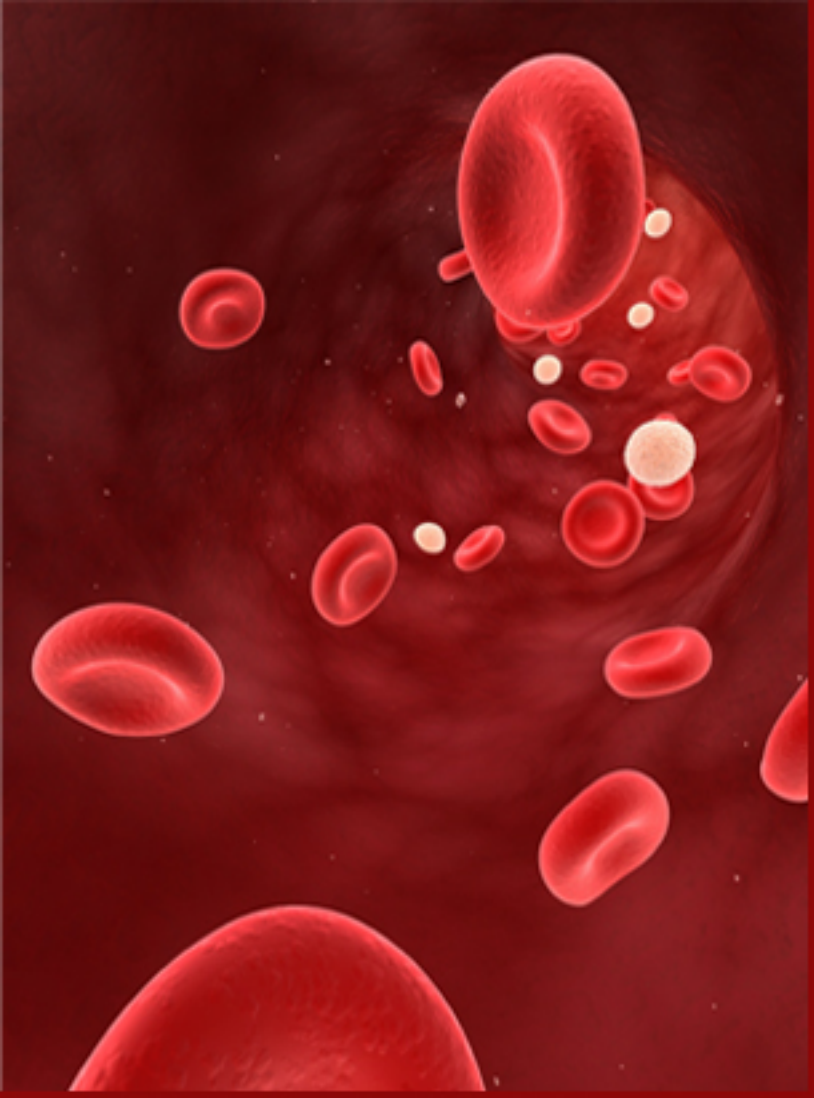

Hematology and Dermatology

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First Edition, 2012

ISBN 978-81-323-1400-4

Published by:
College Publishing House
4735/22 Prakashdeep Bldg,
Ansari Road, Darya Ganj,
Delhi - 110002
Email: info@wtbooks.com

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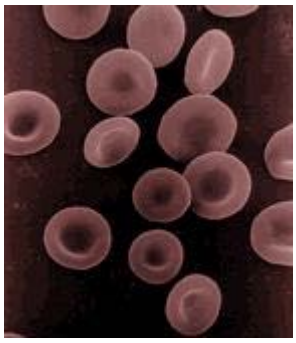
Chapter 1

Red Blood Cell



Human red blood cells (6-8μm)

Red blood cells (also referred to as **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 the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries.

These cells' cytoplasm is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color.

In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles. 2.4 million new erythrocytes are produced per second. 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 blood corpuscles** (an archaic term), **haematids**, **erythroid cells** or **erythrocytes** (from Greek *erythros* for "red" and *kytos* for "hollow", with *cyte* translated as "cell" in modern usage). Packed red blood cells, which are made from whole blood with the plasma removed, are used in transfusion medicine.

History

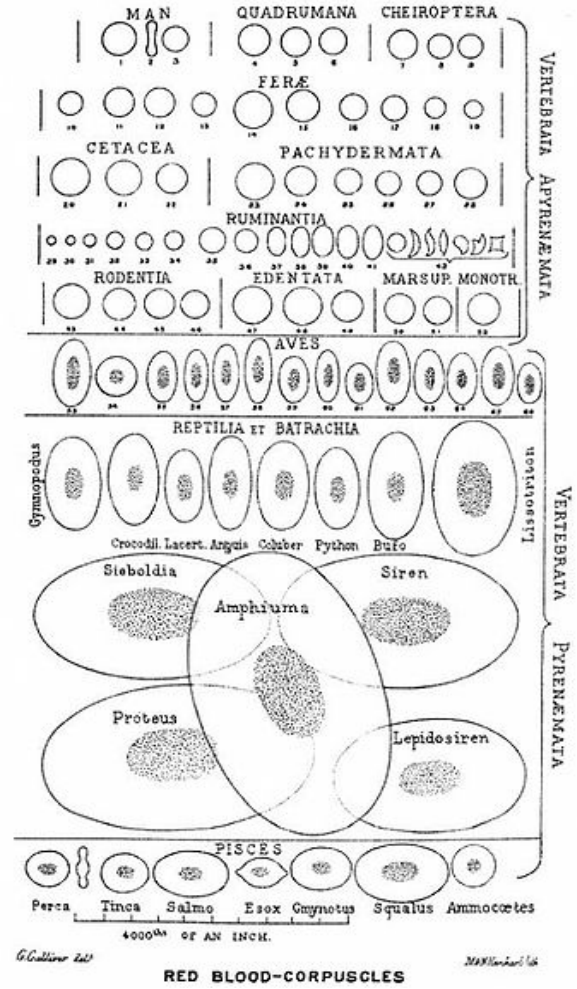
The first person to describe red blood cells was the young Dutch biologist Jan Swammerdam, who had used an early microscope in 1658 to study the blood of a frog.

Unaware of this work, Anton van Leeuwenhoek provided another microscopic description in 1674, this time providing a more precise description of red blood cells, even approximating their size, "25,000 times smaller than a fine grain of sand".

In 1901 Karl Landsteiner published his discovery of the three main blood groups—A, B, and C (which he later renamed to O). Landsteiner described the regular patterns in which reactions occurred when serum was mixed with red blood cells, thus identifying compatible and conflicting combinations between these blood groups. A year later Alfred von Decastello and Adriano Sturli, two colleagues of Landsteiner, identified a fourth blood group—AB.

In 1959, by use of X-ray crystallography, Dr. Max Perutz was able to unravel the structure of hemoglobin, the red blood cell protein that carries oxygen.

Vertebrate erythrocytes



There is an immense size variation in vertebrate erythrocytes, as well as a correlation between cell and nucleus size. Mammalian erythrocytes, which do not contain nuclei, are considerably smaller than those of most other vertebrates.

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.

The sequestration of oxygen carrying proteins inside specialized cells (rather than having them 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.

The only known vertebrates without erythrocytes are the crocodile icefishes (family Channichthyidae); they live in very oxygen rich cold water and transport oxygen freely dissolved in their blood. While they don't use hemoglobin anymore, remnants of hemoglobin genes can be found in their genome.

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.

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.

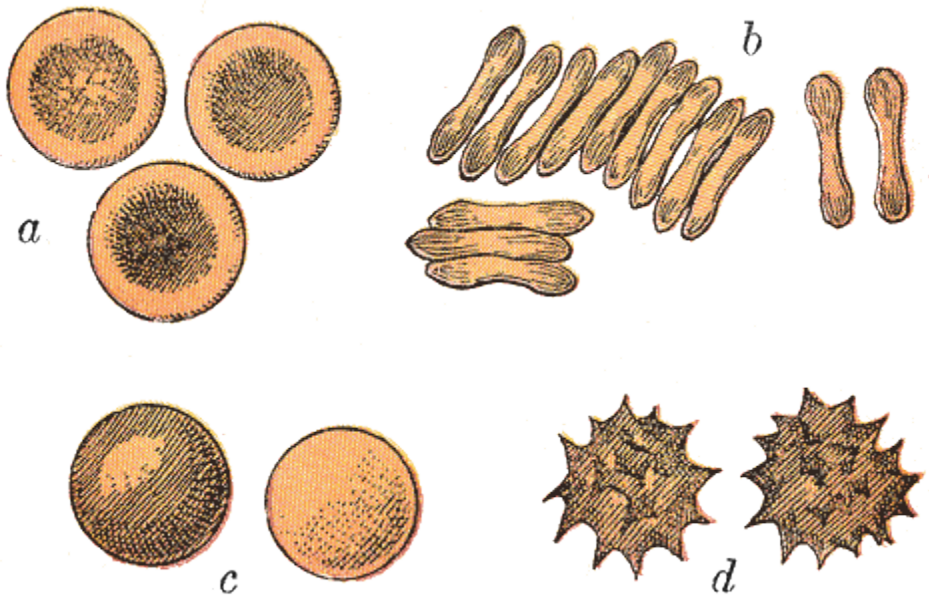
When their hemoglobin molecules are deoxygenated, erythrocytes release S-nitrosothiols which also acts to dilate vessels, thus directing more blood to areas of the body depleted of oxygen.

It has been recently demonstrated that erythrocytes can also synthesize nitric oxide enzymatically, using L-arginine as substrate, just like 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



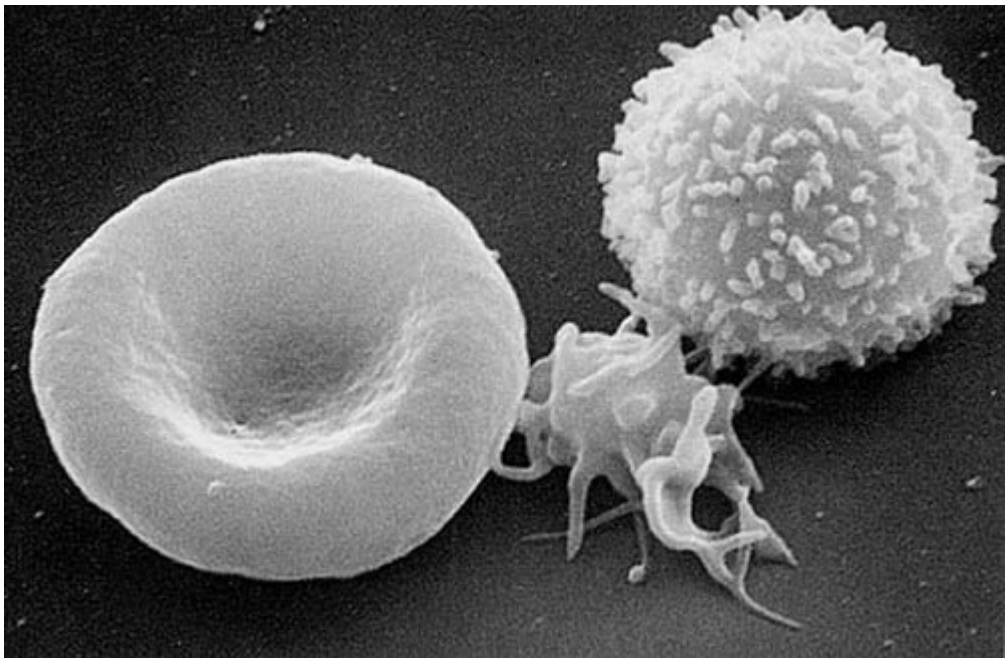
Typical mammalian erythrocytes: (a) seen from surface; (b) in profile, forming rouleaux; (c) rendered spherical by water; (d) rendered crenate by salt. (c) and (d) do not normally occur in the body.

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 lactic acid fermentation of glucose. 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.

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.

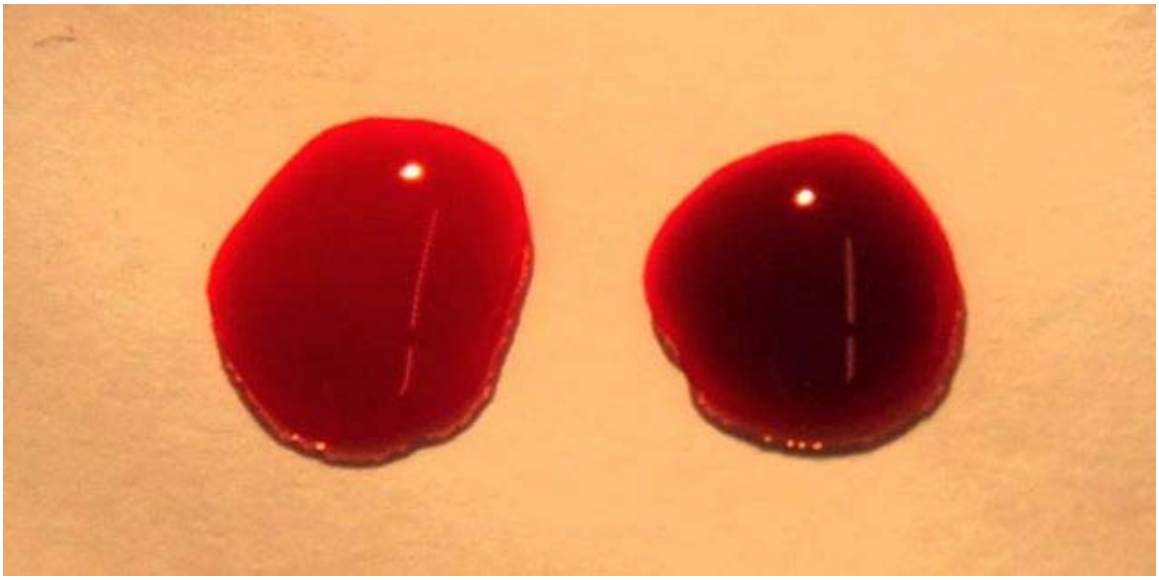
In large blood vessels, red blood cells sometimes occur as a stack, flat side next to flat side. This is known as *rouleaux formation*, and it occurs more often if the levels of certain serum proteins are elevated, as for instance during inflammation.

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.

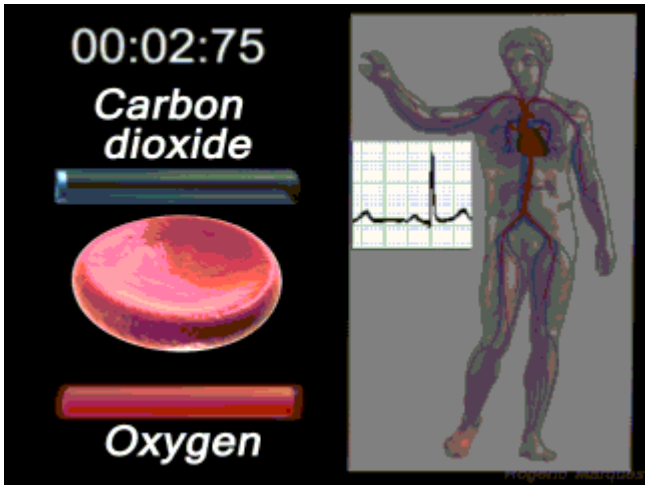


Scanning electron micrograph of blood cells. From left to right: human erythrocyte, thrombocyte (platelet), leukocyte.

Human erythrocytes



Two drops of blood are shown with a bright red oxygenated drop on the left and a deoxygenated drop on the right.



A typical human red blood cell cycle in the circulatory system. This image shows the red blood cell deform as it enters capillaries, as well as changing color as it alternates in states of oxygenation along the circulatory system.

A typical human erythrocyte has a disk diameter of 6–8 μm and a thickness of 2 μm, being much smaller than most other human cells. These cells have a 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 2–3 × 10^{13} (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 human red blood cells for protein biosynthesis.

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, these cells live in blood circulation for about 100 to 120 days. At the end of their lifespan, they become senescent, and are removed from circulation.

Erythropoiesis

Erythropoiesis is the development process in which new erythrocytes are produced, through which each cell matures in 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 erythropoietin (EPO), synthesised by the kidney. Just before and after leaving the bone marrow, the developing cells are known as reticulocytes; these comprise about 1% of circulating red blood cells.

Functional lifetime

This phase lasts about 100–120 days, during which the erythrocytes are continually moving by the blood flow push (in arteries), pull (in veins) and squeezing through microvessels such as capillaries as they compress against each other in order to move.

Senescence

The aging erythrocyte undergoes changes in its plasma membrane, making it susceptible to selective recognition by macrophages and subsequent phagocytosis in the

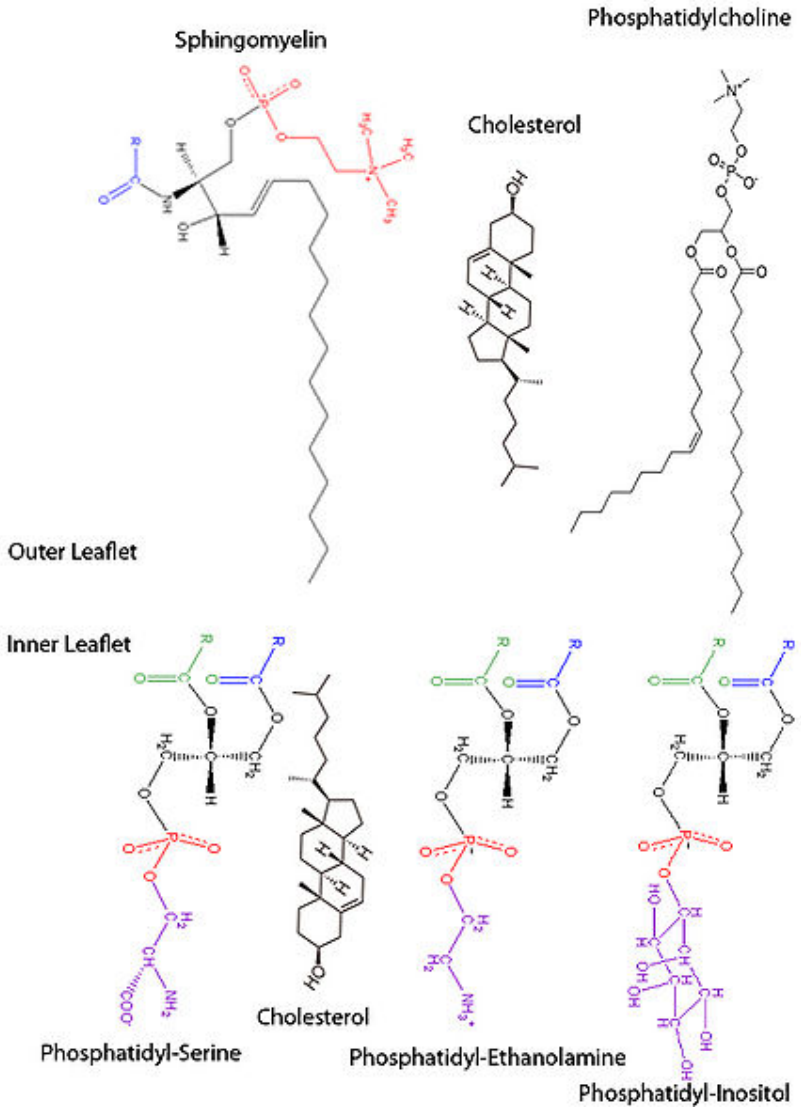
reticuloendothelial system (spleen, liver and bone marrow), thus removing old and defective cells and continually purging the blood. This process is termed eryptosis, or erythrocyte programmed cell death. This process normally occurs at the same rate of production by erythropoiesis, balancing the total 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 important 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.

Membrane 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. In human erythrocytes, like in most mammal erythrocytes, half of the membrane mass is represented by proteins and 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.

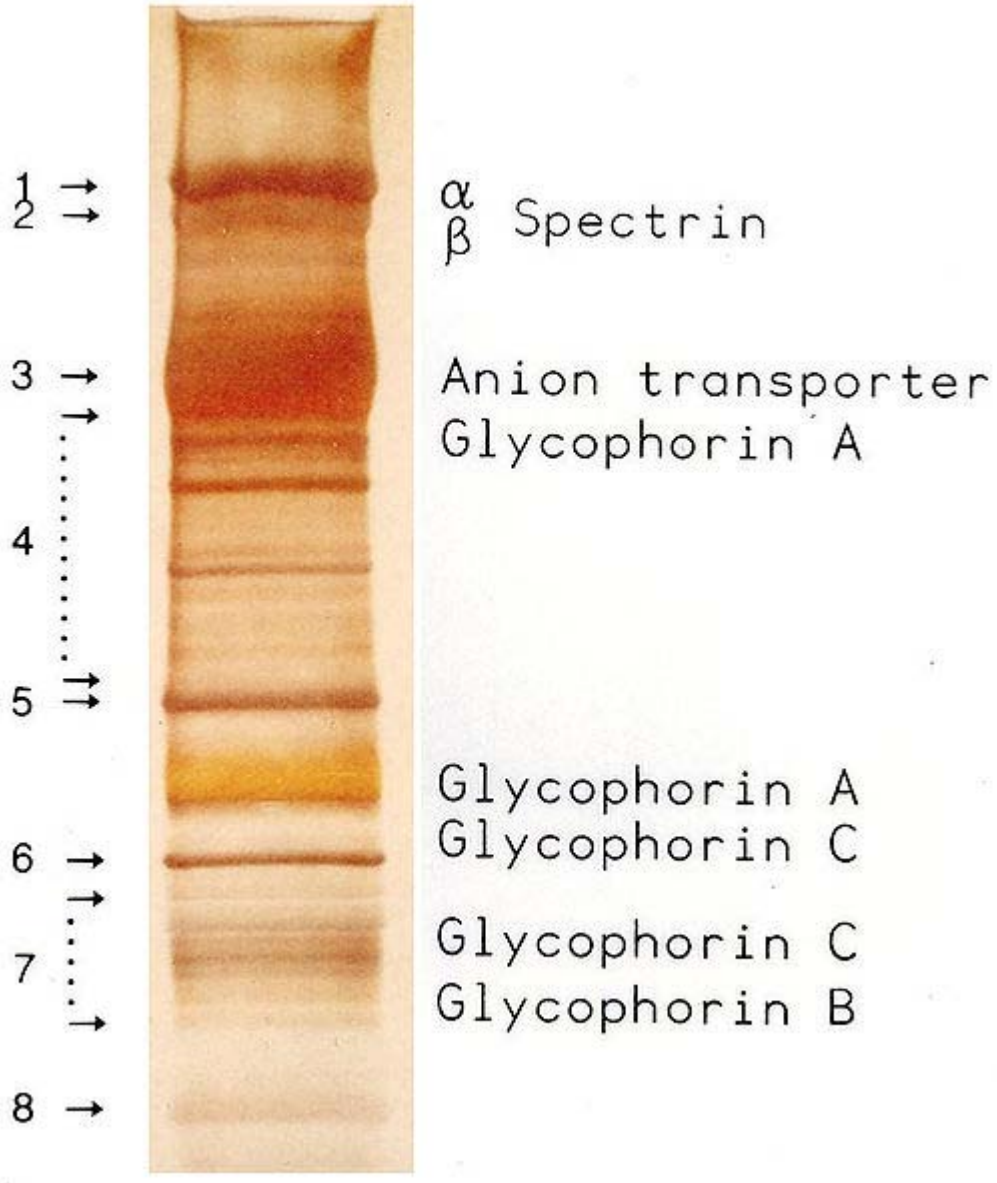
- Premature destruction of thallassemic and sickle red cells has been linked to disruptions of lipid asymmetry leading to exposure of PS on the outer monolayer.

- 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.

Membrane proteins

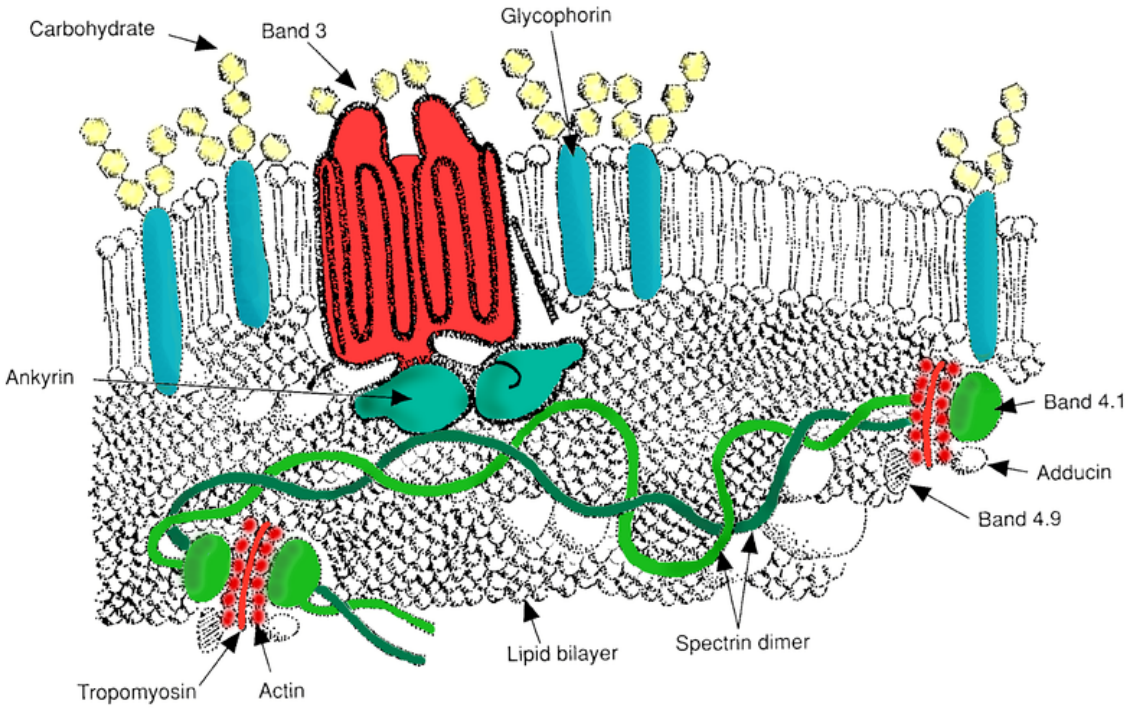


Red blood cell membrane proteins separated by SDS-Page and silverstained

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;

- Adducin - interaction with band 3;
- Dematin- interaction with the Glut1 glucose transporter.

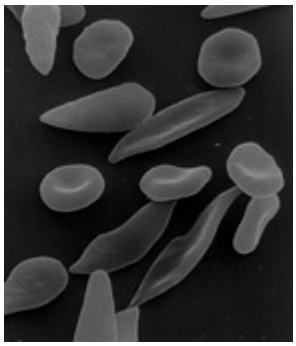
Separation and blood doping

Red blood cells can be obtained from whole blood by centrifugation, which separates the cells from the blood plasma. During plasma donation, the red blood cells are pumped back into the body right away and 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.) 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.

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.

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.

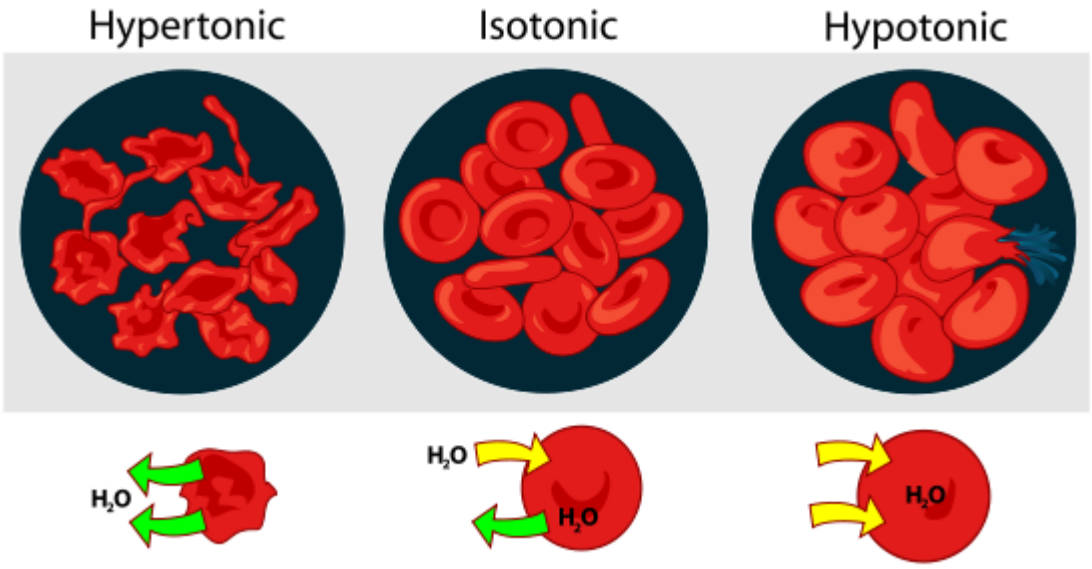
- Thalassemia is a genetic disease that results in the production of an abnormal ratio of hemoglobin subunits.

- Spherocytosis is a genetic disease that causes a defect in the red blood cell's cytoskeleton, causing the red blood cells to be small, sphere-shaped, and fragile instead of donut-shaped and flexible.

- 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

- 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.

- Inherited hemolytic anemias caused by abnormalities of the erythrocyte membrane comprise an important group of inherited disorders. These disorders are characterized by clinical and biochemical heterogeneity and also genetic heterogeneity, as evidenced by recent molecular studies.

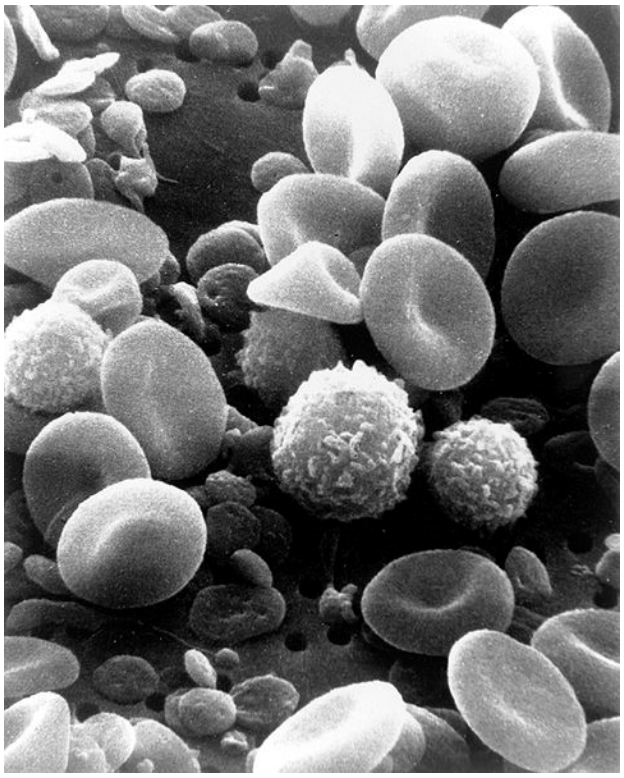
    - The Hereditary spherocytosis (HS) syndromes are a group of inherited disorders characterized by the presence of spherical-shaped erythrocytes on the peripheral blood smear. HS is found worldwide. It is the most common inherited anemia in individuals of northern European descent, affecting approximately 1 in 1000-2500 individuals depending on the diagnostic criteria. The primary defect in hereditary spherocytosis is a deficiency of membrane surface area. Decreased surface area may produced by two different mechanisms: 1) Defects of spectrin, ankyrin, or protein 4.2 lead to reduced density of the membrane skeleton, destabilizing the overlying lipid bilayer and releasing band 3-containing microvesicles. 2) Defects of band 3 lead to band 3 deficiency and loss of its lipid-stabilizing effect. This results in the loss of band 3-free microvesicles. Both pathways result in membrane loss, decreased surface area, and formation of spherocytes with decreased deformability. These deformed erythrocytes become trapped in the hostile environment of the spleen where splenic conditioning inflicts further membrane damage, amplifying the cycle of membrane injury.
    - Hereditary elliptocytosis
    - Hereditary pyropoikilocytosis
    - Hereditary stomatocytosis

- 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. The blood type needs to be determined to prepare for a blood transfusion or an organ transplantation.

Chapter 2

White Blood Cell



A scanning electron microscope image of normal circulating human blood. In addition to the irregularly shaped leukocytes, both red blood cells and many small disc-shaped platelets are visible.

White blood cells, or **leukocytes** (also spelled "leucocytes," "leuco-" being Greek for white), are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.

The number of WBCs in the blood is often an indicator of disease. There are normally between 4×10^9 and 1.1×10^{10} white blood cells in a litre of blood, making up

approximately 1% of blood in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia.

Etymology

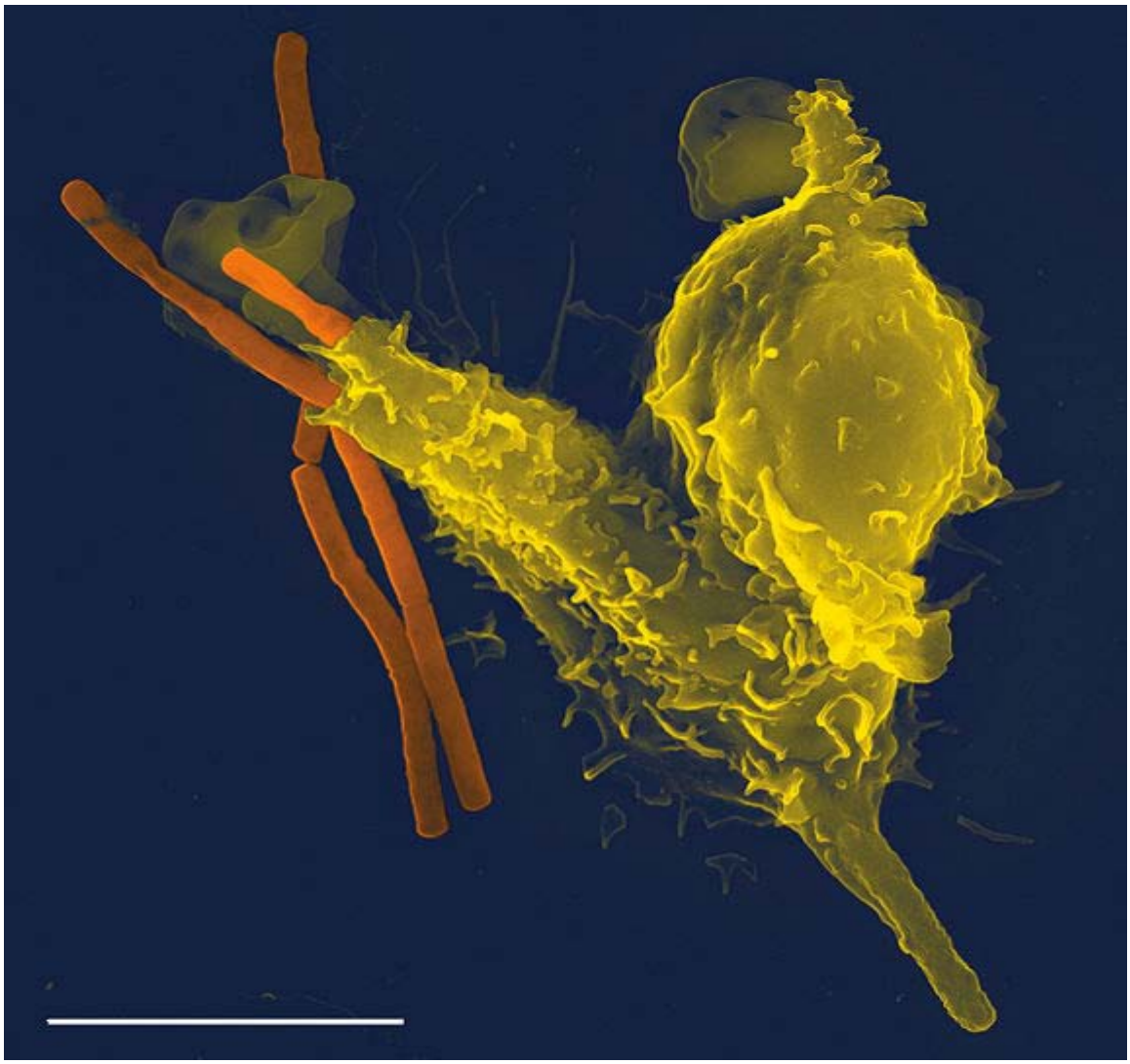
The name "white blood cell" derives from the fact that after centrifugation of a blood sample, the white cells are found in the *buffy coat*, a thin, typically white layer of nucleated cells between the sedimented red blood cells and the blood plasma. The scientific term *leukocyte* directly reflects this description, derived from Greek *λευκό* (white), and *κύτταρο* (cell). Blood plasma may sometimes be green if there are large amounts of neutrophils in the sample, due to the heme-containing enzyme myeloperoxidase that they produce.

Types

There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells are often characterized as granulocytes or agranulocytes:

- **Granulocytes** (polymorphonuclear leukocytes): leukocytes characterised by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes which primarily act in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils, which are named according to their staining properties.
- **Agranulocytes** (mononuclear leucocytes): leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes. The cells include lymphocytes, monocytes, and macrophages.

Neutrophil



Neutrophil engulfing anthrax bacteria

Neutrophils defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are commonly referred to as polymorphonuclear (PMN) leukocytes, although technically PMN refers to all granulocytes. They have a multilobed nucleus which may appear like multiple nuclei, hence the name polymorphonuclear leukocyte. The cytoplasm may look transparent because of fine granules that are pale lilac. Neutrophils are very active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes used in digesting microbes and die after having phagocytosed a few pathogens. Most common cell seen in acute inflammation, comes in and kill foreign substance.They make up 60-70% of total leukocyte count.The life span of neutrophil is about 8 days.

Eosinophil

Eosinophils primarily deal with parasitic infections and an increase in them may indicate such. Eosinophils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. Generally their nucleus is bi-lobed. The cytoplasm is full of granules which assume a characteristic pink-orange color with eosin stain.

Basophil

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilation. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules which hide it. They are characterized by their large blue granules.

Lymphocyte

Lymphocytes are much more common in the lymphatic system. Lymphocytes are distinguished by having a deeply staining nucleus which may be eccentric in location, and a relatively small amount of cytoplasm. The blood has three types of lymphocytes:

- B cells: B cells make antibodies that bind to pathogens to enable their destruction. (B cells not only make antibodies that bind to pathogens, but after an attack, some B cells will retain the ability to produce an antibody to serve as a 'memory' system.)
- T cells:
    - CD4+ (helper) T cells co-ordinate the immune response and are important in the defense against intracellular bacteria. In acute HIV infection, these T cells are the main index to identify the individual's immune system activity. Research has shown that CD8+ cells are also another index to identify human's immune activity.
    - CD8+ cytotoxic T cells are able to kill virus-infected and tumor cells.
    - γδ T cells possess an alternative T cell receptor as opposed to CD4+ and CD8+ αβ T cells and share characteristics of helper T cells, cytotoxic T cells and natural killer cells.
- Natural killer cells: Natural killer cells are able to kill cells of the body which are displaying a signal to kill them, as they have been infected by a virus or have become cancerous.

Monocyte

Monocytes share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an additional role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed, or so that an antibody response may be mounted. Monocytes eventually leave the bloodstream to become tissue macrophages which remove dead cell debris as well as attacking microorganisms. Neither

of these can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically agranulated. They also possess abundant cytoplasm.

Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages.

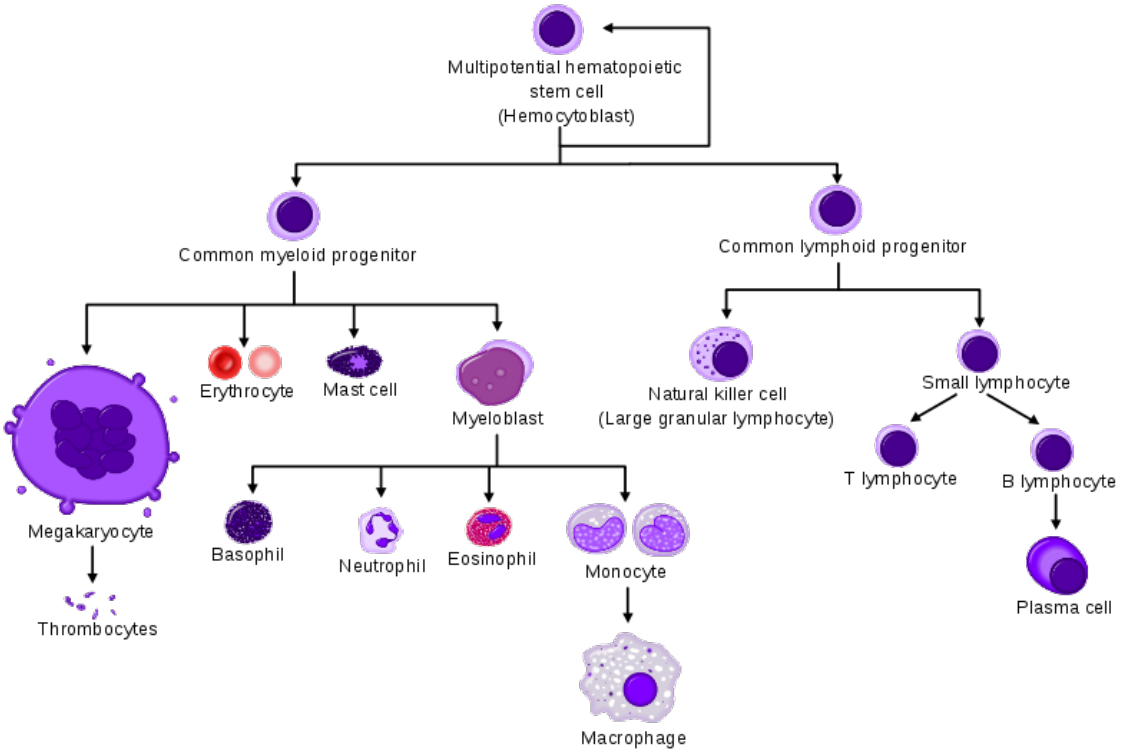
Medication causing leukopenia

Some medications can have an impact on the number and function of white blood cells. Leukopenia is the reduction in the number of white blood cells, which may affect the overall white cell count or one of the specific populations of white blood cells. For example, if the number of neutrophils is low, the condition is known as neutropenia. Likewise, low lymphocyte levels are termed lymphopenia. Medications which can cause leukopenia include clozapine, an antipsychotic medication with a rare adverse effect leading to the total absence of all granulocytes (neutrophils, basophils, eosinophils). Other medications include immunosuppressive drugs, such as sirolimus, mycophenolate mofetil, tacrolimus, and cyclosporine. Interferons used to treat multiple sclerosis, like Rebif, Avonex, and Betaseron, can also cause leukopenia.

Fixed leukocytes

Some leukocytes migrate into the tissues of the body to take up a permanent residence at that location rather than remaining in the blood. Often these cells have specific names depending upon which tissue they settle in, such as fixed macrophages in the liver which become known as Kupffer cells. These cells still serve a role in the immune system.

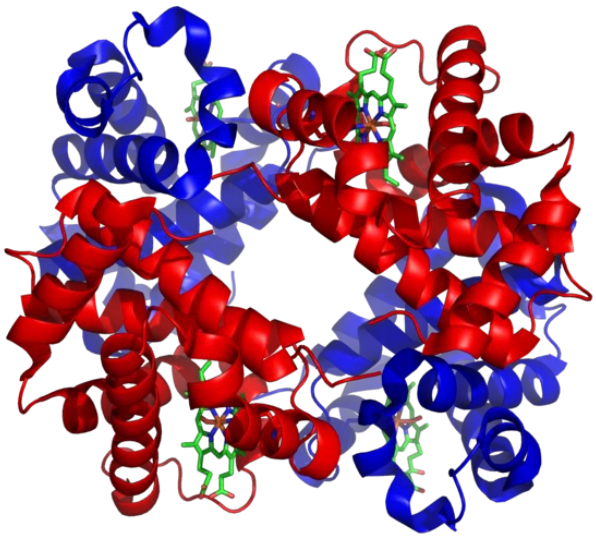
- Histiocytes
- Dendritic cells (Although these will often migrate to local lymph nodes upon ingesting antigens)
- Mast cells
- Microglia



HSC=Hematopoietic stem cell, Progenitor=Progenitor cell, L-blast=Lymphoblast, Lymphocyte, Mo-blast=Monoblast, Monocyte, Myeloblast, Pro-M=Promyelocyte, Myelocyte, Meta-M=Metamyelocyte, Neutrophil, Eosinophil, Basophil, Pro-E=Proerythroblast, Baso-E=Basophilic erythroblast, poly-E=Polychromatic erythroblast, Ortho-E=Orthochromatic erythroblast, Erythrocyte, Promegakaryocyte, Megakaryocyte, Platlet

Chapter 3

Hemoglobin

Hemoglobin, human, adult (heterotetramer, $(\alpha\beta)_2$)		
		
Structure of human hemoglobin. The protein's α and β subunits are in red and blue, and the iron-containing heme groups in green. From PDB 1GZX *Proteopedia Hemoglobin*		
Protein type		metalloprotein, globulin
Function		oxygen-transport
Cofactor(s)		heme (4)
Subunit Name	Gene	Chromosomal Locus
Hb-α1	HBA1	Chr. 16 p13.3

Hb-α2	HBA2	Chr. 16 p13.3
Hb-β	HBB	Chr. 11 p15.5

Hemoglobin (also spelled **haemoglobin** and abbreviated **Hb** or **Hgb**) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (except the fish family Channichthyidae) and the tissues of some invertebrates. Hemoglobin in the blood is what transports oxygen from the lungs or gills to the rest of the body (i.e. the tissues) where it releases the oxygen for cell use, and collects carbon dioxide to bring it back to the lungs.

In mammals the protein makes up about 97% of the red blood cells' dry content, and around 35% of the total content (including water). Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin, which increases the total blood oxygen capacity seventyfold.

Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.

Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobins may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leghemoglobin, is used to scavenge oxygen, to keep it from poisoning anaerobic systems, such as nitrogen-fixing nodules of leguminous plants.

Research history

The oxygen-carrying protein hemoglobin was discovered by Hünefeld in 1840. In 1851, Otto Funke published a series of articles in which he described growing hemoglobin crystals by successively diluting red blood cells with a solvent such as pure water, alcohol or ether, followed by slow evaporation of the solvent from the resulting protein solution. Hemoglobin's reversible oxygenation was described a few years later by Felix Hoppe-Seyler.

In 1959 Max Perutz determined the molecular structure of hemoglobin by X-ray crystallography. This work resulted in his sharing with John Kendrew the 1962 Nobel Prize in Chemistry.

The role of hemoglobin in the blood was elucidated by physiologist Claude Bernard. The name *hemoglobin* is derived from the words *heme* and *globin*, reflecting the fact that each subunit of hemoglobin is a globular protein with an embedded heme (or haem) group. Each heme group contains one iron atom, that can bind one oxygen molecule through ion-induced dipole forces. The most common type of hemoglobin in mammals contains four such subunits.

Genetics

Hemoglobin consists mostly of protein (the "globin" chains), and these proteins, in turn, are composed of sequences of amino acids. These sequences are linear, in the manner of letters in a written sentence or beads on a string. In all proteins, it is the variation in the type of amino acids in the protein sequence of amino acids, which determine the protein's chemical properties and function. This is true of hemoglobin, where the sequence of amino acids may affect crucial functions such as the protein's affinity for oxygen.

There is more than one hemoglobin gene. The amino acid sequences of the globin proteins in hemoglobins usually differ between species, although the differences grow with the evolutionary distance between species. For example, the most common hemoglobin sequences in humans and chimpanzees are nearly identical, differing by only one amino acid in both the alpha and the beta globin protein chains. These differences grow larger between less closely related species.

Even within a species, different variants of hemoglobin always exist, although one sequence is usually a "most common" one in each species. Mutations in the genes for the hemoglobin protein in a species result in hemoglobin variants. Many of these mutant forms of hemoglobin cause no disease. Some of these mutant forms of hemoglobin, however, cause a group of hereditary diseases termed the *hemoglobinopathies*. The best known hemoglobinopathy is sickle-cell disease, which was the first human disease whose mechanism was understood at the molecular level. A (mostly) separate set of diseases called thalassemias involves underproduction of normal and sometimes abnormal hemoglobins, through problems and mutations in globin gene regulation. All these diseases produce anemia.

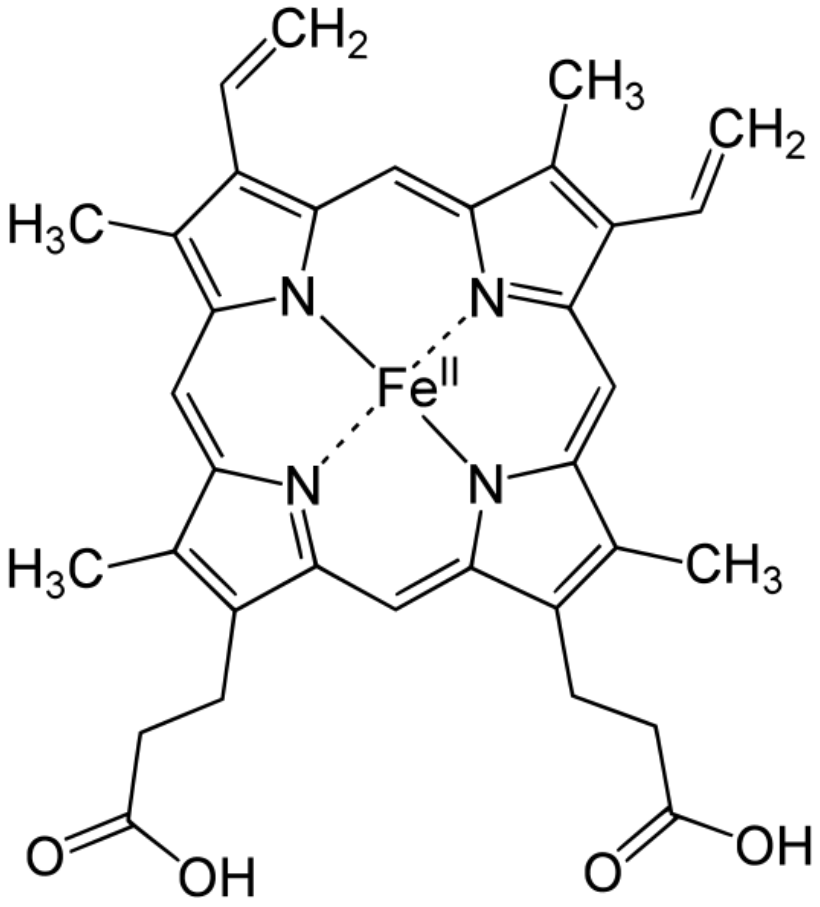
Variations in hemoglobin amino acid sequences, as with other proteins, may be adaptive. For example, recent studies have suggested genetic variants in deer mice that help explain how deer mice that live in the mountains are able to survive in the thin air that accompanies high altitudes. A researcher from the University of Nebraska-Lincoln found mutations in four different genes that can account for differences between deer mice that live in lowland prairies versus the mountains. After examining wild mice captured from both highlands and lowlands, it was found that: the genes of the two breeds are “virtually identical–except for those that govern the oxygen-carrying capacity of their hemoglobin”.

"The genetic difference enables highland mice to make more efficient use of their oxygen", since less is available at higher altitudes, such as those in the mountains. Mammoth hemoglobin featured mutations that allowed for oxygen delivery at lower temperatures, thus enabling mammoths to migrate to higher latitudes during the Pleistocene.

Synthesis

Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

Structure



Heme b group

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin. This folding pattern contains a pocket that strongly binds the heme group.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of the F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure.

The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporally turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable to bind oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin type is a tetramer (which contains 4 subunit proteins) called **hemoglobin A**, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of about 17,000 daltons, for a total molecular weight of the tetramer of about 68,000 daltons (64,458 g/mol). Thus, 1 g/dL = 0.01551 mmol/L. Hemoglobin A is the most intensively studied of the hemoglobin molecules.

In human infants, the hemoglobin molecule is made up of 2 α chains and 2 gamma chains. The gamma chains are gradually replaced by β chains as the infant grows.

The four polypeptide chains are bound to each other by salt bridges, hydrogen bonds, and the hydrophobic effect. There are two kinds of contacts between the α and β chains: $\alpha_1\beta_1$ and $\alpha_1\beta_2$.

In general, hemoglobin can be saturated with oxygen molecules (oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin). *Oxyhemoglobin* is formed during physiological respiration when oxygen binds to the heme component of the protein hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized in aerobic glycolysis and in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH.

Hemoglobin exists in two forms, a **taut form** (T) and a **relaxed form** (R). Various factors such as low pH, high CO_2 and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. The opposite of these aforementioned factors at the level of the lung capillaries favors the relaxed form which can better bind oxygen.

Deoxyhemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This difference is used for measurement of the amount of oxygen in patient's blood by an instrument called pulse oximeter.

Iron's oxidation state in oxyhemoglobin

Assigning oxygenated hemoglobin's oxidation state is difficult because oxyhemoglobin ($Hb\text{-}O_2$), by experimental measurement, is diamagnetic (no net unpaired electrons), yet the low-energy electron configurations in both oxygen and iron are paramagnetic (suggesting at least one unpaired electron in the complex). The lowest-energy form of oxygen, and the lowest energy forms of the relevant oxidation states of iron, are these:

- Triplet oxygen, the lowest energy molecular oxygen species, has two unpaired electrons in antibonding π* molecular orbitals.
- Iron(II) tends to exist in a high-spin configuration where unpaired electrons exist in E_g antibonding orbitals.
- Iron(III) has an odd number of electrons, and thus must have one or more unpaired electrons, in any energy state.

All of these structures are paramagnetic (have unpaired electrons), not diamagnetic. Thus, a non-intuitive (e.g., a higher-energy for at least one species) distribution of electrons in

the combination of iron and oxygen must exist, in order to explain the observed diamagnetism and no unpaired electrons.

The three logical possibilities to produce diamagnetic (no net spin) Hb-O_2 are:

1. Low-spin Fe^{2+} binds to singlet oxygen. Both low-spin iron and singlet oxygen are diamagnetic. However, the singlet form of oxygen is the higher-energy form of the molecule.
2. Low-spin Fe^{3+} binds to $\cdot O_2^-$ (the superoxide ion) and the two unpaired electrons couple antiferromagnetically, giving diamagnetic properties.
3. Low-spin Fe^{4+} binds to peroxide, O_2^{2-}. Both are diamagnetic.

Direct experimental data:

- X-ray photoelectron spectroscopy suggests iron has an oxidation state of approximately 3.2
- infrared stretching frequencies of the O-O bond suggests a bond length fitting with superoxide (a bond order of about 1.6, with superoxide being 1.5).

Thus, the nearest formal oxidation state of iron in Hb-O_2 is the +3 state, with oxygen in the -1 state (as superoxide $\cdot O_2^-$). The diamagnetism in this configuration arises from the single unpaired electron on superoxide aligning antiferromagnetically from the single unpaired electron on iron, to give no net spin to the entire configuration, in accordance with diamagnetic oxyhemoglobin from experiment.

The second choice of the three logical possibilities above for diamagnetic oxyhemoglobin being found correct by experiment, is not surprising: singlet oxygen (possibility #1) and large separations of charge (possibility #3) are both unfavorably high-energy states. Iron's shift to a higher oxidation state in Hb-O_2 decreases the atom's size, and allows it into the plane of the porphyrin ring, pulling on the coordinated histidine residue and initiating the allosteric changes seen in the globulins.

Early postulates by bio-inorganic chemists claimed that possibility #1 (above) was correct and that iron should exist in oxidation state II. This seemed particularly likely since the iron oxidation state III as methemoglobin, when **not** accompanied by superoxide $\cdot O_2^-$ to "hold" the oxidation electron, was known to render hemoglobin incapable of binding normal triplet O_2 as it occurs in the air. It was thus assumed that iron remained as Fe(II) when oxygen gas was bound in the lungs. The iron chemistry in this previous classical model was elegant, but the required presence of the required diamagnetic high-energy singlet oxygen was never explained. It was classically argued that the binding of an oxygen molecule placed high-spin iron(II) in an octahedral field of strong-field ligands; this change in field would increase the crystal field splitting energy, causing iron's electrons to pair into the low-spin configuration, which would be diamagnetic in Fe(II). This forced low-spin pairing is indeed thought to happen in iron when oxygen binds, but is not enough to explain iron's change in size. Extraction of an

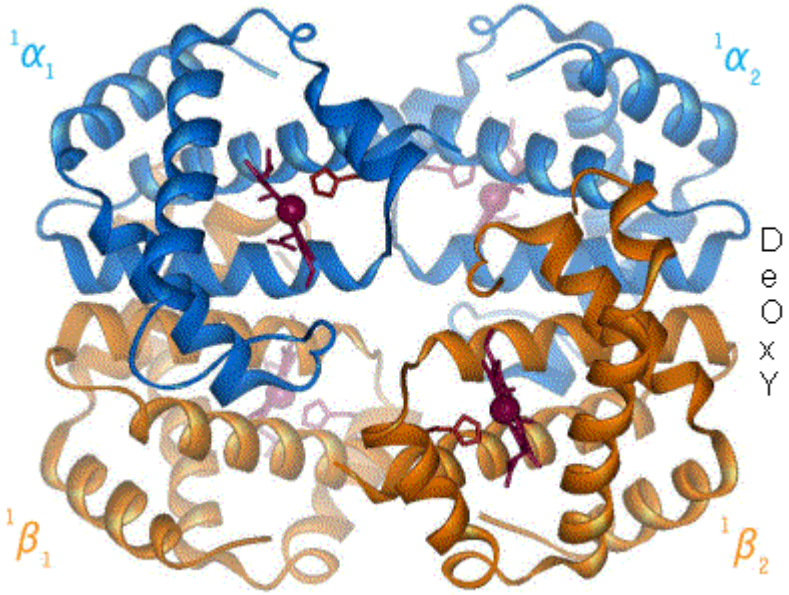
additional electron from iron by oxygen is required to explain both iron's smaller size and observed increased oxidation state, and oxygen's weaker bond.

It should be noted that the assignment of a whole-number oxidation state is a formalism, as the covalent bonds are not required to have perfect bond orders involving whole electron-transfer. Thus, all three models for paramagnetic Hb-O_2 may contribute to some small degree (by resonance) to the actual electronic configuration of Hb-O_2. However, the model of iron in Hb-O_2 being Fe(III) is more correct than the classical idea that it remains Fe(II).

