Haemoglobin

Haemoglobin is a pigment found in red blood cells that binds to oxygen. It is the protein that transports oxygen from the lungs to the tissues while returning carbon dioxide from the tissues to the lungs. To function optimally, haemoglobin must strongly bind to oxygen in the oxygen-rich atmosphere of the lungs and be able to quickly release oxygen in the tissues' more oxygen-poor environment.

Structure Of Haemoglobin

The red oxygen carrying pigment in the RBCs of vertebrates is haemoglobin. It consists of the protein Globin (polypeptide) united with the pigment Haem.

1. **Haem:** It is an iron containing porphyrin, called iron-protoporphyrin IX. The porphyrin nucleus is tetrapyrrole i.e. it consists of 4' pyrrole rings' joined together by 4 methane (= CH-) bridges. The pyrrole rings are numbered I, II, III, and IV; the carbon atoms of the methane bridges are labeled α, β, γ and δ; the position on pyrrole rings, to which side chains are attached are numbered 1 to 8. The side chains at 1, 3, 5 and 8 position are methyl ($-CH_3$); 2 and 4 are vinyl ($-CH = CH_2$); 6 and 7 are propionic acid ($-CH_2-CH_2-COOH$).

2. **The Iron:** The iron in hemoglobin is present as ferrous (Fe^{++}) iron. Every pyrrole ring's 'N' has an iron linked to it. One oxygen molecule and each Fe^{++} combine loosely and reversibly. Oxygenation is the process of combining oxygen with hemoglobin. Because iron remains in the Fe^{++} state in the hemoglobin, oxygen is transported as molecular oxygen.

3. **Globin:** The protein haemoglobin is made up (primarily) of 4 polypeptides. As proteins are built by taking amino acids and stringing them together into long chains called polypeptides, each of the 4 polypeptides is necessary to make one full haemoglobin molecule. Typically, when a protein is made up of multiple polypeptides, each polypeptide is simply called a protein subunit. However, in the case of haemoglobin, the subunits are each called globin. In other words, a globin is the same thing as an individual polypeptide of haemoglobin. The 4 globins are of two types. 2 of them with identical amino acid sequence are called alpha-globins (α-globins), while the other 2 also have identical amino acid sequences and are called beta-globins (β-globins). Every haemoglobin molecule contains 2 α - globins and 2 - β globins. Each polypeptide chain is associated with one haem group. Thus, there are 4 haem to the one molecule of haemoglobin.

4 globins and 4 haeme groups together make a haemoglobin molecule.

Like other proteins, haemoglobin has a primary, secondary, tertiary, and even quaternary structure.

The Primary Structure relates the order of the amino acid sequence in the α and β aglobins chain The alpha globin chain is composed of 141 amino acids and the beta globin chain is composed of 146 amino acids. The α and β globins are not that different, they are quite similar with the teensiest bit different. Tiny differences in primary structure of polypeptides can cause dramatic differences in protein function. This is best exemplified by disorder sickle-cell anemia.

Similar secondary and tertiary structures, each with eight helical segments, are shared by the beta and alpha globin proteins in a hemoglobin molecule.

Hydrogen bonds serve to stabilize the α-helix and β-sheet conformations, which are examples of the secondary structure found in proteins. Along with brief non-helical regions, the majority of the amino acids in hemoglobin form alpha helices. No beta strands or disulfide linkages are present in hemoglobin.

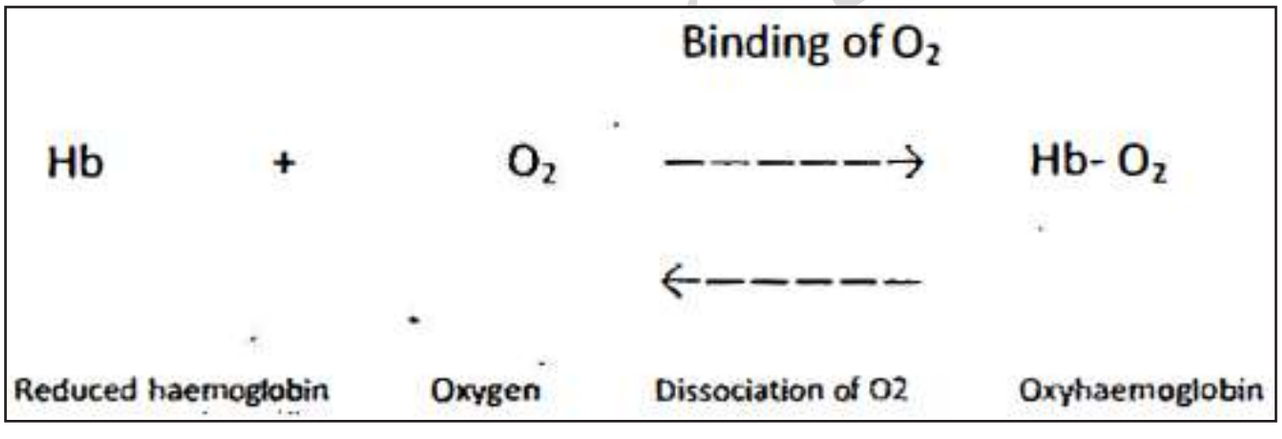
The whole tri-dimensional structure of a polypeptide chain is called the tertiary structure of hemoglobin. It results from the molecular interactions between distant molecules in a non-linear sequence that secondary structure brings closer.

Quaternary Structure: Although the secondary and tertiary structure of various haemoglobin subunits are similar, reflecting extensive homology in amino acid composition, the variations in amino acid composition that do exist impart marked differences in haemoglobin's oxygen carrying properties. In addition, the quaternary structure of haemoglobin leads to physiologically important allosteric interactions between the subunits.

OXYGEN TRANSPORT:

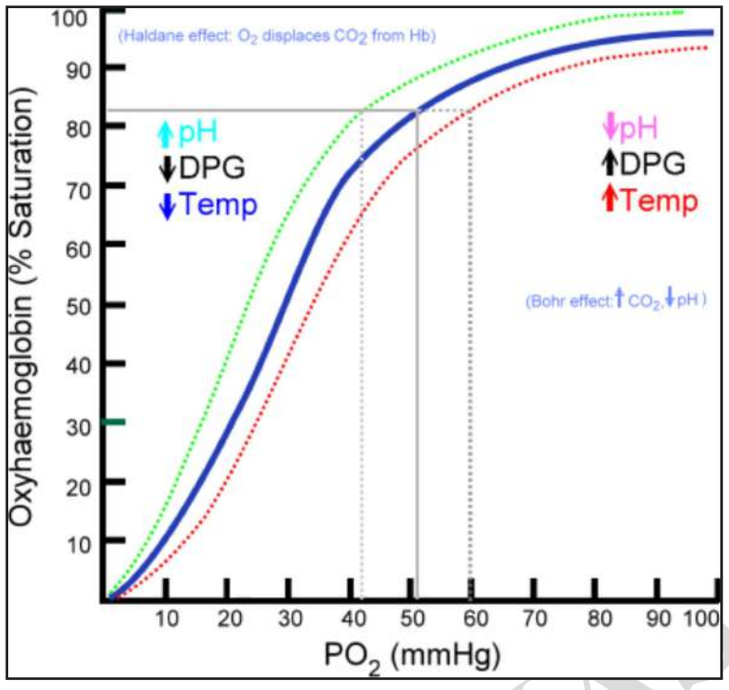
Oxygen does not dissolve easily in water, and therefore only about 1.5% of the O_2 is dissolved in blood plasma, which is mostly water.

About 98.5% of blood O_2 is bound to haemoglobin in red blood cells. The heme portion of haemoglobin contains four atoms of iron, each capable of binding to a molecule of O_2. Oxygen and haemoglobin bind in an easily reversible reaction to form oxyhaemoglobin:



Each 100ml of oxygenated blood contains the equivalent of 20ml of gaseous O_2. The amount dissolved in the plasma is 0.3 mL and the amount bound to haemoglobin is 19.7 mL.

The Relation between Haemoglobin and Oxygen partial pressure: The most important factor that determines how much O_2 binds to haemoglobin is the Po_2. The higher the Po_2 the more O_2 combines with Hb. When reduced haemoglobin (Hb) is completely converted to oxyhaemoglobin (Hb-O_2), the haemoglobin is said to be fully saturated; when haemoglobin consists of a mixture of Hb and Hb-O_2, it is partially saturated. **Oxygen-haemoglobin dissociation curve:** The relation between the percent saturation of haemoglobin and Po_2 is illustrated in the oxygen haemoglobin dissociation curve.

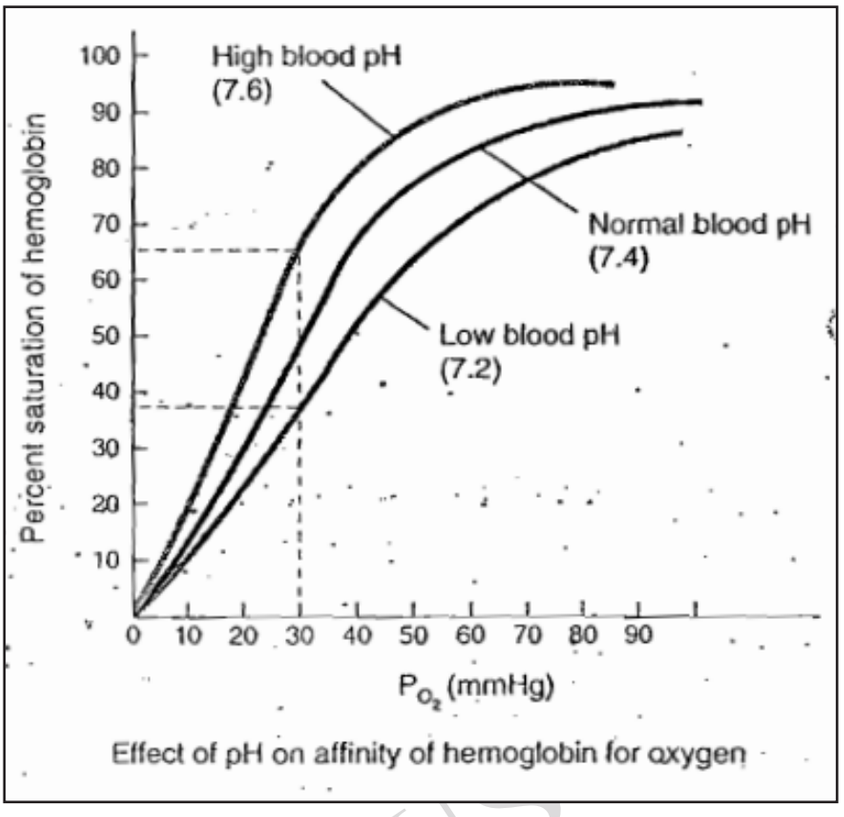


The more O_2 that binds to hemoglobin, therefore, the higher the Po_2, until all of the accessible hemoglobin molecules are saturated. Consequently,

- In pulmonary capillaries, where Po_2 is high, a lot of O_2 binds to haemoglobin.
- In tissue capillaries, where the Po_2 is lower, haemoglobin does not hold as much O_2 and the O_2 is unloaded via diffusion into tissue cells.
- When the Po_2 is between 60 and 100 mmHg, haemoglobin is 90% or more saturated with O_2. Thus, blood picks up a nearly full load of O_2 from the lungs even when the Po_2 of alveolar air is as low as 60 mmHg.
- In active tissue such as contracting muscles, Po_2 may drop well below 40 mmHg. Then, a large percentage of the O_2 is released from haemoglobin, providing more O_2 to metabolic metabolically active tissue.

