Ministry of Public Health of Ukraine
Poltava State Medical University
Department of biological and bioorganic chemistry

Biochemistry of blood.
Blood plasma proteins: proteins of acute phase of inflammation, secretory and indicatory proteins.
Respiratory function of erythrocytes. Pathological forms of hemoglobins.
Nonprotein nitrogenous organic compounds of blood plasma (residual nitrogen).

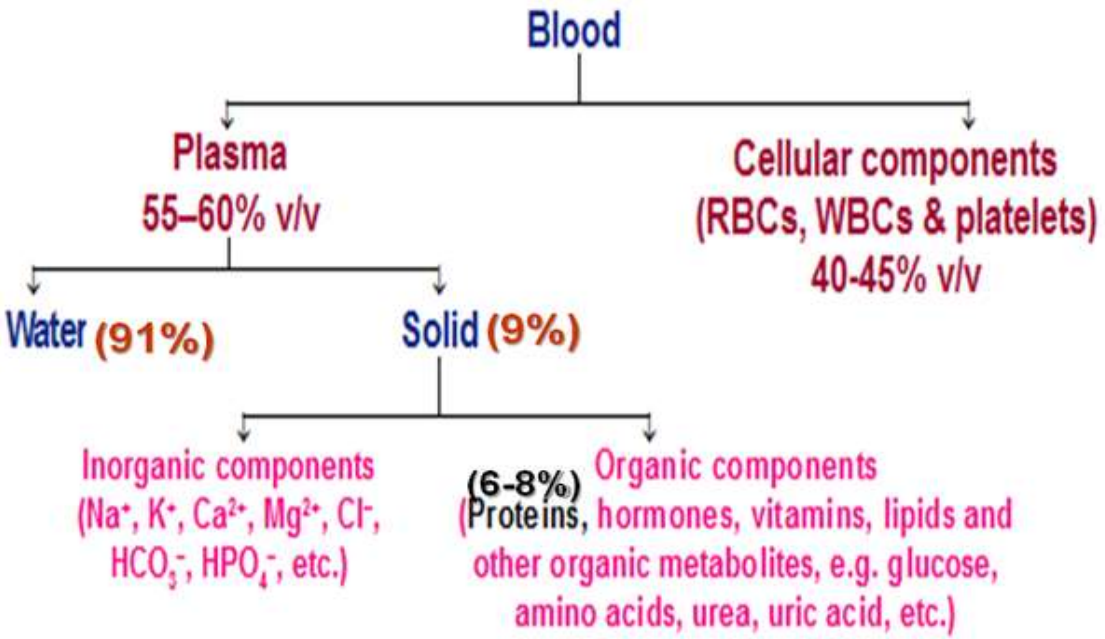
Assoc. Prof. Bilets M.V.

Lecture plan

- Proteins of blood plasma
- Enzymes of blood plasma
- The kallikrein-kinin system.
- Respiratory function of erythrocytes
- Acid-base balance of the organism.
- Nonprotein nitrogenous organic compounds of blood plasma.

Blood

- Blood is a specialized body fluid. It has four main components: **plasma**, **erythrocytes (**red blood cells), **leukocytes (**white blood cells), and **thrombocytes (**platelets).



https://www.quora.com/What-are-the-chief-components-of-blood-plasma

Blood functions:

Transport:

Blood transports:

- Gases, like oxygen (O_2) and carbon dioxide (CO_2), between the lungs and rest of the body
- Nutrients from the digestive tract and storage sites to the rest of the body
- Waste products to be detoxified or removed by the liver and kidneys
- Hormones from the glands in which they are produced to their target cells

Protection:

- Leukocytes, or white blood cells, destroy invading microorganisms and cancer cells
- Antibodies and other proteins destroy pathogenic substances
- Platelet factors initiate blood clotting and help minimise blood loss

Regulation

- pH by interacting with acids and bases
- Water balance by transferring water to and from tissues

Blood plasma

- **Blood plasma** is a yellowish liquid component of blood that holds the blood cells of whole blood in suspension. It is the liquid part of the blood that carries cells and proteins throughout the body.
- Plasma constitutes 55% of total blood volume. Composed of 90% water, salts, lipids and hormones, it is especially rich in proteins (including its main protein albumin), immunoglobulins, clotting factors and fibrinogen.
- **Serum** is the fluid and solute component of blood which does not play a role in clotting. It may be defined as blood plasma without fibrinogens.

Blood plasma proteins

- Plasma proteins are most often divided into fractions: albumins, α_1-globulins, α_2-globulins, β-globulins and γ-globulins, depending on their charge and size.

Fractions of plasma proteins

Fraction
Albumins: Albumin, pre-albumin (transthyretin)
α_1-globulins: Thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein
α_2-globulins: Haptoglobin, macroglobulin, ceruloplasmin
β-globulins: Transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C_3 and C_4 components of the complement system
γ-globulins: IgG, IgM, IgA, IgD, IgE

https://www.slideshare.net/YESANNA/plasma-proteins-70169448

Blood plasma protein concentration

- **Total blood plasma protein concentration: 65.0-85.0 g/l**
- **Albumins: 35.0-50.0 g/l**
- **Globulins: 23.0-35.0 g/l**
- **Protein coefficient (albumin/globulin index): 1.5-2.3 g/l**

Dysproteinemia is a change in the ratio of individual protein fractions. The total protein content remains normal.
Causes:
- Dysfunctions of the kidneys, the A / G ratio decreases due to the loss of albumin to a greater extent;
- Liver dysfunctions. The A / G ratio decreases due to a decrease in the synthesis of albumins and globulins;
- Infectious diseases, accompanied by an increase in antibodies.

Paraproteinemia is the appearance of proteins that do not exist normally.
- Interferon - a specific protein synthesized in the body as a result of the penetration of various viruses;
- C-reactive protein - appears in the blood during the acute period of the disease (acute phase protein) or during the exacerbation of the chronic process (pneumonia, rheumatism, etc.);
- Myeloma proteins - with multiple myeloma;
- Macroglobulins - with Waldenstrom's macroglobulinemia;

Hypoproteinemia - a decrease in the concentration of total blood protein.
It is observed with bleeding, malignant neoplasms, kidney and liver diseases, starvation, etc.

Hyperproteinemia - an increase in the concentration of total blood protein.
Relative hyperproteinemia is associated with water loss and, as a result, an increase in the concentration of total protein (diarrhea, vomiting, diabetes mellitus and diabetes insipidus, cholera, dysentery).
Absolute hyperproteinemia occurs due to increased production of proteins, for example, the formation of γ-globulins in infectious diseases.

Albumins

- Albumin is a family of globular proteins, the most common of which are the serum albumins. All the proteins of the albumin family are water-soluble. Albumins are commonly found in blood plasma and differ from other blood proteins in that they are not glycosylated.

Albumins make up approximately 2/3 of the total protein.

Human albumin is a small globular protein (molecular weight: 66.5 kDa), consisting of a single chain of 585 amino acids organized in three repeated homolog domains.

Albumin is synthesized by liver hepatocytes and rapidly excreted into the bloodstream at the rate of about 10 gm to 15 gm per day.

Albumin functions:

Maintenance of oncotic blood plasma pressure. Therefore, with a decrease in the content of albumin in the plasma, the oncotic pressure drops, and the fluid leaves the bloodstream into the tissues.
"Hungry" edema develops. Albumin provides about 80% of the plasma oncotic pressure. It is the albumin that is easily lost in the urine in kidney disease.
Therefore, they play an important role in the drop in oncotic pressure in such diseases, which leads to the development of edema.

