5
Blood System

Definition : Blood is a ***specialised fluid connective tissue*** in which there is liquid intercellular substance (plasma) and formed elements -(RBC, WBC and platelets) suspended in the plasma which circulates in closed system of blood vessels. It is red, thick and slightly alkaline.

Composition of blood

Blood is composed of-

A. ***Cellular substances :*** 45% (42-45%)
   i. Erythrocytes or red blood corpuscles (RBC)
   ii. Leukocytes or white blood corpuscles (WBC)
   iii. Platelets or thrombocytes

B. ***Liquid intercellular substances :*** i.e, plasma - 55% (55 - 58%) :

   Plasma contains -
   i. *Liquid :* (91 - 92 %) - water
   ii. *Solids :* (8 - 9%) :
      a. *Inorganic constituents (0.9%)* : Sodium, potassium, calcium, magnesium, phos-phorus, iron, copper etc.
      b. *Organic constituents :*
         i. *Proteins - 7.5% :* serum albumin, serum globulin, fibrinogen, prothrombin, etc.
         ii. *Non-Protein nitrogenous substances (NPN) :* Urea, uric acid, xanthine, hypoxanthine, creatine, creatinine ammonia, amino acids etc.
         iii. *Fats :* Neutral fat, phospholipid, choles-terol, cholesterides etc.
         iv. *Carbohydrates :* Glucose, sucrose, etc.
         v. *Other substances :* Internal secretions, anti-bodies and various enzymes (amylases, proteases, lipases, phospha-tases, etc.)
         vi. *Colouring matters :* Yellow colours of plasma is due to small amount of bilirubin, carotine and xanthophyllin.

Properties of blood

i.	Blood volume	: 5 - 6 litres
ii.	Normal reaction	: Slightly alkaline, pH = 7.36 - 7.45
iii.	Specific gravity	: 1.052 - 1.060
iv.	Viscosity	: 4.5 times more viscus than water.
v.	Temperature	: 36-38°C
vi.	Osmotic pressure	: Average 25 mm of Hg.
vii.	Taste	: Salty.

viii. Colour : Red, due to presence of haemoglobin inside RBC.

Viscosity of blood

Viscosity of blood is the ratio of time of flow of a given volume of blood with same volume of distilled water, while passing through a specially prepared tube known as viscometer. It is about 4.5 times more viscus than water.

The ratio of relative viscosities of water, plasma and whole blood is roughly- 1 : 1.8 : 4.5 respectively.

It depends upon :
i. The amount of plasma protein
ii. Number and volume of cellular elements.
iii. CO_2 tension
iv. Temperature.

Viscosity rises for following causes :
i. Acidosis
ii. Hyper calcaemia
iii. Hyper glycaemia
iv. Polycythemia
v. Cyanosis
vi. Diabetes mellitus

Viscosity is reduced in :
i. Anaemia
ii. Fever
iii. Exercise
iv. Oedema
v. Lymphatic leukaemia
vi. Malaria
vii. Rise of temperature.

Specific gravity

Blood : 1.052 - 1.060
Plasma : 1.025 - 1.030
Cells : 1.088.

Specific gravity of blood rises in :
i. Lose of water from the body such as excessive sweating, diarrhoea, cholera etc.
ii. When water intake is inadequate.
iii. Exudation of fluid into tissue of serous cavity due to inflammation, surgical operation, burn etc.

Specific gravity falls in:
i. When a large quantity of water is taken.

ii. Injection of saline, glucose into vein.
iii. Immediately after severe haemorrhage, there is shift of tissue fluid into blood causing haemodilatation.

Hydrostatic pressure

In arterial end of capillary : 32 mm of Hg.
In venous end of capillary : 15 mm of Hg.