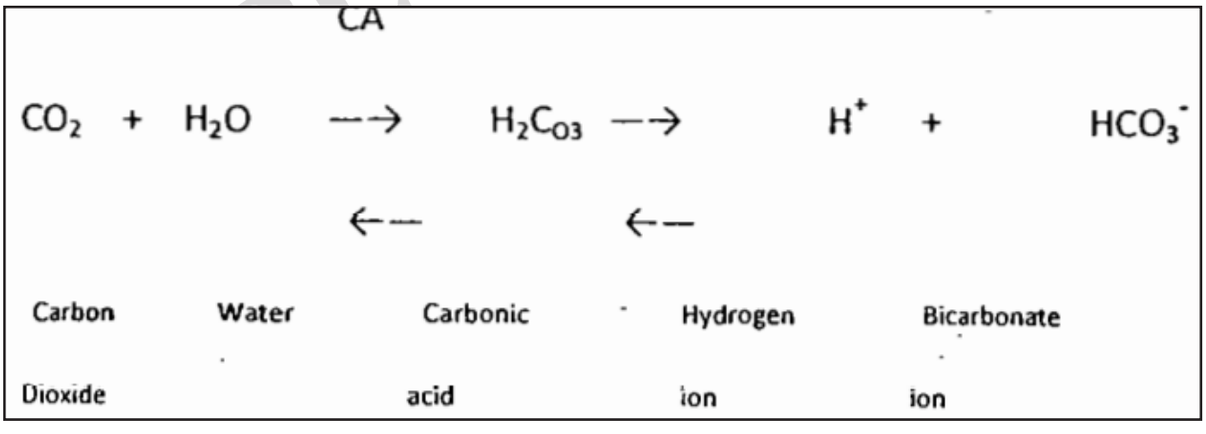
Other elements influencing the affinity of hemoglobin for oxygen include: A number of different parameters affect the tightness or affinity with which hemoglobin hinds oxygen, even though Po_2 is the primary factor that determines the percent O_2 saturation of hemoglobin. Essentially, these variables cause the entire curve to move, either to the right (lower affinity) or left (greater affinity).

Acidity (pH): As acidity increases (pH decreases), the affinity of haemoglobin for O_2 decreases, and O_2 dissociates more readily from haemoglobin. In other words, increasing acidity enhances the unloading of oxygen from haemoglobin. The main acids produced by metabolically active tissue are lactic acid and carbonic acid When. pH decreases, the entire oxygen haemoglobin dissociation curve shifts to the right at any given Po_2 PoHb is less saturated with O_2, a change termed the Bohr Effect. The Bohr Effect works both ways: An increase in FH in blood causes O_2 to unload from haemoglobin, and the binding of O_2 to haemoglobin causes unloading of H^+ from haemoglobin. The explanation for the Bohr Effect is that haemoglobin can act as a buffer for hydrogen ions (H^+) But when H^+ binds to amino acids in haemoglobin, they slightly alter its structure and thereby decrease its oxygen-carrying capacity. Thus, lowered pH drives O_2 off haemoglobin, making more, available for tissue cells. By contrast, elevated pH increases the affinity of haemoglobin for and shifts the oxygen-haemoglobin dissociation curve to the left.

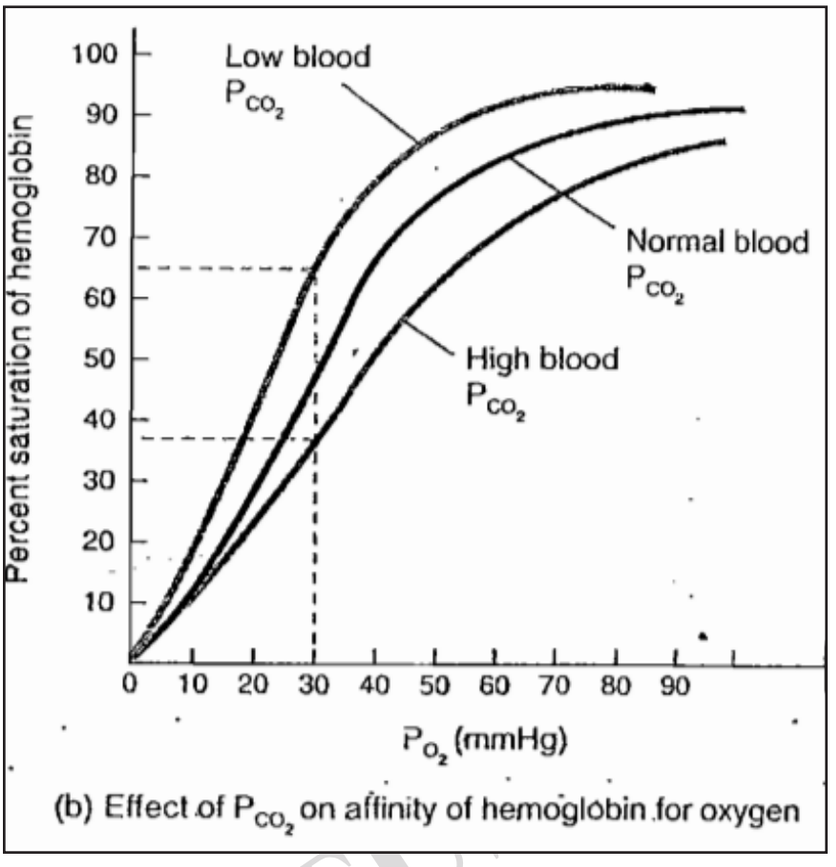


Effect of pH on affinity of hemoglobin for oxygen

Partial pressure of carbon dioxide: CO_2 also can bind to haemoglobin and the effect is similar to that of H^+ (shifting the curve to the right). As P_{co2} rises, haemoglobin releases O_2 more readily. P_{co2} and PH are related factors because low blood pH (acidity) results from high P_{co2}. AS CO_2 enters the blood, much of it is temporary converted to carbonic acid ($H2C_{03}$), a reaction catalyzed by an enzyme in red blood cells called carbonic anhydrase (CA):



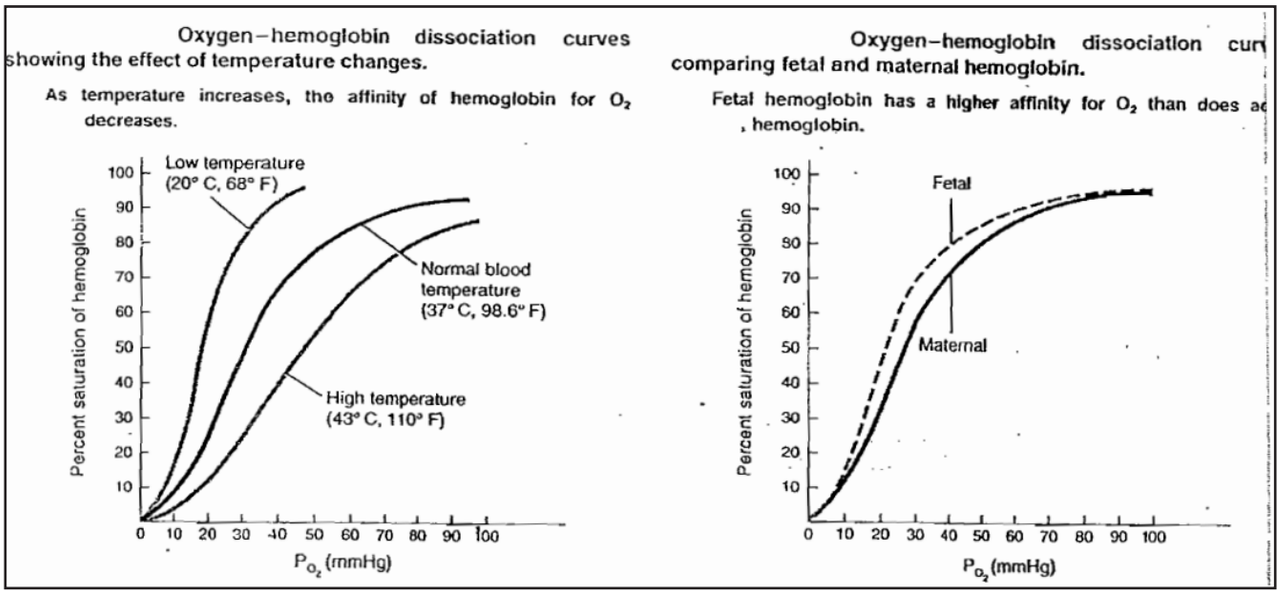
The carbonic acid thus formed in red blood cells dissociates into hydrogen ions and bicarbonate ions. As the H^+ concentration increases, pH decreases. Thus, an increased P_{co2} produces a more acidic environment, which helps release O_2 from haemoglobin. During exercise, lactic acid - a byproduct of anaerobic metabolism within muscles - also decreases blood pH. Decreased P_{co2} (and elevated pH) shifts the saturation curve to the left.



(b) Effect of P_{CO_2} on affinity of hemoglobin for oxygen

BPG: 2, 3-bisphosphoglycerate (BPG), a material present in red blood cells, lowers hemoglobin's affinity for oxygen and aids in unloading. from hemoglobin, O_2. Haemoglobin binds O_2 less firmly at the heme group sites when BPG attaches to the terminal amino groups of the two beta globin chains, causing haemoglobin to mix with it. More O_2 is released from hemoglobin at higher BPG levels. Thyroxin, human growth hormone, adrenaline, and the combination of epinephrine and testosterone are among the hormones that promote the production of BPG. Those who live at higher elevations also have greater BPG levels.

Temperature: Within limits, as temperature increases, so does the amount of O_2 released from haemoglobin. Metabolically active cells require more O_2 and liberate more acids and heat. The acids and heat, in turn, promote release of O_2 from oxyhaemoglobin.