Binding for ligands other than oxygen

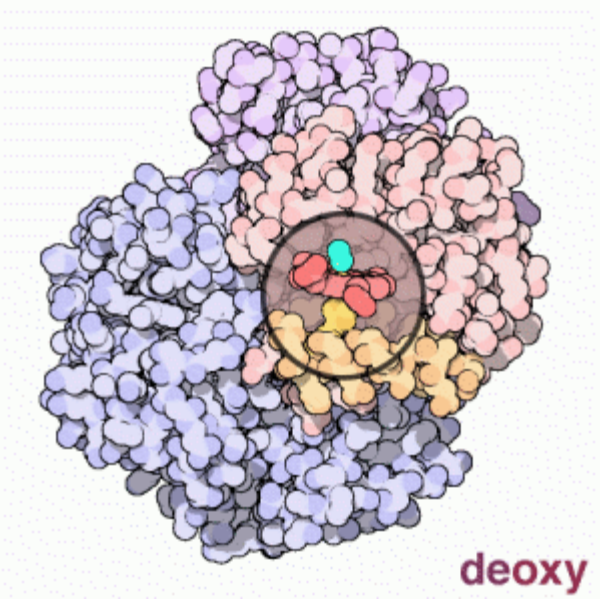
Besides the oxygen ligand, which binds to hemoglobin in a cooperative manner, hemoglobin ligands also include competitive inhibitors such as carbon monoxide (CO) and allosteric ligands such as carbon dioxide (CO_2) and nitric oxide (NO). The carbon dioxide is bound to amino groups of the globin proteins as carbaminohemoglobin, and is thought to account for about 10% of carbon dioxide transport in mammals. Nitric oxide is bound to specific thiol groups in the globin protein to form an S-nitrosothiol which dissociates into free nitric oxide and thiol again, as the hemoglobin releases oxygen from its heme site. This nitric oxide transport to peripheral tissues is hypothesised to assist oxygen transport in tissues, by releasing vasodilatory nitric oxide to tissues in which oxygen levels are low.

Cooperative



A schematic visual model of oxygen-binding process, showing all four monomers and hemes, and protein chains only as diagramatic coils, to facilitate visualization into the molecule. Oxygen is not shown in this model, but, for each of the iron atoms, it binds to the iron (red sphere) in the flat heme. For example, in the upper left of the four hemes shown, oxygen binds at the left of the iron atom shown in the upper left of diagram. This

causes the iron atom to move backward into the heme which holds it (the iron moves upward as it binds oxygen, in this illustration), tugging the histidine residue (modeled as a red pentagon on the right of the iron) closer, as it does. This, in turn, pulls on the protein chain holding the histidine.



Another view of how binding and release of ligands induces a conformational (structural) change in hemoglobin. Only one of the four heme groups is shown, but more of the electron cloud of the protein chain is included in this diagram, as compared with above. The binding and release of oxygen (shown now in green) illustrates the structural differences between oxy- and deoxyhemoglobin, respectively. The histidine, which is pulled by motion of the iron atom, is shown here in yellow.

When oxygen binds to the iron complex, it causes the iron atom to move back toward the center of the plane of the porphyrin ring. At the same time, the imidazole side-chain of the histidine residue interacting at the other pole of the iron is pulled toward the porphyrin ring. This interaction forces the plane of the ring sideways toward the outside of the tetramer, and also induces a strain in the protein helix containing the histidine as it moves nearer to the iron atom. This strain is transmitted to the remaining three monomers in the tetramer, where it induces a similar conformational change in the other heme sites such that binding of oxygen to these sites becomes easier.

In the tetrameric form of normal adult hemoglobin, the binding of oxygen is, thus, a cooperative process. The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygens bound influencing the shape of the binding sites for the next oxygens, in a way favorable for binding. This positive cooperative binding is achieved through steric conformational changes of the hemoglobin protein complex as discussed above; i.e., when one subunit protein in hemoglobin becomes oxygenated, a conformational or structural change in the whole complex is initiated, causing the other subunits to gain an increased affinity for oxygen. As a

consequence, the oxygen binding curve of hemoglobin is sigmoidal, or *S*-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding.

The dynamic mechanism of the cooperativity in hemoglobin and its relation with the low-frequency resonance has been discussed.

Competitive

Hemoglobin's oxygen-binding capacity is decreased in the presence of carbon monoxide because both gases compete for the same binding sites on hemoglobin, carbon monoxide binding preferentially in place of oxygen.

The binding of oxygen is affected by molecules such as carbon monoxide (CO) (for example, from tobacco smoking, car exhaust, and incomplete combustion in furnaces). CO competes with oxygen at the heme binding site. Hemoglobin binding affinity for CO is 250 times greater than its affinity for oxygen, meaning that small amounts of CO dramatically reduce hemoglobin's ability to transport oxygen. When hemoglobin combines with CO, it forms a very bright red compound called carboxyhemoglobin, which may cause the skin of CO poisoning victims to appear pink in death, instead of white or blue. When inspired air contains CO levels as low as 0.02%, headache and nausea occur; if the CO concentration is increased to 0.1%, unconsciousness will follow. In heavy smokers, up to 20% of the oxygen-active sites can be blocked by CO.

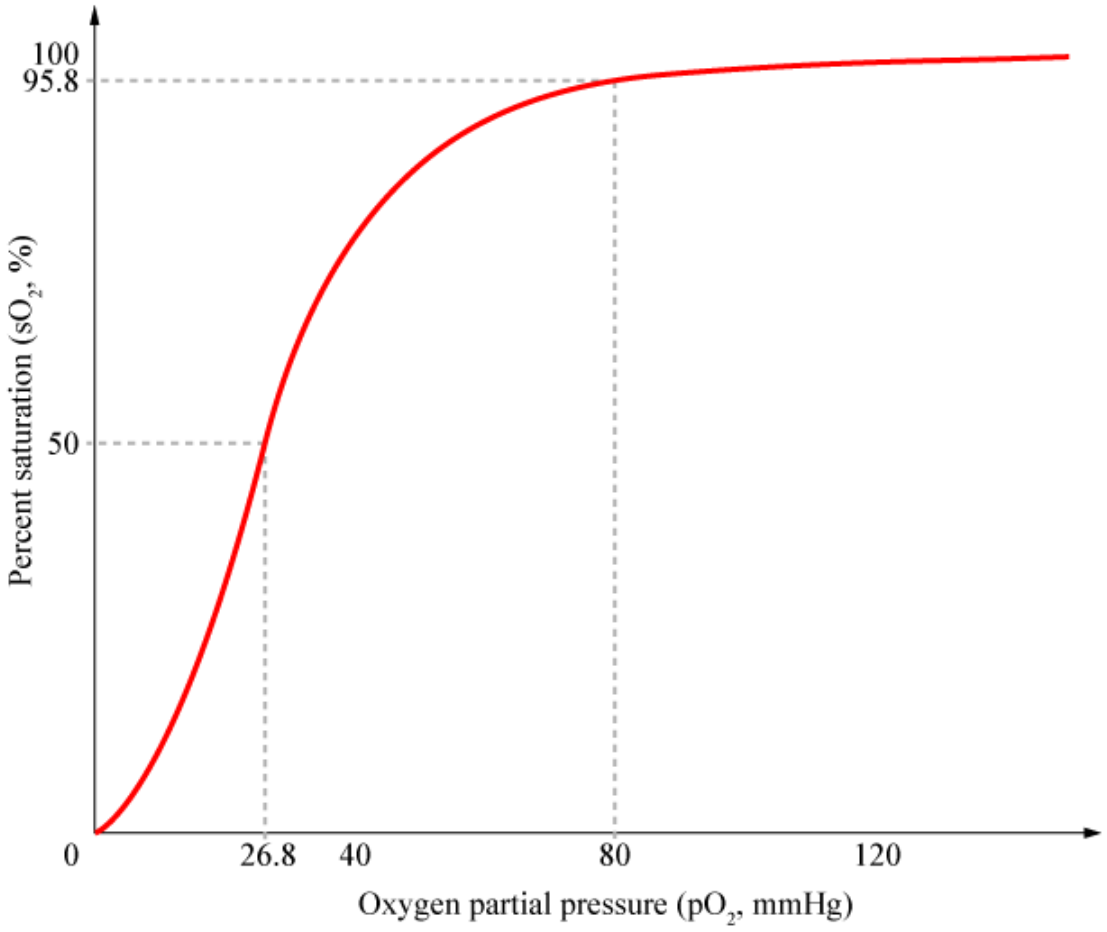
In similar fashion, hemoglobin also has competitive binding affinity for cyanide (CN^-), sulfur monoxide (SO), nitric oxide (NO), and sulfide(S^{2-}), including hydrogen sulfide (H_2S). All of these bind to iron in heme without changing its oxidation state, but they nevertheless inhibit oxygen-binding, causing grave toxicity.

The iron atom in the heme group must initially be in the ferrous (Fe^{2+}) oxidation state to support oxygen and other gases' binding and transport (it temporarily switches to ferric during the time oxygen is bound, as explained above). Initial oxidation to the ferric (Fe^{3+}) state without oxygen converts hemoglobin into "hemiglobin" or methemoglobin (pronounced "MET-hemoglobin"), which cannot bind oxygen. Hemoglobin in normal red blood cells is protected by a reduction system to keep this from happening. Nitric oxide is capable of converting a small fraction of hemoglobin to methemoglobin in red blood cells. The latter reaction is a remnant activity of the more ancient nitric oxide dioxygenase function of globins.

Allosteric

Carbon *di*oxide occupies a different binding site on the hemoglobin. Carbon dioxide is more readily dissolved in deoxygenated blood, facilitating its removal from the body after the oxygen has been released to tissues undergoing metabolism. This increased affinity for carbon dioxide by the venous blood is known as the Haldane effect. Through the enzyme carbonic anhydrase, carbon dioxide reacts with water to give carbonic acid, which decomposes into bicarbonate and protons:

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



The sigmoidal shape of hemoglobin's oxygen-dissociation curve results from cooperative binding of oxygen to hemoglobin.

Hence blood with high carbon dioxide levels is also lower in pH (more acidic). Hemoglobin can bind protons and carbon dioxide, which causes a conformational change in the protein and facilitates the release of oxygen. Protons bind at various places on the protein, while carbon dioxide binds at the α-amino group. Carbon dioxide binds to hemoglobin and forms carbaminohemoglobin. This decrease in hemoglobin's affinity for oxygen by the binding of carbon dioxide and acid is known as the Bohr effect (shifts the O_2-saturation curve to the *right*). Conversely, when the carbon dioxide levels in the blood decrease (i.e., in the lung capillaries), carbon dioxide and protons are released from hemoglobin, increasing the oxygen affinity of the protein.

It is necessary for hemoglobin to release the oxygen that it binds; if not, there is no point in binding it. The sigmoidal curve of hemoglobin makes it efficient in binding (taking up O_2 in lungs), and efficient in unloading (unloading O_2 in tissues).

In people acclimated to high altitudes, the concentration of 2,3-Bisphosphoglycerate (2,3-BPG) in the blood is increased, which allows these individuals to deliver a larger amount of oxygen to tissues under conditions of lower oxygen tension. This phenomenon, where molecule Y affects the binding of molecule X to a transport molecule Z, is called a *heterotropic* allosteric effect.

A variant hemoglobin, called fetal hemoglobin (HbF, $\alpha_2\gamma_2$), is found in the developing fetus, and binds oxygen with greater affinity than adult hemoglobin. This means that the oxygen binding curve for fetal hemoglobin is left-shifted (i.e., a higher percentage of hemoglobin has oxygen bound to it at lower oxygen tension), in comparison to that of adult hemoglobin. As a result, fetal blood in the placenta is able to take oxygen from maternal blood.

Hemoglobin also carries nitric oxide in the globin part of the molecule. This improves oxygen delivery in the periphery and contributes to the control of respiration. NO binds reversibly to a specific cysteine residue in globin; the binding depends on the state (R or T) of the hemoglobin. The resulting S-nitrosylated hemoglobin influences various NO-related activities such as the control of vascular resistance, blood pressure and respiration. NO is not released in the cytoplasm of erythrocytes but transported by an anion exchanger called AE1 out of them.

A study was performed to examine the influence of the form of hemoglobin (Hb) on the partitioning of inhaled volatile organic compounds (VOCs) into [human and animal] blood. Benzene was the prototypic VOC used in the investigations for this research due to the similar properties it shares with many other VOCs. To be specific, this study analyses the influence of the water solubility of Hb on the partitioning coefficient (PC) of a VOC as compared to the influence of the "species" or form of Hb. The different forms of blood used include: human hemoglobin (HbA), rat Hb, and sickle-cell hemoglobin (HbS). Rat Hb contains little water and is in a quasi-crystalline form, found inside the red blood cells (RBC), meaning they are more hydrophobic than human Hb, which are water-soluble. Sickle-cell hemoglobin (HbS) is water-soluble, however it can become water-insoluble, forming hydrophobic polymers, when deoxygenated. The findings state that the benzene PC for rat Hb was much higher than human that for Hb; however, the tests that measured the PCs of the oxygenated and deoxygenated forms of HbA and HbS did not differ, indicating that the affinity of benzene was not affected by the water solubility of Hb.

Types in humans

Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants.

In the embryo:

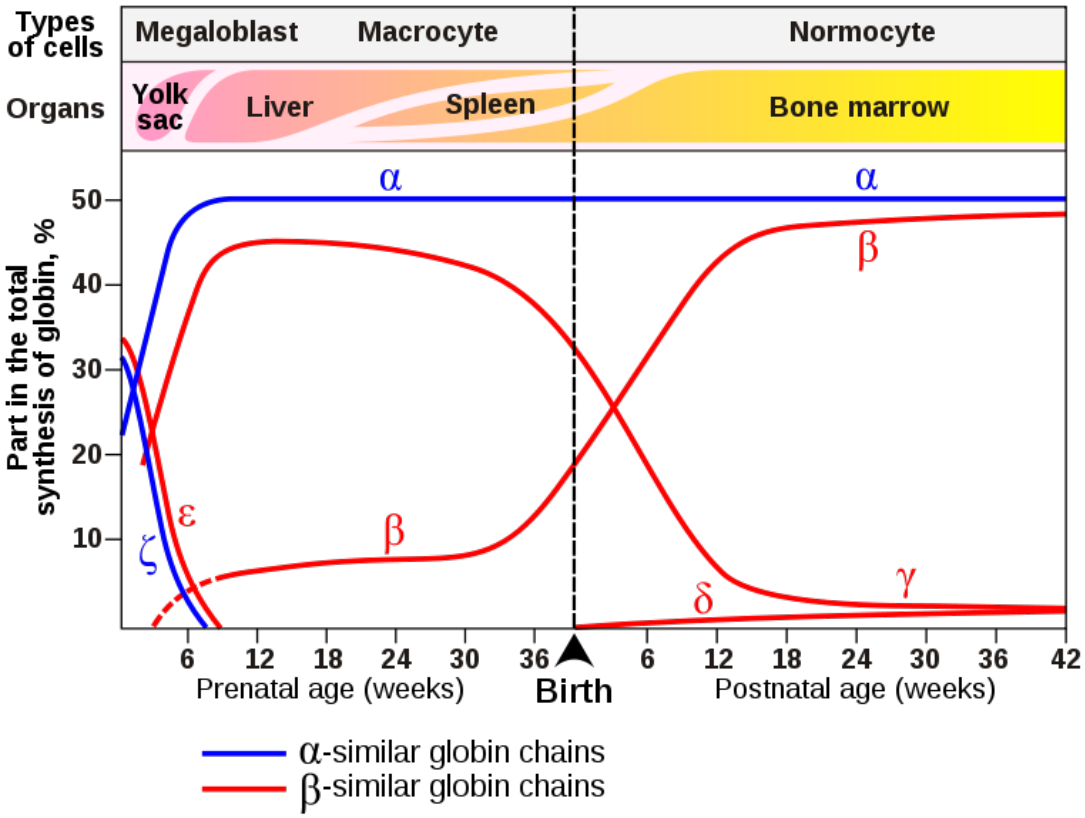
- Gower 1 ($\zeta_2\varepsilon_2$)
- Gower 2 ($\alpha_2\varepsilon_2$) (PDB 1A9W)
- Hemoglobin Portland ($\zeta_2\gamma_2$)

In the fetus:

- Hemoglobin F ($\alpha_2\gamma_2$) (PDB 1FDH)

In adults:

- Hemoglobin A ($\alpha_2\beta_2$) (PDB 1BZ0) - The most common with a normal amount over 95%
- Hemoglobin A_2 ($\alpha_2\delta_2$) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%
- Hemoglobin F ($\alpha_2\gamma_2$) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia.



Gene expression of hemoglobin before and after birth. Also identifies the types of cells and organs in which the gene expression (data on *Wood W.G.*, (1976). **Br. Med. Bull. 32, 282.**)

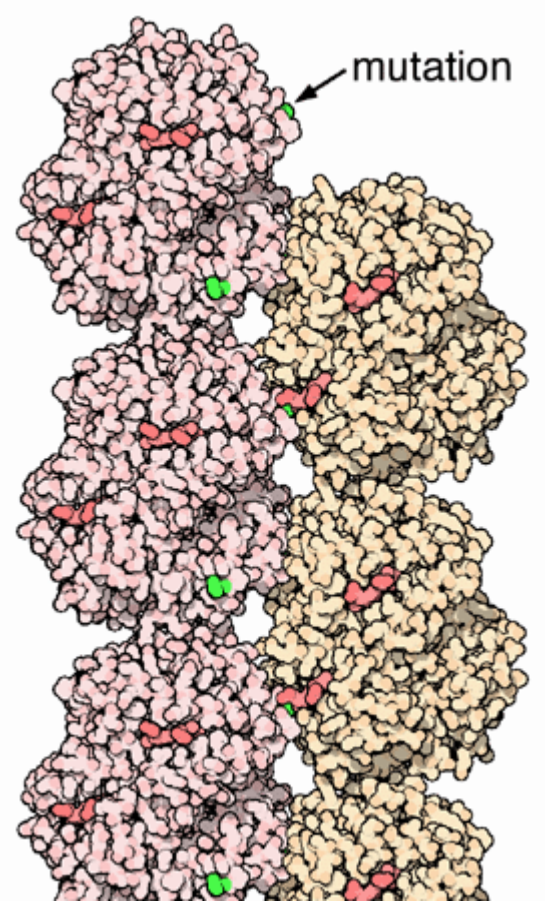
Variant forms that cause disease:

- Hemoglobin H (β_4) - A variant form of hemoglobin, formed by a tetramer of β chains, which may be present in variants of α thalassemia.
- Hemoglobin Barts (γ_4) - A variant form of hemoglobin, formed by a tetramer of γ chains, which may be present in variants of α thalassemia.
- Hemoglobin S ($\alpha_2\beta^S_2$) - A variant form of hemoglobin found in people with sickle cell disease. There is a variation in the β-chain gene, causing a change in the properties of hemoglobin, which results in sickling of red blood cells.
- Hemoglobin C ($\alpha_2\beta^C_2$) - Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin E ($\alpha_2\beta^E_2$) - Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin AS - A heterozygous form causing Sickle cell trait with one adult gene and one sickle cell disease gene
- Hemoglobin SC disease - Another heterozygous form with one sickle gene and another encoding Hemoglobin C.

Degradation in vertebrate animals

When red cells reach the end of their life due to aging or defects, they are broken down, the hemoglobin molecule is broken up and the iron gets recycled. When the porphyrin ring is broken up, the fragments are normally secreted in the bile by the liver. This process also produces one molecule of carbon monoxide for every molecule of heme degraded. This is one of the few natural sources of carbon monoxide production in the human body, and is responsible for the normal blood levels of carbon monoxide even in people breathing pure air. The other major final product of heme degradation is bilirubin. Increased levels of this chemical are detected in the blood if red cells are being destroyed more rapidly than usual. Improperly degraded hemoglobin protein or hemoglobin that has been released from the blood cells too rapidly can clog small blood vessels, especially the delicate blood filtering vessels of the kidneys, causing kidney damage.

Role in disease



In sickle cell hemoglobin (HbS) glutamic acid in position 6 (in beta chain) is mutated to valine. This change allows the deoxygenated form of the hemoglobin to stick to each other.

Hemoglobin deficiency can be caused either by decreased amount of hemoglobin molecules, as in anemia, or by decreased ability of each molecule to bind oxygen at the same partial pressure of oxygen. Hemoglobinopathies (genetic defects resulting in abnormal structure of the hemoglobin molecule) may cause both. In any case, hemoglobin deficiency decreases blood oxygen-carrying capacity. Hemoglobin deficiency is, in general, strictly distinguished from hypoxemia, defined as decreased partial pressure of oxygen in blood, although both are causes of hypoxia (insufficient oxygen supply to tissues).

Other common causes of low hemoglobin include loss of blood, nutritional deficiency, bone marrow problems, chemotherapy, kidney failure, or abnormal hemoglobin (such as that of sickle-cell disease).

High hemoglobin levels may be caused by exposure to high altitudes, smoking, dehydration, or tumors.

The ability of each hemoglobin molecule to carry oxygen is normally modified by altered blood pH or CO_2, causing an altered oxygen-hemoglobin dissociation curve. However, it can also be pathologically altered in, e.g., carbon monoxide poisoning.

Decrease of hemoglobin, with or without an absolute decrease of red blood cells, leads to symptoms of anemia. Anemia has many different causes, although iron deficiency and its resultant iron deficiency anemia are the most common causes in the Western world. As absence of iron decreases heme synthesis, red blood cells in iron deficiency anemia are *hypochromic* (lacking the red hemoglobin pigment) and *microcytic* (smaller than normal). Other anemias are rarer. In hemolysis (accelerated breakdown of red blood cells), associated jaundice is caused by the hemoglobin metabolite bilirubin, and the circulating hemoglobin can cause renal failure.

Some mutations in the globin chain are associated with the hemoglobinopathies, such as sickle-cell disease and thalassemia. Other mutations, as discussed at the beginning, are benign and are referred to merely as hemoglobin variants.

There is a group of genetic disorders, known as the *porphyrias* that are characterized by errors in metabolic pathways of heme synthesis. King George III of the United Kingdom was probably the most famous porphyria sufferer.

To a small extent, hemoglobin A slowly combines with glucose at the terminal valine (an alpha aminoacid) of each β chain. The resulting molecule is often referred to as Hb A_{1c}. As the concentration of glucose in the blood increases, the percentage of Hb A that turns into Hb A_{1c} increases. In diabetics whose glucose usually runs high, the percent Hb A_{1c} also runs high. Because of the slow rate of Hb A combination with glucose, the Hb A_{1c} percentage is representative of glucose level in the blood averaged over a longer time (the half-life of red blood cells, which is typically 50–55 days).

Glycosylated hemoglobin is the form of hemoglobin to which glucose is bound. The binding of glucose to amino acids in the hemoglobin takes place spontaneously (without the help of an enzyme) in many proteins, and is not known to serve a useful purpose. However, the binding to hemoglobin does serve as a record for average blood glucose levels over the life time of red cells, which is approximately 120 days. The levels of glycosylated hemoglobin are therefore measured in order to monitor the long-term control of the chronic disease of type 2 diabetes mellitus (T2DM). Poor control of T2DM results in high levels of glycosylated hemoglobin in the red blood cells. The normal reference range is approximately 4–5.9 %. Though difficult to obtain, values less than 7 % are recommended for people with T2DM. Levels greater than 9 % are associated with poor control of the glycosylated hemoglobin, and levels greater than 12 % are associated with very poor control. Diabetics who keep their glycosylated hemoglobin levels close to 7 % have a much better chance of avoiding the complications that may accompany diabetes (than those whose levels are 8 % or higher).

Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia. This elevation may be caused by congenital heart

disease, cor pulmonale, pulmonary fibrosis, too much erythropoietin, or polycythemia vera.

Elevation in levels of hemoglobin were found in one study of the yogic practice of Yoga Nidra (yogic sleep) for half an hour daily.

Diagnostic uses

Hemoglobin concentration measurement is among the most commonly performed blood tests, usually as part of a complete blood count. For example it is typically tested before or after blood donation. Results are reported in g/L, g/dL or mol/L. 1 g/dL equals about 0.6206 mmol/L. Normal levels are:

- Men: 13.8 to 18.0 g/dL (138 to 182 g/L, or 8.56 to 11.3 mmol/L)
- Women: 12.1 to 15.1 g/dL (121 to 151 g/L, or 7.51 to 9.37 mmol/L)
- Children: 11 to 16 g/dL (111 to 160 g/L, or 6.83 to 9.93 mmol/L)
- Pregnant women: 11 to 12 g/dL (110 to 120 g/L, or 6.83 to 7.45 mmol/L)

Normal values of hemoglobin in the 1st and 3rd trimesters of pregnant women must be at least 11 g/dL and at least 10.5 g/dL during the 2nd trimester.

If the concentration is below normal, this is called anemia. Anemias are classified by the size of red blood cells, the cells that contain hemoglobin in vertebrates. The anemia is called "microcytic" if red cells are small, "macrocytic" if they are large, and "normocytic" otherwise.

Hematocrit, the proportion of blood volume occupied by red blood cells, is typically about three times the hemoglobin level. For example, if the hemoglobin is measured at 17, that compares with a hematocrit of 51.

Long-term control of blood sugar concentration can be measured by the concentration of Hb A_{1c}. Measuring it directly would require many samples because blood sugar levels vary widely through the day. Hb A_{1c} is the product of the irreversible reaction of hemoglobin A with glucose. A higher glucose concentration results in more Hb A_{1c}. Because the reaction is slow, the Hb A_{1c} proportion represents glucose level in blood averaged over the half-life of red blood cells, is typically 50–55 days. An Hb A_{1c} proportion of 6.0% or less show good long-term glucose control, while values above 7.0% are elevated. This test is especially useful for diabetics.

The functional magnetic resonance imaging (fMRI) machine uses the signal from deoxyhemoglobin, which is sensitive to magnetic fields since it is paramagnetic.

Analogues in non-vertebrate organisms

A variety of oxygen-transport and -binding proteins exist in organisms throughout the animal and plant kingdoms. Organisms including bacteria, protozoans, and fungi all have

hemoglobin-like proteins whose known and predicted roles include the reversible binding of gaseous ligands. Since many of these proteins contain globins and the heme moiety (iron in a flat porphyrin support), they are often called hemoglobins, even if their overall tertiary structure is very different from that of vertebrate hemoglobin. In particular, the distinction of "myoglobin" and hemoglobin in lower animals is often impossible, because some of these organisms do not contain muscles. Or, they may have a recognizable separate circulatory system but not one that deals with oxygen transport (for example, many insects and other arthropods). In all these groups, heme/globin-containing molecules (even monomeric globin ones) that deal with gas-binding are referred to as oxyhemoglobins. In addition to dealing with transport and sensing of oxygen, they may also deal with NO, CO_2, sulfide compounds, and even O_2 scavenging in environments that must be anaerobic. They may even deal with detoxification of chlorinated materials in a way analogous to heme-containing P450 enzymes and peroxidases.

The giant tube worm *Riftia pachyptila* showing red hemoglobin-containing plumes

The structure of hemoglobins varies across species. Hemoglobin occurs in all kingdoms of organisms, but not in all organisms. Primitive species such as bacteria, protozoa, algae, and plants often have single-globin hemoglobins. Many nematode worms, molluscs, and crustaceans contain very large multisubunit molecules, much larger than those in vertebrates. In particular, chimeric hemoglobins found in fungi and giant annelids may contain both globin and other types of proteins.

One of the most striking occurrences and uses of hemoglobin in organisms is in the giant tube worm (*Riftia pachyptila*, also called Vestimentifera), which can reach 2.4 meters

length and populates ocean volcanic vents. Instead of a digestive tract, these worms contain a population of bacteria constituting half the organism's weight. The bacteria react with H_2S from the vent and O_2 from the water to produce energy to make food from H_2O and CO_2. The worms end with a deep red fan-like structure ("plume"), which extends into the water and absorbs H_2S and O_2 for the bacteria, and CO_2 for use as synthetic raw material similar to photosynthetic plants. The structures are bright-red due to their containing several extraordinarily complex hemoglobins that have up to 144 globin chains, each including associated heme structures. These hemoglobins are remarkable for being able to carry oxygen in the presence of sulfide, and even to carry sulfide, without being completely "poisoned" or inhibited by it as hemoglobins in most other species are.

Other oxygen-binding proteins

Myoglobin: Found in the muscle tissue of many vertebrates, including humans, it gives muscle tissue a distinct red or dark gray color. It is very similar to hemoglobin in structure and sequence, but is not a tetramer; instead, it is a monomer that lacks cooperative binding. It is used to store oxygen rather than transport it.

Hemocyanin: The second most common oxygen-transporting protein found in nature, it is found in the blood of many arthropods and molluscs. Uses copper prosthetic groups instead of iron heme groups and is blue in color when oxygenated.

Hemerythrin: Some marine invertebrates and a few species of annelid use this iron-containing non-heme protein to carry oxygen in their blood. Appears pink/violet when oxygenated, clear when not.

Chlorocruorin: Found in many annelids, it is very similar to erythrocruorin, but the heme group is significantly different in structure. Appears green when deoxygenated and red when oxygenated.

Vanabins: Also known as **vanadium chromagens**, they are found in the blood of sea squirts. There were once hypothesized to use the rare metal vanadium as an oxygen binding prosthetic group. However, although they do contain vandadium by preference, they apparently bind little oxygen, and thus have some other function, which has not been elucidated (sea squirts also contain some hemoglobin). They may act as toxins.

Erythrocruorin: Found in many annelids, including earthworms, it is a giant free-floating blood protein containing many dozens—possibly hundreds—of iron- and heme-bearing protein subunits bound together into a single protein complex with a molecular mass greater than 3.5 million daltons.

Pinnaglobin: Only seen in the mollusc *Pinna squamosa*. Brown manganese-based porphyrin protein.

Leghemoglobin: In leguminous plants, such as alfalfa or soybeans, the nitrogen fixing bacteria in the roots are protected from oxygen by this iron heme containing oxygen-binding protein. The specific enzyme protected is nitrogenase, which is unable to reduce nitrogen gas in the presence of free oxygen.

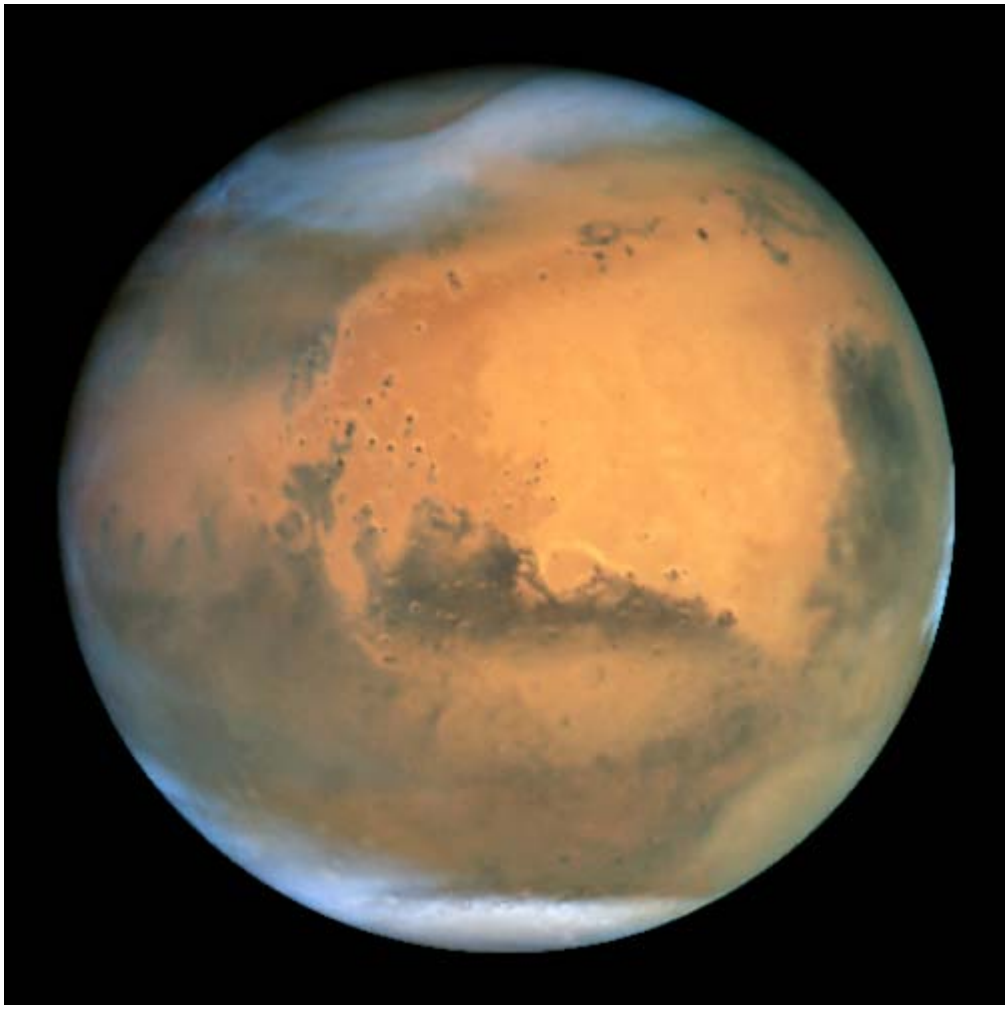
Coboglobin: A synthetic cobalt-based porphyrin. Coboprotein would appear colorless when oxygenated, but yellow when in veins.

Presence in nonerythroid cells

Some nonerythroid cells (i.e., cells other than the red blood cell line) contain hemoglobin. In the brain, these include the A9 dopaminergic neurons in the substantia nigra, astrocytes in the cerebral cortex and hippocampus, and in all mature oligodendrocytes. It has been suggested that brain hemoglobin in these cell may enable the "storage of oxygen to provide a homeostatic mechanism in anoxic conditions, which is especially important for A9 DA neurons that have an elevated metabolism with a high requirement for energy production". It has been noted further that "A9 dopaminergic neurons may be at particular risk since in addition to their high mitochondrial activity they are under intense oxidative stress caused by the production of hydrogen peroxide via autoxidation and/or monoamine oxidase (MAO)-mediated deamination of dopamine and the subsequent reaction of accessible ferrous iron to generate highly toxic hydroxyl radicals". This may explain the risk of these cells for degeneration in Parkinson's disease. The presence of iron from hemoglobin in these cells also results in the post-mortem darkness of these cells, which is the origin of the Latin name, substantia *nigra*.

Outside the brain, hemoglobin has non-oxygen-carrying functions as an antioxidant and a regulator of iron metabolism in macrophages, alveolar cells, and mesangial cells in the kidney.

In history, art and music



The planet Mars

Historically, the color of blood was associated with rust, as ancient Romans associated the planet Mars with the god of war since Mars is orange-red. The color of Mars is due to iron-oxygen in the Martian soil, but the red in blood is not due to the iron in hemoglobin and its oxides, which is a common misconception. The red is due to the porphyrin moiety of hemoglobin to which the iron is bound, not the iron itself, although the ligation and redox state of the iron can influence the pi to pi* electronic transitions of the porphyrin and hence its optical characteristics.

Heart of Steel (Hemoglobin) (2005) by Julian Voss-Andreae. The images show the 5' (1.60 m) tall sculpture right after installation, after 10 days, and after several months of exposure to the elements.

Artist Julian Voss-Andreae created a sculpture called "Heart of Steel (Hemoglobin)" in 2005, based on the protein's backbone. The sculpture was made from glass and weathering steel. The intentional rusting of the initially shiny work of art mirrors hemoglobin's fundamental chemical reaction of oxygen binding to iron.

Rock band Placebo recorded a song called "Haemoglobin" with the lyrics "Haemoglobin is the key to a healthy heartbeat".

Chapter 4

Platelet

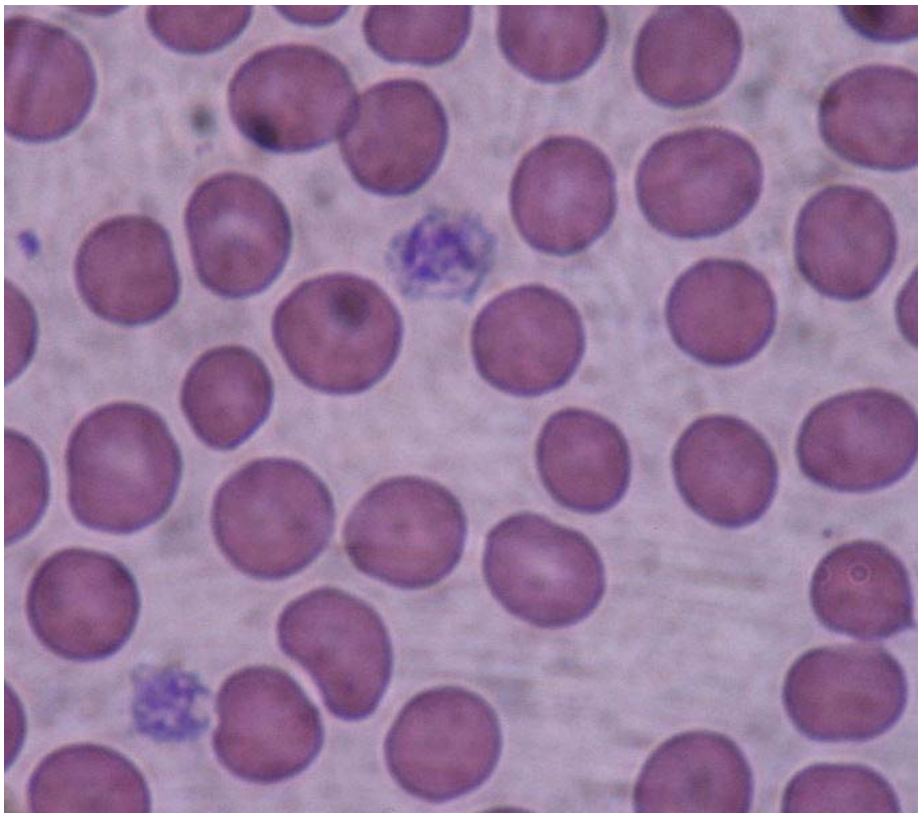


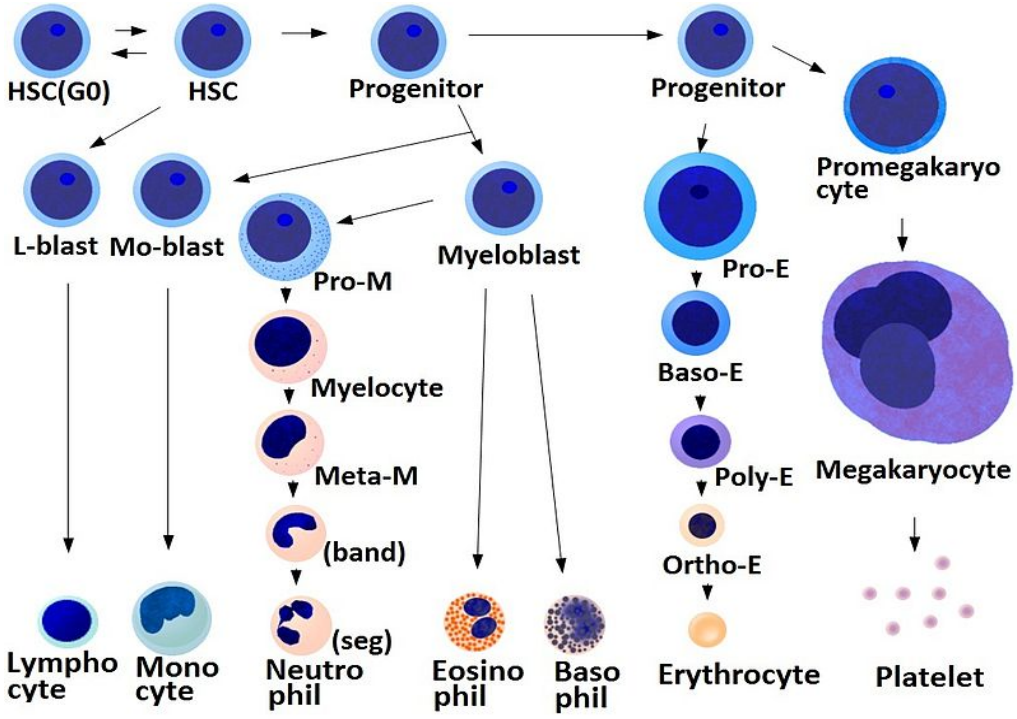
Image from a light microscope (40x) from a peripheral blood smear surrounded by red blood cells. One platelet can be seen in the upper left side of the image (purple) and is significantly smaller in size than the red blood cells (stained pink) and the two large platelets (stained purple).

Platelets, or **thrombocytes** (from Greek θρόμβος, "clot" and κύτος, "cell"), are small, regularly-shaped clear cell fragments (i.e. cells that do not have a nucleus containing DNA), 2-3 μm in diameter, which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is normally just 5 to 9 days. Platelets play a fundamental role in hemostasis and are a natural source of growth factors. They circulate in the blood of mammals and are involved in hemostasis, leading to the formation of blood clots.

If the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic purpura (TTP) that typically cause thromboses, or clots, instead of bleeding.

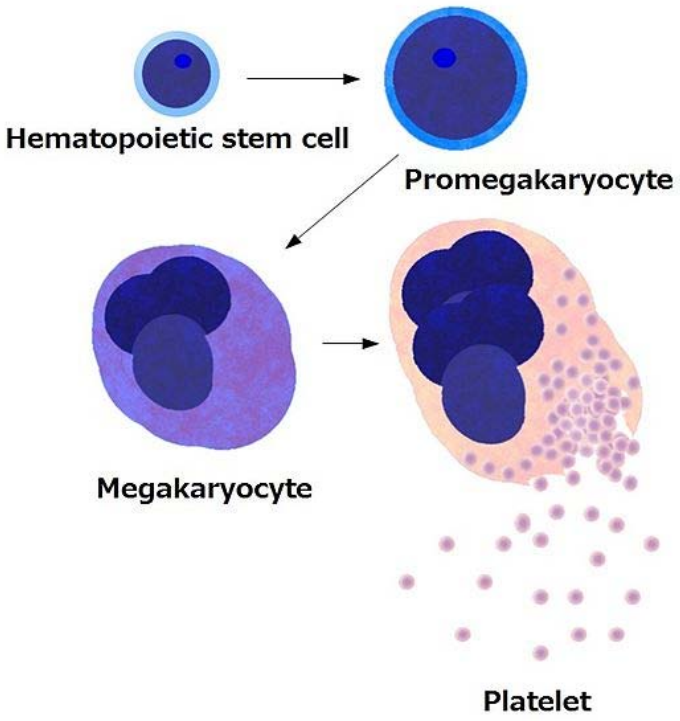
Platelets release a multitude of growth factors including Platelet-derived growth factor (PDGF), a potent chemotactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through Platelet-rich plasma (PRP) has been used as an adjunct to wound healing for several decades.

Kinetics



HSC=Hematopoietic stem cell , Progenitor=Progenitor cell , L-blast=Lymphoblast , Lymphocyte , Mo-blast=Monoblast , Monocyte , Myeloblast , Pro-M=Promyelocyte , Myelocyte , Meta-M=Metamyelocyte , Neutrophil , Eosinophil , Basophil , Pro-E=Proerythroblast , Baso-E=Basophilic erythroblast , poly-E=Polychromatic erythroblast

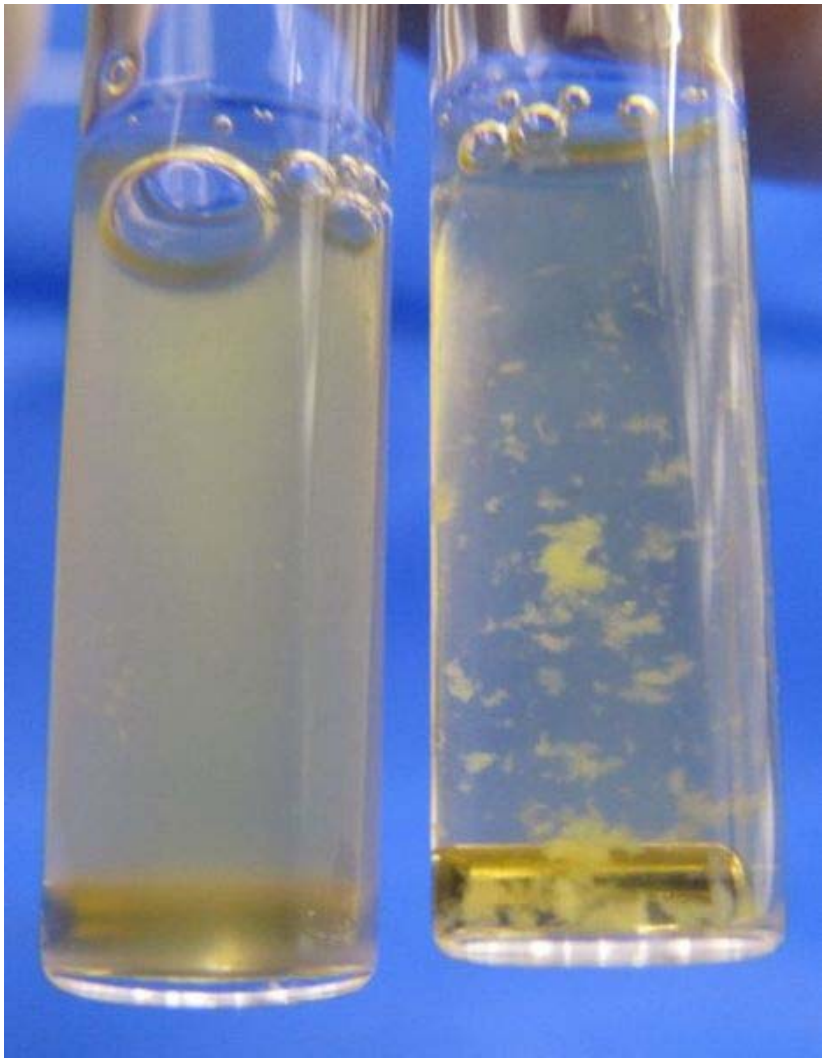
, Ortho-E=Orthochromatic erythroblast , Erythrocyte , Promegakaryocyte , Megakaryocyte , Platlet



Blood cell lineage

- Platelets are produced in blood cell formation (thrombopoiesis) in bone marrow, by budding off from megakaryocytes.
- The physiological range for platelets is 150-400 x 10^9 per liter.
- Around 1 x 10^{11} platelets are produced each day by an average healthy adult.
- The lifespan of circulating platelets is 5 to 9 days.
- Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys.
- Each megakaryocyte produces between 5,000 and 10,000 platelets.
- Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver.
- A reserve of platelets are stored in the spleen and are released when needed by sympathetically-induced splenic contraction.

Thrombus formation



Aggregation of platelets. Platelet rich human blood plasma (left vial) is a turbid liquid. Upon addition of ADP, platelets are activated and start to aggregate, forming white flakes (right vial).

The function of platelets is the maintenance of hemostasis. This is achieved primarily by the formation of thrombi, when damage to the endothelium of blood vessels occurs. On the converse, thrombus formation must be inhibited at times when there is no damage to the endothelium.

Activation

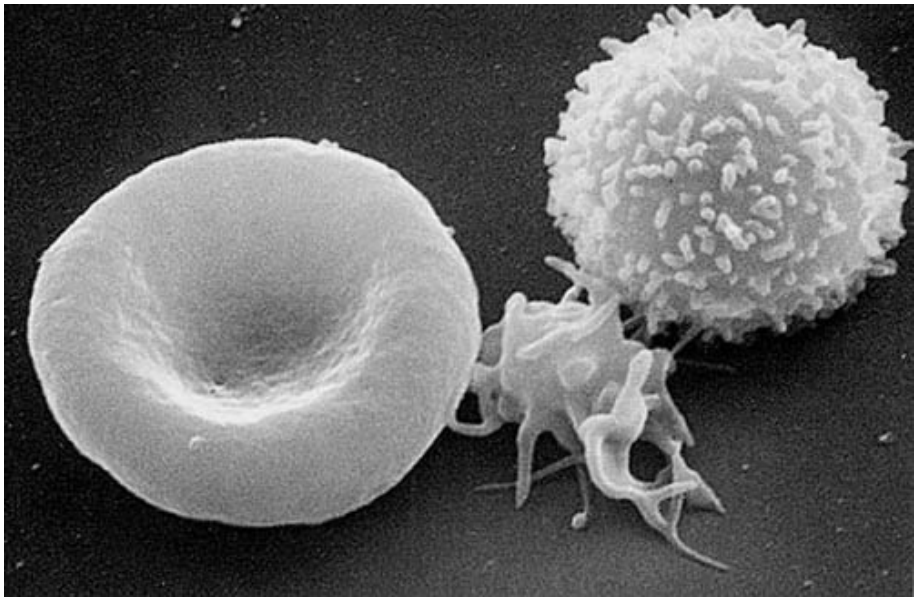
The inner surface of blood vessels is lined with a thin layer of endothelial cells that, in normal hemostasis, acts to inhibit platelet activation by producing nitric oxide, endothelial-ADPase, and PGI_2. Endothelial-ADPase clears away the platelet activator, ADP.

Endothelial cells produce a protein called von Willebrand factor (vWF), a cell adhesion ligand, which helps endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen is not exposed to the bloodstream. vWF is secreted constitutively into the plasma by the endothelial cells, and is stored in granules within the endothelial cell and in platelets.

When the endothelial layer is injured, collagen, vWF and tissue factor from the subendothelium is exposed to the bloodstream. When the platelets contact collagen or vWF, they are activated (e.g. to clump together). They are also activated by thrombin (formed with the help of tissue factor). They can also be activated by a negatively-charged surface, such as glass.

Platelet activation further results in the scramblase-mediated transport of negatively-charged phospholipids to the platelet surface. These phospholipids provide a catalytic surface (with the charge provided by phosphatidylserine and phosphatidylethanolamine) for the tenase and prothrombinase complexes. Calcium ions are essential for binding of these coagulation factors.

Shape change



Scanning electron micrograph of blood cells. From left to right: human erythrocyte, activated **thrombocyte** (platelet), leukocyte.

Activated platelets change in shape to become more spherical, and pseudopods form on their surface. Thus they assume a stellate shape.

Granule secretion

Platelets contain alpha and dense granules. Activated platelets excrete the contents of these granules into their canalicular systems and into surrounding blood. There are three types of granules:

- dense (or delta) granules (containing ADP or ATP, calcium, and serotonin)
- lambda granules - similar to lysosomes and contain several hydrolytic enzymes.
- Alpha granules (containing platelet factor 4, transforming growth factor-β1, platelet-derived growth factor, fibronectin, B-thromboglobulin, vWF, fibrinogen, and coagulation factors V and XIII).

Thromboxane A2 synthesis

Platelet activation initiates the arachidonic acid pathway to produce TXA_2. TXA_2 is involved in activating other platelets and its formation is inhibited by COX inhibitors, such as aspirin.

Adhesion and aggregation

Platelets aggregate, or clump together, using fibrinogen and vWF as a connecting agent. The most abundant platelet aggregation receptor is glycoprotein IIb/IIIa (gpIIb/IIIa); this is a calcium-dependent receptor for fibrinogen, fibronectin, vitronectin, thrombospondin, and von Willebrand factor (vWF). Other receptors include GPIb-V-IX complex (vWF) and GPVI (collagen).

Activated platelets will adhere, via glycoprotein (GP) Ia, to the collagen that is exposed by endothelial damage. Aggregation and adhesion act together to form the platelet plug. Myosin and actin filaments in platelets are stimulated to contract during aggregation, further reinforcing the plug.

Platelet aggregation is stimulated by ADP, thromboxane, and α2 receptor-activation, but inhibited by other inflammatory products like PGI2 and PGD2. Platelet aggregation is enhanced by exogenous administration of anabolic steroids.

Wound repair

The blood clot is only a temporary solution to stop bleeding; vessel repair is therefore needed. The aggregated platelets help this process by secreting chemicals that promote the invasion of fibroblasts from surrounding connective tissue into the wounded area to completely heal the wound or form a scar. The obstructing clot is slowly dissolved by the fibrinolytic enzyme, plasmin, and the platelets are cleared by phagocytosis.

Other functions

- Clot retraction

- Pro-coagulation
- Inflammation
- Cytokine signalling
- Phagocytosis

Cytokine signaling

In addition to being the chief cellular effector of hemostasis, platelets are rapidly deployed to sites of injury or infection, and potentially modulate inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators . Platelets also secrete platelet-derived growth factor (PDGF).

Role in disease

High and low counts

A normal platelet count in a healthy individual is between 150,000 and 450,000 per µl (microlitre) of blood (150–450 x 10^9/L). Ninety-five percent of healthy people will have platelet counts in this range. Some will have statistically abnormal platelet counts while having no demonstrable abnormality. However, if it is either very low or very high, the likelihood of an abnormality being present is higher.

Both thrombocytopenia and thrombocytosis may present with coagulation problems. In general, low platelet counts increase bleeding risks; however there are exceptions. For example, immune heparin-induced thrombocytopenia and thrombocytosis (high counts) may lead to thrombosis, although this is mainly when the elevated count is due to myeloproliferative disorder.

Low platelet counts are, in general, not corrected by transfusion unless the patient is bleeding or the count has fallen below 5 x 10^9/L. Transfusion is contraindicated in thrombotic thrombocytopenic purpura (TTP), as it fuels the coagulopathy. In patients undergoing surgery, a level below 50 x 10^9/L is associated with abnormal surgical bleeding, and regional anaesthetic procedures such as epidurals are avoided for levels below 80-100.

Normal platelet counts are not a guarantee of adequate function. In some states, the platelets, while being adequate in number, are *dysfunctional*. For instance, aspirin irreversibly disrupts platelet function by inhibiting cyclooxygenase-1 (COX1), and hence normal hemostasis. The resulting platelets are unable to produce new cyclooxygenase because they have no DNA. Normal platelet function will not return until the use of aspirin has ceased and enough of the affected platelets have been replaced by new ones, which can take over a week. Ibuprofen, another NSAID, does not have such a long duration effect, with platelet function usually returning within 24 hours, and taking ibuprofen before aspirin will prevent the irreversible effects of aspirin. Uremia, a consequence of renal failure, leads to platelet dysfunction that may be ameliorated by the administration of desmopressin.

Medications

Oral agents, often used to alter/suppress platelet function: aspirin, clopidogrel, cilostazol, ticlopidine.

Intravenous agents, often used to alter/suppress platelet function: abciximab, eptifibatide, tirofiban.

Diseases

Disorders leading to a reduced platelet count:

- Thrombocytopenia
  - Idiopathic thrombocytopenic purpura - also known as immune thrombocytopenic purpura (ITP)
  - Thrombotic thrombocytopenic purpura
  - Drug-induced thrombocytopenic purpura (for example heparin-induced thrombocytopenia (HIT))
- Gaucher's disease
- Aplastic anemia
- Onyalai

Alloimmune disorders

- Fetomaternal alloimmune thrombocytopenia
- Some transfusion reactions

Disorders leading to platelet dysfunction or reduced count:

- HELLP syndrome
- Hemolytic-uremic syndrome
- Chemotherapy
- Dengue

Disorders featuring an elevated count:

- Thrombocytosis, including essential thrombocytosis (elevated counts, either reactive or as an expression of myeloproliferative disease); may feature dysfunctional platelets

Disorders of platelet adhesion or aggregation:

- Bernard-Soulier syndrome
- Glanzmann's thrombasthenia
- Scott's syndrome
- von Willebrand disease

- Hermansky-Pudlak Syndrome
- Gray platelet syndrome

Disorders of platelet metabolism

- Decreased cyclooxygenase activity, induced or congenital
- Storage pool defects, acquired or congenital

Disorders that indirectly compromise platelet function:

- Haemophilia

Disorders in which platelets play a key role:

- Atherosclerosis
- Coronary artery disease, CAD and myocardial infarction, MI
- Cerebrovascular disease and Stroke, CVA (cerebrovascular accident)
- Peripheral artery occlusive disease (PAOD)
- Cancer
- Malaria

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected
Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected
Bernard-Soulier	unaffected	unaffected	prolonged	decreased

syndrome

Discovery

Brewer traced the history of the discovery of the platelet. Although red blood cells had been known since van Leeuwenhoek (1632–1723), it was the German anatomist Max Schultze (1825–1874) who first offered a description of the platelet in his newly-founded journal *Archiv für mikroscopische Anatomie*. He describes "spherules" to be much smaller than red blood cells that are occasionally clumped and may participate in collections of fibrous material. He recommends further study of the findings.

Giulio Bizzozero (1846–1901), building on Schultze's findings, used "living circulation" to study blood cells of amphibians microscopically *in vivo*. He is especially noted for discovering that platelets clump at the site of blood vessel injury, a process that precedes the formation of a blood clot. This observation confirmed the role of platelets in coagulation.

In transfusion medicine

Platelet concentrate

Platelets are either isolated from collected units of whole blood and pooled to make a therapeutic dose or collected by apheresis, sometimes concurrently with plasma or red blood cells. The industry standard is for platelets to be tested for bacteria before transfusion to avoid septic reactions, which can be fatal. Recently the AABB Industry Standards for Blood Banks and Transfusion Services (5.1.5.1) has allowed for use of pathogen reduction technology as an alternative to bacterial screenings in platelets.

Pooled whole-blood platelets, sometimes called "random" platelets, are made primarily by two methods. In the US, a unit of whole blood is placed into a large centrifuge in what is referred to as a "soft spin." At these settings, the platelets remain suspended in the plasma. The platelet-rich plasma (PRP) is removed from the RBCs, then centrifuged at a faster setting to harvest the platelets from the plasma. In other regions of the world, the unit of whole blood is centrifuged using settings that cause the platelets to become suspended in the "buffy coat" layer, which includes the platelets and the white blood cells. The "buffy coat" is isolated in a sterile bag, suspended in a small amount of red blood cells and plasma, then centrifuged again to separate the platelets and plasma from the red and white blood cells. Regardless of the initial method of preparation, multiple platelets may be combined into one container using a sterile connection device to manufacture a single product with the desired therapeutic dose.

Apheresis platelets are collected using a mechanical device that draws blood from the donor and centrifuges the collected blood to separate out the platelets and other components to be collected. The remaining blood is returned to the donor. The advantage to this method is that a single donation provides at least one therapeutic dose, as opposed to the multiple donations for whole-blood platelets. This means that a recipient is not exposed to as many different donors and has less risk of transfusion-transmitted disease and other complications. Sometimes a person such as a cancer patient that requires routine transfusions of platelets will receive repeated donations from a specific donor to further minimize the risk. Pathogen reduction of platelets using for example, riboflavin and UV light treatments can also be carried out to reduce the infectious load of pathogens contained in donated blood products, thereby reducing the risk of transmission of transfusion transmitted diseases.

Platelets are not cross-matched unless they contain a significant amount of red blood cells (RBCs), which results in a reddish-orange color to the product. This is usually associated with whole-blood platelets, as apheresis methods are more efficient than "soft spin" centrifugation at isolating the specific components of blood. An effort is usually made to issue type specific platelets, but this is not as critical as it is with RBCs.

Platelets collected by either method have a very short shelf life, typically five days. This results in frequent problems with short supply, as testing the donations often requires up to a full day. Since there are no effective preservative solutions for platelets, they lose potency quickly and are best when fresh.

Platelets are stored under constant agitation at 20-24 °C. Storage at room temperature provides an environment where any bacteria that are introduced to the blood component

during the collection process may proliferate and subsequently cause bacteremia in the patient. Regulations are in place in the United States that require products to be tested for the presence of bacterial contamination before transfusion.

Platelets, either apheresis or random-donor platelets, can be processed through a volume reduction process. In this process, the platelets are spun in a centrifuge and the excess plasma is removed, leaving 10 to 100 ml of platelet concentrate. Volume-reduced platelets are normally transfused only to neonatal and pediatric patients when a large volume of plasma could overload the child's small circulatory system. The lower volume of plasma also reduces the chances of an adverse transfusion reaction to plasma proteins. Volume reduced platelets have a shelf life of only four hours.