Function of blood :

1. *Transport of respiratory gasses :* It carries O_2 from alveoli of the lungs to the tissues and eleminates CO_2 from the tissue to the alveoli of the lungs.
2. *Transport of nutrients :* It carries digestive food materials absorbed by the intestine to the tissue cells i.e. glucose, amino-acid, fatty acid etc.
3. *Acts as vehicle :* Hormones, enzymes, vitamins & other chemicals are brought to their places of activity through blood stream.
4. *Regulation of body temperature :* Blood has high water content, water make it very suitable for this property due to
   a. High specific heat
   b. High thermal conductivity
   c. High latent heat of vaporization.
5. *Regulation of water and electrolytes balance:* Fluid portion of blood is interchangable with the intercellular fluid of the tissue, thus maintains water and electrolyte balance.
6. *Maintenance of acid base balance :* By efficient buffering power and with the help of kidney, skin, lungs etc blood maintains acid base balance.
7. *Defensive function :*
   a. WBC by its phagocytic properties engulf bacteria and foreign particles.
   b. It develops antibodies which combat toxic agent.
8. *Excretory function :* The metabolic end products and other waste products are carried by blood to the organ of excretion i.e. Kidney. Lungs, Skin etc.
9. *Regulation of blood pressure :* It regulate blood pressure by changing volume and viscosity of blood.
10. *It maintains colloidal osmotic pressure :* By the action of plasma proteins.
11. *Prevent haemorrhage :* By the process of coagulation it prevent haemorrhage.

Plasma

The fluid portion of the blood, the *plasma*, is a remarkable solution containing an immense number of ions, inorganic molecules, and organic molecules that are in transit to various parts of the body or aid in the transport of other substances. The normal plasma volume is about 5% of body weight, or roughly 3500 mL in a 70-kg man. Plasma clots on standing, remaining fluid only if an anticoagulant is added. If whole blood is allowed to clot and the clot is removed, the remaining fluid is called *serum*. Serum has essentially the same composition as plasma except that its flbrinogen and clotting factors II, V, and VIII have been removed and it has a higher serotonin content because of the breakdown of platelets during clotting.

(Ref. Ganong 22th Edition; Page-539)

Measurement of plasma volume

Indicators Used : Plasma volume can be measured by using indicators are-

1. Evans blue dye (also called, T-1824)
2. Serum albumin labelled with radio-active iodine (^{125}I-albumin).

(Ref. Guyton & Hall-11th Edition; Page-296)

Procedure : Suppose 10 ml of venous blood of a subject is taken in a heparinized tube. This serves as the control sample. 5 ml of a 5% solution of Even's blue (T-1824) in diluted water is then injected intravenously. 10 minute after the injection another 10 ml of sample is withdrawn from the vein into another heparinized tube. The haematocrit of both sample are determined. The optical density of the dye stained plasma is estimated. 0.01 ml of the dye is then diluted to 5 ml (dilution is 1 : 500) with the control plasma and its optical density is measured.

So plasma volume (ml)

$$= \text{Dye solution injected} \times \text{dye Solution stadarisation} \times \frac{\text{Density of standard}}{\text{Density of unknown}}$$

Calculation of interstitial fluid volume

Interstitial fluid volume cannot be measured directly but it can be calculated as-

$$\text{Interstitial fluid volume} = \text{Extracellular fluid volume} - \text{Plasma volume}$$

Measurement of blood volume

The avreage blood volume of a normal adult man is approximately 5,000 ml. On the average approximtely 3000 ml of this is plasma and remainder 2000 ml is blood cells.

Here plasma volume is obtained same as ECF.

$$\text{Total blood volume} = \frac{\text{Plasma volume}}{1 - \text{Hematocrit}}$$

For example, if plasma volume is 3 liters and hematocrit is 0.40, then total blood volume would be calculated as

$$\text{Total blood volume} = \frac{3}{1 - 0.40}$$

$$= 5 \text{ liters}$$

Another way to measure blood volume is to inject into the

which is normally only onethird saturated with iron, now becomes almost fully bound with iron, so that the transferrin accepts almost no new iron from the mucosal cells. Then, as a final stage of this process, the buildup of excess iron in the mucosal cells themselves depresses active absorption of iron from the intestinal lumen.

2. When the body already has excess stores of iron, the liver decreases its rate of formation of *apotransferrin,* thus reducing the concentration of this iron-transporting molecule in the plasma and the bile. Therefore, less iron is then absorbed by the intestinal apotransferrin mechanism, and less iron can also be transported away from the intestinal epithelial cells in the plasma by plasma transferrin.

Yet, despite these feedback control mechanisms for regulating iron absorption, when a person eats extremely large amounts of iron compounds, excess iron does enter the blood and can lead to massive deposition of hemosiderin in the reticuloendothelial cells throughout the body. At times, this can be very damaging.

