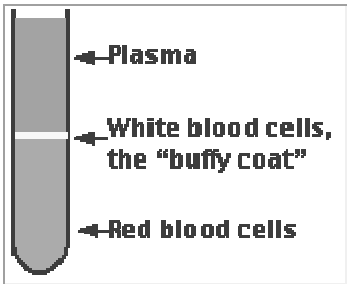
Blood

Blood is a liquid tissue. Suspended in the watery **plasma** are seven types of cells and cell fragments: **Red blood cells** (**RBC**s) or **erythrocytes**, **platelets** or **thrombocytes** and five kinds of **white blood cells** (**WBC**s) or **leukocytes**. Three kinds of **granulocytes neutrophils**, **eosinophils** and **basophils** and Two kinds of leukocytes without granules in their cytoplasm **lymphocytes** and **monocytes**

If one takes a sample of blood, treats it with an agent to prevent clotting, and spins it in a centrifuge, the red cells settle to the bottom. The white cells settle on top of them forming the "buffy coat".
The fraction occupied by the red cells is called the **hematocrit**. Normally it is approximately 45%. Values much lower than this are a sign of **anaemia**.

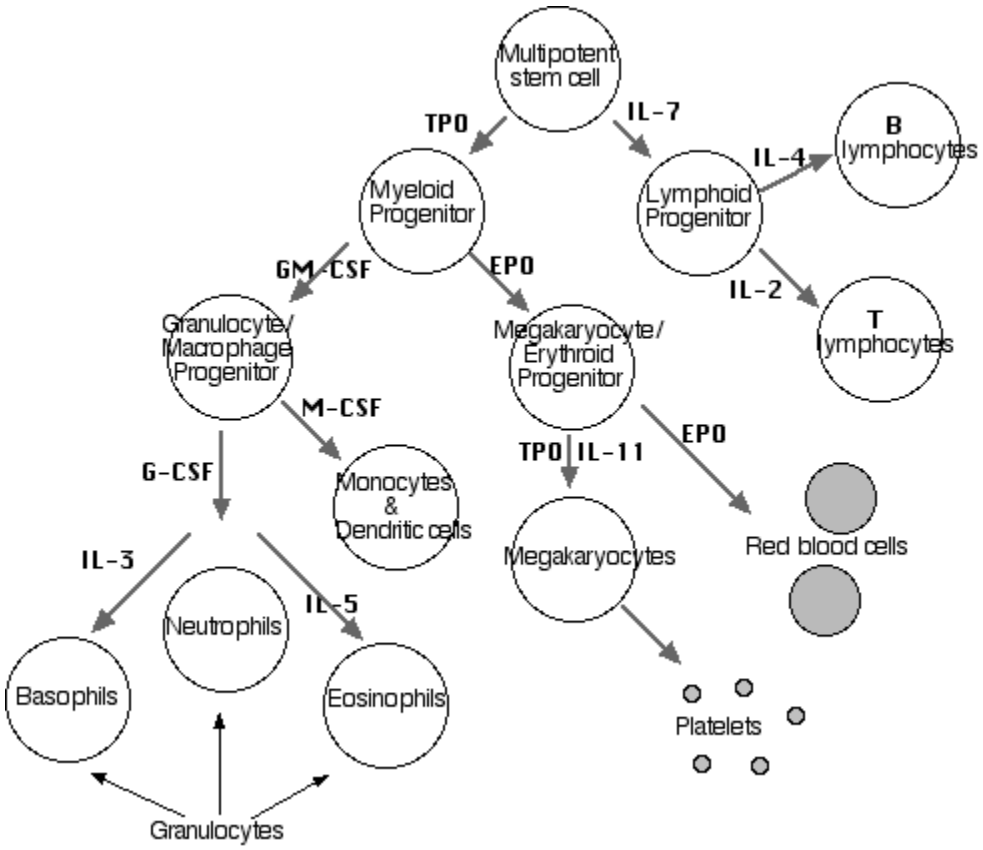


Functions of the Blood

Blood performs two major functions: It transports the following through the body: oxygen and carbon dioxide, food molecules (glucose, lipids, amino acids), ions (e.g., Na^+, Ca^{2+}, HCO_3^-), wastes (e.g., urea), hormones and heat. It plays a defence of the body against infections and other foreign materials. All the WBCs participate in these defences.