Transport function. Albumin transports many substances in the blood, especially those that are poorly soluble in water: free fatty acids, fat-soluble vitamins, steroids, hormones (thyroxine, triiodothyronine, cortisol), metabolites (uric acid, bilirubin), some ions (Ca2 +, Mg2 +). For the binding of calcium in the albumin molecule there are special calcium-binding centers. In complex with albumin, many drugs are transported, for example, acetylsalicylic acid, penicillin.

Albumin is a ***reserve of free amino acids*** in the body, formed as a result of proteolytic cleavage of these proteins.

Buffer function of albumin – they are components of the buffer system of blood plasma, as they contain a large amount of charged (negative) amino acids.

Globulins

- All globulins fall into one of the following four categories :

Alpha 1 globulins

Alpha 2 globulins

Beta globulins

Gamma globulins (one group of gamma globulins is the immunoglobulins, which are also known as "antibodies")

- Globulins exist in various sizes. The lightest globulins are the alpha globulins, which typically have molecular weights of around 93 kDa, while the heaviest class of globulins are the gamma globulins, which typically weigh about 1193 kDa. Being the heaviest, the gamma globulins are among the slowest to segregate in gel electrophoresis.

Alpha 1 globulins

- **Alpha-1-antitrypsin** is the major component of circulating alpha-1-globulin, and is a potent inhibitor of many proteases, including trypsin, chymotrypsin, elastase, and collagenase.
- This heterogenous glycoprotein has a molecular weight of 54,000 and consists of a single polypeptide chain with four carbohydrate side chains, mannose, galactose, n-acetyl glucosamine, and sialic acid.
- Alpha-1-antitrypsin is synthesized in hepatocytes, has a half-life of approximately one week, and appears in many bodily secretions, including bile, milk, saliva, semen, cervical fluid, and lymph.
- Levels increase in the presence of inflammation, malignant neoplasms, and pregnancy. Alpha-1-antitrypsin is a major agent in limiting potential bodily injury from inflammatory and destructive agents, and appears to inactivate proteolytic enzymes by combining with them at lysine linkages.
- Deficiency in this protein is responsible for some cases of pulmonary emphysema. Smoking promotes the oxidation of a methionine residue of α1-antiproteinase which inactivates it. This is the reason why smokers who have low levels of this glycoprotein are predisposed to proteolytic destruction of lung tissue and the production of lung emphysema and pulmonary obstructive chronic disease (POCD).

- **Orosomucoid.** Also called α1 acid glycoprotein, has a high proportion of carbohydrates. The concentration of this protein increases during inflammatory processes, along with the C-reactive protein (so called because it reacts with the pneumococcal polysaccharide C).
- **Prothrombin.** This is the precursor to thrombin, the enzyme that catalyzes the conversion of fibrinogen to fibrin, the final step of blood coagulation.
- **Transcortin.** This is the protein responsible for the transport of cortisol, a hormone produced in the adrenal gland cortex.

Alpha 2 globulins

Ceruloplasmin. This is a protein with a mass of 151 kDa, Which contains six copper atoms per molecule and has an intense blue color. It functions as a copper carrier and is an enzyme which exhibits ferroxidase activity.

In Wilson's disease, an autosomal recessive genetic disorder, ceruloplasmin levels in plasma are decreased. The copper accumulates in the brain and liver causing neurological symptoms and liver disease.

α_2 macroglobulin is a 720-kDa homotetramer. It is mainly produced by the liver, and also locally synthesized by macrophages fibroblasts, and adrenocortical cells.
Transports 10% of the total zinc present in plasma and functions as a protease inhibitor. It functions as an inhibitor of fibrinolysis by inhibiting plasmin and kallikrein. It functions as an inhibitor of coagulation by inhibiting thrombin. Alpha-2-macroglobulin may act as a carrier protein because It also binds to numerous growth factors and cytokines, such as platelet-derived growth factor, basic fibroblast growth factor, TGF-β, insulin, and IL-1β.

No specific deficiency with associated disease has been recognized, and no disease state is attributed to low concentrations of alpha-2-macroglobulin. The concentration of alpha-2-macroglobulin rises 10-fold or more in the nephrotic syndrome when other lower molecular weight proteins are lost in the urine. The loss of alpha-2-macroglobulin into urine is prevented by its large size. The net result is that alpha-2-macroglobulin reaches serum levels equal to or greater than those of albumin in the nephrotic syndrome, which has the effect of maintaining oncotic pressure.

Haptoglobin is a protein that binds to hemoglobin. Hemoglobin normally circulates in plasma in low amounts due to its release by eventual red bloocell hemolysis. This protein–protein association prevents free circulating Hb from being excreted in the urine (free hemoglobin in plasma can cross the kidney filter).

Haptoglobin, in its simplest form, consists of two alpha and two beta chains, connected by disulfide bridges. The chains originate from a Common precursor protein, which is proteolytically cleaved during protein synthesis.

Beta globulins

Transferrin. This protein binds two atoms of iron and serves as an iron carrier in plasma. In cases of iron deficiency or during normal pregnancy, transferrin levels increase significantly. It is decreased in pernicious anemia, chronic infections, and liver failure.
Transferrin consists of a polypeptide chain containing 679 amino acids and two carbohydrate chains. The protein is composed of alpha helices and beta sheets that form two domains. The N- and C-terminal sequences are represented by globular lobes and between the two lobes is an iron-binding site.
They are produced in the liver and contains binding sites for two Fe^{3+} atoms. Human transferrin is encoded by the *TF* gene and produced as a 76 kDa glycoprotein.

β_2 microglobulin. This is a small 11.7-kDa protein found in cell membranes. It is part of the major histocompatibility complex (MHC) class I.

Sex hormone-binding globulin (**SHBG**) or **sex steroid-binding globulin** (**SSBG**) is a glycoprotein that binds to androgens and estrogens. Other steroid hormones such as progesterone, cortisol, and other corticosteroids are bound by transcortin.

Plasmin is an important enzyme present in blood that degrades many blood plasma proteins, including fibrin clots.

Plasmin is a serine protease that acts to dissolve fibrin blood clots. Apart from fibrinolysis, plasmin proteolyses proteins in various other systems: It activates collagenases, some mediators of the complement system, and weakens the wall of the Graafian follicle, leading to ovulation. Plasmin is also integrally involved in inflammation. It cleaves fibrin, fibronectin, thrombospondin, laminin, and von Willebrand factor. Plasmin, like trypsin, belongs to the family of serine proteases.

Plasmin is released as a zymogen called plasminogen (PLG) from the liver into the systemic circulation.
C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose circulating concentrations rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q.
According to other sources of information, C-reactive protein belongs to alpha 2 globulins

CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes).