(Ref. Guyton & Hall-11th ed, Page 426)

Erythrocytic sedimentatin rate (ESR)

Definition : When the blood is mixed with a suitable anticoagulant and is made to stand vertically, red blood corpuscles settle down to the bottom. The rate at which this sedimentation of red cells take place is known as erythrocytic sedimentatin rate (ESR).

Normal count :

1. Westergren method :
   Male : O to 6 mm in Ist hour
   Female : O to 12 mm in Ist hour.
2. Wintrobe method :
   Male : O to 12 mm in Ist hour.
   Female : O to 18 mm in Ist hour.

Importance of ESR :

1. To see the prognosis of disease.
2. To assay the condition of some chronic inflammatory diseases, such as :
   i. Pulmonary tuberculosis
   ii. Pulmonary embolism
   iii. Myocardial infarction
   iv. Coronary thrombosis
   v. Rheumatic arthritis
   vi. Carcinoma.
3. To see the therapaeutic effects of drugs.

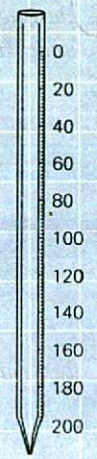


Fig. 5-2. *Westergren ESR tube*

ESR increases in

A. *Physiological conditions :*
   i. During menstruation
   ii. During pregnancy
   iii. In high atmosphere pressure.

B. *Pathological conditions :*
   1. Multiple myeloma
   2. Tuberculosis
   3. Rheumatic arthritis
   4. Chronic inflammation
   5. Malignancy
   5. Coronary thrombosis
   7. Haemorrhage
   8. Pregnancy
   9. Toxaemic condition
   10. SLE.
   11. Other types of tissue necrosis

ESR falls in :

A. Phygiological condition :
   i. In high altitude
   ii. In dehydration
   iii. In defect of rouleaux formation.

B. Pathological condition :
   i. Haemocromatosis
   ii. Polycythemia vera.

Estimation of ESR

There are two methods for estimatin of ESR :

i. Westergren method.
ii. Wintrobe method.

Westergen method

Materials required :

1. Westergren tube : It is a straight glass tube, 30 cm. in length and 2.5 mm in diameter. It is calibrated in m.m. from 0 to 200. Both ends of tube are open.
2. Westergren tube-rack.
3. Test tube
4. Reagent : 3.8% sodium citrate solution (anticoagulant).
5. Blood collecting meterials.

Steps :

1. Take 0.4 ml reagent in a test tube.
2. Draw venous blood and add 1.6 ml blood in the test tube.
3. Draw mixed blood in the westergren tube by sucking by the mouth upto the mark 'O'.
4. Place the tube in the rack in vertical position with the zero mark above.
5. Take the reading after one hour by noting down the level of lower end of the clear plasma.

Precautions to be taken :

1. The tube must be in perfect vertical position.

2. The reagent and the blood must be mixed in correct proportions.
3. The test must be made within one hour after the collection of the blood.

Wintrobe Method

Materials required :

1. Wintrobe tube : It is a straight tube, 110 mm in length and 2.5 mm in diameter. It is graduated in mm with double rows, 0 - 100 and 100 - 0; 0 - 100 for ESR and 100 - 0 for PCV. One end of the tube is open and another is closed.
2. Reagent : Paul-Heller mixture- 6 mg of Ammonium oxalate and 4 mg of potassium oxalate for 5 ml of blood.
3. Test tube.
4. Blood collecting materials.

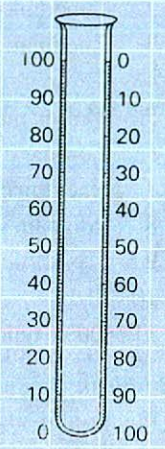


Fig.5-3. *Wintrobe ESR tube.*

Steps :

1. Collect one to five ml of blood and transfer to the tube containing the anticoagulant in the proportion of 1-2 mg of dry powder for each ml. of blood.
2. Draw one ml of blood in a capillary tube and fill the wintrobe tube upto 100 mark (0) from the bottom.
3. Place the tube in vertical position and take the reading after one hour.

