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Chapter 1

Composition and function of normal blood

Composition and function of normal blood

Blood consists of a pale yellow , coagulable fluid called plasma in which various types of blood cells are suspended. The cells comprise the erythrocytes, granulocytes, monocytes ,lymphocytes and platelets.[1]

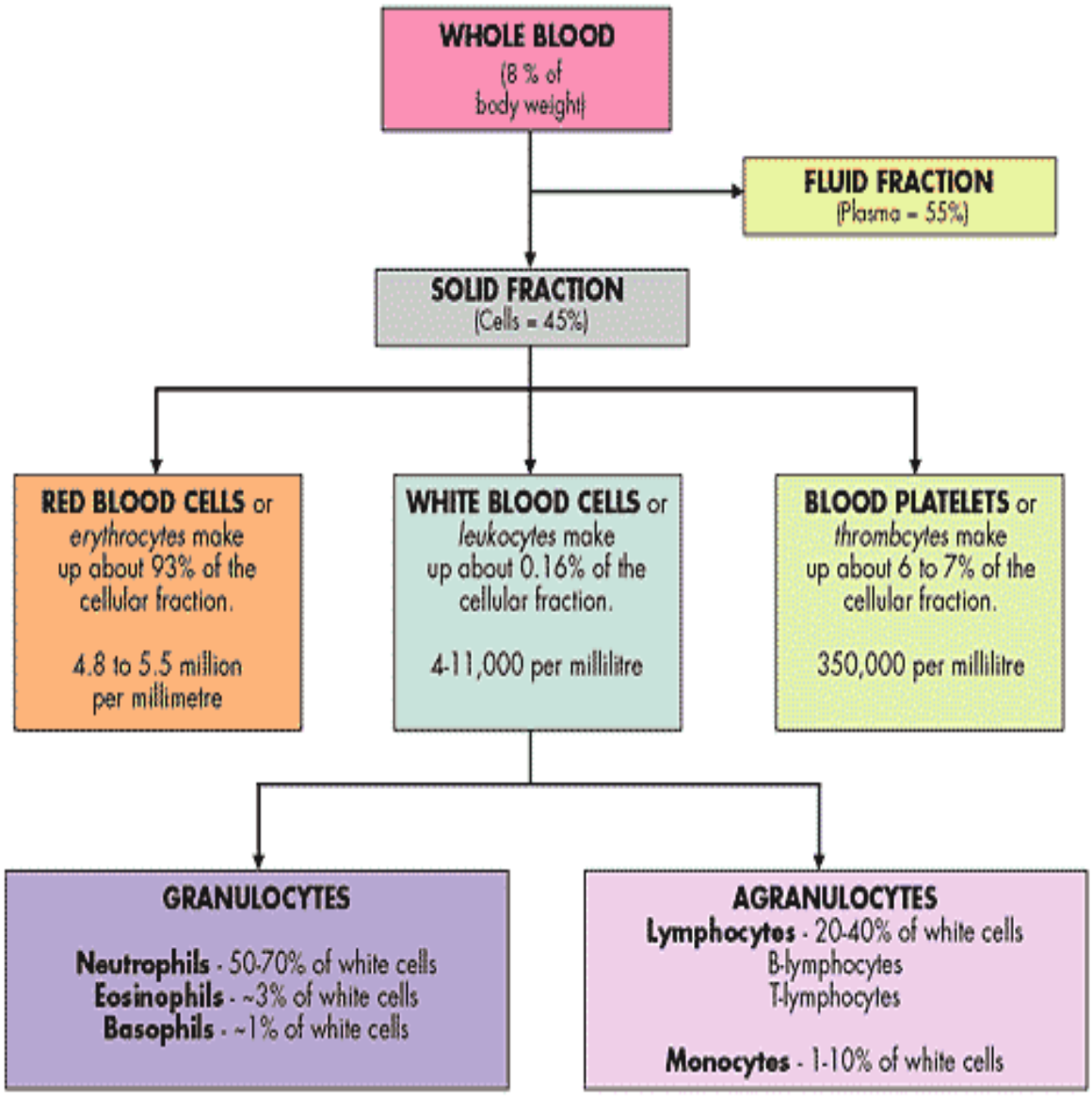


Fig (1-1) composition of the blood

1- Blood plasma :

plasma is the medium in which numerous substances such as hormones, minerals, vitamins , amino acids, sugars and excretory products are transported to and from various parts of the body. This fluid contains about 9% of solids , about four fifth of which consists of protein. There are many different plasma proteins and these have been separated into various fractions using techniques such as salt precipitation, electrophoresis and ultracentrifugation. Plasma proteins are classically divided into three main fractions :

1. globulins
2. fibrinogen
3. Albumin

A- Globulin : (MW 330000)

The globulin fraction is divided into α^1, α^2, β^1, β^2 and γ globulins. The globulins are investigated by zone electrophoresis of serum on filter paper , cellulose acetate or agarose gel using a barbiton buffer (ph 8.6 , ionic strength 0.05 – 0.1 mol /l). At a ph 8.6 the proteins except the immunoglobulin become –ve negatively charged and migrate towards the anode . The γ globulins are electrically neutral but move towards the cathode because of the effect of electroendosmosis .

B- Fibrinogen : (MW 330000)

fibrinogen is formed almost exclusively in the liver and the precursor of the fibrin of the blood clot. It is also largely responsible for the viscosity of the plasma ; solutions of fibrinogen are a bout six times as viscous as solutions of albumin of the same concentration.

C- Albumin: (MW 68000)

Albumin is the most a abundant plasma protein and synthesized mainly in the liver. It play an important role in maintaining the distribution of water between the blood and the tissue fluids. It is responsible for a bout 80 % of the total colloid osmotic pressure of

plasma partly because of its abundance and partly because of its low MW. It exerts 2 – 3 times the osmotic pressure of globulins per unit weight.

Disease processes are often reflected in change in plasma protein. A common , non specific change is (the acute phase response) to tissue inflammation and damage within 1 to 2 days arise occurs in α^1globulins, α^2 globulins , fibrinogen and C- reactive protein (γ- mobility). Subsequently C3 - C4 and ceruloplasmin rise followed by immunoglobulins, albumin ,prealbumin and transferring fall. The acute phase response is reflected in arise in the erythrocyte sedimentation rate (ESR) which is largely caused by arise in fibrinogen and α^2 macroglobulins.[(2)]

Table 1.1 Concentration of serum proteins in g/l at various ages[1]. Values represent 95% confidence limits

Age	Total Protein	Albumin	α_1 globulin	α_2 globulin	β globulin	γ globulin
Birth	46–70	32–48	1–3	2–6	3–6	6–12
3 months	45–65	32–48	1–3	3–7	3–7	2–7
1 year	54–75	37–57	1–3.4	2.8–11	3.8–10	2–9
2 years–Adult	53–80	33–58	1–3	4–10	3–12	4–14
Adult	64–78	36–52	1–4	4–8	5–12	7–15

2-Blood cells

Blood may be considered as a connective tissue consisting of formed elements suspended in plasma. The three types of formed elements are the RBC's (erythrocytes), WBC's (leukocytes), and platelet. A cubic millimeter of blood normally contains about: 5 million RBC's, 7000 WBCs, and 250,000 platelet.

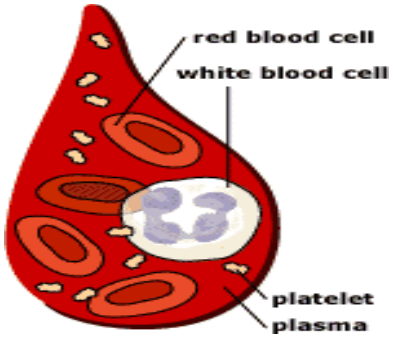


Fig (1-2) blood cells

Red Blood Cells (or erythrocytes):

Mature red blood cells are biconcave discs packed with hemoglobin. Its shape and content are adapted to transport oxygen. Their thanes enables them to bent easily hence passing through narrow passageways and its biconcave shape increases its surface area for gas exchange. The average volume of a RBC is $83\mu m^3$.Specific gravity of red blood cells is about 1.10. As red blood cells develop they lose their nuclei, in addition to their mitochondria, and ribosomes they have a 120 day life span. As they move through the cardiovascular system at times they are pounding against artery walls or are squeezing through tiny capillaries. They are normally destroyed in the liver or spleen. Most of its components are recycled for future use. The red cells contain a very high concentration of hemoglobin and really nothing else. The hemoglobin molecule is composed of four polypeptide chains. Hemoglobin exists in two functional forms of oxyhemoglobin and deoxyhemoglobin. Since carbon monoxide (CO) binds more strongly to heme groups than oxygen, poisoning occurs. When red blood cells are placed in distilled water or a hypotonic solution, water enters and the cell swells. Swelling occurs until a critical point is reached where the membrane abruptly becomes permeable to hemoglobin, which leaves it almost completely and enters the external medium (hemolysis). If the cell is given subsequent exposure to hypertonic saline will shrink the cell back towards the original volume. Erythropoietin is the hormone that is responsible for stimulating red blood cell production in the bone marrow.

White blood cells (or leucocytes or leukocytes):

White blood cells are larger but less numerous than the red blood cells. These nucleated cells protect against injury, infection, and cancer by either engulfing foreign agents or releasing biochemical that destroy them. The five varieties of WBCs are:

Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes.

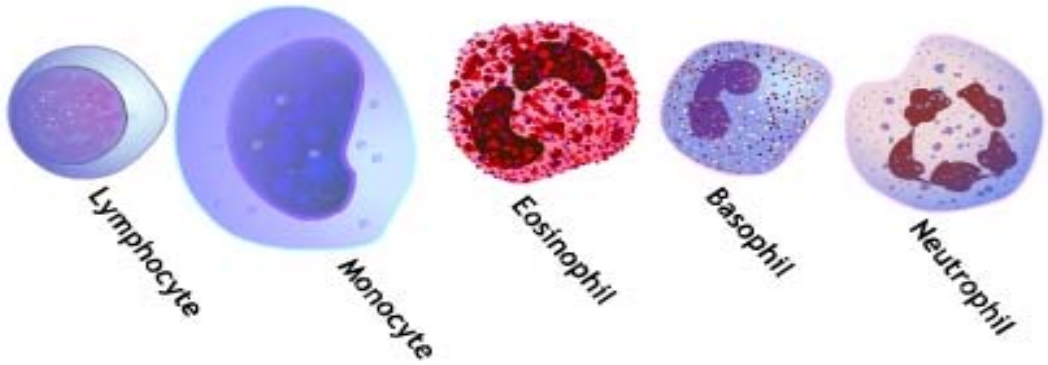


Fig (1-3) types of the white blood cells

WBC originate in the bone marrow and liver for about one year, spending only 3 or 4 days in the blood. Elevated or diminished numbers of WBC can provide clues regarding the type of infection or disease present.

Platelets (thrombocytes)

Platelets are small, colorless cell fragments responsible for blood clotting. They originate as part of a huge bone marrow cell called a megakaryocytes. They last for about one week. In healthy circulatory system they travel freely within the vessels and last for about one week. [3]

Erythrocytes

(Red blood corpuscles)

erythrocytes are highly differentiated cells which have no nuclei or cytoplasmic organelles although these cells are responsible for the red color of blood. Single erythrocytes are pale reddish yellow when examined in transmitted light. Normal erythrocytes are circular biconcave discs with a mean diameter of 7.2 µm (range 6.7 - 7.7µm) in dried fixed smears and a bout 7.5 µm in the living state. The normal

erythrocytes count is usually lower in women than in men; Women 4.5 to 5 million per cubic millimeter of blood (4.5×10^{12} / L to 5×10^{12} / L) Men 5 to 5.5 million per cubic millimeter of blood (5×10^{12} / L to 5.5×10^{12} / L) The erythrocytes are formed in the red bone marrow. Red bone marrow is present in the cancellous bone which is found in the extremities of long bones and between layers of compact bone in flat and irregular bones such as the sternum, skull, ribs and vertebrate. As red cells don't contain ribosomes , they can't synthesize new protein to replace essential molecules (e.g. enzymes, structural proteins) which become denaturated in the course of time. Red cells therefore have limited life span of 110 – 120 days , at the end of which they are ingested and degraded by the phagocytic cells of the marrow , spleen , liver and other organs.

Composition of Red blood cells

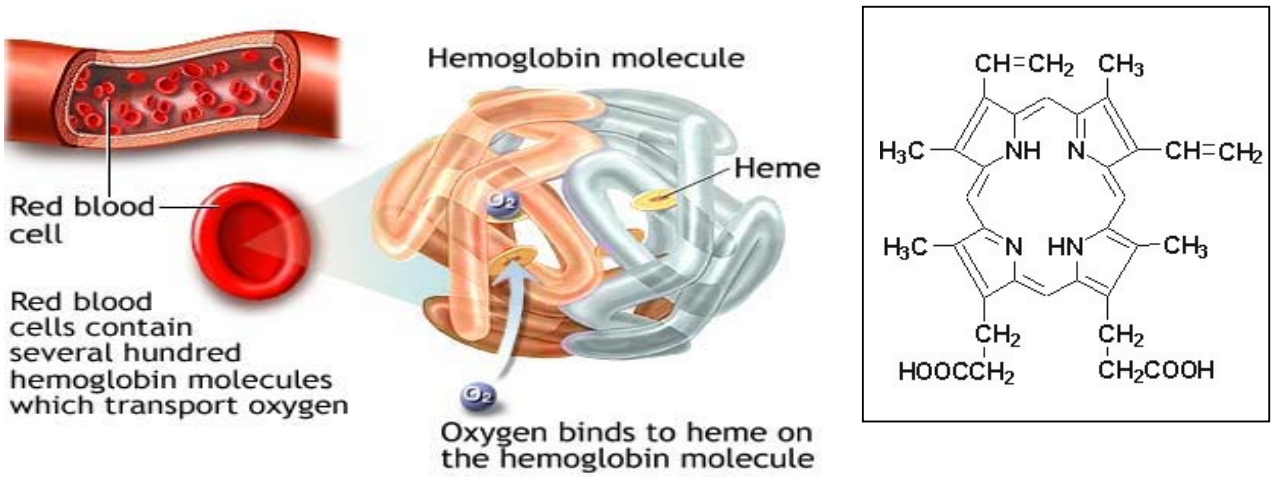
1- Water ***65%***

2- Solids **35%** → 32 % Hb hemoglobin

→ 3 % other solids as carbohydrates, lipids

Hemoglobin structure, synthesis, and breakdown :-

Hemoglobin is the oxygen – carrying protein within the erythrocytes of the blood. It is made of heme and globin , and the red pigment of the heme provides the red colour of blood. The molecular structure of hemoglobin consists of four globin (polypeptide chains) and four heme (protoporphyrin) groups each containing one atom of iron hence four iron molecules. The specific amino acids sequence of the globin chain determines the classification or type of hemoglobin present and the way in which the chain folds to the heme pocket. The structure of hemoglobin is directly related to the function (uptake and release of oxygen) therefore, one change in the amino acid sequence of a polypeptide globin chain may affect the function.



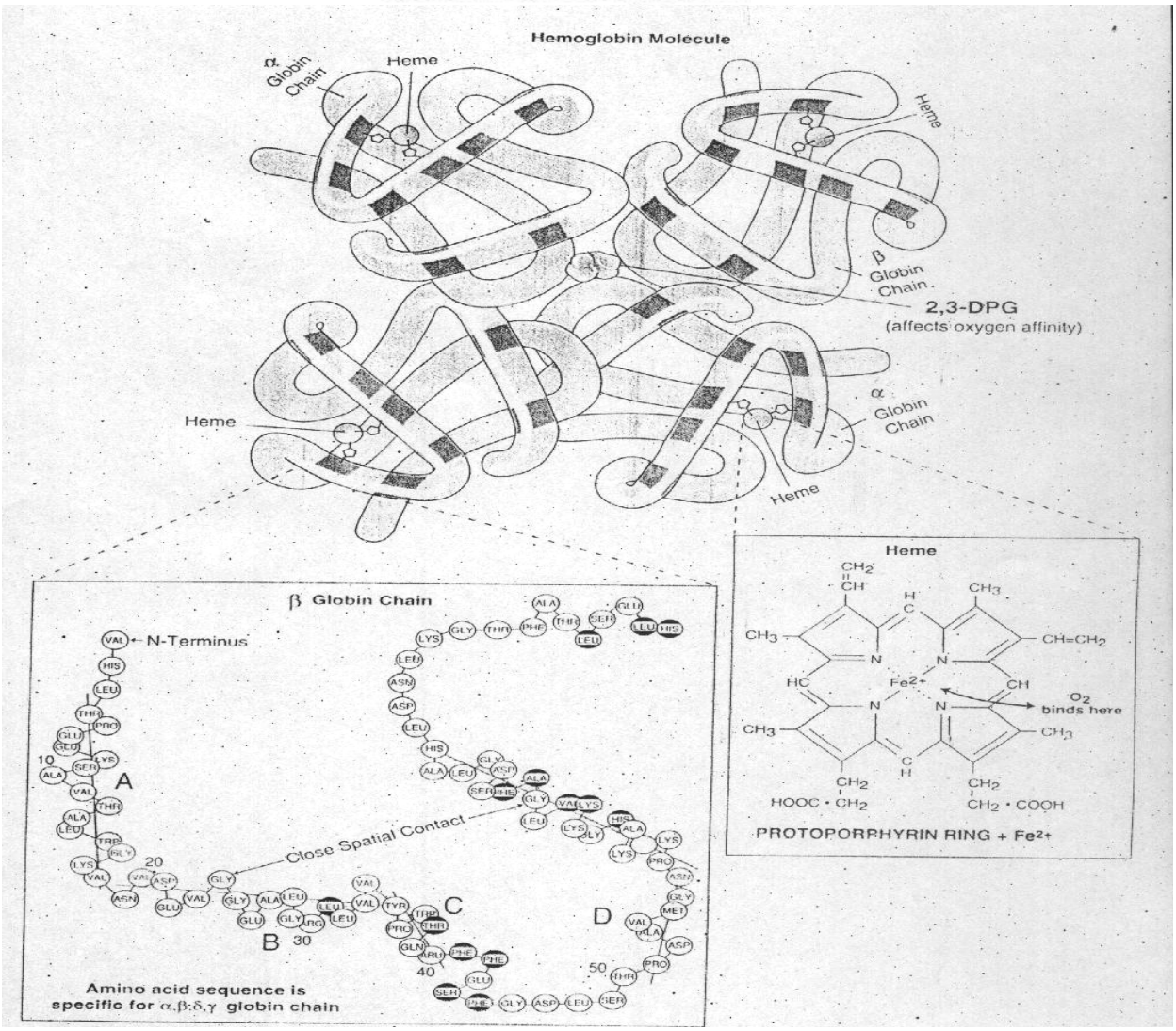


Fig (1-4) hemoglobin molecule structure. the molecular structure of hemoglobin consists of four globin (polypeptide chains) and four heme (protoporphyrin) groups each containing one atom of iron hence four iron molecules.

Hemoglobin synthesis :

65% of hemoglobin synthesis occurs in the immature nucleated red blood cells and 35% occurs in the reticluocytes (immature a nucleate red blood cells) .

this synthesis occurs in the mitochondria (heme) and in the ribosomes (globin) in the cytoplasm. The condensation of glycine and succinyl CO-A is the first step in heme synthesis, and this is limited by the action of amino levulinic acid (ALA) enzyme . pyridoxal phosphate (vit B6) is a cofactor for this reaction, which results in the synthesis of the protoporphyrin ring which combines with iron (fe^2) to form heme which then combine with the globin chains produced by the polyribosomes in the plasma. Four globin chains combine with four heme to become the hemoglobin molecule.