The Formation of Blood Cells

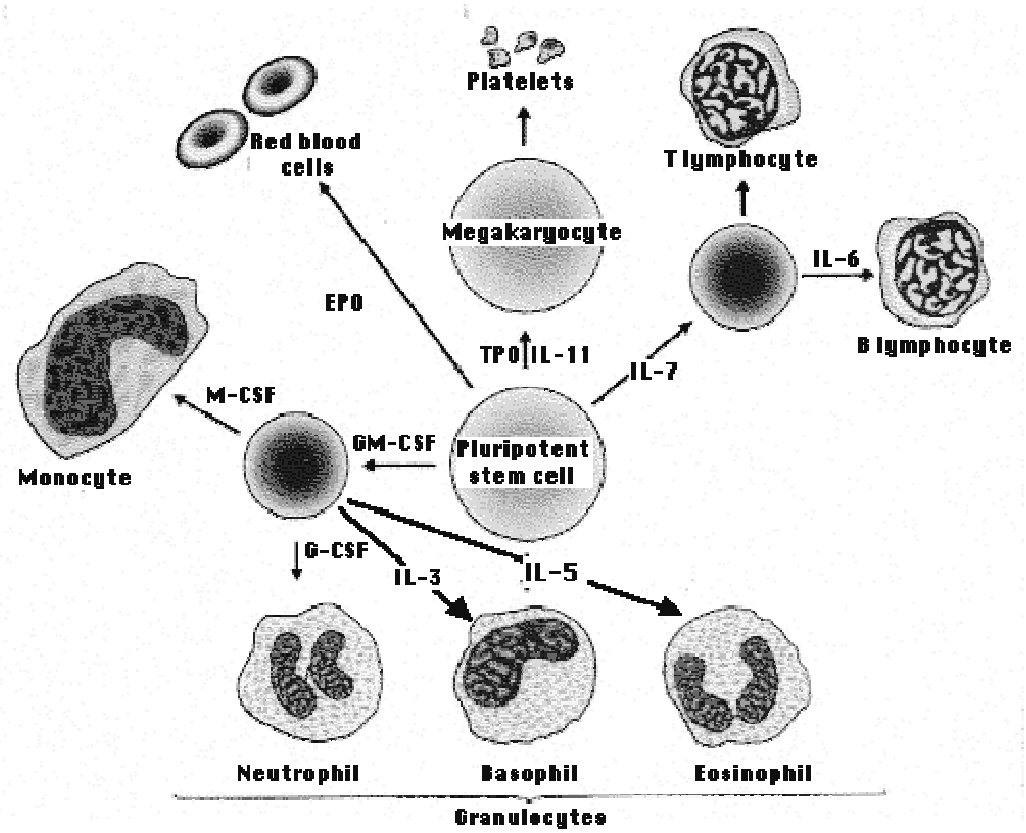
All the various types of blood cells are produced in the **bone marrow** (some 10^{11} of them each day in an adult human!). They arise from a single type of cell called a **hematopoietic stem cell** — an "adult" **multipotent stem cell**. These stem cells are very rare (only about one in 10,000 bone marrow cells). They are attached (probably by **adherens junctions**) to **osteoblasts** lining the inner surface of bone cavities. They express a cell-surface protein designated **CD34** and produce, by mitosis, two kinds of progeny. More stem cells (A mouse that has had all its blood stem cells killed by a lethal dose of radiation can be saved by the injection of a single living stem cell!). Cells that begin to differentiate along the paths leading to the various kinds of blood cells. Which path is taken is regulated by the need for more of that type of blood cell which is, in turn, controlled by appropriate **cytokines** and/or hormones.



Formation of Blood Cells

Examples:

1. **Interleukin-7** (**IL-7**) is the major cytokine in stimulating bone marrow stem cells to start down the path leading to the various **lymphocytes** (mostly **B cells** and **T cells**).
2. **Erythropoietin** (**EPO**), produced by the kidneys, enhances the production of **red blood cells** (RBCs).
3. **Thrombopoietin** (**TPO**), assisted by Interleukin-11 (**IL-11**), stimulates the production of **megakaryocytes**. Their fragmentation produces **platelets**.
4. **Granulocyte-macrophage colony-stimulating factor** (**GM-CSF**), as its name suggests, sends cells down the path leading to both those cell types. In due course, one path or the other is taken.
    a. Under the influence of **granulocyte colony-stimulating factor** (**G-CSF**), they differentiate into **neutrophils**.
    b. Further stimulated by interleukin-5 (**IL-5**) they develop into **eosinophils**.
    c. Interleukin-3 (**IL-3**) participates in the differentiation of most of the white blood cells but plays a particularly prominent role in the formation of **basophils** (responsible for some **allergies**).
    d. Stimulated by **macrophage colony-stimulating factor** (**M-CSF**) the granulocyte/macrophage progenitor cells differentiate into **monocytes, macrophages**, and **dendritic cells** (DCs).



Examples of Formation of Blood Cells

Transplants of Haematopoietic Stem Cells

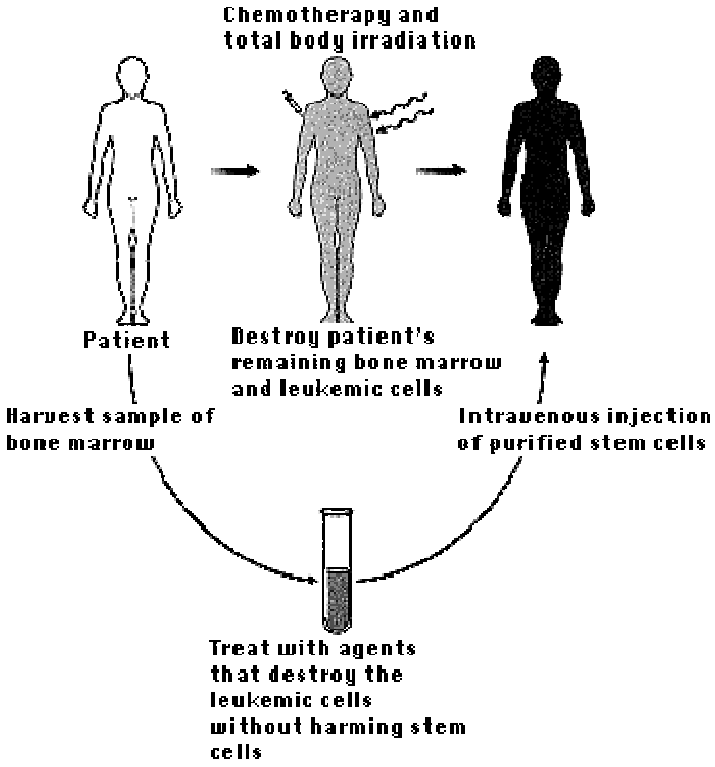
Haematopoietic stem cells are the cells from which all the types of blood cells are made. They reside in the bone marrow and often bone marrow is harvested to secure them. However, a few infusions of granulocyte colony-stimulating factor (**G-CSF**) — available thanks to **recombinant DNA technology** — causes them to be released into the blood where they can more easily be collected. They are increasingly being used to treat **multiple myeloma**, a cancer of **plasma cells**; various **leukemias** and some other types of cancer. High doses of chemotherapy and radiation can be used to kill off the cancerous cells in a patient, but they also destroy the patient's bone marrow, and the patient will die without a transplant of haematopoietic stem cells.