Factors influence the ESR

The factors involved in the rate of fall of the RBC influence the ESR. These are :

1. *Factors which increase rouleaux formation and viscosity of plasma enhance ESR :*
   a. Increase plasma fibrinogen.
   b. Increase plasma globulins.
   c. Decrease plasma albumin.
   d. Decrease plasma Hb. conc. (Anaemia)
   e. Increase dilution of blood.
   f. Decrease number of RBC.
2. *Technical and instrumental factors also influence ESR :*
   a. Position of ESR tube : Properly vertical tube gives accurate reading. Any degree of inclination increase ESR.
   b. Quantity of anticoagulant used : Excess potassium oxalate and sodium citrate increase and decrease the ESR value respectively.
   c. Delay in the test : If the test is delayed after collection of blood, ESR becomes high.
   d. Room temperature : With the rise of room temperature ESR is raised.
   e. Method of determination : The length and diameter of the ESR tube influence the value. Hence the value is more in wintrobe method than the westergren method.

Rouleaux formation

It is a special process of sedimentation of RBC by which RBC settle one at the top of the others, so that the ratio of mass and surface area increased. It depends upon-

i. Fibrinogen & globulin number
ii. Tissue destruction
iii. Albumin number.

Anaemia

Definition : Anaemia may be defined as a reduction of haemoglobin concentration per unit volume of peripheral blood below normal for the age and sex of the patien which is usually but not invariably associated with the reduction of RBC.

(Ref. Clinical pathology)

Cause of anaemia :

1. Excessive blood loss due to acute or chronic haemorrhage
2. Reduced production of RBC due to lack of some factors which are necessary for RBC production.
3. Excessive blood cell destruction in comparision to the production of blood cells
4. Destruction of bone marrow.

Site of observation of Anaemia :

1. Lower palpebral conjunctivae
2. Dorsal surface of the tongue
3. Oral mucous membrane
4. Skin of the hands
5. Skin of the sole of the feet.

Classification of anaemia

A. ***Pathogenetic & Aetiological :*** (According to the cause of anaemia)

1. *Anaemia due to blood loss :*
   a. Acute post haemorrhagic : May be due to trauma, accident & causes normocytic or slight macrocytic normochromic anaemia.
   b. Chronic post haemorrhagic e.g chronic blood loss due to hookworm infestation. Initially causes normocytic normochromic anaemia & later, hypochromic microcytic anaemia.
2. *Anaemia due to impaired red cell formation :*
   a. Anaemia due to disturbance of bone marrow function *due to deficiency of erythropoietic factors :*
      i. Iron deficiency anaemia (commonest)
      ii. Megaloblastic anaemia
      iii. Anaemia associated with scurvy

iv. Anaemia due to protein malnutrition (rare).

b. Anaemia due to disturbance of bone marrow function not *due to deficiency of substances essential for erythropoiesis :*

i. Anaemia associated with infection, renal failure, liver disease, disseminated malignancy, collagen disease, bone marrow infiltration, leukaemia, malignant lymphoma, multiple myeloma, myelosclerosis etc.

ii. Aplastic anaemia.

iii. Sideroblastic anaemia.

iv. Anaemia associated with myxoedema & hypopituitarism.

v. Congenital diserythropoietic anaemia.

3. *Anaemia due to increased red cell destruction :*

a. Haemolytic anaemia due to corpuscular defect.

b. Haemolytic anaemia due to abnormal haemolytic mechanism (extracorpuscular defect).

B. ***Morpholoical :*** (Based on the characteristics of the red cell as determined by blood examination mainly MCV & MCHC).

1. *Normocytic normochromic anaemia* e.g. aplastic anaemia, acute post haemorrhagic anaemia.
2. *Microcytic hypochromic* e.g. Iron deficiency anaemia, Thalassaemia.
3. *Macrocytic anaemia* e.g. Megaloblastic anaemia.
4. *Simple microcytic.*

Effect of anaemia :

One of the major effects of anaemia is greartly increased work load on the heart. Due to increased venous return caused by-i. decreased viscosity of blood. ii. Diminished transport of oxygen by the blood causes tissue vessels to dilate.