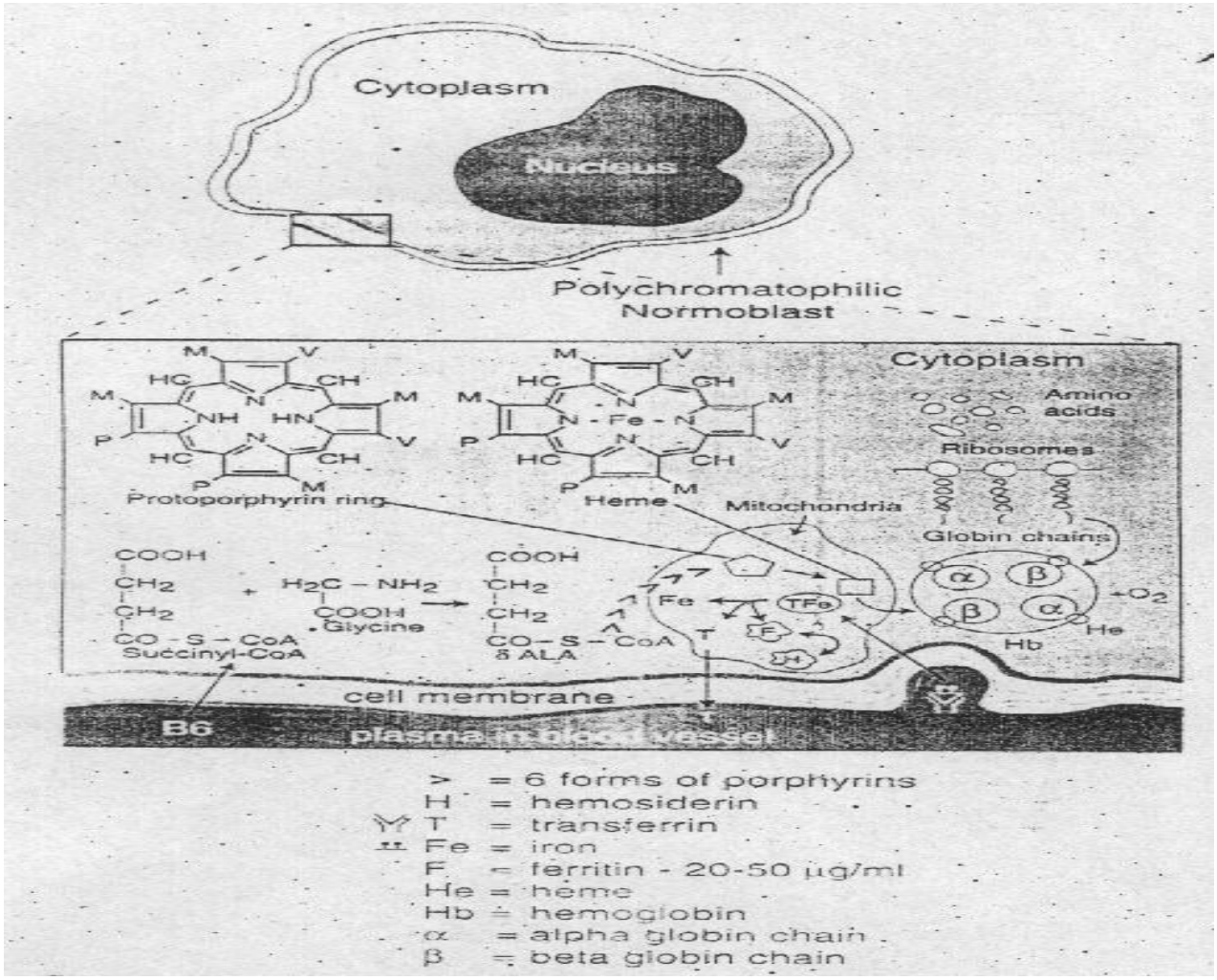


Fig (1-5) hemoglobin synthesis

Hemoglobin catabolism: Hemolysis (red blood cells breakdown or lysis)

Hemoglobin catabolism or breakdown occurs through two types of hemolysis

1- Intravascular hemolysis :

This type of hemolysis occurs within the blood stream and results in the immediate release of free hemoglobin into the plasma. This hemoglobin lose via excretion through the kidney in to urine.

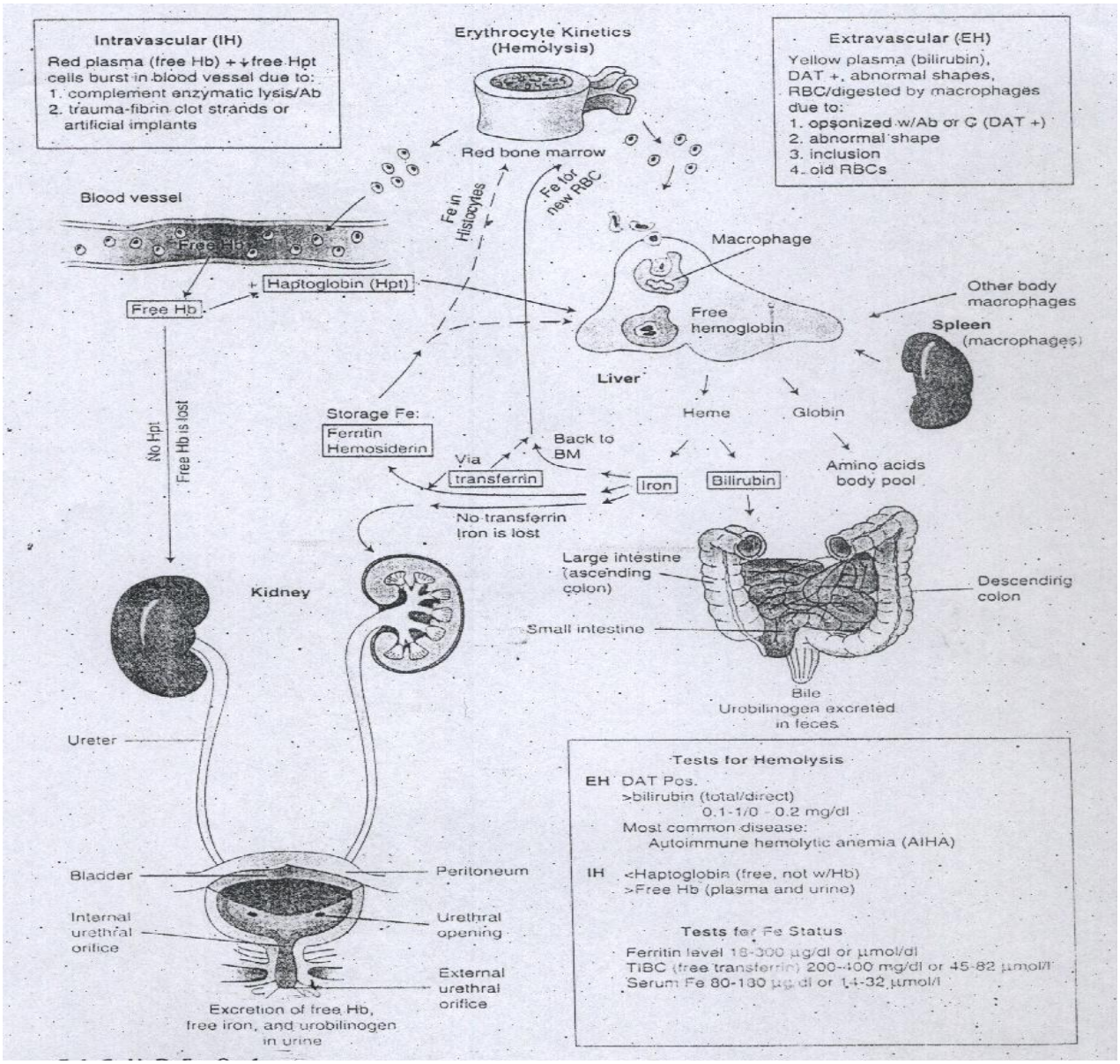


Fig (1-6) intravascular hemolysis versus extravascular hemolysis

2- Extravascular hemolysis:

Extravascular hemolysis occurs when a damaged or abnormal red blood cells is phagocytized by a macrophage usually in liver or spleen and the product of heme catabolism is a yellow, toxic substance called billirubin which further catabolized in the liver and excreted through the kidneys in the urine and through the digestive tract in the feces. The globin chains are broken down and returned to the amino acids pool of the body when heme is broken down, the iron (fe^{2}) is bound to a protein transferrin and is carried to the bone marrow for the ongoing production of new erythrocytes.

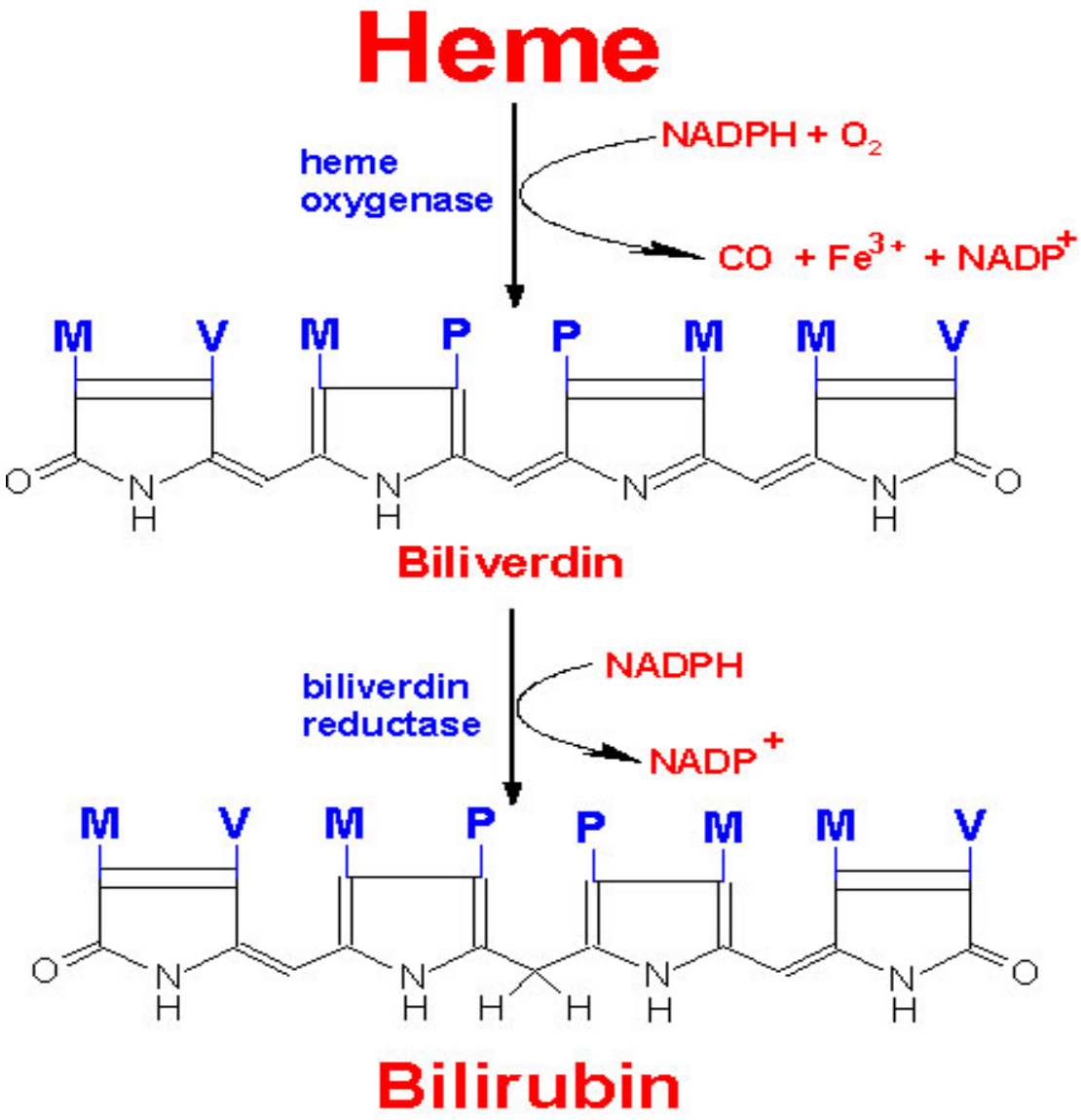


Fig (1-7) hemoglobin breakdown

Classification of the hemoglobin :

The classification of hemoglobin is determined by the globin chain present. Each globin chain has a different amino acid sequence which determines how it will function. The most common globin chain are the alpha (α), beta (β), delta (Δ), and gamma (γ) chains .Hemoglobin is capable of binding with substances other than oxygen "**oxyhemoglobin**" Once the oxygen has dissociated with the hemoglobin in the tissue, it is described as **"deoxyhemoglobin " (Hb)** and is found primarily in the venous circulation. Oxyhemoglobin and deoxyhemoglobin are normal functional states of hemoglobin and are associated with oxygen transport from the lungs to the body tissues. If carbon monoxide (CO) is present, this gas will preferentially bind hemoglobin molecule in place of oxygen to form **carboxyhemoglobin (Hbco)**. (CO) has a 210x greater affinity for the hemoglobin molecule. If sulfur is present during oxidation of hemoglobin , it is incorporated into the heme ring of the hemoglobin producing a green color. This form of hemoglobin is **sulfhemoglobin (SHb)** which isn't capable of oxygen transport and is irreversible. Cyanosis (blue skin due to poor oxygenation of the blood result in formation of (SHb). The iron (fe) molecule associated with hemoglobin is in the reduced ferrous state (fe^{2}) when oxidized to the ferric state (fe^{3}), hemoglobin is unable to bind with O2 and is called " **methemoglbin** " (Hi) which formed in the blood at low level throughout the process of normal oxygen transport.

Abnormal hemoglobins may results from genetic alternation of the amino acid sequence during synthesis of one of the globin chains.

Erythrocytes function :

The function of erythrocytes is to transport oxygen (O2) to maintain functional hemoglobin, and to maintain cell shape. Hemoglobin carries O2 from the lung to the body organs and tissues of the body. Where it must release the O2 and carry small amount of carbon dioxide (CO2) back where the process repeats itself. The erythrocytes are able to perform this function due to the O2 carrying protein hemoglobin (Hb) which is synthesized within the erythrocyte. [(5)]

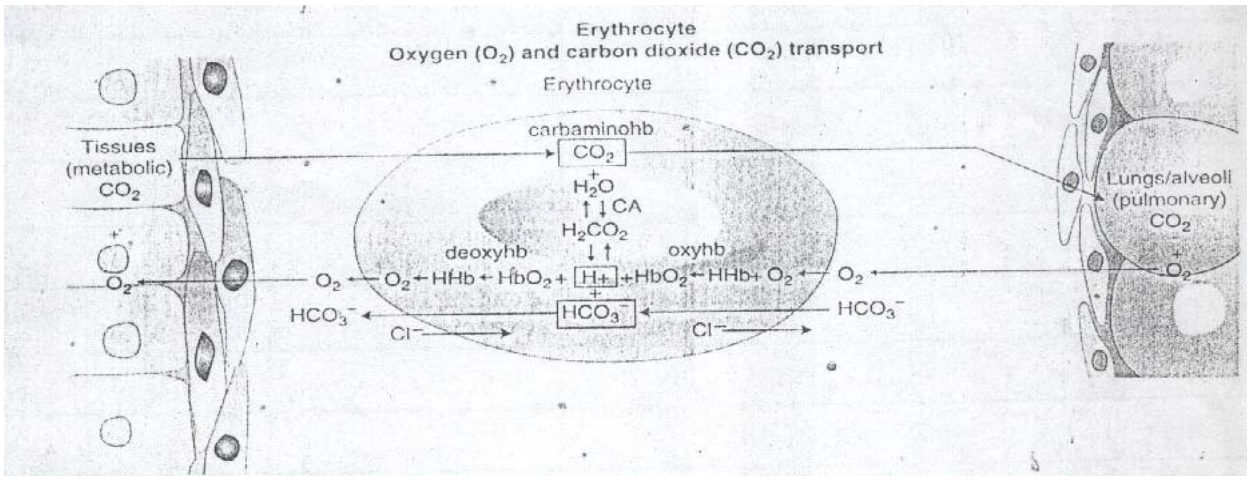


Fig (1-8) oxygen and carbon dioxide transport.

RETICULOCYTES

These are the immediate precursors of the red cells. They are rounded a nucleate cells which are about 20% larger in volume than mature red cells and appear faintly polychromatic when stained by a Romanowsky method. **(6)**

Reticulocytes are rounded cells with a tortuous surface and that in addition to ribosomes they contain mitochondria and autophagic vacuoles Circulating reticulocytes mature into red cells over a period of 1-2 days during which there is a progressive degradation of ribosomes and mitochondria and the acquisition of the biconcave shape. Reticulocytes actively synthesize hemoglobin and non-hemoglobin proteins. They contain enzymes of the Embden-Meyerhof pathway and the pentose phosphate shunt and, unlike the mature red cells, can also derive energy aerobically via the Krebs cycle which operates in the mitochondria and oxidises pyruvate to CO2 and water. In normal adults, reticulocytes comprise 0.8-2.6% of the total circulating erythrocyte plus reticulocyte population in males and 1.0 - 3.7% in females. **(7)**

It is more useful to express them as the total number per liter of blood, i.e. as an absolute rcticulocyte count. The latter is directly proportional both to the rate of effective erythropoiesis and to the average maturation time of blood reticulocytes. In normal adults the absolute reticulocyte count is 18-158 $x10^9$/l.**(8)**

GRANULOCYTES

(POLYMORPHONUCLEAR LEUCOCYTES)

These cells contain characteristic cytoplasmic granules and a segmented nucleus. The latter consists of two or more nuclear masses (nuclear segments) joined together by fine strands of nuclear chromatin.

The nuclear masses contain moderate quantities of condensed chromatin. The granulocytes are subdivided into neutrophil, eosinophil and basophil granulocytes according to the staining reactions of the granules.

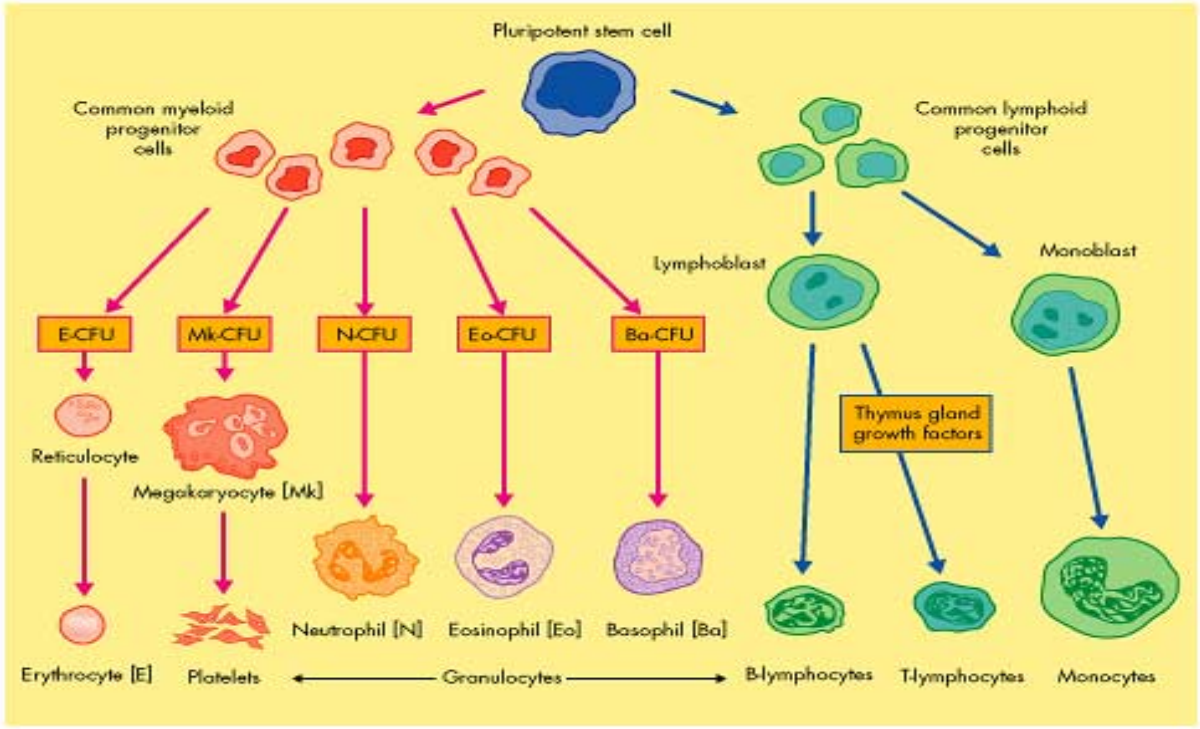


Fig (1-9) origins and maturation of the blood cells

Neutrophil granulocytes

Morphology and composition

Neutrophil granulocytes have a mean volume of 500 fl and, in dried fixed smears, have a diameter of 9-15/µm. Their cytoplasm is slightly acidophilic and contains many very fine granules which stain with neutral dyes; the granules stain a faint purple color with Romanowsky stains .The nucleus usually contains two to five nuclear segments; .the. average, values for the proportions of cells with two, three, four and five or more

segments are 32, 46, 19 and 3% respectively. Neutrophil granules show a considerable heterogeneity with respect to their ultrastructure.[6]

Some granules arc electron-dense and ellipsoidal; they are referred to as primary granules. Others are less electron-dense and are very pleomorphic; they are termed specific, granules. The primary granules, which are formed at the promyelocyte stage, are 0.5-1.0 µm in their long axis and contain myeloperoxidase, acid phosphatase, β-galactosidase, esterase, elastase, collagenase and cationic proteins. The specific granules are formed in the myelocyte and meta-myelocyte stages. They vary considerably in size being frequently quite small (0.2-0.5 µm long) and contain lysozyme (muramidase), amino peptidase and lactoferrin. The alkaline phosphatase activity of neutrophils appears to be present within membrane bound intracytoplasmic vesicles which have been called phosphosomes. In addition to the various organelles mentioned above, the cytoplasm contains a centrosome, a poorly-developed Golgi apparatus, microtubules and microfilaments, a few small mitochondria, a few ribosomes, a little endoplasmic reticulum and numerous glycogen particles.

Number and life-span

In the blood, the neutrophil granulocytes are distributed between a circulating granulocyte pool (CGP) and a marginated granulocyte pool (MGP).[9]

Functions of neutrophils [10]

These cells are highly motile. They move towards, phagocytose and degrade "various types of particulate material such a bacteria and damaged tissue cells. Neutrophils are attracted to sites of infection or inflammation as a result of chemotactic gradients generated around such sites.

The chemotactic factors include activated complement components(C3a,C5a, C567), lymphokines released from activated lymphocytes, kallikrein , products of certain bacteria and a factor released by neutrophils containing phagosomes. The arrival of neutrophils is probably facilitated by an increased permeability of adjoining blood vessels as a result of the action of anaphylatoxins such as C3a. [11]

Eosinphil granulocytes

Morphology and composition

Eosinophil granulocytes have a diameter of 12-17jum_injfixed smears. Their cytoplasm is packed with large rounded granules which stain reddish-Orange with Romanowsky stains . The proportions of tells with one,. two, three and four nuclear segments are 6, 68, 22 and 4%, respectively. Two types of eosinophil granules can be distinguished by electron microscopy: a few rounded homogeneously electron-dense granules and many rounded, elongated or oval crystalloid containing granules .The homogeneous granules contain a sulphated acid rnucosubstace and high levels of acid phosphates. Both homogeneous and crystalloid containing granules contain an arginine- and zinc-rich basic protein, a peroxidase (distinct from neutrophil peroxidase) and aryl sulphatase. Eosinophil granules also contain phospholipase B and D, β-glycerophosphatase, histaminase, kininase, Ribonuclease , β – glucuronidase cathepsin, PGE1 and PGE2 but probably not lysozyme. Eosinophils possess surface receptors for IgG-Fc, C4, C3b and C3d. [12]

Number and life-span :

Eosinophil granulocytes leave the circulation in a random manner with a Time of about 4.5-8 h; they probably survive in the tissues for 8-12 days.