These can be:

(a) Autologous — from hematopoietic stem cells that were removed from the patient before cancer therapy began, stored alive, and, if there were cancer cells in the bone marrow (the case with multiple myeloma and leukemias), treated to "purge" them. Most failures of autologous stem cell transplants occur because of failure to get all the cancer cells out of the harvested cells rather than failure to eliminate them from the patient.

(b) Allogeneic — hematopoietic stem cells removed from someone else, often a close relative. Another source of hematopoietic stem cells is **cord blood** — blood drained (through the umbilical cord) from the **placenta** of newborn infants. Allogeneic stem cells avoid the problem of lurking residual cancer cells but should be closely matched to the **major histocompatibility loci** (MHC) of the patient. If not, the donor cells will attack the recipient causing often-fatal **graft-versus-host disease** (GVHD).

If the patient's own marrow was not completely destroyed, the donor lymphocytes and the patient's lymphocytes can exist together. Then a later infusion of the donor's T cells may be able to kill off all the patient's remaining malignant cells leaving the patient with a bone marrow that produces donor-type cells exclusively. So haematopoietic stem cell transplants (HSCT) can be life-saving but create their own problems. (Another example: an "immediate"-type allergy like **hay fever or asthma** of the donor can create the same allergy in the recipient.). Autologous hematopoietic stem cell transplants also show promise of being an effective treatment for the autoimmune disorder **systemic lupus erythematosus** (SLE)



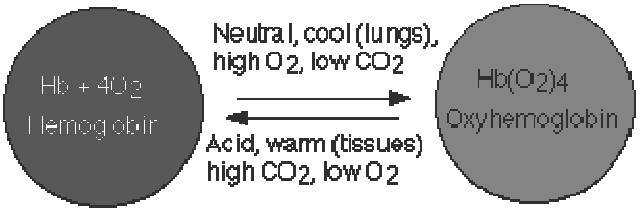
Transplants of Haematopoietic Stem Cells

Red Blood Cells (Erythrocytes)

The most numerous type in the blood. Women average about 4.8 million of these cells per cubic millimeter (mm^3; which is the same as a microliter [µl]) of blood. Men average about 5.4 x 10^6 per µl. These values can vary over quite a range depending on such factors as health and altitude. (Peruvians living at 18,000 feet may have as many as 8.3 x 10^6 RBCs per µl.). RBC precursors mature in the bone marrow closely attached to a macrophage. They manufacture haemoglobin until it accounts for some 90% of the dry weight of the cell. The nucleus is squeezed out of the cell and is ingested by the macrophage. No-longer-needed proteins are expelled from the cell in vesicles called **exosomes**. Thus RBCs are terminally differentiated; that is, they can never divide. They live about 120 days and then are ingested by phagocytic cells in the liver and spleen. Most of the iron in their haemoglobin is reclaimed for reuse. The remainder of the heme portion of the molecule is degraded into **bile pigments** and excreted by the liver. Some 3 million RBCs die and are scavenged by the liver each second. Red blood cells are responsible for the transport of **oxygen** and **carbon dioxide**.

(a) Oxygen Transport

In adult humans the haemoglobin (Hb) molecule consists of four polypeptides: two **alpha (α) chains** of 141 amino acids and two **beta (β) chains** of 146 amino acids. Each of these is attached the **prosthetic group haeme**. There is one atom of iron at the centre of each haeme. One molecule of oxygen can bind to each haeme.



The reaction is reversible. Under the conditions of lower temperature, higher pH, and increased oxygen pressure in the capillaries of the lungs, the reaction proceeds to the right. The purple-red deoxygenated haemoglobin of the venous blood becomes the bright-red **oxyhaemoglobin** of the arterial blood. Under the conditions of higher temperature, lower pH, and lower oxygen pressure in the tissues, the reverse reaction is promoted and oxyhaemoglobin gives up its oxygen.

(b) Carbon Dioxide Transport

Carbon dioxide (CO_2) combines with water forming carbonic acid, which dissociates into a hydrogen ion (H^+) and a **bicarbonate ions**:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

95% of the CO_2 generated in the tissues is carried in the red blood cells: It probably enters (and leaves) the cell by diffusing through transmembrane channels in the plasma membrane. (One of the proteins that forms the channel is the **D antigen** that is the most important factor in the **Rh system** of blood groups.). Once inside, about one-half of the CO_2 is directly bound to haemoglobin (at a site different from the one that binds oxygen). The rest is converted — following the equation above — by the enzyme **carbonic anhydrase** into bicarbonate ions that diffuse back out into the plasma and hydrogen ions (H^+) that bind to the protein portion of the haemoglobin (thus having no effect on pH). Only about 5% of the CO_2 generated in the tissues dissolves directly in the plasma. (A good thing, too: if all the CO_2 we make were carried this way, the pH of the blood would drop from its normal 7.4 to an instantly-fatal 4.5!). When the red cells reach the lungs, these reactions are reversed and CO_2 is released to the air of the alveoli.

(c) Anaemia

Anaemia is a shortage of RBCs and/or the amount of haemoglobin in them. Anaemia has many causes. One of the most common is an inadequate intake of **iron** in the diet.

(d) Blood Groups

Blood groups are created by molecules present on the surface of red blood cells (and often on other cells as well).

(i) The ABO Blood Groups

The **ABO blood groups** were the first to be discovered (in 1900) and are the most important in assuring safe blood transfusions.

The table shows the four ABO **phenotypes** ("blood groups") present in the human population and the **genotypes** that give rise to them.

Blood	Antigens	Antibodies in	Genotypes

Group	on RBCs	Serum	
A	**A**	Anti-B	*AA* or *AO*
B	**B**	Anti-A	*BB* or *BO*
AB	**A** and **B**	Neither	*AB*
O	Neither	Anti-A and anti-B	*OO*

When **red blood cells** carrying one or both **antigens** are exposed to the corresponding **antibodies**, they agglutinate; that is, clump together. People usually have antibodies against those red cell antigens that they lack.