Acute-phase proteins

- **Acute-phase proteins (APPs)** are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction (also called acute-phase response). The acute-phase reaction characteristically involves fever, acceleration of peripheral leukocytes, circulating neutrophils and their precursors.
- In response to injury, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL1, and IL6, and TNFα. The liver responds by producing many acute-phase reactants. At the same time, the production of a number of other proteins is reduced; these proteins are, therefore, referred to as "negative" acute-phase reactants. Increased acute-phase proteins from the liver may also contribute to the promotion of sepsi
- "Negative" acute-phase proteins decrease in inflammation. Examples include albumin, transferrin, transthyretin, retinol-binding protein, antithrombin, transcortin. The decrease of such proteins may be used as markers of inflammation. The physiological role of decreased synthesis of such proteins is generally to save amino acids for producing "positive" acute-phase proteins more efficiently. Theoretically, a decrease in transferrin could additionally be decreased by an upregulation of transferrin receptors, but the latter does not appear to change with inflammation.
- While the production of C3 (a complement factor) increases in the liver, the plasma concentration often lowers because of an increased turn-over, therefore it is often seen as a negative acute-phase protein.
- Measurement of acute-phase proteins, especially C-reactive protein, is a useful marker of inflammation. It correlates with the erythrocyte sedimentation rate (ESR), however not always directly. This is due to the ESR being largely dependent on elevation of fibrinogen, an acute phase reactant with a half-life of approximately one week. This protein will therefore remain higher for longer despite removal of the inflammatory stimuli. In contrast, C-reactive protein (with a half-life of 6–8 hours) rises rapidly and can quickly return to within the normal range if treatment is employed. For example, in active systemic lupus erythematosus, one may find a raised ESR but normal C-reactive protein.

"Positive" acute-phase proteins:

Protein	Immune system function
C-reactive protein	Opsoni on microbes[(not an acute-phase reactant in mice)
Serum amyloid P component	Opsonin
Serum amyloid A	•Recruitment of immune cells to inflammatory sites •Induction of enzymes that degrade extracellular matrix
Complement factors	Opsonization, lysis and clumping of target cells. Chemotaxis
Mannan-binding lectin	Mannan-binding lectin pathway of complement activation
Fibrinogen, prothrombin, factor VIII, von Willebrand factor	Coagulation factors trapping invading microbes in blood clots. Some cause chemotaxis
Plasminogen activator inhibitor-1 (PAI-1)	Prevents the degradation of blood clots by inhibiting tissue Plasminogen Activator(tPA)
Alpha 2-macroglobulin	•Inhibitor of coagulation by inhibiting thrombin. •Inhibitor of fibrinolysis by inhibiting plasmin
Ferritin	Binding iron, inhibiting microbe iron uptake
Hepcidin	Stimulates the internalization of ferroportin, preventing release of iron bound by ferritin within intestinal enterocytes and macrophages
Ceruloplasmin	Oxidizes iron, facilitating for ferritin, inhibiting microbe iron uptake
Haptoglobin	Binds hemoglobin, inhibiting microbe iron uptake and prevents kidney damage
Orosomucoid (Alpha-1-acid glycoprotein, AGP)	Steroid carrier
Alpha 1-antitrypsin	Serpin, downregulates inflammation
Alpha 1-antichymotrypsin	Serpin, downregulates inflammation

Enzymes of blood plasma

- Blood plasma contains many enzymes, which are classified into **functional and non-functional plasma enzymes/**
- **Functional plasma enzymes:**

Present in plasma in higher concentrations in comparison to tissues;

Their substrates are always present in the blood;

Synthesized by liver; Decrease in liver diseases;

Examples: clotting factors e.g. prothrombin, Lipoprotein lipase and pseudocholine esterase.

- **Non-functional plasma enzymes:**

Normally, present in plasma in very low concentrations in comparison to tissues;

Their substrates are absent from the blood;

Synthesized by different organs e.g. liver, heart, brain and skeletal muscles; Different enzymes increase in different organ diseases;

Examples: alanineaminotransferase, aspartateaminotransferase, creatinekinase, lactatedehydrogenase, alkaline phosphatase, acid phosphatase and amylase.

Enzymes of blood plasma

- **Sources of non-functional plasma enzymes :**

1. Increase in the rate of enzyme synthesis) e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver diseases.

2. Obstruction of normal pathway e.g. obstruction of bile ducts increases alkaline phosphatase.

3. Increased permeability of cell membrane as in tissue hypoxia.

4. Cell damage with the release of its content of enzymes into the blood e.g. myocardial infarction and viral hepatitis.

- **Medical importance of non-functional plasma enzymes :**

Measurement of non-functional plasma enzymes is important for:

1.Diagnosis of diseases as diseases of different organs cause elevation of different plasma enzymes.

2. Prognosis of the disease; we can follow up the effect of treatment by measuring plasma enzymes before and after treatment.

Examples of medically important non-functional plasma enzymes :

1.Amylase and lipase enzymes increase in diseases of the pancreas as acute p

ancreatitis.

2. Creatine kinase (CK) enzyme increases in heart, brain and skeletal muscle diseases.

3. Lactate dehydrogenase (LDH) enzyme increases in heart, liver and blood diseases.

4. Alanine transaminase (ALT) enzyme, it is also called serum glutamic pyruvic transaminase (SGPT). It increases in liver and heart diseases.

5. Aspartate transaminase (AST) enzyme, it is also called serum glutamic oxalacetic transaminase (SGOT). It increases in liver and heart diseases.

6. Acid phosphatase enzyme increases in cancer prostate.

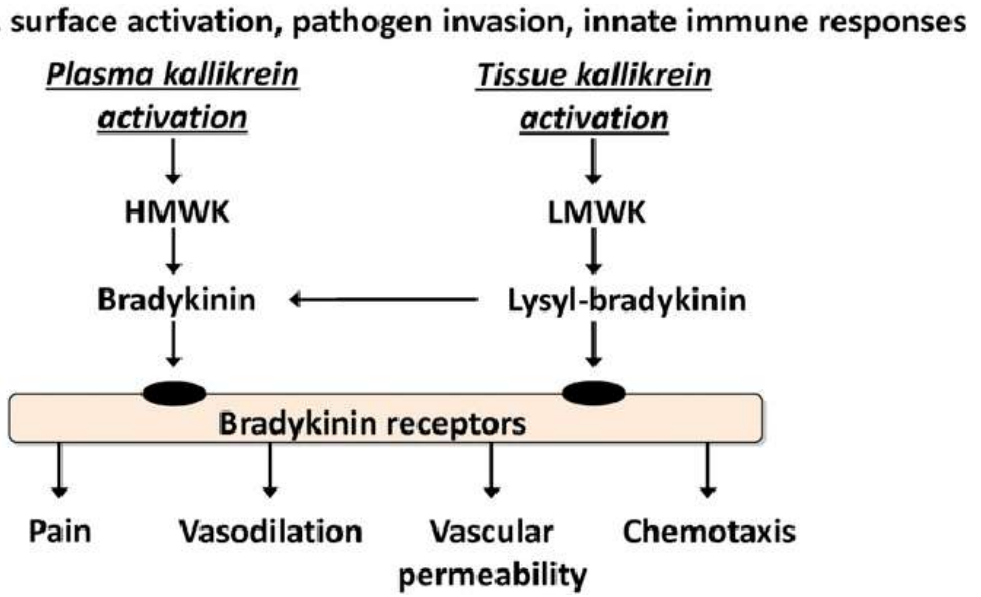
7. Alkaline phosphatase enzyme increases in obstructive liver diseases, bone diseases and hyperparathyroidism.

Kallikrein- kinin system

- **The kallikrein - kinin system** consists of blood proteins that play a role in inflammation, blood pressure control, coagulation and pain.
- **Kallikreins (tissue and plasma kallikrein)** are serine proteases that liberate kinins from the kininogens, which are plasma proteins that are converted into vasoactive peptides. Prekallikrein is the precursor of plasma kallikrein. It can only activate kinins after being activated itself by factor XIIa or other stimuli.
- Its important mediators **bradykinin** and **kallidin** are vasodilators and act on many cell types.clinical symptoms include marked weakness, tachycardia, fever, leukocytosis, acceleration of ESR.
- **High-molecular weight kininogen (HMWK)** and **low-molecular weight kininogen (LMWK)** are precursors of the polypeptides. They have no activity of themselves.
- HMWK is produced by the liver together with **prekallikrein** It acts mainly as a cofactor on coagulation and inflammation, and has no intrinsic catalytic activity.
- LMWK is produced locally by numerous tissues, and secreted together with tissue kallikrein.