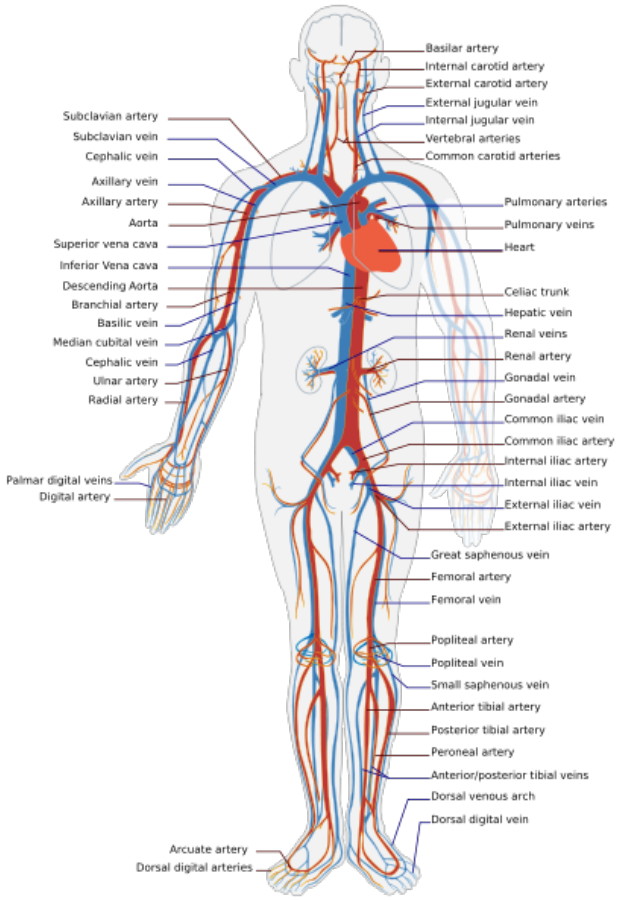
Other species

Nucleated thrombocytes of nonmammalian vertebrates differ from the mammalian thrombocytes not only in having a nucleus and resembling B lymphocytes, but also these nucleated thrombocytes do not aggregate in response to ADP, serotonin and adrenaline (although they do aggregate with thrombin).

Chapter 5

Blood Vessel

Blood vessel



Simple diagram of the human circulatory system.

Latin *vas sanguineum*

The **blood vessels** are the part of the circulatory system that transport blood throughout the body. There are three major types of blood vessels: the arteries, which carry the blood away from the heart; the capillaries, which enable the actual exchange of water and chemicals between the blood and the tissues; and the veins, which carry blood from the capillaries back toward the heart.

Anatomy

The arteries and veins have different structures, veins having two layers and arteries having three.

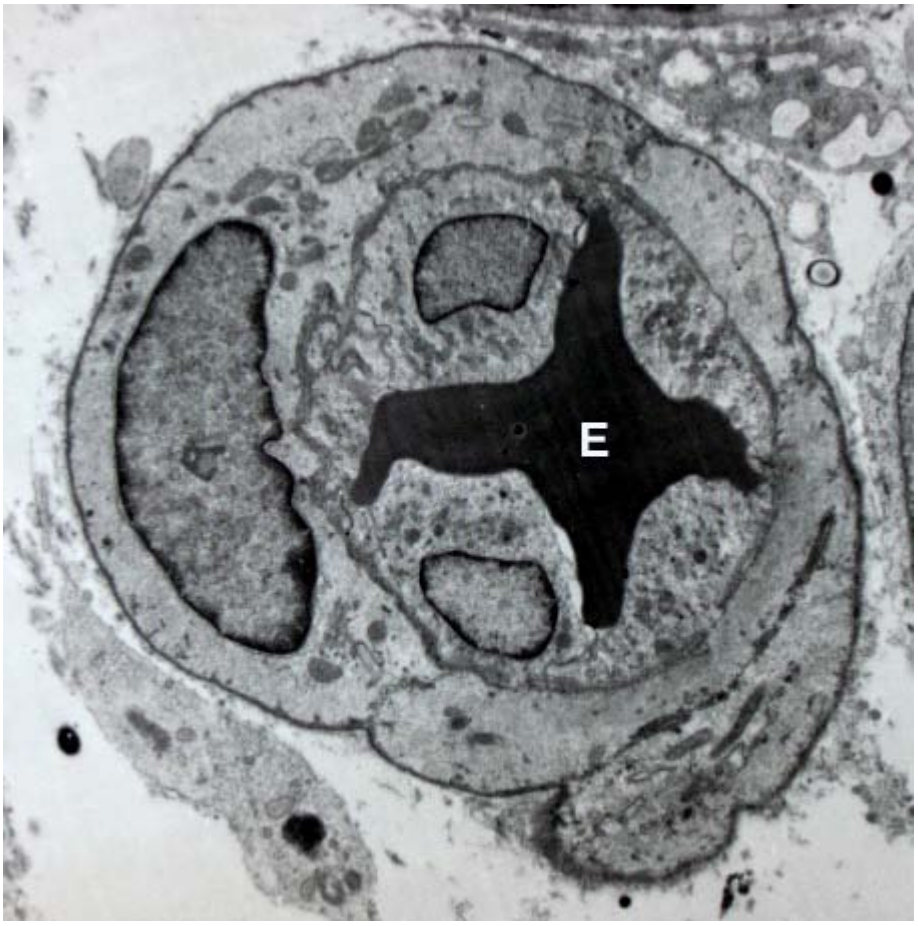
- *Tunica intima* (the thinnest layer): a single layer of simple squamous endothelial cells glued by a polysaccharide intercellular matrix, surrounded by a thin layer of subendothelial connective tissue interlaced with a number of circularly arranged elastic bands called the *internal elastic lamina*.
- *Tunica media* (the thickest layer): circularly arranged elastic fiber, connective tissue, polysaccharide substances, the second and third layer are separated by another thick elastic band called external elastic lamina. The tunica media may (especially in arteries) be rich in vascular smooth muscle, which controls the caliber of the vessel.
- *Tunica adventitia*: entirely made of connective tissue. It also contains nerves that supply the vessel

as well as nutrient capillaries (vasa vasorum) in the larger blood vessels.

Capillaries consist of little more than a layer of endothelium and occasional connective tissue.

When blood vessels connect to form a region of diffuse vascular supply it is called an anastomosis (pl. anastomoses). Anastomoses provide critical alternative routes for blood to flow in case of blockages.

Types



Blood vessel with an erythrocyte (red blood cell, E) within its lumen, endothelial cells forming its *tunica intima* (inner layer), and pericytes forming its *tunica adventitia* (outer layer).

There are various kinds of blood vessels:

- Arteries
  - Aorta (the largest artery, carries blood out of the heart)
  - Branches of the aorta, such as the carotid artery, the subclavian artery, the celiac trunk, the mesenteric arteries, the renal artery and the iliac artery.
- Arterioles
- Capillaries (the smallest blood vessels)
- Venules
- Veins
  - Large collecting vessels, such as the subclavian vein, the jugular vein, the renal vein and the iliac vein.
  - Venae cavae (the 2 largest veins, carry blood into the heart)

They are roughly grouped as *arterial* and *venous*, determined by whether the blood in it is flowing *away from* (arterial) or *toward* (venous) the heart. The term "arterial blood" is nevertheless used to indicate blood high in oxygen, although the pulmonary artery carries "venous blood" and blood flowing in the pulmonary vein is rich in oxygen. This is because they are carrying the blood to and from the lungs, respectively, to be oxygenated.

Physiology

Blood vessels do not actively engage in the transport of blood (they have no appreciable peristalsis), but arteries - and veins to a degree - can regulate their inner diameter by contraction of the muscular layer. This changes the blood flow to downstream organs, and is determined by the autonomic nervous system. Vasodilation and vasoconstriction are also used antagonistically as methods of thermoregulation.

Oxygen (bound to hemoglobin in red blood cells) is the most critical nutrient carried by the blood. In all arteries apart from the pulmonary artery, hemoglobin is highly saturated (95-100%) with oxygen. In all veins apart from the pulmonary vein, the hemoglobin is desaturated at about 75%. (The values are reversed in the pulmonary circulation.)

The blood pressure in blood vessels is traditionally expressed in millimetres of mercury (1 mmHg = 133 Pa). In the arterial system, this is usually around 120 mmHg systolic (high pressure wave due to contraction of the heart) and 80 mmHg diastolic (low pressure wave). In contrast, pressures in the venous system are constant and rarely exceed 10 mmHg.

Vasoconstriction is the constriction of blood vessels (narrowing, becoming smaller in cross-sectional area) by contracting the vascular smooth muscle in the vessel walls. It is regulated by vasoconstrictors (agents that cause vasoconstriction). These include paracrine factors (e.g. prostaglandins), a number of hormones (e.g. vasopressin and angiotensin) and neurotransmitters (e.g. epinephrine) from the nervous system.

Vasodilation is a similar process mediated by antagonistically acting mediators. The most prominent vasodilator is nitric oxide (termed endothelium-derived relaxing factor for this reason).

Permeability of the endothelium is pivotal in the release of nutrients to the tissue. It is also increased in inflammation in response to histamine, prostaglandins and interleukins, which leads to most of the symptoms of inflammation (swelling, redness and warmth).

Role in disease

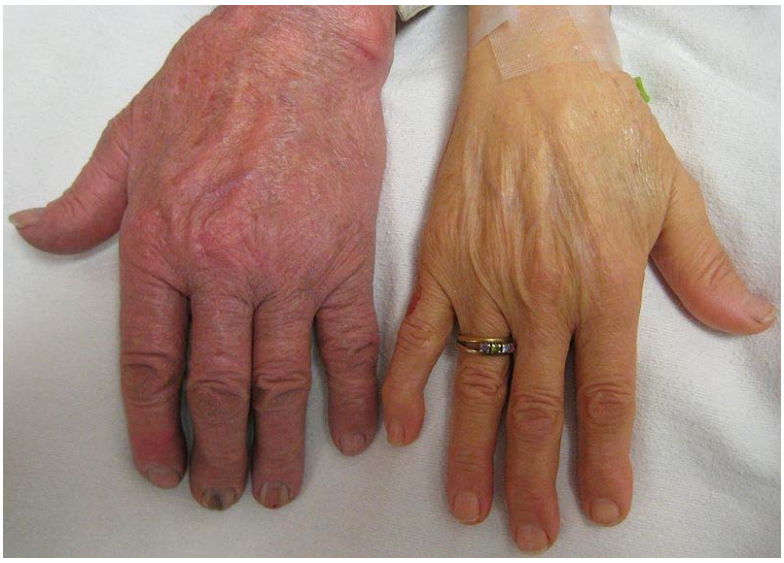
Blood vessels play a huge role in virtually every medical condition. Cancer, for example, cannot progress unless the tumor causes angiogenesis (formation of new blood vessels) to supply the malignant cells' metabolic demand. Atherosclerosis, the formation of lipid lumps (atheromas) in the blood vessel wall, is the most common cardiovascular disease, the main cause of death in the Western world.

Blood vessel permeability is increased in inflammation. Damage, due to trauma or spontaneously, may lead to haemorrhage due to mechanical damage to the vessel endothelium. In contrast, occlusion of the blood vessel by atherosclerotic plaque, by an embolised blood clot or a foreign body leads to downstream ischemia (insufficient blood supply) and possibly necrosis. Vessel occlusion tends to be a positive feedback system; an occluded vessel creates eddies in the normally laminar flow or plug flow blood currents. These eddies create abnormal fluid velocity gradients which push blood elements such as cholesterol or chylomicron bodies to the endothelium. These deposit onto the arterial walls which are already partially occluded and build upon the blockage.

Chapter 6

Anemia

Anemia



The pale hand of a woman with severe anemia (right) in comparison to the normal hand of her husband (left).

ICD-10	D50.-D64.
ICD-9	280-285
DiseasesDB	663
MedlinePlus	000560
eMedicine	med/132 emerg/808 emerg/734
MeSH	D000740

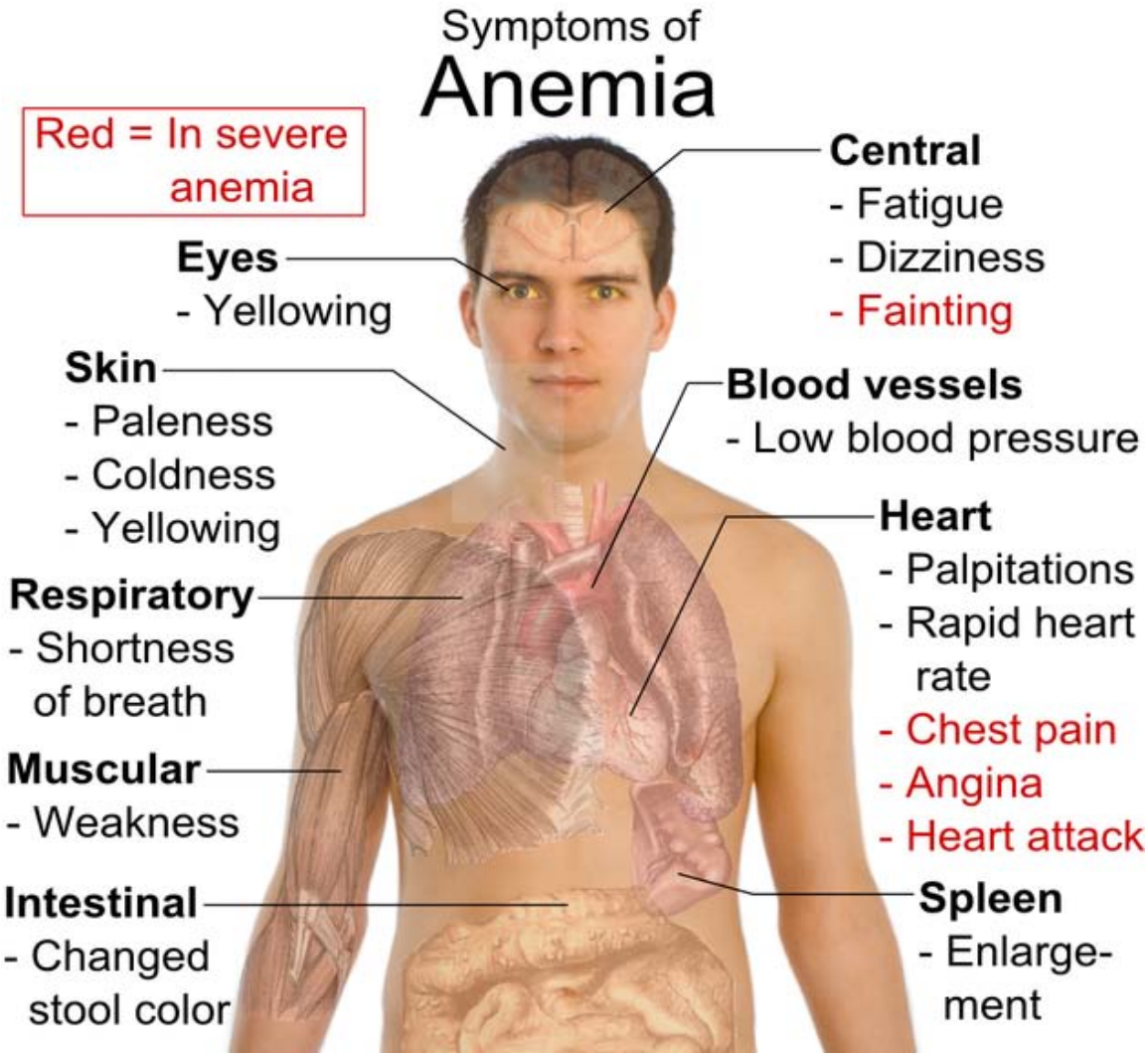
Anemia is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood. However, it can include decreased oxygen-binding ability of each hemoglobin molecule due to deformity or lack in numerical development as in some other types of hemoglobin deficiency.

Because hemoglobin (found inside RBCs) normally carries oxygen from the lungs to the tissues, anemia leads to hypoxia (lack of oxygen) in organs. Because all human cells depend on oxygen for survival, varying degrees of anemia can have a wide range of clinical consequences.

Anemia is the most common disorder of the blood. There are several kinds of anemia, produced by a variety of underlying causes. Anemia can be classified in a variety of ways, based on the morphology of RBCs, underlying etiologic mechanisms, and discernible clinical spectra, to mention a few. The three main classes of anemia include excessive blood loss (acutely such as a hemorrhage or chronically through low-volume loss), excessive blood cell destruction (hemolysis) or deficient red blood cell production (ineffective hematopoiesis).

There are two major approaches: the "kinetic" approach which involves evaluating production, destruction and loss, and the "morphologic" approach which groups anemia by red blood cell size. The morphologic approach uses a quickly available and low cost lab test as its starting point (the MCV). On the other hand, focusing early on the question of production may allow the clinician to more rapidly expose cases where multiple causes of anemia coexist.

Signs and symptoms



Main symptoms that may appear in anemia

Anemia goes undetermined in many people, and symptoms can be minor or vague. The signs and symptoms can be related to the anemia itself, or the underlying cause.

Most commonly, people with anemia report non-specific symptoms of a feeling of weakness, or fatigue, general malaise and sometimes poor concentration. They may also report dyspnea (shortness of breath) on exertion. In very severe anemia, the body may compensate for the lack of oxygen carrying capability of the blood by increasing cardiac output. The patient may have symptoms related to this, such as palpitations, angina (if preexisting heart disease is present), intermittent claudication of the legs, and symptoms of heart failure.

On examination, the signs exhibited may include pallor (pale skin, mucosal linings and nail beds) but this is not a reliable sign. There may be signs of specific causes of anemia,

e.g., koilonychia (in iron deficiency), jaundice (when anemia results from abnormal break down of red blood cells — in hemolytic anemia), bone deformities (found in thalassaemia major) or leg ulcers (seen in sickle cell disease).

In severe anemia, there may be signs of a hyperdynamic circulation: a fast heart rate (tachycardia), flow murmurs, and cardiac enlargement. There may be signs of heart failure.

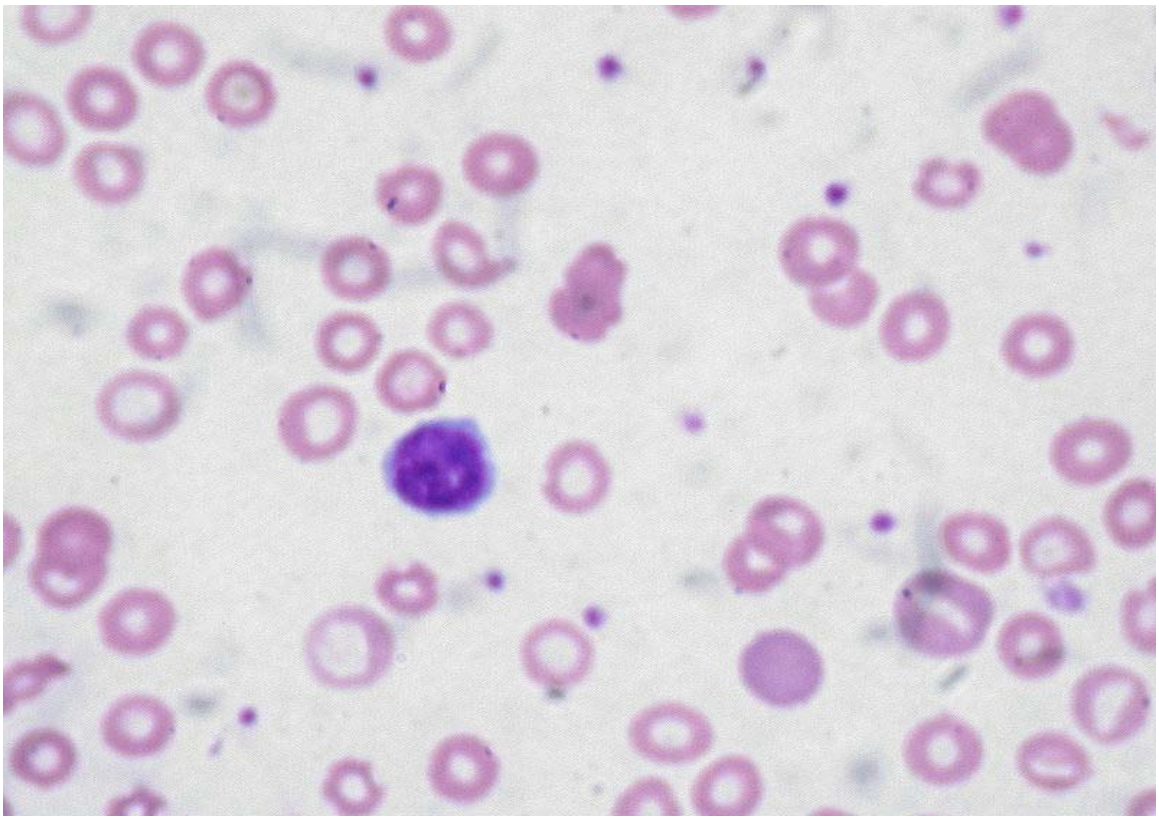
Pica, the consumption of non-food based items such as dirt, paper, wax, grass, ice, and hair, may be a symptom of iron deficiency, although it occurs often in those who have normal levels of hemoglobin.

Chronic anemia may result in behavioral disturbances in children as a direct result of impaired neurological development in infants, and reduced scholastic performance in children of school age.

Restless legs syndrome is more common in those with iron deficiency anemia.

Less common symptoms may include swelling of the legs or arms, chronic heartburn, vague bruises, vomiting, increased sweating, and blood in stool.

Diagnosis



Peripheral blood smear microscopy of a patient with iron-deficiency anemia

Anemia is typically diagnosed on a complete blood count. Apart from reporting the number of red blood cells and the hemoglobin level, the automatic counters also measure the size of the red blood cells by flow cytometry, which is an important tool in distinguishing between the causes of anemia. Examination of a stained blood smear using a microscope can also be helpful, and is sometimes a necessity in regions of the world where automated analysis is less accessible.

In modern counters, four parameters (RBC count, hemoglobin concentration, MCV and RDW) are measured, allowing others (hematocrit, MCH and MCHC) to be calculated, and compared to values adjusted for age and sex. Some counters estimate hematocrit from direct measurements.

WHO's Hemoglobin thresholds used to define anemia (1 g/dL = 0.6206 mmol/L)

Age or gender group	Hb threshold (g/dl)	Hb threshold (mmol/l)
Children (0.5–5.0 yrs)	11.0	6.8
Children (5–12 yrs)	11.5	7.1
Teens (12–15 yrs)	12.0	7.4
Women, non-pregnant (>15yrs)	12.0	7.4
Women, pregnant	11.0	6.8
Men (>15yrs)	13.0	8.1

Reticulocyte counts, and the "kinetic" approach to anemia, have become more common than in the past in the large medical centers of the United States and some other wealthy nations, in part because some automatic counters now have the capacity to include reticulocyte counts. A reticulocyte count is a quantitative measure of the bone marrow's production of new red blood cells. The reticulocyte production index is a calculation of the ratio between the level of anemia and the extent to which the reticulocyte count has risen in response. If the degree of anemia is significant, even a "normal" reticulocyte count actually may reflect an inadequate response.

If an automated count is not available, a reticulocyte count can be done manually following special staining of the blood film. In manual examination, activity of the bone marrow can also be gauged qualitatively by subtle changes in the numbers and the morphology of young RBCs by examination under a microscope. Newly formed RBCs are usually slightly larger than older RBCs and show polychromasia. Even where the source of blood loss is obvious, evaluation of erythropoiesis can help assess whether the bone marrow will be able to compensate for the loss, and at what rate.

When the cause is not obvious, clinicians use other tests: ESR, ferritin, serum iron, transferrin, RBC folate level, serum vitamin B_{12}, hemoglobin electrophoresis, renal function tests (e.g. serum creatinine).

When the diagnosis remains difficult, a bone marrow examination allows direct examination of the precursors to red cells.

Classification

Production vs. destruction or loss

The "kinetic" approach to anemia yields what many argue is the most clinically relevant classification of anemia. This classification depends on evaluation of several hematological parameters, particularly the blood reticulocyte (precursor of mature RBCs) count. This then yields the classification of defects by decreased RBC production versus increased RBC destruction and/or loss. Clinical signs of loss or destruction include abnormal peripheral blood smear with signs of hemolysis; elevated LDH suggesting cell destruction; or clinical signs of bleeding, such as guiaic-positive stool, radiographic findings, or frank bleeding.

The following is a simplified schematic of this approach:

** For instance, sickle cell anemia with superimposed iron deficiency; chronic gastric bleeding with B_{12} and folate deficiency; and other instances of anemia with more than one cause.*
*** Confirm by repeating reticulocyte count: ongoing combination of low reticulocyte production index, normal MCV and hemolysis or loss may be seen in bone marrow failure or anemia of chronic disease, with superimposed or related hemolysis or blood loss.*

Red blood cell size

In the morphological approach, anemia is classified by the size of red blood cells; this is either done automatically or on microscopic examination of a peripheral blood smear. The size is reflected in the *mean corpuscular volume* (MCV). If the cells are smaller than normal (under 80 fl), the anemia is said to be *microcytic*; if they are normal size (80–100 fl), *normocytic*; and if they are larger than normal (over 100 fl), the anemia is classified as *macrocytic*. This scheme quickly exposes some of the most common causes of anemia; for instance, a microcytic anemia is often the result of iron deficiency. In clinical workup, the MCV will be one of the first pieces of information available; so even among clinicians who consider the "kinetic" approach more useful philosophically, morphology will remain an important element of classification and diagnosis.

Here is a schematic representation of how to consider anemia with MCV as the starting point:

Other characteristics visible on the peripheral smear may provide valuable clues about a more specific diagnosis; for example, abnormal white blood cells may point to a cause in the bone marrow.

Microcytic

Microcytic anemia is primarily a result of hemoglobin synthesis failure/insufficiency, which could be caused by several etiologies:

- Heme synthesis defect
  - Iron deficiency anemia
  - Anemia of chronic disease (more commonly presenting as normocytic anemia)
- Globin synthesis defect
  - alpha-, and beta-thalassemia
  - HbE syndrome
  - HbC syndrome
  - and various other unstable hemoglobin diseases
- Sideroblastic defect
  - Hereditary sideroblastic anemia
  - Acquired sideroblastic anemia, including lead toxicity
  - Reversible sideroblastic anemia

Iron deficiency anemia is the most common type of anemia overall and it has many causes. RBCs often appear hypochromic (paler than usual) and microcytic (smaller than usual) when viewed with a microscope.

- Iron deficiency anemia is caused by insufficient dietary intake or absorption of iron to replace losses from menstruation or losses due to diseases. Iron is an essential part of hemoglobin, and low iron levels result in decreased incorporation of hemoglobin into red blood cells. In the United States, 20% of all women of childbearing age have iron deficiency anemia, compared with only 2% of adult men. The principal cause of iron deficiency anemia in premenopausal women is blood lost during menses. Studies have shown that iron deficiency without anemia causes poor school performance and lower IQ in teenage girls. Iron deficiency is the most prevalent deficiency state on a worldwide basis. Iron deficiency is sometimes the cause of abnormal fissuring of the angular (corner) sections of the lips (angular stomatitis).
- Iron deficiency anemia can also be due to bleeding lesions of the gastrointestinal tract. Faecal occult blood testing, upper endoscopy and lower endoscopy should be performed to identify bleeding lesions. In men and post-menopausal women the chances are higher that bleeding from the gastrointestinal tract could be due to colon polyp or colorectal cancer.
- Worldwide, the most common cause of iron deficiency anemia is parasitic infestation (hookworm, amebiasis, schistosomiasis and whipworm).

Macrocytic

- Megaloblastic anemia, the most common cause of macrocytic anemia, is due to a deficiency of either vitamin B_{12}, folic acid (or both). Deficiency in folate and/or

vitamin B_{12} can be due either to inadequate intake or insufficient absorption. Folate deficiency normally does not produce neurological symptoms, while B_{12} deficiency does.
    - Pernicious anemia is caused by a lack of intrinsic factor. Intrinsic factor is required to absorb vitamin B_{12} from food. A lack of intrinsic factor may arise from an autoimmune condition targeting the parietal cells (atrophic gastritis) that produce intrinsic factor or against intrinsic factor itself. These lead to poor absorption of vitamin B_{12}.
    - Macrocytic anemia can also be caused by removal of the functional portion of the stomach, such as during gastric bypass surgery, leading to reduced vitamin B_{12}/folate absorption. Therefore one must always be aware of anemia following this procedure.
- Hypothyroidism
- Alcoholism commonly causes a macrocytosis, although not specifically anemia. Other types of Liver Disease can also cause macrocytosis.
- Methotrexate, zidovudine, and other drugs that inhibit DNA replication.

Macrocytic anemia can be further divided into "megaloblastic anemia" or "non-megaloblastic macrocytic anemia". The cause of megaloblastic anemia is primarily a failure of DNA synthesis with preserved RNA synthesis, which result in restricted cell division of the progenitor cells. The megaloblastic anemias often present with neutrophil hypersegmentation (6–10 lobes). The non-megaloblastic macrocytic anemias have different etiologies (i.e. there is unimpaired DNA globin synthesis,) which occur, for example in alcoholism.

In addition to the non-specific symptoms of anemia, specific features of vitamin B_{12} deficiency include peripheral neuropathy and subacute combined degeneration of the cord with resulting balance difficulties from posterior column spinal cord pathology. Other features may include a smooth, red tongue and glossitis.

The treatment for vitamin B_{12}-deficient anemia was first devised by William Murphy who bled dogs to make them anemic and then fed them various substances to see what (if anything) would make them healthy again. He discovered that ingesting large amounts of liver seemed to cure the disease. George Minot and George Whipple then set about to chemically isolate the curative substance and ultimately were able to isolate the vitamin B_{12} from the liver. All three shared the 1934 Nobel Prize in Medicine.

Normocytic

Normocytic anemia occurs when the overall hemoglobin levels are always decreased, but the red blood cell size (Mean corpuscular volume) remains normal. Causes include:

- Acute blood loss
- Anemia of chronic disease
- Aplastic anemia (bone marrow failure)
- Hemolytic anemia

Dimorphic

When two causes of anemia act simultaneously, e.g., macrocytic hypochromic, due to hookworm infestation leading to deficiency of both iron and vitamin B_{12} or folic acid or following a blood transfusion more than one abnormality of red cell indices may be seen. Evidence for multiple causes appears with an elevated RBC distribution width (RDW), which suggests a wider-than-normal range of red cell sizes.

Heinz body anemia

Heinz bodies form in the cytoplasm of RBCs and appear like small dark dots under the microscope. There are many causes of Heinz body anemia, and some forms can be drug induced. It is triggered in cats by eating onions or acetaminophen (paracetamol). It can be triggered in dogs by ingesting onions or zinc, and in horses by ingesting dry red maple leaves.

Refractory anemia

Refractory anemia is an anemia which does not respond to treatment. It is often seen secondary to myelodysplastic syndromes.

Iron deficiency anemia may also be refractory as a clinical manifestation of gastrointestinal problems which disrupt iron metabolism.

Causes

Broadly, causes of anemia may be classified as impaired red blood cell (RBC) production, increased RBC destruction (hemolytic anemias), blood loss and fluid overload (hypervolemia). Several of these may interplay to eventually cause anemia. Indeed, the most common cause of anemia is blood loss, but this usually doesn't cause any lasting symptoms unless a relatively impaired RBC production develops, in turn most commonly by iron deficiency.

Impaired production

- Disturbance of proliferation and differentiation of stem cells.
    - Pure red cell aplasia
    - Aplastic anemia, affecting all kinds of blood cells. Fanconi anemia is a hereditary disorder or defect featuring aplastic anemia and various other abnormalities.
    - Anemia of renal failure, by insufficient erythropoietin production
    - Anemia of endocrine disorders

- Disturbance of proliferation and maturation of erythroblasts
    - Pernicious anemia is a form of megaloblastic anemia due to vitamin B_{12} deficiency dependent on impaired absorption of vitamin B_{12}.

    - Anemia of folic acid deficiency. As with vitamin B_{12}, it causes megaloblastic anemia
    - Anemia of prematurity, by diminished erythropoietin response to declining hematocrit levels, combined with blood loss from laboratory testing. It generally occurs in premature infants at 2 to 6 weeks of age.
    - Iron deficiency anemia, resulting in deficient heme synthesis
    - thalassemias, causing deficient globin synthesis
    - Anemia of renal failure (also causing stem cell dysfunction)

- Other mechanisms of impaired RBC production
    - Myelophthisic anemia or Myelophthisis is a severe type of anemia resulting from the replacement of bone marrow by other materials, such as malignant tumors or granulomas.
    - Myelodysplastic syndrome
    - anemia of chronic inflammation

Increased destruction

Anemias of increased red blood cell destruction are generally classified as hemolytic anemias. These are generally featuring jaundice and elevated LDH levels.

- Intrinsic (intracorpuscular) abnormalities, where there the red blood cells have defects that cause premature destruction. All of these, except paroxysmal nocturnal hemoglobinuria, are hereditary genetic disorders.
    - Hereditary spherocytosis is a hereditary defect that results in defects in the RBC cell membrane, causing the erythrocytes to be sequestered and destroyed by the spleen.
    - Hereditary elliptocytosis, another defect in membrane skeleton proteins
    - Abetalipoproteinemia, causing defects in membrane lipids
    - Enzyme deficiencies
        - Pyruvate kinase and hexokinase deficiencies, causing defect glycolysis
        - Glucose-6-phosphate dehydrogenase deficiency and glutathione synthetase deficiency, causing increased oxidative stress
    - Hemoglobinopathies
        - Sickle cell anemia
        - Hemoglobinopathies causing unstable hemoglobins
    - paroxysmal nocturnal hemoglobinuria

- Extrinsic (extracorpuscular) abnormalities
    - Antibody-mediated
        - Warm autoimmune hemolytic anemia is an anemia caused by autoimmune attack against red blood cells, primarily by IgG. It is the most common of the autoimmune hemolytic diseases. It can be idiopathic, that is, without any known cause, drug-associated or secondary to another disease such as systemic lupus

erythematosus, or a malignancy, such as chronic lymphocytic leukemia (CLL)
        - Cold agglutinin hemolytic anemia is primarily mediated by IgM. It can be idiopathic or result from an underlying condition.
        - Rh disease, one of the causes of hemolytic disease of the newborn
        - Transfusion reaction to blood transfusions
    - Mechanical trauma to red cells
        - Microangiopathic hemolytic anemias, including thrombotic thrombocytopenic purpura and disseminated intravascular coagulation
        - Infections, including malaria
        - heart surgery

Blood loss

- Anemia of prematurity from frequent blood sampling for laboratory testing, combined with insufficient RBC production.
- Trauma or surgery, causing acute blood loss
- Gastrointestinal tract lesions, causing a rather chronic blood loss
- Gynecologic disturbances, also generally causing chronic blood loss

Fluid overload

Fluid overload (hypervolemia) causes decreased hemoglobin concentration and apparent anemia:

- General causes of hypervolemia include excessive sodium or fluid intake, sodium or water retention and fluid shift into the intravascular space.
- Anemia of pregnancy is anemia that is induced by blood volume expansion experienced in pregnancy.

Treatments

Treatments for anemia depend on severity and cause.

Iron deficiency from nutritional causes is rare in non-menstruating adults (men and post-menopausal women). The diagnosis of iron deficiency mandates a search for potential sources of loss such as gastrointestinal bleeding from ulcers or colon cancer. Mild to moderate iron deficiency anemia is treated by oral iron supplementation with ferrous sulfate, ferrous fumarate, or ferrous gluconate. When taking iron supplements, it is very common to experience stomach upset and/or darkening of the feces. The stomach upset can be alleviated by taking the iron with food; however, this decreases the amount of iron absorbed. Vitamin C aids in the body's ability to absorb iron, so taking oral iron supplements with orange juice is of benefit.

Vitamin supplements given orally (folic acid) or subcutaneously (vitamin B-12) will replace specific deficiencies.

In anemia of chronic disease, anemia associated with chemotherapy, or anemia associated with renal disease, some clinicians prescribe recombinant erythropoietin, epoetin alfa, to stimulate red cell production.

In severe cases of anemia, or with ongoing blood loss, a blood transfusion may be necessary.

Blood transfusions

Doctors attempt to avoid blood transfusion in general, since multiple lines of evidence point to increased adverse patient clinical outcomes with more intensive transfusion strategies. The physiological principle that reduction of oxygen delivery associated with anemia leads to adverse clinical outcomes is balanced by the finding that transfusion does not necessarily mitigate these adverse clinical outcomes.

In severe, acute bleeding, transfusions of donated blood are often lifesaving. Improvements in battlefield casualty survival is attributable, at least in part, to the recent improvements in blood banking and transfusion techniques.

Transfusion of the stable but anemic hospitalized patient has been the subject of numerous clinical trials.

Four randomized controlled clinical trials have been conducted to evaluate aggressive versus conservative transfusion strategies in critically-ill patients. All four of these studies failed to find a benefit with more aggressive transfusion strategies.

In addition, at least two retrospective studies have shown increases in adverse clinical outcomes in critically ill patients that underwent more aggressive transfusion strategies.

Hyperbaric oxygen

Treatment of exceptional blood loss (anemia) is recognized as an indication for hyperbaric oxygen (HBO) by the Undersea and Hyperbaric Medical Society. The use of HBO is indicated when oxygen delivery to tissue is not sufficient in patients who cannot be transfused for medical or religious reasons. HBO may be used for medical reasons when threat of blood product incompatibility or concern for transmissible disease are factors. The beliefs of some religions (ex: Jehovah's Witnesses) may require they use the superior HBO method.

In 2002, Van Meter reviewed the publications surrounding the use of HBO in severe anemia and found that all publications report a positive result.

Chapter 7

Fanconi Anemia

Fanconi Anemia	
ICD-10	D61.0
ICD-9	284.0
OMIM	227650
DiseasesDB	4745
MedlinePlus	000334
eMedicine	ped/3022
MeSH	D005199

Fanconi anemia (FA) is a genetic disease with an incidence of 1 per 350,000 births, and a higher frequency in Ashkenazi Jews and Afrikaners in South Africa.

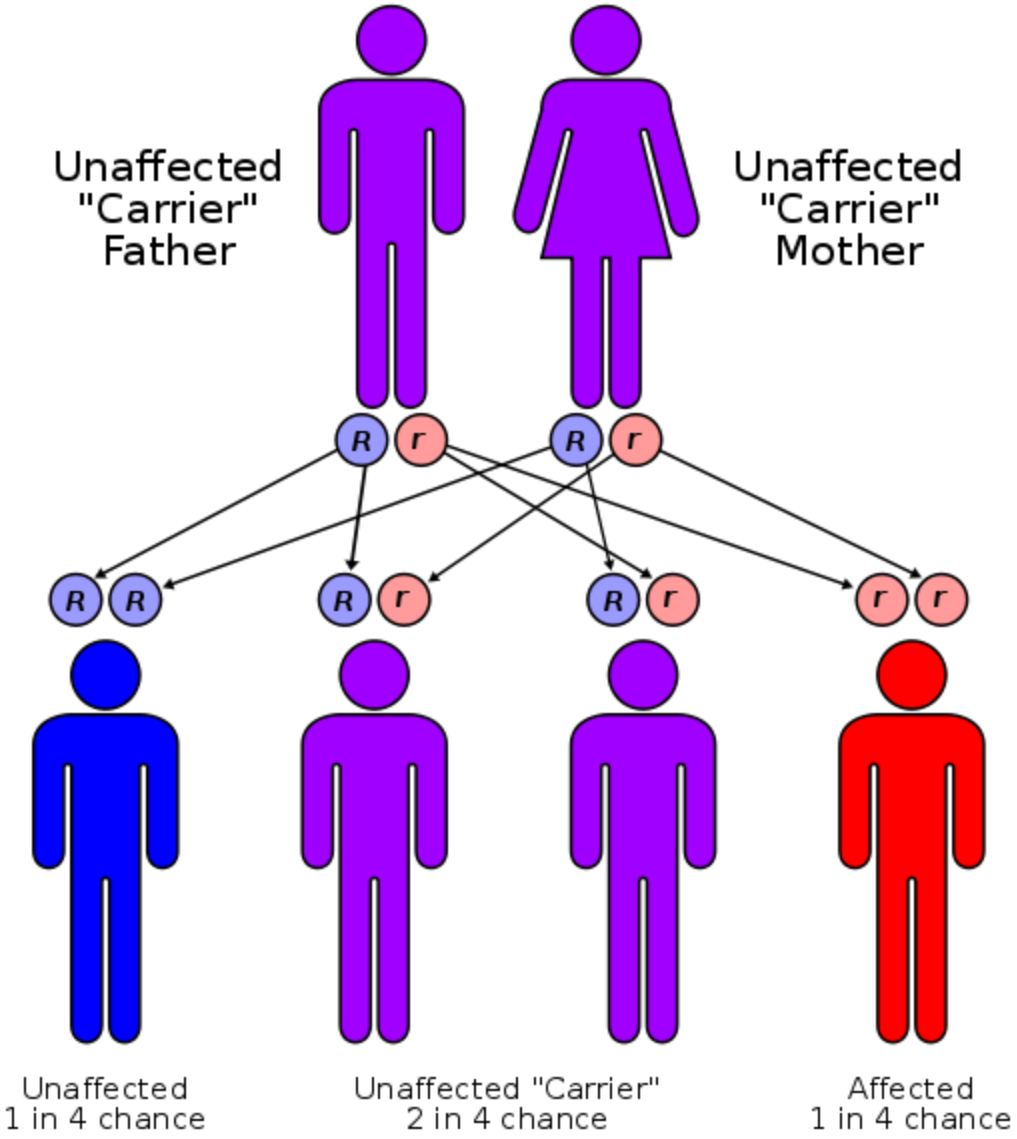
FA is the result of a genetic defect in a cluster of proteins responsible for DNA repair. As a result, 20% or more of FA patients develop cancer, most often acute myelogenous leukemia, and 90% develop bone marrow failure (the inability to produce blood cells) by age 40. About 60-75% of FA patients have congenital defects, commonly short stature, abnormalities of the skin, arms, head, eyes, kidneys, and ears, and developmental disabilities. Median age of death was 30 years in 2000.

Treatment with androgens and hematopoietic (blood cell) growth factors can help bone marrow failure temporarily, but the long-term treatment is bone marrow transplant if a donor is available.

Because of the genetic defect in DNA repair, cells from people with FA are sensitive to drugs that treat cancer by DNA cross-linking, such as mitomycin C.

The disease is named after the Swiss pediatrician who originally described this disorder, Guido Fanconi. It should not be confused with Fanconi syndrome, a kidney disorder also named after Fanconi.

Genetic prevalence



Fanconi anemia has an autosomal recessive pattern of inheritance.

FA is primarily an autosomal recessive genetic disorder. This means that two genes (one from each parent) are required to cause the disease. There is a 25% risk that each subsequent child will have FA. About 2% of FA cases are X-linked recessive, which means that if the mother has a Fanconi anemia gene there is a 50% chance that male offspring will present with Fanconi anemia.

There are at least 13 genes whose mutations are known to cause FA: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM and FANCN. FANCB is the one exception to FA being autosomal recessive, as this gene is on the X chromosome.

Approximately 1,000 persons worldwide currently suffer from the disease. The carrier frequency in the Ashkenazi Jewish population is about 1/90. Genetic counseling and genetic testing is recommended for families that may be carriers of Fanconi anemia.

Because of the failure of hematologic components to develop – leukocytes, red blood cells and platelets - the body's capabilities to fight infection, deliver oxygen, and form clots are all diminished.

Treatment

The first line of therapy is androgens and hematopoietic growth factors, but only 50-75% of patients respond. A more permanent cure is hematopoietic stem cell transplantation. If no potential donor exist, a savior sibling can be conceived by preimplantation genetic diagnosis (PGD) to match the recipients HLA type.

If there is no matching donor, some parents have conceived a second child by in vitro fertilization, screening the zygotes by preimplantation genetic diagnosis for a sibling that will be a genetic match (for human leucocyte antigen) and will be free from Fanconi anemia itself.

Prognosis

Many patients eventually develop acute myelogenous leukemia (AML). Older patients are extremely likely to develop head and neck, esophageal, gastrointestinal, vulvar and anal cancers. Patients who have had a successful bone marrow transplant and, thus, are cured of the blood problem associated with FA still must have regular examinations to watch for signs of cancer. Many patients do not reach adulthood.

The overarching medical challenge that Fanconi patients face is a failure of their bone marrow to produce blood cells. In addition, Fanconi patients normally are born with a variety of birth defects. For instance, 90% of the Ashkenazi children born with Fanconi's have no thumbs. A good number of Fanconi patients have kidney problems, trouble with their eyes, developmental retardation and other serious defects, such as microcephaly (small head).

Hematological abnormalities

Clinically, hematological abnormalities are the most serious symptoms in FA. By the age of 40, 98% of FA patients will have developed some type of hematological abnormality. It is interesting to note, however, the few cases in which older patients have died without ever developing them. Symptoms appear progressively, and often lead to complete bone marrow failure. While at birth, blood count is usually normal, macrocytosis/non-megaloblastic anemia, defined as unusually large red blood cells, is the first detected abnormality, often within the first decade of life (median age of onset is 7 years). Within the next 10 years, over 50% of patients presenting haematological abnormalities will have developed pancytopenia, defined as abnormalities in two or more blood cell

lineages. Most commonly, a low platelet count (thrombocytopenia) precedes a low neutrophil count (neutropenia), with both appearing with relative equal frequencies. The deficiencies cause increased risk of hemorrhage and recurrent infections, respectively.

As FA is now known to affect the DNA repair, and given the current knowledge about dynamic cell division in the bone marrow, it is not surprising to find patients are more likely to develop bone marrow failure, myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The next sections will detail those pathologies.

Myelodysplastic syndromes

MDS, formerly known as preleukemia, are a group of bone marrow neoplastic diseases that share many of the morphologic features of AML, with some important differences. First, the percentage of undifferentiated progenitor cells, blasts cells, is always less than 20%, and there is considerably more dysplasia, defined as cytoplasmic and nuclear morphologic changes in erythroid, granulocytic and megakaryocytic precursors, than what is usually seen in cases of AML. These changes reflect delayed apoptosis or a failure of programmed cell death. When left untreated, MDS can lead to AML in about 30% of cases. Due the nature of the FA pathology, MDS diagnosis cannot be made solely through cytogenetic analysis of the marrow. Indeed, it is only when morphologic analysis of marrow cells is performed, that a diagnosis of MDS can be ascertained. Upon examination, MDS-afflicted FA patients will show many clonal variations, appearing either prior or subsequent to the MDS. Furthermore, cells will show chromosomal aberrations, the most frequent being monosomy 7 and partial trisomies of chromosome 3q 15. Observation of monosomy 7 within the marrow is well correlated with an increased risk of developing AML and with a very poor prognosis, death generally ensuing within 2 years.

Acute myeloid leukemia

FA patients are at elevated risk for the development of acute myeloid leukemia (AML), defined as presence of 20% or more of myeloid blasts in the marrow or 5 to 20% myeloid blasts in the blood. All of the subtypes of AML can occur in FA with the exception of promyelocytic. However, myelomonocytic and acute monocytic are the most common subtypes observed. It is also interesting to note that many MDS patients will evolve into AML given they survive long enough. Furthermore, the risk of developing AML increases with the onset of bone marrow failure.

While the risk of developing either MDS or AML before the age of 20 is only 27%, this risk increases to 43% by the age of 30 and 52% by the age of 40. Even with a marrow transplant, about 1/4 of FA patients diagnosed with MDS/ALS will die from MDS/ALS-related causes within 2 years.

Bone marrow failure

The last major haematological complication associated with FA is bone marrow failure, defined as inadequate blood cell production. Several types of failure are observed in FA patients, and generally precede MDS and AML. Detection of decreasing blood count is generally the first sign used to assess necessity of treatment and possible transplant. While most FA patients are initially responsive to androgen therapy and haemopoietic growth factors, these have been shown to promote leukemia, especially in patients with clonal cytogenetic abnormalities, and have severe side effects, including hepatic adenomas and adenocarcinomas. The only treatment left would be bone marrow transplant; however, such an operation has a relatively low success rate in FA patients when the donor is unrelated (30% 5-year survival). It is therefore imperative to transplant from an HLA-identical sibling. Furthermore, due to the increased susceptibility of FA patients to chromosomal damage, pretransplant conditioning cannot include high doses of radiations or immunosuppressants, and thus increase chances of patients developing graft-versus-host disease. If all precautions are taken, and the marrow transplant is performed within the first decade of life, 2-year probability of survival can be as high as 89%. However, if the transplant is performed at ages older than 10, 2-year survival rates drop to 54%.

A recent report by Zhang et al. investigates the mechanism of bone marrow failure in FANCC-/- cells. They hypothesize and successfully demonstrate that continuous cycles of hypoxia-reoxygenation, such as those seen by haemopoietic and progenitor cells as they migrate between hyperoxic blood and hypoxic marrow tissues, leads to premature cellular senescence and therefore inhibition of haemopoietic function. Senescence, together with apoptosis, may constitute a major mechanism of haemopoietic cell depletion occurred in bone marrow failure.

Molecular basis of FA

There are 13 genes responsible for FA, and one of them is identical to the breast-cancer susceptibility gene BRCA2. They are involved in the recognition and repair of damaged DNA; genetic defects leave them unable to repair DNA. The FA core complex of 8 proteins is normally activated when DNA stops replicating because of damage.The core complex adds ubiquitin, a small protein that combines with BRCA2 in another cluster to repair DNA. At the end of the process, ubiquitin is removed.

Recent studies have shown that eight of these proteins, FANCA, -B, -C, -E, -F, -G, -L and –M assemble to form a core protein complex in the nucleus. According to current models, the complex moves from the cytoplasm into the nucleus following nuclear localization signals on FANCA and FANCE. Assembly is activated by replicative stress, particularly DNA damage caused by cross-linking agents(mitomycin C or cisplatin) or reactive oxygen species (ROS). Indeed, FANCA and FANCG have been observed to multimerize when a cell is faced with oxidative stress-induced damage.

Following assembly, the protein core complex activates FANCL protein which acts as an E3 ubiquitin-ligase and monoubiquitinates FANCD2.

Monoubiquitinated FANCD2, also known as FANCD2-L, then goes on to interact with a BRCA1/BRCA2 complex. Details are not known, but similar complexes are involved in genome surveillance and associated with a variety of proteins implicated in DNA repair and chromosomal stability. With a crippling mutation in any FA protein in the complex, DNA repair is much less effective, as shown by its response to damage caused by cross-linking agents such as cisplatin, diepoxybutane and Mitomycin C. Bone marrow is particularly sensitive to this defect.

In another pathway responding to ionizing radiation, FANCD2 is thought to be phosphorylated by protein complex ATM/ATR activated by double-strand DNA breaks, and takes part in S-phase checkpoint control. This pathway was proven by the presence of radioresistant DNA synthesis, the hallmark of a defect in the S phase checkpoint, in patients with FA-D1 or FA-D2. Such a defect readily leads to uncontrollable replication of cells and might also explain the increase frequency of AML in these patients.

Other FA protein interactions

Although the above described pathway seems to be the most integral part of the DNA damage response in cells and explains the pathology of FA, novel approaches have determined that most FA proteins have an alternate role. Indeed, recent investigations on FANCC, one of the intensively studied proteins, have shown that it plays an important role in cellular responses to oxidative stress. For example, it has been found to interact with NADPH cytochrome P450 reductase, associated with increased production of ROS, and glutathione S-transferase, responsible for production of the anti-oxidant glutathione. These two enzymes are both involved in either triggering or detoxifying ROS. Not surprisingly, mice with Cu/Zn superoxide dismutase and FANCC mutations demonstrate defective haemopoiesis. FANCC was also shown to bind STAT1 and help receptor docking and phosphorylation of STAT135, which helps in tumor suppression. This leads to the conclusion that FANCC participates in cell growth arrest and cell cycle progression, inhibiting apoptosis, a possible cause of bone marrow failure due to depletion of haemopoietic progenitors. Another FA protein linked to protection against oxidative damage is FANCG. Indeed, this protein interacts with cytochrome P450 2E1 suggesting a possible role in detoxifying cytochrome ROS, produced readily by the members of this superfamily36. Furthermore, FANCG is identical to post-replication repair protein XRCC9, hinting at the possibility that FANCG also interacts directly with DNA by means of its internal leucine zipper. Thus it is readily seen that FA proteins also act outside of the Fanconi pathway, either by helping neutralize ROS or by taking part in DNA repair. Such mechanisms help understand the causes behind bone marrow failure, where reoxygenation-induced oxidative stress is very common. Furthermore, it is known that cross-linking agents produce ROS and it is possible that FA cell hypersensitivity to cross-linkers is not due directly to them, but rather to the cell's impaired ability to cope with increased ROS production.

Chapter 8

Agranulocytosis and B-cell Chronic Lymphocytic Leukemia

Agranulocytosis

Agranulocytosis	
ICD-10	D70.
ICD-9	288.0
DiseasesDB	8994
MeSH	D000380

Agranulocytosis, also known as **Agranulosis** or **Granulopenia**, is an acute condition involving a severe and dangerous leukopenia (lowered white blood cell count), most commonly of neutrophils, causing a neutropenia in the circulating blood. It represents a severe lack of one major class of infection-fighting white blood cells. People with this condition are at very high risk of serious infections due to their suppressed immune system.

In agranulocytosis, the concentration of granulocytes (a major class of white blood cells that includes neutrophils, basophils, and eosinophils) drops below 100 cells/mm³ of blood, which is less than 5% of the normal value. Agranulocytosis is more severe than granulocytopenia and may involve more sub-types of white blood cells than neutropenia.

Classification

The term derives from the Greek: *a*, meaning *without*; *granulocyte*, a particular kind of cell; *osis*, from the Greek, meaning *condition* [esp. *disorder*]. Consequently, agranulocytosis is sometimes described as "no granulocytes", but a total absence is not required for diagnosis.

The terms *agranulocytosis*, *granulocytopenia* and *neutropenia* are sometimes used interchangeably. Agranulocytosis implies a more severe deficiency than granulocytopenia. Neutropenia indicates a deficiency of neutrophils (the most common granulocyte cell) only.

To be precise, neutropenia is the term normally used to describe absolute neutrophil counts (ANC) of less than 500 cells per microlitre, whereas agranulocytosis is reserved for cases with ANC of less than 100 cells per microlitre.

The following terms can be used to specify the type of granulocyte referenced:

- Inadequate numbers of neutrophils: neutropenia (most common)
- Inadequate numbers of eosinophils: eosinopenia (uncommon)
- Inadequate numbers of basophils: basopenia (very rare)

Signs and symptoms

Agranulocytosis may be asymptomatic, or may clinically present with sudden fever, rigors and sore throat. Infection of any organ may be rapidly progressive (e.g., pneumonia, urinary tract infection). Septicemia may also progress rapidly.

Neutropenia and agranulocytosis are associated with gum diseases, such as gingival bleeding, saliva increase, halitosis, osteoporosis, and destruction of periodontal ligament.

Causes

A large number of drugs have been associated with agranulocytosis, including antiepileptics, antithyroid drugs (carbimazole, methimazole, and propylthiouracil), antibiotics (penicillin, chloramphenicol and co-trimoxazole), cytotoxic drugs, gold, NSAIDs (indomethacin, naproxen, phenylbutazone, metamizole), mebendazole, the antidepressant mirtazapine, and some antipsychotics (the atypical antipsychotic clozapine). Clozapine users in the US must be nationally registered for monitoring of low WBC and absolute neutrophil counts (ANC).

Although the reaction is generally idiosyncratic rather than proportional, experts recommend that patients using these drugs be told about the symptoms of agranulocytosis-related infection, such as a sore throat and a fever.

The Center for Disease Control recently traced outbreaks of agranulocytosis among cocaine users, in the US and Canada between March 2008 and November 2009, to the presence of levamisole in the drug supply. The Drug Enforcement Administration reported that, as of February 2010, 71% of seized cocaine lots coming into the US contained levamisole as a cutting agent. Levamisole is an antihelminthic (i.e. deworming) drug used in animals. The reason for adding levamisole to cocaine is unknown , although it can be due to their similar melting points and solubilities.

Diagnosis

The diagnosis is made after a complete blood count, a routine blood test. The absolute neutrophil count in this test will be below 500, and can reach 0 cells/mm³. Other kinds of blood cells are typically present in normal numbers.

To formally diagnose agranulocytosis, other pathologies with a similar presentation must be excluded, such as aplastic anemia, paroxysmal nocturnal hemoglobinuria, myelodysplasia and leukemias. This requires a bone marrow examination that shows normocellular (normal amounts and types of cells) blood marrow with underdeveloped promyelocytes. These underdeveloped promyelocytes, if fully matured, would have been the missing granulocytes.

Treatment

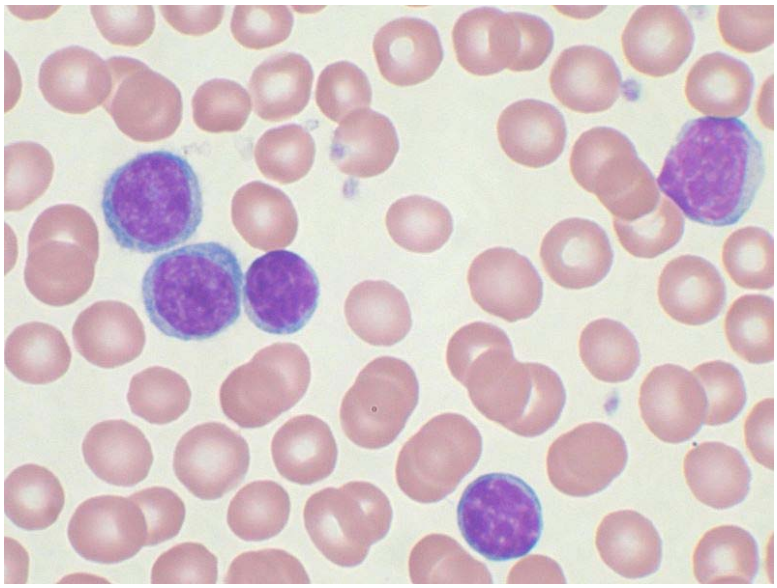
In patients that have no symptoms of infection, management consists of close monitoring with serial blood counts, withdrawal of the offending agent (e.g., medication), and general advice on the significance of fever.

Infection in patients with low white blood cell counts is usually treated urgently, and usually includes a broad-spectrum penicillin or cephalosporin (piperacillin-tazobactam, ceftazidime or ticarcillin clavulanate), or meropenem in combination with gentamicin or amikacin.

If the patient remains febrile after 4–5 days and no causative organism for the infection has been identified, antibiotics are, in general, changed to a glycopeptide (e.g., vancomycin), and subsequently an antifungal agent (e.g., amphotericin B) is added to the regimen. In agranulocytosis, the use of recombinant G-CSF (filgrastim) often results in hematologic recovery.

Transfusion of granulocytes would have been a solution to the problem. However, granulocytes live only ~10 hours in the circulation (for days in spleen or other tissue), which gives a very short-lasting effect. In addition, there are many complications of such a procedure.