The critical principle to be followed is that transfused blood must not contain red cells that the **recipient's** antibodies can clump. Although theoretically it is possible to transfuse group O blood into any recipient, the antibodies in the donated plasma can damage the recipient's red cells. Thus all transfusions should be done with exactly-matched blood. Bacteria living in the intestine, and probably some foods, express **epitopes** similar to those on A and B. We synthesize antibodies against these if we do not have the corresponding epitopes; that is, if our immune system sees them as "foreign" rather than "self".

(ii) The Rh System

Rh antigens are transmembrane proteins with loops exposed at the surface of red blood cells. They appear to be used for the transport of carbon dioxide and/or ammonia across the plasma membrane. They are named for the rhesus monkey in which they were first discovered. There are a number of Rh antigens. Red cells that are "Rh positive" express the one designated **D**. About 15% of the population have no RhD antigens and thus are "Rh negative". The major importance of the Rh system for human health is to avoid the danger of RhD incompatibility between mother and foetus. During birth, there is often a leakage of the baby's red blood cells into the mother's circulation. If the baby is Rh positive (having inherited the trait from its father) and the mother Rh-negative, these red cells will cause her to develop antibodies against the RhD antigen. The antibodies, usually of the **IgG class,** do not cause any problems for that child, but can cross the placenta and attack the red cells of a subsequent Rh^+ foetus. This destroys the red cells producing **anaemia** and jaundice. The disease, called **erythroblastosis fetalis** or **hemolytic disease of the newborn**, may be so severe as to kill the foetus or even the newborn infant. It is an example of an antibody-mediated cytotoxicity disorder.

Although certain other red cell antigens (in addition to Rh) sometimes cause problems for a foetus, an **ABO** incompatibility does not. It turns out that most anti-A or anti-B antibodies are of the **IgM** class and these do **not** cross the placenta. In fact, an **Rh^-/type O** mother carrying an **Rh^+/type A, B, or AB** foetus is resistant to sensitization to the Rh antigen. Presumably her anti-A and anti-B antibodies destroy any foetal cells that enter her blood before they can elicit anti-Rh antibodies in her.

This phenomenon has led to an extremely effective preventive measure to avoid Rh sensitization. Shortly after each birth of an Rh^+ baby, the mother is given an injection of anti-Rh antibodies. The preparation is called **Rh immune globulin** (**RhIG**) or **Rhogam**. These passively acquired antibodies destroy any foetal cells that got into her circulation before they can elicit an active immune response in her. Rh immune globulin came into common use in the United States in 1968, and within a decade the incidence of Rh hemolytic disease became very low.

(iii) Other Blood Groups

Several other blood group antigens have been identified in humans. They, too, may sometimes cause transfusion reactions and even and hemolytic disease of the newborn
in cases where there is no ABO or Rh incompatibility.

White Blood Cells (Leukocytes)

White blood cells are much less numerous than red (the ratio between the two is around 1:700) and have nuclei. They participate in protecting the body from infection. They consist of **lymphocytes** and **monocytes** with relatively clear cytoplasm, and three types of **granulocytes**, whose cytoplasm is filled with granules.

(a) Lymphocytes

There are several kinds of lymphocytes (although they all look alike under the microscope), each with different functions to perform . The most common types of lymphocytes are **B lymphocytes** ("B cells"). These are responsible for making antibodies and **T lymphocytes** ("T cells"). There are several subsets of these:

1. **Inflammatory T cells** that recruit macrophages and neutrophils to the site of infection or other tissue damage
2. **Cytotoxic T lymphocytes** (CTLs) that kill virus-infected and, perhaps, tumour cells
3. **Helper T cells** that enhance the production of antibodies by B cells

Although bone marrow is the ultimate source of lymphocytes, the lymphocytes that will become T cells migrate from the bone marrow to the **thymus** where they mature. Both B cells and T cells also take up residence in lymph nodes, the spleen and other tissues where they encounter antigens; continue to divide by mitosis; and mature into fully functional cells.

(b) Monocytes

Monocytes leave the blood and become **macrophages** and **dendritic cells**. Macrophages are large, phagocytic cells that engulf foreign material (antigens) that enter the body dead and dying cells of the body.

(c) Neutrophils

The most abundant of the WBCs. This photomicrograph shows a single neutrophil surrounded by red blood cells. Neutrophils squeeze through the capillary walls and into infected tissue where they kill the invaders (e.g., bacteria) and then engulf the remnants by **phagocytosis**. This is a never-ending task, even in healthy people: Our throat, nasal passages, and colon harbour vast numbers of bacteria. Most of these are **commensals**, and do us no harm. But that is because neutrophils keep them in check. However, heavy doses of radiation, chemotherapy and many other forms of stress can reduce the numbers of neutrophils so that formerly harmless bacteria begin to proliferate. The resulting **opportunistic infection** can be life-threatening.

(d) Eosinophils

The number of eosinophils in the blood is normally quite low (0–450/µl). However, their numbers increase sharply in certain diseases, especially infections by parasitic worms. Eosinophils are cytotoxic, releasing the contents of their granules on the invader.

(e) Basophils

The number of basophils also increases during infection. Basophils leave the blood and accumulate at the site of infection or other inflammation. There they discharge the contents of their granules, releasing a variety of mediators such as: **histamine, serotonin, prostaglandins and leukotrienes** which increase the blood flow to the area and in other ways add to the

inflammatory process. The mediators released by basophils also play an important part in some allergic responses such as hay fever and an **anaphylactic response** to insect stings.