Functions: [13,14]

Eosinophils share several functions with neutrophils: both, cell types are motile, respond to specific chemotactic agents and phagocytose and kill similar types of microorganisms. Eosinophils tend to be somewhat slower at ingesting and killing bacteria than neutrophils but appear to be metabolically more active than these cells. Eosinophils also have a role in regulating immediate- type hypersensitivity reactions. In these reactions chemical mediators of anaphylaxis are released from mast cells and basophils as a result Of the interaction between specific antigen and IgE on the surface of these cells. The three best-known chemical mediators are histamine, slow-reacting substance of anaphylaxis and an eosinophil chemotactic factor. The latter, and probably also histamine, attracts eosinophils to the site of the activated mast cells or basophils.

The eosinophils then release a substance which inhibits further histamine release. Eosinophils also release histaminase and arylsulphatase which inactivate histamine and slow-reacting substance, respectively. [15]

Basophil granulocytes :

In Romanowsky-stained blood smears, basophil granulocytes have an average diameter of about 127µm and display large rounded purplish black cytoplasmic granules. Some of these granules lie over the nucleus. The nucleus usually has two segments. They appear to undergo varying degrees of extraction during processing for electron microscopy and characteristically show a particulate substructure with each particle measuring about 20 nm in diameter . [16]

The granules contain histamine (which is synthesized by the cell), kallikrein, peroxidase, neutral esterases and proteases, serine hydrolase and three sulphated mucopolysaccharides (chondroitin sulphate, dermatin sulphate and heparin sulphate).

Basophils possess receptors for IgE, IgG, C and contain platelet-activating factor (which causes platelets to aggregate and release their contents) and histamine at their cell surface and an eosinophil chemotactic factor (ECF-A). Both basophils and mast cells play a key role in immediate-type hypersensitivity reactions. When IgE-coated basophils react with specific antigen, they rapidly degranulate (by exoplasmosis), release histamine and ECF-A and generate and release slow-reacting substance of anaphylaxis. Basophils may also be involved in the histamine mediated killing of intestinal parasites and, by virtue of their heparin content, in triglyceride metabolism.

MONOCYTES: [17]

These are the largest leucocytes in peripheral blood. In stained smears, they vary considerably in diameter (15-30µm) and in morphology. The nucleus is large and eccentric and may be rounded, kidney or horseshoe-shaped or lobulated, The nuclear chromatin has a skein like or lacy Appearance. Under the electron microscope, monocyte granules are seen to vary considerably in size and shape and to be more or less

homogeneously electron-dense. Some of these granules contain acid phosphatase and peroxidase; the composition of the remainder is unknown. The peroxidase-positive granules are characteristically smaller than those of neutrophils Their cytoplasm contains appreciable amounts of rough endoplasmic reticulum, moderate numbers of dispersed ribosomes, a well-developed Golgi apparatus, several mitochondria and bundles of microfibrils. The nucleus has moderate Quantities of heterochromatin and although nucleoli are not usually detectable by light microscopy, they are frequently seen by electron microscopy. Blood monocytes are distributed, as are neutrophils, between a circulating and a marginated pool there are, on average, 3.6 times more marginated than circulating cells. Monocytes are actively motile cells which respond to chemotactic stimuli and phagocytose particulate material in a manner similar to that described for neutrophil granulocytes. Monocytes and monocyte-derived macrophages are particularly conspicuous in sites of chronic inflammation. Macrophages appear to play important roles in various aspects of the immune response, including the processing of antigen to a form recognizable by lymphocytes and the degradation of excess antigen.

LYMPHOCYTES

Most of the lymphocytes in normal blood are small Lymphocytes have an average volume of approximately 180 fl and in stained smears have a diameter which varies from about 7 to 12 µm. the nucleus is round or slightly indented and there is considerable condensation of nuclear chromatin. The cytoplasm, which sometimes merely consists of a narrow rim around the nucleus, may contain a few azurophilic granules. **(18)**

Ultra structural studies reveal that small lymphocytes contain a few scattered monoribosomes an inactive Golgi apparatus, a few mitochondria. a few lysosomal granules, and a small nucleolus. In Romanowsky-stained smears, large lymphocytes are about 12-16 µm in diameter and contain more cytoplasm and less condensed chromatin than small lymphocytes. The concentration of lymphocytes in the blood is age-dependent. Lymphocytes recirculate: they leave the blood through the endothelial cells of post-capillary venules of lymphoid organs and eventually find their way back into lymphatic channels and re-enter the blood via the thoracic duct. The life-span of lymphocytes varies

considerably. The average life-span in humans appears to be about four years but some cells survive for over .10 years. Although mature lymphocytes are morphologically similar to one another they can be divided into two major functionally dissimilar groups, designated B-lymphocytes (B-cells) and T-lymphocytes (T-cells). Some characteristics of these two types of cell, including their various functions. T-cells are further divided into several functionally different groups, including helper cells (which promote the functions of B-cells and are required for the maturation of other kinds of T-cells) and suppressor/cytotoxic cells (which inhibit the-functions of other lymphocytes and also have cytotoxic capability against foreign or virus-infected cells). [19]

PLATELETS: [20]

Morphology and composition

Platelets are small fragments of megakaryocyte cytoplasm with an average volume of 7-8 fl. When seen in Romanowsky-stained blood smears, most platelets have a diameter of 2-3 μm They may be found lying singly but show a tendency to form small clumps. Platelets have an irregular outline, stain light blue and contain a number of small azurophilic granules which are usually concentrated at the centre. Newly-formed platelets are larger than more mature ones. platelets are shaped like biconvex discs and contain mitochondria, granules, two systems of cytoplasmic membranes (a surface-connected canalicular system and a dense tubular system), microfilaments, microtubules and clumps of glycogen molecules The microfilaments are situated between various organelles and may be attached to specific proteins at the inner surface of the cell membrane. The equatorial bundle of microtubules is situated in an organelle-free sol-gel zone just beneath the cell membrane and appears to be connected to this membrane by filaments. When platelets change shape during activation, the microtubules break their connections with the Cell membrane, the platelet granules also become concentrated at the centre of the cell.

The cell membrane of the platelet is extensively invaginated to form a surface connected canalicular system. This canalicular system provides a large surface area through which various substances, including the contents of platelet granules. This system is the main site

of synthesis of thromboxane A2 which plays an important role in the reactions leading to the release of the contents of platelet granules.[21]

Number and life-span

The normal range for the platelet count in peripheral blood is about 160-450 x 10/l ; slightly lower values are seen during the first three months of life. The platelet counts of women are slightly higher than those of men. There are also slight racial variations in the normal platelet count.[22]

Functions: [23]

Large quantities of energy are used during various platelet functions. This energy is mainly derived from the metabolism of glucose by the glycolytic pathway and tricarboxylic acid cycle. The energy is held as ATP within a metabolic pool which is distinct from the storage pool of adenine nucleotides situated in the dense bodies. Platelets play an essential rote in the haemostatic mechanism. When endothelial cells of vessel walls are damaged and denuded, platelets adhere to subendothelial connective tissue (basement membrane and microfibrils of elastin) via a specific receptor on the platelet membrane. This adhesion requires calcium ions and von Willebrand factor. Platelets also adhere to collagen via other specific membrane receptors. Adhesion is followed within seconds by the transformation of the platelet from its original discoid shape to a spiny sphere (a potentially reversible process) and within a few minutes by the release of the contents of some platelet granules.

The ADP released from the dense bodies, and possibly also traces of thrombin generated by the activation of the clotting cascade, cause an interaction of other platelets with the adherent platelets and with each other (secondary platelet aggregation) with further re-lease of ADP from the aggregating platelets. Aggregation induced by ADP (and by adrenaline and collagen) is preceded by an alteration of the cell membrane leading to calcium-dependent binding of fibrinogen to specific platelet receptors associated with membrane glycoprotein Ilb-IIIa; binding of fibrinogen to platelets may provide a recognition site for platelet-platelet interaction during aggregation.[24]

The process of secondary aggregation continues until a platelet plug occludes the damaged vessel. The formation of a fibrin clot around the platelet plug is initiated by the activation of factor XII by contact with subendothelial structures. In addition to their primary role in homeostasis, platelets have several other functions. They participate in the generation of the inflammatory response by releasing factors which increase vascular permeability and attract granulocytes. platelets remove the pharmacologically active substance 5HT from their microenvironment by taking it up and concentrating it in the dense granules; they thus serve as 'detoxifying' cells. Platelets also have a limited capacity for phagocytosis. Finally, platelets play a role in pathological processes such as thrombosis and the rejection of transplants and have also been implicated by some workers in the pathogenesis of atherosclerosis.

Blood Groups, Blood Typing and Blood Transfusions: [25]

Patients who loose blood in large amounts need blood transfusion. The patient to whom the blood is given is called a recipient, and the person who gives the blood is called a blood donor. A donor soon makes some more blood to replace the blood he has given and he can safely give blood every 6 months. Mixing blood from two individuals can lead to blood clumping or agglutination. The clumped red cells can crack and cause toxic reactions. This can have fatal consequences. Karl Landsteiner discovered that blood clumping was an immunological reaction which occurs when the receiver of a blood transfusion has antibodies against the donor blood cells. The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cells and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules. The blood group you belong to depends on what you have inherited from your parents. There are more than 20 genetically determined blood group systems known today, but the AB0 and Rh systems are the most important ones used for blood transfusions. Not all blood groups are compatible with each other. Mixing incompatible blood groups leads to blood clumping or agglutination, which is dangerous for individuals.

ABO system:

Individuals are divided into four major groups, A, B, AB and 0 on the basis of. the type of antigen present on the membrane of their red blood cells and the type of antibody present or inherited naturally in their plasma. Individuals may contain the A, B, or both A and B antigenic substances, or else lack these substances (type O). individual who lacks one or more of these antigens will spontaneously develop the corresponding antibodies (agglutinins) shortly after birth. Thus a person with A type blood will naturally produce anti-B agglutinins, a person with B blood will produce anti-A agglutinins, and a person with O blood will produce anti-A and anti-B agglutinins; but a person with AB blood will not produce any agglutinins in this blood group system. Since these agglutinins are always present in the blood, in transfusion the donor blood must be compatible with the recipient's blood, i.e., the donor's blood must not contain antigen corresponding to the recipient's antibody. Other blood group.

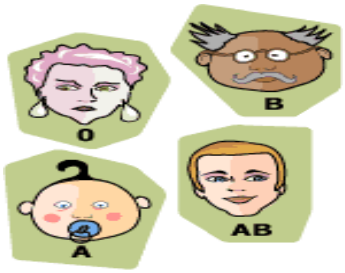


Fig (1-10) ABO system

Rh factor blood grouping system

Many people also have a so called Rh factor on the red blood cell's surface. It is named after the rhesus monkeys because it was first studied in the blood of these animals, it is a system composed of many antigens; D is by far the most active antigen and those who have it are called Rh+ (D positive) These agglutinins are only found due to a genetic defect as a sequel of lacking agglutinogen on RBCs (D negative).Those who haven't are called Rh- (D negative). A person with Rh- blood does not have Rh antibodies naturally in the blood plasma (as one can have A or B antibodies, for instance). But a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from

a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh+ blood can receive blood from a person with Rh- blood without any problems.

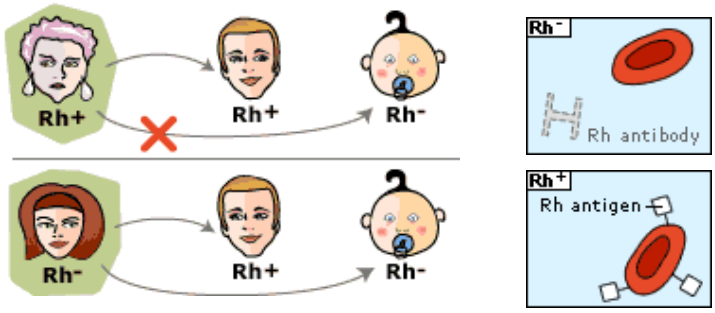


Fig (1-11) Rh factor blood grouping system

What is happening when the blood clumps or agglutinates?

For a blood transfusion to be successful, AB0 and Rh blood groups must be compatible between the donor blood and the patient blood. If they are not, the red blood cells from the donated blood will clump or agglutinate. The agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. The agglutinated red blood cells also crack and its contents leak out in the body. The red blood cells contain hemoglobin which becomes toxic when outside the cell. This can have fatal consequences for the patient.

Now, what is the meaning of antigen, antibody and the possible reaction which can occur between them?

You must know that, specific modified plasma γ-globulin which are present naturally in blood or are produced there, are referred to as antibodies (Ab), because they are concerned in the immunological reactions, referred to as immunoglobulins, "Ig or agglutinin". On the other hand, any protein that is when injected into an animal, induces the formation of antibody against it is referred to as antigen (Ag) "agglutinogen". When both antigen and its antibody meet together a reaction occurs between them leads to coagulation of these proteins and is referred to as; precipitation reaction in vitro, and agglutination reaction in vivo. The name agglutinin and agglutinogen is referred to the agglutination reaction. The plasma in the transfusion is usually so diluted in the recipient that it rarely causes agglutination reaction. However, when the recipient's plasma has agglutinin against the

donor's red cells, they attack them and the cells agglutinate and hemolyze. We came back to the previously mentioned four groups; they are divided on the basis of the presence or absence of the most important and best known antigens (or agglutinogens) which are agglutinogen A and B. This is because the agglutinin is formed shortly after birth and stimulated by immunization with A or B antigens. Antigens and antibodies react by forming a physical bond between the antigen determinant group and the antibody-combining site. This reaction may be :

Specific reaction : If Ab reacts with Ag inducing its formation.

Cross matching reaction: If Ab reacts with Ag that didn't induce its formation .

Both agglutinogen A and B are glycoproteins that differ only in carbohydrate (CHO) moiety:

Type A;

Individuals with type A have agglutinogen A on the red cells and an appreciable titer of agglutinins against antigen B called anti B agglutinin.

Type B;

Individuals with type B have agglutinogen B on the red cells and agglutinins against antigen A called anti A agglutinin.

Type AB

Individuals with type A and B have agglutinogens A and B on the red cells and no circulating agglutinins.

Type O;

Individuals with type O have no agglutinogens and have both A and B agglutinins against antigens A and B.

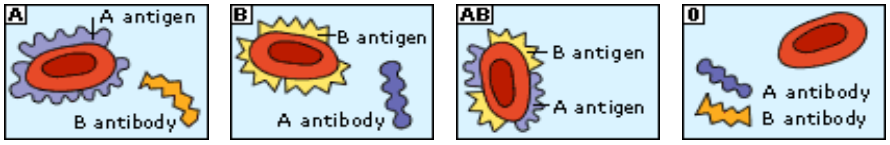


Fig (1-12) different blood groups

Later it was found that there are two types of agglutinogen A, A| (prevalent) and A2 (rare); therefore there are 6 groups instead of 4 which are:

Type A1.

Type A2.

Type B.

Type A1B.

Type A2B.

Type O.

Transfusion Reactions;

The patient can take a large amount of blood of his type by replaced transfusion. This doesn't mean, however, that blood should ever be transferred without being cross matching except in the most extreme emergencies, since the possibility of reactions or sensitization due to incompatibilities other than those due to ABO always exists. Dangerous hemolytic transfusion reactions occur when blood is transferred into an individual with an incompatible blood type, i.e. an individual who has agglutinins against the red cells in the transfusion, i.e., if a patient receives blood against which he has antibodies (incompatible), rapid destruction of the transfused red cells may result. This is accompanied by fever, shivering nausea, increase in pulse rate and fall in blood pressure. Subsequently, jaundice and hemoglobinuria may be observed. Patients with blood type AB can be given blood of any type because they haven't anti A or anti B to attack the red cells of the transfused blood, therefore, they are called; Universal recipients. Patients with blood type O contain both types of agglutinins therefore they should be given only blood of their type while they can give blood to anyone because they have no antigen to be attacked. They are called; Universal donors.

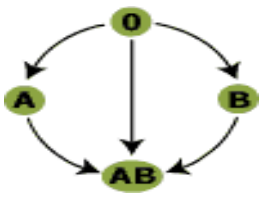


Fig (1-13) transfusion reaction

Blood Group	Antigens	Antibodies	Can give blood to	Can receive blood from
AB	A and B	None	AB	AB, A, B, 0
A	A	B	A and AB	A and 0
B	B	A	B and AB	B and 0
0	None	A and B	AB, A, B, 0	0

Chapter2 Hematopoiesis

Hematopoiesis

Hematopoiesis is the production of blood. Hematopoiesis results from a unique interaction of the pluripotent stem cells of the body, the microenvironment (stromal .cells include, fibroblasts, macrophages, endothelial cells, adipocytes/fat cells) in which hematopoiesis is taking place, and the growth factor or regulatory proteins (Fig. 2-1). These components of hematopoiesis work in a unique balance to produce normal healthy blood. This process takes place initially in the embryonic yolk sac, followed by the fetal liver and spleen, and hematopoiesis finally establishes within the bone marrow, where it continues to produce blood throughout life. during a bone marrow transplant, the donor cells are administered to the patient through the peripheral blood. The hematopoietic stem cells migrate to the bone marrow, called homing, and locate in the microenvironment for proper development, .if hematopoietic stem cells are transplanted into another area of the body, they will! not develop due to a lack of the appropriate environmental influences. This explains why any alteration in this microenvironment due to genetic bone marrow cell abnormalities, radiation, toxic chemicals, or infiltration of the bone marrow with malignant cells will significantly affect hematopoiesis.

Scheme of Hematopoiesis: (26)

The scheme of hematopoiesis demonstrating the various cells and their major regulatory proteins is presented in (figure 2-1). The initial cell of blood origin is called the pluripotent stem cell (PSC). This cell makes up a significant percentage (approximately 10%) of the umbilical cord blood cells of a newborn and less than 1% of the blood cells of an adult. This is the parent cell to a variety of 'cells within the body. Due to the enriched population of these PSCs, the cord blood is under investigation as an excellent source of cells for bone marrow transplantation. The direction of development and differentiation is determined by the protein factors that interact with the PSC. These factors can be stimulatory or inhibitory, and the cellular response to these factors depends on the presence of functional proteins and receptors on the cell that will allow the factor to bind to the cell and induce a

reaction. One observation to explain hematopoietic regulation is the competition of protein regulatory factors (cytokines) for the stem cell receptors. The cytokine in highest concentration would bind an increased number of receptors and influence the direction of lineage-specific differentiation.

Specific Growth of Factors and Differentiation of Cells

Specific growth factors such as stem cell factor (SCF, also known as kit ligand and the Steel factor), interleukin-1 (IL-1), 3 (IL-3), and 6 (T1.-6) influence the PSC to proliferate and differentiate into the hematopoietic stem cell (HSC). The HSC is destined to become only a blood eel! and differentiates further into the specific lymphoid or myeloid (bone marrow) cell lines. The PSC, under the influence of myeloid growth factors, will begin to proliferate and transform into the colony forming unit- granulocyte, erythrocyte, monocyte, and megakaryocyte (CFU-GEMM). This myeloid progenitor cell (self-renewing cell) is committed to the myeloid hematopoietic cell line. The CFU-GEMM cell is destined to become a granulocyte, erythrocyte, monocyte, or megakaryocyte (platelet precursor). If influenced by lymphoid growth factors such as IL-7 and SCF, the P.SC will proliferate and differentiate into a lymphoid stem cell (LSC), which is found primarily in the lymphatic system of tile body instead of the bone marrow. This lymphoid progenitor cell is committed to the lymphoid hematopoietic cell line. The LSC will become either a B lymphocyte, a T lymphocyte, a killer (K) lymphocyte, or a natural killer (NK) lymphocyte. The lymphoid cells are considered to be the result of extramedullary hematopoiesis. The CFU-GEMM progenitor cell then responds to specific cell line growth factors listed below to become the corresponding cell shown in (Figure 2-1). The myeloid growth factors are called colony-stimulating factors (CSFs), with a cell line designation such as G-CSF for granulocyte growth factors or M-CSF for monocyte growth factors. This balance of growth factors and cells produced is in response to the demands of the body. If an individual is anemic and in need of erythrocytes, the growth factor that increases the proliferation and differentiation of erythroid cells will be produced. If the individual has a bacterial infection, the growth factors that stimulate the granulocytic (bacterial fighting) cells to proliferate and differentiate will be produced in higher concentrations. granulocyte

monocyte (CFU-GM), colony-forming unit-eosinophil (CFU-Eo), colony forming unit basophil (CFU-Baso), colony-forming unit-megakaryocyte (CFU-Mega), or burst-forming unit-erythroid (BFU-E).