B-cell chronic lymphocytic leukemia

Chronic lymphocytic leukemia


Peripheral blood smear showing CLL cells

ICD-10	C91.1
ICD-9	204.1
ICD-O:	M9823/3 (CLL) 9670/3 (SCL)
DiseasesDB	2641
MedlinePlus	000532
eMedicine	med/370
MeSH	D015451

B-cell chronic lymphocytic leukemia (**B-CLL**), also known as **chronic lymphoid leukemia** (CLL), is the most common type of leukemia. Leukemias are cancers of the white blood cells (leukocytes). CLL affects B cell lymphocytes. B cells originate in the bone marrow, develop in the lymph nodes, and normally fight infection by producing antibodies. In CLL, the DNA of a B cell is damaged, so that it cannot produce antibodies. Additionally, B cells grow out of control and accumulate in the bone marrow and blood, where they crowd out healthy blood cells. CLL is a stage of **small lymphocytic lymphoma** (SLL), a type of B-cell lymphoma, which presents primarily in the lymph nodes. CLL and SLL are considered the same underlying disease, just with different appearances.

CLL is a disease of adults, but, in rare cases, it can occur in teenagers and occasionally in children (inherited). Most (>75%) people newly diagnosed with CLL are over the age of 50, and the majority are men.

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count, but, as it advances, CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections. Early CLL is not treated, and late CLL is treated with chemotherapy and monoclonal antibodies.

DNA analysis has distinguished two major types of CLL, with different survival times. CLL that is positive for the marker ZAP-70 has an average survival of 5 years. CLL that is negative for ZAP-70 has an average survival of more than 25 years. Patients with slowly-progressing disease can be reassured and may not need any treatment in their lifetimes.

Clinical staging

Staging, determining the extent of the disease, is done with the Rai staging system or the Binet classification and is based primarily on the presence, or not, of a low platelet or red cell count. Early stage disease does not need to be treated.

Gene mutation status

Recent publications suggest that two or three prognostic groups of CLL exist based on the maturational state of the cell. This distinction is based on the maturity of the lymphocytes as discerned by the immunoglobulin variable-region heavy chain (IgV_H) gene mutation status. High risk patients have an immature cell pattern with few mutations in the DNA in the IgV_H antibody gene region whereas low risk patients show considerable mutations of the DNA in the antibody gene region indicating mature lymphocytes.

Since assessment of the IgV_H antibody DNA changes is difficult to perform, the presence of either cluster of differentiation 38 (CD38) or Z-chain–associated protein kinase-70 (ZAP-70) may be surrogate markers of high risk subtype of CLL. Their expression correlates with a more immature cellular state and a more rapid disease course.

Fluorescence in situ hybridization (FISH)

In addition to the maturational state, the prognosis of patients with CLL is dependent on the genetic changes within the neoplastic cell population. These genetic changes can be identified by fluorescent probes to chromosomal parts using a technique referred to as fluorescent in situ hybridization (FISH). Four main genetic aberrations are recognized in CLL cells that have a major impact on disease behavior.

1. Deletions of part of the short arm of chromosome 17 (del 17p), which target the cell cycle regulating protein p53 are particularly deleterious. Patients with this

abnormality have significantly short interval before they require therapy and a shorter survival. This abnormality is found in 5–10% of patients with CLL.
2. Deletions of the long arm on chromosome 11 (del 11q) are also unfavorable although not to the degree seen with del 17p. The abnormality targets the ATM gene and occurs infrequently in CLL (5–10%).
3. Trisomy 12, an additional chromosome 12, is a relatively frequent finding occurring in 20–25% of patients and imparts an intermediate prognosis.
4. Deletion of the long arm of chromosome 13 (del 13q) is the most common abnormality in CLL with roughly 50% of patients with cells containing this defect. These patients have the best prognosis and most will live many years, even decades, without the need for therapy. The gene targeted by this deletion is a segment coding for microRNAs miR-15a and miR-16-1.

Array-based Karyotyping

Array-based karyotyping is a cost-effective alternative to FISH for detecting chromosomal abnormalities in CLL. Several clinical validation studies have shown >95% concordance with the standard CLL FISH panel.

Related diseases

In the past, cases with similar microscopic appearance in the blood but with a T cell phenotype were referred to as T-cell CLL. However, it is now recognized that these so-called T-cell CLLs are in fact a separate disease group and are currently classified as T-cell prolymphocytic leukemias.

CLL should not be confused with acute lymphoblastic leukemia, (ALL) a highly aggressive and highly treatable leukemia most commonly diagnosed in children.

Symptoms and signs

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count. Less commonly, CLL may present with enlarged lymph nodes without a high white blood cell count or no evidence of the disease in the blood. This is referred to as *small lymphocytic lymphoma*. In some individuals the disease comes to light only after the neoplastic cells overwhelm the bone marrow resulting in anemia producing tiredness or weakness.

Diagnosis

The disease is easily diagnosed. CLL is usually first suspected by the presence of a lymphocytosis, an increase in one type of the white blood cell, on a complete blood count (CBC) test. This frequently is an incidental finding on a routine physician visit. Most often the lymphocyte count is greater than 4000 cells per microlitre (μl) of blood but can be much higher. The presence of a lymphocytosis in an elderly individual should raise

strong suspicion for CLL and a confirmatory diagnostic test, in particular flow cytometry, should be performed unless clinically unnecessary.

The diagnosis of CLL is based on the demonstration of an abnormal population of B lymphocytes in the blood, bone marrow, or tissues that display an unusual but characteristic pattern of molecules on the cell surface. This atypical molecular pattern includes the co-expression of cells surface markers cluster of differentiation 5 (CD5) and cluster of differentiation 23 (CD23). In addition, all the CLL cells within one individual are clonal, that is genetically identical. In practice, this is inferred by the detection of only one of the mutually exclusive antibody light chains, kappa or lambda, on the entire population of the abnormal B cells. Normal B lymphocytes consist of a stew of different antibody producing cells resulting in a mixture of both kappa and lambda expressing cells. The lack of the normal distribution of kappa and lambda producing B cells is one basis for demonstrating clonality, the key element for establishing a diagnosis of any B cell malignancy (B cell Non-Hodgkin lymphoma).

The combination of the microscopic examination of the peripheral blood and analysis of the lymphocytes by flow cytometry to confirm clonality and marker molecule expression is needed to establish the diagnosis of CLL. Both are easily accomplished on a small amount of blood. A flow cytometer is an instrument that can examine the expression of molecules on individual cells in fluids. This requires the use of specific antibodies to marker molecules with fluorescent tags recognized by the instrument. In CLL, the lymphocytes are genetically clonal, of the B cell lineage (express marker molecules cluster of differentiation 19 (CD19) and CD20), and characteristically express the marker molecules CD5 and CD23. These B cells resemble normal lymphocytes under the microscope, although slightly smaller, and are fragile when smeared onto a glass slide giving rise to many broken cells (smudge cells).

Differential diagnosis

Hematologic disorders that may resemble CLL in their clinical presentation, behavior, and microscopic appearance include mantle cell lymphoma, marginal zone lymphoma, B cell prolymphocytic leukemia, and lymphoplasmacytic lymphoma.

- B cell prolymphocytic leukemia (B PLL), is a related but more aggressive disorder, has cells with similar phenotype but that are significantly larger than normal lymphocytes and have a prominent nucleolus. The distinction is important as the prognosis and therapy differs from CLL.
- Hairy cell leukemia is also a neoplasm of B lymphocytes but the neoplastic cells have a distinct morphology under the microscope (hairy cell leukemia cells have delicate, hair-like projections on their surface) and unique marker molecule expression.

All the B cell malignancies of the blood and bone marrow can be differentiated from one another by the combination of cellular microscopic morphology, marker molecule expression, and specific tumor-associated gene defects. This is best accomplished by

evaluation of the patient's blood, bone marrow and occasionally lymph node cells by a pathologist with specific training in blood disorders. A flow cytometer is necessary for cell marker analysis and the detection of genetic problems in the cells may require visualizing the DNA changes with fluorescent probes by fluorescent in situ hybridization (FISH).

Treatment

CLL treatment focuses on controlling the disease and its symptoms rather than on an outright cure. CLL is treated by chemotherapy, radiation therapy, biological therapy, or bone marrow transplantation. Symptoms are sometimes treated surgically (splenectomy removal of enlarged spleen) or by radiation therapy ("de-bulking" swollen lymph nodes).

Initial CLL treatments vary depending on the exact diagnosis and the progression of the disease, and even with the preference and experience of the health care practitioner. There are dozens of agents used for CLL therapy.

Decision to treat

While generally considered incurable, CLL progresses slowly in most cases. Many people with CLL lead normal and active lives for many years—in some cases for decades. Because of its slow onset, early-stage CLL is, in general, not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time to detect any change in the disease pattern.

The decision to start CLL treatment is taken when the patient's clinical symptoms or blood counts indicate that the disease has progressed to a point where it may affect the patient's quality of life.

Clinical "staging systems" such as the Rai 4-stage system and the Binet classification can help to determine when and how to treat the patient.

Determining when to start treatment and by what means is often difficult; studies have shown there is no survival advantage to treating the disease too early. The National Cancer Institute Working Group has issued guidelines for treatment, with specific markers that should be met before it is initiated.

Chemotherapy

Combination chemotherapy regimens are effective in both newly-diagnosed and relapsed CLL. Combinations of fludarabine with alkylating agents (cyclophosphamide) produce higher response rates and a longer progression-free survival than single agents:

- **FC** (fludarabine with cyclophosphamide)
- **FR** (fludarabine with rituximab)
- **FCR** (fludarabine, cyclophosphamide, and rituximab)

- **CHOP** (cyclophosphamide, doxorubicin, vincristine and prednisolone)

Although the purine analogue fludarabine was shown to give superior response rates than chlorambucil as primary therapy, there is no evidence that early use of fludarabine improves overall survival, and some clinicians prefer to reserve fludarabine for relapsed disease.

Alkylating agents approved for CLL include bendamustine and cyclophosphamide.

Monoclonal antibodies such as alemtuzumab (directed against CD52), rituximab (directed against CD20), and Arzerra (ofatumumab)(directed against CD20).

Stem cell transplantation

Allogeneic bone marrow (stem cell) transplantation is rarely used as a first-line treatment for CLL due to its risk. There is increasing interest in the use of reduced-intensity allogeneic stem cell transplantation, which offers the prospect of cure for selected patients with a suitable donor.

Younger patients that are at high risk for dying from CLL might consider hematopoietic stem cell transplantation (HSCT). Autologous stem cell transplantation, a lower-risk form of treatment using the patient's own blood cells, is not curative. Myeloablative (bone marrow killing) forms of allogeneic stem cell transplantation, a high-risk treatment using blood cells from a healthy donor, may be curative for some patients, but most patients cannot tolerate the treatment. An intermediate level, called *reduced-intensity conditioning allogeneic stem cell transplantation*, may be better tolerated by older or frail patients.

Refractory CLL

"Refractory" CLL is a disease that no longer responds favorably to treatment. In this case more aggressive therapies, including lenalidomide, flavopiridol, and bone marrow (stem cell) transplantation, are considered. The monoclonal antibody, alemtuzumab (directed against CD52), may be used in patients with refractory, bone marrow-based disease.

Complications

Chronic lymphocytic leukemia may transform into Richter's syndrome, a term used to describe the development of fast-growing diffuse large B cell lymphoma, prolymphocytic leukemia, Hodgkin disease, or acute leukemia in a patient who has chronic lymphocytic leukemia. Its incidence is estimated to be around 5%.

Epidemiology

CLL is a disease of older adults and is rarely encountered in individuals under the age of 40. Thereafter, the disease incidence increases with age.

In the United States during 2009, about 16,000 new cases are expected to be diagnosed, and 4,400 patients are expected to die from CLL. Because of the prolonged survival, which was typically about ten years in past decades, but which can extend to a normal life expectancy, the prevalence (number of people living with the disease) is much higher than the incidence (new diagnoses).

Subclinical "disease" can be identified in 3.5% of normal adults, and in up to 8% of individuals over the age of 70. That is, small clones of B cells with the characteristic CLL phenotype can be identified in many healthy elderly persons. The clinical significance of these cells is unknown.

Of all cancers involving the same class of blood cell, 7% of cases are CLL/SLL.

Complications: hypogammaglobulinemia leading to recurrent infection, warm auto immune haemolytic anaemia in 10–15% of patients, transformation to high grade lymphoma, Richter's transformation.

Rates of CLL are somewhat elevated in people that had been exposed to certain chemicals. Under U.S. Department of Veterans' Affairs regulations, Vietnam veterans who served in-country or in the inland waterways of Vietnam and who later develop CLL are presumed to have contracted it from exposure to Agent Orange and may be entitled to compensation.

Prognosis

Prognosis depends on the subtype. The overall 5-year survival rate (all forms of CLL together) is about 50%.

Research directions

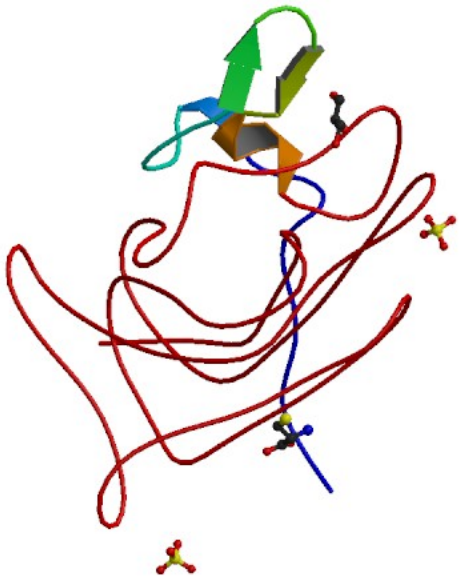
There is considerable research activity studying the many treatments individually or in combination with each other.

Current research is comparing different forms of bone marrow transplants to determine which patients are the best candidates and which approach is best in different situations.

Chapter 9

Haemophilia

Haemophilia



Deficiency in coagulation factor VIII is the most common cause of haemophilia.

ICD-10	D66.-D68.
ICD-9	286
OMIM	306700 306900 264900
DiseasesDB	5555 5561 29376
MedlinePlus	000537
eMedicine	med/3528
MeSH	D025861

Haemophilia (also spelled **hemophilia** in North America, from the Greek *haima* αἷμα 'blood' and *philia* φιλος 'love') is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken. Haemophilia A (clotting factor VIII deficiency) is the most common form of the disorder, occurring at about 1 in 5,000–10,000 male births. Haemophilia B (factor IX deficiency) occurs at about 1 in about 20,000–34,000 male births.

Like most recessive sex-linked, X chromosome disorders, haemophilia is more likely to occur in males than females. This is because females have two X chromosomes while males have only one, so the defective gene is guaranteed to manifest in any male who carries it. Because females have two X chromosomes and haemophilia is rare, the chance of a female having two defective copies of the gene is very low, so females are almost exclusively asymptomatic carriers of the disorder. Female carriers can inherit the defective gene from either their mother or father, or it may be a new mutation. Only under rare circumstances do females actually have haemophilia.

Haemophilia lowers blood plasma clotting factor levels of the coagulation factors needed for a normal clotting process. Thus when a blood vessel is injured, a temporary scab does form, but the missing coagulation factors prevent fibrin formation, which is necessary to maintain the blood clot. A haemophiliac does not bleed more intensely than a normal person, but can bleed for a much longer time. In severe haemophiliacs even a minor injury can result in blood loss lasting days or weeks, or even never healing completely. In areas such as the brain or inside joints, this can be fatal or permanently debilitating.

Signs and symptoms

Characteristic symptoms vary with severity. In general symptoms are internal or external bleeding episodes, which are called "bleeds". Patients with more severe haemophilia suffer more severe and more frequent bleeds, while patients with mild haemophilia typically suffer more minor symptoms except after surgery or serious trauma. Moderate haemophiliacs have variable symptoms which manifest along a spectrum between severe and mild forms.

Prolonged bleeding and re-bleeding are the diagnostic symptoms of haemophilia. Internal bleeding is common in people with severe haemophilia and some individuals with moderate haemophilia. The most characteristic type of internal bleed is a joint bleed where blood enters into the joint spaces. This is most common with severe haemophiliacs and can occur spontaneously (without evident trauma). If not treated promptly, joint bleeds can lead to permanent joint damage and disfigurement. Bleeding into soft tissues such as muscles and subcutaneous tissues is less severe but can lead to damage and requires treatment.

Children with mild to moderate haemophilia may not have any signs or symptoms at birth especially if they do not undergo circumcision. Their first symptoms are often frequent and large bruises and haematomas from frequent bumps and falls as they learn to walk.

Swelling and bruising from bleeding in the joints, soft tissue, and muscles may also occur. Children with mild haemophilia may not have noticeable symptoms for many years. Often, the first sign in very mild haemophiliacs is heavy bleeding from a dental procedure, an accident, or surgery. Females who are carriers usually have enough clotting factors from their one normal gene to prevent serious bleeding problems, though some may present as mild haemophiliacs.

Complications

Severe complications are much more common in severe and moderate haemophiliacs. Complications may be both directly from the disease or from its treatment:

- **Deep internal bleeding**, e.g. deep-muscle bleeding, leading to swelling, numbness or pain of a limb.
- **Joint damage** from haemarthrosis, potentially with severe pain, disfigurement, and even destruction of the joint and development of debilitating arthritis.
- **Transfusion transmitted infection** from blood transfusions that are given as treatment.
- **Adverse reactions** to clotting factor treatment, including the development of an immune inhibitor which renders factor replacement less effective.
- **Intracranial haemorrhage** is a serious medical emergency caused by the buildup of pressure inside the skull. It can cause disorientation, nausea, loss of consciousness, brain damage, and death.

Life expectancy

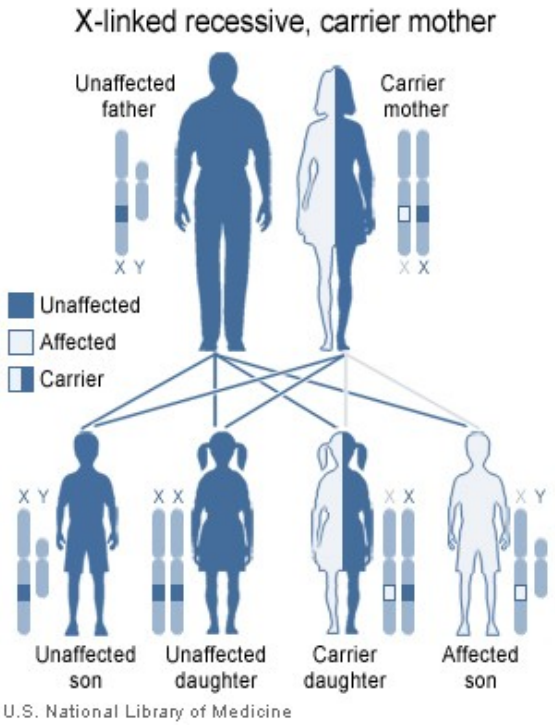
Like most aspects of the disorder, life expectancy varies with severity and adequate treatment. People with severe haemophilia who don't receive adequate, modern treatment have greatly shortened lifespans and often do not reach maturity. Prior to the 1960s when effective treatment became available, average life expectancy was only 11 years. By the 1980s the life span of the average haemophiliac receiving appropriate treatment was 50–60 years. Today with appropriate treatment, males with haemophilia typically have a near normal quality of life with an average lifespan approximately 10 years shorter than an unaffected male.

Since the 1980s the primary leading cause of death of people with severe haemophilia has shifted from haemorrhage to HIV/AIDS acquired through treatment with contaminated blood products. The second leading cause of death related to severe haemophilia complications is intracranial haemorrhage which today accounts for one third of all deaths of patients with haemophilia. Two other major causes of death include: hepatitis infections causing cirrhosis and, obstruction of air or blood flow due to soft tissue haemorrhage.

Causes

- Haemophilia A is a recessive X-linked genetic disorder involving a lack of functional clotting Factor VIII and represents 80% of haemophilia cases.
- Haemophilia B is a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. It comprises approximately 20% of haemophilia cases.
- Haemophilia C is an autosomal genetic disorder (i.e. *not* X-linked) involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive: heterozygous individuals also show increased bleeding.

Genetics



X-linked recessive inheritance

Females possess two X-chromosomes, males have one X and one Y chromosome. Since the mutations causing the disease are X-linked, a woman carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other chromosome should express itself to produce the necessary clotting factors, due to X inactivation. However, the Y-chromosome in men has no gene for factors VIII or IX. If the genes responsible for production of factor VIII or factor IX present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so the deficient gene is not masked and he will develop the illness.

Since a male receives his single X-chromosome from his mother, the son of a healthy female silently carrying the deficient gene will have a 50% chance of inheriting that gene

from her and with it the disease; and if his mother is affected with haemophilia, he will have a 100% chance of being a haemophiliac. In contrast, for a female to inherit the disease, she must receive two deficient X-chromosomes, one from her mother and the other from her father (who must therefore be a haemophiliac himself). Hence haemophilia is far more common among males than females. However, it is possible for female carriers to become mild haemophiliacs due to lyonisation (inactivation) of the X chromosomes. Haemophiliac daughters are more common than they once were, as improved treatments for the disease have allowed more haemophiliac males to survive to adulthood and become parents. Adult females may experience menorrhagia (heavy periods) due to the bleeding tendency. The pattern of inheritance is criss-cross type. This type of pattern is also seen in colour blindness.

A mother who is a carrier has a 50% chance of passing the faulty X chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters. A son cannot inherit the defective gene from his father.

Genetic testing and genetic counselling is recommended for families with haemophilia. Prenatal testing, such as amniocentesis, is available to pregnant women who may be carriers of the condition.

As with all genetic disorders, it is of course also possible for a human to acquire it spontaneously through mutation, rather than inheriting it, because of a new mutation in one of their parents' gametes. Spontaneous mutations account for about 33% of all cases of haemophilia A. About 30% of cases of haemophilia B are the result of a spontaneous gene mutation.

If a female gives birth to a haemophiliac child, either the female is a carrier for the disease or the haemophilia was the result of a spontaneous mutation. Until modern direct DNA testing, however, it was impossible to determine if a female with only healthy children was a carrier or not. Generally, the more healthy sons she bore, the higher the probability that she was not a carrier.

If a male is afflicted with the disease and has children with a female who is not even a carrier, his daughters will be carriers of haemophilia. His sons, however, will not be affected with the disease. The disease is X-linked and the father cannot pass haemophilia through the Y chromosome. Males with the disorder are then no more likely to pass on the gene to their children than carrier females, though all daughters they sire will be carriers and all sons they father will not have haemophilia (unless the mother is a carrier).

Severity

There are numerous different mutations which cause each type of haemophilia. Due to differences in changes to the genes involved, patients with haemophilia often have some level of active clotting factor. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1-5% active factor have moderate haemophilia,

and those with mild haemophilia have between 5-40% of normal levels of active clotting factor.

Diagnosis

Haemophilia A can be mimicked by von Willebrand disease.

- von Willebrand Disease could significantly affect as many as 1 in 10,000 people.
- von Willebrand Disease type 2A, where decreased levels of von Willebrand Factor can lead to premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 2A is inherited in an autosomal dominant fashion.
- von Willebrand Disease type 2N, where von Willebrand Factor cannot bind Factor VIII, autosomal recessive inheritance. (i.e.; both parents need to give the child a copy of the gene).
- von Willebrand Disease type 3, where lack of von Willebrand Factor causes premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 3 is inherited in an autosomal recessive fashion.

Additionally, severe cases of vitamin K deficiency can present similar symptoms to haemophilia. This is due to the fact that vitamin K is necessary for the human body to produce several protein clotting factors. This vitamin deficiency is rare in adults and older children but is common in newborns. Infants are born with naturally low levels of vitamin K and do not yet have the symbiotic gut flora to properly synthesise their own vitamin K. Bleeding issues due to vitamin K deficiency in infants is known as "haemorrhagic disease of the newborn", to avoid this complication newborns are routinely injected with vitamin K supplements.

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected

Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected
Bernard-Soulier syndrome	unaffected	unaffected	prolonged	decreased

Management

Commercially produced factor concentrates such as "Advate", a recombinant Factor VIII produced by Baxter International, come as a white powder in a vial which must be mixed with sterile water prior to intravenous injection.

Though there is no cure for haemophilia, it can be controlled with regular infusions of the deficient clotting factor, i.e. factor VIII in haemophilia A or factor IX in haemophilia B.

Factor replacement can be either isolated from human blood serum, recombinant, or a combination of the two. Some haemophiliacs develop antibodies (inhibitors) against the replacement factors given to them, so the amount of the factor has to be increased or non-human replacement products must be given, such as porcine factor VIII.

If a patient becomes refractory to replacement coagulation factor as a result of circulating inhibitors, this may be partially overcome with recombinant human factor VII (NovoSeven), which is registered for this indication in many countries.

In early 2008, the US Food and Drug Administration (FDA) approved Xyntha (Wyeth) anti-haemophilic factor, genetically engineered from the genes of Chinese hamster ovary cells. Since 1993 (Dr. Mary Nugent) recombinant factor products (which are typically cultured in Chinese hamster ovary (CHO) tissue culture cells and involve little, if any human plasma products) have been available and have been widely used in wealthier western countries. While recombinant clotting factor products offer higher purity and safety, they are, like concentrate, extremely expensive, and not generally available in the developing world. In many cases, factor products of any sort are difficult to obtain in developing countries.

In Western countries, common standards of care fall into one of two categories: prophylaxis or on-demand. Prophylaxis involves the infusion of clotting factor on a regular schedule in order to keep clotting levels sufficiently high to prevent spontaneous bleeding episodes. On-demand treatment involves treating bleeding episodes once they arise. In 2007, a clinical trial was published in the *New England Journal of Medicine* comparing on-demand treatment of boys (< 30 months) with haemophilia A with prophylactic treatment (infusions of 25 IU/kg body weight of Factor VIII every other day) in respect to its effect on the prevention of joint-diseases. When the boys reached 6 years of age, 93% of those in the prophylaxis group and 55% of those in the episodic-therapy group had a normal index joint-structure on MRI. Prophylactic treatment, however, resulted in average costs of $300,000 per year. The author of an editorial published in the same issue of the *NEJM* supports the idea that prophylactic treatment not only is more effective than on demand treatment but also suggests that starting after the first serious joint-related haemorrhage may be more cost effective than waiting until the fixed age to begin. This study resulted in the first (October 2008) FDA approval to label any Factor VIII product to be used prophylactically. As a result, the factor product used in the study (Bayer's Kognate) is now labelled for use to prevent bleeds, making it more likely that insurance carries in the US will reimburse consumers who are prescribed and use this product prophylactically. Despite Kognate only recently being "approved" for this use in the US, it and other factor products have been well studied and are often prescribed to treat Haemophilia prophylactically to prevent bleeds, especially joint bleeds.

Preventive exercises

It is recommended that people affected with haemophilia do specific exercises to strengthen the joints, particularly the elbows, knees, and ankles. Exercises include

elements which increase flexibility, tone, and strength of muscles, increasing their ability to protect joints from damaging bleeds. These exercises are recommended after an internal bleed occurs and on a daily basis to strengthen the muscles and joints to prevent new bleeding problems. Many recommended exercises include standard sports warm-up and training exercises such as stretching of the calves, ankle circles, elbow flexions, and quadriceps sets.

Alternative medicine

While not a replacement for traditional treatments, preliminary scientific studies indicate that hypnosis and self-hypnosis can be effective at reducing bleeds and the severity of bleeds and thus the frequency of factor treatment. Herbs which strengthen blood vessels and act as astringents may benefit patients with haemophilia, however there are no peer reviewed scientific studies to support these claims. Suggested herbs include: Bilberry (Vaccinium myrtillus), Grape seed extract (Vitis vinifera), Scotch broom (Cytisus scoparius), Stinging nettle (Urtica dioica), Witch hazel (Hamamelis virginiana), and yarrow (Achillea millefolium).

Contraindications

Anticoagulants such as Heparin and Warfarin are contraindicated for people with haemophilia as these can aggravate clotting difficulties. Also contraindicated are those drugs which have "blood thinning" side effects. For instance, medications which contain aspirin, ibuprofen, or naproxen sodium should not be taken because they are well known to have the side effect of prolonged bleeding.

Also contraindicated are activities with a high likelihood of trauma, such as motorcycling and skateboarding. Popular sports with very high rates of physical contact and injuries such as American football, hockey, boxing, wrestling, and rugby should be avoided by people with haemophilia. Other active sports like soccer, baseball, and basketball also have a high rate of injuries, but have overall less contact and should be undertaken cautiously and only in consultation with a doctor.

Epidemiology

Haemophilia is rare, with only about 1 instance in every 10,000 births (or 1 in 5,000 male births) for haemophilia A and 1 in 50,000 births for haemophilia B. About 18,000 people in the United States have haemophilia. Each year in the US, about 400 babies are born with the disorder. Haemophilia usually occurs in males and less often in females. It is estimated that about 2500 Canadians have haemophilia A, and about 500 Canadians have haemophilia B.

History

"About seventy or eighty years ago, a woman by name of Smith, settled in the vicinity of Plymouth, New Hampshire, and transmitted the following idiosyncrasy to her descendants. It is one, she

observed, to which her family is unfortunately subject, and had been the source not only of great solicitude, but frequently the cause of death. If the least scratch is made on the skin of some of them, as mortal a hemorrhagy will eventually ensue as if the largest wound is inflicted. (…) So assured are the members of this family of the terrible consequences of the least wound, that they will not suffer themselves to be bled on any consideration, having lost a relation by not being able to stop the discharge occasioned by this operation."

John C. Otto, 1803

Scientific discovery

The first written account of haemophilia occurred in the 2nd century in the Babylonian Talmud. In it Rabbi Judah haNasi, redactor of the Mishneh, wrote: "If she circumcised her first child and he died, and a second one also died, she must not circumcise her third child." This passage refers to both the prolonged bleeding caused by circumcision and to the maternal inheritance of the disease. The first medical professional to describe a disease was Albucasis. In the tenth century he described families whose males died of bleeding after only minor traumas. While many other such descriptive and practical references to the disease appear throughout historical writings, scientific analysis did not begin until the start of the nineteenth century.

In 1803, Dr. John Conrad Otto, a Philadelphian physician, wrote an account about "a hemorrhagic disposition existing in certain families" in which he called the affected males "bleeders." He recognised that the disorder was hereditary and that it affected mostly males and was passed down by healthy females. His paper was the second paper to describe important characteristics of an X-linked genetic disorder (the first paper being a description of colour blindness by John Dalton who studied his own family). Otto was able to trace the disease back to a woman who settled near Plymouth in 1720. The idea that affected males could pass the trait onto their unaffected daughters was not described until 1813 when John Hay published an account in The New England Journal of Medicine.

A Finnish Doctor in 1924 discovered a heredity bleeding disorder similar to Haemophilia localised in a group of islands (called the "Aland Islands") which are located to the southwest of Finland. This bleeding disorder is called "Von Willebrand Disease".

The term "haemophilia" is derived from the term "haemorrhaphilia" which was used in a description of the condition written by Friedrich Hopff in 1828, while he was a student at the University of Zurich. In 1937, Patek and Taylor, two doctors from Harvard, discovered anti-haemophilic globulin. In 1947, Pavlosky, a doctor from Buenos Aires, found haemophilia A and haemophilia B to be separate diseases by doing a lab test. This test was done by transferring the blood of one haemophiliac to another haemophiliac. The fact that this corrected the clotting problem showed that there was more than one form of haemophilia.

European royalty

Queen Victoria passed haemophilia on to some of her descendants.

Haemophilia has featured prominently in European royalty and thus is sometimes known as "the royal disease". Queen Victoria passed the mutation to her son Leopold and, through some of her daughters, to various royals across the continent, including the royal families of Spain, Germany, and Russia. In Russia, Tsarevich Alexei Nikolaevich, son of Nicholas II, was a descendant of Queen Victoria through his mother Empress Alexandra and suffered from haemophilia.

Ryan White was an American haemophiliac who became infected with HIV/AIDS through contaminated blood products.

It was claimed that Rasputin was successful at treating the Tsarevich's haemophilia. At the time, a common treatment administered by professional doctors was to use aspirin, which worsened rather than lessened the problem. It is believed that, by simply advising against the medical treatment, Rasputin could bring visible and significant improvement to the condition of Alexei.

In Spain, Queen Victoria's youngest daughter, Princess Beatrice, had a daughter Victoria Eugenie of Battenberg, who later became Queen of Spain. Two of her sons were haemophiliacs and both died from minor car accidents: Her eldest son, Prince Alfonso of Spain, Prince of Asturias, died at the age of 31 from internal bleeding after his car hit a telephone booth. Her youngest son, Infante Gonzalo, died at age 19 from abdominal bleeding following a minor car accident where he and his sister hit a wall while avoiding a cyclist. Neither appeared injured or sought immediate medical care and Gonzalo died two days later from internal bleeding.

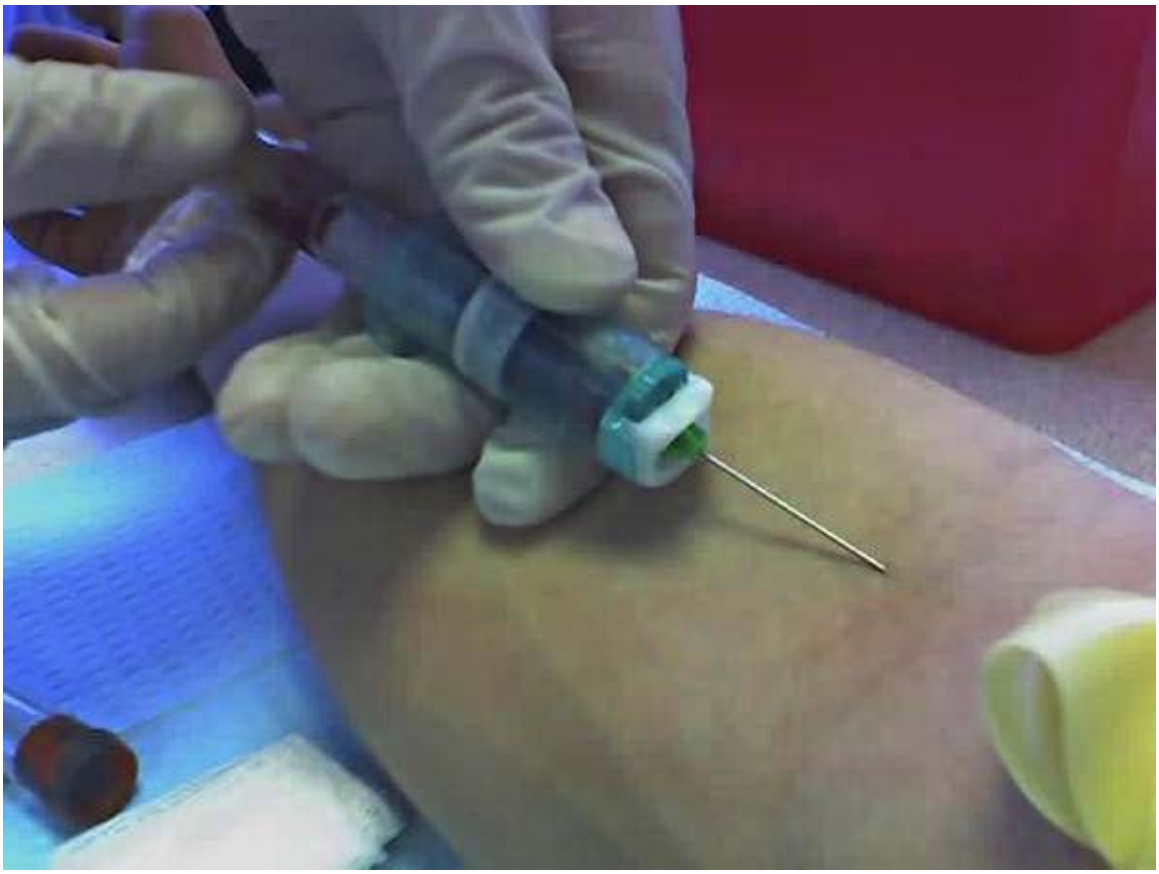
Blood contamination issues

Prior to 1985, there were no laws enacted within the U.S. to screen blood. As a result, many haemophilia patients who received untested and unscreened clotting factor prior to 1992 were at an extreme risk for contracting HIV and hepatitis C via these blood products. It is estimated that more than 50% of the haemophilia population, over 10,000 people, contracted HIV from the tainted blood supply in the United States alone.

As a direct result of the contamination of the blood supply in the late 1970s and early/mid 1980s with viruses such as hepatitis and HIV, new methods were developed in the production of clotting factor products. The initial response was to heat-treat (pasteurise) plasma-derived factor concentrate, followed by the development of monoclonal factor concentrates, which use a combination of heat treatment and affinity chromatography to inactivate any viral agents in the pooled plasma from which the factor concentrate is derived. The Lindsay Tribunal in Ireland investigated, among other things, the slow adoption of the new methods.

Chapter 10

Blood Test



A venipuncture performed using a vacutainer

A **blood test** is a laboratory analysis performed on a blood sample that is usually extracted from a vein in the arm using a needle, or via fingerprick.

Blood tests are used to determine physiological and biochemical states, such as disease, mineral content, drug effectiveness, and organ function. They are also used in drug tests. Although the term *blood test* is used, most routine tests (except for most haematology) are done on plasma or serum, instead of blood cells.

Extraction

Venipuncture is useful as it is a relatively non-invasive way to obtain cells and extracellular fluid (plasma) from the body for analysis. Since blood flows throughout the body, acting as a medium for providing oxygen and nutrients, and drawing waste products back to the excretory systems for disposal, the state of the bloodstream affects, or is affected by, many medical conditions. For these reasons, blood tests are the most commonly performed medical tests.

Phlebotomists, laboratory technicians and nurses are those charged with patient blood extraction. However, in special circumstances, and emergency situations, paramedics and physicians sometimes extract blood. Also, respiratory therapists are trained to extract arterial blood for arterial blood gases.

Types of blood tests

Biochemical analysis

A basic metabolic panel measures sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), magnesium, creatinine, and glucose. It also sometimes includes calcium.

Some blood tests, such as those that measure glucose, cholesterol, or for determining the existence or lack of STD, require fasting (or no food consumption) eight to twelve hours prior to the drawing of the blood sample.

For the majority of blood tests, blood is usually obtained from the patient's vein. However, other specialized blood tests, such as the Arterial blood gas, require blood extracted from an artery. Blood gas analysis of arterial blood is primarily used to monitor carbon dioxide and oxygen levels related to pulmonary function, but it is also used to measure blood pH and bicarbonate levels for certain metabolic conditions.

While the regular glucose test is taken at a certain point in time, the glucose tolerance test involves repeated testing to determine the rate at which glucose is processed by the body.

T-cells and white blood cells are often counted.

Normal ranges

Test	Low	High	Unit	Comments
Sodium (Na)	136	145	mmol/L	
Potassium (K)	3.5	5.5	mmol/L	
Urea	2.5	6.4	mmol/L	BUN - blood urea nitrogen
Urea	15	40	mg/dL	
Creatinine - male	62	115	μmol/L	

Creatinine - female	53	97	μmol/L
Creatinine - male	0.7	1.3	mg/dL
Creatinine - female	0.6	1.1	mg/dL
Glucose (fasting)	3.9	5.8	mmol/L
Glucose (fasting)	70	105	mg/dL

Molecular profiles

- Protein electrophoresis (general technique -- not a specific test)
- Western blot (general technique -- not a specific test)
- Liver function tests
- Polymerase chain reaction (DNA). DNA testing is today possible with even very small quantities of blood: this is commonly used in forensic science, but is now also part of the diagnostic process of many disorders.
- Northern blot (RNA)
- Sexually transmitted diseases

Cellular evaluation

- Full blood count (or "complete blood count")
- Hematocrit and MCV ("mean corpuscular volume")
- Erythrocyte sedimentation rate (ESR)
- Cross-matching. Determination of blood type for blood transfusion or transplants
- Blood cultures are commonly taken if infection is suspected. Positive cultures and resulting sensitivity results are often useful in guiding medical treatment.

Future alternatives

Saliva tests

In 2008, scientists announced that the more cost effective saliva tests could eventually replace some blood tests, as saliva contains 20% of the proteins found in blood.

Micro emulsion

February 2011: Canadian researchers have developed a microchip for blood tests. It is called micro-emulsion, a droplet of blood captured inside a layer of another substance. It can control the exact size and spacing of the droplets. The new test could improve the efficiency, accuracy and speed of laboratory tests while also doing it cheaply. The microchip costs $25, whereas the robotic dispensers currently in use cost around $10,000.

Chapter 11

Anticoagulant

An **anticoagulant** is a substance that prevents coagulation; that is, it stops blood from clotting. A group of pharmaceuticals called anticoagulants can be used *in vivo* as a medication for thrombotic disorders. Some chemical compounds are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment.

As medications

Anticoagulants reduce blood clotting. This prevents deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke.

Coumadins (Vitamin K antagonists)

These oral anticoagulants are a class of pharmaceuticals that antagonize the effects of vitamin K. Examples include warfarin. It takes at least 48 to 72 hours for the anticoagulant effect to develop. Where an immediate effect is required, heparin must be given concomitantly. These anticoagulants are used to treat patients with deep-vein thrombosis (DVT), pulmonary embolism (PE), atrial fibrillation (AF), and mechanical prosthetic heart valves.

Adverse effects

Patients aged 80 years or more may be especially susceptible to bleeding complications with a rate of 13 bleeds per 100 person-years.

These oral anticoagulants are used widely as poisons for mammalian pests, especially rodents.

Depletion of vitamin K by coumarin therapy increases risk of arterial calcification and heart valve calcification, especially if too much vitamin D is present.

Available agents

- Warfarin (Coumadin) *This is the main agent used in the U.S. and UK*
- Acenocoumarol and phenprocoumon *This is used more commonly outside the U.S. and the UK*

- Brodifacoum *Rat poison, not used medically*
- Phenindione

Heparin and derivative substances

Heparin is a biological substance, usually made from pig intestines. It works by activating antithrombin III, which blocks thrombin from clotting blood. Heparin can be used *in vivo* (by injection), and also *in vitro* to prevent blood or plasma clotting in or on medical devices. Vacutainer brand test tubes containing heparin are usually colored green.

Low molecular weight heparin

Low molecular weight heparin is a more highly processed product that is useful as it does not require monitoring of the APTT coagulation parameter (it has more predictable plasma levels) and has fewer side effects.

Synthetic pentasaccharide inhibitors of factor Xa

- Fondaparinux is a synthetic sugar composed of the five sugars (pentasaccharide) in heparin that bind to antithrombin. It is a smaller molecule than low molecular weight heparin.
- Idraparinux

Major pharmaceutical Heparin recall due to contamination

In March 2008 major recalls of Heparin were announced by pharmaceuticals due to a suspected and unknown contamination of the raw Heparin stock imported from China . The contaminant was later found to be a non-naturally occurring compound called oversulfated chondroitin sulfate . The U.S. Food and Drug Administration was quoted as stating that at least 19 deaths were believed linked to a raw Heparin ingredient imported from the People's Republic of China, and that they had also received 785 reports of serious injuries associated with the drug's use. According to the New York Times: 'Problems with heparin reported to the agency include difficulty breathing, nausea, vomiting, excessive sweating and rapidly falling blood pressure that in some cases led to life-threatening shock'.

Direct thrombin inhibitors

Another type of anticoagulant is the direct thrombin inhibitor. Current members of this class include argatroban, lepirudin, bivalirudin, and dabigatran. An oral direct thrombin inhibitor, ximelagatran (Exanta) was denied approval by the Food and Drug Administration (FDA) in September 2004 and was pulled from the market entirely in February 2006 after reports of severe liver damage and heart attacks.

Other types of anticoagulants

Many other anticoagulants exist, for use in Research & Development, and more or less uses as drug candidates or diagnostics

- **Batroxobin**, a toxin from a snake venom that clots platelet-rich plasma without affecting platelets functions (lyses fibrinogen).
- **Hementin** is an anticoagulant protease from the salivary glands of Haementeria ghilianii

Food supplements

Food supplements with blood thinning effect include Nattokinase and Lumbrokinase

General indications

Therapeutic uses of anticoagulants include atrial fibrillation, pulmonary embolism (PE), deep vein thrombosis (DVT), or venous thromboembolism (VTE), congestive heart failure, stroke, myocardial infarction, genetic or acquired hypercoagulability

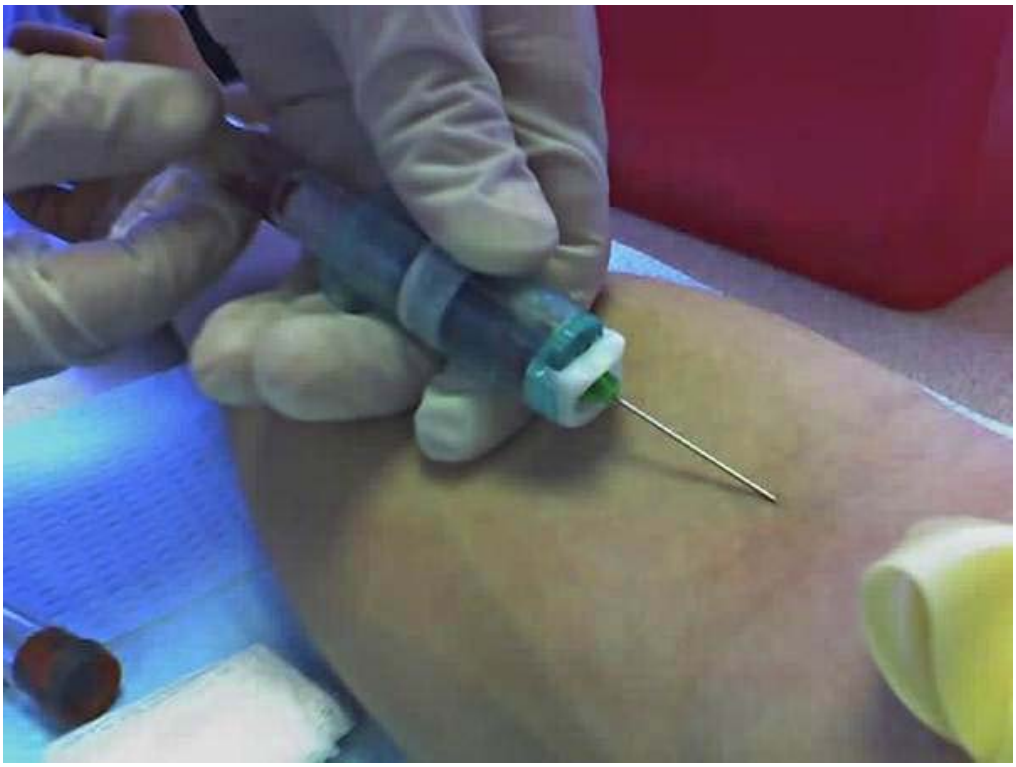
Anticoagulants outside the body

Laboratory instruments, test tubes, blood transfusion bags, and medical and surgical equipment will get clogged up and become nonoperational if blood is allowed to clot. Chemicals can be added to stop blood clotting. Apart from heparin, most of these chemicals work by binding calcium ions, preventing the coagulation proteins from using them.

- EDTA is denoted by mauve or purple caps on Vacutainer brand test tubes. This chemical strongly and irreversibly binds calcium. It is in a powdered form.
- Citrate is usually in blue Vacutainer tube. It is in liquid form in the tube and is used for coagulation tests, as well as in blood transfusion bags. It gets rid of the calcium, but not as strongly as EDTA. Correct proportion of this anticoagulant to blood is crucial because of the dilution. It can be in the form of sodium citrate or ACD.
- Oxalate has a mechanism similar to that of citrate. It is the anticoagulant used in fluoride (grey top) tubes.

Chapter 12

Venipuncture



Venipuncture using the BD Vacutainer system. This picture shows a multiple-use BD Vacutainer device that is no longer used. It has been replaced by a single use BD Vacutainer device.

In medicine, **venepuncture**,**venopuncture** or **venipuncture** is the process of obtaining intravenous access for the purpose of intravenous therapy or obtaining a sample of venous blood. This procedure is performed by medical laboratory scientists, medical practitioners, some EMTs, paramedics, phlebotomists and other nursing staff. Venepuncture is one of the most routinely performed invasive procedures and is carried out for two reasons, to obtain blood for diagnostic purposes or to monitor levels of blood components (Lavery & Ingram 2005). Blood analysis is one of the most important diagnostic tools available to clinicians within healthcare. Its data is relied upon in the clinical setting for interpretation of a myriad of clinical signs and symptoms and developing skills in venepuncture can facilitate holistic and timely treatment.

Blood is most commonly obtained from the median cubital vein, which lies within the cubital fossa anterior to the elbow. This vein lies close to the surface of the skin, and there is not a large nerve supply.

Minute quantities of blood may be taken by fingersticks sampling and collected from infants by means of a heel stick or from scalp veins with a winged infusion needle.

Phlebotomy (incision into a vein) is also the treatment of certain diseases such as hemochromatosis and primary and secondary polycythemia.

Equipment

There are many ways in which blood can be drawn from a vein. The best method varies with the age of the patient, equipment available and tests required.

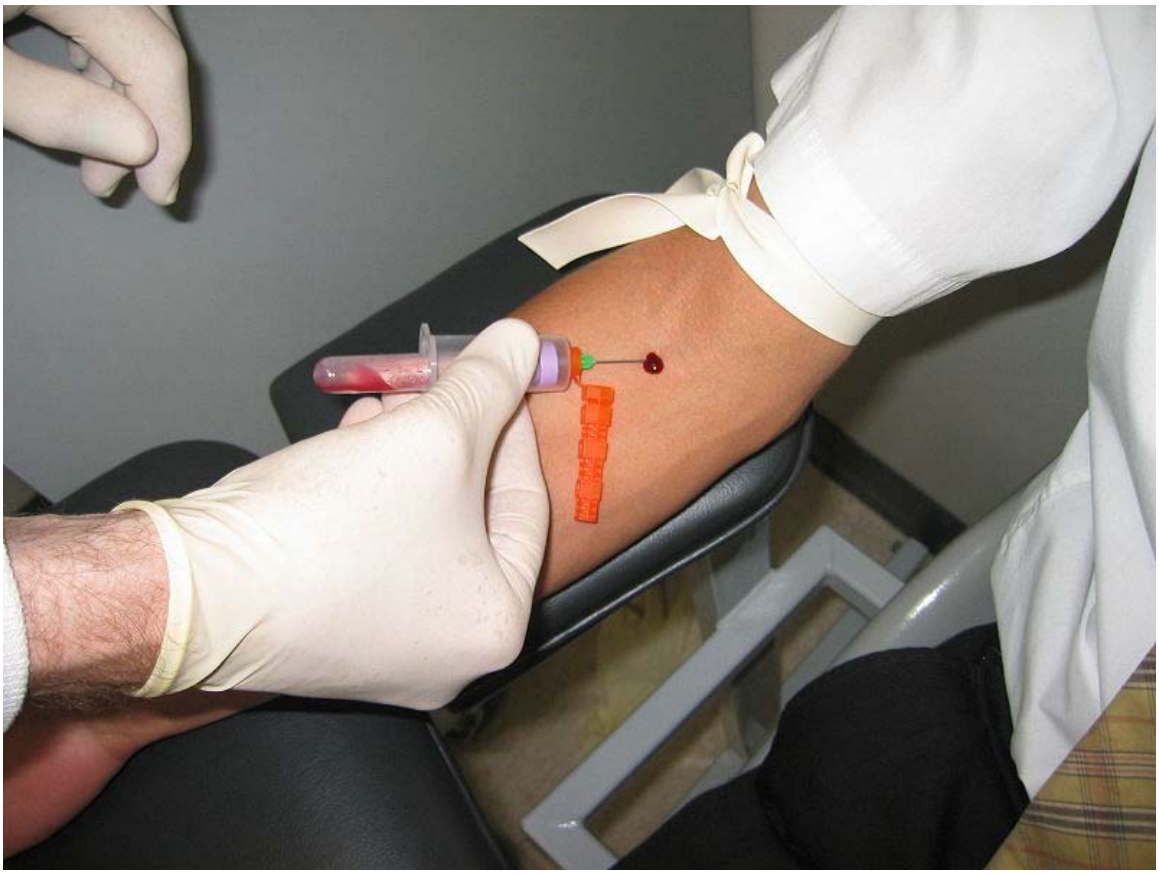
Most blood collection in the US and UK is done with an evacuated tube system, (two common systems are Vacutainer (Becton, Dickinson and company) and Vacuette (Greiner Bio-One GmBH). The equipment consisting of a plastic hub, a hypodermic needle, and a vacuum tube. Under certain circumstances, a syringe may be used, often with a butterfly needle, which is a plastic catheter attached to a short needle. In the developing world, a needle and syringe are still the most common method of drawing blood.

Additives and order of draw

The tubes in which blood is transported back to the laboratory contain a variety of additives or none at all. It is important to know which tube the individual laboratory requires for which test as reagents vary between laboratories and may be affected by different additives. In general whole blood needs to be mixed with EDTA which chelates calcium to prevent it clotting, unless the clotting time is the test to be measured in which citrates are used. The majority of biochemistry tests are performed on serum and so either a plain tube or a clotting accelerator is used. This clotting accelerator can interfere with some assays and so a plain tube is recommended in these cases but will obviously delay the result. Some assays may also require whole blood but are interfered with by EDTA and in this case Lithium Heparin is an alternative.

With the vacuum tube system, the needle pierces the top of the sample tube and will potentially come into contact with the additives in the tube. As it is a hollow needle some of this can be carried into the next tube and contaminate it. The most likely additive to cause trouble is EDTA which will affect the coagulation time assays and by chelating some of the metal ions may interfere with some of the biochemistry results(especially potassium). Thus EDTA samples should be drawn last in most cases and plain tubes drawn first.

Venipuncture with evacuated or vacuum tubes



Blood draw filling a purple top vacuum tube

Vacuum tubes were first marketed by U.S. company BD (Becton, Dickinson and Company) under the trade name Vacutainer tubes. Today, many companies sell vacuum tubes as the patent for this device is in the public domain. Some models are a type of test tube that contains a vacuum that automatically aspirates blood into itself. The tubes are made of glass or plastic. The tubes are attached to a needle and hub.

Multiple vacuum tubes can be attached to and removed in turn from a single needle, allowing multiple samples to be obtained from a single procedure. This is possible due to a Japanese invention called the multiple sample sleeve, which is basically a plastic cap fitting over the posterior end of the needle cannula, thus keeping blood from draining onto both health care worker and patient.

BD used this invention to create a system that included the vacuum pressurized sleeve, and a cannula with sleeve attached to a holder that holds the needle/sleeve combination, and guides the negatively pressured collection vial that is inserted once the needle is in the patient. Unfortunately, the sleeve that stops blood from moving until its seal is perforated by the collection tube's insertion, also prevents flashback, the tell-tale sign the vein has been entered properly. If the phlebotomist chooses to use a butterfly needle, the "flash" is still present, and easily visible to the phlebotomy tech as well as the patient.

They are commonly used in US, UK, and Australian hospitals, private doctors' offices and community labs, and are available in various sizes to suit the age of the patient and the type of sample needed.

There are several sized needles for a phlebotomist to choose from. The most commonly used are as follows: a 21g (green top) needle, a 22g (black top) needle, a 21g (green label) butterfly needle, a 23g (blue label) butterfly needle, and a 25g (orange label) butterfly needle (however this needle is only used in pediatrics or extreme cases as it is so small that it can often result in hemolizing the blood sample, thereby invalidating the test). Most lay people are under the impression that using a butterfly needle is easier on their veins and less painful. Using a finer needle is less painful. However, the hazard of using butterfly needles lies in the fact that as the blood flows through the rubber tubing, it cools significantly, thereby clotting faster. In clotting in the tube, it stops up and reduces or as in most cases, completely stops. When this happens, it is necessary to re-stick a patient in order to obtain the blood sample. This is not the case when using the more common needles. Since the tubing is not a part of the needle, the blood goes through the needle directly into the tube. This method results in a faster, cleaner sample with less chance of hemolization. (When a blood sample is hemolyzed, it means the red cells have ruptured to the extent that they impart a pink/red color to the blood plasma, which is normally pale yellow.)

Venipuncture in children

Use of Lidocaine Iontophoresis is an effective method for reducing pain and alleviating distress during venipuncture in pediatric patients. Rapid dermal anesthesia can be achieved by local anesthetic infiltration, but it may evoke anxiety in children frightened by needles or distort the skin, making vascular access more difficult and increasing the risk of needle exposure to health care workers. Dermal anesthesia can also be achieved without needles by the topical application of local anesthetics (e.g., EMLA®, ASTRA Pharmaceutical, Sodertalje, Sweden) or by lidocaine iontophoresis. By contrast, noninvasive dermal anesthesia can be established in 5–15 min without distorting underlying tissues by lidocaine iontophoresis, where a direct electrical current facilitates dermal penetration of positively charged lidocaine molecules when placed under the positive electrode.

One study did conclude that the iontophoretic administration of lidocaine was safe and effective in providing dermal anesthesia for venipuncture in children 6–17 years old. This technique may not be applicable to all children. Future studies may provide information on the minimum effective iontophoretic dose for dermal anesthesia in children and the comparison of the anesthetic efficacy and satisfaction of lidocaine iontophoresis with topical anesthetic creams and subcutaneous infiltration.

Venipuncture with needle and syringe

Some health care workers prefer to use a syringe-needle technique for Venipuncture. Sarstedt manufactures a blood-drawing system S-Monovette that uses this principal. This

method can be preferred on elderly patients, oncology patients, severely burned patients, obese patients or patients with unreliable or fragile veins. Because syringes are manually operated, the amount of suction applied may be easily controlled. This is particularly helpful with patients that have small veins that collapse under the suction of an evacuated tube. In children or other circumstances where the quantity of blood gained may be limited it can be helpful to know how much blood can be obtained before distributing it amongst the various additives that the laboratory will require.

Blood cultures

There are times where a patient may require a blood culture collection. The culture will determine if the patient has pathogens in the blood. Normally blood is sterile. When drawing blood from cultures using a sterile solution such as Betadine rather than alcohol.

Using sterile gloves, do not wipe away the surgical solution, touch the puncture site, or in any way compromise the sterile process. It is vital that the procedure is performed in as sterile a manner as possible as the persistent presence of skin commensals in blood cultures could indicate endocarditis but they are most often found as contaminants. It is encouraged to use an abrasive method of skin preparation. This removes the upper layers of dead skin cells along with their contaminating bacteria. Povidone-iodine has traditionally been used but in the UK a 2% chlorhexidine in 70% ethanol or isopropyl alcohol solution is preferred and time must be allowed for it to dry. The tops of any containers used when drawing a blood culture should also be disinfected using a similar solution. Some labs will actively discourage iodine use where iodine is thought to degrade the rubber stopper through which blood enters the bottle, thus allowing contaminates to enter the container.

The blood is collected into special transport bottles, which are like vacuum tubs but shaped differently. The blood culture bottle contains transport media to preserve any microorganisms present while they are being transported to the laboratory for cultures. Because it is unknown whether the pathogens are anaerobic (living without oxygen) or aerobic (living with oxygen), blood is collected to test for both. The aerobic bottle is filled first, and then the anaerobic bottle is filled. However if the collection is performed using a syringe, the anaerobic bottle is filled first. If a butterfly collection kit is used, the aerobic bottle is filled first, so that any air in the tubing is released into the oxygen-containing bottle.