Platelets

Platelets are cell fragments produced from **megakaryocytes**. Blood normally contains 150,000–350,000 per microliter (µl) or cubic millimeter (mm^3). This number is normally maintained by a homeostatic (negative-feedback) mechanism. If this value should drop much below 50,000/µl, there is a danger of uncontrolled bleeding because of the essential role that platelets have in blood clotting. Some causes include certain drugs and herbal remedies and autoimmunity.

When blood vessels are damaged, fibrils of **collagen** are exposed. **von Willebrand factor** links the collagen to platelets forming a plug of platelets there. The bound platelets release **ADP** and **thromboxane A2** which recruit and activate still more platelets circulating in the blood. (This role of thromboxane accounts for the beneficial effect of low doses of aspirin — a cyclooxygenase inhibitor — in avoiding heart attacks.) **ReoPro®** is a **monoclonal antibody** directed against platelet receptors. It inhibits platelet aggregation and appears to reduce the risk that "reamed out" coronary arteries (after coronary angioplasty) will plug up again.

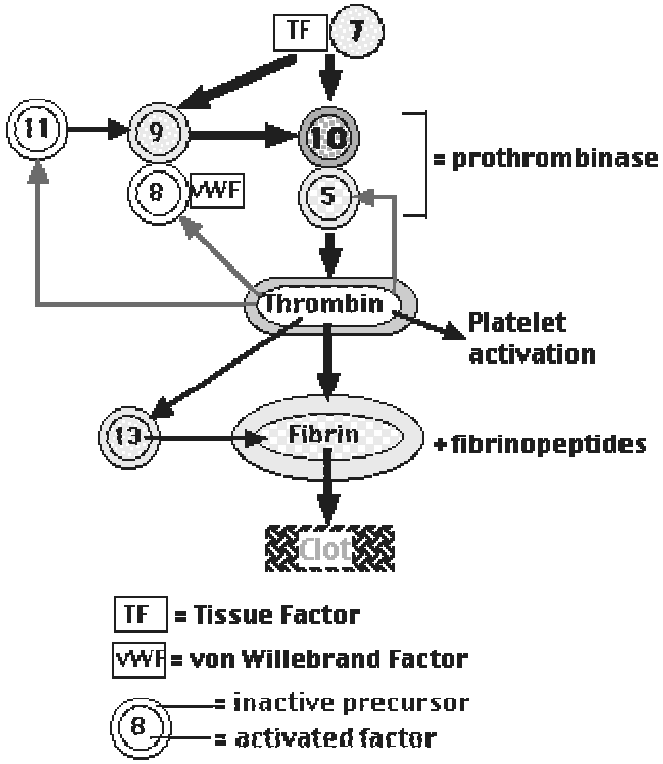
Blood Clotting

When blood vessels are cut or damaged, the loss of blood from the system must be stopped before **shock** and possible death occur. This is accomplished by solidification of the blood, a process called **coagulation** or clotting. A blood clot consists of a **plug of platelets** enmeshed in a network of insoluble **fibrin** molecules. Platelet aggregation and fibrin formation both require the proteolytic enzyme **thrombin**. Clotting also requires: calcium ions (Ca^{2+})(which is why blood banks use a chelating agent to bind the calcium in donated blood so the blood will not clot in the bag). about a dozen other protein **clotting factors**. Most of these circulate in the blood as inactive precursors. They are activated by proteolytic cleavage becoming, in turn, active **proteases** for other factors in the system. By tradition, these factors are designated by Roman numerals. I find this somewhat confusing and will use Arabic numerals instead.

(a) Initiating the Clotting Process

Damaged cells display a surface protein called **tissue factor** (**TF**). Tissue factor binds to activated Factor **7**. The **TF-7 heterodimer** is a protease with two substrates: Factor **10** and Factor **9.**

Factor 10 binds and activates Factor **5**. This heterodimer is called **prothrombinase** because it is a protease that converts **prothrombin** (also known as Factor II) to **thrombin**. Thrombin has several different activities. Two of them are: proteolytic cleavage of **fibrinogen** (aka "Factor I") to form soluble molecules of **fibrin** and a collection of small **fibrinopeptides**, activation of Factor **13** which forms covalent bonds between the soluble fibrin molecules converting them into an insoluble meshwork — the **clot**. (Thrombin and activated Factors 10 ("Xa") and 11 ("XIa") are **serine proteases**.)



Mechanism of Blood Clotting

(b) Amplifying the Clotting Process

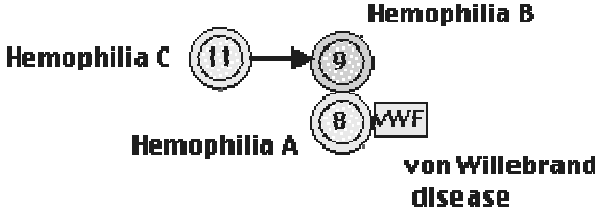
The clotting process also has several **positive feedback loops** which quickly magnify a tiny initial event into what may well be a lifesaving plug to stop bleeding. The TF-7 complex (which started the process) also activates Factor **9**. Factor 9 binds to Factor **8**, a protein that circulates in the blood stabilized by another protein, **von Willebrand Factor** (**vWF**). This complex activates more Factor **10**. As thrombin is generated, it activates more Factor **5**, Factor **8**, and Factor **11**. Factor 11 amplifies the production of activated Factor **9**. Thus what may have begun as a tiny, localized event rapidly expands into a cascade of activity.

Bleeding Disorders

A deficiency of a clotting factor can lead to uncontrolled bleeding. The deficiency may arise because not enough of the factor is produced or a mutant version of the factor fails to perform properly.

Examples include **von Willebrand disease**, haemophilia **A** for **factor 8** deficiency haemophilia **B** for **factor 9** deficiency, haemophilia **C** for factor **11** deficiency

In some cases of von Willebrand disease, either a **deficient level** or a **mutant version** of the factor eliminates its protective effect on factor 8. The resulting low level of factor 8 mimics haemophilia A.