Iron deficiency anaemia

Aetiology & pathognesis of iron deficiency anaemia. :

Major aetiologic factors :

1. In females in the reproductive period of life :
   a. Menstruation
   b. Pregnancy
   c. Pathological blood loss
   d. Defective diet.
2. In adult males & post-menopausal females :
   a. Pathological blood loss.
3. In infants & children :
   a. Defective diet
   b. Diminished iron stores at birth.

Pathogenesis : When the supply of iron to the bone marrow is insufficient for the requirements of Hb synthesis, iron deficiency anaemia takes place.

Iron deficiency may be regarded as developing in two stages

1. Progressive depletion of the available tissue iron stores.
2. Ultimate exhaustion of the available tissue iron stores, followed by the development of anaemia.

There are 4 major factors in the pathogenesis of iron deficiency anaemia :

1. An increase physiological demand for iron.
2. Loss of blood.
3. Inadequate iron intake.
4. Impaired absorption of iron.

Increase physiological demand :

1. In children : During the period of growth there is a progressive increase in the blood volume & consequently in the total amount of Hb in the body, this result in an increase demand for iron by the marrow for hemoglobin synthesis.
2. In female ; During the reproductive life due to menstruction, (blood loss- 15-30 mg/month), pregnancy (requires 500-600 mg for foetus), parturition, and lactation(180 mg).

Blood loss :

1. From GIT : Bleeding peptic ulcer, Hook worm infestation,Carcinoma stomach, Rupture of oesophageal varies, Hiatus hernia, Haemorrhoids, Carcinoma colon, Ulcerative colitis, Chronic aspirin ingestion & Piles.
2. From respiratory tract : Any condition associated with haemoptysis e.g Pulmonary tuberculosis., Bronchogenic carcinoma.
3. From urinary tract : Any condition associated with haematuria e.g calculi in urinary tract.
4. From uterus : menorrhagia, uterine fibroid, Carcinoma cervix or uterus.
5. Wide spread bleeding disorder e.g Haemophilia, purpua etc.

Inadequate intake of iron :

1. In infant : milk injury, improper feeding.
2. In adult : Anorexia, poverty, dietary dislike.

Impaired absorption :

Coeliac disease, Sprue, Diarrhoea. Achlorhydria, Total or partial gastrectomy, Atopic gastritis, following gastroenterostomy.

Aplastic anaemia

Definition : Aplastic anaemia may be defined as a disorder characterised by the occurance of anaemia, leucopenia and thrombocytopenia, resulting from aplasia of the bone marrow.

Classification :

A. Primary or idiopathic

B. Secondary or symptomatic : caused by physical or chemical agents.

C. Miscellenous :
   1. Familial hypoplastic anaemia (Fanconi's syndrome).
   2. Aplastic anaemia associated with infective hepatitis.
   3. Aplastic anaemia associated with pancreatic insufficiency.
   4. Aplastic anaemia associated with paroxysmal nocturnal haemoglobinuria.

D. Pure red cell aplasia (erythroblastopenia) :
   1. Congenital
   2. Acquired (with or without associated thymoma).

Aetiology

a. Drugs :
   i. Drugs which regularly cause bone marrow depression: N-mustard, busulphan, chloram bucil, vincristine etc.
   ii. Drugs which rarely cause bone marrow depression: Oxyphenbutazone, chloram phenicol, tolbutamide, chlordizaepoxide, aloxidone.

b. Chemicals : Benzene, TNT, lindane, DDT, chlordane.

c. Physical agents : X-ray, gamma-ray, neutrons, a & b ray.

Megaloblastic anaemia

Definition : The anaemias which are characterised by distinctive cytologic and functional abnormalities in peripheral blood and bone marrow cells due to impaired DNA synthesis as a result of vitamin B_{12} or folic acid deficiency or both are known as megaloblastie anaemias.

Causes :

A. *Causes of vitamin B_{12} deficincy :*
   1. Less intake :
      Nutritional deficiency.
   2. Impaired absorption :
      a. Gastric :
         i. Pernicious anaemia - due to lack of intrinsic factor of castle.
         ii. Total or partial gastrectomy.
      b. Intestinal causes :
         i. Coeliac disease
         ii. Tropical sprue
         iii. Fish tape (Dyphylobothrium latum) worm infestation.
      c. Chronic pancreatic disease.
      d. Interference with normal absorption by bacteria or parasites.
   3. Drugs :
      a. Para amino salicylic acid
      b. Metformin
      c. Neomycine.
   4. Vit. B_{12} deficiency in child hood :
      a. Juvenile pernicious anaemia
      b. Congenital intrinsic factor deficiency.