Specially designed blood culture collection bottles eliminate the need for either the syringe or butterfly collection method. These specially designed bottles have long necks that fit into the evacuated tubes holders that are use for regular Venipuncture collection. These bottles also allow for collection of other blood specimens via evacuated tubes, to be collected without additional Venipuncture.

The amount of blood that is collected is critical for the optimal recovery of microorganisms. Up to 10mL of blood is typical, but can vary according to the recommends of the manufacturer of the collection bottle. Collection from infants and

children are 1 to 5 mL. If too little blood is collected, the ratio of blood –to-nutrient broth will inhibit the growth of microorganisms. If too much blood is collected from the patient, the patient risks a hospital-induced anemia and the ratio of blood-to-nutrient broth will tilt in the opposite direction, which also is not conductive to optimal growth.

The bottles are then incubated in specialized units for 24 hours before a lab technician studies and/or tests it. This step allows the very small numbers of bacteria (potentially 1 or 2 organisms) to multiply to a level which is sufficient for identification +/-antibiotic resistance testing. Modern blood culture bottles have an indicator in the base which changes color in the presence of bacterial growth and can be read automatically by machine. (For this reason the barcoded stickers found on these bottles should not be removed as they are used by the laboratorys automated systems.)

Taking blood samples from animals

Blood samples from living laboratory animals may be collected using following methods:

A) *Blood collection not requiring anesthesia*: Saphenous vein (rat, mice, guinea pig); Dorsal pedal vein (rat, mice).

B) *Blood collection requiring anesthesia (local/general anesthesia)*: Tail vein (rat, mice); Tail snip (mice); Orbital sinus (rat, mice); Jugular vein (rat, mice); Temporary cannula (rat, mice); Blood vessel cannulation (guinea pig, ferret); Tarsal vein (guinea pig); Marginal ear vein/artery (rabbit).

C) *Terminal procedure*: Cardiac puncture (rat, mice, guinea pig, rabbit, ferret); Orbital sinus (rat, mice); Posterior vena cava (rat, mice).

The volume of the blood sample collection is very important in experimental animals. All nonterminal blood collection without replacement of fluids is limited up to 10% of total circulating blood volume in healthy, normal, adult animals on a single occasion and collection may be repeated after 3 to 4 weeks. In case repeated blood samples are required at short intervals, a maximum of 0.6 ml/kg/day or 1.0% of an animal's total blood volume can be removed every 24 hour.The estimated blood volume in adult animals is 55 to 70 ml/kg body weight. Care should be taken for older and obese animals. If blood collection volume exceeds more than 10% of total blood volume, fluid replacement may be required. Lactated Ringer's solution (LRS) is recommended as the best fluid replacement by National Institutes of Health (NIH). If the volume of blood collection exceeds more than 30% of the total circulatory blood volume, adequate care should be taken so that the animal does not suffer from hypovolemia.

Blood alcohol tests

It is generally not advisable to use isopropyl alcohol to cleanse the venipuncture site when obtaining a specimen for a blood alcohol test. Using soap and hot water or a povidone iodine swab are advisable alternatives to isopropyl alcohol in this case.

Chapter 13

Dermatology

Dermatologist	
Occupation	
Names	Doctor, Medical Specialist
Type	Specialty
Activity sectors	Medicine
Description	
Education required	Doctor of Medicine
Fields of employment	Hospitals, Clinics

Dermatology is the branch of medicine dealing with the skin and its diseases, a unique specialty with both medical and surgical aspects. A dermatologist takes care of diseases, in the widest sense, and some cosmetic problems of the skin, scalp, hair, and nails.

Etymology

Coined in English 1819, the word *dermatology* originated in the form of the words *dermologie* (in French, 1764) and, a little later, *dermatologia* (in Latin, 1777). The term derives from the Greek "δέρματος" (*dermatos*), genitive of "δέρμα" (*derma*), "skin" (from "δέρω" – *dero*, "to flay") + "-logy, "the study of", a suffix derived from "λόγος" (*logos*), amongst others meaning "speech, oration, discourse, quote, study, calculation, reason", in turn from "λέγω" – *lego*, "to say", "to speak".

History

Readily visible alterations of the skin surface have been recognized since the dawn of history, with some being treated, and some not. In 1801 the first great school of dermatology became a reality at the famous Hôpital Saint-Louis in Paris, while the first textbooks (Willan's, 1798–1808) and atlases (Alibert's, 1806–1814) appeared in print during the same period of time. In 1952, Dermatology was greatly advanced by Dr. Norman Orentreich's pioneering work in hair transplantation.

Training

After earning a medical degree (M.D. or D.O.), the length of training for a general dermatologist in the United States is a total of four years. This training consists of an initial medical or surgical intern year followed by a three-year dermatology residency. Following this training, one- or two- year post-residency fellowships are available in immunodermatology, phototherapy, laser medicine, Mohs micrographic surgery, cosmetic surgery or dermatopathology. For the past several years, dermatology residency positions in the United States have been the most competitive to obtain.

Subspecialties

Cosmetic dermatology

Dermatologists have been leaders in the field of cosmetic surgery. Some dermatologists complete fellowships in surgical dermatology. Many are trained in their residency on the use of botox, fillers, and laser surgery. Some dermatologists perform cosmetic procedures including liposuction, blepharoplasty, and face lifts. Most dermatologists limit their cosmetic practice to minimally invasive procedures. Despite an absence of formal guidelines from the American Board of Dermatology, many cosmetic fellowships are offered in both surgery and laser medicine.

Dermatopathology

A dermatolopathologist is a pathologist or dermatologist who specializes in the pathology of the skin. This field is shared by dermatologists and pathologists. Usually a dermatologist or pathologist will complete one year of dermatopathology fellowship. This usually includes six months of general pathology, and six months of dermatopathology. Alumni of both specialties can qualify as dermatopathologists. At the completion of a standard residency in dermatology, many dermatologists are also competent at dermatopathology. Some dermatopathologists qualify to sit for their examinations by completing a residency in dermatology and one in pathology.

Immunodermatology

This field specializes in the treatment of immune-mediated skin diseases such as lupus, bullous pemphigoid, pemphigus vulgaris, and other immune-mediated skin disorders. Specialists in this field often run their own immunopathology labs.

Mohs surgery

The dermatologic subspecialty called Mohs surgery focuses on the excision of skin cancers using a tissue-sparing technique that allows intraoperative assessment of 100% of the peripheral and deep tumor margins developed in the 1930s by Dr. Frederic E. Mohs. The procedure is defined as a type of CCPDMA processing. Physicians trained in this technique must be comfortable with both pathology and surgery, and dermatologists

receive extensive training in both during their residency. Physicians who perform Mohs surgery can receive training in this specialized technique during their dermatology residency, but many will seek additional training either through preceptorships to join the American Society for Mohs Surgery or through formal one- to two-year Mohs surgery fellowship training programs administered by the American College of Mohs Surgery.

Pediatric dermatology

Physicians can qualify for this specialization by completing both a pediatric residency and a dermatology residency. Or they might elect to complete a post-residency fellowship. This field encompasses the complex diseases of the neonates, hereditary skin diseases or genodermatoses, and the many difficulties of working with the pediatric population.

Teledermatology

Teledermatology is a form of dermatology where telecommunication technologies are used to exchange medical information via all kinds of media (audio, visual and also data communication, but typically photos of dermatologic conditions) usually made by non-dermatologists for evaluation off-site by dermatologists). This subspecialty deals with options to view skin conditions over a large distance to provide knowledge exchange, to establish second-opinion services for experts or to use this for follow-up of individuals with chronic skin conditions.

Therapies

Therapies provided by dermatologists include, but not restricted to:

- Cosmetic filler injections
- Hair removal with laser or other modalities
- Hair transplantation – a cosmetic procedure practiced by many dermatologists.
- Intralesional treatment – with steroid or chemotherapy.
- Laser therapy – for both the management of birth marks, skin disorders (like vitiligo), tattoo removal, and cosmetic resurfacing and rejuvenation.
- Photodynamic therapy – for the treatment of skin cancer and precancerous growths.
- Phototherapy – including the use of narrowband UVB, broadband UVB, psoralen and UVB.
- Tattoo removal with laser
- Tumescent liposuction – liposuction was invented by a gynecologist. A dermatologist (Dr. Jeffrey A. Klein) adapted the procedure to local infusion of dilute anesthetic called tumescent liposuction. This method is now widely practiced by dermatologists, plastic surgeons and gynecologists.
- Cryosurgery – for the treatment of warts, skin cancers, and other dermatosis.
- Radiation therapy – although rarely practiced by dermatologists, many dermatologist continue to provide radiation therapy in their office.

- Vitiligo surgery – Including procedures like autologous melanocyte transplant, suction blister grafting and punch grafting.
- Allergy testing – 'Patch testing' for contact dermatitis.
- Systemic therapies – including antibiotics, immunomodulators, and novel injectable products.
- Topical therapies – dermatologists have the best understanding of the numerous products and compounds used topically in medicine.

Most dermatologic pharmacology can be categorized based on the Anatomical Therapeutic Chemical Classification System, specifically the ATC code D.

Chapter 14

Teledermatology

Teledermatology is a subspecialty in the medical field of dermatology and probably one of the most common applications of telemedicine and e-health. In teledermatology, telecommunication technologies are used to exchange medical information (concerning skin conditions and tumours of the skin) over a distance using audio, visual and data communication. Applications comprise health care management such as diagnoses, consultation and treatment as well as (continuous) education.

The dermatologists Perednia and Brown were the first to coin the term “teledermatology” in 1995. In a scientific publication, they described the value of a teledermatologic service in a rural area underserved by dermatologists.

Modes of data transmission

Teledermatology (as telemedicine) is practised on the basis of two concepts: **Store and forward (SAF)** and **real time/live interactive teledermatology**. Hybrid modes also exist (combining SAF and real time applications).

The SAF method is most commonly used in teledermatology: It involves sending (forwarding) digital images associated with (anonymous) medical information to the data storage unit of a consulted specialist. It can be as easy as sending an email with a digital image of a lesion to seek advice for a skin condition. Advantages of this method are that it does not demand the presence of both parties at the same time and does not usually require expensive equipment.

In real-time/ live interactive teledermatology applications, provider and individuals usually interact via live videoconferencing. It may also involve remote surgery and the use of telerobotic microscopes in dermatopathology. This mode generally requires more sophisticated and costly technology than used in the SAF mode. Both participants must be available at the same time.

Areas of application

Health care management

Direct consultation involves an individual with a skin condition contacting a dermatologist via telecommunication to request diagnosis and treatment. In this field, mobile applications of teledermatology gain importance.

Telediagnosis in the absence of personal contact with health care workers to the individual is complex. It requires active participation of the individual and without appropriate guidance may lead to improper management. However, as a triage tool, leading the individual directly to the appropriate specialist for his/her disease, it could be very valuable in the near future.

Specialist referral is a major area of application in teledermatology A general practitioner (or other medical professional) that sees the individual consults a specialist/ specialised centre via telecommunication in order to get a second opinion. The specialist then helps the GP in rendering a diagnosis, providing management options et cetera.

Home telehealth/telehomecare involves an individual with a chronic condition being examined and managed remotely at home. An important field of interest of telehomecare in dermatology is the follow-up treatment of individuals with skin conditions requiring regular follow-up such as crural ulcers. Crural ulcers are a common skin condition that needs follow up visits up to twice a week demanding significant time commitments by the individuals in addition to causing a financial burden on the health care system. Teledermatology can help to reduce the time and costs involved in the follow up of such conditions.

Education and information

Medical education/continuous education are a major advantage of telemedicine/e-health. Numerous universities offer online courses, computer based training and Web applications in this field principally aimed at medical students. Specialist training courses via internet are also available, particularly in dermoscopy.

General medical/health information may be accessed by non-professionals, such as individuals affected by a skin condition, and their relatives, through the internet. They are also able to join peer support groups with others affected by the same condition.

Domains with special interest

Teledermoscopy

In teledermoscopy, digital dermoscopic lesion images (with or without clinical images) are transmitted electronically to a specialist for examination.

Dermoscopy (dermatoscopy, epiluminescence microscopy) is the technical field of using an epiluminescence microscope for viewing skin lesions in magnification in-vivo. It is particularly useful in the early detection of malignant skin lesions (i.e., melanoma). Digital dermoscopic images can be taken with a digital camera attached to a dermatoscope or special video cameras suited for dermoscopy, e.g. the FotoFinder. Since dermoscopy is based on examination of a two-dimensional image it is very well suited for digital imaging and teledermatology.

Teledermatopathology

Teledermatopathology is the transmission of dermatopathologic images either in real-time with the aid of a robotic microscope or using a store-and-forward system (transmission as a single file). In the latter method (SAF) a rather new development is the introduction of virtual slide systems (VSS).

Virtual slides are made by digitally scanning an entire glass slide at a high resolution and then sending the images to a storage system. These can be then assessed on a computer screen similar to conventional microscopy, allowing the pathologist to maneuver around the image and view every part of the slide at any magnification.

Teledermoscopically-aided dermatopathology

This is the transmission of crucial medical data and dermoscopic as well as clinical images to a pathologist who renders the conventional histopathologic diagnosis.

In the everyday clinical setting, skin biopsies are taken by the physician directly responsible for the individual and are assessed by a dermatopathologist. This pathologist has most likely never seen the clinical aspect of the lesion and might not have any information about the person. These limitations can be overcome by teledermoscopically-aided dermatopathology whereby a patient history and clinical data may increase the sensitivity of diagnosis.

Additionally it has been shown that provision of such data may improve the level of diagnostic confidence held by the assessing dermatopathologists.

Mobile Teledermatology

Mobile telemedicine is a system in which at least one participant (the person seeking advice or the doctor, for instance) uses wireless or mobile equipment (i.e. mobile phones, handheld devices), in contrast to conventional stationary telemedicine platforms. Travellers who develop skin lesions as well as doctors who are on the move in hospital/non-hospital area can benefit from this new development in teledermatology. In order to facilitate access to medical advice and enable individuals to play a more active role in managing their own health status, mobile teledermatology seems to be especially suited for patient filtering or triage. (i.e. referral based on the severity and character of

their skin condition). Another possible practical application is for follow-up of individuals with chronic skin conditions mentioned above.

Suitability of cases for Teledermatology

Not all cases are suitable for teledermatology.The type of cases suited for teledermatology is a topic, which requires more studies. Some studies have observed that eczema and follicular lesions were diagnosed with relatively more certainty,while in some other studies it was seen that diagnoses were made with more certainty in cases like viral warts, herpes zoster, acne vulgaris, irritant dermatitis, vitiligo, and superficial bacterial and fungal infections. Unlike in western studies where pigmented lesions suspicious of melanomas are one of the most referred cases for teledermatology (with or without teledermatoscopy), Asian studies have fewer cases referred based on the suspicion of melanoma.

Chapter 15

Cutaneous Conditions

There are many conditions of or affecting the human integumentary system—the organ system that comprises the entire surface of the body and includes skin, hair, nails, and related muscle and glands.

History

In 1572, Geronimo Mercuriali of Forlì, Italy, completed *De morbis cutaneis* (translated "On the diseases of the skin"). It is considered the first scientific work dedicated to dermatology.

Epidemiology

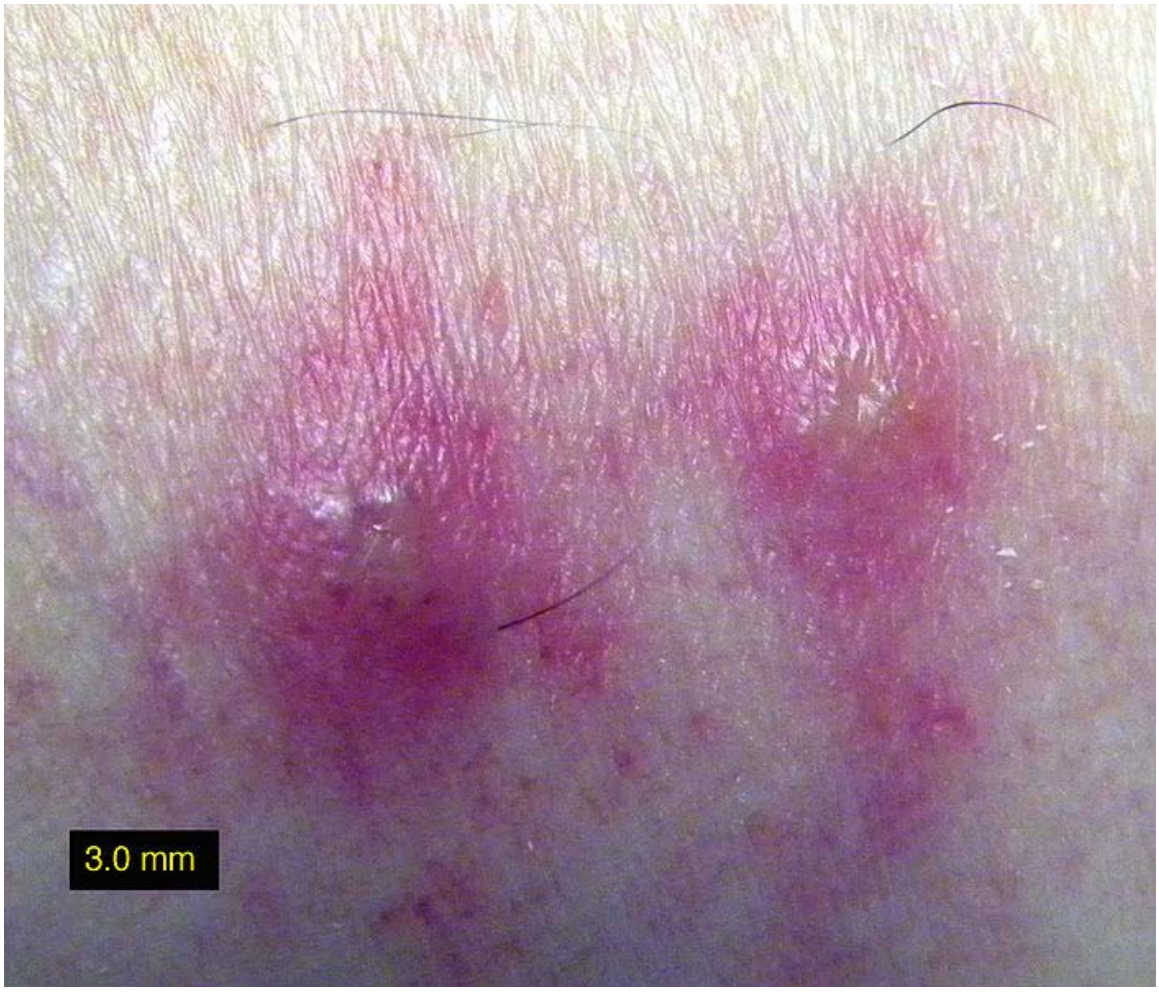
In World War I, over two million days of service are estimated to have been lost by reason of skin diseases alone.

Approach to diagnoses

The physical examination of the skin and its appendages, as well as the mucous membranes, forms the cornerstone of an accurate diagnosis of cutaneous conditions. Most of these conditions present with cutaneous surface changes term "lesions," which have more or less distinct characteristics. Often proper examination will lead the physician to obtain appropriate historical information and/or laboratory tests that are able to confirm the diagnosis. Upon examination, the important clinical observations are the (1) morphology, (2) configuration, and (3) distribution of the lesion(s). With regard to morphology, the initial lesion that characterizes a condition is known as the "primary lesion," and identification of such a lesions is the most important aspect of the cutaneous examination. Over time, these primary lesions may continue to develop or be modified by regression or trauma, producing "secondary lesions." However, with that being stated, the lack of standardization of basic dermatologic terminology has been one of the principal barriers to successful communication among physicians in describing cutaneous findings. Nevertheless, there are some commonly accepted terms used to describe the macroscopic morphology, configuration, and distribution of skin lesions, which are listed below.

Morphology

Primary lesions



Chigger bites on human skin showing characteristic welts

- **Macule** - A macule is a change in surface color, without elevation or depression and, therefore, nonpalpable, well or ill-defined, variously sized, but generally considered less than either 5 or 10mm in diameter at the widest point.
- **Patch** - A patch is a large macule equal to or greater than either 5 or 10mm, depending on one's definition of a macule. Patches may have some subtle surface change, such as a fine scale or wrinkling, but although the consistency of the surface is changed, the lesion itself is not palpable.
- **Papule** - A papule is a circumscribed, solid elevation of skin with no visible fluid, varying in size from a pinhead to either less than 5 or 10mm in diameter at the widest point.
- **Plaque** - A plaque has been described as a broad papule, or confluence of papules equal to or greater than 1 cm, or alternatively as an elevated, plateau-like lesion that is greater in its diameter than in its depth.

- **Nodule** - A nodule is morphologically similar to a papule, but is greater than either 5 or 10mm in both width and depth, and most frequently centered in the dermis or subcutaneous fat. The depth of involvement is what differentiates a nodule from a papule.
- **Vesicle** - A vesicle is a circumscribed, fluid-containing, epidermal elevation generally considered less than either 5 or 10mm in diameter at the widest point.
- **Bulla** - A bulla is a large vesicle described as a rounded or irregularly shaped blister containing serous or seropurulent fluid, equal to or greater than either 5 or 10mm, depending on one's definition of a vesicle.
- **Pustule** - A pustule is a small elevation of the skin containing cloudy or purulent material usually consisting of necrotic inflammatory cells.
- **Cyst** - A cyst is an epithelial-lined cavity containing liquid, semi-solid, or solid material.
- **Erosion** - An erosion is a discontinuity of the skin exhibiting incomplete loss of the epidermis, a lesion that is moist, circumscribed, and usually depressed.
- **Ulcer** - An ulcer is a discontinuity of the skin exhibiting complete loss of the epidermis and often portions of the dermis and even subcutaneous fat.
- **Fissure** - A fissure is a crack in the skin that is usually narrow but deep.
- **Wheal** - A wheal is a rounded or flat-topped, pale red papule or plaque that is characteristically evanescent, disappearing within 24 to 48 hours.
- **Telangiectasia** - A telangiectasia represents an enlargement of superficial blood vessels to the point of being visible.
- **Burrow** - A burrow appears as a slightly elevated, grayish, tortuous line in the skin, and is caused by burrowing organisms.

Secondary lesions

- **Scale** - dry or greasy laminated masses of keratin that represent thickened stratum corneum.
- **Crust** - dried serum, pus, or blood usually mixed with epithelial and sometimes bacterial debris.
- **Lichenification** - epidermal thickening characterized by visible and palpable thickening of the skin with accentuated skin markings.
- **Excoriation** - a punctate or linear abrasion produced by mechanical means (often scratching), usually involving only the epidermis but not uncommonly reaching the papillary dermis.
- **Induration** - dermal thickening causing the cutaneous surface to feel thicker and firmer.
- **Atrophy** - refers to a loss of tissue, and can be epidermal, dermal, or subcutaneous. With epidermal atrophy, the skin appears thin, translucent, and wrinkled. Dermal or subcutaneous atrophy is represented by depression of the skin.

Configuration

- Agminate

- Annular
- Arciform or arcuate
- Circinate
- Digitate
- Discoid
- Figurate
- Guttate
- Herpetiform
- Linear
- Nummular
- Reticular or reticulated
- Serpiginous or gyrate
- Targetoid
- Verrucous

Distribution

- Generalized
- Symmetric
- Flexural
- Extensor
- Intertriginous
- Palmoplantar
- Periorificial
- Periungual
- Alopecia
- Blaschkoid
- Photodistributed
- Zosteriform or dermatomal

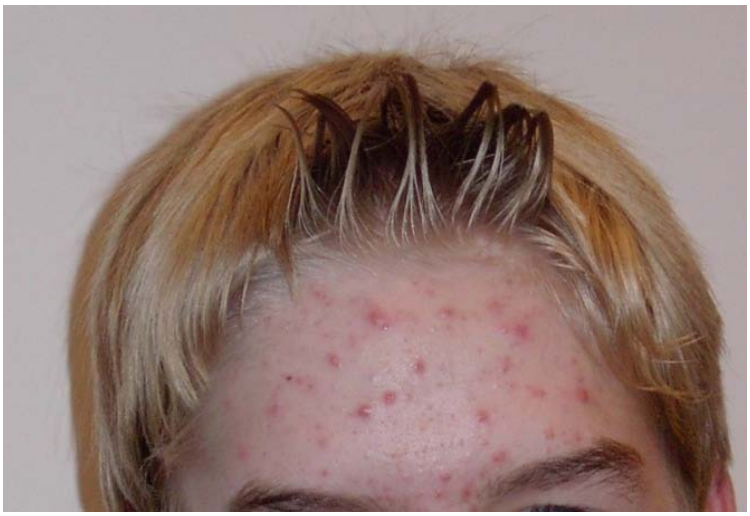
Other terms collarette Combined (conjoint) terms (maculopapular, papuloerosive, papulopustular, papulovesicular, papulosquamous, tuberoulcerative, vesiculobullous, vesiculopustular) are used to describe eruptions that evolve from one type of lesion to the next

Comedo Confluent Eczema Granuloma Livedo Mamillated Morbilliform Stellate

Other terms include purpura, erythema, horn, and poikiloderma.

Chapter 16

Acne Vulgaris

Acne vulgaris


Acne of a 14-year-old male during puberty

ICD-10	L70.0
ICD-9	706.1
DiseasesDB	10765
MedlinePlus	000873
eMedicine	derm/2
MeSH	D000152

Acne vulgaris (or **acne**) is a common human skin disease, characterized by areas of skin with seborrhea (scaly red skin), comedones (blackheads and whiteheads), papules (pinheads), pustules (pimples), nodules (large papules) and possibly scarring. Acne affects mostly skin with the densest population of sebaceous follicles; these areas include the face, the upper part of the chest, and the back. Severe acne is inflammatory, but acne can also manifest in noninflammatory forms. The lesions are caused by changes in

pilosebaceous units, skin structures consisting of a hair follicle and its associated sebaceous gland, changes that require androgen stimulation.

Acne occurs most commonly during adolescence, and often continues into adulthood. In adolescence, acne is usually caused by an increase in male sex hormones, which people of both genders accrue during puberty. For most people, acne diminishes over time and tends to disappear — or at the very least decrease — after one reaches one's early twenties. There is, however, no way to predict how long it will take to disappear entirely, and some individuals will carry this condition well into their thirties, forties, and beyond.

Some of the large nodules were previously called "cysts" and the term *nodulocystic* has been used to describe severe cases of inflammatory acne. The "cysts," or boils that accompany cystic acne, can appear on the buttocks, groin, and armpit area, and anywhere else where sweat collects in hair follicles and perspiration ducts. Cystic acne affects deeper skin tissue than does common acne.

Aside from scarring, its main effects are psychological, such as reduced self-esteem and, according to at least one study, depression or suicide. Acne usually appears during adolescence, when people already tend to be most socially insecure. Early and aggressive treatment is therefore advocated by some to lessen the overall impact to individuals.

Terminology

The term *acne* comes from a corruption of the Greek ἀκμή (*akmē*), literally "point, edge", but in the sense of a "skin eruption" in the writings of Aëtius Amidenus. Used by itself, the term "acne" refers to the presence of pustules and papules. The most common form of acne is known as acne vulgaris, meaning "common acne". Many teenagers get this type of acne. Use of the term "acne vulgaris" implies the presence of comedones.

The term "acne rosacea" is a synonym for rosacea, however some individuals may have almost no acne comedones associated with their rosacea and prefer therefore the term rosacea. Chloracne is associated with exposure to polyhalogenated compounds.

Signs and symptoms

Typical features of acne include: seborrhea (scaly red skin), comedones (blackheads and whiteheads), papules (pinheads), pustules (pimples), nodules (large papules) and, possibly scarring. It presents somewhat differently in people with dark skin.

Scars

Acne scars are the result of inflammation within the dermis brought on by acne. The scar is created by the wound trying to heal itself resulting in too much collagen in one spot.

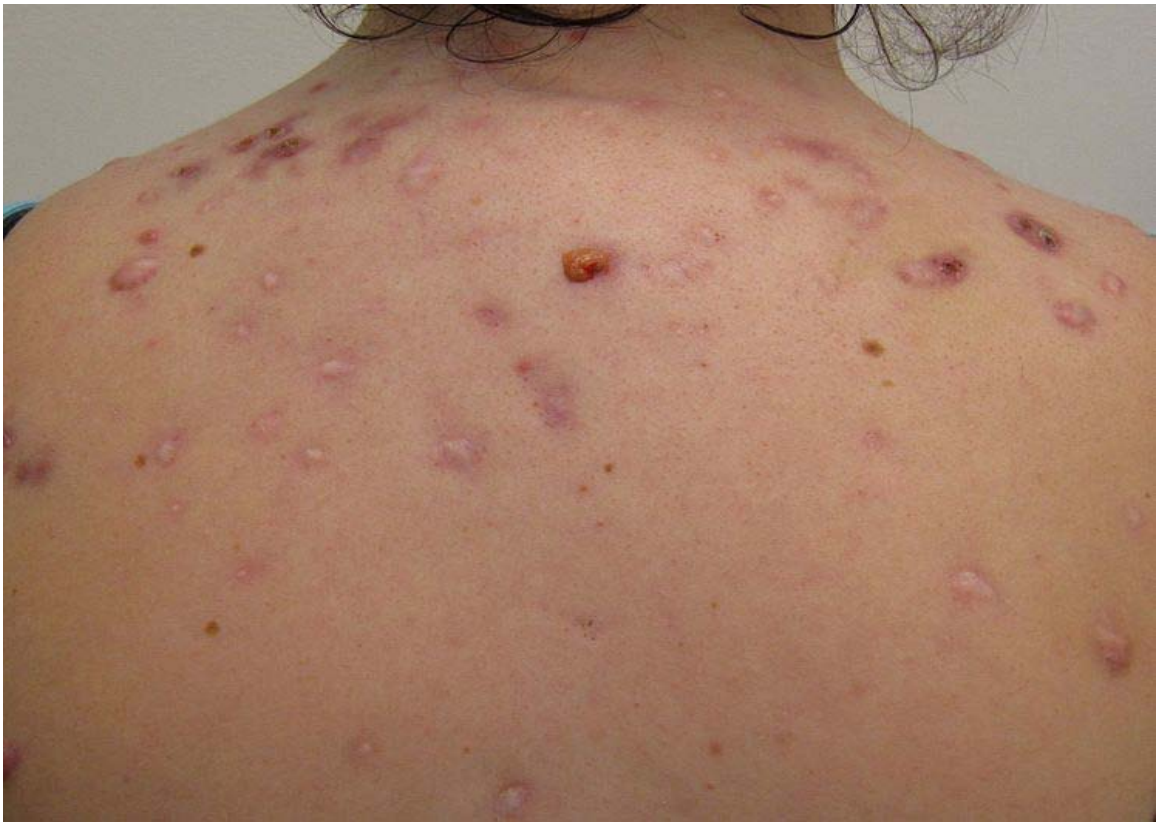
Physical acne scars are often referred to as "Icepick" scars. This is because the scars tend to cause an indentation in the skin's surface. There are a range of treatments available.

Although quite rare, the medical condition Atrophia Maculosa Varioliformis Cutis also results in "acne-like" depressed scars on the face.

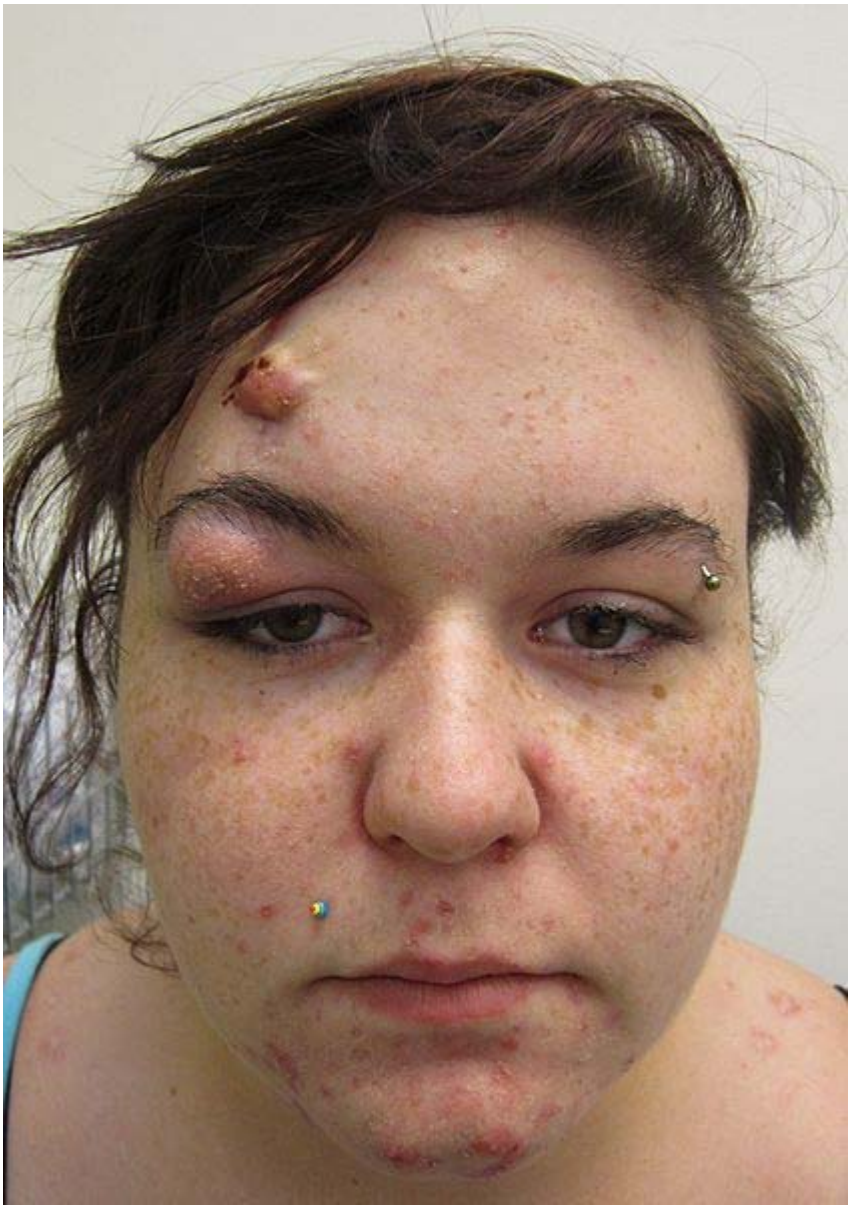
- *Ice pick scars*: Deep pits, that are the most common and a classic sign of acne scarring.
- *Box car scars*: Angular scars that usually occur on the temple and cheeks, and can be either superficial or deep, these are similar to chickenpox scars.
- *Rolling scars*: Scars that give the skin a wave-like appearance.
- *Hypertrophic scars*: Thickened, or keloid scars.

Pigmentation

Pigmented scars is a slightly misleading term, as it suggests a change in the skin's pigmentation and that they are true scars; however, neither is true. Pigmented scars are usually the result of nodular or cystic acne (the painful 'bumps' lying under the skin). They often leave behind an inflamed red mark. Often, the pigmentation scars can be avoided simply by avoiding aggravation of the nodule or cyst. Pigmentation scars nearly always fade with time taking between three months to two years to do so, although can last forever if untreated.



Cystic acne on the back



A severe case of cystic acne

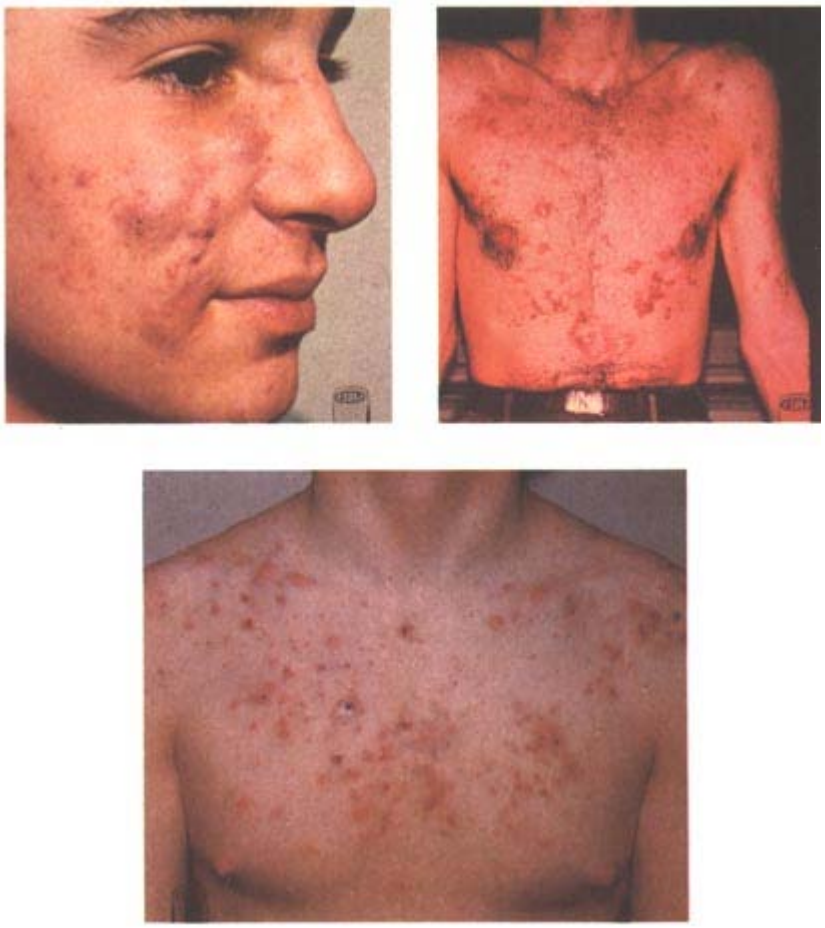


FIGURE 85.—Acne vulgaris. A, Cystic acne of face. B, Subsiding tropical acne of trunk. C, Extensive acne of chest and shoulders.

Different types of Acne Vulgaris: A: Cystic acne on the face, B: Subsiding tropical acne of trunk, C: Extensive acne on chest and shoulders.

Cause

Acne develops as a result of blockages in follicles. Hyperkeratinization and formation of a plug of keratin and sebum (a microcomedo) is the earliest change. Enlargement of sebaceous glands and an increase in sebum production occur with increased androgen (DHEA-S) production at adrenarche. The microcomedo may enlarge to form an open comedone (blackhead) or closed comedone (milia). Comedones are the direct result of sebaceous glands' becoming clogged with sebum, a naturally occurring oil, and dead skin cells. In these conditions, the naturally occurring largely commensal bacterium *Propionibacterium acnes* can cause inflammation, leading to inflammatory lesions (papules, infected pustules, or nodules) in the dermis around the microcomedo or comedone, which results in redness and may result in scarring or hyperpigmentation.

Hormonal

Hormonal activity, such as menstrual cycles and puberty, may contribute to the formation of acne. During puberty, an increase in male sex hormones called androgens cause the follicular glands to grow larger and make more sebum. Use of anabolic steroids may have a similar effect. Several hormones have been linked to acne: the androgens testosterone, dihydrotestosterone (DHT) and dehydroepiandrosterone sulfate (DHEAS), as well as insulin-like growth factor 1 (IGF-I).

Development of acne vulgaris in later years is uncommon, although this is the age group for rosacea, which may have similar appearances. True acne vulgaris in adult women may be a feature of an underlying condition such as pregnancy and disorders such as polycystic ovary syndrome or the rare Cushing's syndrome. Menopause-associated acne occurs as production of the natural anti-acne ovarian hormone estradiol fails at menopause. The lack of estradiol also causes thinning hair, hot flushes, thin skin, wrinkles, vaginal dryness, and predisposes to osteopenia and osteoporosis as well as triggering acne (known as acne climacterica in this situation).

Genetic

The tendency to develop acne runs in families. For example, school aged boys with acne often have other members in their family with acne, as well. A family history of acne is associated with an earlier occurrence of acne and an increased number of retentional acne lesions.

Psychological

While the connection between acne and stress has been debated, scientific research indicates that "increased acne severity" is "significantly associated with increased stress levels." The National Institutes of Health (USA) list stress as a factor that "can cause an acne flare." A study of adolescents in Singapore "observed a statistically significant positive correlation […] between stress levels and severity of acne." It is also not clear whether acne causes stress and thus perpetuates itself to some extent.

Infectious

Bacteria in the pores, *Propionibacterium acnes (P. acnes)* is the anaerobic bacterium that causes acne. *In vitro*, resistance of *P. acnes* to commonly used antibiotics has been increasing.

Diet

A high glycemic load diet and cow's milk have been associated with worsening acne. Other associations such as chocolate and salt are not supported by the evidence.

Diagnosis

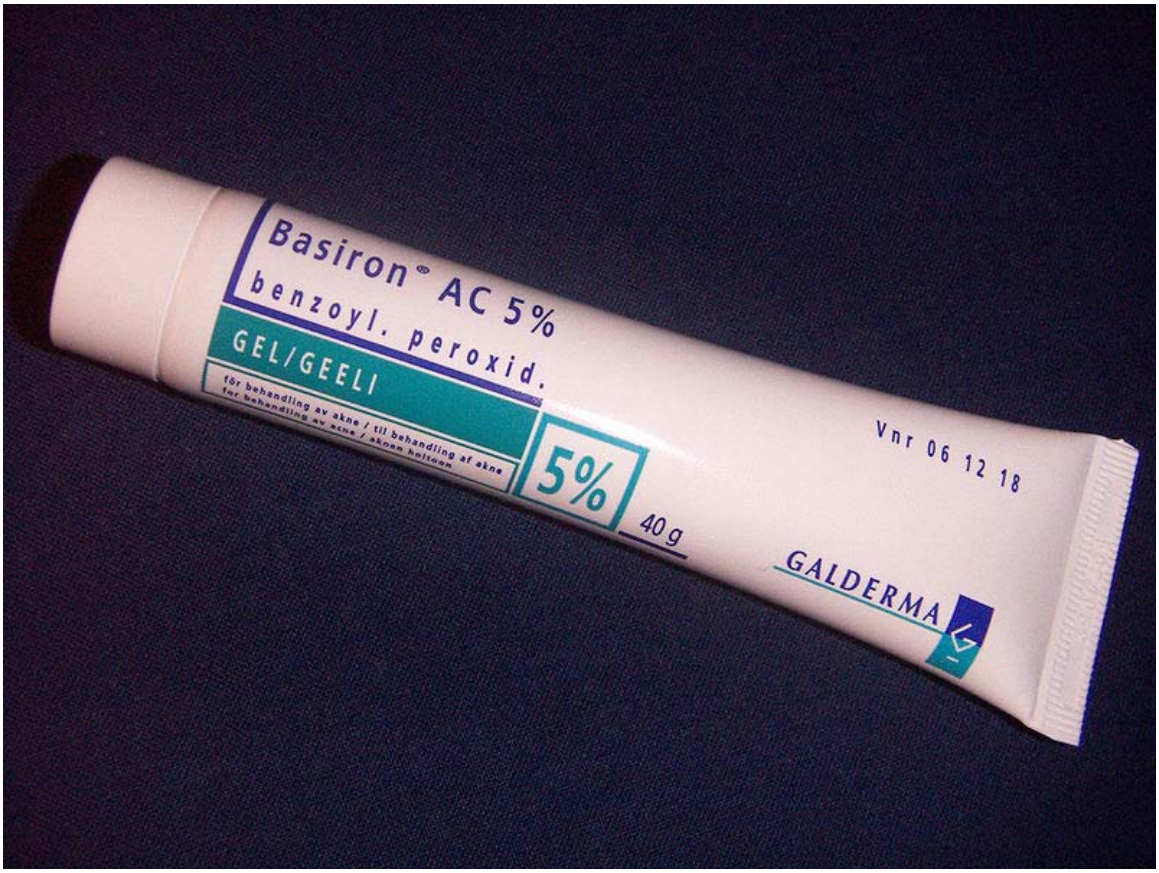
There are multiple grading scales for grading the severity of acne vulgaris, three of these being:

- Leeds acne grading technique: Counts and categorises lesions into inflammatory and non-inflammatory (ranges from 0-10.0).
- Cook's acne grading scale: Uses photographs to grade severity from 0 to 8 (0 being the least severe and 8 being the most severe).
- Pillsbury scale: Simply classifies the severity of the acne from 1 (least severe) to 4 (most severe).

Differential

- Keratosis pilaris
- Rosacea
- Chloracne

Management



Benzoyl peroxide cream

Many different treatments exist for acne including benzoyl peroxide, antibiotics, retinoids, antiseborrheic medications, salicylic acid, alpha hydroxy acid, azelaic acid, nicotinamide, and kera-tolytic soaps. They are believed to work in at least 4 different ways including: normalising shedding into the pore to prevent blockage, killing *Propionibacterium acnes*, anti-inflammatory effects, hormonal manipulation.

Medications

Benzoyl peroxide

Benzoyl peroxide is a first-line treatment for mild and moderate acne vulgaris due to its effectiveness and mild side-effects (primarily an irritant dermatitis). It normally causes just dryness of the skin, slight redness, and occasional peeling as part of some side-effects. This topical does increase sensitivity to the sun as indicated on the package, so sunscreen should be used during the treatment to prevent sunburn. Benzoyl peroxide has been found to be nearly as effective as antibiotics with all concentrations 2.5%, 5.0%, and 10% equally effective. Unlike antibiotics, benzoyl peroxide does not appear to generate bacterial resistance.

Antibiotics

Antibiotics are reserved for more severe cases. With increasing resistance of *P. acnes* worldwide, they are becoming less effective. Commonly used antibiotics, either applied topically or taken orally, include erythromycin, clindamycin, and tetracyclines such as minocycline.

Hormones

In females, acne can be improved with hormonal treatments. The common combined estrogen/progestogen methods of hormonal contraception have some effect, but the antiandrogen cyproterone in combination with an oestrogen (*Diane 35*) is particularly effective at reducing androgenic hormone levels. Diane-35 is not available in the USA, but a newer oral contraceptive containing the progestin drospirenone is now available with fewer side-effects than Diane 35 / Dianette. Both can be used where blood tests show abnormally high levels of androgens, but are effective even when this is not the case. Along with this, treatment with low-dose spironolactone can have anti-androgenetic properties, especially in patients with polycystic ovarian syndrome.

Topical retinoids

A group of medications for normalizing the follicle cell life-cycle are topical retinoids such as tretinoin (Retin-A), adapalene (Differin), and tazarotene (Tazorac). Like isotretinoin, they are related to vitamin A, but they are administered as topicals and, in general, have much milder side-effects. They can, however, cause significant irritation of the skin. The retinoids appear to influence the cell creation and death life-cycle of cells in the follicle lining. This helps prevent the hyperkeratinization of these cells that can create a blockage. Retinol, a form of vitamin A, has similar, but milder, effects and is used in

many over-the-counter moisturizers and other topical products. Effective topical retinoids have been in use for over 30 years, but are available only on prescription, so are not as widely used as the other topical treatments. Topical retinoids often cause an initial flare-up of acne and facial flushing.

Oral retinoids

A daily oral intake of vitamin A derivative isotretinoin (marketed as Roaccutane, Accutane, Amnesteem, Sotret, Claravis, Clarus) over a period of 4–6 months can cause long-term resolution or reduction of acne. It is believed that isotretinoin works primarily by reducing the secretion of oils from the glands, however some studies suggest that it affects other acne-related factors as well. Isotretinoin has been shown to be very effective in treating severe acne and can either improve or clear well over 80% of patients. The drug has a much longer effect than anti-bacterial treatments and will often cure acne for good. The treatment requires close medical supervision by a dermatologist because the drug has many known side-effects (many of which can be severe). About 25% of patients may relapse after one treatment. In those cases, a second treatment for another 4–6 months may be indicated to obtain desired results. It is often recommended that one let a few months pass between the two treatments, because the condition can actually improve somewhat in the time after stopping the treatment and waiting a few months also gives the body a chance to recover. On occasion, a third or even a fourth course is used, but the benefits are often less substantial. The most common side-effects are dry skin and occasional nosebleeds (secondary to dry nasal mucosa). Oral retinoids also often cause an initial flare-up of acne within a month or so, which can be severe. There are reports that the drug has damaged the liver of patients. For this reason, it is recommended that patients have blood samples taken and examined before and during treatment. In some cases, treatment is terminated or reduced due to elevated liver enzymes in the blood, which might be related to liver damage. Others claim that the reports of permanent damage to the liver are unsubstantiated, and routine testing is considered unnecessary by some dermatologists. Blood triglycerides also need to be monitored. However, routine testing are part of the official guidelines for the use of the drug in many countries. Some press reports suggest that isotretinoin may cause depression, but, as of September 2005, there is no agreement in the medical literature as to the risk. The drug also causes birth defects if women become pregnant while taking it or take it while pregnant. For this reason, female patients are required to use two separate forms of birth control or vow abstinence while on the drug. Because of this, the drug is supposed to be given to females as a last resort after milder treatments have proven insufficient. Restrictive rules for use were put into force in the USA beginning in March 2006 to prevent misuse, causing occasioned widespread editorial comment.

Anti-inflammatories

Nicotinamide, (vitamin B_3) used topically in the form of a gel, has been shown in a 1995 study to be of comparable efficacy to topical clindamycin used for comparison. The property of topical nicotinamide's benefit in treating acne seems to be its anti-inflammatory nature. It is also purported to result in increased synthesis of collagen,

keratin, involucrin and flaggrin, and may also, according to a cosmetic company, be useful for reducing skin hyperpigmentation (acne scars), increasing skin moisture and reducing fine wrinkles.

Naproxen or ibuprofen are used for some moderate acne cases for their anti-inflammatory effects.

Mandelic acid has been noted to be an effective topical treatment for mild to moderate acne. It is considered to be a gentler alternative to popular alpha hydroxy acids, such as glycolic acid and lactic acid.

Procedures

Dermabrasion

Dermabrasion is a cosmetic medical procedure in which the surface of the skin is removed by abrasion (sanding). It is used to remove sun-damaged skin and to remove or lessen scars and dark spots on the skin. The procedure is very painful and usually requires a general anaesthetic or "twilight anaesthesia", in which the patient is still partly conscious. Afterward, the skin is very red and raw-looking, and it takes several months for the skin to regrow and heal. Dermabrasion is useful for scar removal when the scar is raised above the surrounding skin, but is less effective with sunken scars.

In the past, dermabrasion was done using a small, sterilized, electric sander. In the past decade, it has become more common to use laser dermabrasion using CO_2, Er:YAG laser or a combination of both for the treatment of acne scars. Indications for CO_2 laser treatment include previous non erythematous and non-proliferative hypertrophic scars, atrophic acne scars and burn scars. Laser dermabrasion is much easier to control, much easier to gauge, and is practically bloodless compared to classic dermabrasion.

Microdermabrasion comes from the above mentioned technique dermabrasion. Microdermabrasion is a more natural skin care that is a gentler, less invasive technology for doing an exfoliation on the skin. The goal of the microdermabrasion is to eliminate the superficial layer of the skin called the epidermis. If the surface of the abraded skin is touched, a roughness of the skin will be noticed. The roughness is keratinocytes, which are better hydrated than the surface corneocytes. Keratinocytes appear in the basal layer from the proliferation of keratinocyte stem cells. They are pushed up through the cells of the epidermis, experiencing gradual specialization until they reach the stratum corneum where they form a layer of dead, flattened, strongly keratinized cells called squamous cells. This layer creates an efficient barrier to the entry of foreign matter and infectious elements into the body and reduces moisture loss. Keratinocytes are shed and restored continuously from the stratum corneum.

The time of transit from basal layer to shedding is generally one month. Corneocytes are cells derived from keratinocytes in the late stages of terminal specialization of squamous epithelia. The microdermabrasion is done to eliminate some of the corneocytes. These

cells are responsible for the impermeability of the skin. The minimizing or elimination of scars, skin lesions, blotchiness and stretch marks from the skin can be an easy process with the use of skin exfoliation. The result depends on how well the procedure known as "skin remodeling" works. Results are optimal and fewer treatments are needed with more recent and/or superficial scars. Still, microdermabrasion can be used on scars that showed up during puberty or many years later.

Phototherapy

Blue and red light

Light exposure has long been used as a short term treatment for acne. Recently, visible light has been successfully employed to treat mild to moderate acne (phototherapy or deep penetrating light therapy) - in particular intense violet light (405-420 nm) generated by purpose-built fluorescent lighting, dichroic bulbs, LEDs or lasers. Used twice weekly, this has been shown to reduce the number of acne lesions by about 64% and is even more effective when applied daily. The mechanism appears to be that a porphyrin (Coproporphyrin III) produced within *P. acnes* generates free radicals when irradiated by 420 nm and shorter wavelengths of light. Particularly when applied over several days, these free radicals ultimately kill the bacteria. Since porphyrins are not otherwise present in skin, and no UV light is employed, it appears to be safe, and has been licensed by the U.S. FDA.

It seems that the treatment works even better if used with a mixture of the violet light and red visible light (660 nanometer), resulting in a 76% reduction of lesions after three months of daily treatment for 80% of the patients; and overall clearance was similar or better than benzoyl peroxide. Unlike most of the other treatments, few if any negative side-effects are typically experienced, and the development of bacterial resistance to the treatment seems very unlikely. After treatment, clearance can be longer-lived than is typical with topical or oral antibiotic treatments; several months is not uncommon. The equipment or treatment, however, is relatively new and reasonably expensive to buy initially, although the total cost of ownership can be similar to many other treatment methods (such as the total cost of benzoyl peroxide, moisturizer, washes) over a couple of years of use.

Photodynamic therapy

In addition, basic science and clinical work by dermatologists Yoram Harth and Alan Shalita and others has produced evidence that intense blue/violet light (405-425 nanometer) can decrease the number of inflammatory acne lesion by 60-70% in four weeks of therapy, in particular, when the *P. acnes* is pretreated with delta-aminolevulinic acid (ALA), which increases the production of porphyrins. However this photodynamic therapy is controversial and not published in a peer-reviewed journal. A phase II trial, while it showed improvement occurred, failed to show improved response compared to the blue/violet light alone.

Laser treatment

Laser surgery has been in use for some time to reduce the scars left behind by acne, but research has been done on lasers for prevention of acne formation itself. The laser is used to produce one of the following effects:

- to burn away the follicle sac from which the hair grows
- to burn away the sebaceous gland, which produces the oil
- to induce formation of oxygen in the bacteria, killing them

Since lasers and intense pulsed light sources cause thermal damage to the skin, there are concerns that laser or intense pulsed light treatments for acne will induce hyperpigmented macules (spots) or cause long-term dryness of the skin.

In the FDA has approved the use of a cosmetic laser for the treatment of acne. However, efficacy studies have used very small sample sizes for periods of six months or less, and have shown contradictory results. Also, laser treatment being relatively new, protocols remain subject to experimentation and revision, and treatment can be quite expensive. Also, some Smoothbeam laser devices had to be recalled due to coolant failure, which resulted in painful burn injuries to patients.

Surgery

For people with cystic acne, boils can be drained through surgical lancing.

Other

- Aloe vera: there are treatments for acne mentioned in Ayurveda using herbs such as Aloe vera, Neem, Haldi (Turmeric) and Papaya. There is limited evidence from medical studies on these products.
- Tea tree oil (melaleuca oil) has been used with some success, where it is comparable to benzoyl peroxide but without excessive drying, kills *P. acnes*, and has been shown to be an effective anti-inflammatory in skin infections.

Prognosis

Acne usually improves around the age of 20 but may persist into adulthood.

Epidemiology

Acne affects 40 to 50 million people in the United States (16%), and approximately 3 to 5 million in Australia (23%). It affects people of all racial and ethnic groups.

History

- 1970s: Tretinoin (original Trade Name Retin A) was found effective for acne. This preceded the development of oral isotretinoin (sold as Accutane and Roaccutane) in 1980.
- 1980s: Accutane is introduced in the United States, and later found to be a teratogen, highly likely to cause birth defects if taken during pregnancy. In the United States, more than 2,000 women became pregnant while taking the drug between 1982 and 2003, with most pregnancies ending in abortion or miscarriage. About 160 babies with birth defects were born.

Research

A vaccine against inflammatory acne has been tested successfully in mice, but it is not certain that it would work similarly in humans.

A 2007 microbiology article reporting the first genome sequencing of a *Propionibacterium acnes* bacteriophage (PA6) said this "should greatly enhance the development of a potential bacteriophage therapy to treat acne and, therefore, overcome the significant problems associated with long-term antibiotic therapy and bacterial resistance."

Chapter 17

Hidradenitis Suppurativa

Hidradenitis suppurativa	
ICD-10	L73.2
ICD-9	705.83
DiseasesDB	5892
eMedicine	emerg/259 med/2717 derm/892
MeSH	D017497

Hidradenitis suppurativa is a skin disease that most commonly affects areas bearing apocrine sweat glands or sebaceous glands, such as the underarms, breasts, inner thighs, groin and buttocks.

Overview

The non-contagious disease manifests as clusters of chronic abscesses, epidermoid cyst, sebaceous cysts, pilonidal cyst or multilocalised infections, which can be as large as baseballs or as small as a pea, that are extremely painful to the touch and may persist for years with occasional to frequent periods of inflammation, culminating in incision and drainage of pus, often leaving open wounds that will not heal. The simple procedure of incision and drainage provides some relief from severe, often debilitating, pressure pain. Flare-ups may be triggered by perspiration, hormonal changes (such as monthly cycles in women), humidity and heat, and clothing friction. Persistent lesions may lead to scarring and the formation of sinus tracts, or tunnels connecting the abscesses or infections under the skin. At this stage, complete healing is usually not possible, and progression varies from person to person, with some experiencing remission anywhere from months to years at a time, while others may worsen and require multiple surgeries in order to live comfortably. Wound dehiscence, a premature "bursting" open of a wound often complicates the healing process. Occurrences of bacterial infections and cellulitis (deep tissue inflammation) may occur at these sites. HS pain and depression can be difficult to manage.

HS often goes undiagnosed for years because patients are too ashamed to speak with anyone. When they do see a doctor or medical practitioner, the disease is frequently misdiagnosed or prescribed treatments are ineffective, temporary and sometimes even harmful. There is no known cure nor any consistently effective treatment. Carbon dioxide laser surgery is currently considered the last resort for those who have advanced to its highest stage, where the affected areas are excised, and the skin is grafted. Surgery doesn't always alleviate the condition, however, and can be very expensive.

Several articles and clinics consider this disease as widely misdiagnosed, due to the misunderstanding of the causes and progression of the disease. HS is neither the biblical stigmata, leprosy nor caused by poor hygiene. HS is often called an 'orphan illness', due to little research being conducted on the disease at this time. Because HS is considered a rare disease, its incidence rate is not well known, but has been estimated as being between 1:24 (4.1%) and 1:600 (0.2%).

Areas of Involvement in Men and Women with Hidradenitis Suppurativa

HS Patients (n=164, 121 females, 43 males)	Female	Male	p (Statistical Significance)
Axillae	70 (58%)	30 (70%)	NS (not significant)
Mammary and inter-mammary	31 (26%)	2 (5%)	0.006
Inguino-femoral	111 (92%)	32 (74%)	0.007
Perianal and Perineal	40 (33%)	24 (56%)	0.01
Buttocks	30 (25%)	21 (50%)	0.006

- Note that for the study involved in the Table above was a personal series of 164 patients. Of the 164 patients with HS, 76% were in Hurley's Stage I, 20% were in Hurley's Stage II, and 4% were in the final phase of the disease, Hurley's Stage III.