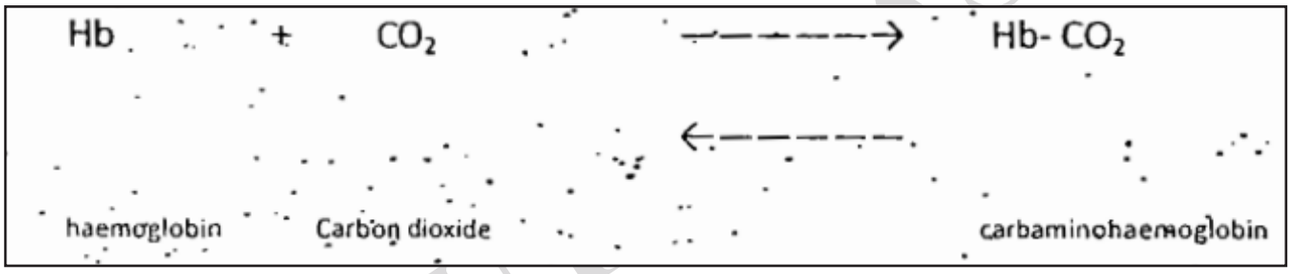
Air Affinity of fetal and adult hemoglobin: The structure and affinity of fetal hemoglobin (Hb-F) for O_2 are different from those of adult hemoglobin (Hb-A). HD-F binds BPG less firmly, which results in a larger affinity for O_2. Therefore, Hb-F can carry up to 30% more O_2 than maternal Hb-A when Po_2 is low. O_2 is easily transmitted from maternal blood to fetal blood as it passes via the placenta. This is crucial because fetal hemoglobin's higher affinity for oxygen would prevent the fetus from experiencing hypoxia due to the placenta's relatively low O_2 saturation in maternal blood.

CARBON DIOXIDE TRANSPORT

Under normal resting conditions, each 100 ml of deoxygenated blood contains the equivalent of 53 mL of gaseous CO_2 which is transported in the blood in three main forms:

Dissolved CO_2: Blood plasma dissolves the least amount of CO_2—roughly 9%. It diffuses into alveolar air upon entering the lungs and is expelled.

Carbamino compounds: In the blood, around 13% of amino groups from proteins and amino acids combine to generate carbamino compounds. Since hemoglobin is the most common protein in blood, the majority of CO_2 delivered in this way is attached to hemoglobin. Carbaminohaemoglobin (Hb-CO_2) is the name for hemoglobin that has bonded CO_2.



PCO_2 has a major impact on the synthesis of carbaminohaemoglobin. The comparatively high PCO_2 in tissue capillaries encourages the production of carbaminohaemoglobin. However, PCO_2 is comparatively low in pulmonary capillaries, and CO_2 easily separates from globin and diffuses into the alveoli.

The greatest percentage of CO_2 about 78% is transported in blood plasma as bicarbonate ions (HCO_3). As CO_2 diffuses into systemic capillaries and enters red blood cells, it reacts with water in the presence of the enzyme carbonic anhydrase (CA) to form, carbonic acid, which dissociates into H^+ and HCO_3:

The Chloride Shift: When blood flows through the systemic capillaries, a significant amount of carbonic acid is created due to a catalyst produced by carbonic anhydrase in red blood cells. This equation illustrates how the accumulation of carbonic acid concentrations within red blood cells promotes the breakdown of these molecules into hydrogen ions (protons, which add to the acidity of a solution) and HCO_3 (bicarbonate):

$$H_2CO_3 \longrightarrow H^+ HCO_3$$

When hydrogen ions (H^+) from the breakdown of carbonic acid combine with deoxyhaemoglobin in red blood cells, they are largely buffered. Even though unbuffered hydrogen ions are more able to diffuse bicarbonate out of the plasma than do H^+, they are still free to diffuse all of the red blood cells. The inside of the red blood cell acquires a net positive charge as a result of the hydrogen ions "trapping" within it due to their attachment to hemoglobin and the outward diffusion of bicarbonate. This draws in chloride ions (Cl), which enter red blood cells at the same time that HCO_3 leaves. The term "chloride shift" refers to this anions-to-anions exchange that occurs as blood passes through tissue capillaries. The % has an impact on the quantity of CO_2 that can be carried in the blood. Oxygen saturation saturation of hemoglobin The Haldane effect states that the blood's capacity increases with decreasing levels of oxyhaemoglobin (Hb-O_2).

A reverse chloride shift operates in the pulmonary capillaries to convert carbonic acid to H_2O and CO_2 gas, which is eliminated in the exhaled air. The P_{co2}, carbonic acid, H^+ and bicarbonate concentrations in the systemic arteries are thus maintained relatively constant by normal ventilation.

TYPES OF HAEMOGLOBIN

Normal haemoglobin types include:

Hb A - makes up about 95%-98% of Hb found in adults. It contains two alpha (a) protein chains and two beta (B) protein chains.

Hb A_2- makes up about 2%-3% of Hb. It has two alpha (a) and two delta (6) protein chains.

Hb F - makes up to 2% of Hb found in adults. It has two alpha (a) and two gamma (y) protein chains and is the primary haemoglobin produced by the fetus during pregnancy. Its production usually falls to a low level shortly after birth. It has greater affinity for oxygen and can take much larger volume of oxygen than HbA at low oxygen pressure. This facilitates the movement of oxygen from maternal to foetal circulation.

COMMON HAEMOGLOBIN VARIANTS

Haemoglobin S: Individuals with sickle cell disease primarily carry this type of hemoglobin. Sickle cell disease affects 0.15 percent of African Americans, and 8% of Americans of African heritage have one of their two beta genes mutated to sickle Hb. Two normal alpha (α) chains and two aberrant beta (β) chains are present in individuals with hemoglobin S illness. When red blood cells are subjected to low oxygen levels, as occurs during exercise, the presence of hemoglobin S causes the cells to distort and take on a sickle shape. In addition to reducing the red blood cells' ability to carry oxygen and shortening their life, sickled red blood cells can obstruct tiny blood veins, causing discomfort and poor circulation. A single beta (β^s) copy does not cause symptoms unless it is combined with another haemoglobin mutation, such as that causing HBC (β^c).

Haemoglobin C: About 2-3% of people of West African descent are heterozygotes for haemoglobin C (have one copy of β^c). Haemoglobin C disease (seen in homozygotes - those with two copies of β^c) is rare and relatively mild. It usually causes a minor amount of hemolytic anemia and a mild to moderate enlargement of the spleen.

Haemoglobin E: Throughout the world, hemoglobin E is among the most prevalent beta chain hemoglobin variations. In Southeast Asia, it is particularly common in people of Southeast Asian heritage as well as in Cambodia, Laos, and Thailand. Individuals with two copies of β^E, or homozygous for Hb E, typically exhibit microcytic red blood cells, minor splenic enlargement, and mild hemolytic anemia. Haemoglobin E gene mutations other than the orre for beta thalassemia trait are required for symptoms to manifest when a single copy of the gene is present.

Hemoglobin H: Hb H is an aberrant hemoglobin found in some cases of alpha thalassemia. It is made up of four beta (β) globin chains and is formed due to a paucity of alpha (α) chains. While each beta (β) globin chain is normal, the tetramer of 4 beta chains does not operate properly. It has a higher affinity for oxygen, retaining it rather than releasing it to the tissues and cells.

MYOGLOBIN

Myoglobin and haemoglobin are hemeproteins whose physiological significance is mostly due to their capacity to bind molecular oxygen. Myoglobin is a 153-amino acid single-chain globular protein with a heme (iron-containing porphyrin) prosthetic group in the center that forms the basis for the remaining apoprotein structure.

It has a molecular weight of 16,700 daltons and is the principal oxygen-carrying pigment found in muscular tissues. The oxygen pressure in the surrounding tissue has no effect on myoglobin's binding to oxygen. Organisms with high myoglobin concentrations can hold their breath for longer periods of time.

Structure: The monomeric heme protein called myoglobin is mostly present in muscle tissue, where it acts as an intracellular oxygen storage facility. A porphyrin ring with an iron center is present in myoglobin. A distal histidine group is present on the other face and is not connected to the iron, whereas a proximal histidine group is directly coupled to the iron center. Myoglobin's tertiary structure resembles that of a normal globular protein that dissolves in water. Because a large percentage (75%) of its secondary structure is a-helical, its secondary structure is unique. A myoglobin polypeptide is comprised of. 8 separate right handed a-helices, designated A through H, that are connected by short non helical regions. Amino acid R-groups packed into the interior of the molecule are predominantly hydrophobic in character while those exposed on the surface of the molecule are generally hydrophilic, thus making the molecule relatively water soluble.

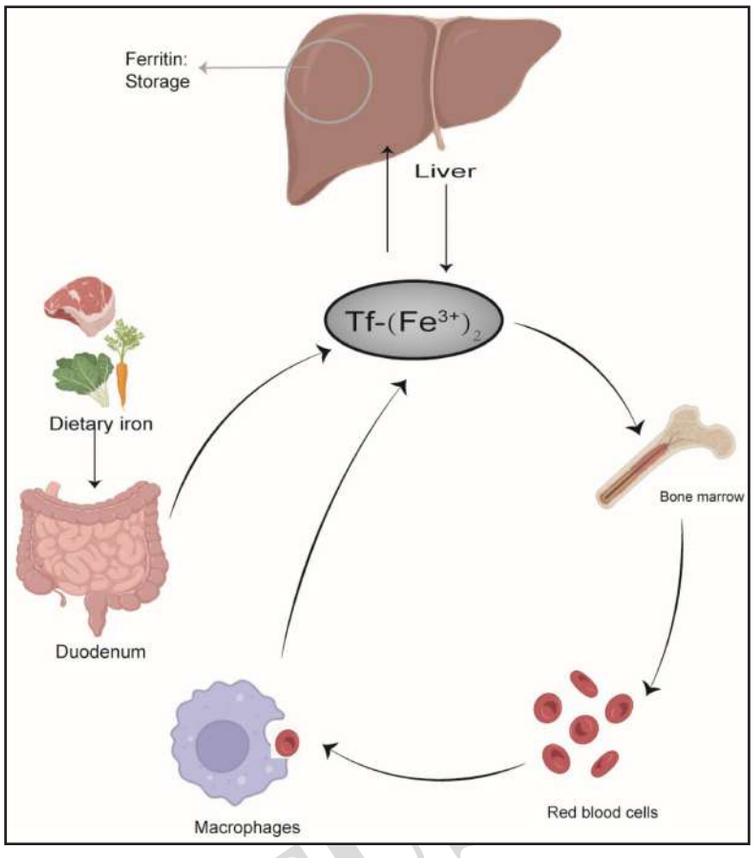
IRON METABOLISM

Iron plays a crucial role in the synthesis of hemoglobin as well as other vital components in the body (e.g., myoglobin, cytochromes, cytochrome oxidase, peroxidase, catalase).

The usual amount of iron in the body is 4 to 5 grams, of which approximately 65% is found in hemoglobin. About 4 percent is stored for later use, primarily in the reticuloendothelial system and liver parenchymal cells, primarily in the form of ferritin. 1 percent is in the form of various heme compounds that promote intracellular oxidation. 0.1% is combined with the protein transferrin in the blood plasma.