Depending on the specific stimulation, the CFU-GEMM will become a colony forming unit. The CFU-GM can be directed to differentiate into a neutrophilic granulocyte or a monocyte. Current theory suggests that only the neutrophilic granulocyte and the monocyte have a common precursor cell, the CFU-GM. The other two granulocytic cells, the eosinophilic and basophilic granulocytes, have their own committed CFU-Eo and CFU-Baso. Under the influence of colony-stimulating factor-granulocyte monocyte (GM-CSF), the CFU-GEMM will become the CFU-GM. This progenitor cell then responds to G-CSF to become the colony-forming unit-granulocyte (CFU-G), which is the neutrophilic granulocytic committed blast cell called the myeloblast. This cell is not classified as a progenitor cell due to the inability to reproduce itself. This cell is only capable of dividing, further differentiating into the promyelocyte, myelocyte, and metamyelocyte and maturing into a final functional segmented neutrophilic granulocyte. The polysegmented neutrophilic granulocyte, which is also called the polymorphonuclear (Poly, PMN, or Seg) cell, functions to destroy bacteria invading the body. If the predominating growth factor influencing the CFU-GM is the M-CSF, the CFU-GM becomes the monoblast or colony-forming unit-monocyte (CFU-M). This cell is not a progenitor cell and will divide and mature into the promonocyte and final functional cell, the monocyte. The monocyte is an extremely important component in the body's response to infection. The monocyte is a major phagocytic cell of the body that not only destroys the invading organism or substance but also relays information about the invader to other cells of the body.

If the CFU-GEMM responds to IL-5 and unknown eosinophil CSFs, it will become the CFU-Eo. This is a committed progenitor cell that will develop only into a promyelocyte, an eosinophilic myelocyte, an eosinophilic metamyelocyte, and a segmented eosinophil. Some believe that this cell derives from the CFU-GM; however, this diagram will support the evidence of a CPU unique to the eosinophil. The CFU-Eo is involved in hypersensitivity reactions and is seen in association with allergies, colds, and parasitic infections.

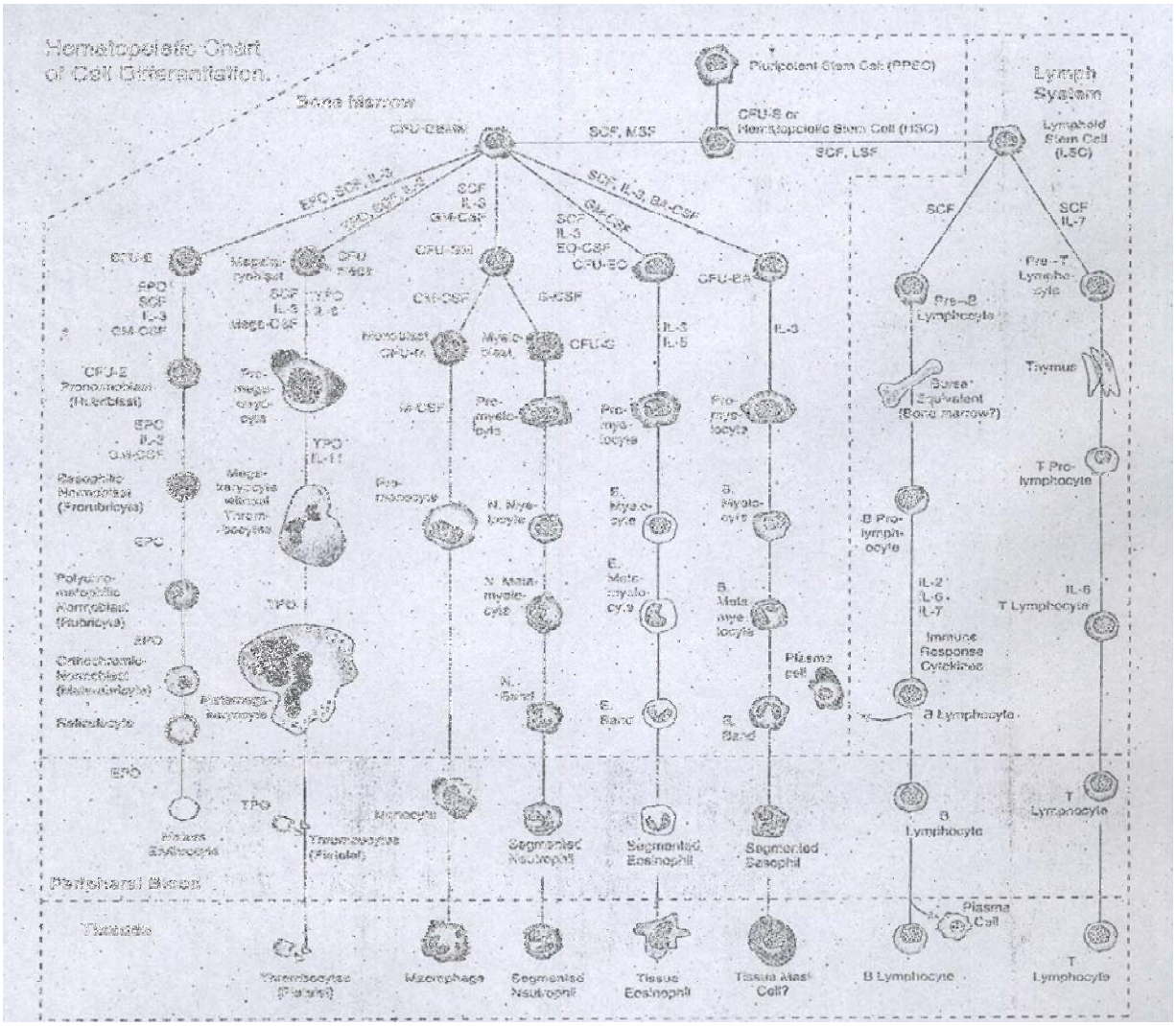


Fig (2-1) scheme of hematopoiesis with regulators

GENERAL CHARACTERISTICS OF HAEMOPOIESIS : [27]

The formation of blood cells of all types involves two processes:

1. the progressive development of structural and functional characteristics specific for a given cell type (i.e. cytodifferentiation).
2. cell proliferation. The latter serves to amplify the number of mature cells produced from one precursor cell which has become committed to any particular blood cell production line.

HAEMOPOIETIC STEM CELLS AND OTHER MORPHOLOGICALLY UNRECOGNIZED PRECURSORS : [28_29]

The haemopoietic cells can be divided into two categories:

the early precursors which have not yet been recognized morphologically with certainty but which can be studied by functional tests (described as the 'morphologically unrecognized precursors'); and

the morphologically recognizable precursors. The morphologically unrecognized precursors also consist of two categories:

A) - haemopoietic stem cells which have both the ability to develop into at least four types of blood cells and an extensive capacity to maintain their own numbers by cell proliferation;

B) - cells which are committed to three, two or one haemopoietic differentiation pathway but which do not have a substantial capacity for self-renewal.

Studies in experimental animals and in humans have clearly shown the presence of a multipotent myeloid stem cell whose progeny develop into erythrocytes, granulocytes, monocytes and megakaryocytes.

this stem cell is therefore frequently referred to as the colony forming unit in spleen or CFU-S. Subsequently, these stem cells became assayable in semi-solid culture media in which they give rise to colonies containing cells of all four myeloid lineages, (i.e. granulocyte-erythrocyte - macrophage - megakaryocyte (GEMM) colonies.

The myeloid stem cell is itself derived from a pluripotent haemopoietic stem cell which also gives rise to the lymphoid stem cell.

ERYTHROPOIESIS: [30]

There are several generations of morphologically unrecognized cells which are committed to erythropoiesis. These cells have been defined and studied operationally in terms of the characteristics of the erythroid colonies they generate in appropriate semi-solid media. The most immature of such cells are referred to as the erythroid burst-forming units (BFU-E) and the most mature as the erythroid colony-forming units (CFU-E).

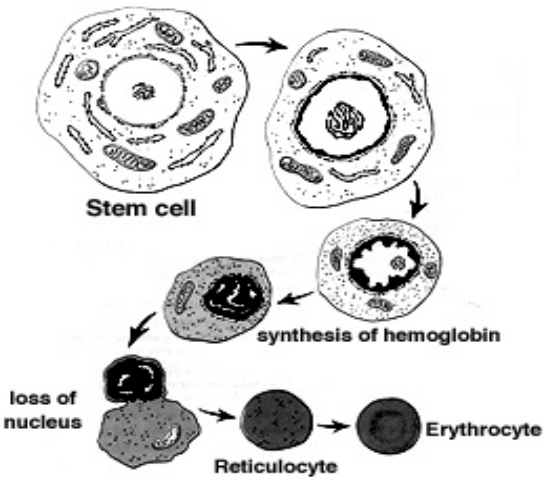


Fig (2-2) Erythropoiesis

There is a morphologically unrecognized precursor cell which is responsive to erythropoietin in vivo and this cell is referred to as the erythropoietin-responsive cell (ERC); the ERC may correspond to the CFU-E or a slightly earlier cell. Under the influence of erythropoietin, the ERCs develop into proerythroblasts which are the earliest morphologically recognizable red cell precursors in the marrow. The proerythroblasts then progress through If several morphologically-defined etiological classes. These are, in order of increasing maturity, the basophilic erythroblasts, the early polychromatic erythroblasts, the late polychromatic erythroblasts and the marrow and blood reticulocytes. Cell division occurs in the proerythroblasts, basophilic erythroblasts and early polychromatic erythroblasts but not in more mature cells. By contrast, the results of cytodifferentiation can be seen in all classes of morphologically recognizable precursors, both proliferating and non-proliferating. There are, on average, four cell divisions in the morphologically-recognizable precursor pool so that one proerythroblast may give rise to 2 or 16 red cells. In normal adults, the time taken for a proerythroblast to mature into marrow reticulocytes and for these reticulocytes to enter the circulation is about 7 days ; of this , about 2.5 days is spent in the marrow reticulocytes pool. The time taken for blood reticulocytes to mature into erythrocytes is 1-2 days.

In normal individuals erythrocytes circulate for 110 – 120 days before they are removed and broken down by cells of the mononuclear phagocyte system.

Some of the erythropoietic cells do not develop successfully into erythrocytes but are recognized as being abnormal and phagocytosed by the bone marrow macrophages. This loss of potential erythrocytes is referred to as ineffective erythropoiesis. The extent of ineffective erythropoiesis is small in normal marrow but is substantial in certain diseases.

Regulation of erythropoiesis : [31]

The rate of red cell production is primarily regulated by the hormone erythropoietin whose main action is to stimulate the rate of conversion of ERC to pronormoblasts. The plasma level of this hormone is inversely related to the capacity of the blood to deliver oxygen to tissues. thus, in most anaemic states there is an increased level of erythropoietin, which in turn causes an enhancement of the rate of erythropoiesis The kidneys are the organs mainly concerned with erythropoietin production in adults. The secretions of various endocrine glands also mfluenjp erythropoiesis and patients with hypofunction of some endocrine glands develop a moderate anaemia

NEUTROPHIL GRANULOCYTOPOIESIS AND MONOCYTOPOIESIS

There are several generations of morphologically unrecognized precursor cells concerned with the production of neutrophil granulocytes and macrophages. These have been denned on me basis of their ability to form colonies of granulocvtes, macrophages or both when grown in vitro in semi-solid media containing appropriate colony-stimulating factors or in vivo within diffusion chambers implanted intraperitoneally in mice. Cells giving rise to granulocyte or macrophage colonies in vitro are described as colony-forming units in culture (CFU-C) or, more specifically, granulocyte-macrophage colony-forming units (CFU-GM). The available data suggest that the multipotent myeloid stem cell generates a bipotent granulocyte-macrophage progenitor cell and that the latter develops into CFU-C which are irreversibly committed to mature either into neutrophils or into macrophages. The earliest morphologically recognizable precursors in the neutrophil and monocyte-macrophage series are the myeloblasts and monoblasts respectively.

Neutrophil granulocytopoiesis : [30,32]

The morphologically-recognizable cells of the neutrophil series are, in order of increasing maturity, the myeloblasts, neutrophil promyelocytes, neutrophil myelocytes, neutrophil metamyelocytes, juvenile neutrophils and the marrow neutrophil granulocytes. Cell division occurs up to and including the myelocyte stage; more mature cells are non-dividing. Cytodifferentia-tion occurs both in the proliferating and non-proliferating cells. The time taken for a myeloblast to mature into a marrow granulocyte and for the latter to enter the circulation is 10-12 days; about half of this time is spent in the proliferating cell pool. In normal individuals the blood neutrophils leave the circulation with a T½ of 7.2 h. There is no evidence for a substantial degree of ineffective neutrophil granulocytopoiesis in normal marrow.

Monocytopoiesis: (mononuclear phagocyte system)[33]

The morphologically-recognizable precursors of the blood monocytes are, in order of increasing maturity, the monoblasts, promonocytes and marrow monocytes; only the first two of these cell types undergo division. The blood monocytes leave the circulation with an average T½ of 71 h and transform into tissue macrophages. In the normal steady state there is a constant loss of tissue macrophages (e.g. by shedding of alveolar macrophages), which is balanced by the formation of new macrophages from blood monocytes and to a small extent from the division of some existing macrophages. The system of cells con-cerned with macrophage production is called the mononuclear phagocyte system. At sites of inflammation, macrophages may transform into epithelioid cells or develop into multinucleate giant cells. In the bone marrow, some monocyte-derived macrophages develop into osteoclasts.

Regulation of neutrophil granulocytopoiesis and monocytopoiesis : [34]

The regulation of the production of neutrophils is closely linked to that of the production of monocytes. The colony-stimulating factors (CSF) required for the formation of neutrophil and macrophage colonies in vitro appear to be also involved .in the formation of these cells in vivo. These factors consist of a family of glycoproteins produced by monocytes,

bone marrow macrophages and other types of cells such as fibroblasts and phytohaemagglutinin-stimulated lymphocytes. CSFs regulate the proliferation of CFU-C and promote their conversion to myeloblasts or monoblasts. Some CSF preparations stimulate the production of granulocyte colonies, and others that of macrophage colonies. The available data suggest that the bone marrow macrophages play a key role in regulating the production of their own precursors as well as of granulocytes. They do this by producing both CSF as well as prostaglandin E (PGE), an inhibitor of CFU-C. Any stimulation of CSF production is followed by a CSF-mediated stimulation of PGE synthesis which has the effect of returning the cell system to its original steady state. Granulocytes contain inhibitors of the proliferation of granulocytopoietic cells. They also contain lactoferrin, a metal-binding glycoprotein which depresses the formation of CSF by some monocytes and macrophages.16 These inhibitory substances may participate in the regulation of the production of monocytes and granulocytes in vivo by providing a negative feedback between the mass of mature granulocytes and the rate of production of new cells. Mature granulocytes in blood and tissue might also influence cell production in the marrow by removing or inactivating bacteria and their products, with the consequence that these do not reach macrophages and stimulate them to produce CSF. A reduction in the number of granulocytes in the body would lead to an increase in the bacterial antigenic load reaching the bone marrow macrophages, an increased production of CSF and, eventually, to an increased production of granulocytes.

EOSINOPHIL GRANULOCYTOPOIESIS :

There is a morphologically-unrecognized precursor committed to eosinophil granulocytopoiesis which can be detected by its ability to form colonies consisting of eosinophil granulocytes when grown in a semi-solid medium containing a specific eosinophil colony-stimulating factor (produced by antigen-stimulated non-adherent spleen cells).[(35)] This precursor cell is described as the eosinophil colony-forming unit or CFU-Eo. The earliest morphologically recognizable eosinophil precursor is the eosinophil promyelocyte. The other more mature cells of the eosinophil series are the eosinophil myelocytes, eosinophil metamyelocytes, eosinophil band cells and marrow eosinophil

granulocytes. The CFU-Eo, eosinophil promyelocytes and eosinophil myelocytes undergo cell division; the metamyelocytes, band cells and granulocytes are non-dividing cells

Regulation of eosinophil granulocytopoiesis: [36]

In experimental animals, the development of an eosinophilia in response to parasitic infections occurs in two phases: an initial inductive phase which is dependent on T-lymphocytes and a subsequent phase of increased proliferation of eosinophil precursors which is dependent on a humoral factor. It appears that parasite antigens stimulate sensitized lymphocytes to release a factor which increases eosinophil granulocytopoiesis by directly or indirectly acting on CFU-Eo and the morphologically recognizable eosinophil precursors. Other studies have shown that the specific depletion of mature eosinophils by the administration of anti-eosinophil serum generates a low molecular weight substance which stimulates eosinophil granulocytopoiesis both in vivo and in vitro; this substance has been termed eosinophilopoietin. [37]

PRODUCTION OF BASOPHIL GRANULOCYTES AND MAST CELLS

The basophil granulocyte is derived from the multipotent haemopoietic stem cell. In the postnatal period, basophil granulocytopoiesis occurs in the bone marrow. The morphologically-recognizable precursors of basophil granulocytes are rounded in shape and may be subdivided into basophil myelocytes, which have round or oval nuclei, and basophil metamyelocytes, which have C-shaped, unsegmented nuclei. Characteristically, in Romanowsky-stained smears both these cell types have large basophilic cytoplasmic granules which often overlie and obscure the nucleus. However, the granules are water-soluble and so their contents may be extracted during fixation and staining. With basic dyes such as toluidine blue or methylene blue, the more mature granules stain metachromatically (i.e. a reddish-violet). The ultrastructure of the granules in basophil promyelocytes and myelocytes is similar to that of the granules of mature basophil granulocytes except that the intra-granular particles are finer.[38]

Recent studies have shown that the tissue mast cells are also derived from CFU-S and that the latter develop within the marrow into undifferentiated precursors of mast cells These mast cell precursors circulate in the peripheral blood and migrate into tissues where they

proliferate and mature into mast cells. There are some data suggesting that both the basophil granulocyte and the mast cell might share a common bipotent morphologically unrecognized precursor cell (derived from the CFU-S). However, the exact relationship between the morphologically unrecognized precursors which are committed to the production of basophils and mast cells and those committed to the production of neutrophils, macrophages or eosinophils is still uncertain.[39]

Mast cells vary considerably in size (diameter of 5-25 μm in smears) and can be distinguished from basophils by their generally larger size, tendency to have an elongated or ovoid shape, and the fact that the coarse, purplish-black to reddish-purple granules (Romanowsky stain) are less water-extractable and seldom overlie the nucleus . The nucleus of the mast cell is small, round or oval, contains less condensed chromatin than that of a basophil and stains more or less uniformly. It is centrally or, occasionally, eccentrically placed. Mast cells contain histamine, heparin, hydrolytic enzymes (e.g. chloroacetate esterase) and surface receptors for IgE but, unlike basophil granulocytes , they also contain 5-hydroxytryptamine (serotonin) and do not contain peroxidase. Mast cells (like basophils) are PAS-positive. The mast cell granules vary markedly in their ultrastructure and may be dense and amorphous, show central condensations or contain crystals or lamellar structures arranged in various ways (e.g. scrolls or whorls and parallel lamellae) .[39]

MEGAKARYOCYTOPOIESIS: [30,40]

The morphologically-unrecognized early progenitor cells committed to megakaryocytopoiesis are called the megakaryocyte colony-forming units (CFU-Meg). The latter are derived from the multipotent myeloid stem cells and develop into the earliest morphologically-recognizable member of the megakaryocytopoietic system, the megakaryoblast. The CFU-Meg is denned operationally in terms of its ability to form a small colony of megakaryocytes when grown in suitable semi-solid medium in the presence of megakaryocyte colony-stimulating- factors (e.g. mitogen-stimulated spleen cell supernatants or the conditioned medium from a myelomonocytic leukaemia cell line). The megakaryocytes in these colonies show more or less normal maturation and shed

platelets. The CFU-Meg are a diploid cell population in which DNA synthesis and nuclear division (karyokinesis) is followed by cell division (cytokinesis).

Four types of megakaryocytes can be recognized in Romanowsky-stained marrow smears These are, in increasing order of maturity, megakaryoblasts (group I megakaryocytes), promegakaryocytes (group II megakaryocytes), granular megakaryocytes (group III megakaryocytes) and 'bare nuclei'. DNA synthesis occurs in 44% of megakaryoblasts, 18% of promegakaryocytes and in only 2% of granular megakaryocytes. This DNA synthesis is not associated with cytokinesis and results in the production of mononucleate polyploid cells. The DNA content of a megakaryoblast ranges from 4-32 c and that of a promegakaryocyte or granular megakaryocyte from 8 -64 c; cells with higher DNA content are larger than those with lower content (lc = the haploid DNA content, i.e. the DNA content of a spermatozoon). The time taken for a megakaryoblast to mature into a platelet-producing granular megakaryocyte may be about six days. Although the majority of the megakaryocytes are found in the marrow parenchyma, some whole cells enter the circulation via the marrow sinusoids . Most of the circulating megakaryocytes are trapped in the lungs and some of the pulmonary megakaryocytes appear to produce platelets.

PLASMA CELLS: [41]

The mature plasma cells seen in Romanowsky-stained smears of normal bone marrow may vary markedly in their morphology. The majority are 14-20 μm in diameter and have deep-blue cytoplasm with a paler paranuclear zone corresponding to the site of the Golgi apparatus; the cytoplasm may have one or more vacuoles .The nucleus is eccentric and small relative to the volume of the cytoplasm and contains moderate amounts of condensed chromatin . The characteristic cartwheel appearance of the nucleus is only seen in histological sections, not in smears. A small proportion of normal plasma cells show a variety of additional cytological features and are then sometimes given different names. Some plasma cells contain Russell bodies which are very large acidophilic cytoplasmic inclusions which stain by the periodic-acid-Schiff reaction; there is usually only one Russell body per cell. Mott cells (grape cells or morular cells) are plasma cells containing several smaller, slightly basophilic, rounded inclusions. Some plasma cells have a

reticulated appearance due to the presence of many pleomorphic inclusions and others are described as 'flaming cells' as they are eosinophilic at their periphery (occasionally the entire cytoplasm may take on an eosinophilic hue). Rarely, a plasma cell may contain azurophilic rods with a crystalline ultrastructure.