Other names for HS

Hidradenitis suppurativa has been referred to by multiple names in the literature, as well as in various cultures. Some of these are also used to describe different diseases, or specific instances of this disease.

- Acne conglobata - not really a synonym - this is a similar process but in classic acne areas of chest and back
- Acne Inversa (AI) - a proposed new term which has not gained widespread favour.

- Apocrine Acne - a misnomer, out-dated, based on the disproven concept that apocrine glands are primarily involved. Though many do suffer with apocrine gland infection, so thought should be given to using HS with subtext re: glands involved.
- Apocrinitis - another misnomer, out-dated, based on the disproven concept that apocrine glands are primarily involved
- Fox-den disease - a catchy term not used in medical literature, based on the deep fox den / burrow - like sinuses
- Hidradenitis Supportiva - a misspelling
- Pyodermia fistulans sinifica - an older term, considered archaic now
- Velpeau's disease - commemorating the French surgeon who first described the disease in 1833
- Verneuil's disease - recognizing the French surgeon whose name is most often associated with the disorder as a result of his 1854-1865 studies

Historical Overview of Hidradenitis Suppurativa

- In 1839, the first description of Hidradenitis Suppurativa was identified and published by Velpeau.
- In 1854, Verneuil described Hidradenitis Suppurativa as "Hidrosadénite Phlegmoneuse". This is how HS obtained it's alternate name "Verneuil's disease".
- In 1922, Schiefferdecker hypothesized a pathogenic link between "Acne inversa" and human apocrine sweat glands.
- In 1956, Pillsbury wrote and published a medical journal article discussing Hidradenitis Suppurativa, describing the disease's main characteristics, dubbing them the "Acne triad: hidradenitis suppurativa,perifolliculitis capitis abscendens et suffodiens". Pillsbury's research study was one of the first peer-reviewed journal articles to appear publicly with many details of Hidradenitis Suppurativa, which are still used and relied on today in the medical realm of research on this disease.
- In 1975, Plewig and Kligman, following Pillsbury's research path, modified the "Acne triad", replacing it with the "Acne tetrad: acne triad, plus pilonidal sinus". Plewig and Kligman's research follows in Pillsbury's footsteps, offering explanations of the symptoms associated with Hidradenitis Suppurativa.
- Finally in 1989, Plewig and Steger's research led them to rename Hidradenitis Suppurativa, calling it "Acne Inversa" - which not still used today in medical terminology; although some individuals still use this outdated term.

A surgeon from Paris, Velpeau described an unusual inflammatory process with formation of superficial axillary, sub-mammary and perianal abscesses in 1839. One of his colleagues also located in Paris, named Verneuil, coined the term “hidrosadénite phlegmoneuse” approximately 15 years later. This name for the disease reflects the former pathogenetic model of acne inversa, which is considered inflammation of sweat glands as the primary cause of Hidradenitis Suppurativa. In 1922 Schiefferdecker suspected a pathogenic association between acne inversa and apocrine sweat glands. In 1956 Pillsbury postulated follicular occlusion as the cause of acne inversa, which they

grouped together with acne conglobata and perifolliculitis capitis abscendens et suffodiens (dissecting cellulitis of the scalp) as the "acne triad". Plewig and Kligman added another element to their acne triad, pilonidal sinus. Plewig et al. noted that this new "acne tetrad" includes all the elements found in the original "acne triad", in addition to a fourth element, pilonidal sinus. In 1989 Plewig and Steger introduced the term "acne inversa", indicating a follicular source of the disease and replacing older terms such as "Verneuil disease".

Historical View of Hidradenitis Suppurativa

Author	Year	Findings
Valpeau	1839	First description of the Hidradenitis Suppurativa
Verneuil	1854	"Hidrosadénite Phlegmoneuse"
Pillsbury	1956	Acne triad (hidradenitis suppurativa, perifolliculitis capitis abscendens et suffodiens)
Plewig & Kligman	1975	Acne tetrad (acne triad + pilonidal sinus)
Plewig & Steger	1989	Acne inversa

Dermatohistological View of Hidradenitis Suppurativa

Author	Year	Major Features
Plewig & Steger	1989	Initial hyperkeratosis of the follicular infundibulum. Bacterial super-infection and follicle rupture. Granulomatous inflammatory reaction of the connective tissue. Apocrine and eccrine sweat glands secondarily involved.
Yu & Cook	1990	Cysts and sinus tracts lined with epithelium, in part with hair shafts. Inflammation of apocrine sweat glands only if eccrine sweat glands and hair follicles are also inflamed.
Boer & Weltevreden	1996	Primary inflammation of the follicular infundibulum. Apocrine sweat glands are secondarily involved.

Stages

HS presents itself in three stages. Due to the large spectrum of clinical severity, to severe repercussions on quality of life and to the variety of available, a reliable method for evaluating HS severity is required. It should take into account the number, type and size of lesions, evolution, pain and repercussions for the quality of life of the patient. Such a comprehensive instrument does not yet exist, but there have been two successful attempts proposed to classify patients with HS according to the severity of their disease.

Hurley's Staging System

This is historically the first classification system proposed, and is still in use for the classification of patients with skin/dermatologic diseases (i.e. psoriasis, HS, acne). Hurley separated patients into three groups based largely on the presence and extent of cicatrization and sinuses. It has been used as a basis for clinical trials in the past and is a useful basis to approach therapy for patients. These three stages are based on Hurley's staging system, which is simple and relies on the subjective extent of the diseased tissue the patient has. Hurley's three stages of Hidradenitis Suppurativa are as follows:

Stage	Characteristics
I	Solitary or multiple isolated abscess formation without scarring or sinus tracts. (A few minor sites with rare inflammation; may be mistaken for acne.)
II	Recurrent abscesses, single or multiple widely separated lesions, with sinus tract formation. (Frequent inflammation restrict movement and may require minor surgery such as incision and drainage.)
III	Diffuse or broad involvement across a regional area with multiple interconnected sinus tracts and abscesses. (Inflammation of sites to the size of golf balls, or sometimes baseballs; scarring develops, including subcutaneous tracts of infection. Obviously, patients at this stage may be unable to function.)

Sartorious Staging System

The Sartorious staging system is more sophisticated than Hurley's, and is likely to supplant it as a means for conducting clinical trials during research. Sartorius et al. have suggested that the Hurley system is not sophisticated enough to assess treatment effects in clinical trials. The need for uniform outcome variables when reporting treatment effects has led to the proposition of a score by Sartorious and colleagues. This classification allows for better dynamic monitoring of the disease severity in individual patients and therefore forms a complimentary system to the Hurley classification. They suggest a system that incorporates several of the involved areas of consideration:

- Anatomic regions involved (axilla, groin gluteal or other region or infra-mammary region left or right)
- Number and types of lesions involved (abscesses, nodules, fistulas, scars, points for lesions of all regions involved)
- The distance between lesions, in particular the longest distance between two relevant lesions (i.e. nodules and fistulas in each region or size if only one lesion present)
- The presence of normal skin in between lesions (i.e. are all lesions clearly separated by normal skin?)

Points are accumulated in each of the mentioned categories above, and added to give both a regional and total score. In addition, the authors recommend adding a visual analog scale for pain or using the dermatology life quality index (DLQI, or the Skindex) when

assessing HS. This system will likely be the basis of most future clinical trials and research studies.

Causes

As this disease is poorly studied, the causes are controversial and experts disagree.

Hydradenitis suppurativa occurs when apocrine gland become plugged. Lesions occur in areas of the body with numerous apocrine glands such as the axilla, groin, and perianal region. This theory includes most of the following potentials indicators:

- post-pubescent individuals are more likely to exhibit HS
- females are more likely than males
- genetic predisposition among families of Sephardic Jewish, Italian, Greek, Middle Eastern and Northern African Ancestry.
- Research is assessing possible relations with Hashimoto's Thyroiditis, Crohn's Disease, Rheumatoid Arthritis, and Squamous Cell Carcinoma.
- Plugged apocrine (sweat) gland or hair follicle
- excessive sweating
- bacterial infection
- sometimes linked with other auto-immune conditions
- androgen dysfunction
- genetic disorders that alter cell structure
- being overweight makes it worse, however this condition is not caused by obesity and weight loss will improve but not cure it. Patients with more advanced cases may find exercise intolerably painful, which may increase the rate of obesity among sufferers.

The historical understanding of the disease is that there are dysfunctional apocrine glands or dysfunctional hair follicles, possibly triggered by a blocked gland, creating inflammation, pain, and a swollen lesion. More recent studies imply there is an autoimmune component.

HS is **not** caused by any bacterial elements.

Triggering Factors

There are a number of triggering factors that should be taken into consideration, as it is advisable to avoid such triggers.

- Obesity is an exacerbating rather than a triggering factor, through mechanical irritation, occlusion, and maceration.
- Tight clothing, and clothing made of heavy, non-breathable materials.
- Smoking tobacco products.
- Deodorants, depilation products, shaving of the affected area - their association with Hidradenitis suppurativa is still an ongoing debate amongst researchers.

- Drugs, in particular oral contraceptives (i.e. oral hormonal birth control; "the pill") and lithium.
- Hot and especially humid climates (dry/arid climates often cause remission)

Predisposing Factors

- Genetic factors: an autosomal dominant inheritance pattern has been postulated.
- Endocrine factors: sex hormones, principally an excess of androgens, are thought to be involved, although the apocrine glands are not sensitive to these hormones. Women often have outbreaks before menstruation and post-pregnancy, and the disease usually remits during pregnancy and after menopause.

Severe complications

In disease stage III, fistulas left undiscovered, undiagnosed, or untreated, can lead to the development of squamous cell carcinoma, a rare cancer, in the anus or other affected areas. Other stage III chronic sequelae may also include anemia, multilocalised infections, amyloidosis, and arthropathy. Stage III complications have been known to lead to death, but clinical data is still uncertain.

Potential Complications

- Contractures and reduced mobility of the lower limbs and axillae due to fibrosis and scarring. Severe lymphedema may develop in the lower limbs.
- Local and systemic infections (meningitis, bronchitis, pneumonia, etc.), which may even progress to sepsis.
- Interstitial keratitis
- Anal, rectal, or urethral fistulas in anogenital hidradenitis suppurativa
- Normochromic or hypochromic anemia
- Squamous cell carcinoma: this has been found on rare occasions in chronic hidradenitis suppurativa of the anogenital region. The mean time to the onset of this type of lesion is 10 years or more, and the tumors are usually highly aggressive
- Tumors of the lung and oral cavity, probably related to the high level of smoking among these patients, and liver cancer.
- Hypoproteinemia and amyloidosis, which can lead to renal failure and death.
- Seronegative and usually asymmetric arthropathy: pauciarticular arthritis, polyarthritis/polyarthralgia syndrome.

Treatments

Treatments may vary depending upon presentation and severity of the disease. Due to the poorly-studied nature of this disease, the effectiveness of the drugs and therapies listed below is not yet clear, and patients should discuss all options with their doctor or dermatologist. Nearly a quarter of patients state that nothing relieves their symptoms. A list of treatments that are possible treatments for some patients is as follows.

- **Lifestyle**
  - Changes in diet avoiding inflammatory foods, foods high in refined carbohydrates.
  - Warm compresses, hydrotherapy, balneotherapy
  - Icing the inflamed area daily until pain reduction is noticed
  - Weight loss in overweight and obese patients, as well as smoking cessation can improve or even alleviate many symptoms of Hidradenitis suppurativa: Obese and overweight patients should be helped to lose weight in all cases of HS regardless of which stage patient may be in.

- **Medication**
  - Antibiotics Taken orally, these are used for their anti-inflammatory properties rather than to treat infection. The most effective is a combination of Rifampicin [300 mg twice a day] and Clindamycin [300 mg twice a day] given concurrently for 2–3 months. This brings about remission in around three quarters of cases. A few popular anitbiotics used to treat HS include tetracycline, minocycline, and clindamycin.
  - Corticosteroid injections. Also known as intralesional steroids: can be particularly useful for localized disease, if the drug can be prevented from escaping via the sinuses.
  - Vitamin A supplementation
  - Anti-androgen therapy: hormonal therapy with cyproterone acetate and ethinyloestadiol proved effective in randomized control trials. It is of note that dosages reported have been very high.
  - IV or subcutaneous infusion of anti-inflammatory (anti-TNF-alpha) drugs such as infliximab (Remicade), etanercept (Enbrel), and adalimumab. This use of the drugs is not currently Food and Drug Administration (FDA) approved and is somewhat controversial, and therefore may not be covered by insurance.
  - Zinc gluconate taken orally has been shown to induce remission. Recommended dose is at least 30mg taken 3 times daily (90mg/day). Toxicity is known to occur at doses exceeding 1000mg/day.
  - Chlorhexidine (Hibiclens) plus an antibiotic soap for cleansing the skin surface. Hexachlorophene shower with liquid soap like Phisohex, covering sores with Metrolotion after medicated showers. These are considered to be general measures, and are the foundation of any good medical treatment and management plans for HS.
  - Turmeric capsules orally or through topical application. The active ingredient in the tumeric spice is curcumin.
  - Infliximab: a chimeric monoclonal anti-TNF antibody has demonstrated efficacy in treating HS.
  - Topical clindamycin has been shown to have an effect in double-blind placebo controlled studies.

- **Surgical therapy**

When the process becomes chronic, wide surgical excision is the procedure of choice. Wound in the affected area do not heal by secondary intention, and immediate application of a split thickness skin graft is more appropriate.

- **Radiation**

Electron beam radiotherapy has been a successful treatment of hidradenitis, especially in Europe; it is not a common treatment option in most of the United States, as radiation oncologists generally refuse to treat patients with non-malignant diseases because of the potential for secondary radiation-induced tumors in the long term.

Chapter 18

Bullous Pemphigoid and Dermatitis Herpetiformis

Bullous pemphigoid

Bullous pemphigoid	
ICD-10	L12.0
ICD-9	694.5
eMedicine	derm/64
MeSH	D010391

Bullous pemphigoid, also referred to as **BP**, is an acute or chronic autoimmune skin disease, involving the formation of blisters, more appropriately known as bullae, at the space between the skin layers epidermis and dermis.

Pathophysiology

The bullae are formed by an immune reaction, initiated by the formation of IgG autoantibodies targeting the type XVII collagen component of hemidesmosomes. It can also rarely involve the mucous membranes. Following antibody targeting, a cascade of immunomodulators results in a variable surge of neutrophils, lymphocytes and eosinophils coming to the affected area. Unclear events subsequently result in a separation along the dermal-epidermal junction and eventually stretch bullae.

Presentation

Clinically the earliest lesions may appear urticarial (like hives). Tense bullae eventually erupt, most commonly at the inner thighs and upper arms but the trunk and extremities are frequently both involved. Any part of the skin surface can be involved. Milia are more common with epidermolysis bullosa acquisita (EBA), because of the deeper antigenic targets. A more ring-like configuration, with a central depression or centrally

collapsed bullae may indicate linear IgA disease. The disease may be acute, but typically will wax and wane.

Diagnosis

Diagnosis is based on two biopsies of the skin, one submitted for routine H&E staining and one for immunofluorescence studies.

Treatment

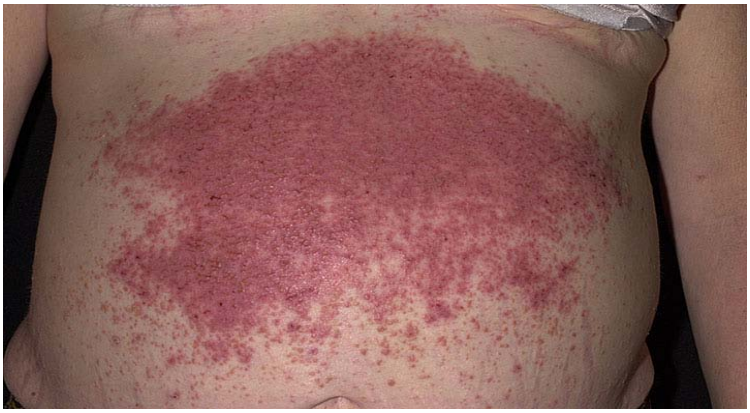
Treatments include Class I topical steroids (clobetasol, halobetasol, etc.) which in some studies have proven to be equally as effective as systemic, or pill, therapy and somewhat safer. However, in difficult to manage or widespread cases, systemic prednisone and powerful steroid-free immunosuppressant medications such as methotrexate, azathioprine or mycophenolate mofetil may be appropriate.

Epidemiology

Many mammals can be afflicted, including dogs, cats, pigs, and horses, as well as humans. Very rarely seen in children, it usually occurs in people 70 years of age and older. It is very rare in dogs; on average, three cases are diagnosed around the world each year.

Dermatitis herpetiformis

Dermatitis herpetiformis



Dermatitis herpetiformis characteristic rash

ICD-10 L12.2, L13.

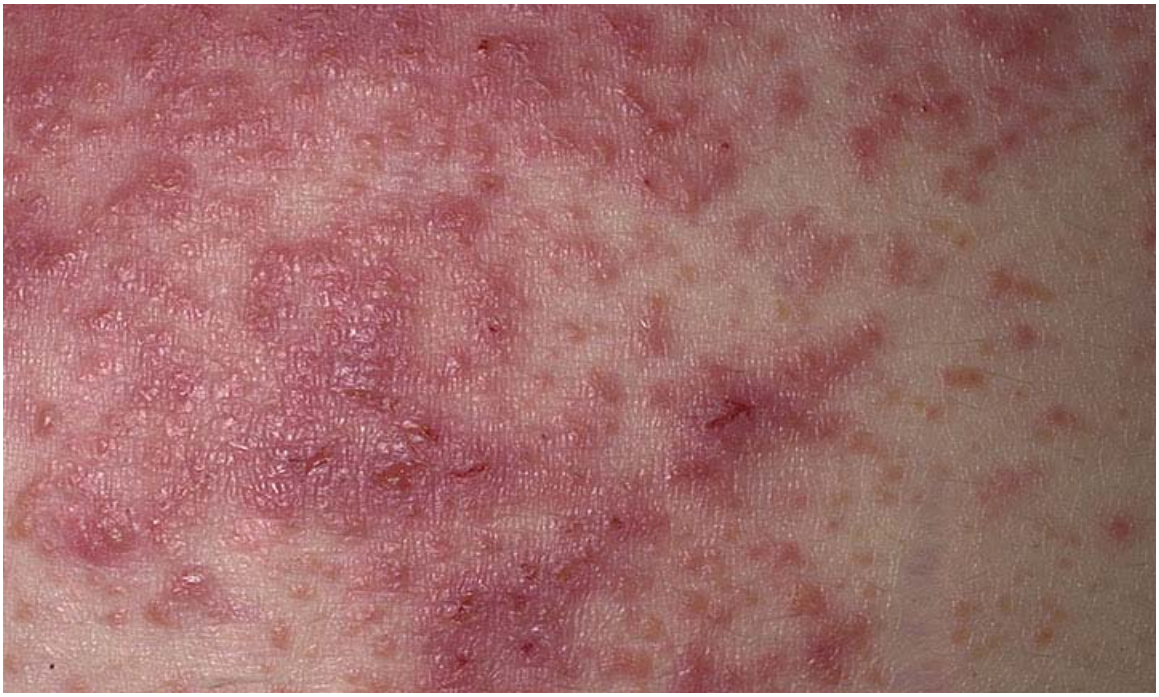
ICD-9	694.0
DiseasesDB	3597
MedlinePlus	001480
eMedicine	derm/95
MeSH	D003874

Dermatitis herpetiformis (DH), or Duhring's Disease, is a chronic blistering skin condition, characterised by blisters filled with a watery fluid. Despite its name, DH is not related to or caused by herpes virus: the name means that it is a skin inflammation having an appearance similar to herpes.

DH was first described by Dr. Louis Duhring in 1884. A connection between DH and gluten intolerance (celiac disease) was recognised in 1967, although the exact causal mechanism is not known.

The age of onset is usually about 15-40, but DH can also affect children and the elderly. Men and women are equally affected. Estimates of DH prevalence vary from 1 in 400 to 1 in 10000.

Symptoms



Characteristic rash

Dermatitis herpetiformis is characterized by intensely itchy, chronic papulovesicular eruptions, usually distributed symmetrically on extensor surfaces (buttocks, back of neck, scalp, elbows, knees, back). The blisters vary in size from very small up to 1 cm across.

The condition is extremely itchy, and the desire to scratch can be overwhelming. This sometimes leading to the blisters being scratched off before they are examined by a doctor. Intense itching or burning sensations are sometimes felt before the blisters appear in a particular area.

Untreated, the severity of DH can vary significantly over time, probably in response to the amount of gluten ingested.

Dermatitis herpetiformis symptoms typically first appear in the early years of adulthood between 20 and 30 years of age.

Although the first signs and symptoms of dermatitis herpetiformis are intensive itching and burning, the first visible signs are the small papules or vesicles that usually look like red bumps or blisters. Sometimes they appear on the face and along the hairline, and occasionally on the shoulders, the lower end of the spinal column and within the mouth. The rash rarely occurs on other mucous membranes excepting the mouth or lips. The symptoms range in severity from mild to serious, but they are likely to disappear if gluten ingestion is avoided and appropriate treatment is administered. However, the consumption of aliments that contain gluten as well as oral contraceptives may exacerbate the symptoms.

Dermatitis herpetiformis symptoms are chronic, and they tend to come and go, mostly in short periods of time. Sometimes, these symptoms may be accompanied by symptoms of coeliac disease, commonly including abdominal pain and fatigue.

The rash caused by dermatitis herpetiformis forms and disappears in three stages. In the first stage the patient may notice a slight discoloration of the skin at the site where the lesions appear. In the next stage, the skin lesions transform into obvious vesicles and papules which are likely to occur in groups. Healing of the lesions is the last stage of the development of the symptoms and it is usually characterized by a change in the skin color. This can result in areas of the skin turning darker or lighter than the color of the skin on the rest of the body. Because of the intense itching, patients usually scratch which leads to the formation of crusts.

Usually, itching and burning are the first symptoms to be experienced in dermatitis herpetiformis and they may occur up to 12 hours before the rash becomes visible. After the blisters develop, itching and burning may persist to up to 10 days when they finally begin to crust.

Inflammation and pus formation within the blisters is not specific.

Diagnosis

Diagnosis is confirmed by a simple blood test for IgA antibodies, and by a skin biopsy in which the pattern of IgA deposits in the dermal papillae, revealed by direct immunofluorescence, distinguishes it from linear IgA bullous dermatosis and other forms of dermatitis. These tests should be done before the patient starts on a gluten-free diet, otherwise they might produce false negatives. If the patient has already started a gluten-free diet, it might be necessary for them to come off it for some weeks before the tests can be done reliably.

Treatment

Dermatitis herpetiformis responds well to medication and changes in diet.

Dapsone is an effective treatment for most patients. DH responds to dapsone so quickly (itching is significantly reduced within 2–3 days) that this response may almost be considered diagnostic. However, dapsone treatment has no effect on any intestinal damage that might be present.

A strict gluten-free diet must therefore also be followed, and this will usually be a lifelong requirement. This will reduce any associated intestinal damage, and the risk of other complications. After some time on a gluten-free diet, the dosage of dapsone can usually be reduced or even stopped, although this can take up to anything from 1 to 3 years.

Dapsone is an antibacterial, and its role in the treatment of DH, which is not caused by bacteria, is poorly understood. It can cause adverse effects on the blood, and regular blood monitoring is required.

Dapsone is the drug of choice, but for patients unable to tolerate dapsone for any reason, the following can be tried, although they are less effective:

- colchicine
- lymecycline
- nicotinamide
- tetracycline
- sulfamethoxypyridazine
- sulfapyridine

Pathology

Although the lesions caused by this condition are unique, they may be mistaken as provoked by another condition. Dermatitis herpetiformis is not a condition that may be self-diagnosed and differential diagnosis is made based on a skin biopsy and immunologic tests.

Pathologically, the first signs of the condition may be observed within the dermis. The changes that can take place at this level may include edema, vascular dilatation and cellular infiltration. Commonly, lymphocytes and eosinophils can be seen. The bullae found in the skin affected by dermatitis herpetiformis are subepidermal and have rounded lateral borders.

When looked at under the microscope, the skin affected by dermatitis herpetiformis presents a collection of cells known as neutrophils. They have an increased prevalence in the areas where the dermis and is the closest to the epidermis.

Direct IMF studies of uninvolved skin show IgA in the dermal papillae and patchy granular IgA along the basement membrane. The jejunal mucosa may show partial villous atrophy but the changes tend to be milder than in coeliac disease.

Immunological studies revealed findings that are similar to coeliac disease in terms of autoantigens. The main autoantigen of dermatitis herpetiformis is epidermal transglutaminase (eTG), a cytosolic enzyme involved in cell envelope formation during keratinocyte differentiation.

Various research studies have pointed out different potential factors that may play a larger or smaller role in the development of dermatitis herpetiformis. The fact that eTG has been found in precipitates of skin-bound IgA from skin affected by this condition has been used to conclude that dermatitis herpetiformis may be caused by a deposition of both IgA and eTG within the dermis. It is estimated that these deposits may resorb after 10 years of following a gluten-free diet. Moreover, it is suggested that this condition is closely linked to genetics. This theory is based on the arguments that individuals with a family history of gluten sensitivity who still consume foods that contain gluten are more likely to develop the condition as a result of the formation of antibodies to gluten. These antibodies cross-react with eTG, and IgA/eTG complexes deposit within the papillary dermis to cause the lesions of dermatitis herpetiformis. These IgA deposits can disappear after long-term (up to 10 years) avoidance of dietary gluten. Genetics are thought to play an important role in the development of the condition, as well as environmental factors. Monozygotic twins, for example, have an increased risk of developing dermatitis herpetiformis. It has been shown that androgen levels may affect the immune system in terms of suppression, leading to a decreased immunity. Some specialists argue that low levels of androgens may trigger this condition.

Complications

DH is an autoimmune disease, and patients with DH are more likely than others to have thyroid problems and intestinal lymphoma.

Dermatitis herpetiformis does not usually cause complications on its own, without being associated with another condition. Complications from this condition however arise from the autoimmune character of the disease as an over-reacting immune system is a sign that

something does not work well and might cause problems to other parts of the body which do not necessarily involve the digestive system.

Gluten intolerance and the body's reaction to it are what make the disease more worrying in what concerns the possible complications. This means that complications that may arise from dermatitis herpetiformis are the same with those resulted from coeliac disease and they include osteoporosis, certain kinds of gut cancer and an increased risk of other autoimmune diseases such as thyroid disease.

The risks of developing complications from dermatitis herpetiformis decrease significantly if the patients follow a gluten-free diet. The disease has been associated with auto-immune thyroid disease, insulin dependent diabetes, lupus erythematosus, Sjögren's syndrome, sarcoidosis, and vitiligo or alopecia areata.

Notable cases

It has been suggested that French revolutionary Jean-Paul Marat suffered from DH, leading him to spend much of his time in, and even work from, a bathtub filled with an herbal mixture that he used to calm the sores.

Chapter 19

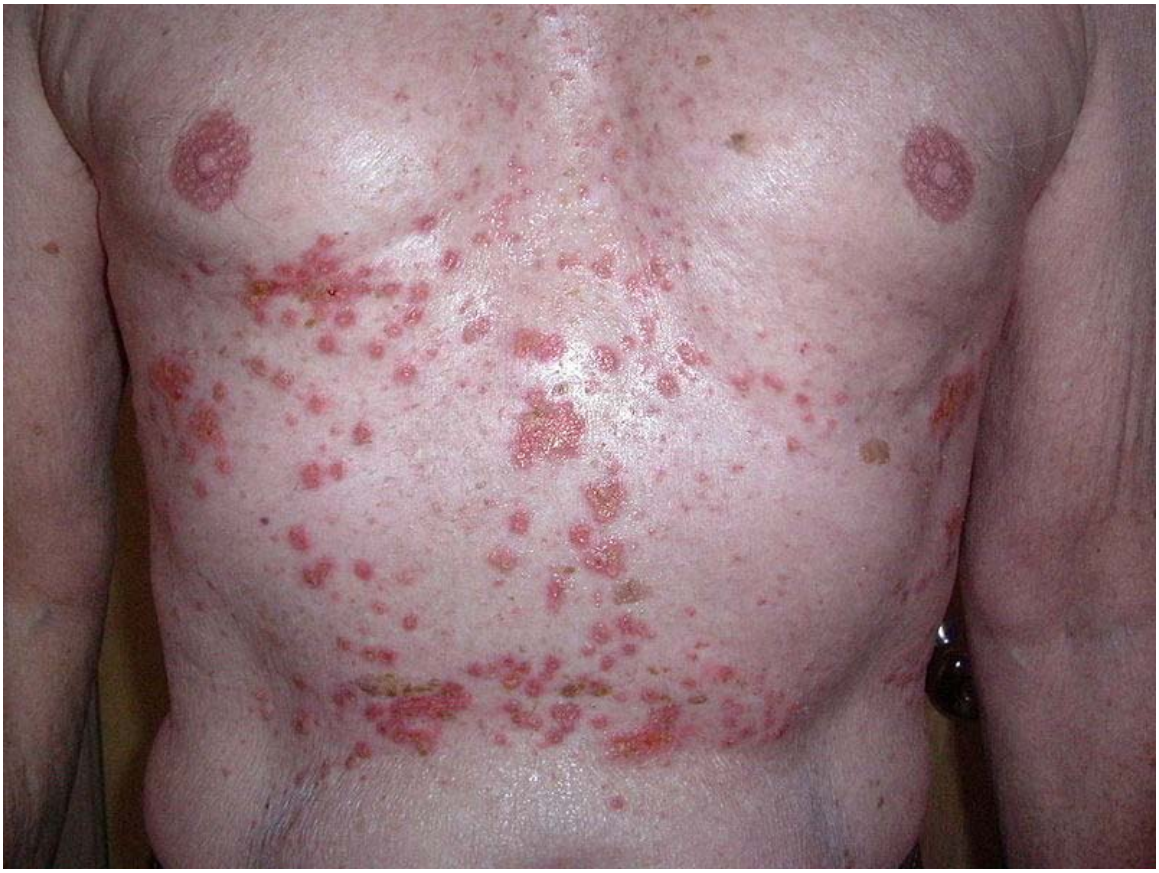
Transient Acantholytic Dermatosis and Pemphigus Vulgaris

Transient acantholytic dermatosis

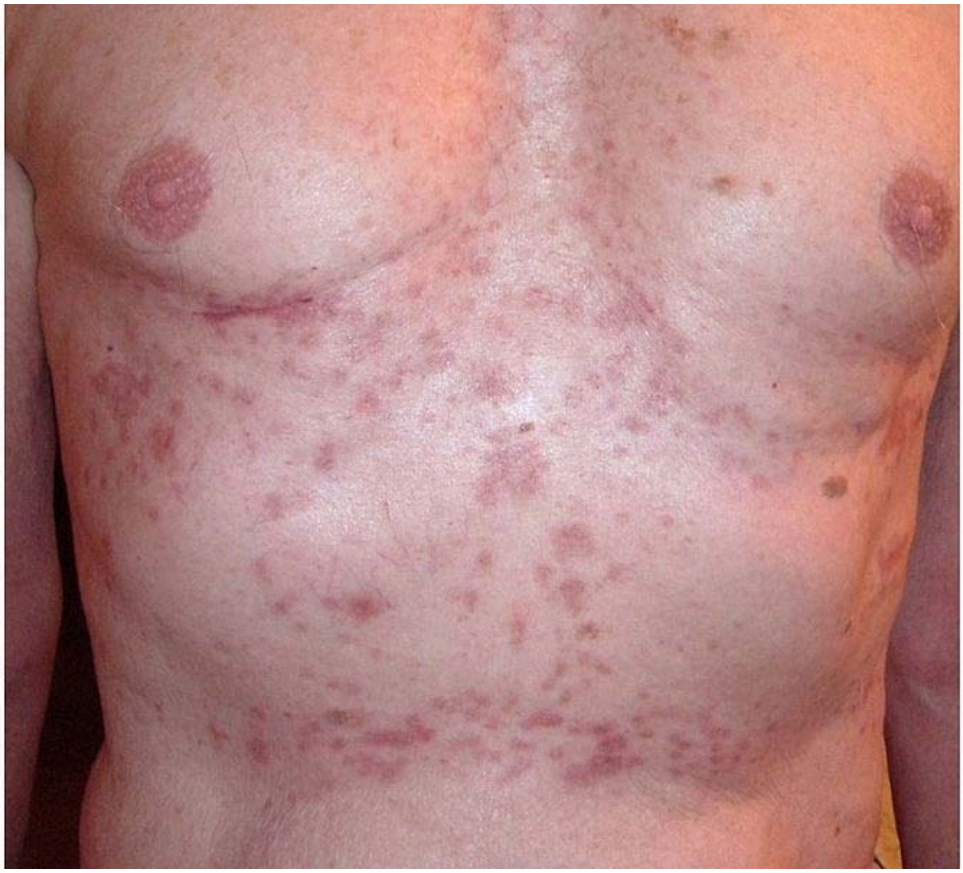
Grover's disease	
ICD-10	L11.1
ICD-9	694.8
DiseasesDB	32883
eMedicine	article/1124347

"In 1970, Ralph Grover presented a series of six patients with a pruritic papular and/or papulovesicular rash upon the trunk that cleared within weeks. Histopathologic analysis revealed a characteristic pattern of acantholysis within the epidermis, and Grover named the disease "transient acantholytic dermatosis". Subsequent reports described an often chronic course, but essentially identical histologic findings and the term "transient and persistent acantholytic dermatosis" was proposed. However, the eponym, "Grover's disease," remains in wide use."**Grover's disease** (also known as "Benign papular acantholytic dermatosis," "Persistent acantholytic dermatosis," and "Transient acantholytic dermatosis") is a polymorphic, pruritic, papulovesicular dermatosis characterized histologically by acantholysis.

Symptoms



Advanced case, third month



Advanced case, fifth month

Grover's disease often starts quite suddenly. It results in very itchy spots on the central back, mid chest and occasionally elsewhere. Frequently, it follows sweating or some unexpected heat stress.

Symptoms of Grover's disease are characterized by an itchy eruption that may last an average of 10–12 months. It is characterized by papules and papulovesicles with excoriations occurring on the chest, back, lower sternum, arms, and thighs. Grover's Disease is mainly seen in males over the age of forty and the papules are most commonly found on the mid chest.

Sometimes the features of Grover's are found in people who do not itch or have a conspicuous rash. Most of the people with Grover's who visit a dermatologist, however, itch a lot. Grover's disease (GD), or transient acantholytic dermatosis, is a pruritic, papulovesicular eruption characterized histopathologically by acantholysis with or without dyskeratosis.

Epidemiology

The prevalence and incidence of Grover's disease have not been firmly established. In a study from Switzerland, Grover's disease was diagnosed in just 24 of more than 30,000 skin biopsies.

It is thought that Grover's disease affects chiefly white adults in the fifth decade or later, and appears to be around 1.6 to 2.1 times more common in men than in women. Grover's disease appears less commonly in darker-skinned individuals, but has been reported.

Etiology

The etiology of Grover's disease is unknown. Suspected triggers of disease activity include heat and sweating, sunlight, ionizing irradiation, end-stage renal disease/hemodialysis, and mechanical irritation or prolonged bed rest.

Some cases of Grover's disease have been associated with medications such as sulfadoxine-pyrimethamine, ribavirin, cetuximab, and interleukin-4 [1,8-15]. One series of 300 patients with Grover's disease reported an association with other coexisting dermatoses including atopic dermatitis, contact dermatitis, and xerosis cutis. Finally, smaller series have detailed an association with pyoderma gangrenosum, bacterial and viral infections, and occasionally, malignancies.

Diagnosis

Grover's may be suspected by its appearance, but since it has such a characteristic appearance under the microscope a shave skin or punch biopsy is often performed. Once confirmed, most cases of Grover's disease last six to twelve months (which is why it was originally called "transient"). Unfortunately it may last much longer.

Treatment

The most important thing about Grover's disease treatment is to remain cool, as further sweating will induce more itchy spots. However, lesions aggravated by sweat usually return to "normal" fairly quickly — avoiding sweat is not a reason to avoid exercise. Minor outbreaks can be controlled with prescription strength topical cortisone creams. More troubling eruptions usually clear up after treatment for one to three months with Accutane or tetracycline. If these fail or the outbreak is severe, PUVA phototherapy treatments, antifungal pills and cortisone injections are alternatives.

A further treatment option is a cream of zinc oxide, talc, and glycerol. This cream helps with the itching and promotes faster healing. In France, where it is available over the counter (OTC), the zinc oxide, talc, and glycerol cream is branded as Aloplastine.

Sometimes, Grover's disease can be complicated by the development of dermatitis. (Non sequitur mixing cause with complications) Although the cause of Grover's is unknown, it may arise in quite dry skin. Many affected individuals are sun damaged.

Some research has correlated damage to the basement membrane and mercury toxicity with Grover's disease. Damage to the basement membrane might be from sun damage, age, or other factors. Mercury in the body causes the cellular reaction that we know as Grover's disease. The recommended treatment explained here is with Chemet. After treatment, subsequent flaring may be caused by the release of mercury stored deeper in the body or from mercury that comes into the body from our environment. Both require the patient to be vigilant about maintaining an internal environment that is as free as possible from mercury.

Pemphigus vulgaris

Pemphigus vulgaris	
ICD-10	L10.0
OMIM	169610
DiseasesDB	9764

Pemphigus vulgaris is a chronic blistering skin disease with skin lesions that are rarely pruritic, but which are often painful.

It is an autoimmune disease caused by antibodies directed against both desmoglein 1 and desmoglein 3 resulting in the loss of cohesion between keratinocytes in the epidermis. It is characterized by extensive flaccid blisters and mucocutaneous erosions. The severity of the disease, as well as the mucosal lesions, is believed to be directly proportional to the levels of desmoglein 3. Milder forms of pemphigus (like foliacious and erythematoses) are more desmoglein 1 heavy. It arises most often in middle-aged or older people, usually starting with a blister that ruptures easily. The lesions can become quite extensive. The pathogenesis of the disease involves autoantibodies against desmosome proteins, separating keratinocytes from the basal layer of the epidermis. On histology, the basal keratinocytes are usually still attached to the basement membrane leading to the appearance and thus the term, "tombstoning". Transudative fluid accumulates in between, forming a blister. This is a contrasting feature from bullous pemphigoid, where the detachment occurs between the epidermis and dermis (subepidermal bullae).

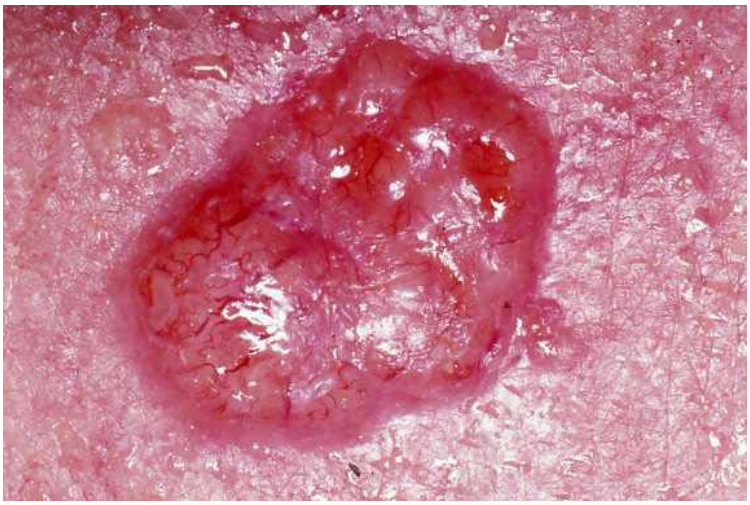
Corticosteroids and other immunosuppressive drugs are the mainstay of treatment. IVIG, rituximab, cellcept, methotrexate, imuran, and cytoxan have also been used with varying degrees of success. It is a difficult disease to control.

Pemphigus vulgaris is easy to confuse with impetigo and candidiasis. The gold standard for diagnosis is a punch biopsy of the lesion with direct immunofluorescent staining. IgG4 is considered pathogenic. The diagnosis can be confirmed by testing for the infections that cause these other conditions, and by a lack of response to antibiotic treatment.

Chapter 20

Skin Neoplasm

Skin cancer



A basal cell carcinoma. Note the pearly appearance and telangiectasia.

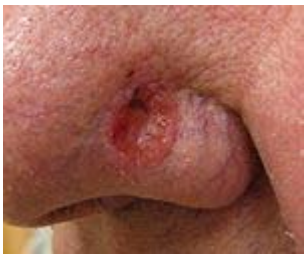
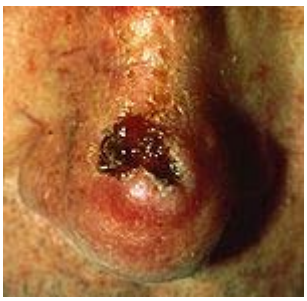
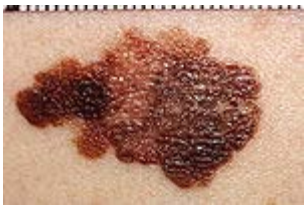
ICD-10	C43.-C44.
ICD-9	172, 173
ICD-O:	8010-8720
MeSH	D012878

Skin neoplasms are skin growths with differing causes and varying degrees of malignancy. The three most common malignant skin cancers are basal cell cancer, squamous cell cancer, and melanoma, each of which is named after the type of skin cell from which it arises. Skin cancer generally develops in the epidermis (the outermost layer of skin), so a tumor is usually clearly visible. This makes most skin cancers detectable in the early stages. Unlike many other cancers, including those originating in the lung, pancreas, and stomach, only a small minority of those afflicted will actually die of the disease. In fact, though it can be disfiguring, except for melanoma, skin cancer is rarely fatal. Skin cancer represents the most commonly diagnosed cancer, surpassing lung,

breasts, colorectal, and prostate cancer. Melanoma is less common than both basal cell carcinoma and squamous cell carcinoma, but it is the most serious—for example, in the UK there are 9,500 new cases of melanoma each year, and 2,300 deaths. It is the most common cancer in the young population (20 – 39 age group). Most cases are caused by long periods of exposure to the sun. Non-melanoma skin cancers are the most common skin cancers. The majority of these are basal cell carcinomas. These are usually localized growths caused by excessive cumulative exposure to the sun and do not tend to spread.

Classification

The three most common types of skin cancers are:

Cancer	Description	Illustration
Basal cell carcinoma	Note the fleshy color, symmetrical nature, and ulceration which are characteristic.	
Squamous cell carcinoma	Commonly presents as a red, crusted, or scaly patch.	
Malignant melanoma	The common appearance is an asymmetrical area, with an irregular border, color variation, and greater than 6 mm diameter.	

Basal cell carcinomas are present on sun-exposed areas of the skin, especially the face. They rarely metastasize and rarely cause death. They are easily treated with surgery or radiation. Squamous cell carcinomas (SCC) are common, but much less common than basal cell cancers. They metastasize more frequently than BCCs. Even then, the metastasis rate is quite low, with the exception of SCCs of the lip, ear, and in immunosuppressed patients. Melanomas are the least frequent of the 3 common skin cancers. They frequently metastasize, and could potentially cause death once they spread.

Less common skin cancers include: Dermatofibrosarcoma protuberans, Merkel cell carcinoma, Kaposi's sarcoma, keratoacanthoma, spindle cell tumors, sebaceous

carcinomas, microcystic adnexal carcinoma, Pagets's disease of the breast, atypical fibroxanthoma, leimyosarcoma, and angiosarcoma.

The BCC and the SCC often carry a UV-signature mutation indicating that these cancers are caused by UV-B radiation via the direct DNA damage. However the malignant melanoma is predominantly caused by UV-A radiation via the indirect DNA damage. The indirect DNA damage is caused by free radicals and reactive oxygen species. Research indicates that the absorption of three sunscreen ingredients into the skin, combined with a 60-minute exposure to UV, leads to an increase of free radicals in the skin, if applied in too little quantities and too infrequently. However, the researchers add that newer creams often do not contain these specific compounds, and that the combination of other ingredients tends to retain the compounds on the surface of the skin. They also add the frequent re-application reduces the risk of radical formation.

Skin cancer as a group

There are three main distinct types of skin cancer: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma. They are individuated for a number of reasons:

- the mechanism that generates the first two forms is different from the mechanism that generates melanoma. The direct DNA damage is responsible for BCC and SCC while the indirect DNA damage causes melanoma.
- the mortality rate of BCC and SCC is around 0.3% causing 2000 deaths per year in the US. In comparison the mortality rate of melanoma is 15-20% and it causes 6500 deaths per year.

Even though it is much less common than BCCs and SCCs, malignant melanoma is responsible for 75% of all skin cancer-related deaths.

While sunscreen has been shown to protect against BCC and SCC it may not protect against malignant melanoma. When sunscreen penetrates into the skin it generates reactive chemicals. The experimental and epidemiological evidence suggests that sunscreen use is correlated with malignant melanoma incidence. This gives rise to questions regarding the possibility that a sunscreen user's lifetime exposure to ultraviolet light may be higher than average. Alternatively, one might question whether sun screens are themselves tumor promoters or carcinogens. Arguably, sunscreen users are the ones most likely to be burned or have been burned by sun light. Similarly, most sunscreens primarily screen UVB, the primary cause of sunburn, while UVA is the primary cause of melanoma. Thus, by limiting the discomfort of sunburn, UVB screening may indirectly result in more UVA exposure. In any case, if some sunscreens promote skin cancer, physical light-scattering sunscreens based in zinc oxide, titanium dioxide or some other natural base are likely safer than chemical blockers such as benzones, etc., as they will be less chemically active.

Signs and symptoms

There are a variety of different skin cancer symptoms. These include changes in the skin that do not heal, ulcering in the skin, discolored skin, and changes in existing moles, such as jagged edges to the mole and enlargement of the mole.

- *Basal cell carcinoma* usually presents as a raised, smooth, pearly bump on the sun-exposed skin of the head, neck or shoulders. Sometimes small blood vessels can be seen within the tumor. Crusting and bleeding in the center of the tumor frequently develops. It is often mistaken for a sore that does not heal. This form of skin cancer is the least deadly and with proper treatment can be completely eliminated, often without scarring.
- *Squamous cell carcinoma* is commonly a red, scaling, thickened patch on sun-exposed skin. Some are firm hard nodules and dome shaped like keratoacanthomas. Ulceration and bleeding may occur. When SCC is not treated, it may develop into a large mass. Squamous cell is the second most common skin cancer. It is dangerous, but not nearly as dangerous as a melanoma.
- Most *melanomas* are brown to black looking lesions. Unfortunately, a few melanomas are pink, red or fleshy in color; these are called amelanotic melanomas. These tend to be more aggressive. Warning signs of malignant melanoma include change in the size, shape, color or elevation of a mole. Other signs are the appearance of a new mole during adulthood or new pain, itching, ulceration or bleeding. An often-used mnemonic is "ABCD", where A= asymmetrical, B= "borders" (irregular= "Coast of Maine sign"), C= "color" (variegated) and D= "diameter" (larger than 6 mm—the size of a pencil eraser).
- *Merkel cell carcinomas* are most often rapidly growing, non-tender red, purple or skin colored bumps that are not painful or itchy. They may be mistaken for a cyst or other type of cancer.

Causes

Skin cancer has many potential causes. Examples include:

1. Smoking tobacco and related products can double the risk of skin cancer.
2. Overexposure to UV-radiation may cause skin cancer either via the direct DNA damage or via the indirect DNA damage mechanism. Overexposure (burning) UVA & UVB have both been implicated in causing DNA damage resulting in cancer. Because UVB is highly absorbed by the atmosphere, UVB between 10AM and 4PM is most intense. Natural (sun) & artificial UV exposure (tanning salons) are possibly associated with skin cancer.
    1. UVB rays primarily affect the epidermis causing sunburns, redness, and blistering of the skin when overexposed. The melanin of the epidermis is activated with UVB just as with UVA; however, the effects are longer lasting with pigmentation continuing over 24 hours.
3. Chronic non-healing wounds, especially burns. These are called Marjolin's ulcers based on their appearance, and can develop into squamous cell carcinoma.

4. Genetic predisposition, including "Congenital Melanocytic Nevi Syndrome". CMNS is characterized by the presence of "nevi" or moles of varying size that either appear at or within 6 months of birth. Nevi larger than 20 mm (3/4") in size are at higher risk for becoming cancerous.
5. Human papilloma virus (HPV) is often associated with squamous cell carcinoma of the genitals, anus, mouth, pharynx, and fingers.
6. Skin cancer is one of the potential dangers of ultraviolet germicidal irradiation.
7. Deficiencies in certain vitamins and minerals.
8. Arsenic poisoning is associated with an increased incidence of squamous cell carcinoma.

A 2010 study has found a relation between HPV infection and incidence of squamous cell carcinoma.

Prevention

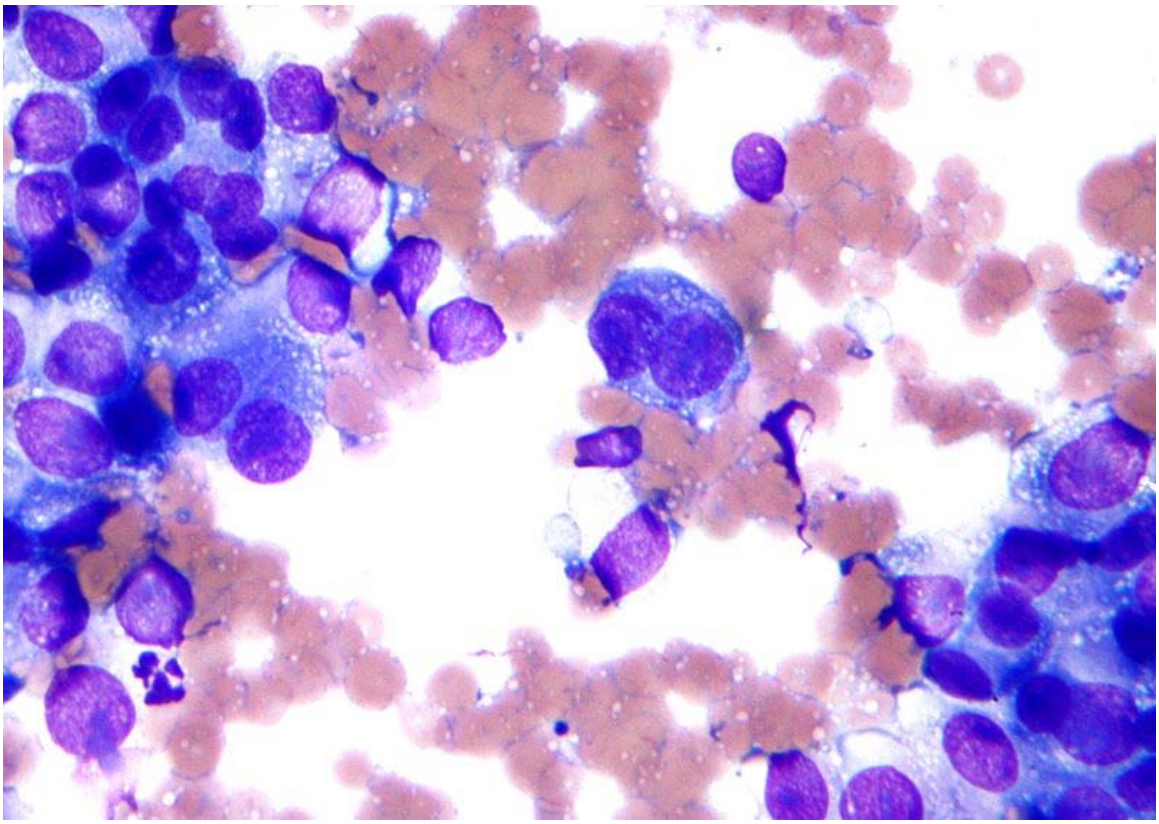
Although it is impossible to completely eliminate the possibility of skin cancer, the risk of developing such a cancer can be reduced significantly with the following steps:

- Avoid the use of tobacco products.
- Reducing overexposure to ultraviolet (UV) radiation, especially in early years
- Avoiding sun exposure during the peak UV times during the day, typically from 10 AM to 3 PM (dependent on country) when the sun is directly overhead
- Wearing protective clothing (long sleeves and hats) when outdoors
- Using a broad-spectrum sunscreen that blocks both UVA and UVB radiation
- Reapply sun block as per the manufacturers directions

Australian scientist Ian Frazer who developed a vaccine for cervical cancer, says that a vaccine effective in preventing for certain types of skin cancer has proven effective on animals and could be available within a decade. The vaccine would only be effective against Squamous Cell Carcinoma.

Primary health care providers should examine their patients during the course of a routine comprehensive physical examination by means of a full body screening (all areas of the body's skin surface are examined, with the use of a special light and a magnifying glass, for abnormal masses, lesions, and cancerous neoplasms like BCC, SCC, and MM). Referrals or visits to a dermatologist will usually include this as a first part of the examination. Many times, hospitals, doctor's offices, and dermatologist's offices will perform these for the general public as part of a mass screening program done at certain times during the year, and these are usually free or are low-priced and thus are often very popular. If necessary, skin cells from the outer epidermis can be scraped off or an actual biopsy performed, and the results examined for pathologies.

Pathology



Micrograph of melanoma. FNA specimen. Field stain.

Squamous cell carcinoma is a malignant epithelial tumor which originates in epidermis, squamous mucosa or areas of squamous metaplasia.

Macroscopically, the tumor is often elevated, fungating, or may be ulcerated with irregular borders. Microscopically, tumor cells destroy the basement membrane and form sheets or compact masses which invade the subjacent connective tissue (dermis). In well differentiated carcinomas, tumor cells are pleomorphic/atypical, but resembling normal keratinocytes from prickle layer (large, polygonal, with abundant eosinophilic (pink) cytoplasm and central nucleus). Their disposal tends to be similar to that of normal epidermis: immature/basal cells at the periphery, becoming more mature to the centre of the tumor masses. Tumor cells transform into keratinized squamous cells and form round nodules with concentric, laminated layers, called "cell nests" or "epithelial/keratinous pearls". The surrounding stroma is reduced and contains inflammatory infiltrate (lymphocytes). Poorly differentiated squamous carcinomas contain more pleomorphic cells and no keratinization.

Management

Treatment is dependent on type of cancer, location of the cancer, age of the patient, and whether the cancer is primary or a recurrence. One should look at the specific type of

skin cancer (basal cell carcinoma, squamous cell carcinoma, or melanoma) of concern in order to determine the correct treatment required. An example would be a small basal cell cancer on the cheek of a young man, where the treatment with the best cure rate (Mohs surgery or CCPDMA) might be indicated. In the case of an elderly frail man with multiple complicating medical problems, a difficult to excise basal cell cancer of the nose might warrant radiation therapy (slightly lower cure rate) or no treatment at all. Topical chemotherapy might be indicated for large superficial basal cell carcinoma for good cosmetic outcome, whereas it might be inadequate for invasive nodular basal cell carcinoma or invasive squamous cell carcinoma.. In general, melanoma is poorly responsive to radiation or chemotherapy.

For low-risk disease, radiation therapy (external beam radiotherapy or brachytherapy), topical chemotherapy (imiquimod or 5-fluorouracil) and cryotherapy (freezing the cancer off) can provide adequate control of the disease; both, however, may have lower overall cure rates than certain type of surgery. Other modalities of treatment such as photodynamic therapy, topical chemotherapy, electrodessication and curettage can be found in the discussions of basal cell carcinoma and squamous cell carcinoma.

Mohs' micrographic surgery (Mohs surgery) is a technique used to remove the cancer with the least amount of surrounding tissue and the edges are checked immediately to see if tumor is found. This provides the opportunity to remove the least amount of tissue and provide the best cosmetically favorable results. This is especially important for areas where excess skin is limited, such as the face. Cure rates are equivalent to wide excision. Special training is required to perform this technique. An alternative method is CCPDMA and can be performed by a pathologist not familiar with Mohs surgery.

In the case of disease that has spread (metastasized), further surgical procedures or chemotherapy may be required.

Scientists have recently been conducting experiments on what they have termed "immune- priming". This therapy is still in its infancy but has been shown to effectively attack foreign threats like viruses and also latch onto and attack skin cancers. More recently researchers have focused their efforts on strengthening the body's own naturally produced "helper T cells" that identify and lock onto cancer cells and help guide the killer cells to the cancer. Researchers infused patients with roughly 5 billion of the helper T cells without any harsh drugs or chemotherapy. This type of treatment if shown to be effective has no side effects and could change the way cancer patients are treated.

A cream used to treat pre-cancerous skin lesions also reverses signs of aging, a study released in April 2009 indicated. In March 2010 academics from Dundee University in Scotland announced they had devised a new, less-painful method of treating skin cancer, which could be administered from the home.

Post surgery reconstruction

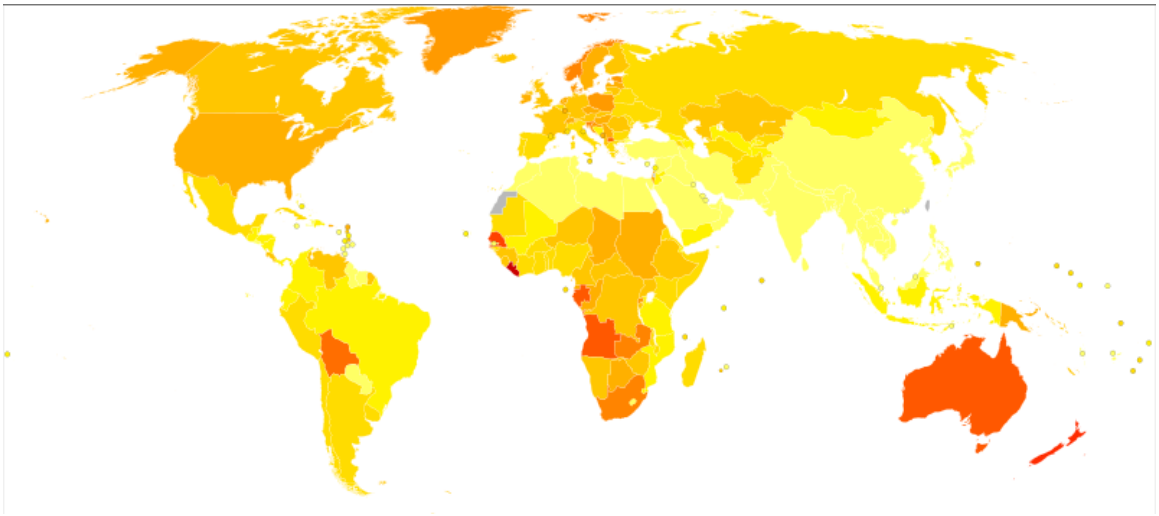
Currently, surgical excision is the most common form of treatment for skin cancers. The goal of reconstructive surgery is restoration of normal appearance and function. The choice of technique in reconstruction is dictated by the size and location of the defect. Excision and reconstruction of facial skin cancers is generally more challenging due to presence of highly visible and functional anatomic structures in the face.

When skin defects are small in size, most can be repaired with simple repair where skin edges are approximated and closed with sutures. This will result in a linear scar. If the repair is made along a natural skin fold or wrinkle line, the scar will be hardly visible. Larger defects may require repair with a skin graft, local skin flap, pedicled skin flap, or a microvascular free flap. Skin grafts and local skin flaps are by far more common than the other listed choices.