Bradykinin (BK), which acts on the B2 receptor and slightly on B1, is produced when kallikrein releases it from HMWK. It is a nonapeptide (9 amino acids) with the amino acid sequence Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg. BK is a proinflammatory peptide, a pain mediator and potent vasodilator, leading to robust accumulation of fluid in the interstitium.
Kallidin (KD) or lysylbradikinin is released from LMWK by tissue kallikrein. It is a decapeptide. KD has the same amino acid sequence as Bradykinin with the addition of a Lysine at the N-terminus, thus is sometimes referred to as Lys-Bradykinin.

HMWK and LMWK are formed by alternative splicing of the same gene.

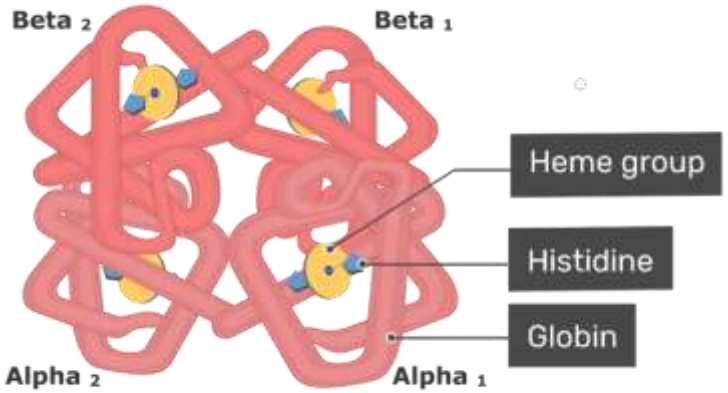


https://www.researchgate.net/figure/Role-of-kallikreins-in-kininogen-fragmentation-In-response-to-trauma-immune-and_fig5_308771191

Hemoglobin

- Hemoglobin is an oxygen-binding protein found in erythrocytes which transports oxygen from the lungs to tissues. Each hemoglobin molecule is a tetramer made of four polypeptide globin chains. Each globin subunit contains a heme moiety formed of an organic protoporphyrin ring and a central iron ion in the ferrous state (Fe2+). The iron molecule in each heme moiety can bind and unbind oxygen, allowing for oxygen transport in the body. The most common type of hemoglobin in the adult is **HbA,** which comprises two alpha-globin (each chain consists of 141 amino acids) and two beta-globin (each chain consists of 146 amino acids) subunits. Different globin genes encode each type of globin subunit.
- **HbA** production explodes after birth and ultimately makes up 95-98% of hemoglobin in adults.
- **HbA2** is a less common adult form of hemoglobin. It comprises two alpha and two delta-globin subunits and makes up 1-3% of hemoglobin in adults
- During pregnancy, the fetus primarily produces **fetal hemoglobin** (HbF). HbF comprises two a and two gamma-globin subunits. HbF has a stronger oxygen affinity than HbA, allowing oxygen to flow from maternal to fetal circulation through the placenta. Production of HbF drops significantly after birth, reaches low, near-adult, levels by two years, and ultimately makes up 2-3% of hemoglobin in adults.
- Heme is an iron **porphyrin compound.** Porphyrin is a **tetrapyrrole structure**.
- Ferrous iron occupies the center of the porphyrin ring and establishes linkages with all the four nitrogens of all the pyrrole rings.

It is also linked to the nitrogen of the imidazole ring of histidine present in the globin part.
Globin part is made of four polypeptide chains, to identical α-chains and two identical β-chains in normal adult hemoglobin.
Each chain contains a “heme” in the so-called ‘heme pocket’. So one Hb molecule possesses four heme units.
Hb molecule contains hydrophobic amino acids inside and hydrophilic ones on the surface.

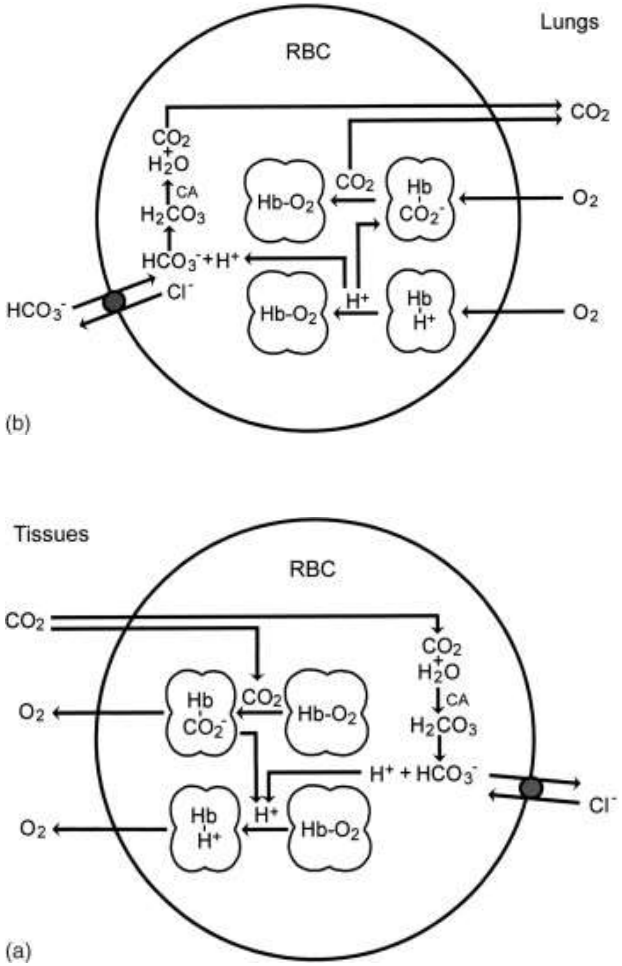


https://www.getbodysmart.com/respiratory-gases-and-their-transport/hemoglobin-structure

Transport of oxygen and carbon dioxide by hemoglobin

- Haemoglobin molecule has high affinity for oxygen and the attachment of oxygen to heme is readily reversible. In the alveoli of lungs, haemoglobin comes to direct contact with a rich supply of oxygen (partial pressure (pO2)=90-100 mm Hg) and is converted to oxyhaemoglobin.
- These oxyhaemoglobin is carried by the arterial circulation to the cells in which there is a low oxygen concentration (partial pressure = 25-40 mm Hg) and a relatively high concentration of carbon dioxide (60 mm Hg) prevails. The oxygen is given up to these cells; the resulting haemoglobin carries some of the carbon dioxide back to the lungs to be expelled, and more oxyhaemoglobin is formed.
- **Oxygenation of hemoglobin depends on:**