B. *Causes of folic acid deficiency :*
   1. Less intake :
      a. Nutritional deficiency.
   2. Impaired absorption :
      a. Coeliac disease
      b. Tropical sprue.
   3. Increased demand :
      a. Pregnancy
      b. Puerperium
      c. Haemolytic anaemia
      d. Leukaemia
      e. Lymphoma
      f. Malignancy
      g. Sideroblastic anaemia
      h. Inflammatory disorders
      i. Hyper thyroidism
      j. Skin disease
   4. Drugs : Anti convulsant, Oral contracetive, Methotrexate, trimethoprim, triamterene, Pyrimethamine.

Haemolytic anaemia

Definition :

A haemolytic anaemia may be defined as an anaemia resulting from an increase in the rate of red cell destruction.

Classification :

A. *Haemolytic Anaemias due to intracorpuscular defects :*
   1. Congenital :
      a. Due to membrane defects :
         i. Hereditary spherocytosis
         ii. Hereditary eliptocytosis.
         iii. Hereditary stomatocytosis.
      b. Due to Hb. defect :
         i. Haemoglobinopathies e.g sickle cell anaemia, unstable Hb. disease.
         ii. Thalassaemia (b-thalassaemia major, Hb–H disease)
      c. Due to enzyme defect :
         i. Non-spherocytic congenital haemo- lytic anaemia
            Due to deficiency of pyruvate kinase
            Due to deficiency of glucose- 6- phosphate dehydrogenase.
         ii. Drug induced haemolytic anaemia & favism.
   2. Acquired :
      a. Paroxysmal nocturnal haemoglobinuria

B. *H. anaemias due to extra corpusclar defects.*

1. Immune mechanisms :
   a. Autoimmune acqired haemolytic anaemia (Warm & cold antibody)
   b. Haemolytic disease of the newborn erythroblastosis foetalis
   c. Incompatible blood transfusion.
   d. Drug induced haemolytic anaemia.
2. Non-immune mechanisms :
   a. Mechanical haemolytic anaemia
      i. Cardiac haemolytic anaemia
      ii. Microangiopathic A.
   b. Misc :
      i. Haemolytic anaemia due to direct action of drugs and chemicals.
      ii. Haemolytic anaemia due to infection.
      iii. Haemolytic anaemia due to burns.
      iv. Lead poisoning.

Pernicious anaemia

Pathogenesis : The fundamental defect in pernicious anaemia is a failure of secretion of intrinsic factor by the stomach due to permanent atrophy of the gastric mucosa. Gastric atrophy is the end result of complex interaction between genetic and autoimmune factors.

Gastric atrophy → Reduced intrinsic factor secretion → failure of absorption of dietary Vit. B_{12} → deficiency of Vit. B_{12} → give rise to following C/F.

Clinical features :

a. *Common :*
   1. Megaloblastic macrocytic anaemia
   2. Glossitis.
   3. Peripheral neurophathy & subacute combined degeneration of spinal cord.

b. *Less common :*
   1. Angina of effort
   2. CCF (Congestive cardiac failure)
   3. Recurrent diarrhoea
   4. Dyspepsia, anorexia & weight loss.
   5. Mental disturbances
   6. Visual disturbances.

Age : Middle & older age groups.

Thalassaemia

Definition : The thalassaemias are a heterogenous group of disorders in which there is a genetically determined reduction in the rate of synthesis of one or more types of normal hemoglobin. polypeptide chains resulting in a decrease production of hemoglobin having the affected chain.

Classification :

A. *Haematological :*
   1. α thalassaemia : affecting the synthesis of a chain.
   2. β Thalassaemia : affecting the synthesis of b chain.
      a. β^+ Thalassaemia : incomplete suppression of beta chain synthesis.
      b. β^o Thalassaemia : complete absence of beta chain synthesis.

B. *Clinical :*
   1. Thalassaemia major or cooley's anaemia : Total suppression of beta chain synthesis and it is the homozygous form of the disease.
   2. Thalassaemia minor or trait : Heterozygous form of the disease.