Skin grafting is patching of a defect with skin that is removed from another site in the body. The skin graft is sutured to the edges of the defect, and a bolster is placed atop the graft for seven to ten days, to immobilize the graft as it heals in place. There are two forms of skin grafting: split thickness and full thickness. In a split thickness skin graft, a shaver is used to shave a layer of skin from the abdomen or thigh. The donor site, regenerates skin and heals over a period of two weeks. In a full thickness skin graft, a segment of skin is totally removed and the donor site needs to be sutured closed. Split thickness grafts can be used to repair larger defects, but the grafts are inferior in their cosmetic appearance. Full thickness skin grafts are more acceptable cosmetically. However, full thickness grafts can only be used for small or moderate sized defects.

Local skin flaps are a method of closing defects with tissue that closely matches the defect in color and quality. Skin from the periphery of the defect site is mobilized and repositioned to fill the deficit. Various forms of local flaps can be designed to minimize disruption to surrounding tissues and maximize cosmetic outcome of the reconstruction. Pedicled skin flaps are a method of transferring skin with an intact blood supply from a nearby region of the body. An example of such reconstruction is a pedicled forehead flap for repair of a large nasal skin defect. Once the flap develops a source of blood supply form its new bed, the vascular pedicle can be detached.

Epidemiology



Age-standardized death from melanoma and other skin cancers per 100,000 inhabitants in 2004.

- no data
- less than 0.7
- 0.7-1.4
- 1.4-2.1
- 2.1-2.8
- 2.8-3.5
- 3.5-4.2
- 4.2-4.9
- 4.9-5.6
- 5.6-6.3
- 6.3-7
- 7-7.7
- more than 7.7

A study of the incidence of non-melanoma skin cancer from 1992 to 2006 in the United States was performed by the dermatologist Howard Rogers, MD, PhD, and his colleagues based on the evaluation of Medicare databases. The results of their research showed that cases of non-melanoma skin cancer rose an average of 4.2% a year.

More than 3.5 million cases of skin cancer are diagnosed annually in the United States, which makes it the most common form of cancer in that country. According to the Skin Cancer Foundation, one in five Americans will develop skin cancer at some point of their lives. The first most common form of skin cancer is basal cell carcinoma, followed by the squamous cell carcinoma. Although the incidence of many cancers in the United States is falling, the incidence of melanoma keeps growing, with approximately 68,729 melanomas diagnosed in 2004 according to reports of the National Cancer Institute.

The survival rate for patients with melanoma depends upon when they start treatment. The cure rate is very high when melanoma is detected in early stages, when it can easily be removed surgically. The prognosis is less favorable if the melanoma has spread to other parts of the body.

In the UK, 84,500 non-melanoma skin cancers were registered in 2007 although a study estimated that at least 100,000 cases are diagnosed each year. Most NMSCs were basal cell carcinomas or squamous cell carcinomas. In 2007, 10,672 cases of malignant melanoma were diagnosed.

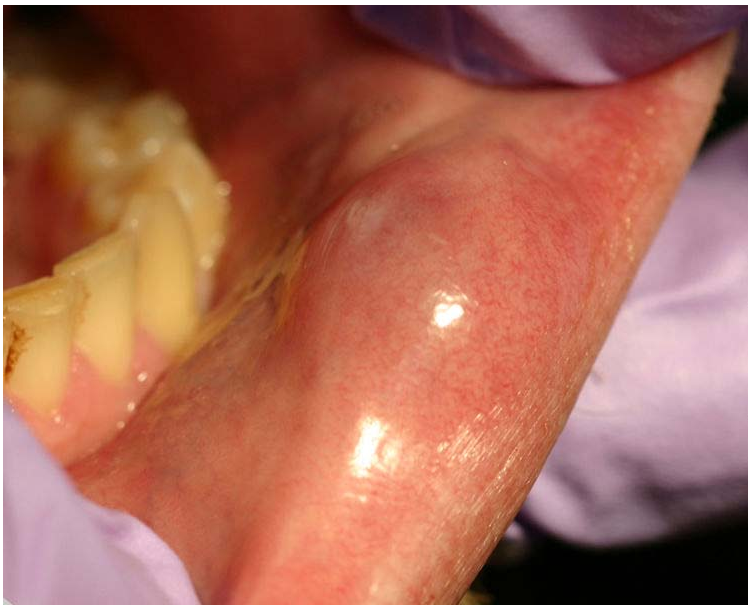
According to the British Association of Dermatologists children, from 0 to 14 years, and teenagers, from 15 to 19 years, exhibit the highest rates of skin cancers of any European country. Furthermore, incidence of melanoma increased four times in UK teenagers from 1978 to 1997.

Australia exhibits one of the highest rates of skin cancer incidence in the world, almost four times the rates registered in the United States, the UK and Canada. Around 434,000 people receive treatment for non-melanoma skin cancers and 10,300 are treated for melanoma. Melanoma is the common type of cancer in people between 15–44 years in Australia.

Chapter 21

Mucous Cyst of the Oral Mucosa and Ameloblastoma

Mucous cyst of the oral mucosa

Mucous cyst of the oral mucosa


A mucocele on the lower lip.

ICD-10	K11.6
ICD-9	527.6
DiseasesDB	30713
eMedicine	derm/274
MeSH	D009078

A "mucous cyst of the oral mucosa" (also known as a "mucocele") is a clinical term that refers to two related phenomena: **mucus extravasation phenomenon**, and **mucus retention cyst**. The former is a swelling of connective tissue consisting of collected mucin due to a ruptured salivary gland duct usually caused by local trauma, in the case of mucus extravasation phenomenon, and an obstructed salivary duct in the case of a mucus retention cyst. The mucocele has a bluish translucent color, and is more commonly found in children and young adults.

It can be considered a polyp or a cyst.

Locations

The most common location to find a mucocele is the surface of the lower lip. It can also be found on the inner side of the cheek (known as the buccal mucosa), on the anterior ventral tongue, and the floor of the mouth. When found on the floor of the mouth, the mucocele is referred to as a ranula. They are rarely found on the upper lip. As their name suggests they are basically mucus lined cysts and they can also occur in the Paranasal sinuses most commonly the frontal sinuses, the frontoethomidal region and also in the maxillary sinus. Sphenoid sinus involvement is extremely rare. When the lumen of the vermiform appendix gets blocked due to any factor, again a mucocele can form.

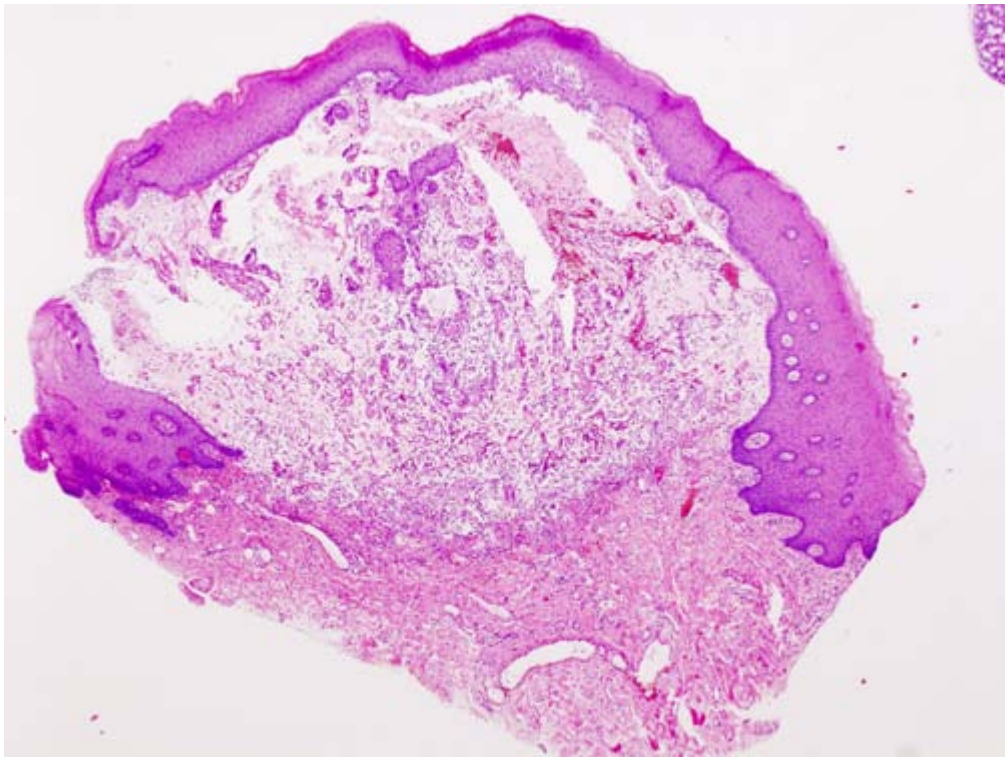
Characteristics

The size of oral mucoceles vary from 1 mm to several centimeters and they usually are slightly transparent with a blue tinge. On palpation, mucoceles may appear fluctuant but can also be firm. Their duration lasts from days to years, and may have recurrent swelling with occasional rupturing of its contents.

Variations

A variant of a mucocele is found on the palate, retromolar pad, and posterior buccal mucosa. Known as a "superficial mucocele", this type presents as single or multiple vesicles and bursts into an ulcer. Despite healing after a few days, superficial mucoceles recur often in the same location.

Histology



Histopathologic image of extravasation type mucocele of the lower lip. H & E stain.

Microscopically, mucoceles appears as granulation tissue surrounding mucin. Since inflammation occurs concurrently, neutrophils and foamy histiocytes usually are present.

Treatment

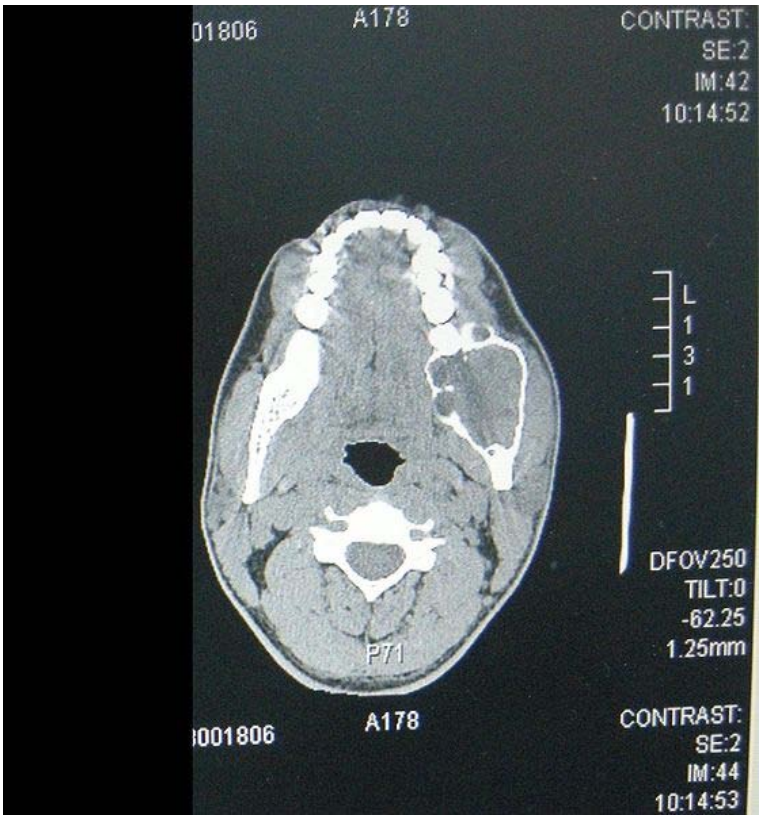
Some mucoceles spontaneously resolve on their own after a short time. Others are chronic and require surgical removal. Recurrence may occur, and thus the adjacent salivary gland is excised as a preventive measure.

Several types of procedures are available for the surgical removal of mucoceles. These include laser and minimally-invasive techniques which means recovery times are reduced drastically.

A non-surgical option that may be effective for a small or newly identified mucocle is to rinse the mouth thoroughly with salt water (one tablespoon of salt per cup) four to six times a day for a few days. This may draw out the fluid trapped underneath the skin without further damaging the surrounding tissue. If the mucocele persists, individuals should see a doctor to discuss further treatment.

Ameloblastoma

Ameloblastoma



A CT scan of a patient suffering from an ameloblastoma

ICD-10	D16.5
ICD-9	213.1
ICD-O:	9310/0
DiseasesDB	31676
MeSH	D000564

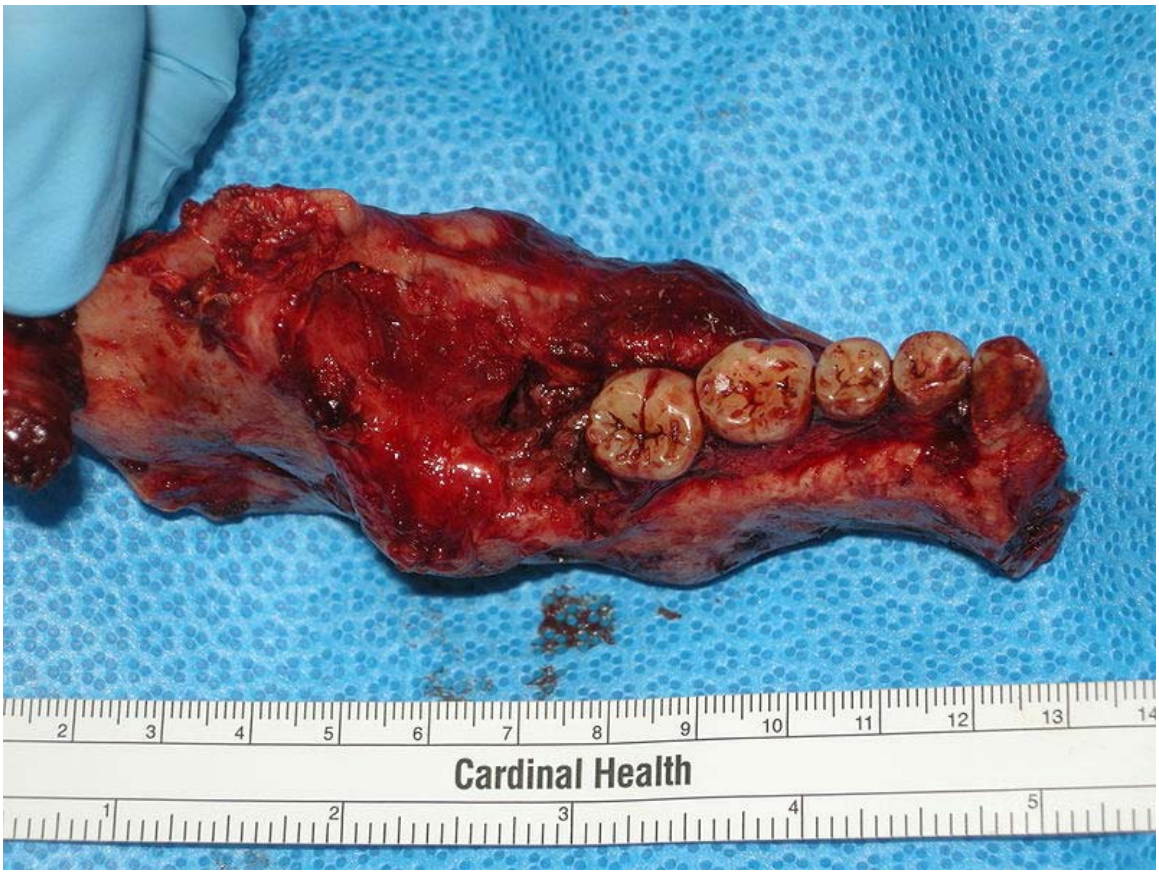
Ameloblastoma (from the early English word *amel*, meaning enamel + the Greek word *blastos*, meaning germ) is a rare, benign tumor of odontogenic epithelium (ameloblasts, or outside portion, of the teeth during development) much more commonly appearing in the mandible than the maxilla. It was recognized in 1827 by Cusack. This type of odontogenic neoplasm was designated as an *adamantinoma* in 1885 by the French physician Louis-Charles Malassez. It was finally renamed to the modern name *ameloblastoma* in 1930 by Ivey and Churchill.

While these tumors are rarely malignant or metastatic (that is, they rarely spread to other parts of the body), and progress slowly, the resulting lesions can cause severe abnormalities of the face and jaw. Additionally, because abnormal cell growth easily infiltrates and destroys surrounding bony tissues, wide surgical excision is required to treat this disorder.

Subtypes

There are three main clinical subtypes of ameloblastoma: unicystic, multicystic, peripheral. The peripheral subtype composes 2% of all ameloblastomas. Of all ameloblastomas in younger patients, unicystic ameloblastomas represent 6% of the cases. A fourth subtype, malignant, has been considered by some oncologic specialists, however, this form of the tumor is rare and may be simply a manifestation of one of the three main subtypes. Ameloblastoma also occurs in long bones, and another variant is Craniopharyngioma (Rathke's pouch tumour, Pituitary Ameloblastoma.)

Clinical features



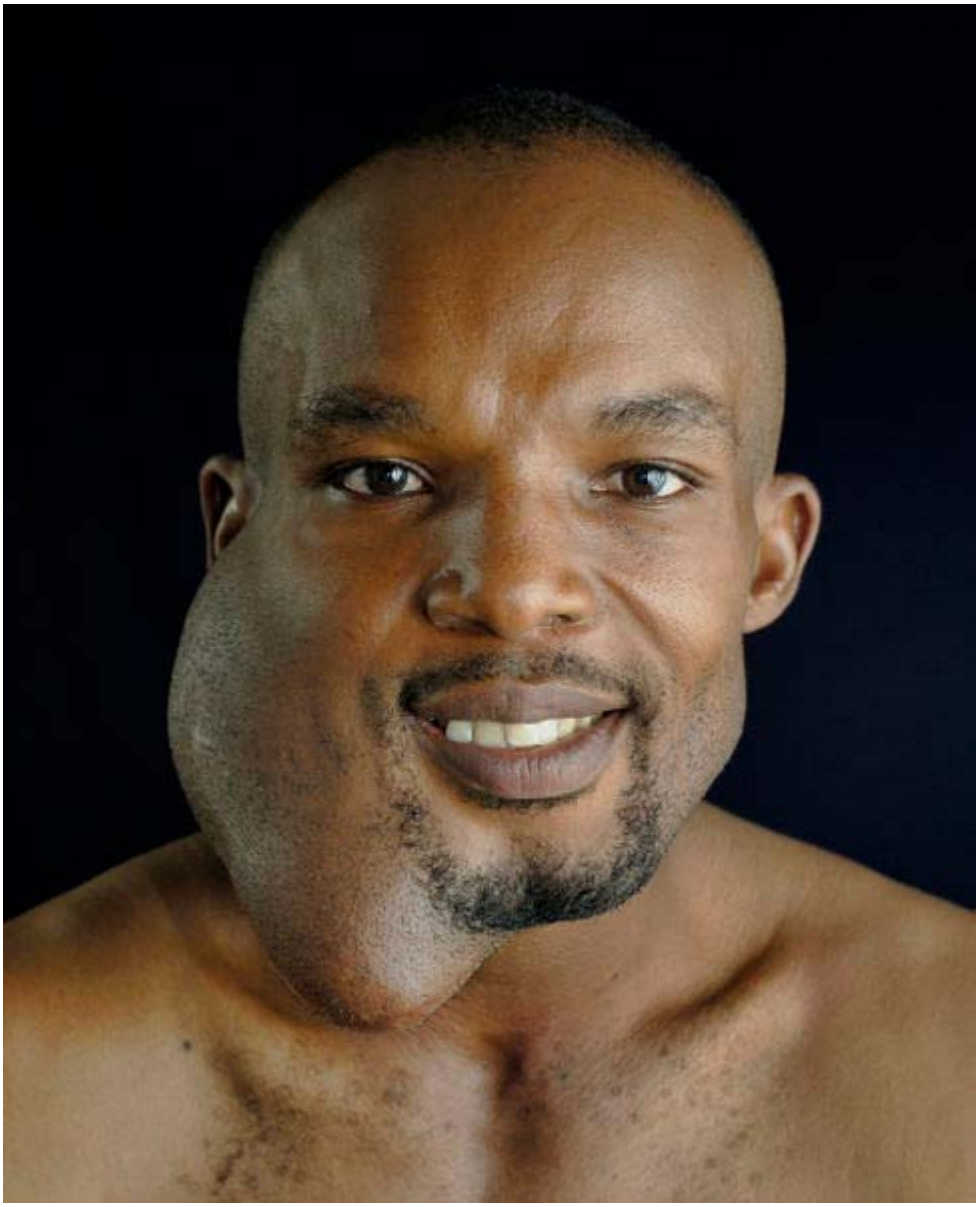
The resected left half of a mandible containing an ameloblastoma, initiated at the third molar

Ameloblastomas are often associated with the presence of unerupted teeth. Symptoms include painless swelling, facial deformity if severe enough, pain if the swelling impinges on other structures, loose teeth, ulcers, and periodontal (gum) disease. Lesions will occur in the mandible and maxilla,although 75% occur in the ascending ramus area and will result in extensive and grotesque deformitites of the mandible and maxilla. In the maxilla it can extend into the maxillary sinus and floor of the nose. The lesion has a tendency to expand the bony cortices because slow growth rate of the lesion allows time for periosteum to develop thin shell of bone ahead of the expanding lesion. This shell of bone cracks when palpated and this phenomenon is referred to as "Egg Shell Cracking" or crepitus, an important diagnostic feature. Ameloblastoma is tentatively diagnosed through radiographic examination and must be confirmed by histological examination (e.g., biopsy). Radiographically, it appears as a lucency in the bone of varying size and features—sometimes it is a single, well-demarcated lesion whereas it often demonstrates as a multiloculated "soap bubble" appearance. Resorption of roots of involved teeth can be seen in some cases, but is not unique to ameloblastoma. The disease is most often found in the posterior body and angle of the mandible, but can occur anywhere in either the maxilla or mandible.

Ameloblastoma is often associated with bony-impacted wisdom teeth—one of the many reasons dentists recommend having them extracted.

Histopathology

Histopathology will show cells that have the tendency to move the nucleus away from the basement membrane. This process is referred to as "Reverse Polarization". The follicular type will have outer arrangement of columnar or palisaded ameloblast like cells and inner zone of triangular shaped cells resembling stellate reticulum in bell stage. The central cells sometimes degenerate to form central microcysts. The plexiform type has epithelium that proliferates in a "Fish Net Pattern". The plexiform ameloblastoma shows epithelium proliferating in a 'cord like fashion', hence the name 'plexiform'. There are layers of cells in between the proliferating epithelium with a well-formed desmosomal junctions, simulating spindle cell layers.



Ameloblastoma

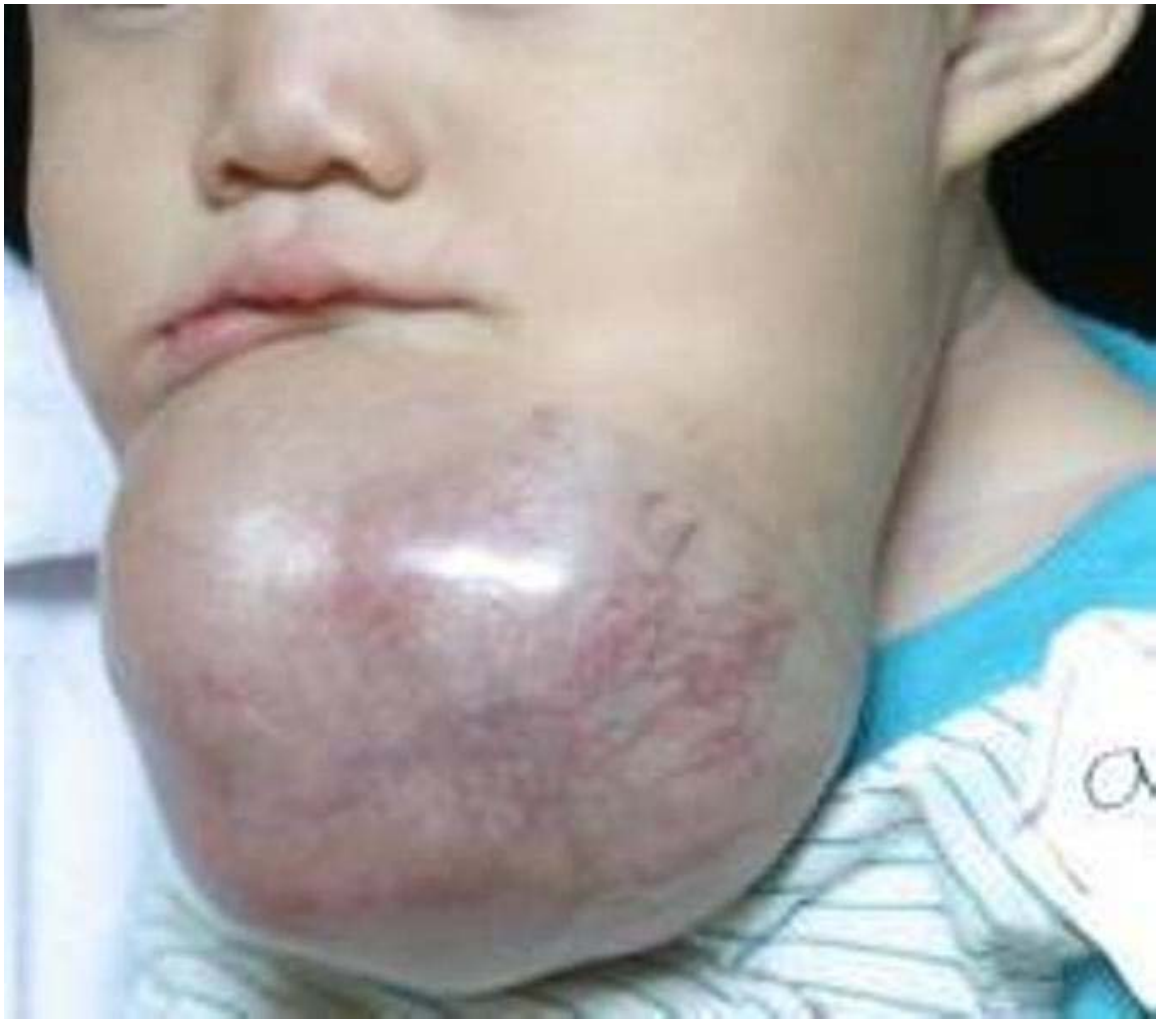
Variants

The six different histopathological variants of ameloblastoma are desmoplastic, granular cell, basal cell, plexiform, follicular, and acanthomatous.

The acanthomatous variant is extremely rare.

One-third of ameloblastomas are plexiform, one-third are follicular. Other variants such as acanthomatous occur in older patients. In one center, desmoplastic ameloblastomas represented about 9% of all ameloblastomas encountered.

Treatment



Tracheal intubation is anticipated to be difficult in this child with a massive ameloblastoma.

While chemotherapy, radiation therapy, curettage and liquid nitrogen have been effective in some cases of ameloblastoma, surgical resection or enucleation remains the most definitive treatment for this condition. In a detailed study of 345 patients, chemotherapy and radiation therapy seemed to be contraindicated for the treatment of ameloblastomas. Thus, surgery is the most common treatment of this tumor. Because of the invasive nature of the growth, excision of normal tissue near the tumor margin is often required. Some have likened the disease to basal cell carcinoma (a skin cancer) in its tendency to spread to adjacent bony and sometimes soft tissues without metastasizing. While not a cancer that actually invades adjacent tissues, ameloblastoma is suspected to spread to adjacent areas of the jaw bone via marrow space. Thus, wide surgical margins that are clear of disease are required for a good prognosis. This is very much like surgical treatment of cancer. Often, treatment requires excision of entire portions of the jaw.

Radiation is ineffective in many cases of ameloblastoma. There have also been reports of sarcoma being induced as the result of using radiation to treat ameloblastoma. Chemotherapy is also often ineffective. However, there is some controversy regarding this and some indication that some ameloblastomas might be more responsive to radiation that previously thought.

While the Mayo Clinic recommends surgery for almost all ameloblastomas, there are situations in which a Mayo Clinic physician might recommend radiation therapy. These include malignancy, inability to completely remove the ameloblastoma, recurrence, unacceptable loss of function, and unacceptable cosmetic damage. In the case of radiotherapy, oncologists at the Mayo Clinic would use intensity-modulated radiotherapy.

Molecular biology

There is evidence that suppression of matrix metalloproteinase-2 may inhibit the local invasiveness of ameloblastoma, however, this was only demonstrated *in vitro*. There is also some research suggesting that $\alpha_5\beta_1$ integrin may participate in the local invasiveness of ameloblastomas.

Recurrence

Recurrence is common, although the recurrence rates for block resection followed by bone graft are lower than those of enucleation and curettage. Follicular variants appear to recur more than plexiform variants. Unicystic tumors recur less frequently than "non-unicystic" tumors. Persistent follow-up examination is essential for managing ameloblastoma. Follow up should occur at regular intervals for at least 10 years. Follow up is important, because 50% of all recurrences occur within 5 years postoperatively. Recurrence within a bone graft (following resection of the original tumor) does occur, but is less common. Seeding to the bone graft is suspected as a cause of recurrence. The recurrences in these cases seem to stem from the soft tissues, especially the adjacent periosteum. Recurrence has been reported to occur as many as 36 years after treatment.

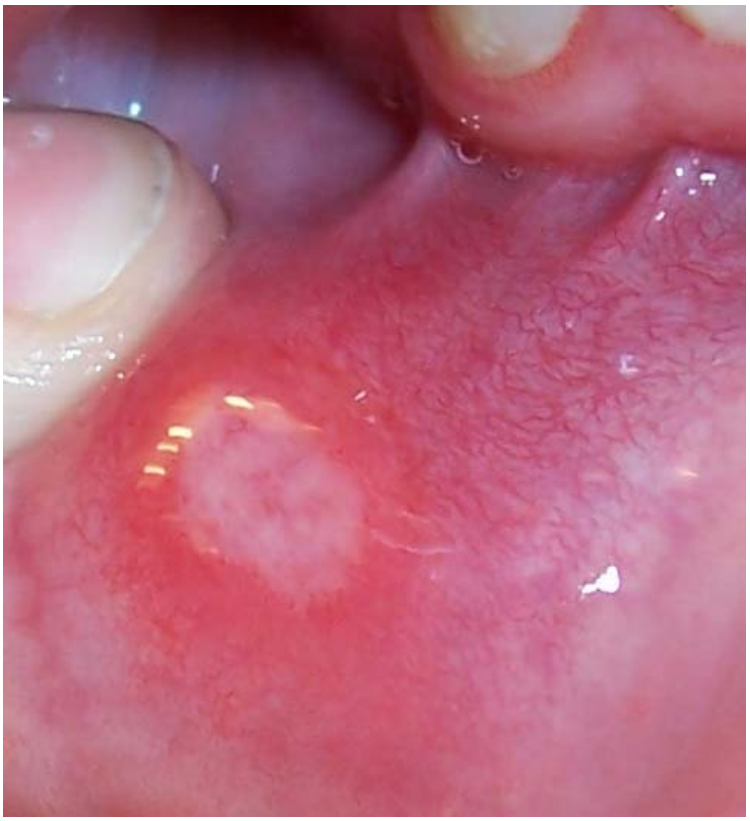
To reduce the likelihood of recurrence within grafted bone, meticulous surgery with attention to the adjacent soft tissues is required.

Epidemiology

The annual incidence rates per million for ameloblastomas are 1.96, 1.20, 0.18 and 0.44 for black males, black females, white males and white females respectively. Ameloblastomas account for about one percent of all oral tumors and about 18% of odontogenic tumors. Men and women tend to be equally affected, although women tend to be 4 years younger than men when tumors first occur and tumors appear to be larger in females.

Chapter 22

Aphthous Ulcer

Aphthous ulcer

Mouth ulcer on the lower lip

ICD-10	K12.0
ICD-9	528.2
MedlinePlus	000998
eMedicine	ent/700 derm/486 ped/2672
MeSH	D013281

An **aphthous ulcer** also known as a **canker sore**, is a type of mouth ulcer, appears as a painful open sore inside the mouth or upper throat characterized by a break in the mucous membrane. Its cause is unknown, but they are not contagious. The condition is also known as **aphthous stomatitis,** and alternatively as **Sutton's Disease**, especially in the case of major, multiple, or recurring ulcers.

The term ***aphtha*** means **ulcer;** it has been used for many years to describe areas of ulceration on mucous membranes. Aphthous stomatitis is a condition characterized by recurrent discrete areas of ulceration that are almost always painful. Recurrent aphthous stomatitis (RAS) can be distinguished from other diseases with similar-appearing oral lesions, such as certain oral bacteria or herpes simplex, by their tendency to recur, and their multiplicity and chronicity. Recurrent aphthous stomatitis is one of the most common oral conditions. At least 10% of the population has it, and women are more often affected than men. About 30–40% of patients with recurrent aphthae report a family history.

Classification

Aphthous ulcers are classified according to the diameter of the lesion.

Minor ulceration

"Minor aphthous ulcers" indicate that the lesion size is between 3 mm (0.1 in)-10 mm (0.4 in). The appearance of the lesion is that of an erythematous halo with yellowish or grayish color. Pain that affects quality of life is the obvious characteristic of the lesion. When the ulcer is white or grayish, the ulcer will be extremely painful and the affected lip may swell. They may last about 2 weeks.

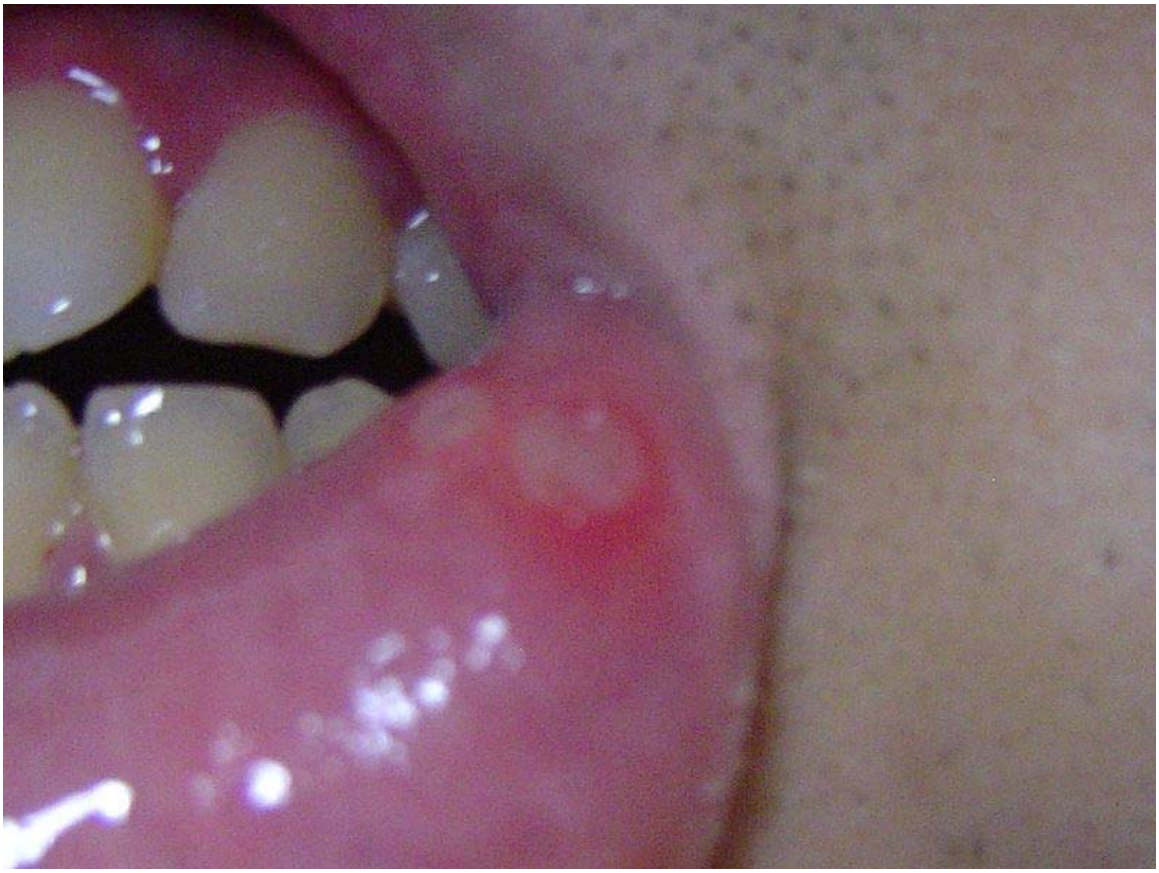
Major ulcerations

Major aphthous ulcers have the same appearance as minor ulcerations, but are greater than 10 mm in diameter and are extremely painful. They usually take more than a month to heal, and frequently leave a scar. These typically develop after puberty with frequent recurrences. They occur on movable non-keratinizing oral surfaces, but the ulcer borders may extend onto keratinized surfaces.

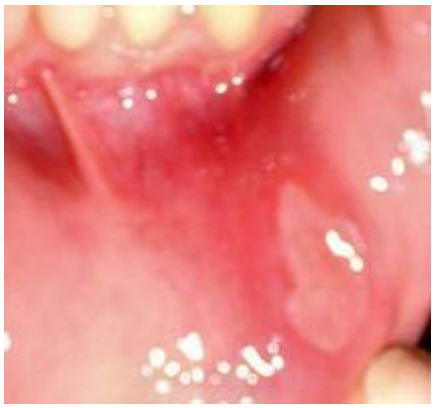
Herpetiform ulcerations

This is the most severe form. It occurs more frequently in females, and onset is often in adulthood. It is characterized by small, numerous, 1–3 mm lesions that form clusters. They typically heal in less than a month without scarring. Supportive treatment is almost always necessary.

Signs and symptoms



Apthous ulcer



Large aphthous ulcer on the lower lip

Aphthous ulcers usually begin with a tingling or burning sensation at the site of the future aphthous ulcer. In a few days, they often progress to form a red spot or bump, followed by an open ulcer.

The aphthous ulcer appears as a white or yellow oval with an inflamed red border. Sometimes a white circle or halo around the lesion can be observed. The gray-, white-, or yellow-colored area within the red boundary is due to the formation of layers of fibrin, a protein involved in the clotting of blood. The ulcer, which itself is often extremely painful, especially when agitated, may be accompanied by a painful swelling of the lymph nodes below the jaw, which can be mistaken for toothache; another symptom is fever. A sore on the gums may be accompanied by discomfort or pain in the teeth.

Causes

The exact cause of many aphthous ulcers is unknown but citrus fruits (e.g., oranges and lemons), physical trauma, stress, lack of sleep, sudden weight loss, food allergies, immune system reactions, and deficiencies in vitamin B_{12}, iron, and folic acid may contribute to their development. Nicorandil and certain types of chemotherapy are also linked to aphthous ulcers. One recent study showed a strong correlation with allergies to cow's milk. Aphthous ulcers are a major manifestation of Behçet disease, and are also common in people with Crohn's disease.

Trauma to the mouth is the most common trigger. Physical trauma, such as that caused by toothbrush abrasions, laceration with sharp or abrasive foods (such as toast, potato chips or other objects), accidental biting (particularly common with sharp canine teeth), after losing teeth, or dental braces can cause aphthous ulcers by breaking the mucous membrane. Other factors, such as chemical irritants or thermal injury, may also lead to the development of ulcers. Using a toothpaste without sodium lauryl sulfate (SLS) may reduce the frequency of aphthous ulcers One smaller study found no connection between SLS in toothpaste and aphthous ulcers. Celiac disease has been suggested as a cause of aphthous ulcers; small studies of patients (33% or 1 out of 3) with Celiac disease did demonstrate a conclusive link between the disease and aphthous ulcers vs control group (23%) but some patients benefited from eliminating gluten from their diets.

There is no indication that aphthous ulcers are related to menstruation, pregnancy, and menopause. Smokers appear to be affected less often.

Prevention

Oral and dental measures

- Regular use of non-alcoholic mouthwash may help prevent or reduce the frequency of sores. In fact, informal studies suggest that mouthwash may help to temporarily relieve pain.
- In some cases, switching toothpastes can prevent aphthous ulcers from occurring, with research looking at the role of sodium dodecyl sulfate (sometimes called sodium lauryl sulfate, or with the acronymes SDS or SLS), a detergent found in most toothpastes. Using toothpaste free of this compound has been found in several studies to help reduce the amount, size, and recurrence of ulcers.

- Dental braces are a common physical trauma that can lead to aphthous ulcers and the dental bracket can be covered with wax to reduce abrasion of the mucosa. Avoidance of other types of physical and chemical trauma will prevent some ulcers, but, since such trauma is usually accidental, this type of prevention is not usually practical.

Nutritional therapy

- Zinc deficiency has been reported in people with recurrent aphthous ulcers. The few small studies looking into the role of zinc supplementation have mostly reported positive results particularly for those people with deficiency, although some research has found no therapeutic effect.

Treatment

A number of different treatments exist for apthous ulcers including: analgesics, anesthetics agents, antiseptics, anti-inflammatory agents, steroids, sucralfate, tetracycline suspension, and silver nitrate.

Amlexanox paste has been found to speed healing and alleviate pain.

Vitamin B_{12} has been found to be effective in treating recurrent aphthous ulcers, regardless of whether there is a vitamin deficiency present.

While dietary supplements of L-lysine can be effective in treating cold sores/herpetic lesions, there is no evidence of an impact on canker sores.

Suggestions to reduce the pain caused by an ulcer include: avoiding spicy food, rinsing with salt water or over-the-counter mouthwashes, proper oral hygiene and non-prescription local anesthetics. Active ingredients in the latter generally include benzocaine, benzydamine or choline salicylate.

Anesthetic mouthwashes containing benzydamine hydrochloride have not been shown to reduce the number of new ulcers or significantly reduce pain, and evidence supporting the use of other topical anesthetics is very limited, though some individuals may find them effective. In general, their role is limited; their duration of effectiveness is, in general, short and does not provide pain control throughout the day. Such medications may also cause complications in children.

Evidence is limited for the use of antimicrobial mouthwashes but suggests that they may reduce the painfulness and duration of ulcers and increase the number of days between ulcerations, without reducing the number of new ulcers.

Milk of magnesia is useful against aphthous ulcers when used topically.

Corticosteroid preparations containing hydrocortisone hemisuccinate or triamcinolone acetonide to control symptoms are effective in treating aphthous ulcers.

The application of silver nitrate will cauterize the sore; a single treatment decreases pain but does not affect healing time though in children it can cause tooth discoloration if the teeth are still developing. The use of tetracycline is controversial, as is treatment with levamisole, colchicine, gamma-globulin, dapsone, estrogen replacement and monoamine oxidase inhibitors.

While commonly used, Magic mouthwash, a combination of a number of ingredients including viscous lidocaine, benzocaine, milk of magnesia, kaolin-pectate, chlorhexidine, or diphenhydramine, has little evidence to support its use in the treatment of aphthous ulcers.

There is the hypothesis that pasteurized goat milk can help with disease symptoms. At the current time, a clinical trial is conducted to check these claims.

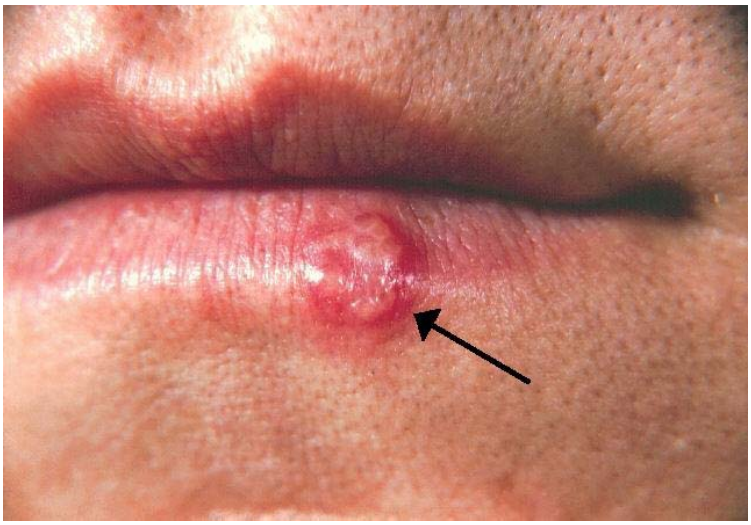
Epidemiology

Canker sores are a very common oral lesion. Epidemiological studies show an average prevalence between 15% and 30%. Canker sores tend to afflict women more than men and people less than 45 years old. Canker sores occur most frequently among 16- to 25-year-olds, and they rarely occur in anyone over 55. The frequency of canker sores varies from less than 4 episodes per year (85% of all cases) to more than one episode per month (10% of all cases) including people suffering from continuous RAS.

Chapter 23

Herpes Simplex

Herpes simplex



Herpes labialis of the lower lip. Note the blisters in a group marked by an arrow.

ICD-10	A60., B00., G05.1, P35.2
ICD-9	054.0, 054.1, 054.2, 054.3, 771.2
DiseasesDB	5841 33021
eMedicine	med/1006
MeSH	D006561

Herpes simplex (Ancient Greek: ἕρπης - herpes, lit. "creeping") is a viral disease caused by both herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). Infection with the herpes virus is categorized into one of several distinct disorders based on the site of infection. Oral herpes, the visible symptoms of which are colloquially called *cold sores* or *fever blisters*, infects the face and mouth. Oral herpes is the most common form of infection. Genital herpes, known simply as *herpes*, is the second most common form of herpes. Other disorders such as herpetic whitlow, herpes gladiatorum, ocular herpes

(keratitis), cerebral herpes infection encephalitis, Mollaret's meningitis, neonatal herpes, and possibly Bell's palsy are all caused by herpes simplex viruses.

Herpes viruses cycle between periods of active disease—presenting as blisters containing infectious virus particles—that last 2–21 days, followed by a remission period. Genital herpes, however, is often asymptomatic, though viral shedding may still occur. After initial infection, the viruses are transported along sensory nerves to the sensory nerve cell bodies, where they become latent and reside life-long. Causes of recurrence are uncertain, though some potential triggers have been identified, including immunosuppressant drugs (see below). The previously latent virus then multiplies new virus particles in the nerve cell and these are transported along the axon of each neuron to the nerve terminals in the skin, where they are released. Over time, episodes of active disease reduce in frequency and severity.

Herpes simplex is most easily transmitted by direct contact with a lesion or the body fluid of an infected individual. Transmission may also occur through skin-to-skin contact during periods of asymptomatic shedding. Barrier protection methods are the most reliable method of preventing transmission of herpes, but they merely reduce rather than eliminate risk. Oral herpes is easily diagnosed if the patient presents with visible sores or ulcers. Early stages of orofacial herpes and genital herpes are harder to diagnose; laboratory testing is usually required.

A cure for herpes has not yet been developed. Once infected, the virus remains in the body for life. However, after several years, some people will become perpetually asymptomatic and will no longer experience outbreaks, though they may still be contagious to others. Treatments with antivirals can reduce viral shedding and alleviate the severity of symptomatic episodes. Vaccines are in clinical trials but have not demonstrated effectiveness. It should not be confused with conditions caused by other viruses in the *herpesviridae* family such as herpes zoster, which is caused by varicella zoster virus. The differential diagnosis includes hand, foot and mouth disease due to similar lesions on the skin.

Classification

Herpes simplex is divided into two types: HSV type 1 and HSV type 2. HSV1 primarily causes mouth, throat, face, eye, and central nervous system infections, while HSV2 primarily causes anogenital infections. However, each may cause infections in all areas.

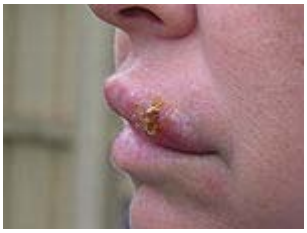
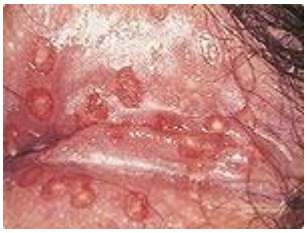
Signs and symptoms

HSV infection causes several distinct medical disorders. Common infection of the skin or mucosa may affect the face and mouth (orofacial herpes), genitalia (genital herpes), or hands (herpes whitlow). More serious disorders occur when the virus infects and damages the eye (herpes keratitis), or invades the central nervous system, damaging the brain (herpes encephalitis). Patients with immature or suppressed immune systems, such as newborns, transplant recipients, or AIDS patients are prone to severe complications

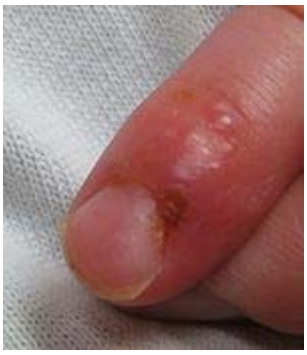
from HSV infections. HSV infection has also been associated with cognitive deficits of bipolar disorder, and Alzheimer's disease, although this is often dependent on the genetics of the infected person.

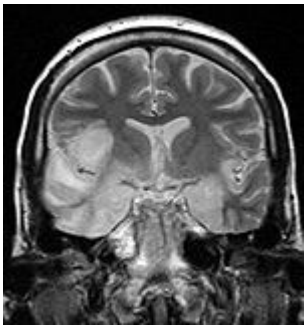
In all cases HSV is never removed from the body by the immune system. Following a primary infection, the virus enters the nerves at the site of primary infection, migrates to the cell body of the neuron, and becomes latent in the ganglion. As a result of primary infection, the body produces antibodies to the particular type of HSV involved, preventing a subsequent infection of that type at a different site. In HSV-1 infected individuals, seroconversion after an oral infection will prevent additional HSV-1 infections such as whitlow, genital herpes, and keratitis. Prior HSV-1 seroconversion seems to reduce the symptoms of a later HSV-2 infection, although HSV-2 can still be contracted. Most indications are that an HSV-2 infection contracted prior to HSV-1 seroconversion will also immunize that person against HSV-1 infection.

Many people infected with HSV-2 display no physical symptoms—individuals with no symptoms are described as asymptomatic or as having subclinical herpes.

Condition	Description	Illustration
Herpetic gingivostomatitis	Herpetic gingivostomatitis is often the initial presentation during the first herpes infection. It is of greater severity than herpes labialis which is often the subsequent presentations.	
Herpes labialis	Infection occurs when the virus comes into contact with oral mucosa or abraded skin.	
Herpes genitalis	When symptomatic, the typical manifestation of a primary HSV-1 or HSV-2 genital infection is clusters of inflamed papules and vesicles on the outer surface of the genitals resembling cold sores.	

Herpetic whitlow	Herpes whitlow is a painful infection that typically affects the fingers or thumbs. Occasionally infection occurs on the toes or on the nail cuticle.
Herpes gladiatorum	Individuals that participate in contact sports such as wrestling, rugby, and soccer sometimes acquire a condition caused by HSV-1 known as herpes gladiatorum, *scrumpox*, *wrestler's herpes*, or *mat herpes*, which presents as skin ulceration on the face, ears, and neck. Symptoms include fever, headache, sore throat and swollen glands. It occasionally affects the eyes or eyelids.
Herpetic keratoconjunctivitis	Primary infection typically presents as swelling of the conjunctiva and eyelids (blepharoconjunctivitis), accompanied by small white itchy lesions on the surface of the cornea.
Herpesviral encephalitis	A herpetic infection of the brain that is thought to be caused by the retrograde transmission of virus from a peripheral site on the face following HSV-1 reactivation, along the trigeminal nerve axon, to the brain. HSV is the most common cause of viral encephalitis. When infecting the brain, the virus shows a preference for the temporal lobe.
Herpesviral meningitis	HSV-2 is the most common cause of Mollaret's meningitis, a type of recurrent viral meningitis.
Neonatal herpes simplex	Neonatal HSV infection is a rare but serious condition, usually caused by vertical transmission of HSV (type 1 or 2) from mother to newborn.
During immunodeficiency	In patients with a weakened immune system, herpes simplex can cause unusual lesions in the skin. One of the most striking is the appearance of clean linear erosions in skin creases, with the appearance of a knife





	cut.
Herpetic sycosis	Herpetic sycosis is a recurrent or initial herpes simplex infection affecting primarily the hair follicle.
Eczema herpeticum	Infection with herpesvirus in patients with chronic atopic dermatitis may result in spread of herpes simples throughout the eczematous areas.
Herpes esophagitis	Symptoms may include painful swallowing (odynophagia) and difficulty swallowing (dysphagia). It is often associated with impaired immune function (e.g. HIV/AIDS, immunosuppression in solid organ transplants).

Bell's palsy

Although the exact cause of Bell's palsy, a type of facial paralysis, is unknown it may be related to reactivation of herpes simplex virus type 1. This theory has been contested, however since HSV is detected in large numbers of individuals who never experienced facial paralysis, and higher levels of antibodies for HSV are not found in HSV-infected individuals with Bell's palsy compared to those without. Regardless antivirals have been found to not improve outcomes.

Alzheimer's disease

HSV-1 has been proposed as a possible cause of Alzheimer's disease. In the presence of a certain gene variation (APOE-epsilon4 allele carriers), HSV-1 appears to be particularly damaging to the nervous system and increases one’s risk of developing Alzheimer’s disease. The virus interacts with the components and receptors of lipoproteins, which may lead to the development of Alzheimer's disease. Without the presence of the gene allele, HSV type 1 does not appear to cause any neurological damage and thus increase the risk of Alzheimer’s. Herpes simplex virus type 1 DNA is localized within the beta-amyloid plaques that characterize Alzheimer's disease. It suggests that this virus is a major cause of the plaques and hence probably a significant aetiological factor in Alzheimer's disease.

Pathophysiology

Herpes is contracted through direct contact with an active lesion or body fluid of an infected person. Herpes transmission occurs between discordant partners; a person with a history of infection (HSV seropositive) can pass the virus to an HSV seronegative person. The only way to contract Herpes simplex virus 2 is through direct skin-to-skin contact with an infected individual. To infect a new individual, HSV travels through tiny breaks

in the skin or mucous membranes in the mouth or genital areas. Even microscopic abrasions on mucous membranes are sufficient to allow viral entry.

HSV asymptomatic shedding occurs at some time in most individuals infected with herpes. It can occur more than a week before or after a symptomatic recurrence in 50% of cases. Virus enters into susceptible cells via entry receptors such as nectin-1, HVEM and 3-O sulfated heparan sulfate. Infected people that show no visible symptoms may still shed and transmit virus through their skin; asymptomatic shedding may represent the most common form of HSV-2 transmission. Asymptomatic shedding is more frequent within the first 12 months of acquiring HSV. Concurrent infection with HIV increases the frequency and duration of asymptomatic shedding. There are indications that some individuals may have much lower patterns of shedding, but evidence supporting this is not fully verified; no significant differences are seen in the frequency of asymptomatic shedding when comparing persons with one to twelve annual recurrences to those who have no recurrences.

Antibodies that develop following an initial infection with a type of HSV prevents reinfection with the same virus type—a person with a history of orofacial infection caused by HSV-1 cannot contract herpes whitlow or a genital infection caused by HSV-1. In a monogamous couple, a seronegative female runs a greater than 30% per year risk of contracting an HSV infection from a seropositive male partner. If an oral HSV-1 infection is contracted first, seroconversion will have occurred after 6 weeks to provide protective antibodies against a future genital HSV-1 infection.

Diagnosis

Primary orofacial herpes is readily identified by clinical examination of persons with no previous history of lesions and contact with an individual with known HSV-1 infection. The appearance and distribution of sores in these individuals typically presents as multiple, round, superficial oral ulcers, accompanied by acute gingivitis. Adults with non-typical presentation are more difficult to diagnose. Prodromal symptoms that occur before the appearance of herpetic lesions help differentiate HSV symptoms from the similar symptoms of other disorders, such as allergic stomatitis. When lesions do not appear inside the mouth, primary orofacial herpes is sometimes mistaken for impetigo, a bacterial infection. Common mouth ulcers (aphthous ulcer) also resemble intraoral herpes, but do not present a vesicular stage.

Genital herpes can be more difficult to diagnose than oral herpes since most HSV-2-infected persons have no classical symptoms. Further confusing diagnosis, several other conditions resemble genital herpes, including fungal infection, lichen planus, atopic dermatitis, and urethritis. Laboratory testing is often used to confirm a diagnosis of genital herpes. Laboratory tests include: culture of the virus, direct fluorescent antibody (DFA) studies to detect virus, skin biopsy, and polymerase chain reaction (PCR) to test for presence of viral DNA. Although these procedures produce highly sensitive and specific diagnoses, their high costs and time constraints discourage their regular use in clinical practice.

Until recently, serological tests for antibodies to HSV were rarely useful to diagnosis and not routinely used in clinical practice. The older IgM serologic assay could not differentiate between antibodies generated in response to HSV-1 or HSV-2 infection. However, the new Immunodot glycoprotein G-specific (IgG) HSV test is more that 98% specific at discriminating HSV-1 from HSV-2. It is the opinion of some modern medical professionals that the new IgG test should always be clinically preferred to the old IgM test, however not all doctors appear to be informed of the availability of the newer, reliable IgG tests.

Prevention

Barrier protection, such as a condom, can reduce the risk of herpes transmission

As with almost all sexually transmitted infections, women are more susceptible to acquiring genital HSV-2 than men. On an annual basis, without the use of antivirals or condoms, the transmission risk of HSV-2 from infected male to female is approximately 8-10%. This is believed to be due to the increased exposure of mucosal tissue to potential infection sites. Transmission risk from infected female to male is approximately 4-5% annually. Suppressive antiviral therapy reduces these risks by 50%. Antivirals also help prevent the development of symptomatic HSV in infection scenarios—meaning the infected partner will be seropositive but symptom free—by about 50%. Condom use also reduces the transmission risk by 50%. Condom use is much more effective at preventing male to female transmission than vice-versa. The effects of combining antiviral and condom use is roughly additive, thus resulting in approximately a 75% combined reduction in annual transmission risk. These figures reflect experiences with subjects having frequently recurring genital herpes (>6 recurrences per year). Subjects with low recurrence rates and those with no clinical manifestations were excluded from these studies.

However, asymptomatic carriers of the HSV-2 virus are still contagious. In many infections, the first symptom a person will have of their own infection is the horizontal transmission to a sexual partner or the vertical transmission of neonatal herpes to a newborn at term. Since most asymptomatic individuals are unaware of their infection, they are considered at high risk for spreading HSV.

Barrier methods

Condoms offer moderate protection against HSV-2 in both men and women, with consistent condom users having a 30% lower risk of HSV-2 acquisition compared with those who never use condoms The virus cannot pass through a latex condom, but a condom's effectiveness is limited because it does not prevent skin contact or bodily fluid contact with the scrotum, anus, buttocks, upper thighs or area immediately surrounding the penis, all of which are susceptible to infection with and transmission of the virus. Preventing contact with these areas during sex, in addition to wearing a condom, should theoretically provide enhanced protection against herpes. The use of condoms or dental dams also limits the transmission of herpes from the genitals of one partner to the mouth of the other (or vice versa) during oral sex. When one partner has a herpes simplex infection and the other does not, the use of antiviral medication, such as valaciclovir, in conjunction with a condom, further decreases the chances of transmission to the uninfected partner. Topical microbicides which contain chemicals that directly inactivate the virus and block viral entry are being investigated.