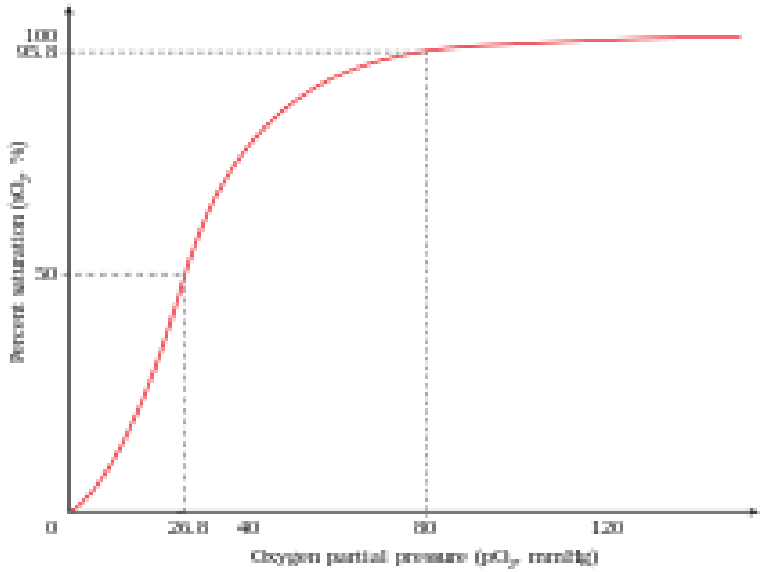
- partial pressure of oxygen and CO2 (the partial pressure of O2 favors oxygenation, partial pressure of CO2 favors dissociation);
- pH (The addition of H+ results in a decrease in oxygen affinity of Hb (Bohr effect));
- concentration of 2,3 diphosphoglycerate (promotes hemoglobin transition from a high-oxygen-affinity state to a low-oxygen-affinity state)



https://www.sciencedirect.com/topics/medicine-and-dentistry/bohr-effect

Cooperativity effect of hemoglobin

- One oxygen molecule can bind to the ferrous iron of a heme molecule in each of the four chains of a hemoglobin molecule.
- Deoxy-hemoglobin has a relatively low affinity for oxygen, but when one molecule binds to a single heme, the oxygen affinity increases, allowing the second molecule to bind more easily, and the third and fourth even more easily.
- The oxygen affinity of 3-oxy-hemoglobin is ~300 times greater than that of deoxy-hemoglobin.
- This behavior leads the affinity curve of hemoglobin to be sigmoidal.
- By the same process, the ability for hemoglobin to lose oxygen increases as fewer oxygen molecules are bound.



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

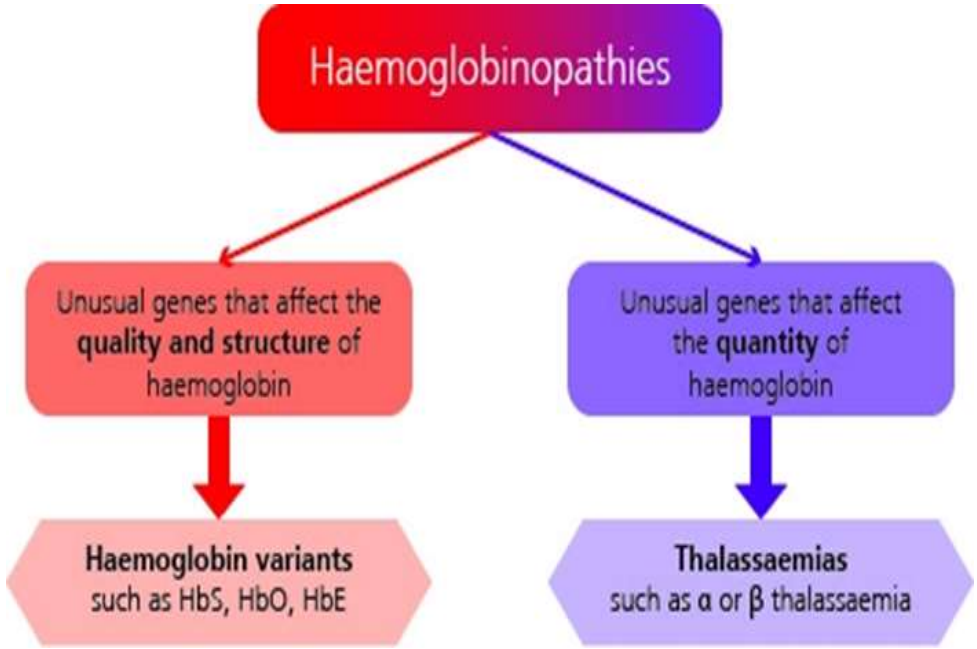
https://en.wikipedia.org/wiki/Cooperativity#:~:text=An%20example%20of%20positive%20cooperativity%20is%20the%20binding%20of%20oxygen%20to%20hemoglobin.&text=This%20behavior%20leads%20the%20affinity,fewer%20oxygen%20molecules%20are%20bound.

Forms of hemoglobin

- **O*xyhemoglobin (oxy-Hb)* –** hemoglobin with **O2**. Oxygenated Hb is in a relaxed state i.e, ‘R’ state. R state characterized by the removal of valine residue from heme pocket of β-subunit; broken salt bridges; cannot bind BPG; FFe++ comes in the plane of porphyrin ring; Heme-Heme interaction increases the affinity to O2; Histidines ofβ-chains release protons (H+).
- ***Deoxyhemoglobin (deoxy-Hb)*** - In “T” form i.e. Taut form, salt bridges plenty and intact, valine residue covers heme pocket of β-chain and does not allow entry of O2; β-chain histidine residue protonated (H+ added).
- **Carbaminohemoglobin (carbhemoglobin, carbohemoglobin)** is a compound of hemoglobin and **carbon dioxide**, and is one of the forms in which carbon dioxide exists in the blood. Twenty-three percent of carbon dioxide is carried in blood this way (70% is converted into bicarbonate by carbonic anhydrase and then carried in plasma, 7% carried as free CO2, in solution, or plasma
- **Carboxyhemoglobin (Hb+ carbo monoxide):** Firmer combination, not reversible, the affinity of Hb to **CO** in 210 times more than)2; inhibits cytochrome oxidase of electron transport chain
- **Sulfhaemoglobin:** Greenish pigment; formed when **H2S** reacts with Oxy-Hb, seen in severe constipation, certain types of bacteria.
- ***Methemoglobin (met-Hb*** Methemoglobin (methaemoglobin) is a hemoglobin in the form of metalloprotein, in which the iron in the heme group is in the **Fe3+** (ferric) state, not the Fe2+ (ferrous) of normal hemoglobin.
- Methemoglobin cannot bind oxygen, which means it cannot carry oxygen to tissues. It is bluish chocolate-brown in color. In human blood a trace amount of methemoglobin is normally produced spontaneously, but when present in excess the blood becomes abnormally dark bluish brown.
- The NADH-dependent enzyme methemoglobin reductase is responsible for converting methemoglobin back to hemoglobin.
- **Glycated hemoglobin (HbA1c)** is a form of hemoglobin (Hb) that is chemically linked to a glucose.
- The formation of the sugar-hemoglobin linkage indicates the presence of excessive sugar in the bloodstream, often indicative of diabetes mellitus.

Hemoglobinopathy and hemoglobin variants

- **Haemoglobinopathies** are a group of recessively inherited genetic conditions affecting the haemoglobin component of blood. They are caused by a genetic change (mutation) in the haemoglobin. More than 1,000 mutations have been identified that result in either haemoglobin variants or thalassaemias.
- Most of these unusual genes are clinically insignificant. However, there is a genetic relevance to some haemoglobinopathies which, when combined with other variants or thalassaemias, may cause a significant clinical condition resulting in illness and potential death.
- The most significant haemoglobinopathies result in either a change in the structure and quality of the haemoglobin or a reduction in the quantity of haemoglobin produced.
- Haemoglobinopathies are not gender (x) linked, more prevalent in some parts of the world
- Sickle cell disease is most common in West Africa and India. Thalassaemia major is more common in Asia and Mediterranean countries.
- The likelihood of a person being a carrier of a haemoglobinopathy depends on ancestry. The type of mutation varies between ethnic groups.
- It is possible to inherit mutations in both alpha and beta globin genes at the same time.
- It is also possible (although rare) for an individual to have a ‘de novo’ haemoglobin mutation. This is a genetic mutation that is not directly inherited from parents but is present only in that individual.