In Haemophilia A and B, The genes encoding factors 8 and 9 are on the X chromosome. Thus their inheritance is X-linked.Like other X-linked disorders, haemophilia A and B are found almost exclusively in males because they inherit just a single X chromosome, and if the gene for factor 8 (or 9) on it is defective, they will suffer from the disease. There are many different mutant versions of the genes for factors 8 and 9. Although some produce only a minor effect on the function of their protein, others fail to produce any functioning clotting factor.

Treating Haemophilia A and B

Factor **8** and **9** can be extracted from donated blood, usually pooled from several thousand donors, and purified. Injections of this material can halt episodes of bleeding in haemophiliacs and have allowed countless young men to live relatively normal lives. However, in the early 1980s, blood contaminated with the human immunodeficiency virus (**HIV**) was unknowingly used to manufacture preparations of factors 8 and 9. In some areas, 90% or more of the haemophiliacs became infected by these contaminated preparations. Many have since died of **AIDS**.

The future now looks brighter because: all donated blood is now tested to see if the donor has been infected with HIV (as well as hepatitis B and C); plasma-derived preparations of factors 8 and 9 are now treated with heat and/or solvents to destroy any viruses that might be present; **recombinant factor 8** and **recombinant factor 9** made by genetic engineering are now available.

These recombinant factors are made by inserting the DNA encoding the human protein into mammalian cells grown in culture. *E. coli* cannot be used because these factors are **glycoproteins**, and E. coli lacks the machinery to attach carbohydrate properly.

And the team that brought us **Dolly** reported in the 19 December 1997 issue of **Science** that they have succeeded in cloning female sheep **transgenic** for the human **factor 9** gene. The human gene is coupled to the **promoter** for the ovine (sheep) milk protein beta-lactoglobulin. When the lambs mature, it is hoped that they will secrete large amounts of human factor 9 in their milk, which can then be purified for human therapy. Several attempts have also been made to try to cure haemophilia by **gene therapy**. It is difficult to see how even the most worried critics of genetic engineering can fail to approve its potential to save the lives of thousands of haemophiliacs in the years to come.

People with liver failure can be cured with a liver transplant. On the rare occasions when the patient has happened to be a haemophiliac (A,B or C), the transplant cured not only the patient's liver disease but cured his haemophilia as well!

Controlling Clotting

While the ability to clot is essential to life, the process must be carefully regulated. Inappropriate clot formation, especially in the brain or lungs, can be life-threatening.

(a) Antithrombin III

As its name suggests, this plasma protein (a **serpin**) inhibits the formation of thrombin. It does so by binding to and thus **in**activating prothrombin, factor 9 and factor 10. Some surgical patients, especially those receiving hip or heart valve replacements, and people at risk of ischemic stroke (clots in the brain), are given heparin. **Heparin** is a mixture of polysaccharides that bind to antithrombin III, inducing an **allosteric** change that greatly **enhances** its inhibition of thrombin synthesis.

(b) Protein C

With its many clotting promoting activities, it is probably no accident that thrombin sits at the centre of the control mechanism. Excess thrombin binds to cell-surface receptors called **thrombomodulin**. The resulting complex activates a plasma protein called **Protein C** and its cofactor **Protein S**. Together these inhibit further thrombin formation directly — by inactivating Factor **5** and indirectly — by inactivating Factor **8**. Some inherited disorders that predispose to spontaneous clots, especially in the leg veins: inherited deficiency of **Protein C** or **Protein S**; inherited mutation in the **Factor 5** gene producing a protein that no longer responds to the inhibitory effect of Protein C. **Recombinant Protein C** is now available to treat people threatened with inappropriate clotting, e.g., as a result of widespread infection (sepsis).

(c) Vitamin K

Vitamin K is a cofactor needed for the synthesis (in the liver) of factors **2** (prothrombin), **7**, **9**, and **10** proteins **C** and **S**. So a **deficiency** of Vitamin K predisposes to bleeding. Conversely, blocking the action of vitamin K helps to prevent inappropriate clotting. **Warfarin** (aka coumadin) is sometimes prescribed as a "blood thinner" because it is an effective vitamin K **antagonist**. (Warfarin is also used as a rat poison because it can cause lethal (internal) bleeding when eaten.)

Dissolving Clots

Plasma contains **plasminogen**, which binds to the fibrin molecules in a clot. Nearby healthy cells release **tissue plasminogen activator** (**TPA**), which also binds to fibrin and, as its name suggests, activates plasminogen forming **plasmin**. Plasmin (another **serine protease**) proceeds to digest fibrin, thus dissolving the clot. **Recombinant human TPA** is now produced by **recombinant DNA technology.** Injected within the first hours after a heart attack, it has saved many lives by dissolving the clot blocking the **coronary artery** and restoring blood flow before the heart muscle becomes irreversibly damaged. It is also used for people who suffer an **ischemic stroke**; that is, a **clot** in the brain. (It must not, of course, be used for hemorrhagic strokes, that is, a burst blood vessel!)

Plasma

Plasma is the straw-coloured liquid in which the blood cells are suspended.

Composition of blood plasma	
Component	**Percent**
Water	~92
Proteins	6–8
Salts	0.8
Lipids	0.6
Glucose (blood sugar)	0.1

Plasma transports materials needed by cells and materials that must be removed from cells:various ions (Na^+, Ca^{2+}, HCO_3^-, etc, glucose and traces of other sugars, amino acids, other organic acids, cholesterol and other lipids, hormones and urea and other wastes. Most of these materials are in transit from a place where they are added to the blood (a "source") exchange organs like the intestine depots of materials like the liver
to places ("sinks") where they will be removed from the blood, every cell and exchange organs like the kidney, and skin.