ABSORPTION OF IRON FROM THE INTESTINAL TRACT

Every portion of the small intestine absorbs iron. The mature enterocytes' apical membrane is where dietary iron is absorbed. Moderate levels of apotransferrin are secreted by the liver into the bile, which then enters the duodenum via the bile duct. In this case, the apotransferrin binds to both free iron and specific iron complexes like myoglobin and hemoglobin. Transferrin is the name given to this combination. It then attaches itself to receptors in the intestinal epithelial cells' membranes. Then, by pinocytosis, the transferrin molecule, carrying its iron store, is - absorbed into the epithelial cells and later released into the blood . capillaries beneath these cells in the form of plasma transferrin. Iron absorption from the intestines is extremely slow, at a maximum rate of only a few milligrams per day. This means that - even when tremendous quantities of iron. are. present in the food, only small proportions can be absorbed.



TRANSPORT AND STORAGE OF IRON

- Iron is rapidly combined with the beta globulin apotransferrin in the blood plasma upon absorption from the small intestine to create transferrin, which is subsequently carried by the plasma. Since the iron is only weakly bound by the transferrin, any tissue in the body that has transferrin receptors can absorb it.
- After being absorbed from the small intestine, iron quickly combines with the beta globulin apotransferrin in the blood plasma to form transferrin, which is then transported by the plasma. Any tissue in the body with transferrin receptors can absorb iron because it is only weakly bound by transferrin.
- Hemosterin is the very insoluble form of iron found in smaller amounts in the storage pool. This is particularly true if the body contains more iron overall than the apoferritin storage pool can hold. Hemosiderin aggregates within cells to create sizable clusters, which resemble huge particles when viewed under a microscope. Ferritin particles, on the other hand, are typically only visible in the cytoplasm of cells under an electron microscope due to their small size and dispersion.
- Liver parenchymal tissuens especially rich in transferrin receptors, and stores large quantities of iron in response to increased intracellular iron levels the liver synthesizes and releases hepcidin, which has a dual role. It stimulates increased iron storage by reticuloendothelial macrophages and decreases tron export from enterocytes.
- Most of the circulating iron is used by the bone marrow-to generate haemoglobinfor red blood cells.
- Around 10-15% is utilized by muscle fibers to generate myoglobin.
- The largest pool of iron storage cells is usually made up of circulating red blood cells. Red blood cells are consumed by reticuloendothelial macrophages as they become senescent, and transferrin is then used to redistribute the iron in the cells to other tissues. The iron export protein ferroportin allows re-

ticuloendothelial macrophages to release iron into the plasma. Iron that has been released is oxidized by ceruloplasmin into the ferric (Fe3+) form, which enables the iron to attach to transferrin.

DAILY LOSS OF IRON

An average male excretes 0.6 mg of iron daily, mostly in his feces. Further amounts of iron are lost during bleeding. An additional menstrual loss of blood causes a woman's long-term iron loss to average approximately 1.3 mg per day.

REGULATION OF TOTAL BODY IRON BY CONTROLLING RATE OF ABSORPTION

When the body has become saturated with iron so that essentially all apoferritin in the iron storage areas is. already combined with iron, the rate of additional iron absorption from the intestinal tract becomes greatly decreased. On the other hand, the rate of absorption might increase five times or more the typical rate when the stocks of iron have been depleted. Thus, total body iron is regulated mainly by altering the rate of absorption.

HAEMOSTASIS AND BLOOD COAGULATION

Haemastasis is the term for the prevention of blood loss. Haemostasis is done by multiple methods in the event of a vascular rupture or severance.

1. Vascular constriction
2. Formation of a platelet plug
3. Formation of a blood clot as a result of blood coagulation, and
4. Fibrous Organization or dissolution of the Blood clot.

VASCULAR CONSTRICTION

The smooth muscle in the walls of arteries and arterioles is arranged in a circle and contracts instantly when they are injured. For many minutes to several hours, such a vascular spasm lessens blood loss; during this time, the other haemostatic processes kick in. Damage to the smooth muscle, chemicals produced from activated platelets, and reflexes triggered by pain receptors are likely the causes of the spasm.

FORMATION OF PLATELET PLUG

If the cut in the blood vessel is very small-indeed, many very small vascular holes do develop throughout the body each day-the cut is often sealed by a platelet plug, rather than by a blood clot.

- When platelets come in contact with a damaged vascular surface, especially with collagen fibers in the vascular wall, the platelets themselves immediately change their own characteristics drastically. They begin to swell; they assume irregular forms with numerous irradiating pseudo pods protruding from their surfaces; they become sticky so that they adhere to collagen in the tissues and to a protein called von Willebrand factor that leaks into the traumatized tissue from the plasma. This is called platelet adhesion.

- Due to adhesion, the platelets secrete large quantities of ADR Their contractile proteins contract forcefully and cause the release of granules that contain multiple active factors and their enzymes form thromboxane A_2. This phase is called platelet release reaction. The ADP and thromboxane in turn act on nearby platelets to activate them as well, and the stickiness of these additional platelets causes them to adhere to the original activated platelets.

- Further, at the site of any opening in a blood vessel wall, the damaged vascular wall activates successively increasing numbers of platelets that themselves attract more and more additional platelets, thus forming a platelet plug. The gathering of platelets is called Platelet aggregation. The platelet plug is at

first a loose plug, but it is usually successful in blocking blood loss if the vascular opening is small.

BLOOD COAGULATION

The blood clot's creation is the third method of hemostasis. If there has been significant trauma to the vascular wall, the clot starts to form in 15 to 20 seconds, and if there has been just minor stress, it takes 1 to 2 minutes.

Clotting Factors in-Blood and Their Synonyms.

Clotting Factor	Synonyms
Fibrinogen	Factor 1
Prothrombin	Factor II
Tissue factor	Factor III; tissue thromboplastin
Calcium	Factor IV
Factor V	Proaccelerin; labile factor; Ac-globulin (Ac-G)
Factor VII	Serum prothrombin conversion accelerator (SPCA); proconvertin; stable factor
Factor VIII	Antihemophilic factor (AHF); antihemophilic globulin (AHG); antihemophilic factor A
Factor IX	Plasma thromboplastin component (PTC); Christmas factor; antihemophilic factor B
Factor X	Stuart factor; Stuart-Prower factor
Factor XI	Plasma thromboplastin antecedent (PTA); antihemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald-factor; HMWK (high-molecular-weight) kininogen
Platelets	

MECHANISM OF BLOOD COAGULATION

All research workers in the field of blood coagulation agree that clotting takes place in three essential steps:

(A) In response to rupture of the vessel or damage to the blood itself, a complex cascade of chemical reactions occurs in the blood involving more than a dozen blood coagulation factors. The net result is formation of a complex of activated substances collectively called prothrombin activator or prothrombinase.

(B) The prothrombin activator catalyzes conversion of prothrombin into thrombin.

(C) The thrombin acts as an enzyme to convert fibrinogen into fibrin fibers that enmesh platelets, blood cells, and plasma to form the clot.

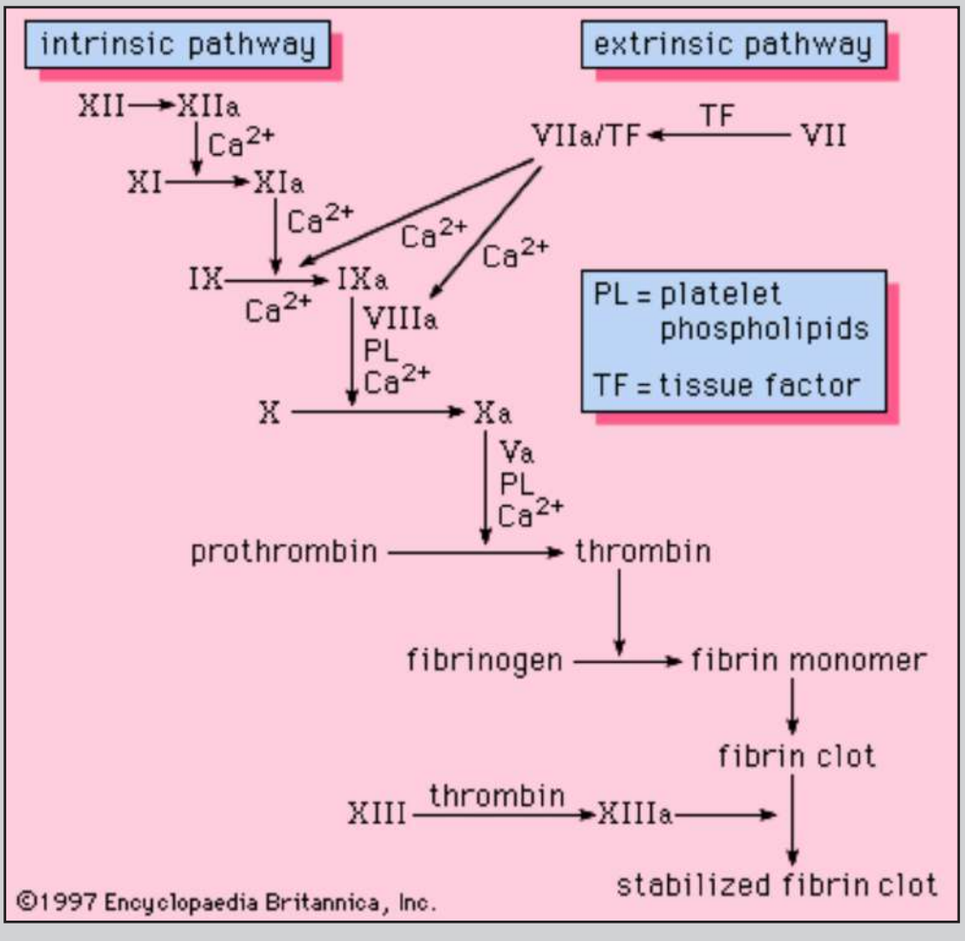
(A) Formation of Prothrombin Activator (Initiation of Coagulation): Prothrombin activator is generally considered to be formed in two ways, although, in reality, the two ways interact constantly with each other: (1) by the extrinsie pathway that begins with traûma to the vascular wall and surrounding tissues and (2) by the intrinsic pathway that begins in the blood itself. Blood-clotting factors, a group of distinct plasma proteins, are important players in both the intrinsic and extrinsic modes. The majority of them are proteolytic enzymes in their dormant state. Their enzymatic activity initiate the series of cascading reactions of the clotting process when they are transformed to the active forms.