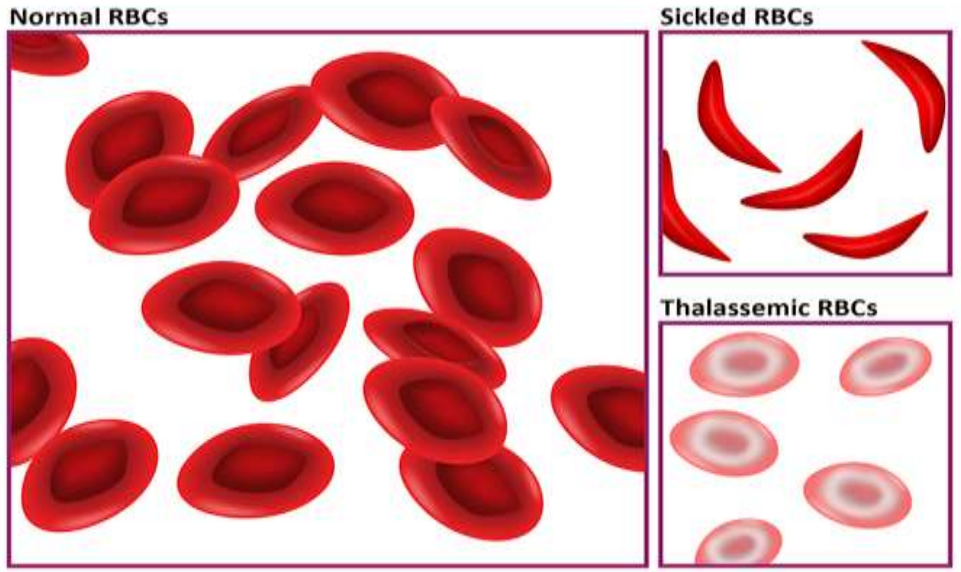


https://www.gov.uk/government/publications/handbook-for-sickle-cell-and-thalassaemia-screening/understanding-haemoglobinopathies

Hemoglobinopathy and hemoglobin variants

- Hemoglobin variants are mutant forms of hemoglobin in a population (usually of humans), 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.
- Normal hemoglobin types are: **Hemoglobin A** (Hb A), which is 95-98% of hemoglobin found in adults, **Hemoglobin A2** (Hb A2), which is 2-3% of hemoglobin found in adults, and **Hemoglobin F** (Hb F), which is found in adults up to 2.5% and is the primary hemoglobin that is produced by the fetus during pregnancy.
- **Hemoglobin S** (Hemoglobin S, HbS) is a special mutant form of hemoglobin that is formed in patients with sickle cell anemia and is prone to crystallization instead of the formation of a normal quaternary structure and dissolution in the erythrocyte cytoplasm. There is a single amino acid substitution in hemoglobin S compared to normal hemoglobin A: L-glutamic acid in the 6th position of the β-chain of globin replaces L-valine. As a result of this replacement, the solubility of hemoglobin drops sharply.
- **Hemoglobin C** (HbC) is an abnormal hemoglobin in which glutamic acid residue at the 6th position of the β-globin chain is replaced with a lysine residue due to a point mutation in the HBB gene.
- **Hemoglobin E** (HbE) is an abnormal hemoglobin with a single point mutation in the β chain. At position 26 there is a change in the amino acid, from glutamic acid to lysine (E26K). Hemoglobin E is very common among people of Southeast Asian including Northeast Indian, East Asian descent.

- Thalassemia is a disease inherited in a recessive manner, which is based on a decrease in the synthesis of polypeptide chains, which is part of normal hemoglobin.
- There are two main types, alpha thalassemia and beta thalassemia.



https://imaratx.com/our-programs/

Acid-base balance

- The acid-base balance of the organism is one of the most important and most strictly stabilized parameters of homeostasis. The relationship between hydrogen and hydroxyl substances in the internal environment of the body depends on the activity of redox mechanisms, metabolic factors, body resources, functions of organs and systems, the constancy of water and electrolytic metabolism, the activity and excitability of biological membranes, etc. The activity of the reaction of the environment affects the ability of hemoglobin to bind oxygen and give it to tissues.
- It is customary to evaluate the active reaction of the medium by the content in the liquid medium.
- pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution.

$$H_2O \rightleftharpoons H^+ + OH^-$$

$$pH = - \log[H^+]$$

"Acidic" $\Rightarrow$ pH<7

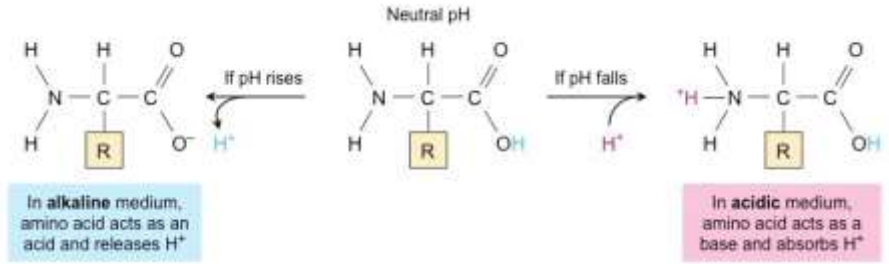
"Alkaline" $\Rightarrow$ pH>7

https://projects.ncsu.edu/project/hubbepaperchem/Defnitns/PH.htm

- The pH value is one of the most "severe" blood parameters and normally fluctuates in humans within very narrow limits - **the arterial blood pH is 7.35–7.45; venous - 7.32-7.42.** More significant changes in blood pH are associated with pathological metabolic disorders. In other biological fluids and in cells, the pH can differ from the pH of the blood.

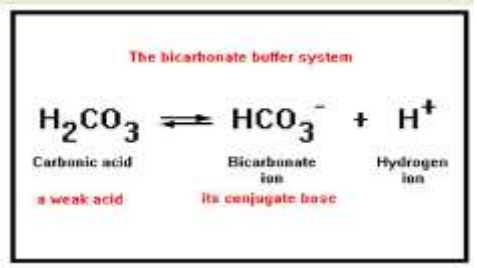
- Shifts in blood pH to simplify the structure leads to a change in the properties of enzymes, the permeability of biological membranes, causes dysfunction of the cardiovascular, respiratory and other systems; a shift of 0.3 can cause coma, and a shift of 0.4 is often incompatible with life.

- The acid-base state is maintained by powerful homeostatic mechanisms. They are based on the peculiarities of the buffer systems of the blood and physiological processes in which the external respiration systems, kidneys, liver, gastrointestinal tract, etc. take part.



