; since its red blood cells have no A or B antigens and are Rh-negative, no other blood type will reject it.

Identification of ABO and Rh gene frequencies among human populations has various benefits in transfusion medicine, transplantation and disease risk.[18]

ABO and Rh blood type donation showing matches between donor and recipient types

		Donors							
		O+	**A+**	**B+**	**AB+**	**O−**	**A−**	**B−**	**AB−**
	O+	✔				✔			
	A+	✔	✔			✔	✔		
	B+	✔		✔		✔		✔	
Recipients	**AB+**	✔	✔	✔	✔	✔	✔	✔	✔
	O−					✔			
	A−					✔	✔		
	B−					✔		✔	
	AB−					✔	✔	✔	✔

Genetics



A and B are codominant, giving the AB phenotype.

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three types of alleles inferred from classical genetics: i, I^A, and I^B. The gene encodes a glycosyltransferase—that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens. The gene is located on the long arm of the ninth chromosome (9q34).

The I^A allele gives type A, I^B gives type B, and i gives type O. As both I^A and I^B are dominant over i, only ii people have type O blood. Individuals with I^AI^A or I^Ai have type A blood, and individuals with I^BI^B or I^Bi have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship: codominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi,I^Ai) The *cis-AB* phenotype has a single enzyme that creates both A and B antigens. The resulting red blood cells do not usually express A or B antigen at the same level that would be expected on common group A_1 or B red

blood cells, which can help solve the problem of an apparently genetically impossible blood group.

Blood group inheritance

Blood type		**O**	**A**		**B**		**AB**
	Genotype	***ii* (OO)**	**$I^A i$ (AO)**	**$I^A I^A$ (AA)**	**$I^B i$ (BO)**	**$I^B I^B$ (BB)**	**$I^A I^B$ (AB)**
O	***ii* (OO)**	O OO OO OO OO	O or A AO OO AO OO	A AO AO AO AO	O or B BO OO BO OO	B BO BO BO BO	A or B AO BO AO BO
A	**$I^A i$ (AO)**	O or A AO AO OO OO	O or A AA AO AO OO	A AA AA AO AO	O, A, B or AB AB AO BO OO	B or AB AB AB BO BO	A, B or AB AA AB AO BO
	$I^A I^A$ (AA)	A AO AO AO AO	A AA AO AA AO	A AA AA AA AA	A or AB AB AO AB AO	AB AB AB AB AB	A or AB AA AB AA AB
B	**$I^B i$ (BO)**	O or B BO BO OO OO	O, A, B or AB AB BO AO OO	A or AB AB AB AO AO	O or B BB BO BO OO	B BB BB BO BO	A, B or AB AB BB AO BO
	$I^B I^B$ (BB)	B BO BO BO BO	B or AB AB BO AB BO	AB AB AB AB AB	B BB BO BB BO	B BB BB BB BB	B or AB AB BB AB BB
AB	**$I^A I^B$ (AB)**	A or B AO AO BO BO	A, B or AB AA AO AB BO	A or AB AA AA AB AB	A, B or AB AB AO BB BO	B or AB AB AB BB BB	A, B, or AB AA AB AB BB

The table above summarizes the various blood groups children may inherit from their parents. Genotypes are shown in the second column and in small print for the offspring: AO and AA both test as type A; BO and BB test as type B. The four possibilities represent the combinations obtained when one allele is taken from each parent; each has a 25% chance, but some occur more than once.

Blood group inheritance by phenotype only

Blood type	O	A	B	AB
O	O	O or A	O or B	A or B
A	O or A	O or A	O, A, B or AB	A, B or AB
B	O or B	O, A, B or AB	O or B	A, B or AB
AB	A or B	A, B or AB	A, B or AB	A, B or AB

Historically, ABO blood tests were used in parental testing, but in 1957 only 50% of American men falsely accused were able to use them as evidence against paternity. Occasionally, the blood types of children are not consistent with expectations—for example, a type O child can be born to an AB parent—due to rare situations, such as Bombay phenotype and cis AB.

Subgroups

The A blood type contains about twenty subgroups, of which A1 and A2 are the most common (over 99%). A1 makes up about 80% of all A-type blood, with A2 making up almost all of the rest. These two subgroups are interchangeable as far as transfusion is concerned, but complications can sometimes arise in rare cases when typing the blood.

With the development of DNA sequencing, it has been possible to identify a much larger number of alleles at the ABO locus, each of which can be categorized as A, B, or O in terms of the reaction to transfusion, but which can be distinguished by variations in the DNA sequence. There are six common alleles in white individuals of the ABO gene that produce one's blood type:

A	**B**	**O**
A101 (A1)	*B101* (B1)	*O01* (O1)
A201 (A2)		*O02* (O1v)
		O03 (O2)

The same study also identified 18 rare alleles, which generally have a weaker glycosylation activity. People with weak alleles of A can sometimes express anti-A antibodies, though these are usually not clinically significant as they do not stably interact with the antigen at body temperature.

Cis AB is another rare variant, in which A and B genes are transmitted together from a single parent.