Extrinsic Pathway for Initiating Clotting

The extrinsic pathway of blood clotting has fewer steps than the intrinsic pathway and occurs rapidly. It is so named because a tissue protein called Tissue factor (TF), also, known as thromboplastin, leaks into blood from cells outside (extrinsic to) blood vessels and initiates the formation of prothrombin activator. TF is composed especially of phospholipids from the membranes of the tissue plus a lipoprotein complex and is released from the surface of damaged cells. In the presence of calcium ions, TF begins a sequence of reaction that ultimately activates clotting factor X. Once factor is activated, it combines with factor V in the presence of calcium ions to form the active enzyme Prothrombin activator or completing the extrinsic pathway. Prothrombinase,

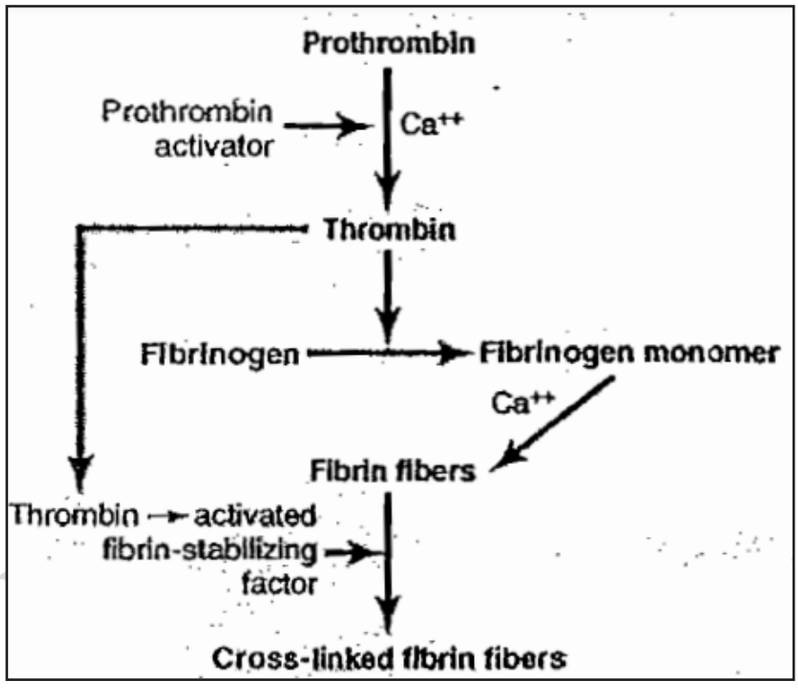
Intrinsic Pathway for Initiating Clotting

The intrinsic pathway of blood clotting is more complex than the extrinsic pathway, and it occurs more slowly, usually requiring several minutes. The intrinsic pathway is so named because its activators are either in direct contact with blood or contained within (intrinsic to) the blood; outside tissue damage is not needed. If endothelial cells become roughened or damaged, blood can come in contact with collagen fibers in the connective tissue around the endothelium of the blood vessel. In addition trauma to endothelial cells causes damage to platelets, resulting in the release of phospholipids by the platelets. Contact with collagen fibers activates clotting factor XII which begins a sequence of reactions that eventually activates clotting factor X. Platelet phospholipids and calcium ions can also participate in the activation of factor X. Once factor X is activated, it combines with factor V to form the active enzyme Prothrombin activator or Prothrombinase, completing the intrinsic pathway.



The role of calcium ions in the intrinsic and extrinsic pathways is as follows: all blood-clotting reactions require calcium ions for promotion or acceleration, with the exception of the first two steps in the intrinsic pathway. Consequently, neither pathway of blood clotting works in the absence of calcium ions.

(B) Prothrombin to Thrombin Conversion and the Role of Vitamin K: Prothrombin is converted to thrombin by the prothrombin activator when there is enough ionic calcium present. Normal plasma contains prothrombin, an alpha2-globulin and plasma protein. It is a brittle protein that readily disassembles into smaller molecules, thrombin being one of them. The liver continuously produces thrombin, which is then utilized by the body as a blood coagulant. In the event that the liver is unable to manufacture prothrombin, the plasma's prothrombin concentration eventually drops too low to support healthy blood coagulation. For the liver to properly produce prothrombin and a few other clotting components, vitamin K is necessary. Therefore, a prothrombin level that is so low as to induce bleeding tendencies can be caused by either a deficiency of vitamin K or the existence of liver disease that inhibits normal prothrombin synthesis.



(C). Conversion of fibrinogen to fibrin (Formation of clot): In a further 10 to 15 seconds, the thrombin forces the fibrinogen molecules to polymerize into fibrin threads. A protein enzyme with limited proteolytic activity is called thrombin. It works by dissolving four low molecular weight peptides from each fibrinogen molecule to create a single fibrin monomer molecule that can spontaneously polymerize with additional fibrin monomer molecules to generate fibrin fibers. Consequently, a large number of fibring monomer molecules polymerize into long fibrin fibers in a matter of seconds, which make up the blood clot's reticulum.

Blood Clot: The clot is made up of a network of fibrin threads that ensnared plasma, platelets, and blood cells. These fibers run in all directions. The fibrin fibers also stick to blood vessel surfaces that are compromised; as a result, the blood clot sticks to any vascular opening and stops additional blood loss.

Clot Retraction-Serum. A clot starts to constrict a few minutes after it forms, and it normally expresses the majority of its fluid within 20 to 60 minutes. Because all of the fibrinogen and the majority of the other clotting factors have been eliminated, the fluid that has been expressed is referred to as serum; this is how serum differentiates from plasma. Because serum lacks these components, it is unable to clot. Clot retraction requires platelets to take place. Consequently, the inability to retract the clot suggests that there may not be as many platelets in the bloodstream. The edges of the shattered blood vessel are drawn together when the clot retracts, further enhancing the final condition of hemostasis.

INTRAVASCULAR ANTICOAGULANTS

The most important factors for preventing clotting in the normal vascular system are:

- The endothelial cell surface's smoothness, which inhibits contact-induced activation of the intrinsic clotting system.
- A layer of mucopolysaccharide called glycocalyx, which has been adsorbed to the surfaces of endothelial cells, covers the endothelium and acts as a barrier to clotting factors and platelets, limiting the activation of clotting.
- An related protein with the endothelium membrane is thrombomodulin, which binds thrombin. The interaction of thrombin with thrombomodulin inhibits thrombin, which slows down the clotting process.
- Additionally, a plasma protein called protein C is activated by the thrombomodulin-thrombin complex. This protein functions as an anticoagulant by deactivating active Factors V and VIII.
- The most important anticoagulant in the blood itself is antithrombin, which blocks the action of factors XII, XI, IX, X, and II (thrombin).
- Another potent anticoagulant made by basophils and mast cells is heparin. It enhances antithrombin's ability to inhibit thrombin when combined with it. In order to avoid intravascular coagulation, heparin is frequently utilized as a pharmacological agent in medical practice at significantly greater concentrations.

ANTICOAGULANTS FOR CLINICAL USE

In some thromboembolic conditions, it is desirable to delay the coagulation process. Various anticoagulants have been developed for this purpose. The ones most useful clinically are heparin and the coumarins.

Heparin: Heparin is manufactured in nearly pure form by extracting it from various animal tissues. The blood-clotting time increases from a normal of approximately 6 minutes to 30 or more minutes upon injection of very tiny doses, approximately 0.5 to 1 mg/kg of body weight. Additionally, this rapid change in clotting time prevents or delays the progression of a thromboembolic disease. Heparin's effects last for 1.5 to 4 hours on average. An enzyme called heparinase in the blood destroys the administered heparin.

Coumarins: Prothrombin and Factors VII, IX, and X, which are all produced by the liver, show a decline in plasma levels when a coumarin like warfarin is administered to a patient. This suggests that warfarin has a strong inhibitory impact on the liver's production of these substances. Warfarin blocks the function of vitamin K by competing with it for reactive sites in the enzyme activities that are necessary to generate prothrombin and the other three clotting factors. The coagulant activity of the blood falls to roughly 50% of normal after 12 hours and to roughly 20% of normal after 24 hours following the administration of an effective dose of warfarin.Put another way, the coagulation process is not stopped right once; instead, it must wait for the prothrombin and other coagulation components that are already present in the plasma to naturally consume. Once coumarin medication is stopped, normal coagulation normally returns within three days.

BLOOD GROUPS AND RH FACTOR

Blood types differ in their antigenic and immunological characteristics, causing antibodies in one blood type's plasma to react with antigens on the surfaces of another blood type's red blood cells. There are two specific kinds of antigens:

- The O-A-B system of antigens and

- The Rh system.

CLASSICAL ABO BLOOD TYPES

The most well known and medically important blood types are in the ABO group. They were discovered in 1900 and 1901 at the University of Vienna by Karl Landsteiner in the process of trying to learn why blood transfusions sometimes cause death and at other. times save a patient. In 1930, he belatedly received the Nobel Prize for this discovery.

A and B Antigens- Agglutinogens: Two antigens (also called agglutinogens because they often cause blood cell agglutination)-type A and type B-occur on, the surfaces of the red blood cells in a large proportion of human beings. Because of the way these agglutinogens are inherited, people may have neither of them on their cells, they may have one, or they may have both simultaneously.

Major O-A-B Blood Types: The blood is normally classified into four major O-A-B blood types depending on the presence or absence of the two agglutinogens, the A and B agglutinogens.

- When neither A nor B agglutinogen is present, the blood is type O.
- When only type A agglutinogen is present, the blood is type A.
- When only type B agglutinogen is present, the blood is type B.
- When both A and B agglutinogens are present, the blood is type AB.

Genetic Determination of the Agglutinogens: The O-A-B blood type is determined by two genes, one on each of two paired chromosomes. There are three forms of these genes: type O, type A, or type B. However, only one type of each of the two chromosomes can be present. There is no discernible type O agglutinogen produced by the type O gene on the cells because it is either completely or nearly devoid of function. On the other hand, strong agglutinogens are produced on the cells by the type A and type B genes.

Blood Types with Their Genotypes and Their Constituent Agglutinogens and Agglutinins

Genotypes	Blood Types	Agglutinogens	Agglutinins
100	O		Anti-A and Anti-B
OA or AA	A	A	Anti-B
OB or BB	A	B	Anti-A
AB	AB	A and B	

The six potential gene combinations are AA, BB, AB, OO, OA, and OB. Every individual belongs to one of the six genotypes, which are combinations of these genes. Because genotype OO does not create any agglutinogens, the blood type of that individual is OA. Blood type A is produced by individuals with genotypes OA or AA, meaning they have type A blood. Type B blood is given by genotypes OB and BB, and type AB blood is given by genotype AB.

Relative Frequencies of the Different Blood Types: In India, the prevalence of the different blood types is approximately:

O	31%
A	21%
B	39%
AB	9%

Agglutinins: The same bone marrow and lymph node cells that create antibodies to all other antigens

also produce agglutinins, which are gamma globulins like practically other antibodies. IgM and IgG immunoglobulin molecules make up the majority of them. There are essentially no agglutinins in the plasma right after birth. After birth, an infant starts producing agglutinins two to eight months later. Typically, a maximum titer is obtained between the ages of 8 and 10, after which it steadily decreases over the course of the remaining years of life.

In the plasma, antibodies referred to as anti-A agglutinins form when a person's red blood cells lack type A agglutinogen. Antibodies referred to as anti-B agglutinins also form in the plasma when type 8 agglutinogen is absent from red blood cells. Thus,

- Type O blood, although containing no agglutinogens, does contain both anti-A and anti-B agglutinins.
- Type A blood contains type A agglutinogens and anti-B agglutinins.
- Type B blood contains type B agglutinogens and anti-A agglutinins.
- Type AB blood contains both A and B agglutinogens but no agglutinins.