Buffer systems

- Buffer systems are systems in which there is a significant (and nearly equivalent) amount of a weak acid and its conjugate base — or a weak base and its conjugate acid — present in solution.
- This coupling provides a resistance to change in the solution's pH.
- When strong acid is added, it is neutralized by the conjugate base.
- When strong base is added, it is neutralized by the weak acid.
- However, too much acid or base will exceed the buffer's capacity, resulting in significant pH changes.
- **Buffer systems of blood:** ***bicarbonate, plasma proteins, phosphate, hemoglobin buffers.***
- **Bicarbonate-Carbonic Acid Buffer**
- The bicarbonate buffer system is an acid-base homeostatic mechanism involving the balance of carbonic acid (H2CO3), bicarbonate ion (HCO3-), and carbon dioxide (CO2) in order to maintain pH in the blood Catalyzed by carbonic anhydrase, carbon dioxide (CO2) reacts with water (H2O) to form carbonic acid (H2CO3-), which in turn rapidly dissociates to form a bicarbonate ion (HCO3-) and a hydrogen ion (H+):
- Bicarbonate ions and carbonic acid are present in the blood in a 20:1 ratio if the blood pH is within the normal range. With 20 times more bicarbonate than carbonic acid, this capture system is most efficient at buffering changes that would make the blood more acidic. This is useful because most of the body's metabolic wastes, such as lactic acid and ketones, are acids. Carbonic acid levels in the blood are controlled by the expiration of CO_2 through the lungs. In red blood cells, carbonic anhydrase forces the dissociation of the acid, rendering the blood less acidic. Because of this acid dissociation, CO_2 is exhaled (see equations above). The level of bicarbonate in the blood is controlled



http://easy-saad.blogspot.com/2018/05/buffer-abuffer-is-aqueous-solution-that.html?spr

Buffer systems

- **Phosphate Buffer:**
- Phosphates are found in the blood in two forms: **sodium dihydrogen phosphate ($Na_2H_2PO_4^-$)**, which is a weak acid, and **sodium monohydrogen phosphate (Na_2HPO4^{2-})**, which is a weak base. There ratio 1:4
- **Plasma proteins Buffer:**
- Nearly all proteins can function as buffers. Proteins are made up of amino acids, which contain positively charged amino groups and negatively charged carboxyl groups. The charged regions of these molecules can bind hydrogen and hydroxyl ions, and thus function as buffers. Buffering by proteins accounts for two-thirds of the buffering power of the blood and most of the buffering within cells.
- **Hemoglobin Buffer:**
- Hemoglobin is the principal protein inside of red blood cells and accounts for one-third of the mass of the cell. During the conversion of CO2 into bicarbonate, hydrogen ions liberated in the reaction are buffered by hemoglobin, which is reduced by the dissociation of oxygen. This buffering helps maintain normal pH. The process is reversed in the pulmonary capillaries to re-form CO2, which then can diffuse into the air sacs to be exhaled into the atmosphere. This process is discussed in detail in the chapter on the respiratory system.

- acid-base balance indicators

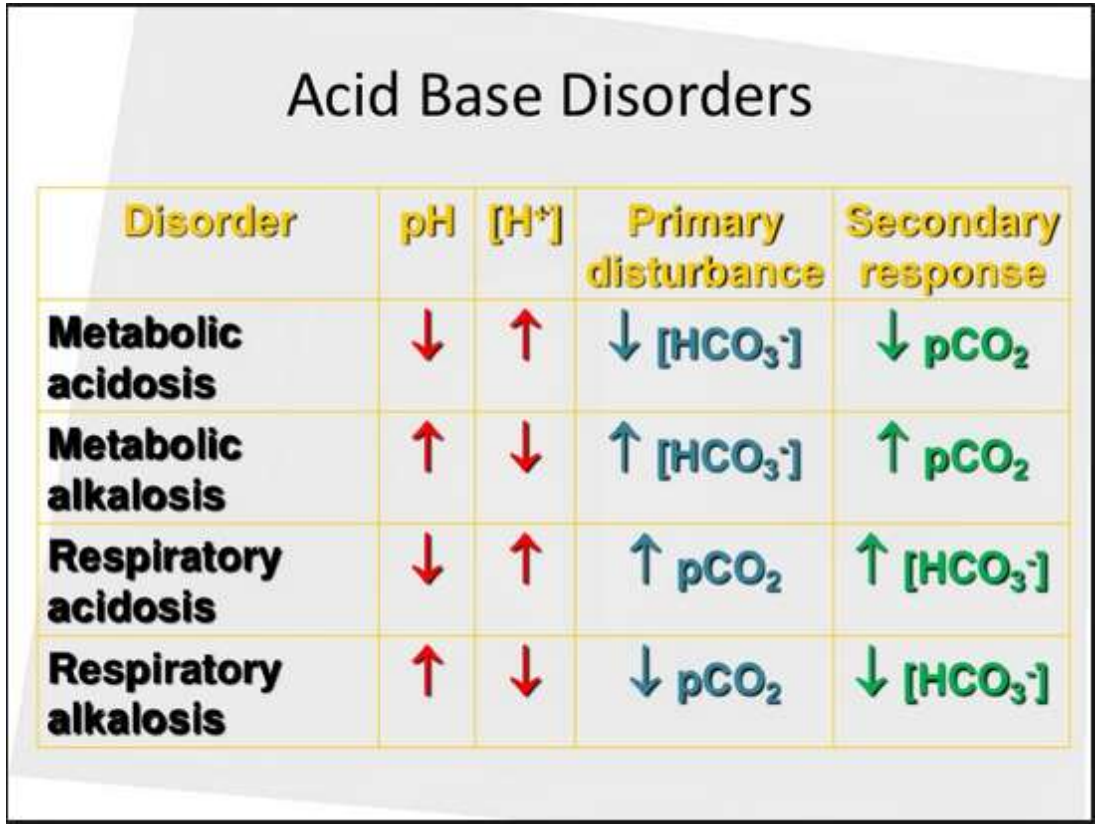
Indicator	**Characteristic**	**Normal rate**
PH	The decimal logarithm of the reciprocal of the hydrogen ion activity in a solution	7,35-7,45
pCO_2	dissolved carbon dioxide concentration (partial pressure of CO_2).	4,5-6,1 кPa (35-45 mmHg)
pO_2	dissolved oxygen concentration (partial pressure of oxygen).	9,4-14,7 кPa (100-108 mmHg)
Standard bicarbonate (SB)	Blood plasma concentration of HCO_3^-	22,0-26,0 mmol/l
Buffer base (BB)	The sum of all bases	46,0-26,0 mmol/l
Base excess (BE)	The number of bases that can be added or taken to the blood so that the pH is 7.4 at t° = 37 ° C pCO_2 = 40 mmHg.	„+”3,0 – „–”3,0 mmol/l

Disorders of acid–base balance

- **Acidaemia -** an arterial pH below the normal range (pH7.45).
- **Alkalaemia** - an arterial pH above the normal range (pH>7.45).
- **Acidosis** - a process lowering pH. This may be caused by a fall in serum bicarbonate and/or a rise in the partial pressure of carbon dioxide (PaCO2).
- **Alkalosis -** a process raising pH. This may be caused by a rise in serum bicarbonate and/or a fall in PaCO2 .

Disorders of acid–base balance are classified according to their cause, and the direction of the pH change:

- **respiratory acidosis** (causes: chronic airway conditions, like asthma, injury to the chest, obesity, which can make breathing difficult)
- **metabolic acidosis** (causes: ketone bodies, lactic acid increasing, renal deseases) **respiratory alkalosis** (causes:hyperventilation, high fever,lack of oxygen,being in high altitudes)
- **metabolic alkalosis** (causes: excess vomiting, which causes electrolyte loss, overuse of diuretics, a large loss of potassium or sodium in a short amount of time, accidental ingestion of bicarbonate, which can be found in baking soda)



https://mbbch.com/health/acid-base-balance/

Non-protein nitrogen-containing blood substances.