When examined with the electron microscope, the cytoplasm of the mature plasma cell is seen to be packed with flattened sacs of rough endoplasmic reticulum (RER) which are frequently aligned parallel to each other, in a concentric arrangement or in whorls; the sacs contain an amorphous material which is thought to be immunoglobulin. The cytoplasm also contains a well-developed Golgi zone , some rounded primary lysosomes (which vary considerably in size) and moderate numbers of mitochondria. Russell bodies and the inclusions within Mott cells and 'reticulated cells' consist of masses of homogeneous electron-dense material which are usually lined by RER ; these inclusions are thought to result from the condensation of immunoglobulin within distended cisternae of the RER.

The nuclei of plasma cells contain a variable quantity of condensed chromatin (which is generally, but not always, proportional to the degree of cytoplasmic maturity) and frequently contain a well-developed nucleolus.

OSTEOBLASTS AND OSTEOCLASTS: [42]

Groups of osteoblasts and individual osteoclasts can occasionally be seen in Romanowsky-stained normal marrow smears. Osteoblasts appear oval or elongated and are 25-50 μm in diameter. They have a single small eccentric nucleus with one to three nucleoli and abundant blue-staining cytoplasm, frequently with somewhat indistinct margins. Although these cells superficially resemble plasma cells, they are larger and their Golgi zone is not immediately adjacent to the nucleus. Furthermore, the nucleus of an osteoblast does not show the heavily-stained coarse clumps of condensed chromatin which are characteristically seen in plasma cells.

Osteoclasts are giant multinucleate cells with abundant pate-staining cytoplasm containing many fine azurophilic granules. The individual nuclei within a single cell are small, round or oval, uniform in size, and have a single prominent nucleolus. There is usually no overlap between adjacent nuclei within the same cell. Osteoclasts must be distinguished

from the other polyploid giant cells in the marrow, the megakaryocytes. Unlike osteoclasts, the latter (when normal) have a single large segmented and lobulated nucleus.

Chapter 3

Blood Coagulation

Blood Coagulation, Fibrinolysis

The ability of the body to control the flow of blood following vascular injury is paramount to continued survival. The process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue, is termed Hemostasis. Hemostasis, composed of 4 major events that occur in a set order following the loss of vascular integrity:

1. The initial phase of the process is vascular constriction. This limits the flow of blood to the area of injury.

2. Next, platelets become activated by thrombin and aggregate at the site of injury, forming a temporary, loose platelet plug. The protein fibrinogen is primarily responsible for stimulating platelet clumping. Platelets clump by binding to collagen that becomes exposed following rupture of the endothelial lining of vessels. Upon activation, platelets release adenosine-5'-diphosphate, ADP and TXA_2 (which activate additional platelets), serotonin, phospholipids, lipoproteins, and other proteins important for the coagulation cascade. In addition to induced secretion, activated platelets change their shape to accommodate the formation of the plug.

3. To insure stability of the initially loose platelet plug, a fibrin mesh (also called the clot) forms and entraps the plug. If the plug contains only platelets it is termed a white thrombus; if red blood cells are present it is called a red thrombus.

4. Finally, the clot must be dissolved in order for normal blood flow to resume followingtissue repair. The dissolution of the clot occurs through the action of plasmin.[(43)] Two pathways lead to the formation of a fibrin clot: the intrinsic and extrinsic pathway. Although they are initiated by distinct mechanisms, the two converge on a common pathway that leads to clot formation. The formation of a red thrombus or a clot in response to an abnormal vessel wall in the absence of tissue injury is the result of the intrinsic pathway. Fibrin clot formation in response to tissue injury is the result of

the extrinsic pathway. Both pathways are complex and involve numerous different proteins termed. [44]

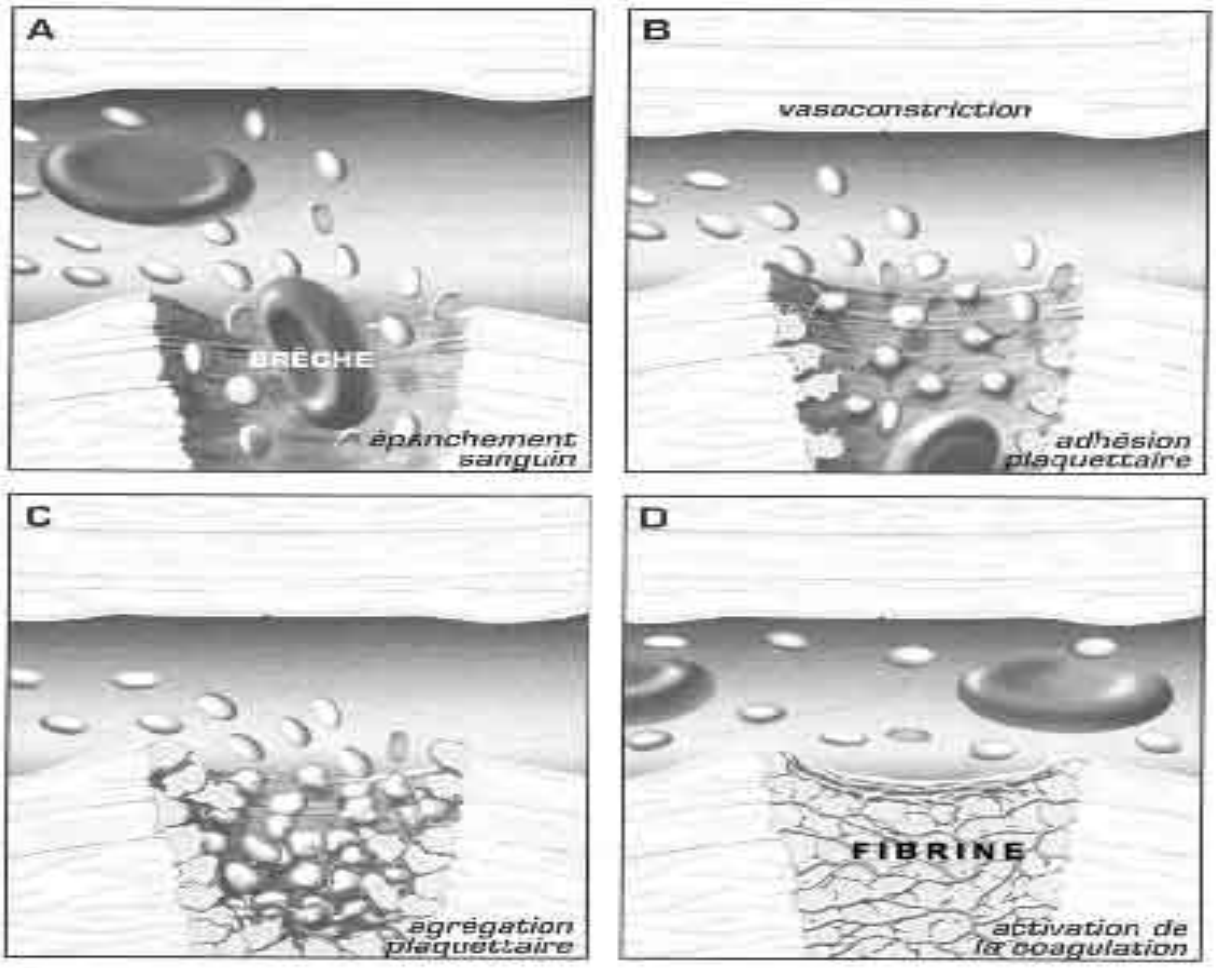


Fig (3-1)blood Hemostasis

Platelet Activation and von Willebrand Factor (vWF): [45]

In order for hemostasis to occur, platelets must adhere to exposed collagen, release the contents of their granules, and aggregate. The adhesion of platelets to the collagen exposed on endothelial cell surfaces is mediated by von Willebrand factor (vWF). Inherited deficiencies of vWF are the causes of von Willebrand disease (vWD), The function of vWF is to act as a bridge between a specific glycoprotein on the surface of platelets (GPIb/IX) and collagen fibrils. In addition to its role as a bridge between platelets and exposed collagen on endothelial surfaces, vWF binds to and stabilizes coagulation factor VIII. Binding of factor VIII by vWF is required for normal survival of factor VIII in the circulation.

von Willebrand factor is a complex multimeric glycoprotein that is produced by and stored in the α-granules of platelets. It is also synthesized by megakaryocytes and found associated with subendothelial connective tissue.

The initial activation of platelets is induced by thrombin binding to specific receptors on the surface of platelets, thereby initiating a signal transduction cascade. The thrombin receptor is coupled to a G-protein that, in turn, activates phospholipase C-γ (PLC-γ).

PLC- γ hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) leading to the formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces the release of intracellular Ca^{2+} stores, and DAG activates protein kinase C (PKC).

The collagen to which platelets adhere as well as the release of intracellular Ca^{2+} leads to the activation of phospholipase A_2 (PLA_2), which then hydrolyzes membrane phospholipids, leading to liberation of arachidonic acid. The arachidonic acid release leads to an increase in the production and subsequent release of thromboxane A_2 (TXA_2). This is another platelet activator that functions through the PLC- γ pathway. Another enzyme activated by the released intracellular Ca^{2+} stores is myosin light chain kinase (MLCK). Activated MLCK phosphorylates the light chain of myosin which then interacts with actin, resulting in altered platelet morphology and motility.

One of the many effects of PKC is the phosphorylation and activation of a specific 47,000-Dalton platelet protein. This activated protein induces the release of platelet granule contents; one of which is ADP.

ADP further stimulates platelets increasing the overall activation cascade; it also modifies the platelet membrane in such a way as to allow fibrinogen to adhere to two platelet surface glycoproteins, GPIIb and GPIIIa, resulting in fibrinogen-induced platelet aggregation. Activation of platelets is required for their consequent aggregation to a platelet plug. However, equally significant is the role of activated platelet surface phospholipids in the activation of the coagulation cascade. .

Primary Factors: [46]

Factor	Trivial Name(s)	Pathway	Characteristic
Prekallikrein	Fletcher factor	Intrinsic	
High molecular weight kininogen (HMWK)	contact activation cofactor; Fitzgerald, Flaujeac Williams factor	Intrinsic	
I	Fibrinogen	Both	-
II	Prothrombin	Both	Contains N-term. *gla* segment
III	Tissue Factor	Extrinsic	-
IV	Calcium	Both	-
V	Proaccelerin, labile factor, accelerator (Ac-) globulin	Both	Protein cofactor
VI (Va)	Accelerin	-	This is Va, redundant to Factor V
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin	Extrinsic	Endopeptidase with *gla* residues
VIII	Antihemophiliac factor A, antihemophilic globulin (AHG)	Intrinsic	Protein cofactor
IX	Christmas Factor, antihemophilic factor B,plasma thromboplastin component (PTC)	Intrinsic	Endopeptidase with *gla* residues
X	Stuart-Prower Factor	Both	Endopeptidase with *gla* residues
XI	Plasma thromboplastin antecedent (PTA)	Intrinsic	Endopeptidase
XII	Hageman Factor	Intrinsic	Endopeptidase
XIII	Protransglutaminase, fibrin stabilizing factor (FSF), fibrinoligase	Both	Transpeptidase

Table (3-1) primary clotting factors

The Clotting Cascades: [47]

The clotting cascades: The intrinsic cascade is initiated when contact is made between blood and exposed endothelial cell surfaces. The extrinsic pathway is initiated upon vascular injury which leads to exposure of tissue factor (TF) (also identified as factor III), a subendothelial cell-surface glycoprotein that binds phospholipid. The green dotted arrow represents a point of cross-over between the extrinsic and intrinsic pathways. The two pathways converge at the activation of factor X to Xa. Factor Xa has a role in the further activation of factor VII to VIIa as depicted by the green arrow. Active factor Xa hydrolyzes and activates prothrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Ultimately the role of thrombin is to convert fribrinogen to fibrin and to activate factor XIII to XIIIa. Factor XIIIa (also termed transglutaminase) cross-links fibrin polymers solidifying the clot. HK = high molecular weight kininogen.

PK = prekallikrein. PL = phospholipid.

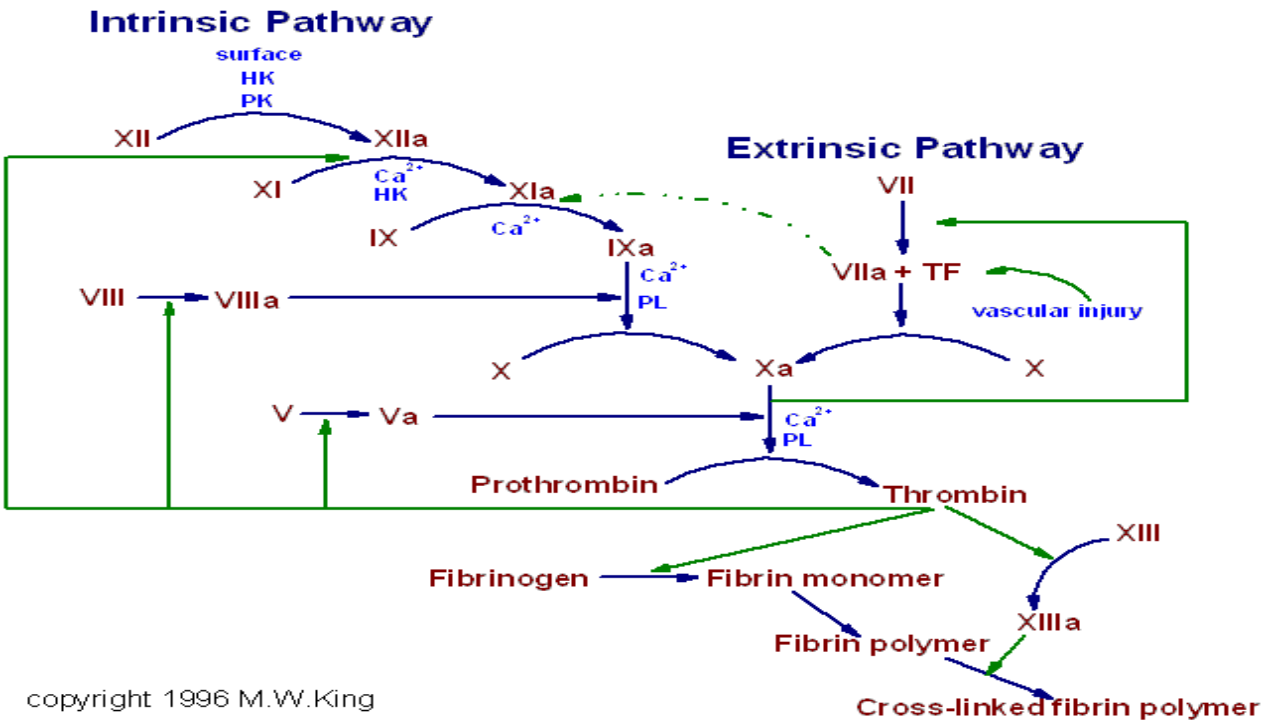


Fig (3-2) clotting cascade

The Intrinsic Clotting Cascade: [48]

The intrinsic pathway requires the clotting factors VIII, IX, X, XI, and XII. Also required are the proteins prekallikrein and high-molecular-weight kininogen, as well as calcium ions and phospholipids secreted from platelets. Each of these pathway constituents leads to the conversion of factor X (inactive) to factor Xa (a signifies active). Initiation of the intrinsic pathway occurs when prekallikrein, high-molecular-weight kininogen, factor XI and factor XII are exposed to a negatively charged surface. This is termed the contact phase. Exposure of collagen to a vessel surface is the primary stimulus for the contact phase. The assemblage of contact phase components results in conversion of prekallikrein to kallikrein, which in turn activates factor XII to factor XIIa. Factor XIIa can then hydrolyze more prekallikrein to kallikrein, establishing a reciprocal activation cascade. Factor XIIa also activates factor XI to factor XIa and leads to the release of bradykinin, a potent vasodilator, from high-molecular-weight kininogen.

In the presence of Ca^{2+}, factor XIa activates factor IX to factor IXa. Factor IX is a proenzyme that contains vitamin K-dependent g-carboxyglutamate (*gla*) residues, whose serine protease activity is activated following Ca^{2+} binding to these gla residues. Several of the serine proteases of the cascade (II, VII, IX, and X) are gla-containing proenzymes. Active factor IXa cleaves factor X at an internal arg-ile bond leading to its activation to factor Xa.

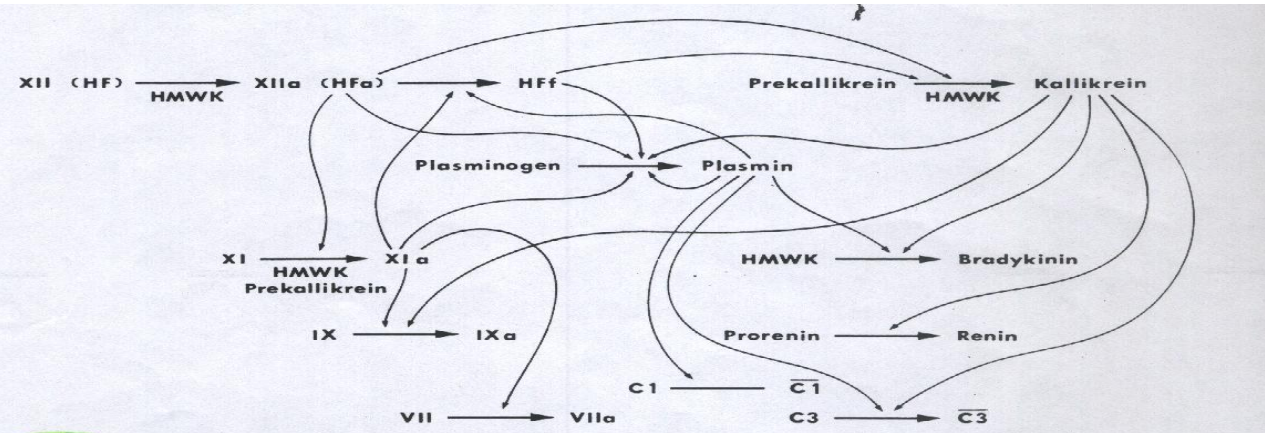


Fig (3-3) the interaction between the contact phase of the intrinsic pathway of blood coagulation and the kinin-generating

The activation of factor Xa requires assemblage of the tenase complex (Ca2+ and factors VIIIa, IXa and X) on the surface of activated platelets. One of the responses of platelets to activation is the presentation of phosphatidylserine and phosphatidylinositol on their surfaces. The exposure of these phospholipids allows the tenase complex to form. The role of factor VIII in this process is to act as a receptor, in the form of factor VIIIa, for factors IXa and X. Factor VIIIa is termed a cofactor in the clotting cascade. The activation of factor VIII to factor VIIIa (the actual receptor) occurs in the presence of minute quantities of thrombin. As the concentration of thrombin increases, factor VIIIa is ultimately cleaved by thrombin and inactivated. This dual action of thrombin, upon factor VIII, acts to limit the extent of tenase complex formation and thus the extent of the coagulation cascade.

Extrinsic Clotting Cascade:

Activated factor Xa is the site at which the intrinsic and extrinsic coagulation cascades converge. The extrinsic pathway is initiated at the site of injury in response to the release of tissue factor (factor III). Tissue factor is a cofactor in the factor VIIa-catalyzed activation of factor X. Factor VIIa, a *gla* residue containing serine protease, cleaves factor X to factor Xa in a manner identical to that of factor IXa of the intrinsic pathway. The activation of factor VII occurs through the action of thrombin or factor Xa. The ability of factor Xa to activate factor VII creates a link between the intrinsic and extrinsic pathways. An additional link between the two pathways exists through the ability of tissue factor and factor VIIa to activate factor IX. The formation of complex between factor VIIa and tissue factor is believed to be a principal step in the overall clotting cascade. Evidence for this stems from the fact that persons with hereditary deficiencies in the components of the contact phase of the intrinsic pathway do not exhibit clotting problems. A major mechanism for the inhibition of the extrinsic pathway occurs at the tissue factor--factor VIIa--Ca^{2+}--Xa complex. The protein, lipoprotein-associated coagulation inhibitor, LACI specifically binds to this complex. LACI is also referred to as extrinsic pathway inhibitor, EPI or tissue factor pathway inhibitor, TFPI

and was formerly named anticonvertin. LACI is composed of 3 tandem protease inhibitor domains. Domain 1 binds to factor Xa and domain 2 binds to factor VIIa only in the presence of factor Xa. .

Activation of Prothrombin to Thrombin: [49]

The common point in both pathways is the activation of factor X to factor Xa. Factor Xa activates prothrombin (factor II) to thrombin (factor IIa). Thrombin, in turn, converts fibrinogen to fibrin. The activation of thrombin occurs on the surface of activated platelets and requires formation of a prothrombinase complex. This complex is composed of the platelet phospholipids, phosphatidylinositol and phosphatidylserine, Ca^{2+}, factors Va and Xa, and prothrombin. Factor V is a cofactor in the formation of the prothrombinase complex, similar to the role of factor VIII in tenase complex formation. Like factor VIII activation, factor V is activated to factor Va by means of minute amounts and is inactivated by increased levels of thrombin. Factor Va binds to specific receptors on the surfaces of activated platelets and forms a complex with prothrombin and factor Xa.