Landsteiner's Law: Based on the above-mentioned facts, Karl Landsteiner in 1900framed a law, called 'Landsteiner's Law'. It has two major components:

- If an agglutinogen is present in the RBCs of an individual, the corresponding agglutinin must be absent from the plasma;
- If the agglutinogen is absent in the individual RBCS, the corresponding agglutinin must be present in the plasma.

Exception to the second part is: absence of Rh, M and N agglutinogens from the RBCs which are not accompanied by presence in the plasma of anti-Rh, anti-M or anti-N agglutinins.

RHESUS (Rh) BLOOD GROUP

When transfusing blood, the Rh blood type system is equally as significant as the O-A-B blood type system. The primary distinction between the Rh and O-A-B systems is that the latter never experiences spontaneous agglutinin development, whereas the former does. In the O-A-B system, the plasma agglutinins that trigger transfusion reactions grow spontaneously. Rather, before enough agglutinins are produced to trigger a severe transfusion reaction, the individual must first be extensively exposed to a Rh antigen, such as by transfusion of blood bearing the Rh antigen.

Rh antigens: Rh factors are the names given to each of the six common forms of Rh antigens. C, D, E, c, d, and e are the designations for these sorts. A person without the G antigen always possesses the C antigen, while a person with a cantigen does not have the cantigen. The D-d and E-e antigens work in the same way. Every individual possesses one of the three pairs of antigens due to the way these elements are inherited.

The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens.

"Rh-Positive" and "Rh-Negative" People: Individuals who possess type D antigen are classified as Rh positives, whereas those who lack type D antigen are classified as Rh negatives. Though the reactions are typically far milder, it should be mentioned that some of the other Rh antigens might also produce transfusion reactions in Rh-negative individuals.

Genetic determination of Rh antigens: The activities of the genes found in chromosomes result in blood type antigens. The antigen D gene is also known as D. When D is missing from a chromosome, the alternate form (Allelomorph) known as "d" takes its place. Both the mother and the father can pass on the

Rh gene. The resulting gene composition (genotype) of the progeny is DD if gene Dis is carried by both sperm and ovum; Dd if the gametes carry D and D, respectively; and dd if both gametes carry D.

DD (homozygous) and Dd (heterozygous) are both Rh positive; dd (homozygous) is Rh negative. Of 85% Rh positive 35% are DD, 48% Dd and 2% have some other genotype containing D.

Relative frequencies of Rh factor: About 85 per cent of all white people are Rh positive and 15 per cent, Rh negative. In American blacks, the percentage of Rh-positives is about 95, whereas in African blacks, it is virtually 100 per cent.

Formation of Anti-Rh Agglutinins (Rh Immune response): When Rh-negative individuals get an injection of red blood cells containing Rh factor, anti-Rh agglutinins form gradually and reach their maximal concentration of agglutinins approximately two to four months later. Some persons experience this immunological reaction to a far greater degree than others. An individual who is Rh-negative will eventually become highly "sensitized" to the Rh factor after being exposed to it several times.

"Hemolytic Disease of the Newborn" (Erythroblastosis Fetalis"): Erythroblastosis fetalis is a disease of the fetus and newborn child characterized by agglutination and phagocytosis of the fetus's red blood cells. In most instances of erythroblastosis fetalis, the mother is Rh negative and the father Rh positive. The baby has inherited the Rh-positive antigen from the father, and the mother develops anti-Rh agglutinins from exposure to the fetus's Rh antigen. In turn, the mother's agglutinins diffuse through the placenta into the fetus and cause red blood cell agglutination.

Incidence of the Disease: Typically, a Rh-negative mother who gives birth to her first Rh-positive kid does not produce enough anti-Rh agglutinins to be harmful. On the other hand, erythroblastosis fetalis manifests in approximately 3 percent of second Rh-positive newborns, 10 percent of third babies, and the incidence increases gradually with consecutive pregnancies.

Effect of the Mother's Antibodies on the Fetus: Anti-Rh antibodies gradually permeate the placental membrane and enter the fetus's bloodstream after they have developed in the mother. There, they cause the blood of the fetus to agglutinate. Hemolyzes after becoming agglutinated red blood cells, releasing hemoglobin into the blood. The hemoglobin is then changed by the fetus's macrophages into bilirubin, which causes the baby's skin to turn yellow (jaundiced). Other bodily cells may potentially be attacked and harmed by the antibodies.

ACID BASE BALANCE

Due to the fact that nearly every enzyme system in the body depends on H^+ concentration for proper functioning, precise H^+ regulation is important. As a result, nearly every bodily function is affected by variations in hydrogen content. The equilibrium of H^+ production or intake and H^+ net elimination from the body is known as the acid-base balance. In controlling the elimination of H^+, the kidneys are crucial. All of this goes well beyond the kidneys' primary function of eliminating H^+ from the extracellular fluid, though, in order to precisely regulate its concentration. Sustaining appropriate H^+ concentrations in extracellular and intracellular fluids is also dependent on a variety of acid-base buffering systems involving the blood, cells, and lungs.

ACIDS AND BASES

Acids: A hydrogen ion is a single free proton released from a hydrogen atom. Molecules containing hydrogen atoms that can release hydrogen ions in solutions are referred to as acids. :

Bases: A base is an ion or a molecule that can accept an H^+. The proteins in the body, also function as bases, because some of the amino acids that make up proteins have net negative charges that readily accept H^+. The protein haemoglobin in the red blood cells and proteins in the other cells of the body are among the most important of the body's bases. The terms base and alkali are often used synonymously.

Alkalosis: The term alkalosis refers to excess removal of H^+ from the body fluids.

Acidosis: The excess addition of H^+ is referred to as acidosis.

NORMAL HYDROGEN ION CONCENTRATION AND PH OF BODY FLUIDS

The blood H^+ concentration is normally maintained within tight limits around a normal value of about 0.00004 mEq/L (40 nEq/L). Normal variations are only about 3 to 5 nEq/L. Because H^+ concentration normally is low, and because these small numbers are cumbersome, it is customary to express H^+ concentration on a logarithm scale, using pH units. pH is inversely related to the H^+ concentration; therefore, a low pH corresponds to a high H^+ concentration, and a high pH corresponds to a low H^+ concentration.

pH and it Concentration of Body Fluids

	H^+ Concentration (mEq/L)	pH
Extracellular fluid		
Arterial blood	4.0×10^{-5}	7.40
Venous blood	4.5×10^{-5}	7.35
Interstitial fluid	4.5×10^{-5}	7.35
Intracellular fluid	1×10^{-3} to 4×10^{-5}	5.0 to 7.4
Urine	3×10^{-2} to 1×10^{-5}	4.5 to 8.0
Gastric HCI	160	0.8

DEFENSES AGAINST CHANGES IN HYDROGEN ION CONCENTRATION

There are three primary systems that regulate the H^+ concentration in the body fluids to prevent acidosis or alkalosis:

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

(2) The respiratory centre, which regulates the removal of CO_2 (and, therefore, H_2CO_3) from the extracellular fluid; and

(3) The kidneys, which can excrete either acid or alkaline urine, thereby readjusting the extracellular fluid H concentration toward normal during acidosis or alkalosis.

CHEMIGAL ACID BASE BUFFER SYSTEMS OF THE BODY FLUIDS.

Buffer is any substance that can reversibly bind H. The general form of the buffering reaction is

$$\text{Buffer} + H^+ \rightleftharpoons H\,\text{Buffer}$$

In this example, a free H^+ combines with the buffer to form a weak acid (H buffer) that can either remain as an unassociated molecule or dissociate back to buffer and H^+. When the H concentration increases, the reaction is forced to the right, and more H^+ binds to the buffer, as long as buffer is available. Conversely, when the H^+ concentration decreases, the reaction shifts coward the left, and H^+ is released from the buffer. In this way, changes in H^+ concentration are minimized.

The three principal buffer systems of the body fluids are:

(a) Bicarbonate Buffer System: The bicarbonate buffer system consists of a water solution that contains two ingredients: (1) a weak acid, H_2CO_3, and (2) a bicarbonate salt, such as $NaHCO_3$. H_2CO_3 is formed in the body by the reaction of CO_2 with H_2O.

$$CO_2 + H_2O \xrightleftharpoons[\text{anhydrase}]{\text{Carbonic}} H_2CO_3$$

H_2CO_3 ionizes weakly to form small amounts of H^+ and HCO_3.

$$H_2CO_3 \rightleftharpoons H + HCO_3$$

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

$$NaHCO_3 \rightleftharpoons Na + HCO_3^-$$

Now, putting the entire system together, we have the following: $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ \underbrace{HCO_3^-}_{Na^+}$

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

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

As a result, more H_2CO_3, is formed causing increased Co_2, and H_2O production. From these reactions, one can see that H^+ afrom the strong acid HCl reacts with HCO_3 to form the very weak acid H_2CO_3, which in turn forms CO_2 and H_2O. The excess CO_2 greatly stimulates respiration, which eliminates the CO_2 from the extracellular fluid

The opposite reactions take place when a strong base, such as sodium hydroxide (NaOH), is added to the bicarbonate buffer solution.

$NaOH + H_2CO_3 \rightarrow NaHCO_3 + H_2O$

In this case, the OH^- from the NaOH combines with H_2CO_3 to form additional HCO_3. Thus, the weak base $NaHCO_3$ replaces the strong base NaOH. At the same time, the concentration of H_2CO_3 decreases (because it reacts with NaOH), causing more CO_2 to combine with H_2O to replace the H_2CO_3.

$$CO_2 + H_2O \longrightarrow \underset{\substack{+\\ NaOH}}{H_2CO_3} \longrightarrow \uparrow H^+ \underset{\substack{+\\ Na}}{HCO_3^-} + H^+$$

The net result; therefore, is a tendency for the CO_2 levels in the blood to decrease, but the decreased CO_2 in the blood inhibits respiration and decreases the rate of CO_2 expiration. The rise in blood HCO_3 that occurs is compensated for by increased renal excretion of HCO_3.

(b) Phosphate buffer system: Although the phosphate buffer system is not important as an extracellular fluid buffer, it plays a major role in buffering renal tubular fluid and intracellular fluids. The main elements of the phosphate buffer system are $H2PO_4$ and HPO_4. When a strong acid such as HCl is added to a mixture of these two substances, the hydrogen is accepted by the base HPO4 and converted to H_2PO_4

The result of this reaction is that the strong acid, HCl, is replaced by an additional amount of a weak acid, NaH_2PO_4, and the decrease in pH is minimized.

When a strong base, such as N_2OH, is added to the buffer system, the OH is buffered by the H_2PO_4 to form additional amounts of $HPO_4 + H_2O$.

In this case, a strong base, NaOH, is traded for a weak base, NaH_2PO_4, causing only a slight increase in pH.