Nitrogen-containing compounds in blood plasma, which remain in it after the precipitation of proteins, are called **“residual nitrogen” (RN).**

These include the final metabolic products: urea - 50%, amino acids - 25%, uric acid, creatine, creatinine, bilirubin, indican, choline, nucleotides, polypeptides.

Normal concentration of RN - 14.2-28.4 mmol/l.
In some diseases, this value increases **(azotemia).**

Types of azotemias:

1. Retention – prerenal, renal and postrenal (cardiovascular failure, lowering blood pressure, decreased renal blood flow).

- **Prerenal** azotemia occurs when fluid isn’t flowing enough through the kidneys. This low flow of fluid creates high-level concentrations of serum creatinine and urea. This type of azotemia is the most common and can usually be reversed.
- **Renal** azotemia (acute kidney failure) typically leads to uremia. It is an intrinsic disease of the kidney, generally the result of kidney parenchymal damage. Causes include kidney failure, glomerulonephritis, acute tubular necrosis, or other kidney disease
- **Postrenal** azotemia – connected with urinary tract obstruction. It can be caused by congenital abnormalities such as vesicoureteral reflux, blockage of the ureters by kidney stones, pregnancy, compression of the ureters by cancer, prostatic hyperplasia, or blockage of the urethra by kidney or bladder stones. Postrenal azotemia can also occur with prerenal azotemia.

2. Production - increased breakdown of tissue proteins: malignant neoplasms, burns, cachexia.

Instead of residual blood nitrogen, the content of urea and creatinine can be determined. Normal: Urea - 3.3-8.3 mmol/l Creatinine-0.044-0.11 mmol/l.

The Urea:Creatinine ratio is a useful measure in determining the type of azotemia. **A normal Urea:Creatinine is equal to 15.**
The Urea:Creatinine in prerenal azotemia is greater than 20. The Urea:Creatinine in renal azotemia is less than 15. The Urea:Creatinine in postrenal azotemia is initially >15.

These types of azotemia may have somewhat different treatments, causes, and outcomes. However, they each can lead to acute kidney injury and failure if it’s left untreated or if it’s not discovered early.

Non-protein nitrogen-containing blood substances.

Component of residual nitrogen	Blood serum concentration	Urine excretion
urea	3.3-8.3 mmol/L	20.0-35.0 g/day
uric acid	0.12-0.46 mmol/L	0.3-0.8 g/day
creatine	0.08-0.11 mmol/L	
creatinine	0.044-0.11 mmol/L	0.8-2.0
ammonia nitrogen	29.4-47.0 mcmol/L	0.5 g/day
indican	1.19-3.13 mcmol/L	0.01 g/day
total bilirubin	8.5-20.5 mcmol/L	
direct bilirubin	1.0-5.0 mcmol/L	
indirect bilirubin	1.7-17.0 mcmol/L	

Catabolism of hemoglobin

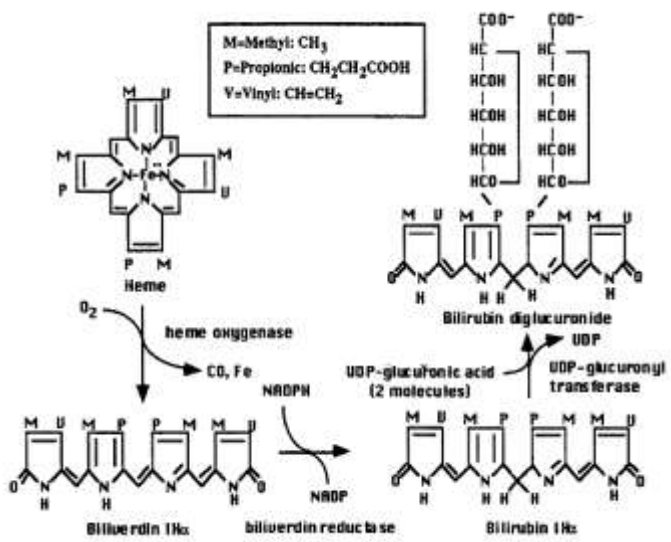
- Red blood cells are continuously undergoing a hemolysis (breaking apart) process. The average life-time of a red blood cell is 120 days. As the red blood cells disintegrate, the hemoglobin is degraded or broken into globin, the protein part, iron and heme . The heme initially breaks apart into biliverdin, a green pigment which is rapidly reduced to bilirubin, an orange-yellow pigment. These processes all occur in the reticuloendothelial cells of the liver, spleen, and bone marrow. The bilirubin is then transported to the liver where it reacts with a solubilizing sugar called glucuronic acid. This more soluble form of bilirubin (conjugated) is excreted into the bile.
- The bile goes through the gall bladder into the intestines where the bilirubin is changed into a variety of pigments. The most important ones are stercobilin, which is excreted in the feces, and urobilinogen, which is reabsorbed back into the blood. The blood transports the urobilinogen back to the liver where it is either re-excreted into the bile or into the blood for transport to the kidneys. Urobilinogen is finally excreted as a normal component of the urine.

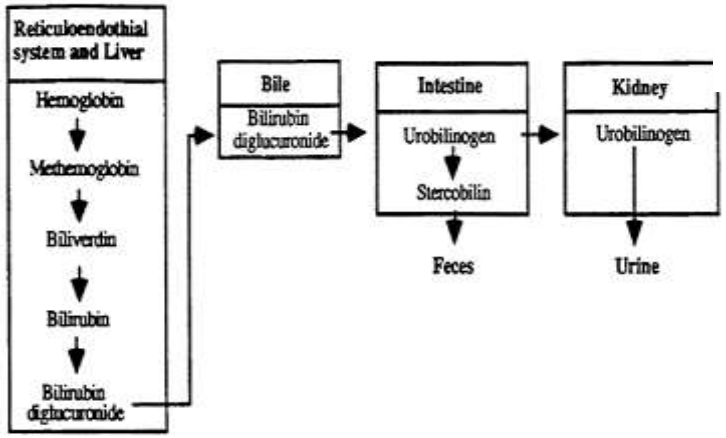
Types of bilirubin

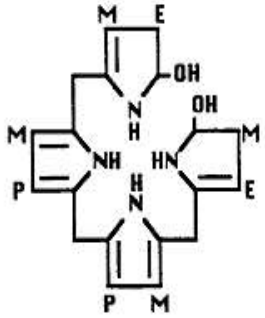
Unconjugated bilirubin (Indirect)	Conjugated bilirubin (Direct)
• Bind to albumen	• Conjugated with glucoronic acid
• Fat soluble	• Water soluble
• Can **cross** blood brain barrier	• Excreted in urine and stool
• **Toxic** in high level to brain	• Not toxic

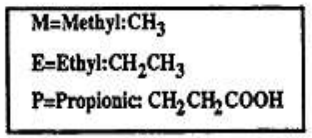
https://www.slideshare.net/sayedahmed90857/neonatal-jaundice-2017

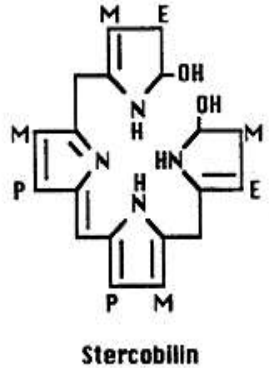
Catabolism of hemoglobin











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