Prothrombin is a 72,000-Dalton, single-chain protein containing ten gla residues in its N-terminal region. Within the prothrombinase complex, prothrombin is cleaved at 2 sites by factor Xa. This cleavage generates a 2-chain active thrombin molecule containing an A and a B chain which are held together by a single disulfide bond.

In addition to its role in activation of fibrin clot formation, thrombin plays an important regulatory role in coagulation. Thrombin combines with thrombomodulin present on endothelial cell surfaces forming a complex that converts protein C to protein Ca. The cofactor protein S and protein Ca degrade factors Va and VIIIa, thereby limiting the activity of these 2 factors in the coagulation cascade.

Thrombin also binds to and leads to the release of G-protein-coupled protease activated receptors (PARs), specifically PAR-1, -3 and -4. The release of these proteins leads to the activation of numerous signaling cascades that in turn increase release of the interleukins, ILs, IL-1 and IL-6, increases secretion of intercellular adhesion molecule-1

(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The thrombin-induced signaling also leads to increased platelet activation and leukocyte adhesion. Thrombin also activates thrombin-activatable fibrinolysis inhibitor (TAFI) thus modulating fibrinolysis (degradation of fibrin clots). TAFI is also known as carboxypeptidase U (CPU) whose activity leads to removal of C-terminal lysines from partially degraded fibrin. This leads to an impairment of plasminogen activation, thereby reducing the rate of fibrin clot dissolution (i.e. fibrinolysis).

Control of Thrombin Levels: [50]

The inability of the body to control the circulating level of active thrombin would lead to dire consequences. There are 2 principal mechanisms by which thrombin activity is regulated. The predominant form of thrombin in the circulation is the inactive prothrombin, whose activation requires the pathways of proenzyme activation described above for the coagulation cascade. At each step in the cascade, feedback mechanisms regulate the balance between active and inactive enzymes.

The activation of thrombin is also regulated by 4 specific thrombin inhibitors. Antithrombin III is the most important since it can also inhibit the activities of factors IXa, Xa, XIa and XIIa. The activity of antithrombin III is potentiated in the presence of heparin by the following means: heparin binds to a specific site on antithrombin III, producing an altered conformation of the protein, and the new conformation has a higher affinity for thrombin as well as its other substrates. This effect of heparin is the basis for its clinical use as an anticoagulant. The naturally occurring heparin activator of antithrombin III is present as heparan and heparan sulfate on the surface of vessel endothelial cells. It is this feature that controls the activation of the intrinsic coagulation cascade. However, thrombin activity is also inhibited by α_2-macroglobulin, heparin cofactor II and α_1-antitrypsin. Although a minor player in thrombin regulation α_1-antitrypsin is the primary serine protease inhibitor of human plasma. Its physiological significance is demonstrated by the fact that lack of this protein plays a causative role in the development of emphysema.

Activation of Fibrinogen to Fibrin: (51)

Fibrinogen (factor I) consists of 3 pairs of polypeptides ([A- α][B-β][γ])$_2$. The 6 chains are covalently linked near their N-terminals through disulfide bonds. The A and B portions of the A- α and B- β chains comprise the **fibrinopeptides**, A and B, respectively. The fibrinopeptide regions of fibrinogen contain several glutamate and aspartate residues imparting a high negative charge to this region and aid in the solubility of fibrinogen in plasma. Active thrombin is a serine protease that hydrolyses fibrinogen at four arg-gly bonds between the fibrinopeptide and the a and b portions of the protein.

Thrombin-mediated release of the fibrinopeptides generates fibrin monomers with a subunit structure (α - β - γ)$_2$. These monomers spontaneously aggregate in a regular array, forming a somewhat weak fibrin clot. In addition to fibrin activation, thrombin converts factor XIII to **factor XIIIa**, a highly specific transglutaminase that introduces cross-links composed of covalent bonds between the amide nitrogen of glutamines and ε-amino group of lysines in the fibrin monomers.

Dissolution of Fibrin Clots (fibrinolysis): (52)

Degradation of fibrin clots is the function of plasmin, a serine protease that circulates as the inactive proenzyme, plasminogen. Any free circulating plasmin is rapidly inhibited by α_2-antiplasmin. Plasminogen binds to both fibrinogen and fibrin, thereby being incorporated into a clot as it is formed. Tissue plasminogen activator (TPA) and, to a lesser degree, urokinase are serine proteases which convert plasminogen to plasmin. Inactive TPA is released from vascular endothelial cells following injury; it binds to fibrin and is consequently activated. Urokinase is produced as the precursor, prourokinase by epithelial cells lining excretory ducts. The role of urokinase is to activate the dissolution of fibrin clots that may be deposited in these ducts. Active TPA cleaves plasminogen to plasmin which then digests the fibrin; the result is soluble degradation product to which neither plasmin nor plasminogen can bind. Following the

release of plasminogen and plasmin they are rapidly inactivated by their respective inhibitors. The inhibition of TPA activity results from binding to specific inhibitory proteins. At least 4 distinct inhibitors have been identified, of which 2:plasminogen activator-inhibitors type 1 (PAI-1) and type 2 (PAI-2) are of greatest physiological significance.

Chapter 4

Blood as a tool in different diseases diagnosis

INTRODUCTION

There are many factors that influence the chemical composition of blood and other body fluids in disease. One general factor includes those instances of increases in blood constituents due to alteration of preamble membranes in the excretory organs such as the lungs, kidneys and liver.(e.g. the accumulation of nitrogenous waste products in certain forms of nephritis and the hyperbilirubinemia)

In other instances , alteration in blood constituents may be due to changes in the rate of formation or in the use of these constituents (e.g. the accumulation of glucose in the blood of diabetic individuals due to a metabolic derangement in the use of this substance). Furthermore , the administration of drugs may alter the concentration of some blood constituents. It is evident that knowledge of the concentration of the various blood constituents is a very important aid to the physician in diagnosis but also in evaluating the results of therapy. Since the success or failure of the physicians efforts and even the life of the patients may depend on accurate determination of the blood levels of the important constituents.

USE OF BIOCHEMICAL TESTS: [53]

Biochemical tests are used extensively in medicine, both in relation to discases that have an obvious maetabolic basis (e.g., diabetes mellitus, hypothyroidism) and those in which biobemical changes are 3 consequence of the disease (e.g., renal failutre, malabsorption). Biochemical tests are used in diagnosis, prognosis, monitoring and screening (F.g.4.1).

Screening	Diagnosis
Detection of Subclinical disease	Confirmation or Rejection of Clinical diagnosis
Monitoring	Prognosis
Natural history of Response to treatment	Information regarding the Likely outcome of disease

1. Diagnosis

Medical diagnosis is based on the partent's history, if availiable, the clinical signs found on examination, the results of investigations and, sometimes, retrospectively on the response to treatment. Frequently, a confident diagnosis can be made on the basis of the history combined with the findings on examination Failing this, it is usually possible to formulate a differential diagnosis, in effect a short-list of possible diagnoses, biochemical and other investigations may then be used to distinguish between them. Investigations may be selected to help either confirm or refute a diagnosis and it is important that the clinician appreciates how well an investigation performs in this context. Making a diagnosis, even if incomplete, such as a diagnosis of hypoglycaemia without knowing its cause, allows treatment to be initiated.

2. Prognosis

Tests used primarily for diagnosis may also provide prognostic information and some are used specifically for this purpose, for example, serial measurements of serum creatinine concentration in progressive renal disease are used to indicate when dialysis may be required. Tests can also predict the risk of developing a particular condition, the serum cholesterol concentration can, for example, predict the risk of coronary artery disease. However, such risks are calculated from statistical data and cannot give a precise prediction for a particular individual.

3. Monitoring

A major use of biochemical tests is to follow the course of an illness and to monitor the effects of treatment. To do this there must be a suitable analyte, for instance, glucose in partients with diabetes mellitus. Biochemical tests may also be used to detect complications of treatment, such as hypokalaemia during treatment with diuretics.

4. Screening

Biochemical tests are widely used to determine whether a condition is present subclinically.

Sampling: [54]

In the analysis of blood constituents the first consideration is collection of the blood specimen. Even if this is not done by the laboratory technician he should be familiar with the technique and principles involved.

Usually venous blood is used . some times small amounts of capillary blood from the fingertip or earlobe will be satisfactory and very rarely arterial blood may be required which taken from the radial brachial or femoral arteries, almost taken for blood gasses determination and when studying the arterial venous difference of the blood glucose.

The specimen can usually be collected into either a glass or plastic container but, in some circumstances, one of these may be preferred or even essential.

The different chemical tests will require different types of samples. The sample provided must be appropriate for the test requested. Many biochemical analyses can be made on either plasma or serum. When The collected blood allowed to stand at room temperature or in water bath at 37°c till clot formation or coagulates in few minutes, when this tube is centrifuged a clear straw colored fluid is gradually squeezed out called serum and the formed elements retracted into a semisolid gelatinous mass. But when a special chemical compounds called anticoagulant was added to the collected blood , the blood will stop clotting and when this tube is centrifuged the clear strew colored fluid obtained is called plasma.

Haemolysis must be avoided when blood is drawn and haemolyzed serum or plasma is unsuitable for analysis. For some tests a small amount of Hemolysis may not be of great importance. Whereas in other tests it must be scrupulously avoided.

Analysis:[55]

For the determination of many blood constituents it is necessary to remove the plasma or serum protiens. A number of methods for preparing protien free filtrates, some methods are for specific determination whereas other are more widly applicable in these methods some substances is added which combines with and precipitates the protien,leaving the desired constituents in solution. The commonly used precipitatants are tungestic acid,

zinc hydroxide and trichloroacetic acid (TCA). The solution is then filtrated or centrifuged to separate the precipitate containing the protein.

Quantaitive analysis of many substances is based on the production of a colored solution by a chemical reaction in such away that the intensity or depth of the color may be used as a measure of the concentration of the substance beimg determined.

In practically all colorimeteric procedures the substances being determined (e.g. glucose, uric acid, cholesterol) are not them selves colored or are only slightly but they form a colored compound through a serious of often complex chemical reactions.

The color formed is then dependant upon the concentration of the eagent as well as other conditions such as the time and temprature of heating or incubation. Hence in comparing the concentration of the sample (unknown) with that of a standard of known concentration.the sample and standard should preerably be run at the same conditions to ensure that the color development and measuring are the same for both. Often the reagents them selves will a certain amount of color to the final solution, even none of the substance being determined is present. This is the reagent blank. In such circumstances the instrument may be set to 100% transmittance with the blank solution containing all of the reagent but none of the substance being determined. This is treated under the same conditions as the sample and standards. Preferably the conditions should be adjusted so that the color due to the blank is minimal.

If readings are made in terms of optical denisty both samples and blank can be read against water and the reading of the blank subtracted from that of the sample before calculation.

BIOCHEMICAL TESTS OF RENAL FUNCTION: [56]

Formal tests of tubular function are used less frequently than those of glomerular function. In glomerular disease, there can either be a change in GFR (Usually a decrease) or glomerular permeability (usually an increase), or both. Tests of glomerular function are, therefore, used either to measure the rate of filtration or to assess permeability. The GFR declines with age, to a greater extent in males than females, and this must be taken into account when interpreting results.

Measurement of glomerular filtration rate (GFR)

Clarance

Because urine is derived from the glomerular filtrate, knowledge of the GFR is extremely important in the assessment of renal function. An estimate of the GFR can be made by measuring the urinary excretion of a substance which is completely filtered from the blood by the glomeruli and which is not secreted, reabsorbed or metabolized by the renal tubules. Experimentally, inulin has been found to meet these requirements. The volume of blood from which inulin is cleared or completely removed in one minute is known as the inulin clearance and is equal to the GFR.

Measurement of inulin clearance requires the infusion of inulin into the blood and is therefore not suitable for routine clinical use. The clearance of creatinine, an endogenous substance normally present in the blood and excreted after glomerular filtration, may be measured instead (Equation 4.1).

$$\text{Clearance} = \frac{UxV}{P} \text{ ml/min.}$$

U = urinary creatinine concentration (µmol/1)

V = urine flow rate (ml/min or (1/2/4h)/1./4/4)

P = plasma creatinine coricentration (µmol/1)

Creatinine is derived largely from the turnover of creatine phosphate in muscle and the daily production is relatively constant, being a function of total muscle mass. A small amount is derived from meat in the diet. Creatinine clearance in adults is normally of the order of 120ml/min, corrected to a standard body surface area of 1.73m2 .

The accurate measurement of creatinine clearance is difficult, especially in outpatients, since it is necessary to obtain a complete and accurately timed sample of urine. The usual collection time is twenty-four hours, but partients may forget the time or may forget to include some urine in the sample. Incontinent patients may find it impossible to make a urine collection. Partients have been known to add water or some other person’s urine to their own collection, hoping to gain the doctor’s approval for having been so prolific.

It may be more convenient and reliable to base the collection period on a patient's normal habits. Thus the time at which the bladder is emptied before returing to bed is noted, any urine passed during the night is collected as is the urine voided when the partient rises. This time is noted and blood sample is taken that morning when the urine is taken to the laboratory for the measurement of plasma creatinine. As long as the time over which the urine collection is made is known, and the collection is complete, any suitable time period can be used Creatinine is actively secreted by the renal tubules and as a result, the creatinine clearance is higher than the true GFR. The difference is of little significance when the GFR is normal but when the GFR is low (< 10ml/min), tubular secretion makes a major contribution to creatinine excretion and the creatinine clearance significantly overestimates the GFR. Creatinine breakdown in the gut also becomes significant when the FGR is very low. Lastly, in the calculation of creatinine clearance, two measurements of creatinine concentration and one of urine volume are required. Each of these has an inherent imprecision which can affect the accuracy of the overall result.

Although measurements of creatinine clearance are made frequently in clinical chemistry laboratories, they are potentially unreliable and should not be carried out unless there is a definite indication. In fact, accurate measurement of the GFR is required infrequently. Indications for its measurement include assessment of potential kidney donors, investigation of patients with minor abnormalities of renal function, and for calculation of the initial dose of a potentially toxic drug that is eliminated from the body by renal excretion. The majority of patients with estabished renal disease do not require repeated measurements of creatinine clearance. In most cases, their renal function can be more reliably monitored by serial.

Plasma creatinine: [57]

The plasma creatinine concentration is the most reliable, simple biochemical test of glomerular function. Ingestion of meat can increase the plasma creatinine concntration by as much as thirty percent seven hours after a normal meal and ideally blood samples should be collected after an overnight fast. Strenuous exercise also causes a transient

slight increase in plasma creatinine concentration. Plasma creatinine concentration is related to muscle bulk and therefore a concentration of 120μmol/1 could be normal for an athletic young man but would suggest renal functional impairment, though not necessarily of clinical significance, in a thin, weventy-year-old woman. Although muscle bulk tends to decline with age, so too does the FGR and hence plasma creatinine concentrations remain fairly constant.

The reference range for plasma creatinine in the adult population is 60-120μmol/1, but the day-to-day variation in an individual is much less than this range. In the clearance formula (see Equation 4.1), the GFR is inversely related to the plasma creatinine concentration. Consequently, a normal plasma creatinine does not necessarily imply normal renal function, although a raised creatinine usually does indicate impaired renal function. Further, a change in plasma creatinine concentration, provided it is outside the limits of analytical error, usually indicates a change in GFR.

Changes in plasma creatinine concentration can occur, independently of renal function, due to changes in muscle mass. Thus a decrease can occur as a result of starvation and in wasting diseases, immediately after surgery and in patients treated with corticosteroids, an increase can occur during refeeding. However, changes in creatinine concentration for these reasons rarely lead to diagnostic confusion.

In pregnancy the GFR increases. This usually more than balances the effect of increased creatinine synthesis during pregnancy and results in a decrease in plasma creatinine concentration.

Plasma urea : [58]

Urea is synthesized in the liver, primarily as a by-product of the deamination of amino acids. Its elimination in the urine represents the major route for nitrogen excretion. It is filtered from the blood at the glomerulus but passive tubular reabsorption occurs to significant extent, especially at low rates of urine flow. Although plasma urea concentration is often used as an index of renal glomerular function, measurement of plasma creatinine provides a more accurate assessment. Urea production is increased by

a high protein intake, in catabolic states, and by the absorption of amino acids and peptides after gastrointestinal haemorrhage. Conversely, production is decreased in partients with a low protein intake and sometimes in partients with liver disease. Plasma urea concentration rises during dehydration as a consequence of tubular reabsorption even when renal function is normal.

Changes in plasma urea are a feature of renal impairment but it is important to consider possible extrarenal influences on urea concentrations before ascribing any changes to an alteration in renal function.

Urea diffuses readily across dialysis memberanes and during renal dialysis a fall in plasma urea concentration is a poor guide to the efficacy of the process in removing other toxic substances from the blood.

BIOCHEMICAL ASSESSMENT OF LIVER FUNCTION: [59]

Bilirubin

Hyperbilirubinaemia is not always present in patients with liver disease nor is it exclusively associated with liver disease. For example, it is not usually present in patients with well compensated cirrhosis but it is a common feature of advanced pancreatic carcinoma.

Unconjugated byperbilirubinaemia:

When an excess of bilirubin is unconjugated the concentration in adults rerely exceeds 100 μmol/1. In the absence of liver disease, unconjugated hyperbilirubinaemia is most often due either to haemolysis or to Gilbert's syndrome, an inherited abnormality of bilirubin metabolism. In haemolysis, hyperbilirubinaemia is due to an increased production of bilirubin which exceeds the capacity of the liver to remove and conjugate the pigment. Nevertheless, more bulirubin is excreted in the bile, the amount of urobilinogen entering the enterohepatic circulation is increased and urinary urobilinogen is increased.

Activity of the hepatic conjugating enzymes is usually low at birth but increases rapidly thereafter, the transient "physiological" jaundice of the newborn reflects this. With excessive haemolysis, as in Rhesus incompatibility, or a lack of enzyme activty. There may be a massive rise in the plasma concentration of unconjugated bilirubin. If bilirubin levels exceed aproximately 340µmol /l, its uptake into the brain may cause sever brain damage (kernicterus).

conjugated byperbilirubinaemia:

This condition is due to leakage of bilirubin from either hepatocytes or the biliary system into the blood stream when its normal route of excretion is blocked. The water-soluble conjugated bilirubin entering the systemic circulation is excreted in the urine, giving it a deep orange brown colour. In complete biliary obstruction, no bilirubin reaches the gut, no bilirubin is formed and the stools are pale in colour.

Hyperbilirubinaemia can be due to an excess of both conjugated and unconjugated bilirubin. It is classified as conjugated if less than 20% is in the unconjugated form. The separate measurement of conjugated and unconjugated bilirubin concentrations is useful in the diagnosis of neonatal jaundice where may be some doubt as to the relative contribution of defective conjugation and other causes, it is less often required in adults.

If the plasma bilirubin concentration is less than 100µmole /l and other tests of liver function are normal, it can be inferred that the raised levels are due to the unconjugated form of the pigment.

A third fraction of bilirubin, consisting of conjugated bilirubin irreversibly bound to albumin, is found in the plasma of patients with long-standing conjugated hyperbilirubinaemia. Its persistence in the plasma during the resolution of liver disease or after the relief of obstruction explains the persistence of jaundice in the absence of bilirubinuria that can occur in these circumstances.

Plasma enzymes:

Enzymes used in the assessment of hepatic function include aspartate and alanine transaminases, alkaline phosphatase and y-glutamyl transpectidase. In general, these enzymes are not specific indicators of liver dysfunction. The hepatic isoenzyme of

alkaline phosphatase is an exception and alanine transaminase is more specific to the liver than aspartate transaminase.

Increasedd transaminase activities reflect cell damage, plasma levels may be twenty times the upper limit of normal (ULN) in partients with hepatitis. In cholestasis, lplasma alkaline phosphatase activity is increased. This is a result of enzyme induction, itself a consequence of cholestasis although the mechanism involved is unknown. In severe obstructive jaundice, the plasma alkaline phosphatase activity may be up to ten times the ULN.

In practice, however, increases in the plasma activities of both enzymes are often present in patients with liver disease, although one may predominate. Thus, in primarily cholestatic disease there may be secondary hepatocellular damage and increased plasma transaminase-activities, while cholestasis frequently occurs in hepatocellular disease.

Increased γ-glutamyl transpeptidase activity is found in both cholestasis and hepatocellular damage, this enzyme is very sensitive but non specific. Thus, although certain patterns of plasma enzyme activities are frequently observed in various types of liver disease, they are not reliably diagnostic.

Plasma enzyme activities are very useful in following the progress of liver disease once the diagnosis has been maded. Falling transaminase activity suggests a decrease in hepatocellular damage and falling alkaline phosphatase activity suggests a resolution of cholestasis. However, in fulminant hepatic failure, a decrease in transaminase activity may be a poor prognostic sign, reflecting almost complete destruction of parenchymal cells.

Plasma proteins:

Albumin is synthesized in the liver and its concentration in the plasma is in part a reflection of the functional capacity of the organ, Plasma albumin concentration is decreased in chronic liver disease, but tends to be normal in the early stages of acute hepatitis due to its long half-life (approximately twenty days). There are many other causes of hypoalbuminaemia, as discussed on pages 211-214 but a normal plasma

albumin concentration in a patient with chronic liver disease does imply adequate synthetic function.

The prothrombin time is a test of plasma clotting activity and reflects the activity of vitamin K-dependent clotting factors synthesized by the liver, of which factor VII has the shortest half0life (four to six hours). An increase in the prothrombin time is often an early feature of acute liver disease, but a prolonged prothrombin time may also reflect vitamin K deficiency. The cause can be determined by administering the vitamin parenterally, in the absence of liver disease, the prothrombin time should return to normal within eighteen hours.