(c) Proteins (Intracellular buffers): Proteins are among the most plentiful buffers in the body because of their high concentrations, especially within the cells. The diffusion of the elements of the bicarbonate

buffer system causes the pH in intracellular fluid to change when there are changes in extracellular pH. For this reason, the buffer systems within the cells help prevent changes in the pH of extracellular fluid but may take several hours to become maximally effective. In the red blood cell, hemoglobin (Hb) is an important buffer, as follows:

$$H^+ + Hb \rightleftharpoons HHb$$

Approximately 60 to 70 per cent of the total chemical buffering of the body fluids is inside the cells, and most of this results from the intracellular proteins.

RESPIRATORY REGULATION OF ACID-BASE CALANCE

The second line of defense against acid-base disruptions is the lung's regulation of the concentration of CO_2 in extracellular fluid. Because respiratory management of acid-base balance operates quickly and prevents excessive changes in H^+ concentration until the kidneys, which respond more slowly, can correct the imbalance, it is a physiologic sort of buffer system. Generally speaking, the respiratory system's total suffering capacity is one to two times more than the sum of all the other chemical buffers in the extracellular fluid's buffering power. This means that this method can often buffer one to two times as much acid or base as chemical buffers can. By removing CO_2 from extracellular fluid, increased ventilation lowers the concentration of H+ through mass action. On the other hand, when ventilation is reduced, CO_2 rises and raises the concentration of H+ in extracellular fluid.

(a) Effect of Alveolar ventilation rate: The rate of alveolar ventilation is the only other element that influences Pco_2 in extracellular fluid, assuming that the metabolic production of CO_2 stays constant. On the other hand, Pco_2 is higher when alveolar ventilation is lower and Pco_2 is lower when alveolar ventilation is higher.

(b) Impact of H^+ ion concentration: The rate of alveolar ventilation not only modifies the Pco2 of bodily fluids, which influences the H^+ concentration, but the H^+ concentration also modifies the rate of alveolar ventilation.

The alveolar ventilation rate rises four to five times faster than normal when the pH drops from 7.4 to 7.0, which is a severely acidic condition. On the other hand, the ventilation rate lowers as the plasma pH exceeds 7.4. This is because the amount of oxygen delivered to the blood falls as the alveolar ventilation rate drops due to an increase in pH (decreased H^+ concentration), and the blood's partial pressure of oxygen (Po_3) also decreases, stimulating the ventilation rate. As a result, the respiratory compensation for a pH rise is not nearly as efficient as the reaction to a sharp pH drop.

RENAL CONTROL OF ACID-BASE BALANCE

The kidneys control acid-base balance by excreting either an acidic or a basic urine. Excreting an acidic urine reduces the amount of acid in extracellular fluid whereas excreting a basic urine removes base from the extracellular fluid. The kidneys regulate extracellular fluid He concentration through three fundamental mechanisms:

(a) Secretion of H^+,

(b) Reabsorption of filtered HCO_3 and

(c) Production of new HCO_3.

(a) Secretion of H+ and (b) Reabsorption of filtered HCO_3: With the exception of the ascending and descending thin limbs of the loop of Henle, almost the whole tubule is involved in the secretion of hydrogen ions and the reabsorption of bicarbonate. An H^+ needs to be released for every bicarbonate that is reabsorbed.

(i) The process of H^+ secretion and bicarbonate reabsorption in the early tubular segments: When CO_2 either diffuses into the tubular cells or is produced by metabolism in the tubular epithelial cells, the secretory process starts. The carbonic anhydrase enzyme causes CO_2 to react with H_2O to generate $H2CO_2$; this then splits into HCO_3 and H^+. The tubular lumen receives the secretion of H^+ from the cell through sodium-hydrogen counter-transport. Na^+ travels along a concentration gradient from the tubule lumen to the inside of the cell. The energy needed to transfer H^+ from the cell's interior to the tubular lumen in the opposite direction is subsequently supplied by the gradient that allows Na^+ to enter the cell. Following H^+ dissociation from H_3CO_2, HCO_3 is produced within the cell and passes through the baso lateral membrane to enter the renal interstitial fluid and the peritubular capillary blood. In summary, an HCO_3 enters the bloodstream for each H^+ released into the tubular lumen. A unique procedure reabsorbing HCO_3 involves first combining with H^+ to generate H_2CO_3, which then turns into CO_2 and H_2O.

Thus, each time an H^+ is formed in the tubular epithelial cells, an HCO_3 is also formed and released back into the blood.

(1) Cellular mechanisms for active secretion ions into the renal tubule; (2) tubular reabsorption of bicarbonate ions by combination with hydrogen ions to form carbonic acid, which dissociates to form carbon dioxide and water; and (3) sodium ion reabsorption in exchange for hydrogen ions secreted. This pattern of hydrogen ion secretion occurs in the proximal convoluted, the thick ascending segment of the loop of Henle, and the early distal tubule.

(ii) The process of H^+ secretion and bicarbonate reabsorption in the late distal and collecting tubules: Beginning in the late distal tubules and continuing through the remainder of the tubular system, the tubular epithelium secretes H^+ by primary active transport. The characteristics of this transport are different from those discussed for the proximal tubule, loop of Henle, and early distal tubule. Hydrogen ion secretion in these cells is accomplished in two steps (1) the dissolved CO_2 in this cell combines with H_2O to form H_2CO_3, and (2) the H_2CO_3 then dissociates into HCO_3, which is reabsorbed into the blood, plus H^+ which is secreted into the tubule by means of the hydrogen-ATPase mechanism. For each secreted, an HCO_3 is reabsorbed, similar to the process in the proximal tubules. The main difference is that moves across the luminal membrane by an active H^+ pump instead of by counter transport, as occurs in the early parts of the nephron.

(c) Production of new HCO_3: Each day the kidney formed about 4820 milliequivalents of bicarbonate (180 L/day × 24 mEq/L); under normal conditions, almost absorbed from the tubules, thereby conserving the primary buffer system of the extracellular reabsorption of bicarbonate and the excretion of H^+ are accomplished through the process of tireden be the tubules. Because the HCO_3 must react with a secreted H^+ to form H_2CO_3 before it can be reabsorbed, 4320 millequivalents of H^+ must be secreted each day just to reabsorb the filtered bicarbonate.

Approximately 80 milliequivalents of nonvolatile acids are also produced daily by the body, primarily through protein metabolism. As a result of not being H_3CO_3, these acids are referred to as nonvolatile as the lungs are unable to expel them. Therefore, each day, 4400 milliequivalents of H^+ are secreted into the tubular fluid. Both the phosphate buffer and the ammonia buffer aid in the daily excretion of the 80 milliequivalents of nonvolatile acid produced by metabolism.

(i) Phosphate Buffer system: The phosphate buffer system is composed of $HPO_4^{\equiv}$ and H2PO4. The process of H^+ secretion into the tubules is the same as described earlier. As long as there is excess HCO_3 in the tubular fluid, most of the secreted H^+ combines with HCO_3. However, once all the HCO_3 has been reabsorbed and is no longer available to combine with H^+ , any excess H^+ can combine with $HPO_4^=$ and other tubular buffers. After the H^+ combines with HPO, to form H_2PO_4, it can be excreted as a sodium salt (NaH_2PO_4), carrying with it the excess hydrogen.

This H^+ excretion sequence differs significantly from the one that was previously discussed. Instead of only replacing filtered HCO_3, the HCO_3 that is produced in the tubular cell and enters the peritubular blood in this instance indicates a net uptake of HCO_3 by the blood. Thus, the overall result is the addition of a new HCO_3 to the blood anytime a H^+ released into the tubular lumen mixes with a buffer other than HCO_3. This illustrates one of the methods by which the kidneys are able to restore the HCO^3 reserves in extracellular fluid.

(ii) Ammonia Buffer system: Ammonia (NH_3) and the ammonium ion (NH_4^+) make up a second buffer system in the tubular fluid that is even more significant quantitatively than the phosphate buffer system. The collecting tubules and proximal tubular segments undergo different processes.

Proximal Tubular Segments: The liver uses glutamine, which is mostly produced through the metabolism of amino acids, to manufacture ammonium ions. The epithelial cells of the proximal tubules, thick ascending limb of the loop of Henle, and distal tubules receive the glutamine that is given to the kidneys. Each glutamine molecule enters the cell and undergoes a series of metabolic processes that result in the formation of two NH_4^+ and two HCO_3. In return for sodium, which is reabsorbed, a counter-transport mechanism secretes NH_4^+ into the tubular lumen. Together with the reabsorbed Na^+ the HCO_3 is carried across the basolateral membrane and absorbed by the peritubular capillaries in the interstitial fluid.

Thus, for each molecure of ultramine metabolized in the proximal tubules, two NH_4 are secreted into the urine and two HCO_2 are reabsorbed into the blood. The HCO_3 generated by this process constitules new picarbonate.

ACID-BASE DISORDERS

Respiratory Acidosis: A disorder characterized by a reduced arterial pH (↑[H^+]); ↑CO_2 tension (Hypercapnia), and a variable ↑ in the plasma (HCO_3).

Changes: ↓ Alveolar ventilation

↓↓

↑ Arterial pCO_2 (due to CO_2 retention)

↓↓

↑H_2CO_3

↓↓

(i) ↑[H^+] → ↓pH (e.g. ≤ 7.2)

(ii) ↑ [HCO_3]

Arterial plasma: pH 7.34; HCO_3 25mEq/L;pCO_2 48 mm Hg

Renal Compensatory Mechanism:

(1) Renal tubular secretion of H^+↑s, removal of H^+ from the body → rise of pH i.e. pH corrected to normal.

(2) ↑HCO_3 reabsorption in spite of plasma HCO_3 → further ↑ in plasma HCO_3 ↑ Cl^- excretion → ↓ plasma Cl^-

Respiratory Alkalosis: A disorder characterized by an elevated arterial pH↓ [H]), a low CO_2, tension (Hypocapnia), and a variable ↓ in plasma [HCO_3^-]

Changes:

↑ Alveolar ventilation

↓↓

↓Arterial PCO_2 (due to CO_2 washout)

↓↓

↓ H_2CO_3

↓↓

(i) ↓ $[H^+]$ → ↑ pH (eg. ≥ 7.6)

(iii) ↓$[HCO_3]$

Arterial plasma: pH 7.53; HCO_3 22mEq / L; pCO_2 27mm Hg

Renal Compensatory Mechanism:

(1) Renal tubular secretion of H^+ ↓s, → retention of H^+ in the body → fall in pH i.e. pH corrected to normal.

(2) ↓HCO_3 reabsorption → ↑ HCO_3 excretion → further ↓s already low plasma HCO_3.

Metabolic Acidosis: A disorder characterized by a reduced arterial pH (↑[H+]), or a reduced plasma $[HCO_3]$. Changes:

Addition of acid c.

Removal of base

↓↓

free H+ → ↓ pH

↓↓

(1) H+ is buffered forming H_2CO_3,

(2) HCO_3 level in plasma fails.