(a) Serum Proteins

Proteins make up 6–8% of the blood. They are about equally divided between **serum albumin** and a great variety of **serum globulins**. After blood is withdrawn from a vein and allowed to

clot, the clot slowly shrinks. As it does so, a clear fluid called serum is squeezed out. Thus **Serum** is blood plasma without fibrinogen and other clotting factors. The serum proteins can be separated by **electrophoresis**.

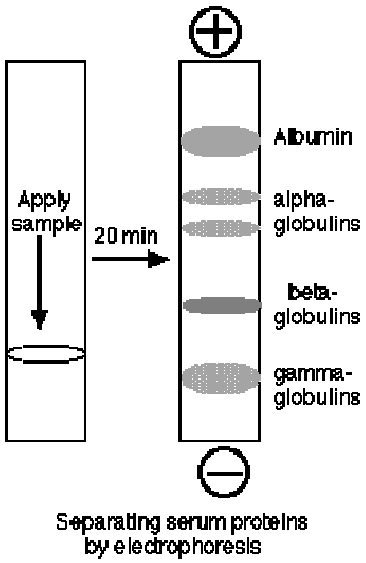
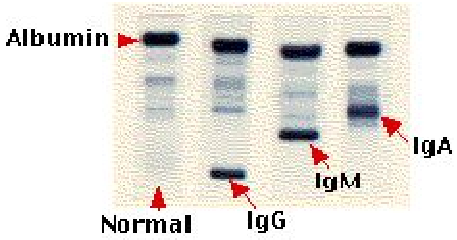


Fig.3.14a. Electrophoresis

A drop of serum is applied in a band to a thin sheet of supporting material, like paper, that has been soaked in a slightly-alkaline salt solution. At pH 8.6, which is commonly used, all the proteins are negatively charged, but some more strongly than others. A direct current can flow through the paper because of the conductivity of the buffer with which it is moistened. As the current flows, the serum proteins move toward the positive electrode. The stronger the negative charge on a protein, the faster it migrates. After a time (typically 20 min), the current is turned off and the proteins stained to make them visible (most are otherwise colourless). The separated proteins appear as distinct bands. The most prominent of these and the one that moves closest to the positive electrode is **serum albumin**. Serum albumin is made in the liver, binds many small molecules for transport through the blood and helps maintain the **osmotic pressure** of the blood. The other proteins are the various serum globulins. They migrate in the order

1. **Alpha globulins** (e.g., the proteins that transport **thyroxine** and **retinol** [vitamin A])
2. **Beta globulins** (e.g., the iron-transporting protein **transferrin**)
3. **Gamma globulins**.

Gamma globulins are the least negatively-charged serum proteins. (They are so weakly charged, in fact, that some are swept in the flow of buffer back toward the negative electrode.). Most antibodies are gamma globulins. Therefore gamma globulins become more abundant following infections or immunizations.



Results of electrophoresis

If a precursor of an antibody-secreting cell becomes cancerous, it divides uncontrollably to generate a **clone** of **plasma cells** secreting a **single kind of antibody molecule**. The image (courtesy of Beckman Instruments, Inc.) shows — from left to right — the electrophoretic separation of:

4. **Normal** human serum with its diffuse band of gamma globulins;
5. Serum from a patient with **multiple myeloma** producing an **IgG myeloma protein;**
6. Serum from a patient with Waldenström's macroglobulinemia where the cancerous clone secretes an **IgM** antibody;
7. Serum with an **IgA** myeloma protein.

Gamma globulins can be harvested from donated blood (usually pooled from several thousand donors) and injected into persons exposed to certain diseases such as chicken pox and hepatitis. Because such preparations of **immune globulin** contain antibodies against most common infectious diseases, the patient gains temporary protection against the disease.

(b) Serum Lipids

Analysis of serum lipids has become an important health measure. The table shows the range of typical values as well as the values above (or below) which the subject may be at increased risk of developing **atherosclerosis.**

LIPID	Typical values (mg/dl)	Desirable (mg/dl)
Cholesterol (total)	170–210	<200
LDL cholesterol	60–140	<100
HDL cholesterol	35–85	>40
Triglycerides	40–160	<160

Total cholesterol is the sum of HDL cholesterol, LDL cholesterol and 20% of the triglyceride value. Note that **high LDL** values are **bad**, but **high HDL** values are **good**. Using the various values, one can calculate a **cardiac risk ratio** = **total cholesterol** divided by **HDL cholesterol**. A cardiac risk ratio greater than 7 is considered a warning.

Blood Transfusions

In the United States, in 2001, some 15 million "units" (~475 ml) of blood were collected from blood donors. Some of these units ("whole blood") were transfused directly into patients (e.g., to replace blood lost by trauma or during surgery). Most were further fractionated into components, including: RBCs. When refrigerated these can be used for up to 42 days. **Platelets** must be stored at room temperature and thus can be saved for only 5 days. **Plasma** can be frozen and stored for up to a year.

(a) Ensuring the Safety of Donated Blood

A variety of infectious agents can be present in blood viruses (e.g., **HIV-1, hepatitis B** and C, **HTLV,** West Nile virus, bacteria like the spirochete of **syphilis**, protozoans like the agents of **malaria** and babesiosis, **prions** (e.g., the agent of **variant Crueutzfeldt-Jakob disease**) and could be transmitted to recipients. To minimize these risks, donors are questioned about their possible exposure to these agents; each unit of blood is tested for a variety of infectious agents. Most of these tests are performed with enzyme immunoassays (EIA)— and detect **antibodies** against the agents. However, it takes a period of time for the immune system to produce antibodies following infection, and during this period ("window"), infectious virus is present in the blood. For this reason, blood is now also checked for the presence of the RNA of these RNA viruses: HIV-1, hepatitis C and West Nile virus by the so-called **nucleic acid-amplification test** (NAT). Thanks to all these precautions, the risk of acquiring an infection from any of

these agents is vanishingly small. Despite this, some people — in anticipation of need — donate their own blood ("autologous blood donation") prior to surgery. Donated blood must also be tested for certain cell-surface antigens that might cause a dangerous transfusion reaction in an improperly-matched recipient.