A polyclonal increase in immunoglobulins is frequently associated with cirrhosis, particularly when the disease is autoimmune in origin, and may cause the total plasma protein to be normal, or even increased, in spite of a low albumin concentration.

Serum protein electrophoresis is of little value in the diagnosis of liver disease, typical patterns may be seen in

Serum enzymes in disease:

Liver disease: [60]

In a patient with jaundice, an increase in aspartate or alanine transaminase levels, often to 10×ULN or more, suggests that the jaundice is due to hepatocellular damage. An increase in alkaline phosphatase of 2-3×ULN or more is characteristic of cholestasis. A high transaminase activity generally precedes any other biochemical change in early hepatitis, including the increase in plasma bilirubin. An increase in liver specific alkaline phosphatase is often the only biochemical abnormality in patients with compensated cirrhosis.

Plasma creatine kinase activity may be very high in children with muscular dystrophy even before the onset of the symptoms through levels tend to fall in the later stages when most of the muscle has been destroyed. There are several forms of muscular dystrophy and an increase of plasma cratine kinase is not always present.

Heart disease: [61]

Plasma enzyme measurements are often requested routinely in patients with suspected myocardial infarction, although it has been estimated that biochemical confirmation of the diagnosis is required in only 15-30% of patients. Many patients with myocardial infarction will have a classical history of crushing central chest pain, perhaps radiating to the arm or jaw, and typical electrocardiographic (ECG) changes. Myocardial infarction can present atypically, however, or may even be clinically silent, particularly in the elderly . The ECG changes may not always be typical particularly with partial thickness infarcts, when there has been a previous infarction and in left bundle branch block.

Significant changes in the plasma activities of creatine kinase, aspartate transaminase and α-hydroxybutyrate dehydrogenase occur following myocardial infarction. Also shown is the time course of the change in the MB isoenzyme of creatinine kinase, this enzyme appears first and is rapidly cleared from the plasma after myocardial infarction. It is obviously vital when interpreting plasma enzyme changes that the time of the samples relative to the time of the suspected infarct is known and is appropriate. Thus the time-window for creating kinase is between twelve and thirty-six hours but little change in a-hydroxybutyrate dehydrogenase would be expected during this time.

Given the lack of tissue specificity of aspartate transaminase, the most useful enzymes for diagnostic purposes in a patient with suspected myocardial infarction are creatine kinase, for early confirmation of the diagnosis, and a-hydroxybutyrate dehydrogenase, for those partients (often with atypical clinical features) who may present several days after the supposed infarct. If a patient has a typical history and fulfills ECG criteria for myocardial infarction, enzyme measurements do not contribute to diagnosis or immediate management although if no increase in creatine kinase has occurred twenty-four hours after the onset of pain, the diagnosis should be reviewed serial measurements can be of value, a persistent increase in creatine kinase, or a further increase, suggests extension of the infarct, which may not be obvious clinically or on the ECG.

The increasing use of thrombolytic therapy early (within twenty-four hours) in myocardial infarction has stimulated efforts to develop a test to diagnose the condition when this is not possible on the basis of the ECG. Sequential measurements of plasma creatine kinase may demonstrate a rise in activity before this exceeds the upper limit of normal but to do this requires the provision of a rapid, twenty-four-hour service for the measurement, which may not be practicable.

Attempts have been made to correlate serial plasma creatine kinase levels with infarct size, but this requires frequent measurements and is of little use in management. The measurement of myoglobin (which diffuses rapidly out of injured cardiac muscle cells) in plasma has been promulgated as an alternative to creating kinase, but has not been shown to have a definite advantage.

Bone disease: [62]

Alkaline phosphatase is secreted by osteoblasts and an increased serum activity is seen in many diseases of bone , osteoporosis is an important exception to this Unless there is coexisting osteomalacia or a fracture occurs, the alkaline phosphatase is normal. In multiple myeloma, despite extensive tumour deposition in bone, the alkaline phosphatase is not raised since there is no concomitant increase in osteoblastic activity.

Serial measurements of plasma alkaline phosphatase are of particular value in the assessment of healing in osteomalacia, rickets, renal osteobdytrophy and in Paget's disease.

Muscle disease : [63]

Enzymes present within striated muscle cells appear in the plasma in certain muscle diseases, including muscular dystrophies (particularly Duchenne type), polymyositis, toxic and other myopathies, and also after trauma and in ischaemia. Enzyme levels tend to be normal in neurogenic muscle disease, that is, syndromes of denervation such as motor neuron disease and lower motor neuron lesions.

The measurement of plasma creatine kinase activity provides the most reliable evidence of muscle disease being more sensitive than, for example, aspartate transaminase of aldolase. However, enzyme measurements do not indicate either the cause or nature of

the diorder. Serial measurements of plasma creatine kinase are valuable in assessing the response to treatment (usually with corticosteroids) of patients with polymyositis.

Malignant disease

Changes in plasma enzyme activities are common in patients with malignant disease. They may be tumour-derived, that is, due to the release of normal or variant enzymes from the tumour itself (for example, acid phosphatase in carcinoma of the prostate, Regan isoenzyme in carcinoma of lung) or tumour-related, that is due to the response of sarrounding tissues to the presence of the tumour (for example, alkaline phosphatase in hepatic and osseous tumours). A raised plasma alkaline phosphatase may be caused by metastases in a patient known to have carcinoma, but the possiblity of metastases from a clinically silent tumour should be considered when a raised alkaline phosphatase is discovered incidentally.

Blood glucose: [64]

The concentration of glucose in the blood is normally subject to rigorous control in healthy subjects whether fasting or in post-prandial state. This control is achieved through the concerted action of various hormones of which the most important are insulin and glucagons.

Insulin is a polypeptide, secreted by the β-cell of the pancreatic islets of langerhans in response to a rise in blood glucose concentration. It promotes the removal of glucose from the blood and its storage in the form of glycogen. If the blood glucose level falls, insulin secretion is inhibited and stored glucose mobilized.

Glucagon is a polypeptide secreted by the α- cell of the pancreatic islets; its secretion is decreased by a rise in the blood glucose concentration. in general its action is oppose those of insulin: it stimulates glycogenolysis and gluconeogenesis and promotes lipolysis and ketogenesis.

In normal adults The fasting blood glucose is usually (70-110) mg/dl, postprandial blood glucose is (110- 140) mg/dl and random blood glucose is up to 180 mg / dl. Distribution of glucose homoeostasis may result in hypoglycaemia or hyperglycaemia.

Hypoglycemia: (lowered blood glucose)

Occurs with fasting blood glucose 40 mg/dl or lower as a result of overdosage of insulin in treatment of diabetes, and insulin secreting tumour of pancreas which may lower glucose level in blood to 0 mg /dl.

Starvation tends to lower the blood glucose but usually the fall, which is most marked during the first day or two, is not great. Starving persons do not die from hypoglycemia. While mild exercise can raise the blood glucose a little, sever exercise may produce a hypoglycemia due to the liver being depleted of its glycogen, from which the blood glucose is maintained. For the same reason, sever liver disease has a similar effect.

Hyperglycaemia :

The highest values for fasting blood glucose are obtained in diabetes, in which it may vary from ≈ 150 – 500 mg /dl and over, according to the severity of the condition. Except in diabetes the fasting blood glucose rarely exceeds 200 mg/dl. Small increase may be found in hyperactivity of the thyroid, pituitary, and adrenal glands. In pancreatitis and pancreatic carcinoma there may be some increase in blood glucose , but it does not exceed 150 mg /dl except in advanced cases. Moderate increases of the blood glucose may be found in infectious diseases and in some intracranial diseases such as meningitis, encephalitis, tumors and hemorrhage. Anesthesia will also cause an increase in the blood glucose depending on the duration and degree, a considerable rise may occur, sometimes exceeding 200 mg/dl due to acidosis, which is produced due to increase glucose formation from glycogen in the liver.

Chapter 5

Blood Disorders

1-The Bleeding Disorders: [65]

Defects in the process of hemostasis, leading to bleeding disorders, have been identified at the level of the proteins of the clotting cascades, platelet activation and function, contact activation and antithrombin function.

Hemophilia A: [66]

Hemophilia A is classic hemophilia (a disease referring to the inability to clot blood). It is an X-linked disorder resulting from a deficiency in factor VIII, a key component of the coagulation cascade. There are severe, moderate and mild forms of hemophilia A that reflect the level of active factor VIII in the plasma. Hemophilia A arises from a variety of mutations. Some 150 different point mutations have been characterized in the factor VIII gene in hemophilia A. Inheritence of the disorder occurs with a frequency of 1:5,000 to 1:10,000 males in all populations. Factor VIII is a cofactor in the activation of factor X to factor Xa in a reaction catalyzed by factor IXa. Activation of factor VIII occurs via proteolytic cleavage by thrombin and factor Xa. Inactivation of factor VIIIa occurs by limited proteolysis by factor Xa or activated protein C. Individuals with deficiencies in factor VIII suffer joint and muscle hemorrhage, easy bruising and prolonged bleeding from wounds. Treatment of hemophilia A is accomplished by infusion of factor VIII concentrates prepared from either human plasma or by recombinant DNA technology.

Hemophilia B:

Hemophilia B results from deficiencies in factor IX. The prevalence of hemophilia B is approximately one-tenth that of hemophilia A. All patients with hemophilia B have prolonged coagulation time and decreased factor IX clotting activity. Like hemophilia A, there are severe, moderate and mild forms of hemophilia B and reflect the factor IX activity in plasma.

At least 300 unique factor IX mutations have been identified, 85% are point mutations, 3% are short nucleotide deletions or insertions and 12% are gross gene alterations.

Disorders of Fibrinogen and Factor XIII: [67]

Several cardiovascular risk factors are associated with abnormalities in fibrinogen. As a result of the acute-phase response or through other poorly understood mechanisms, elevated plasma fibrinogen levels have been observed in patients with coronary artery disease, diabetes, hypertension, peripheral artery disease, hyperlipoproteinemia and hypertriglyceridemia. In addition, pregnancy, menopause, hypercholesterolemia, use of oral contraceptives and smoking lead to increased plasma fibrinogen levels Although rare, there are inherited disorders in fibrinogen. These disorders include afibrinogenemia (a complete lack of fibrinogen), hypofibrinogenemia (reduced levels of fibrinogen) and dysfibrinogenemia (presence of dysfunctional fibrinogen). Afibrinogenemia is characterized by neonatal umbilical cord hemorrhage, ecchymoses, mucosal hemorrhage, internal hemorrhage, and recurrent abortion. The disorder is inherited in an autosomal recessive manner. Hypofibrinogenemia is characterized by fibrinogen levels below 100mg/dL (normal is 250-350mg/dL) and can be either aquired or inherited. Symptoms of hypofibrinogememia are similar to, but less severe than, afibrinogenemia. Dysfibrinogenemias are extremely heterogeneous affecting any of the functional properties of fibrinogen. Clinical consequences of dysfibrinogenemias include hemorrhage, spontaneous abortion and thromboembolism.

Factor XIII is the proenzyme form of plasma transglutaminase and is activated by thrombin in the presence of calcium ions. Active factor XIII catalyzes the cross-linking of fibrin monomers. Factor XIII is a tetramer of two two different peptides, a and b (forming a2b2). Hereditary deficiencies (autosomal recessive) occur resulting in the absence of either subunit. Clinical manifestation of factor XIII deficiency is delayed bleeding although primary hemostasis is normal. Deficiency leads to neonatal umbilical cord bleeding, intracranial hemorrhage and soft tissue hematomas.

Factor XI and Contact Activation

When blood makes contact with negatively charged surfaces it triggers a series of interactions that involve factor XI, prekallikrein and high molecular weight kininogen leading to blood coagulation. This process is referred to as contact activation. Deficiency in factor XI confers an injury-related bleeding tendency. This deficiency was identified in 1953 and originally termed hemophilia C. Factor XI deficiency is very common in Ashkenazic Jews and is inherited as an autosomal disorder with either homozygosity or compound heterozygosity. Three independent point mutations in factor XI have been identified.

Antithrombin Deficiency

Antithrombin functions to inhibit several activated coagulation factors including thrombin, factor IXa and factor Xa, by forming a stable complex with the various factors.. Heparin and heparin sulfates increase the activity of antithrombin at least 1000 fold. Deficiency in antithrombin is seen in approximately 2% of patients with venous thromboembolic disease. Inheritence occurs as an autosomal dominant trait. The prevalence of symptomatic antithrombin deficiency ranges from 1 per 2000 to 1 per 5000 in the general population. Deficiencies results from mutations that affect synthesis or stability of antithrombin or from mutations that affect the protease and/or heparin binding sites of antithrombin. Clinical manifestations of antithrombin deficiency include deep vein thrombosis and pulmonary embolism. Arterial thrombosis is rare in antithrombin deficiency. Thrombosis may occur spontaneously or in association with surgery, trauma or pregnancy. Treatment of acute episodes of thrombosis is by infusion of heparin (for 5-7 days) followed by oral anticoagulant therapy.

Von Willebrand Disease: [68]

von Willebrand disease (vWD) is due to inherited deficiency in von Willebrand factor (vWF). vWD is the most common inherited bleeding disorder of humans. Using sensitive laboratory testing, abnormalities in vWF can be detected in approximately 8000 people per million. Clinically significant vWD occurs in approximately 125 people per million. This is a frequency at least twice that of hemophilia A.

Deficiency of vWF results in defective platelet adhesion and causes a secondary deficiency in factor VIII. The result is that vWF deficiency can cause bleeding that appears similar to that caused by platelet dysfunction or hemophilia. vWD is an extremely heterogeneous disorder that has been classified into several major subtypes. Type I vWD is the most common and is inherited as an autosomal dominant trait. This variant is due to simple quantitative deficiency of all vWF multimers. Type 2 vWD is also subdivided further dependent upon whether the dysfunctional protein has decreased or paradoxically increased function in certain laboratory tests of binding to platelets. Type 3 vWD is clinically severe and is characterized by recessive Inheritence and virtual absence of vWF.

2-Hemochromatosis: [69]

Definition

Hemochromatosis is a disorder that interferes with iron metabolism and results in excess iron deposits throughout the body.

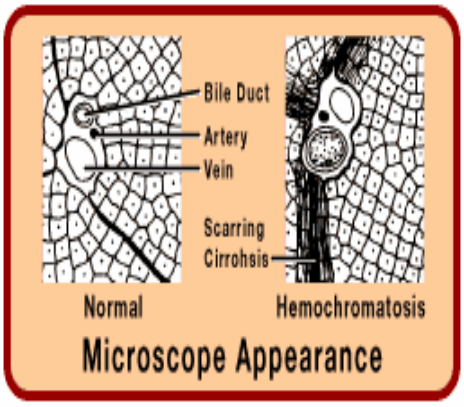


Fig (5-1) Microscopic Appearance of Hemochromatosis

Causes

Primary hemochromatosis is the most common genetic disorder in the US, affecting an estimated 1 of every 200-300 Americans. Similar symptoms may occur from the secondary form of hemochromatosis, which can be caused by other diseases such as thalassemia or sideroblastic anemia. Hemochromatosis may also be caused by having a

large number of blood transfusions, particularly in patients who get them for inherited or pre-malignant anemias. Occasionally, it may be seen with hemolytic anemia, porphyria cutanea tarda, excessive oral iron ingestion, or chronic alcoholism. First, excess iron accumulates in the liver and causes liver enlargement. Then, other organs are affected. The disease may lead to the development of diabetes, skin pigment changes, cardiac problems, arthritis, testicular atrophy, cirrhosis of the liver, liver cancer, hypopituitarism, chronic abdominal pain, severe fatigue, and increased risk of certain bacterial infections.

Hemochromatosis affects men five times more frequently than women. It is particularly common in Caucasians of western European descent. Symptoms are often seen in men between the ages of 30 and 50 and in women over 50, although some people may develop problems by age 20. Alcoholism and a family history of hemochromatosis are risk factors.

Symptoms

1. Joint pain
2. Fatigue
3. Lack of energy
4. Weight loss
5. Generalized darkening of skin color (often referred to as bronzing)
6. Abdominal pain
7. Loss of sexual desire
8. Testicular atrophy
9. Loss of body hair
10. Weakness
11. Heart problems
12. Symptoms related to the onset of diabetes

Treatment

The goal of treatment is to remove excess iron from the body and to give supportive treatment to damaged organs.

Iron removal is done by phlebotomy (removal of blood). One-half liter of blood is removed from the body each week for 2 to 3 years until the iron stores are depleted. After that, less frequent phlebotomy is needed to maintain iron levels within normal limits. The frequency of additional phlebotomy is determined individually for each person based on levels of hemoglobin, serum ferritin, and continuing symptoms. Loss of sexual desire and change in secondary sexual characteristics are improved with testosterone therapy. Management of diabetes, arthritis, liver failure, and heart failure associated with this condition are the same as conventional treatments for these problems. People diagnosed with hemochromatosis must follow a special diet to help maintain a lower serum ferritin. The diet prohibits alcohol consumption, especially for patients who have suffered liver damage. People with hemochromatosis must also avoid iron pills or vitamins containing iron, vitamin supplements, iron cookware, raw seafood (cooked is fine), or fortified processed foods such as 100% iron breakfast cereals. Diet does not prevent, control, or cure hemochromatosis, and phlebotomy is the most efficient method for removing excess iron from the body.

Outlook (Prognosis)

The earlier a diagnosis is made and treatment is implemented, the better. If treatment is started before any organs have been affected, associated diseases such as liver disease, heart disease, arthritis, and diabetes can usually be prevented. The prognosis will be variable for people who already suffer from associated diseases, and it will depend on the degree of the organ damage. Early detection and treatment is key in preventing organ damage and associated diseases. Some of the damage to target organs can be reversed when hemochromatosis is detected early and treated aggressively with phlebotomy. Organizations such as the National Institute of Diabetes and Digestive Kidney Diseases (NIDDK), Centers for Disease Control and Prevention (CDC), and the American Hemochromatosis Society (AHS) are working to promote screening for hemochromatosis to improve early diagnosis and treatment.

Possible Complications

- Liver failure
- Liver cancer

3-Idiopathic Thrombocytopenic Purpura: [70]

Idiopathic thrombocytopenic purpura (ITP) is a bleeding disorder in which the blood does not clot as it should. The bleeding is due to a low number of platelets (PLATE-lets), blood cells that help the blood clot and stop bleeding. People with ITP often have purple bruises that appear on the skin. The bruises mean that bleeding has occurred in small blood vessels under the skin The words idiopathic, thrombocytopenic, and purpura mean: .

Idiopathic means that the cause of the disease or disorder is not known. Thrombocytopenic means there is a lower-than-normal number of platelets in the blood. Purpura (PURR-purr-ah) are purple bruises where bleeding occurs just under the skin. Purple areas may also appear on the mucus membranes (for example, in the mouth). A person with ITP also may have bleeding that looks like tiny red or purple dots on the skin. These dots, often seen on the lower legs, are called petechiae . Petechiae may look like a kind of rash.

With ITP:

People may have nosebleeds, bleeding from the gums when they have dental work done, or other bleeding that is hard to stop.

Women may have heavy menstrual bleeding.

Symptomatic bleeding in the brain is very rare but can be life threatening if it occurs.

ITP is largely an autoimmune disease. The decrease in platelets occurs because the immune system attacks and destroys the body's own platelets, for an unknown reason. Normally, your immune system helps your body fight off infections and diseases. But when the immune system mistakenly attacks some part of a person's own body, this is called an autoimmune disease. Because "idiopathic" means "of unknown cause," a better name for most cases of ITP is immune thrombocytopenic purpura.

What Are Platelets and How Do They Work?

Platelets are small blood cells, or thrombocytes, that are made in your bone marrow (along with other kinds of blood cells). Platelets circulate through the blood vessels and help stop bleeding by sticking together to seal small cuts or breaks in tiny blood vessels.

Types of ITP

There are two types of ITP: acute (temporary or short-term) ITP and chronic (long-lasting) ITP. Acute ITP generally lasts less than 6 months. It mainly occurs in children, both boys and girls, and is the most common type of ITP. It typically occurs following an infection caused by a virus. This type of ITP often goes away on its own within a few weeks or months and does not return. Treatment may not be needed. Chronic ITP is a long-lasting (6 months or longer) type of ITP that mostly affects adults. However, some teenagers and even younger children get this type of ITP. Chronic ITP affects women two to three times more often than men. Treatment depends on how severe the bleeding symptoms are and the platelet count. In mild cases, treatment may not be needed.

Other Names for Idiopathic Thrombocytopenic Purpura

1. Immune thrombocytopenic purpura
2. Autoimmune thrombocytopenic purpura

What Causes Idiopathic Thrombocytopenic Purpura?

In idiopathic thrombocytopenic purpura (ITP), the immune system treats a person's own platelets as if they were invaders in the body, attacking and destroying them. The immune system attacks platelets by making proteins called antibodies. The antibodies bind to platelets (attach) and then are removed by the spleen (an organ that is part of the immune system and helps fight infection).

Normally, the immune system makes antibodies to fight off germs or other harmful things (called antigens) that enter the body. The reason why the immune system decides to attack platelets is not known.

Children who get the acute (temporary) type of ITP often have had a recent viral infection. It is possible that the infection somehow "triggers" or sets off the immune

reaction that leads to ITP in these children. ITP in adults, on the other hand, does not seem to be linked to infections.

What Are the Signs and Symptoms of Idiopathic Thrombocytopenic Purpura?