Arterial plasma: pH 7.28; HCO_3 18mEq/L,pCO_2. 40mm Hg

Respiratory Compensation: ↑ in plasma [H] stimulate respiration arterial pCO_2 → ↑ pH to normal.

Renal Compensation: It causes excretion by following ways:

(1) The anions that replace. HCO_3, in the plasma are filtered each with a cation (mainly Na^+) thus maintaining electrical neutrality.

(2) The renal tubular cells secrete H^+ into the tubular fluid in exchange for Na^+; and for each H^+ secreted, one Na^+ and one HCO^3 are added to blood However, secreted H^+ reacts with buffer systems in the kidney therefore, large amounts of H^+ can be secreted permitting large amounts of HCO_3 to be reabsorbed.

(3) In chronicacidosis, glutamine synthesis in liver is increased and provides the kidneys with an additional source of NH_4^+. The respiratory compensation tends to inhibit the renal response because decrease in pCO_2, and acid secretion, but it also decreases the filtered load of HCO_3 therefore, its net inhibitory effects is slight.

Metabolic Alkalosis: A disorder characterized by a reduced arterial pH (↓ [H]), or an increased plasma [HCO_3].

Change: Addition of acid conc.

Removal of base

↑ free H^+ → ↓ pH

↓↓

(1) H^+ is buffered forming H_2CO_2, on

(2) HCO_3 level in plasma fails.

Arterial plasma: pH 7.28; HCO_3 18mEq/L,pCO_2, 40mm Hg

Respiratory Compensation: ↑ in plasma [H^+] → stimulate respiration → ↓arterial pCO_2 →↑ pH to normal.

Renal Compensation: It causes excretion by following ways:

(1) The anions that replace HCO_3, in the plasma are filtered each with a cation (mainly Na^+) thus maintaining electrical neutrality.

(2) The renal tubular cells secrete H^+ into the tubular fluid in exchange for Na'; and for each H^+ ion secreted, one Na+ and one HCO3 are added to blood However, secreted H+ reacts with buffer systems in the kidney, therefore, large amounts of H+ can be secreted permitting large amounts of HCO3 to be reabsorbed.

(3) In chronic acidosis, glutamine synthesis in liver is increased and provides the kidneys with an additional source of NH_4^+. The respiratory compensation tends to inhibit the renal response because decrease in pCO_2 acid secretion, but it also decreases the filtered load of HCO_3 therefore, its net inhibitory effects is slight.

Metabolic Alkalosis: A disorder characterized by an elevated arterial pH (↓ [H^+]), or an increased plasma [HCO_3].

Changes: ↑s plasma HCO, lever and

↑s pH

Arterial plasma: pH 7.50; HCO_3 30mEq/L, 40mm Hg

Respiratory compensation: ↓ in plasma H_+ in pulmonary ventilation →↑ arterial pCO_2 →↑

$H_2CO_3 \rightleftharpoons H^+ + HCO_3$ bringing pH back to normal and elevating plasma HCO_3 level further.

Renal Compensation: It causes ↑ in HCO_3 excretion of following ways:

(1) More renal H^+ secretions expanded in reabsorbing the increased filtered load of HCO_3 → acidic urine.

(2) The rise in pCO_2 inhibits the compensation by facilitating acid secretion, but its effect is relatively slight.

THERMOREGULATION

CORE TEMPERATURE AND SKIN TEMPERATURE

The temperature of the deep tissues of the body-the "core" of the body-remains very constant, within ±1°F (±0.6°C), day in and day out, except when a person develops a febrile illness. The skin temperature, in contrast to the core temperature, rises and falls with the temperature of the surroundings.

NORMAL CORE TEMPERATURE

No single core temperature can be considered normal, because measurements in many healthy people have shown a range of normal temperatures measured orally, from less than 97°F (36°C) to over 99.5°F (37.5°C). The average normal core temperature is generally considered to be between 98.0° and 98.6°F when measured orally and about 1°F higher when measured rectally.

Lower lethal core temperature is 26°C, this leads to death due to cardiac failure. Upper lethal core temperature is 43.5°C, this leads to death due to heat stroke.

BODY HEAT PRODUCTION AND HEAT LOSS

In health, body temperature is always kept fairly close to normal level by maintaining a balance between heat production and heat loss. When the rate of heat production in the body is greater than the rate at which heat is being lost, heat builds up in the body and the body temperature rises. Conversely, when heat loss is greater, both body heat and body temperature decrease.

Heat Production or Thermogenesis :

- Heat is produced by metabolic activities of the body especially in Liver Heart and Skeletal muscles
    - o Heat production under basal (resting) conditions, called basal metabolic Tate (BMR) is kcal/kg/hour or 37-40 kcal/m2/hour. This output works out at about 1500 kcal/day in females and 1700 kcal/day in males.
- Heat is produced by assimilation of food i.e. specific dynamic action (SDA) of food The SDA of a food is the obligatory energy expenditure that occurs during its assimilation into the body. Maximum heat production is seen after ingestion of proteins.
- Heat is gained by body from the environment from objects hotter than itself:
    - o by direct radiation from the sun or heated ground.
    - o by reflected radiation from the sky.
- Heat is also generated by endocrine mechanisms. Epinephrine and nor-epinephrine produce a rapid but short lived increase in heat production. Thyroid hormones produce a slowly developing but prolonged increase in heat production.
- A source of considerable heat production is a special type of fat, called Brown Fat, which makes up a small percentage of total body fat. It is more abundant in infants but is present in adults also. As brown fat cells contain several small droplets of fat rich in mitochondria, increased fatty acids oxidation in the mitochondria increases heat production (i.e. has a high rate of metabolism).

1. By Radiation: from the body to cooler objects at a distance	50%
2. By Conduction and Convection: to the surrounding atmosphere	20%
3. By Vaporization (evaporation) of water	
(i) Through skin	27%
(ii) Through lungs	2%
4. By excretion in urine and farces (urination and defecation)	1%

REGULATION OF BODY TEMPERATURE

Role of Hypothalamus: The temperature of the body is regulated almost entirely by nervous feedback mechanisms, and almost all these operate through temperature-regulating centers located in the hypothalamus.

The anterior hypothalamic-preoptic area has contain large numbers of heat-sensitive neurons as well as about one third as many cold-sensitive neurons. These neurons are believed to function as temperature sensors for controlling body temperature. The heat-sensitive neurons increase their firing rate 2- to 10-fold in response to a 10°C increase in body temperature. The cold-sensitive neurons, by contrast, increase their firing rate when the body temperature fails.

The Posterior hypothalamus integrates the central and peripheral temperature sensory signals. The temperature sensory signals from the anterior it, hypothalamic preoptic area are also transmitted into this posterior hypothalamic area. Here the signals from the preoptic area and the signals from elsewhere in the body are combined and integrated to control the heat producing and heat conserving reactions of the body.

Role of receptors in Skin and Deep body tissue: Although the signals generated by the temperature receptors of the hypothalamus are extremely powerful in controlling body temperature, receptors in other parts of the body play additional roles in temperature regulation. This is especially true of temperature receptors in the skin and in a few specific deep tissues of the body and skin is endowed with both cold and warmth receptors Deep, body temperature receptors are found mainly in the spinal cord, in the abdominal viscera, and in or around the great veins in the upper abdomen. Both the skin and the deep body receptors are concerned with preventing hypothermia that is, preventing low body temperature.

Role of Endocrines: Various endocrine glands regulating body temperature are:

Adrenal Medulla: Exposure to cold reflexly simulates epinephrine secretion that stimulates metabolism and decreases heat loss. Epinephrine effect is rapid and of short duration.

Thyroid: Exposure to cold via hypothalamic TRH (thyroid releasing hormone) and pituitary thyrotrophin causes stimulation of thyroid, secretion. This increases heat production, mobilizes glycogen and, stimulates gluconeogenesis.

THERMOREGULATORY RESPONSE

1. **Skin blood vessel vasodilatation:** The skin blood vessels enlarge significantly in practically every part of the body. This results from suppression of the posterior hypothalamic sympathetic centers, which constrict blood vessels. The rate at which heat is transferred to the skin can rise up to eight times during full vasodilatation.

2. **Sweating:** Sweating is a result of a higher body temperature. When the body temperature goes over the threshold level of 37°C (98.6°F), there is a rapid increase in the rate of evaporative heat loss from

sweating. Sweating enough to eliminate ten times the basal rate of body heat generation is required for every 1°C increase in body temperature.

3. Decrease in heat production. The mechanisms that cause excess heat production, such as shivering and chemical thermo genesis, are strongly inhibited.

Mechanisms of Temperature Increase When the Temperature Is Too Low: The body's temperature regulating mechanism initiates the exact opposite process when it feels too chilly. They are as follows:

1. Vasoconstriction of the skin on the entire body. The posterior hypothalamic sympathetic centers are stimulated, which is the cause of this.

2. **Piloerection:** Hairs that stand on end are referred to as pilosecrosis. The muscles known as the arrector pili, which are connected to the hair follicles, contract in response to sympathetic stimulation, causing the hairs to stand straight. While this is not significant for humans, in lesser animals the vertical protrusion of the hairs allows them to trap a thick layer of "insulator air" adjacent to the skin, significantly reducing the passage of heat to the surrounding environment.

3. **Increase in thermogenesis (heat production):** Heat production by the metabolic systems is increased by promoting shivering sympathetic excitation of heat production, and thyroxin secretion

   (a) Promoting shivering: Located in the dorsomedial portion of the posterior hypothalamus near the wall of the third ventricle is an area called the primary motor center for shivering. This center becomes activated when the body temperature falls even a fraction of a degree below a critical temperature level.

   (b) Sympathetic excitation of heat production: An increase in either sympathetic stimulation or circulating norepinephrine and epinephrine in the blood can cause an immediate increase in the rate of cellular-metabolism. This effect is called chemical thermogenesis. In adult human beings, who have almost no brown fat, it is rare for chemical thermogenesis to increase the rate of heat production more than 10 to 15 per cent. However, in infants, who do have a small amount of brown fat in the interscapular space, chemical thermogenesis can increase the rate of heat production 100 per cent, which is probably an important factor in maintaining normal body temperature in neonates

   (c) Thyroxin secretion: The increased thyroxin increases the rate of cellular metabolism throughout the body, which is yet another mechanism of chemical thermogenesis. This increase in metabolism does not occur immediately but requires several weeks' exposure to cold to make the thyroid gland hypertrophy and reach its new level of thyroxin secretion.

CONCEPT OF A "SET-POINT FOR TEMPERATURE CONTROL

Heat production and loss rates drastically alter at a critical body core temperature of approximately 98.8°F, or -37.1°C. The body temperature drops and approaches 37.1°C at higher temperatures because the rate of heat loss is higher than the rate of heat production. The body temperature rises and once more reaches 37.1°C at temperatures below this point because the rate of heat production exceeds the rate of heat loss. The temperature control mechanism's "set-point" refers to this critical temperature. In other words, every temperature control system works nonstop to return the body's temperature to this predetermined degree.