Vaccine

Vaccines for HSV are undergoing trials. Once developed, they may be used to help with prevention or minimize initial infections as well as treatment for existing infections.

One vaccine that was under trial was Herpevac, a vaccine against HSV-2. The National Institutes of Health (NIH) in the United States conducted a phase III trials of Herpevac. In 2010, it was reported that, after 8 years of study in more than 8000 women in the United States and Canada, there was no sign of positive results against the sexually transmitted disease caused by HSV-2 (and this despite earlier favorable interim reports).

A laboratory at Harvard Medical School has developed dl5-29 (now known as ACAM-529), a replication-defective mutant virus that has proved successful both in preventing HSV-2/HSV-1 infections, and in combating the virus in already infected hosts, in animal models. It has been shown that the replication-defective vaccine induces strong HSV-2-specific antibody and T-cell responses; protects against challenge with a wild-type HSV-2 virus; greatly reduces the severity of recurrent disease; provides cross-protection against HSV-1, and renders the virus unable to revert to a virulent state or to become latent. His vaccine is now being researched and developed by Accambis (acquired by Sanofi Pasteur in September 2008), and is due to be applied as an Investigational New Drug in 2009.

A private company called BioVex began Phase I clinical trials for ImmunoVEX, another proposed vaccine, in March 2010.

Antivirals

Antivirals may reduce asymptomatic shedding; it is believed asymptomatic genital HSV-2 viral shedding occurs on 20% of days per year in patients not undergoing antiviral treatment, versus 10% of days while on antiviral therapy.

Pregnancy

The risk of transmission from mother to baby is highest if the mother becomes infected at around the time of delivery (30% to 60%), but the risk falls to 3% if it is a recurrent infection, and is less than 1% if there are no visible lesions. To prevent neonatal infections, seronegative women are recommended to avoid unprotected oral-genital contact with an HSV-1 seropositive partner and conventional sex with a partner having a genital infection during the last trimester of pregnancy. A seronegative mother who contracts HSV at this time has up to a 57% chance of conveying the infection to her baby during childbirth, since insufficient time will have occurred for the generation and transfer of protective maternal antibodies before the birth of the child, whereas a woman seropositive for both HSV-1 and HSV-2 has around a 1-3% chance of transmitting infection to her infant. Women who are seropositive for only one type of HSV are only half as likely to transmit HSV as infected seronegative mothers. Mothers infected with HSV are advised to avoid procedures that would cause trauma to the infant during birth (e.g. fetal scalp electrodes, forceps, and vacuum extractors) and, should lesions be present, to elect caesarean section to reduce exposure of the child to infected secretions in the birth canal. The use of antiviral treatments, such as acyclovir, given from the 36th week of pregnancy, limits HSV recurrence and shedding during childbirth, thereby reducing the need for caesarean section.

Acyclovir is the recommended antiviral for herpes suppressive therapy during the last months of pregnancy. The use of valaciclovir and famciclovir, while potentially improving compliance have less well determined safety in pregnancy.

Treatment

There is no method to eradicate herpes virus from the body, but antiviral medications can reduce the frequency, duration, and severity of outbreaks. Analgesics such as ibuprofen and acetaminophen can reduce pain and fever. Topical anesthetic treatments such as prilocaine, lidocaine, benzocaine or tetracaine can also relieve itching and pain.

Antiviral

The antiviral medication acyclovir

There are several antivirals that are effective for treating herpes including: aciclovir (acyclovir), valaciclovir (valacyclovir), famciclovir, and penciclovir. Aciclovir was the first discovered and is now available in generic.

Evidence supports the use of aciclovir and valaciclovir in the treatment of herpes labialis as well as herpes infections in people with cancer. The evidence to support the use of acyclovir in primary herpetic gingivostomatitis is less strong.

Topical

A number of topical antivirals are effective for herpes labialis including acyclovir, penciclovir, and docosanol. Docosanol can be purchased over the counter in Canada and the USA.

Alternative medicine

Certain dietary supplements and alternative remedies are claimed to be beneficial in the treatment of herpes. There is however insufficient evidence to support use of many of these compounds including echinacea, eleuthero, L-lysine, zinc, bee products and aloe vera.

A single study indicates possible benefit from laser treatment.

Prognosis

Following active infection, herpes viruses establish a latent infection in sensory and autonomic ganglia of the nervous system. The double-stranded DNA of the virus is incorporated into the cell physiology by infection of the nucleus of a nerve's cell body. HSV latency is static—no virus is produced—and is controlled by a number of viral genes, including Latency Associated Transcript (LAT).

Many HSV-infected people experience recurrence within the first year of infection. Prodrome precedes development of lesions. Prodromal symptoms include tingling (paresthesia), itching, and pain where lumbosacral nerves innervate the skin. Prodrome may occur as long as several days or as short as a few hours before lesions develop. Beginning antiviral treatment when prodrome is experienced can reduce the appearance and duration of lesions in some individuals. During recurrence, fewer lesions are likely to develop, lesions are less painful and heal faster (within 5–10 days without antiviral treatment) than those occurring during the primary infection. Subsequent outbreaks tend to be periodic or episodic, occurring on average four to five times a year when not using antiviral therapy.

The causes of reactivation are uncertain, but several potential triggers have been documented. A recent study (2009) showed that a protein VP16 plays a key role in reactivation of the dormant virus. Changes in the immune system during menstruation may play a role in HSV-1 reactivation. Concurrent infections, such as viral upper respiratory tract infection or other febrile diseases, can cause outbreaks. Reactivation due to infection is the likely source of the historic terms *cold sore* and *fever blister*.

Other identified triggers include: local injury to the face, lips, eyes, or mouth, trauma, surgery, radiotherapy, and exposure to wind, ultraviolet light, or sunlight.

The frequency and severity of recurrent outbreaks vary greatly between patients. Some individuals' outbreaks can be quite debilitating with large, painful lesions persisting for

several weeks, while others will experience only minor itching or burning for a few days. There is some evidence that genetics plays a role in the frequency of cold sore outbreaks. An area of human chromosome 21 that includes 6 genes has been linked to frequent oral herpes outbreaks. An immunity to the virus is built over time. Most infected individuals will experience fewer outbreaks and outbreak symptoms will often become less severe. After several years, some people will become perpetually asymptomatic and will no longer experience outbreaks, though they may still be contagious to others. Immuno-compromised individuals may experience episodes that are longer, more frequent, and more severe. Antiviral medication has been proven to shorten the frequency and duration of outbreaks. Outbreaks may occur at the original site of the infection or in proximity to nerve endings that reach out from the infected ganglia. In the case of a genital infection, sores can appear at the original site of infection or near the base of the spine, the buttocks, or the back of the thighs. HSV-2 infected individuals are at higher risk for acquiring HIV when practicing unprotected sex with HIV-positive persons, particularly during an outbreak with active lesions.

Epidemiology

Worldwide rates of HSV infection are between 65% and 90%. HSV1 is more common than HSV2 with rates of both increasing as people age. Rates of infection are determined by the presence of antibodies against either viral species.

In the US 17.2% of the population is HSV-2 seropositive with only 14.5% of the seropositive population aware that they are infected.

History

Herpes has been known for at least 2,000 years. It is said that Emperor Tiberius banned kissing in Rome for a time due to so many people having cold sores. In the 16th century *Romeo and Juliet*, it is mentioned that there are blisters "o'er ladies' lips." In 18th century it was so common among prostitutes that it was called "a vocational disease of women."

The term *Herpes Simplex* appeared in Richard Boulton's A System of Rational and Practical Chirurgery in 1713, where the terms *Herpes miliaris* and *Herpes exedens* also appeared.

Herpes was not found to be a virus until the 1940s.

Herpes antiviral therapy began in the early 1960s with the experimental use of medication that interfered with viral replication called deoxyribonucleic acid (DNA) inhibitors. The original use was against normally fatal or disabilitating illness such as adult encephalitis, keratitis, in immunocompromised (transplant) patients, or disseminated herpes zoster. The original compounds used were 5-iodo-2'-deoxyuridine, AKA idoxuridine, IUdR, or(IDU) and 1-β-D-arabinofuranosylcytosine or ara-C, later marketed under the name cytosar or cytorabine. The usage expanded to include topical treatment of herpes simplex, zoster, and varicella. Some trials combined different

antivirals with differing results. The introduction of 9-β-D-arabinofuranosyladenine, AKA ara-A or vidarabine, considerably less toxic than Ara-C, in the mid 1970s, heralded the way for the beginning of regular neonatal antiviral treatment. Vidarabine was the first systemically administered antiviral medication with activity against HSV for which therapeutic efficacy outweighed toxicity for the management of life-threatening HSV disease. Intravenous vidarabine was licensed for use by the U.S. Food and Drug Administration (FDA) in 1977. Other experimental antivirals of that period included: Heparin, trifluorothymidine (TFT), Ribivarin, interferon, Virazole, and 5-methoxymethyl-2'-deoxyuridine (MMUdR). The introduction of 9-(2-hydroxyethoxymethyl)guanine, AKA acyclovir, in the late 1970s raised antiviral treatment another notch and led to vidarabine vs. acyclovir trials in the late 1980s. The lower toxicity and ease of administration over vidarabine has led to acyclovir becoming the drug of choice for herpes treatment after it was licensed by the FDA in 1998. Another advantage in the treatment of neonatal herpes included greater reductions in mortality and morbidity with increased dosages, something that did not occur when compared with increased dosages of vidarabine. On the other side of the equation, acyclovir seems to inhibit antibody response and newborns on acyclovir antiviral treatment experienced a slower rise in antibody titer than those on vidarabine.

Research

Researchers at the University of Florida have made a hammerhead ribozyme that targets and cleaves the mRNA of essential genes in HSV-1. The hammerhead which targets the mRNA of the UL20 gene greatly reduced the level of HSV-1 ocular infection in rabbits and reduced the viral yield in vivo. The gene-targeting approach uses a specially designed RNA enzyme to inhibit strains of the herpes simplex virus. The enzyme disables a gene responsible for producing a protein involved in the maturation and release of viral particles in an infected cell. The technique appears to be effective in experiments with mice and rabbits, but further research is required before it can be attempted in people who are infected with herpes.

Another possibility to eradicate the HSV-1 variant is being pursued by a team at Duke University. By figuring out how to switch all copies of the virus in the host from latency to their active stage at the same time, rather than the way the virus copies normally stagger their activity stage, leaving some dormant somewhere at all times, it is thought that conventional anti-viral drugs can kill the entire virus population completely, since they can no longer hide in the nerve cells. One class of drugs called antagomir could serve this purpose. These are chemically engineered oligonucleotides or short segments of RNA, that can be made to mirror their target genetic material, namely herpes microRNAs. They could be engineered to attach and thus 'silence' the microRNA, thus rendering the virus incapable to keep latent in their host. Professor Cullen believes a drug could be developed to block the microRNA whose job it is to suppress HSV-1 into latency.

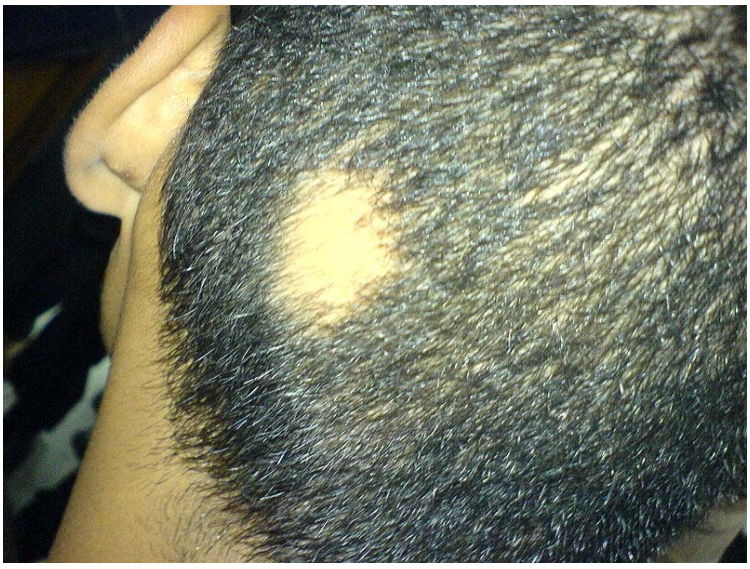
Vironova AB, which is a privately held Swedish biotech company, created an antiviral approach to stop viral growth by inhibiting viral structures from forming, e.g. the capsid.

Correct assembly of structural proteins is essential for the virus to survive the extracellular environment and to become infectious, and is thus a suitable drug target.

Chapter 24

Alopecia Areata

Alopecia areatica



Alopecia areata.

ICD-10	L63.
ICD-9	704.01
OMIM	104000
DiseasesDB	430
MedlinePlus	001450
eMedicine	derm/14
MeSH	D000506

Alopecia areata (**AA**) is a medical condition in which hair is lost from some or all areas of the body, usually from the scalp. Because it causes bald spots on the scalp, especially in the first stages, it is sometimes called **spot baldness**. In 1%–2% of cases, the condition can spread to the entire scalp (Alopecia totalis) or to the entire epidermis (Alopecia

universalis). Conditions resembling AA, and having a similar cause, occur also in other species.

Classification

The most common type of alopecia areata involves hair loss in one or more round spots on the scalp.

- Hair may also be lost more diffusely over the whole scalp, in which case the condition is called **diffuse alopecia areata**.
- **Alopecia areata monolocularis** describes baldness in only one spot. It may occur anywhere on the head.
- **Alopecia areata multilocularis** refers to multiple areas of hair loss.
- The disease may be limited only to the beard, in which case it is called **Alopecia areata barbae**.
- If the patient loses all the hair on his/her scalp, the disease is then called **Alopecia areata totalis**.
- If all body hair, including pubic hair, is lost, the diagnosis then becomes **Alopecia areata universalis**.

Alopecia areata totalis and universalis are rare.

Signs and symptoms

Typical first symptoms are small, soft, bald patches. These can take many shapes, but are most usually round and "coin-shaped". Alopecia areata most often affects the scalp and beard, but may occur on any hair-bearing part of the body. There may be different skin areas with hair loss and regrowth in the same body at the same time. It may also go into remission for a time, or permanently.

The area of hair loss may tingle or be very slightly painful.

The hair tends to fall out over a short period of time, with the loss commonly occurring more on one side of the scalp than the other.

Exclamation point hairs are often present. Exclamation point hairs are hairs that become narrower along the length of the strand closer to the base, producing a characteristic "exclamation point" appearance.

In the case of **healthy hair**, if you were to try to pull some out, none should fall out, and ripped hair should not be distributed evenly across the tugged portion of the scalp. **In cases of alopecia areata** hair will tend to pull out more easily along the edge of the patch where the follicles are already being attacked by the body's immune system than away from the patch where they are still healthy.

Nails may have pitting or trachyonychia.

Patients with alopecia can sometimes present with Ciceromegaly, which is characterized by short stature, hair loss and hypogonadism.

Causes

Alopecia areata is noncommunicable, or not contagious. It occurs more frequently in people who have affected family members, suggesting that heredity may be a factor. Strong evidence that genes may increase risk for alopecia areata was found by studying families with two or more affected members. This study identified at least four regions in the genome that are likely to contain alopecia areata genes. In addition, it is slightly more likely to occur in people who have relatives with autoimmune diseases.

The condition is thought to be an autoimmune disorder in which the body attacks its own hair follicles and suppresses or stops hair growth. There is evidence that T cell lymphocytes cluster around these follicles, causing inflammation and subsequent hair loss. An unknown environmental trigger such as emotional stress or a pathogen is thought to combine with hereditary factors to cause the condition. There are a few recorded cases of babies being born with congenital alopecia areata; however, these are not cases of autoimmune disease because an infant is born without a fully developed immune system.

Treatment

If the affected region is small, it is reasonable to observe the progression of the illness as the problem often spontaneously regresses and the hair may grow back.

In cases where there is severe hair loss, there has been limited success treating alopecia areata with clobetasol or fluocinonide, steroid injections, or cream. Steroid injections are commonly used in sites where there are small areas of hair loss on the head or especially where eyebrow hair has been lost. Some other medications used are minoxidil, elocom ointment (steroid cream) irritants (anthralin or topical coal tar), and topical immunotherapy cyclosporine, each of which are sometimes used in different combinations.

Oral corticosteroids decrease the hair loss, but only for the period during which they are taken, and these drugs have adverse side effects.

For small patches on the beard or head it is possible to suppress with topical tacrolimus ointments like Protopic. Symptoms may remain suppressed until aggravated by stress or other factors.

Initial stages may be kept from increasing by applying topical corticosteroids. However, topical corticosteroids frequently fail to enter the skin deeply enough to affect the hair bulbs, which are the treatment target.

In terms of adapting to the disease rather than treating in an effort to cure, there are also many options available. Wigs are often used by those with Alopecia, particularly

Alopecia Totalis, in which hair is entirely lost from the scalp. Wigs are available at many levels of development and technology, including wigs with suction mechanisms to keep them firmly attached to the scalp.

Prognosis

In most cases that begin with a small number of patches of hair loss, hair grows back after a few months to a year. In cases with a greater number of patches, hair can either grow back or progress to alopecia totalis or, in rare cases, universalis.

Effects of alopecia areata are mainly psychological (loss of self image due to hair loss). However, patients also tend to have a slightly higher incidence of asthma, allergies, atopic dermal ailments, and even hypothyroidism. Loss of hair also means that the scalp sunburns more easily. Loss of nasal hair increases severity of hay fever and similar allergic conditions. Patients may also have aberrant nail formation because keratin forms both hair and nails.

Hair may grow back and then fall out again later. This may not indicate a recurrence of the condition, however, but rather a natural cycle of growth-and-shedding from a relatively synchronised start; such a pattern will fade over time. Episodes of alopecia areata before puberty predispose one to chronic recurrence of the condition.

Psychosocial issues: Alopecia can certainly be the cause of psychological stress. Because hair loss can lead to significant appearance changes, individuals may experience social phobia, anxiety, and depression.

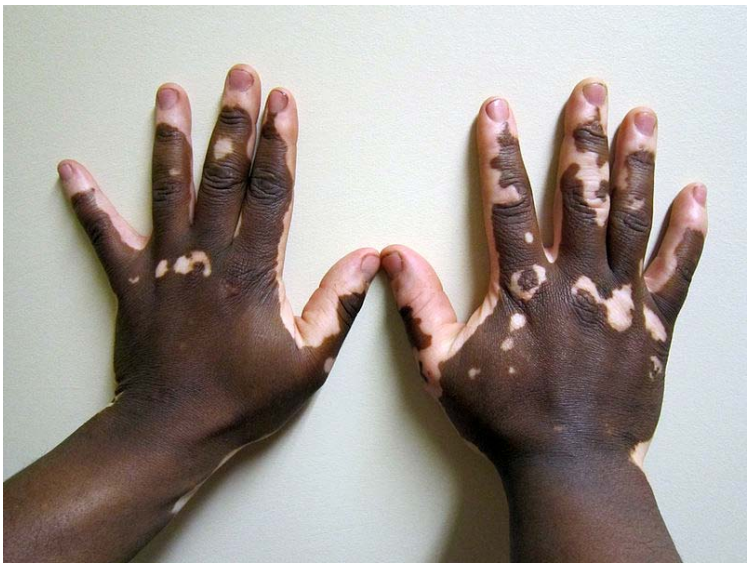
Epidemiology

The condition affects 0.1%–0.2% of humans, occurring in both males and females. Alopecia areata occurs in people who are apparently healthy and have no skin disorder. Initial presentation most commonly occurs in the late teenage years, early childhood, or young adulthood, but can happen with people of all ages.

Chapter 25

Vitiligo

Vitiligo



Vitiligo of the hand in a dark-skinned individual

ICD-10	L80.
ICD-9	709.01
OMIM	193200
DiseasesDB	13965
MedlinePlus	000831
eMedicine	derm/453
MeSH	D014820

Vitiligo is a chronic disorder that causes depigmentation of patches of skin. It occurs when melanocytes, the cells responsible for skin pigmentation, die or are unable to function. The cause of vitiligo is unknown, but research suggests that it may arise from

autoimmune, genetic, oxidative stress, neural, or viral causes. The incidence worldwide is less than 1%. The most common form is non-segmental vitiligo.

Signs and symptoms

The most notable symptom of vitiligo is depigmentation of patches of skin that occurs on the extremities. Although patches are initially small, they often enlarge and change shape. When skin lesions occur, they are most prominent on the face, hands and wrists. Depigmentation is particularly noticeable around body orifices, such as the mouth, eyes, nostrils, genitalia and umbilicus. Some lesions have hyperpigmentation around the edges. Patients who are stigmatised for their condition may experience depression and similar mood disorders.

Non-segmental

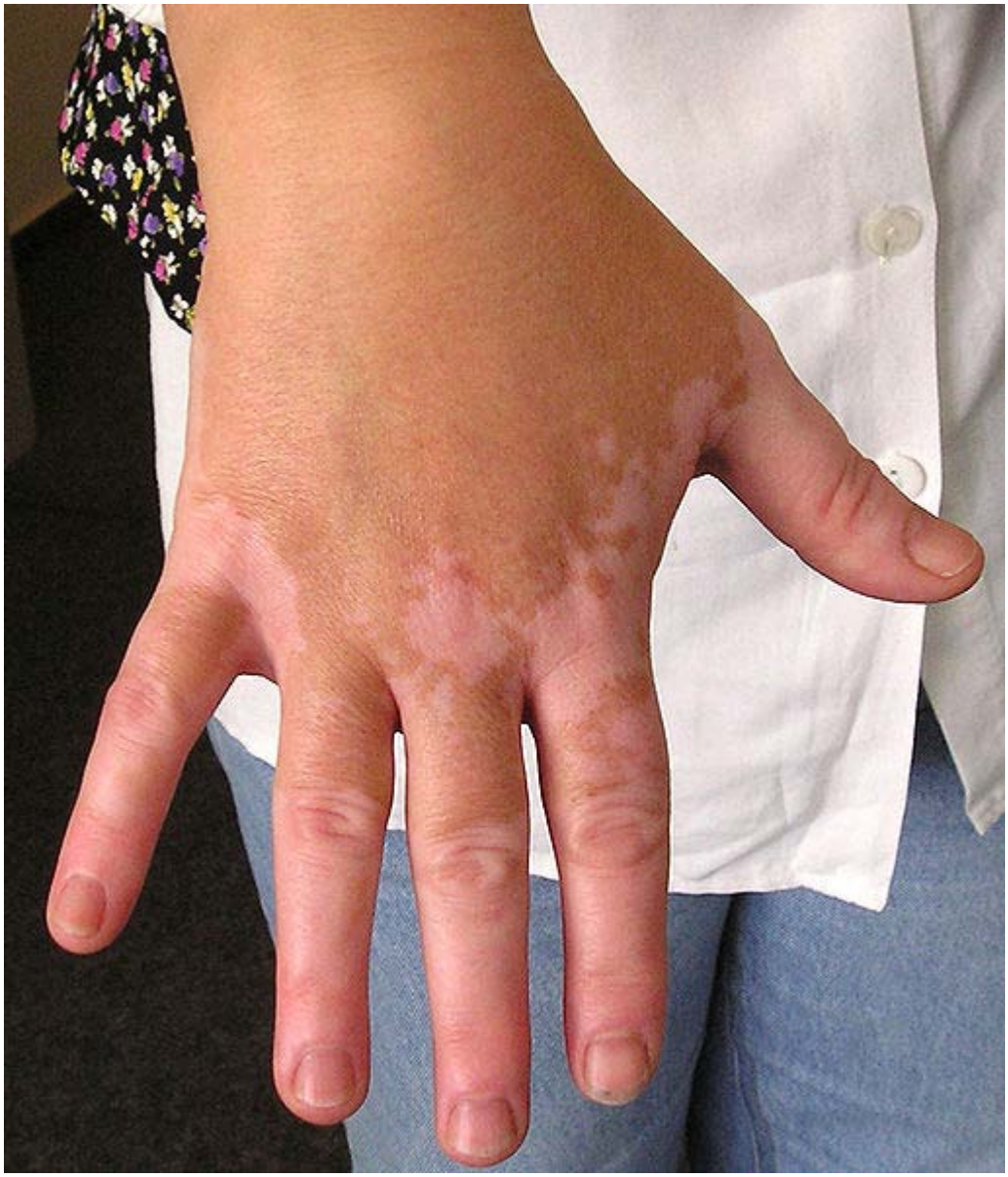
In non-segmental vitiligo (NSV), there is usually some form of symmetry in the location of the patches of depigmentation. New patches also appear over time and can be generalized over large portions of the body or localized to a particular area. Vitiligo where little pigmented skin remains is referred to as *vitiligo universalis*. NSV can come about at any age, unlike segmental vitiligo,which is far more prevalent in teenage years.

Classes of non-segmental Vitiligo include:

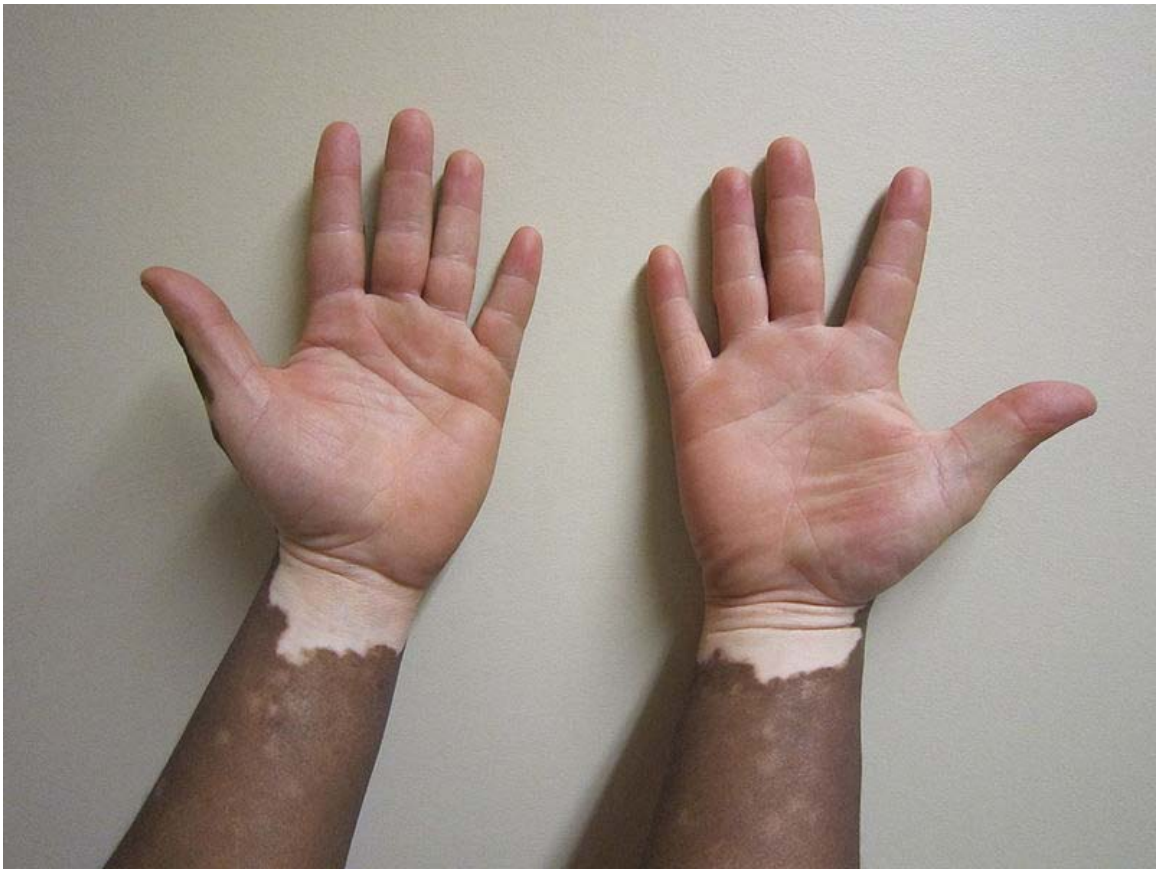
- Generalized Vitiligo: the most common pattern, wide and randomly distributed areas of depigmentation
- Universal Vitiligo: depigmentation encompasses most of the body
- Focal Vitiligo: one or a few scattered macules in one area, most common in children
- Acrofacial Vitiligo: fingers and periorificial areas
- Mucosal Vitiligo: depigmentation of only the mucous membranes

Segmental

Segmental vitiligo (SV) differs in appearance, etiology and prevalence from associated illnesses. Its treatment is different from that of NSV. It tends to affect areas of skin that are associated with dorsal roots from the spine. It spreads much more rapidly than NSV and, without treatment, it is much more stable/ static in course and not associated with auto-immune diseases and a very treatable condition that responds to topical treatment.



Vitiligo in a light-skinned individual



Vitiligo in a dark-skinned individual

Differential diagnosis

Conditions with similar symptoms include:

- Tinea versicolor
- piebaldism
- idiopathic guttate hypomelanosis
- progressive macular hypomelanosis

Pathogenesis

Vitiligo is a disorder characterized by patchy loss of skin pigmentation due to immune attacks on melanocytes, which can be caused by defects in many genes. Variations in genes that are part of the immune system or part of melanocytes have both been associated with vitiligo. The immune system genes are associated with other autoimmune disorders.

In one case, the gene TYR, which makes the melanocyte more susceptible to the immune system in vitiligo, also makes the melanocyte more susceptible to the immune system in

the skin cancer malignant melanoma. So people with vitiligo caused by the TYR gene are less likely to have malignant melanoma.

A genomewide association study found 10 independent susceptibility loci for generalized vitiligo, responsible for 7.4% of the genetic risk. Some patients had vitiligo alone; others had generalized vitiligo with other autoimmune diseases. Most loci were associated with both forms. (The exception was PTPN22, which was only associated with generalized vitiligo.) In the MHC region, which controls the immune system, major association signals were identified in the class I gene region (between HLA-A and HLA-HGC9) and class II gene region (between HLA-DRB1 and HLA-DQA1). Outside the MHC region, association signals were identified near RERE, PTPN22, LPP, IL2RA, GZMB, UBASH3A and C1QTNF6 genes, which are associated with other autoimmune diseases. TYR encodes tyrosinase, which is not a component of the immune system, but is an enzyme of the melanocyte that catalyzes melanin biosynthesis, and a major autoantigen in generalized vitiligo. The major alleles of TYR are associated with vitiligo, and the minor alleles are associated with malignant melanoma. Vitiligo-associated 402R tyrosinase may be more efficiently presented to the immune system. Melanoma-associated 402Q may fail to be identified by the immune system.

The transcriptional profile of melanocytes from vitiligo patients have been studied. Oligonucleotide microarrays containing approximately 16,000 unique genes were used to analyse mRNA expression in melanocytes from vitiligo patients and age-matched healthy controls. In total, 859 genes were identified as differentially expressed.

Vitiligo is sometime associated with autoimmune and inflammatory diseases, commonly thyroid overexpression and underexpression. A study comparing 656 people with and without vitiligo in 114 families found several mutations (single-nucleotide polymorphisms) in the NALP1 gene.The NALP1 gene, which is on chromosome 17 located at 17p13, is on a cascade that regulates inflammation and cell death, including myeloid and lymphoid cells, which are white cells that are part of the immune response. NALP1 is expressed at high levels in T cells and Langerhan cells, white blood cells that are involved in skin autoimmunity.

Among the inflammatory products of NALP1 are caspase 1 and caspase 5, which activate the inflammatory cytokine interleukin-1β. Interleukin-1β is expressed at high levels in patients with vitiligo. There are compounds which inhibit caspase and interleukin-1β, and so might be useful drugs for vitiligo and associated autoimmune diseases. In one of the mutations, the amino acid leucine in the NALP1 protein was replaced by histidine (Leu155->His). The original protein and sequence is highly conserved in evolution, and found in humans, chimpanzee, rhesus monkey, and bush baby, which means that it is an important protein and an alteration is likely to be harmful. Addison's disease (typically an autoimmune destruction of the adrenal glands) may cause vitiligo.

Treatment

There is no cure for vitiligo, but there are a number of treatments that improve the condition. In fair-skinned people, avoiding tanning of normal skin can make patches of vitiligo much less noticeable. Treatment options generally fall into four groups:

Sunblock

A high protection sun-block (factor 20 or above) is applied to areas of vitiligo to prevent sunburn. Affected areas of skin are protected when the sun is strong, especially in the middle of the day by wearing, for example, a wide brimmed hat and long sleeved clothing.

Skin camouflage

In mild cases, vitiligo patches can be hidden with makeup or other cosmetic camouflage solutions. If the affected person is pale-skinned, the patches can be made less visible by avoiding sunlight and sun tanning of unaffected skin.

Reversal

The traditional treatment used by dermatologists is the application of corticosteroid cream.

Studies have shown that immunomodulator creams such as Protopic and Elidel also cause repigmentation in some cases, when used with UVB narrowband treatments.

A 1997 report suggests that combining Vitamin B12 and folic acid supplements with sun exposure caused repigmentation in 52% of cases.

In October 1993, a scientific report was published of successfully transplanting melanocytes to vitiligo affected areas, effectively repigmenting the region. The procedure involved taking a thin layer of pigmented skin from the patient's gluteal region. Melanocytes were then separated out to a cellular suspension that was expanded in culture. The area to be treated was then denuded with a dermabrader and the melanocytes graft applied. Between 70 and 85 percent of patients experienced nearly complete repigmentation of their skin. The longevity of the repigmentation differed from person to person.

Ultraviolet light (UVA) treatments are normally carried out in a hospital clinic. Psoralen and Ultraviolet A light (PUVA) treatment involves taking a drug which makes the skin very sensitive to light. The skin is then exposed to ultraviolet A light (UVA). Treatment is required twice a week for 6–12 months or longer. PUVA may cause side effects such as 'sunburn' type reactions or skin freckling. Narrowband ultraviolet B (UVB) phototherapy is now used more commonly than PUVA as it is less damaging to the skin.

As with PUVA, treatment is carried out twice weekly but there is no requirement to pre-sensitise the skin and the treatment sessions are much shorter.

De-pigmenting

In cases of extensive vitiligo the option to de-pigment the unaffected skin with topical drugs like monobenzone, mequinol or hydroquinone may be considered to render the skin an even colour. The removal of all the skin pigment with monobenzone is permanent and vigorous sun-safety must be adhered to for life to avoid severe sun burn and melanomas. Depigmentation takes about a year to complete.

Notable cases

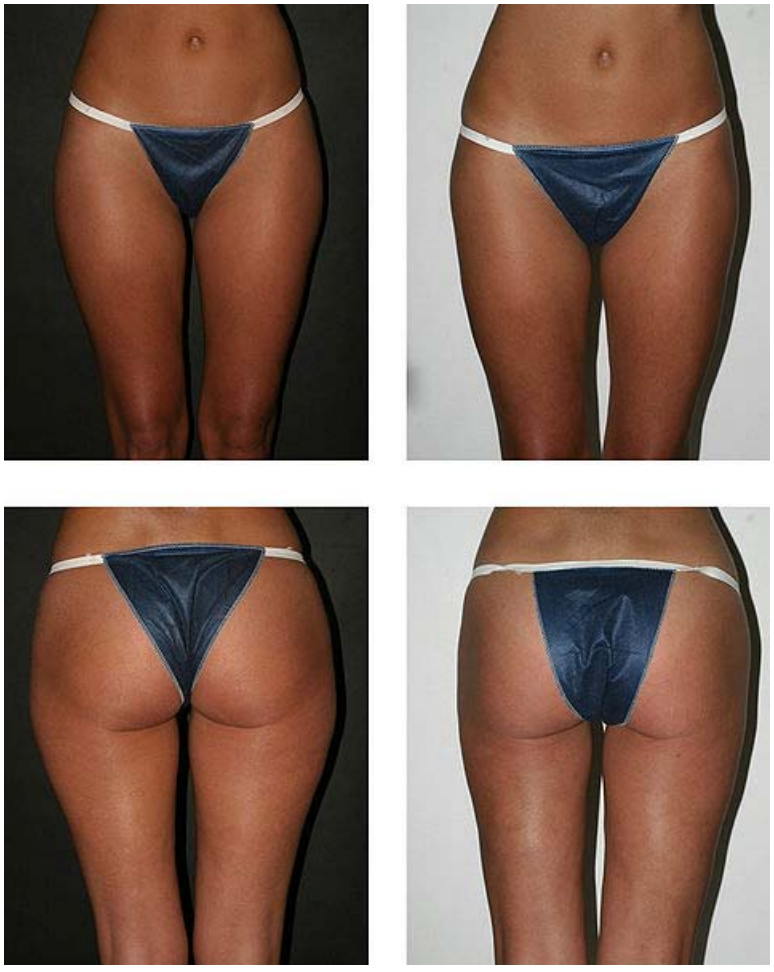
Michael Jackson two years after he was diagnosed with vitiligo universalis, pictured in the early stages of the disease.

- Michael Jackson was diagnosed with vitiligo in 1986. In a 90-minute interview with Oprah Winfrey in February 1993, Jackson claimed that he didn't bleach his skin, stating for the first time that he had vitiligo. A friend claimed he started wearing his signature sequin glove to cover the vitiligo that had begun to appear in the early 80s. It was also confirmed by Jackson during a leaked deposition tape in 1996, that he did not "bleach" his skin. The tape was leaked months after his death in June 2009. According to police reports, 19 tubes of hydroquinone and 18 tubes of benoquin (monobenzone) were found in Michael Jackson's home after his death. In July 2010, Michael Jackson's eldest son Prince Michael Jr is seen in a picture with a patch of de-pigmented skin on his right underarm leading to speculations that he may be affected by vitiligo like his father Michael Jackson.
- Graham Norton, Irish television personality.
- Lee Thomas, a news anchor and entertainment reporter for WJBK (Fox) Detroit.
- Yvette Fielding, British TV presenter, has had vitiligo from age 11; her mother developed it at age 24.
- Bryan Danielson, WWE wrestler.
- John Wiley Price, Dallas County Commissione.
- Amitabh Bachchan, Bollywood actor.
- Scott Jorgensen, WEC fighter.
- Jon Hamm, actor best known for his role in Mad Men.
- John Henson, one of the hosts of ABC's Wipeout has vitiligo and has a white patch of hair on the right side of his head from it.

Chapter 26

Liposuction

Liposuction, also known as **lipoplasty** ("fat modeling"), **liposculpture suction lipectomy** or simply **lipo** ("suction-assisted fat removal") is a cosmetic surgery operation that removes fat from many different sites on the human body. Areas affected can range from the abdomen, thighs and buttocks, to the neck, backs of the arms and elsewhere.



Suction-assisted lipectomy of bilateral outer thighs

Several factors limit the amount of fat that can be safely removed in one session. Ultimately, the operating physician and the patient make the decision. There are negative aspects to removing too much fat. Unusual "lumpiness" and/or "dents" in the skin can be seen in those patients "over-suctioned". The more fat removed, the higher the surgical risk.

While reports of people removing 50 pounds (22.7 kg or around 3.6 stone) of fat has been claimed, the contouring possible with liposuction may cause the appearance of weight loss to be greater than the actual amount of fat removed. The procedure may be performed under general or local ("tumescent") anesthesia. The safety of the technique relates not only to the amount of tissue removed, but to the choice of anesthetic and the patient's overall health. It is ideal for the patient to be as fit as possible before the procedure and not to have smoked for several months.

History

Doctors Giorgio and Arpad Fischer, two Italian-American surgeons working in Rome, Italy, invented the liposuction procedure in 1974. The roots of liposuction, however, date back to the 1920s. Relatively modern techniques for body contouring and removal of fat were first performed by a French surgeon, Charles Dujarier. A tragic case that resulted in gangrene in the leg of a French model in a procedure performed by Dr. Dujarier in 1926 set back interest in body contouring for decades to follow.

Liposuction evolved from work in the late 1960s from surgeons in Europe and was pioneered in the United States by the European surgeon Leon Forrester Tcheupdjian using primitive curettage techniques which were largely ignored, as they achieved irregular results with significant morbidity and bleeding. Modern liposuction first burst on the scene in a presentation by the French surgeon, Dr Yves-Gerard Illouz, in 1982. The "Illouz Method" featured a technique of suction-assisted lipolysis after infusing fluid into tissues using blunt cannulas and high-vacuum suction and demonstrated both reproducible good results and low morbidity. During the 1980s, many United States surgeons experimented with liposuction, developing some variations, and achieving mixed results.

In 1985, Klein and Lillis described the "tumescent technique", which added high volumes of fluid containing a local anesthetic allowing the procedure to be done in an office setting under intravenous sedation rather than general anesthesia. Concerns over the high volume of fluid and potential toxicity of lidocaine with tumescent techniques eventually led to the concept of lower volume "super wet" tumescence.

In the late 1990s, ultrasound was introduced to facilitate the fat removal by first liquefying it using ultrasonic energy. After a flurry of initial interest, an increase in reported complications tempered the enthusiasm of many practitioners.

Technologies involving the use of laser tipped probes (which induce a thermal lipoysis) have been introduced in recent years and are being evaluated to examine any potential benefit over traditional techniques.

Overall, the advantages of 30 years of improvements have been that more fat cells can more easily be removed, with less blood loss, less discomfort, and less risk. Recent developments suggest that the recovery period can be shortened as well. In addition, fat can also be used as a natural filler. This is sometimes referred to as "autologous fat transfer" and in general, for these procedures, fat is removed from one area of the patient's body (for example, the stomach), cleaned, and then re-injected into an area of the body where contouring is desired, for example, to reduce or eliminate wrinkles.

Popularity

Removal of very large volumes of fat is a complex and potentially life-threatening procedure. The American Society of Plastic Surgeons defines "large" in this context as being more than 5 liters (around 8½ pints). Most often, liposuction is performed on the abdomen and thighs in women, and the abdomen and flanks in men. According to the American Society for Aesthetic Plastic Surgery, liposuction was the most common plastic surgery procedure performed in 2006 with 403,684 patients.

Candidacy

Not everyone is a good candidate for liposuction. It is not a good alternative to dieting or exercising. To be a good candidate, one must usually be over 18 and in good general health, have tried a diet and exercise regime, and have found that the last 10 or 15 pounds persist in certain pockets on the body. Diabetes, any infection, heart or circulation problems, generally nullify one's eligibility for the procedure. In older people, the skin is usually less elastic, limiting the ability of the skin to readily tighten around the new shape. Liposuction of the abdominal fat should not be combined with simultaneous tummy tuck procedures due to higher risk of complications and mortality. Laws in Florida prevent practitioners combining liposuction of the upper abdomen and simultaneous abdominoplasty because of higher risks.

Approaches

The basic surgical challenge of any liposuction procedure is:

- To remove the right amount of fat
- To cause the least disturbance of neighboring tissue, such as blood vessels and connective tissue
- To leave the person’s fluid balance undisturbed
- To cause the least discomfort to both patient and surgeon

As techniques have been refined, many ideas have emerged that have brought liposuction closer to being safe, easy, painless, and effective.

Areas of the body where liposuction is performed

- Abdomen
- Hips
- Outer thighs (saddlebags)
- Flanks (love handles)
- Back
- Inner thighs
- Inner knees
- Upper arms
- Submental (chin)
- Gynecomastia (male breast tissue)

Techniques

Power-assisted liposuction Cannula

In general, fat is removed via a cannula (a hollow tube) and aspirator (a suction device). Liposuction techniques can be categorized by the amount of fluid injection and by the mechanism in which the cannula works.

Amount of fluid injection

Dry liposuction

The dry method does not use any fluid injection at all. This method is seldom used today.

Wet liposuction

A small amount of fluid, less in volume than the amount of fat to be removed, is injected into the area. It contains lidocaine as a local anesthetic, adrenaline to contract the blood vessels and thus minimize bleeding, and a salt solution to make the solution isotonic. This fluid helps to loosen the fat cells and reduce bruising. The fat cells are then suctioned out as in the basic procedure.

Super-wet liposuction

Liposuction procedure using the Super-wet technique being performed on female patient

In this method, the infusate volume is in about the same amount as the volume of fat expected to be removed. This is the preferred technique for high-volume liposuction by many plastic surgeons as it better balances haemeostasis and potential fluid overload (as with the tumescent technique). It takes one to three hours, depending on the size of the treated area/ areas. It may require either IV sedation as well as the local lidocaine, or complete anesthesia.

Tumescent liposuction

Tumescent The surgeon injects high volumes of a solution containing a local anesthetic and vasoconstrictor (often lidocaine and epinephrine respectively) directly into the subcutaneous fat to be removed. Due to a potentially large total volume of local anaesthetic injected into the tissue, systemic toxicity from lidocaine is a potentially fatal complication which must be considered with larger volume cases.

Laser assisted liposuction (LAL)

Laser assisted liposuction uses thermal and photomechanical energy to affect the lipolysis. The addition of a laser to traditional liposuction possibly increases skin tightening effects through tissue coagulation. The procedure involves either the use of the Erchonia or Nd:YAG powered devices. The first FDA-approvals came for laser assisted lipolysis units in 2006, but FDA-approved studies using Nd:YAG date back as early as 1994. The efficacy of this technique as opposed to traditional SAL is still being debated.

Mechanism of liposuction

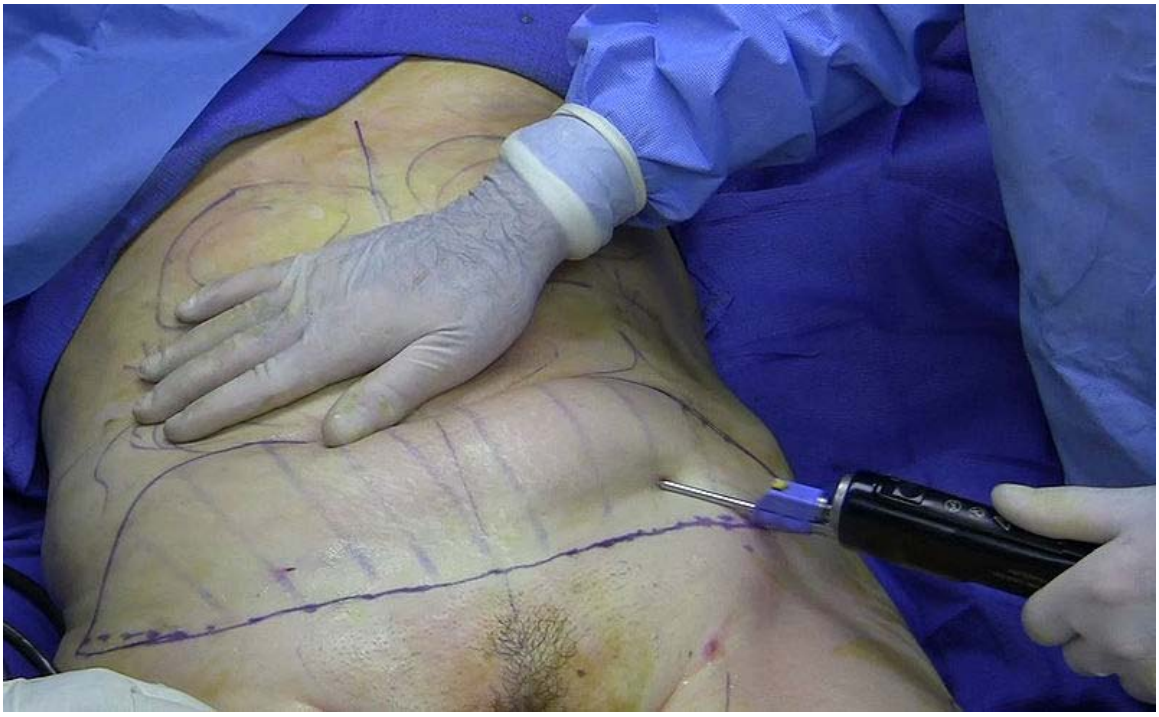
Suction-assisted liposuction (SAL)

Suction-assisted liposuction is the standard method of liposuction. In this approach, a small cannula (like a straw) is inserted through a small incision. It is attached to a vacuum device. The surgeon pushes and pulls it in a forwards and backwards motion, carefully through the fat layer, breaking up the fat cells and drawing them out of the body by suction.

Ultrasound-assisted liposuction (UAL)

In ultrasound-assisted or ultrasonic liposuction, a specialized cannula is used which transmits ultrasound vibrations within the body. This vibration bursts the walls of the fat cells, emulsifying the fat (i.e. liquefying it) and making it easier to suction out. UAL is a good choice for working on more fibrous areas, like the upper back or male breast area. It takes longer than traditional liposuction, but not longer than tumescent liposuction. There is slightly less blood loss. There appears to be slightly more risk of seromas forming (pockets of fluid) which may have to be drained with a needle.

After ultrasonic liposuction, it is necessary to perform suction-assisted liposuction to remove the liquified fat. Ultrasound-assisted liposuction techniques used in the 1980s and 1990s were associated with cases of tissue damage, usually from excessive exposure to ultrasound energy. Third-generation UAL devices address this problem by using pulsed energy delivery and a specialized probe that allows physicians to safely remove excess fat.



A 40-year old woman undergoing a combination liposuction and abdominoplasty. Power-assisted liposuction: the cannula is inserted to about 80% of its full length.

Power-assisted liposuction (PAL)

PAL uses a specialized cannula with mechanized movement, so that the surgeon does not need to make as many manual movements. Otherwise it is similar to traditional SAL.

Twin-cannula (assisted) liposuction (TCAL or TCL)

Twin cannula (assisted) liposuction uses a tube-within-a-tube specialized cannula pair, so that the cannula which aspirates fat, the mechanically reciprocated inner cannula, does not impact the patient's tissue or the surgeon's joints with each and every forward stroke. The aspirating inner cannula reciprocates within the slotted outer cannula to simulate a surgeon's stroke of up to 5 cm (2 in) rather than merely vibrating 1–2 mm (1/4 in) as other power assisted devices, removing most of the labor from the procedure. Superficial or subdermal liposuction is facilitated by the spacing effect of the outer cannula and the fact that the cannulas do not get hot, eliminating the potential for friction burns.

External ultrasound-assisted liposuction (XUAL or EUAL)

XUAL is a type of UAL where the ultrasonic energy is applied from outside the body, through the skin, making the specialized cannula of the UAL procedure unnecessary. It was developed because surgeons found that in some cases, the UAL method caused skin necrosis (death) and seromas, which are pockets of a pale yellowish fluid from the body, analogous to hematomas (pockets of red blood cells).

XUAL is a possible way to avoid such complications by having the ultrasound applied externally. It can also potentially cause less discomfort for the patient, both during the procedure and afterwards; decrease blood loss; allow better access through scar tissue; and treat larger areas. At this time however, it is not widely used and studies are not conclusive as to its effectiveness.

Water-assisted liposuction (WAL)

WAL uses a thin fan-shaped water beam, which loosens the structure of the fat tissue, so that it can be removed by a special cannula. During the liposuction the water is continually added and almost immediately aspirated via the same cannula. WAL requires less infiltration solution and produces less edema from the tumescent fluid. The utility of this technology is under study and is currently not widely used.

Sutures

Since the incisions are small, and the amount of fluid that must drain out is large, some surgeons opt to leave the incisions open, the better to clear the patient's body of excess fluid. They find that the unimpeded departure of that fluid allows the incisions to heal more quickly. Others suture them only partially, leaving space for the fluid to drain out. Others delay suturing until most of the fluid has drained out, about 1 or 2 days. In any case, while the fluid is draining, dressings need to be changed often. After one to three days, small self-adhesive bandages are sufficient.

Preparation

Before receiving any of the procedures, no anticoagulants should be taken for two weeks before the surgery. If general anesthesia or sedation will be used, and the surgery will be in the morning, fasting from midnight the night before is required. If only local anesthesia will be used, fasting is not required. Smoking must be avoided for about two months prior to surgery, as nicotine interferes with circulation and can result in loss of tissue.

The procedure

In all liposuction methods, there are certain things that should be done when having the procedure:

- The candidate and the surgeon will agree ahead of time on exactly which area(s) will be treated and both will discuss what outcome to expect
- A consent form is signed on the day of surgery
- An antibiotic will be given about an hour beforehand, or afterwards
- The targeted areas are marked on the body while the candidate is in a standing position
- Sometimes photos will be taken of the area to be treated, so the patient will have before and after photos

- In the operating room, a sterilizing solution such as Betadine, is applied to the relevant areas
- Local anesthetic is injected and the patient may be given a sedative, either orally, or through an IV injection
- Incisions are small, about a quarter to a third of an inch
- The patient will probably have an IV fluid line, since they will be losing fluid with the fat, and the fluid balance must be kept intact
- There will be some monitoring devices attached to the body to keep track of the blood pressure, heart rate, and blood oxygen level
- The patient will feel only a scraping or rasping sensation from the cannula movement
- Usually the patient can get up, walk around, and go home the same day if they did not receive general anesthesia, although they would need someone else to drive them.

Recovery

Depending on the extent of the liposuction, patients are generally able to return to work between two days and two weeks. A compression garment which can easily be removed by the patient is worn for two to four weeks, this garment must have elasticity and allow for use of bandages. If non-absorbable sutures are placed, they will be removed after five to ten days.

Any pain is controlled by a prescription or over-the-counter medication, and may last as long as two weeks, depending on the particular procedure. Bruising will fade after a few days or maybe as long as two weeks later. Swelling will subside in anywhere from two weeks to two months, while numbness may last for several weeks. Normal activity can be resumed anywhere from several days to several weeks afterwards, depending on the procedure. The final result will be evident anywhere from one to six months after surgery, although the patient will see noticeable difference within days or weeks, as swelling subsides.

The suctioned fat cells are permanently gone. However, if the patient does not maintain a proper diet and exercise regimen, the remaining fat cell neighbors could still enlarge, creating irregularities.

Side effects

A side effect, as opposed to a complication, is medically minor, although it can be uncomfortable, annoying, and even painful.

- Bruising: can be painful in the short term, and should fade after a few weeks.
- Swelling: should subside gradually over a month or two.
- Scars: will vary in size depending on the particular procedure, and should fade over the weeks. Scarring is an individual thing, partly dependent on heredity. For some, scar healing may take as long as a year.

- Pain: should be temporary and controlled by either over-the-counter medication, or by a prescription.
- Numbness: sometimes persists for a few weeks.
- Limited mobility: will depend on the exact procedure.

There could be various factors limiting movement for a short while, such as:

- Wearing a compression garment
- Keeping the head elevated
- Temporary swelling or pain

The surgeon should advise on how soon the patient can resume normal activity.

Possible complications

As with any surgery, there are certain risks, beyond the temporary and minor side effects. The surgeon should mention them during a consultation. Careful patient selection minimizes their occurrence. Their likelihood is somewhat increased when treated areas are very large or numerous and a large amount of fat is removed.

During the 1990s there were some deaths as a result of liposuction, as well as alarmingly high rates of complication. By studying more and educating themselves further, surgeons have reduced complication rates. A study published in *Dermatologic Surgery* (July 2004, pp. 967–978), found that "The overall clinical complication rate [for liposuction] ... was 0.7% (5 out of 702)", the minor complication rate was 0.57%, and the major complication rate was 0.14% with one patient requiring hospitalization.

The more serious possible complications include:

- Allergic reaction to medications or material used during surgery.
- Infection: any time the body is incised or punctured, bacteria can get in and cause an infection. During liposuction, multiple small puncture wounds are made for inserting the cannula, that can vary in size depending on the technique.
- Damage to the skin: most surgeons work on the deeper levels of fat, so as to avoid wounding the skin any more than they must for the insertion of the cannula.
- Sometimes the cannula can damage tissue beneath the skin, which may show up as a spotted appearance on the skin surface.
- Skin necrosis (dead skin) is a rare complication, in which the skin falls off in the necrotic area. The problem can vary in degree. The resulting wound then needs to heal typically requiring extended wound care.
- Puncture of an internal organ: since the surgeon can't see the cannula, sometimes it damages an internal organ, such as the intestines during abdominal liposuction. Such damage can be corrected surgically, although in rare cases it can be fatal. An experienced cosmetic surgeon is unlikely to puncture any internal organ.
- Contour irregularities: sometimes the skin may look bumpy and/or withered, because of uneven fat removal, or poor skin elasticity. Not all patients heal in the

same way, and with older patients the healing may be slower and a bit imperfect. Sometimes a small touch-up procedure can help.
- Thromboembolism and fat embolisation: although liposuction is a low-risk procedure for thromboembolism including pulmonary embolism, the risk can't be ignored.
- Burns: sometimes the cannula movement can cause friction burns to skin or nerves. Also, in UAL, the heat from the ultrasound device can cause injury to the skin or deeper tissue.
- Lidocaine toxicity: when the super-wet or tumescent methods are used, too much saline fluid may be injected, or the fluid may contain too high a concentration of lidocaine. Then the lidocaine may become too much for that particular person's system. Lidocaine poisoning at first causes tingling and numbness and eventually seizures, followed by unconsciousness and respiratory or cardiac arrest.
- Fluid imbalance: since fat contains a lot of fluid and is removed in liposuction, and since the surgeon injects fluid for the procedure, even a very large amount of it for tumescent liposuction, there is a danger of the body's fluid balance being disturbed. This could happen afterwards, after the patient is at home. If too much fluid remains in the body, the heart, lungs and kidneys could be badly affected.

The cosmetic surgeon should give the participant a written list of symptoms to watch for, along with instructions for post-op self-care.

Combined with other procedures

Liposuction and tightening / lifting skin

The removal of quantities of fat from under the skin allows the elastic skin to potentially retract after SAL. Good examples of this effect are seen after liposuction to the arms, stomach areas and breasts. The level of skin retraction following liposuction is affected by the age of the patient, quality of skin, presence of underlying disease or smoking and the presence of previous skin damage such as caused by childbirth and surgery. Liposuction techniques such as subdermal undermining using fine cannulas can stimulate further skin retraction but are more frequently associated with contour irregularity. While subdermal undermining may help the skin contract, patients with severe elasticity loss and heavy stretch marks prior to liposculpture may require removal of redundant skin by surgical means after liposculpture. Usually this can be performed after 6 months.

Surgical lifts such as a rhytidectomy (facelift), mastopexy (breast lift), abdominoplasty (tummy tuck), or lower body lift, thigh lift, or buttock lift can be utilized when sagging skin alone is the issue or after massive weight loss when the combination of large amounts of skin and shrunken fat cause significant skin droop.

Large volume Liposuction (SAL) in combination with other surgery is common, but may have higher complication rates. When done simultaneously, SAL is done minimally in the areas of the undermined tissues to minimize further insult to the blood supply,

however a new techniques in tummytuck surgery involves vigorous liposuction first before excising the redundant skin.

Non-surgical alternatives

Cryolipolysis

Cryolipolysis refers to the external application of controlled cooling to reduce limited fat bulges.

Shapewear

One non-surgical alternative that has gained in popularity is the use of shapewear garments. Although shapewear cannot provide patients with the same level of results as liposuction, body scans have shown that they can remove bulges and slim the waist, hips, and thighs. Most shapewear products are similar to the post-surgical compression garments but unlike the post-surgical garments, shapewear is designed for long-term daily use.

Diet and exercise

Healthy eating habits combined with regular exercise has also been proven to cause weight loss. However, the process can take much longer compared to liposuction. However, losing weight via exercise and eating a healthy diet carries much less risk than liposuction.

Liposuction does not significantly improve the metabolic abnormalities associated with obesity, and does not achieve the general health benefits (such as increased cardiovascular health) associated with weight loss.