The signs and symptoms of idiopathic thrombocytopenic purpura (ITP) are related to increased bleeding due to low numbers of platelets.

Signs include:

1. Bruising (purpura): purplish areas on the skin or mucus membranes (such as in the mouth) due to bleeding. The bruises may occur for no apparent reason.
2. Petechiae: pinpoint red spots on the skin (typically the legs) that often occur in groups and may look like a rash. The spots are due to bleeding under the skin.
3. Bleeding that is hard to stop.
4. Bleeding from gums (for example, when dental work is done).
5. Nosebleeds.
6. Heavy menstrual bleeding in women.
7. Blood in the urine.
8. Blood in the stool (bowel movement).
9. Symptomatic bleeding in the brain is very rare but can be life threatening if it occurs.

A low number of platelets causes no symptoms other than increased risk of bleeding. A low number of platelets is not responsible for pain, fatigue, difficulty with concentration, or any other symptoms.

How Is Idiopathic Thrombocytopenic Purpura Diagnosed?

To diagnose idiopathic thrombocytopenic purpura (ITP), doctors use your medical history, a physical exam, and blood tests. Your medical history includes information about: Your signs and symptoms of bleeding Illnesses you have that could lower your platelet count or cause bleeding medicines or any other supplements or remedies you take that could cause bleeding or lower your platelet count.

Your doctor will do a physical exam and look for signs of bleeding and infection.

Your doctor will also order blood tests to measure the platelet count in your blood. These tests usually include:

A complete blood count, to show the numbers of different kinds of blood cells, including platelets, in a small sample of your blood.

A blood smear, which involves placing some of your blood on a slide. A microscope is then used to look at your platelets and other blood cells.

A blood smear is important to be sure that the platelet count is correct. In healthy people, the platelet count can be falsely low, since the chemical used in the tube during blood collection may cause platelet clumping.

If blood tests show that you have a low number of platelets, you may need additional tests to help with the diagnosis. For example, bone marrow tests may be used to see if enough platelets are being formed in the bone marrow.

In ITP, the red and white blood cell counts are normal.

A low platelet count can occur when the body destroys platelets or doesn't produce enough platelets, or both. In ITP, the platelet count is low because the body is destroying platelets faster than the bone marrow can make new ones.

Some people with mild ITP have few or no signs of bleeding. In that case, they might be diagnosed only after a blood test done for another reason shows that they have a low number of platelets (thrombocytopenia). If other causes for low platelet count are ruled out, ITP may then be diagnosed.

4-Polycythemia Vera: [71]

Definition

Polycythemia vera is an abnormal increase in blood cells (primarily red blood cells) resulting from excess production by the bone marrow.

Alternative Names

Primary Polycythemia; Polycythemia rubra vera; Myeloproliferative disorder; Erythremia; Splenomegalic polycythemia; Vaquez's disease; Osler's disease; Polycythemia with chronic cyanosis - Myelopathic polycythemia; Erythrocytosis megalosplenica; Cryptogenic polycythemia

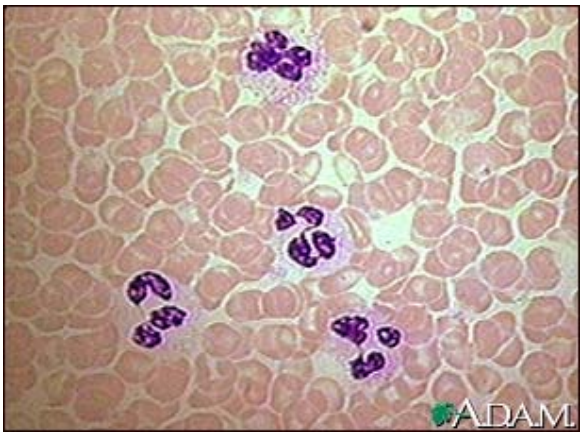


Fig (5-2) microscopic appearance of plycythemia vera

Causes

Polycythemia vera is an acquired disorder of the bone marrow that causes the overproduction of all three blood cell lines: white blood cells, red blood cells, and platelets. It is a rare disease that occurs more frequently in men than women, and rarely in patients under 40 years old. It is not known what causes polycythemia vera. The disease usually develops slowly, and most patients do not experience any problems related to the disease after being diagnosed. However, the abnormal bone marrow cells

may begin to grow uncontrollably in some patients leading to acute myelogenous leukemia. Patients with polycythemia vera also have an increased tendency to form blood clots that can result in strokes or heart attacks. Some patients may experience abnormal bleeding because their platelets are abnormal.

Symptoms

1. Headache
2. Dizziness
3. Itchiness, especially following a warm bath
4. Fullness in the left upper abdomen
5. Red coloration, especially of the face
6. Shortness of breath
7. Breathing difficulty, lying down
8. Symptoms of phlebitis

Note: Symptoms are due to increased blood viscosity and clotting. Additional symptoms that may be associated with this disease:

9. Vision abnormalities
10. Skin spots, red
11. Skin discoloration, bluish
12. Fatigue

Outlook (Prognosis)

Polycythemia vera usually develops slowly, and most patients treated appropriately do not experience any problems related to the disease. However, the abnormal bone marrow cells may begin to grow uncontrollably leading to acute myelogenous leukemia. Patients with polycythemia vera also have an increased tendency to form blood clots that can result in strokes or heart attacks. Some patients may experience abnormal bleeding because their platelets are abnormal.

Possible Complications

- Thrombosis (a cause of stroke and heart attack)
- Peptic ulcer disease

- Gastric bleeding
- Gout
- Leukemia
- Heart failure
- Myelofibrosis

5- Sickle Cell Anemia : [72]

Sickle cell anemia is an inherited blood disease. That means you are born with it and it lasts a lifetime. Sickle cell anemia affects the red blood cells. Normal red blood cells are smooth and round like doughnuts. They move easily through blood vessels to carry oxygen to all parts of the body. In sickle cell anemia, the red blood cells become hard, sticky, and shaped like sickles or crescents. When these hard and pointed red cells go through the small blood vessels, they tend to get stuck and block the flow of blood. This can cause pain, damage, and a low blood count or anemia. The sickle-shaped red blood cells tend to get stuck in narrow blood vessels, blocking the flow of blood.

Anemia is a shortage of red blood cells in your blood. In sickle cell anemia, this shortage of red blood cells occurs because sickle cells do not last very long. It is hard for your body to make new red blood cells fast enough to keep up. Normal red blood cells last about 120 days in the bloodstream. Sickle cells die after only about 10 to 20 days.

Sickle cell trait is different from sickle cell anemia. A person with sickle cell trait does not have the disease but carries the gene that causes the disease. Persons with sickle cell trait can pass the gene to their children.

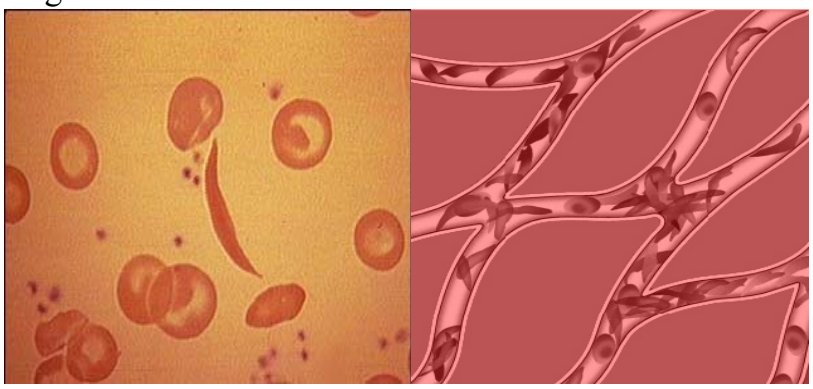


Fig (5-3) sickle cell anemia

Sickle cell anemia is a serious disease and there is no universal cure. Bone marrow transplantation offers a cure, but very few patients have matched donors. Some patients also do not want bone marrow transplants because of the risks involved. Over the past 30 years, doctors have learned a great deal about the disease. They know what causes it, what it does to your body, and how to treat many of the complications. Today, with good health care, many people with the disease: Are in reasonably good health much of the time Live fairly normal lives ,Live 40 to 50 years and longer.

Other Names for Sickle Cell Anemia

Hemoglobin SS Disease

What Causes Sickle Cell Anemia?

People with sickle cell anemia inherit two genes, one from each parent, that are variant (different from normal). The variant genes are call sickle cell genes. The sickle cell genes tell the body to make the variant hemoglobin that results in deformed red blood cells. Hemoglobin is the protein in red blood cells that carries oxygen to all parts of the body.

Children who inherit sickle cell genes from both parents will have sickle cell anemia. Children who inherit the sickle cell gene from only one parent will not have the disease. They will have sickle cell trait. Persons with sickle cell trait:

Generally have no symptoms

Live normal lives

Can pass the sickle cell gene on to their children.

When two people with sickle cell trait have a baby, there is a: One in four chance (25 percent) the baby will inherit two sickle cell genes and have the disease. One in four chance (25 percent) the baby will inherit two normal genes and not have the disease or trait. Two in four chance (50 percent) the baby will inherit one normal gene and one sickle cell gene. The baby will not have the disease, but will have sickle cell trait like the parents. The presence of two sickle cell genes (SS) is needed for sickle cell anemia. If each parent carries one sickle hemoglobin gene (S) and one normal gene (A), with each

pregnancy, there is a 25 percent chance of the child's inheriting two SS genes and having sickle cell anemia; a 25 percent chance of inheriting two AA genes and not having the disease; and a 50 percent chance of being an unaffected carrier (AS) like the parents.

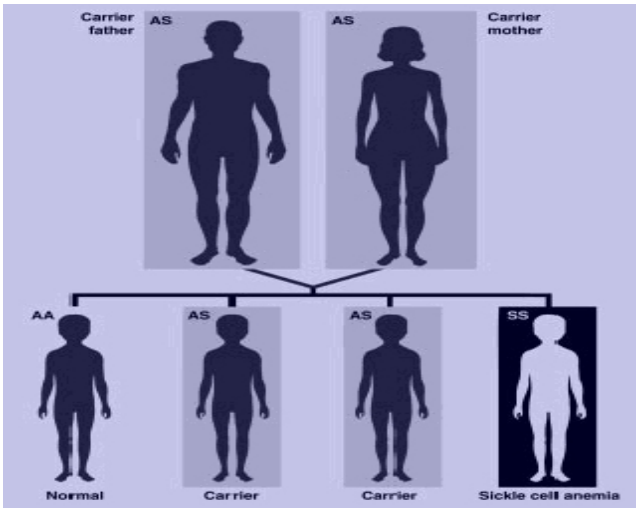


Fig (5-4) Persons with sickle cell trait Can pass the sickle cell gene on to their children

What Are the Signs and Symptoms of Sickle Cell Anemia?

The signs and symptoms of sickle cell anemia are different in each person. Some people have mild symptoms. Others have very severe symptoms and are often hospitalized for treatment.

The most common symptoms or signs are related to:

1. Anemia
2. Pain when sickle-shaped red blood cells block the flow of blood to an organ
3. Other more specific symptoms.
4. The general symptoms or signs of anemia are:
5. Fatigue (feeling very tired)
6. Paleness
7. Yellowing of the skin and eyes (jaundice)
8. Shortness of breath.

Pain is the symptom of sickle cell anemia that most people are familiar with. It occurs in both children and adults. Pain results from blocked blood and oxygen. Painful events or crises may occur in any body organ or joint. Some patients have painful crises less than

once a year. Others may have as many as 15 or even more crises in a year. The pain can be acute (sudden), chronic (long lasting), or a mixture of the two.

Acute pain is the most common type of pain. It is sudden pain that can range from a mild ache to very severe pain. The pain usually lasts from hours to a few days. With complications or poor treatment, the pain can last for weeks.

Chronic pain usually lasts 3 to 6 months or longer. Chronic pain can be hard to bear and mentally draining. This can severely limit daily activities.

Mixed pain is a combination both of acute and chronic pain.

Other more specific symptoms and complications include:

Hand-foot syndrome. When the small blood vessels in hands or feet are blocked, pain and swelling can occur, along with fever. One or both hands and/or feet may be affected at the same time. This may be the first symptom of sickle cell anemia in infants. Pain may be felt in the many bones of the hands and feet. Swelling usually occurs on the back of the hands and feet and moves into the fingers and toes.

Eye problems. The retina is a thin layer of tissue at the back of the eye that receives and processes visual images. When the retina does not get enough blood, it can weaken and cause problems. These problems can be serious enough to cause blindness.

Infections. Both children and adults with sickle cell anemia have a hard time fighting off infections. The spleen is an organ in your body that helps fight infection. In sickle cell anemia, the spleen can become damaged and unable to do its job. Infants and young children with a damaged spleen are more likely to get infections that can kill them within hours or days. Pneumonia is the most common cause of death in young children with sickle cell anemia. Meningitis, influenza, and hepatitis are also common infections in persons with sickle cell anemia.

Acute chest syndrome. This is a life-threatening problem of sickle cell anemia similar to pneumonia. It is caused by infection or by trapped sickle cells in the lung. Patients usually have chest pain, fever, and an abnormal chest x-ray.

Delayed growth and puberty in children. Children with sickle cell anemia often grow more slowly and reach puberty later than other children. Adults with sickle cell anemia often have a slight build. The slow rate of growth is caused by a shortage of red blood cells (anemia). Sores (ulcers) on the legs. Sickle cell ulcers usually begin as small, raised, crusting sores on the lower third of the leg. Leg sores occur more often in males than in females and usually appear between ages 10 and 50. The cause of leg ulcers is unclear. There can be just one ulcer or many. Some heal rapidly but others persist for years or come back after healing.

Stroke. The sickle-shaped red blood cell may stick to the walls of the tiny blood vessels in the brain. This can cause a stroke. This type of stroke occurs mainly in children. The stroke can cause learning disabilities or more severe problems.

Gallstones. Gallstones form in the gallbladder when there is too much bilirubin in the body. Bilirubin is made when red blood cells break down. People with gallstones may have steady pain in the upper right side of the belly, under the right shoulder, or between the shoulder blades that lasts for 30 minutes or more. The pain may occur: After eating fatty meals With nausea, vomiting, fever, sweating, chills, clay-colored stool and yellowish color of the skin or whites of the eyes.

Priapism. Males with sickle cell anemia may have painful and unwanted erections called priapism. This happens because the sickle cells stop blood flow out of an erect penis.

How is Sickle Cell Anemia Diagnosed?

Screening tests are done on newborn infants in most States. These tests can show if the newborn infant has sickle cell anemia or carries the sickle cell trait. Early diagnosis of sickle cell anemia is very important so that children who have sickle cell anemia can get proper treatment.

Forty-four (44) States, the District of Columbia, Puerto Rico, and the Virgin Islands currently screen all newborns for sickle cell anemia. Screening is available by request in the other six (6) States. This screening includes a simple blood test for sickle cell anemia on all newborn infants. This test uses blood from the same blood samples as other routine newborn screening tests.

If the first test shows that the sickle hemoglobin is present, a second blood test is done to confirm the diagnosis. These tests also tell whether the child carries the sickle cell trait. It is also possible to identify sickle cell anemia before birth. This is done by getting a sample of amniotic fluid or tissue taken from the placenta. This test can be done as early as the first few months of pregnancy.

6-Thalassemia:

People with thalassemia have an inherited blood disorder that causes mild or severe anemia (uh-NEE-me-uh). The anemia is due to reduced hemoglobin (he-mo-GLOBE-in) and fewer red blood cells than normal. Hemoglobin is the protein in red blood cells that carries oxygen to all parts of the body.

In people with Thalassemia, the genes that code for hemoglobin are missing or variant (different than the normal genes). Severe forms of thalassemia are usually diagnosed in early childhood and are lifelong conditions.

The two main types of thalassemia, alpha and beta, are named for the two protein chains that make up normal hemoglobin. The genes for each type of thalassemia are passed from parents to their children. There are mild and severe forms of the disease, the latter often called Cooley's anemia. Cooley's anemia is the most common severe form of thalassemia seen in the U.S.

Alpha thalassemia occurs when one or more of the four genes needed for making the alpha globin chain of hemoglobin are variant or missing. Moderate to severe anemia results when more than two genes are affected. Alpha thalassemia major can result in miscarriages.

Beta thalassemia occurs when one or both of the two genes needed for making the beta globin chain of hemoglobin are variant. The severity of illness depends on whether one or both genes are affected, and the nature of the abnormality. If both genes are affected, anemia can range from moderate to severe.

Other Names for Thalassemia:

Alpha Thalassemias

1. Alpha thalassemia "silent carrier"
2. Mild alpha thalassemia, also called alpha thalassemia minor or alpha thalassemia trait
3. Hemoglobin H disease
4. Hydrops fetalis, or alpha thalassemia major

Beta Thalassemias

1. Beta thalassemia minor, also called thalassemia minor or thalassemia trait
2. Beta thalassemia intermedia, also called thalassemia inter media or mild Cooley's anemia
3. Beta thalassemia major, also called thalassemia major or Cooley's anemia
4. Mediterranean anemia
5. Cooley's anemia sometimes is used to refer to any type of thalassemia that requires treatment with regular blood transfusions.

What Causes Thalassemia?

Thalassemia is caused by variant or missing genes that affect how the body makes hemoglobin. Hemoglobin is the protein in red blood cells that carries oxygen. People with thalassemia make less hemoglobin and fewer circulating red blood cells than normal, which results in mild or severe anemia.

There are many possible combinations of variant genes that cause the various types of thalassemia. Thalassemia is always inherited (passed from parents to children). People with moderate to severe forms of thalassemia received variant genes from both parents. A person who inherits a thalassemia gene or genes from one parent and normal genes from the other parent is a carrier (thalassemia trait). Carriers often have no signs of illness other than mild anemia, but they can pass the variant genes on to their children. Hemoglobin includes two kinds of protein chains called alpha globin chains and beta globin chains. If the problem is with the alpha globin part of hemoglobin, the disorder is alpha thalassemia. If the problem is with the beta globin part, it is called beta

thalassemia. There are both mild and severe forms of alpha and beta thalassemia. Severe beta thalassemia is often called Cooley's anemia.

Alpha Thalassemia

There are four genes involved in making the alpha globin part of hemoglobin--two from each parent. Alpha thalassemia occurs when one or more of these genes are variant or missing.

People with only one gene affected are called "silent carriers" and have no sign of illness.

People with two genes affected (alpha thalassemia trait, or alpha thalassemia minor) have mild anemia and are considered carriers.

People with three genes affected (hemoglobin H disease) have moderate to severe anemia.

Until recently, babies with all four genes affected (alpha thalassemia major, or hydrops fetalis) could not survive and usually died before birth. Today, many of these babies can survive with treatment.

If two people with alpha thalassemia trait (carriers) have a child, the baby could have a mild or severe form of alpha thalassemia or could be healthy.

Beta Thalassemia

There are two genes involved in making the beta globin part of hemoglobin--one from each parent. Beta thalassemia occurs when one or both of the two genes are variant.

If one gene is affected, a person is a carrier and has mild anemia. This condition is called beta thalassemia trait, or beta thalassemia minor.

If both genes are variant, a person may have moderate anemia (beta thalassemia intermedia, or mild Cooley's anemia) or severe anemia (beta thalassemia major, or Cooley's anemia).

If two people with beta thalassemia trait (carriers) have a baby, one of three things can happen:

1. The baby could receive two normal genes (one from each parent) and have normal blood (1 in 4 chance).
2. The baby could receive one normal gene from one parent, and one variant gene from the other parent, and have thalassemia trait (2 in 4 chance).
3. The baby could receive two thalassemia genes (one from each parent) and have a moderate to severe form of the disease (1 in 4 chance).

What Are the Signs and Symptoms of Thalassemia?

The symptoms of thalassemia depend on the type and severity of the disease. Symptoms occur when not enough oxygen gets to various parts of the body due to low hemoglobin and a shortage of red blood cells in the blood (anemia). "Silent carriers" and persons with alpha thalassemia trait or beta thalassemia trait (also called carriers) usually have no symptoms. Those with alpha or beta thalassemia trait often have mild anemia that may be found by a blood test.

In more severe types of thalassemia such as Cooley's anemia, signs of the severe anemia are seen in early childhood and may include:

1. Fatigue (feeling tired) and weakness
2. Pale skin or jaundice (yellowing of the skin)
3. Protruding abdomen with enlarged spleen and liver
4. Dark urine
5. Abnormal facial bones and poor growth.
6. Babies with the most severe type of alpha thalassemia (hydrops fetalis) die before birth or soon after birth

How is Thalassemia Diagnosed?

Thalassemia is diagnosed using blood tests, including a complete blood count (CBC) and special hemoglobin studies. A complete blood count provides information about the

amount of hemoglobin and the different kinds of blood cells, such as red blood cells, in a sample of blood. People with thalassemia have less hemoglobin than normal and fewer red blood cells than normal in their blood. Carriers of the trait may have slightly small red blood cells as their only sign. Hemoglobin studies measure the types of hemoglobin in a blood sample. Cooley's anemia is usually diagnosed in early childhood because of signs and symptoms and severe anemia. Some people with milder forms of thalassemia may be diagnosed after a routine blood test shows that they have anemia. Doctors suspect thalassemia if a child has anemia and is a member of an ethnic group that is at risk for thalassemia. To distinguish anemia caused by iron deficiency from anemia caused by thalassemia, tests of the amount of iron in the blood may be done. Family genetic studies are also helpful in diagnosing thalassemia. This involves taking a family history and doing blood tests on family members. Prenatal testing can determine if an unborn baby has thalassemia and how severe it is likely to be.

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