Clinical features :

1. Pallor, diarrhoea & recurrent fever.
2. Hepatomegaly & spleeno-megaly.
3. Slow growth in childhood.
4. Mongoloid face.
5. Severe anaemia.
6. Epistaxis, skin pigmentation, etc.

(Ref. Clinical pathology)

Differences between thalassaemia & iron deficiency anaemia.

Thalassaemia	*Iron-deficiency anaemia*
1. Congenital	1. Acquired
2. Break down of RBC prominent	2. Not present
3. Nucleated RBC plenty	3. A few nucleated RBC in severe case
4. Serum iron increased	4. Serum iron decreased
5. Serum iron binding capacity reduced.	5. Serum iron binding capacity increased.

Haemoglobinopathies

Definition : When possession of Hb variant gives rise to a clearly defined disease state, the affected person is said to have a haemoglobinopathy. The haemoglobinopathies are characterised by the production of structurally abnormal Hb due to abnormalities in the formation of the globin moiety of the molecule.

Types :

1. Hb..S sickle haemoglobinopathies
2. Hb..C sickle haemoglobinopathies
3. Hb..E sickle haemoglobinopathies

4. Hb..D sickle haemoglobinopathies
5. The unstable Hb haemoglobinopathies
6. Haemoglobinopathies associated with polycythemia.

Most of the abnormal Hbs produce no harmful effect and the individual remains asymptomatic and unware of the abnormality within red cells. The abnormal Hbs are inherited as autosomal codominants.

The subjects who inherit one abnormal and one normal gene are called *heterozygotes*. The X subjects who inherits two identical abnormal genes are called *homozygotes*. The *homozygous* state is usually referred to as the 'disease' e.g Hb-C disease and the *heterozygous* state is as the 'trait' e.g sickle cell trait.

(Ref. Clinical Pathology)

Q. ***Write down the laboratory investigation of haemoglobinopathies.***

Ans.

1. *Routine haematological and biochemical test* :
   a. Hb estimation
   b. PCV
   c. TC of RBC
   d. Reticulocyte count
   e. Determination of red cell indices
   f. Examination of the blood film
   g. Bilirubin estimation
   h. Other biochemical tests for the presence of haemolysis.
2. *Test depending on physio-chemical properties of abnormal haemoglobin* :
   a. Sickle test
   b. Hb solubility test
   c. Demonstration of intracellular crystal, Hb H inclusion etc.
   d. Heat instability test
   e. Isopropanolol precipitation test
   f. Oxygen dissociation studies
3. Hb electrophoresis for the demonstration of abnormal Hb
4. Alkali denaturation
5. The acid elution test.

(Ref. Clinical Pathology)

Sickle cell anaemia

The sickle haemoglobinopathies are hereditary disorders in which the red cells contain Hb–S.

Definition : It is a hereditary disorder where there is anaemia due to the presence of Hb–S in red cells.

Types :

1. Sickle cell anaemia (homozygous state)
2. Sickle cell trait (heterozygous state).

Pathogenesis : In sickle cell anaemia red cells containing Hb–S differs from Hb–A in substitution of valine for glutamic acid in the 6th position from the N -terminal end of the beta chain. In the deoxygenated state, the conformational changes in Hb–S are tactoid formation. As a result the red cells containing Hb–S, become rigid and deformed to assume a sickle or crescent shape. Red cells will begin to sickle at an O_2 tension of 50 to 60 mm of Hg. This tension is experienced by the cells in parts of microcirculation and thus sickling occurs in vivo. If the flow rate is rapid, sickling does not become fully established and the cells resume normal shape when they are swept back to areas of the circulation where the oxygen tension is higher. If the flow rate is slow and the cells are delayed in areas where the O_2 tension is low, the cells sickle and there is a great increase in blood viscosity with further slowing of circulation.

The sickling of the red cells in the circulating blood has two major ***pathological effects :***

1. The distorded cells cause a great increase in blood viscosity and block small blood vessels, impairing flow and causing ischaemia and infarction of the tissue.
2. Repeated "sickle-unsickle" cycle leads to loss of fragments of red cell membrane and the cells become spherocytic and fragile.