(b) Blood Substitutes

Years of research have gone into trying to avoid the problems of blood perishability and safety by developing blood substitutes. Most of these have focused on materials that will transport adequate amounts of oxygen to the tissues. Some are totally synthetic substances. Others are derivatives of haemoglobin. Although some have reached **clinical testing,** none has as yet proved acceptable for routine use.

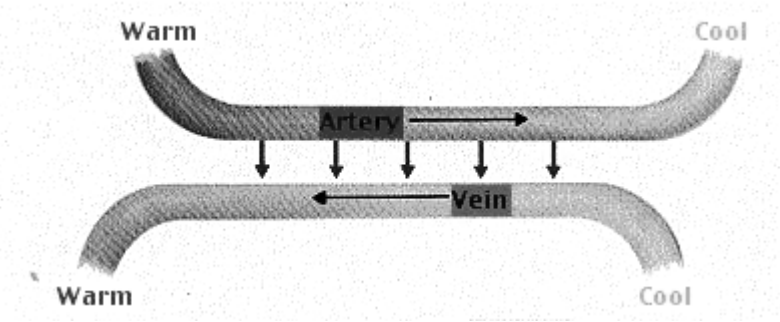
The Transport of Heat

In addition to its role in the transport of materials, the circulatory system is responsible for the distribution of heat throughout the body. This is true both of

1. **Endotherms**, animals — birds and mammals — that generate **internally** the heat needed to maintain their body temperature. Birds and mammals are "warm-blooded' or **homeothermic**, maintaining their body temperature within narrow limits, no matter what the ambient temperature.
2. **Ectotherms**, animals — the other vertebrates and the invertebrates — that secure their heat from their surroundings (e.g., by basking in the sun). Ectotherms are "cold-blooded" or **poikilothermic**.

The major source of heat for endotherms is the metabolism of their internal organs. Over two-thirds of the heat generated in a resting human is created by the organs of the thoracic and abdominal cavities and the brain (which contributes 16% of the total — about the same as all our skeletal muscles when they are at rest). There are several measures that an endothermic animal can take if it begins to lose heat to its surroundings faster than it can generate heat (i.e., it begins to grow cold).

It can increase the metabolic rate of its tissues. Many mammals do this as their surroundings get colder, but it is still uncertain whether humans can. It can increase its physical activity. At rest, muscles make only a small contribution (about 16%) to body heat. During vigorous exercise, this can increase greatly. In the absence of voluntary muscle action, the same effect is achieved by **shivering**. The greater the surface-to-volume ratio of a part of the body, the faster is can transfer heat to its surroundings. This is why you first notice cold in your hands and feet. The loss of heat from the extremities can be sharply reduced by diminishing their blood supply. In extreme cold, for example, the blood supply to the fingers can drop to 1% or so of its normal value. Many animals (including humans) have another way to conserve heat. The arteries of our arms and legs run parallel to a set of deep veins. As warm blood passes down the arteries, the blood gives up some of its heat to the colder blood returning from the extremities in these veins.



Transport of Heat

Such a mechanism is called a **countercurrent heat exchanger**. When heat loss is no problem, most of the venous blood from the extremities returns through veins located near the surface. Countercurrent heat exchangers can operate with remarkable efficiency. A sea gull can maintain a normal temperature in its torso while standing with its unprotected feet in freezing water. When you consider that the blood of fishes passes over the gills which are bathed in the surrounding water, it is easy to see why fishes are "cold-blooded". Nonetheless, some marine fishes (e.g., the tuna) are able to keep their most active swimming muscles warmer than the sea by using a countercurrent heat exchanger. The cold, oxygen-rich arterial blood passes into a series of fine arteries that take the blood into the active muscles. These fine arteries lie side by side with veins draining those muscles. So as the cold blood passes into the muscles, it picks up the heat that had been generated by these muscles and keeps it from being lost to the surroundings. Thanks to this countercurrent heat exchanger, a tuna swimming in the winter can maintain its active swimming muscles 14°C warmer than the surrounding water. Countercurrent exchangers also operate in the **kidney** and are built into the design of **artificial kidneys.**

The circulatory system is also responsible for cooling an animal. If the animal's "core" body temperature gets too high, the blood supply to the surface and extremities is increased enabling heat to be released to the surroundings. If this is insufficient, the animal can evapourate water from the blood — in the form of sweat for those animals with sweat glands. The evapouration of 1 gram of water absorbs some 580 calories of heat. Most endotherms cannot tolerate a rise in body temperature of more than 5°C or so. The brain is the organ most susceptible to damage by a high temperature. Some mammals, dogs for example, have a countercurrent heat exchanger located between the carotid arteries and the vessels that distribute blood to the brain. This heat exchanger transfers some of the heat of the arterial blood to the relatively cool venous blood returning from the nose and mouth. This cools their arterial blood before it reaches the brain. The shifting of blood flow as needed to maintain homeothermy is controlled by temperature receptors in the **hypothalamus** of the brain. One set of receptors here responds to small (0.01°C) increases in the temperature of the blood. When triggered, all the activities by which the body cools itself shunting blood vessels to the skin and extremities, sweating, etc. are brought into play. It is this center that enables us to maintain a constant body temperature (homeothermy) during periods of extreme exertion or in hot surroundings. A second region of the hypothalamus triggers warming responses: shunting blood away from the skin and extremities shivering when the body becomes chilled. It is the hypothalamus that executes the **fever** response. In effect, the hypothalamus is the body's thermostat. The release of prostaglandins during **inflammation** increases the setting; that is, turns the thermostat "up". If the body temperature is not yet there, the body begins shivering violently — causing "chills" — to generate the heat needed. The result is fever when the new set point is reached.