Distribution and evolutionary history

The distribution of the blood groups A, B, O and AB varies across the world according to the population. There are also variations in blood type distribution within human subpopulations.

In the UK, the distribution of blood type frequencies through the population still shows some correlation to the distribution of placenames and to the successive invasions and migrations including Vikings, Danes, Saxons, Celts, and Normans who contributed the morphemes to the placenames and the genes to the population.

The two common O alleles, O01 and O02, share their first 261 nucleotides with the group A allele A01. However, unlike the group A allele, a guanosine base is subsequently deleted. A premature stop codon results from this frame-shift mutation. This variant is found worldwide, and likely predates human migration from Africa. The O01 allele is considered to predate the O02 allele.

Some evolutionary biologists theorize that the I^A allele evolved earliest, followed by *O* (by the deletion of a single nucleotide, shifting the reading frame) and then I^B. This chronology accounts for the percentage of people worldwide with each blood type. It is consistent with the accepted patterns of early population movements and varying prevalent blood types in different parts of the world: for instance, B is very common in populations of Asian descent, but rare in ones of Western European descent. Another theory states that there are four main lineages of the ABO gene and that mutations creating type O have occurred at least three times in humans.

From oldest to youngest, these lineages comprise the following alleles: *A101/A201/O09*, *B101*, *O02* and *O01*. The continued presence of the O alleles is hypothesized to be the result of balancing selection. Both theories contradict the previously held theory that type O blood evolved earliest.

Origin theories

It is possible that food and environmental antigens (bacterial, viral, or plant antigens) have epitopes similar enough to A and B glycoprotein antigens. The antibodies created against these environmental antigens in the first years of life can cross-react with ABO-incompatible red blood cells that it comes in contact with during blood transfusion later in life. Anti-A antibodies are hypothesized to originate from immune response towards influenza virus, whose epitopes are similar enough to the α-D-N-galactosamine on the A glycoprotein to be able to elicit a cross-reaction. Anti-B antibodies are hypothesized to originate from antibodies produced against Gram-negative bacteria, such as *E. coli*, cross-reacting with the α-D-galactose on the B glycoprotein.

HIV can be neutralized in *in vitro* experiments using antibodies against blood group antigens specifically expressed on the HIV-producing cell lines.

However, it is more likely that the force driving evolution of allele diversity is simply negative frequency-dependent selection; cells with rare variants of membrane antigens are more easily distinguished by the immune system from pathogens carrying antigens from other hosts. Thus, individuals possessing rare types are better equipped to detect pathogens. The high within-population diversity observed in human populations would, then, be a consequence of natural selection on individuals.

Normal role in the body

The carbohydrate molecules on the surfaces of red blood cells have roles in cell membrane integrity, cell adhesion, membrane transportation of molecules, and acting as receptors for extracellular ligands, and enzymes. ABO antigens are found having similar roles on epithelial cells as well as red blood cells.

Bleeding and thrombosis (von Willebrand factor)

The ABO antigen is also expressed on the von Willebrand factor (vWF) glycoprotein, which participates in hemostasis (control of bleeding). In fact, having type O blood predisposes to bleeding, as 30% of the total genetic variation

observed in plasma vWF is explained by the effect of the ABO blood group, and individuals with group O blood normally have significantly lower plasma levels of vWF (and Factor VIII) than do non-O individuals. In addition, vWF is degraded more rapidly due to the higher prevalence of blood group O with the Cys1584 variant of vWF (an amino acid polymorphism in VWF): the gene for ADAMTS13 (vWF-cleaving protease) maps to the ninth chromosome (9q34), the same locus as ABO blood type. Higher levels of vWF are more common amongst people who have had ischaemic stroke (from blood clotting) for the first time. The results of this study found that the occurrence was not affected by ADAMTS13 polymorphism, and the only significant genetic factor was the person's blood group.

Disease risks

Compared to O group individuals, non-O group (A, AB, and B) individuals have a 14% reduced risk of squamous cell carcinoma and 4% reduced risk of basal cell carcinoma. Conversely, type O blood is associated with a reduced risk of pancreatic cancer. The B antigen links with increased risk of ovarian cancer. Gastric cancer has reported to be more common in blood group A and least in group O.

According to Glass, Holmgren, et al., those in the O blood group have an increased risk of infection with cholera, and those O-group individuals who are infected have more severe infections. The mechanisms behind this association with cholera are currently unclear in the literature.

ABO hemolytic disease of the newborn

Hemolytic disease of the newborn (ABO)

ABO blood group incompatibilities between the mother and child does not usually cause hemolytic disease of the newborn (HDN) because antibodies to the ABO blood groups are usually of the IgM type, which do not cross the placenta. However, in an O-type mother, **IgG** ABO antibodies are produced and the baby can potentially develop ABO hemolytic disease of the newborn.

Pseudoscience

During the 1930s, connecting blood groups to personality types became popular in Japan and other areas of the world. On the contrary, there are some positive science studies.

Other popular but unsupported ideas include the use of a blood type diet, claims that group A causes severe hangovers, group O is associated with perfect teeth, and those with blood group A2 have the highest IQs. Scientific evidence in support of these concepts is nonexistent.