They are removed by R.E. system and to a lesser extent destroyed in the circulation resulting in both extravascular and intravascular haemolysis leading to anaemia.

Hereditary spherocytosis :

In this condition, the RBC are small in size, spherical in shape rather than biconcave. They can not compressed because they do not have the normal loose bags like structure. There fore, on passing through the small capillaries they easily ruptures and causes anaemia.

Polycythemia

Definition : Polycythaemia is defined as an increase in the concentration of red cells, above the normal for the age & sex of the patient.

Secondary polycythemia : Whenever the tissues become hypoxic because of too little oxygen in the atmosphere, such as at high altitudes, or because of failure of delivery of oxygen to the tissues, as occurs in cardiac failure, the blood-forming organs automatically produce large quantities of extra red blood cells. This condition is called *secondary polvcvthemia*, and the red cell count commonly rises to 6 to 7 million/mm^3, about 30 per cent above normal.

A common type of secondary polycythernia, called *physiologic polycvthemia*, occurs m natives who live at altitudes of 14,000 to 17,000 feet. The blood count is generally 6 to 7 million/mm^3 this is associated with the ability of these people to perform high levels of continuous work even in a rarefied atmosphere.

(Ref. Guyton & Hall-11th ed; Page 427)

Polycythemia Vera (Erythremia) : In addition to those people who have physiologic polycythemia, others have a pathological condition known as *polycythemia vera*, in which the red blood cell count may be 7 to 8 million! mm^3 and the hematocrit 60 to 70 per cent.

Causes of polycythemia vera : Polycythemia vera is caused by a genetic aberration that occurs in the hemocytoblastic cell line that produces the blood cells. The blast cells no longer stop producing red cells when too many cells are already present. This causes excess production of red blood cells in the same manner that a tumor of a breast causes excess production of a specific type of breast cell. It usually causes excess production of white blood cells and platelets as well.

Effect : In polycythemia vera, not only does the *hematocrit increase*, but the *total blood volume also increases*, on some occasions to almost twice the normal level. As a result, the *entire vascular system becomes intensely engorged.* In addition, many of the *capillaries become plugged* by the viscous blood because the viscosity of the blood in polycythemia vera sometimes increases from the normal of 3 times the viscosity of water to 10 times that of water.

(Ref. Guyton & Hall-11th ed; Page 428)

Causes of polycythaemia :

A. *True or absolute polycythaemia :* There is an increase in the total red cell volume of the body relative to body weight.
   1. Idiopathic polycythaemia vera (erythraemia or aquez-Osler disease)
   2. Secondary polycythaemia (erythrocytosis)
      a. Secondary to hypoxia :
         i. High altitude
         ii. Congenital heart disease
         iii. Chronic pulmonary disease
         iv. Miscellaneous (rare)

         Aquired heart disease, disorder associated with alveolar hypoventilation :
         a. Emphysema
         b. Diffuse pulmonary fibrosis, Abnorm alities of pigment metabolism.
      b. Secondry to increased erythropoietin production.
         i. Non neoplastic kidney disease : Cysts, hydronephrosis.
         ii. Tumours : Kidney, liver etc.
         iii. Ovarian tumour
         iv. Leiomyoma of uterus
         v. Pheochromocytoma.
   3. Benign familial polycythaemia.
   4. Polycythaemia associated with haemoglobino pathies (compensatory polycythaemia)

B. *Relative polycythaemia :* Here total red cell volume is within the normal range. But there is increase RBC concentration or increase PCV due to haemo concentration as evidenced by :
   1. Dehydratin : fluid loss or diminished intake.
   2. Redistribution of body fluids
   3. Pseudopolycythaemia (Polycy-thaemia of stress, Spurious polycythaemia)
   4. Adrenal insufficiency : (increase loss of electrolyte Æ increase loss of fluid).
   5. Shock.

Effect of polycythemia:

1. *On circulatory system :*
   a. Because of greatly increased viscosity of the blood in polycythemia, the flow of blood through the vessels is often sluggish. It is obvious that increasing the viscosity tends to decrease the venous return. On the other hand, the blood volume is greatly increased, in polycythemia, which tends to increase the venous return. Actually, the cardiac out put in polycythemia is not far from normal because these two factors more or less neutralize each other.
   b. The arterial pressure is normal in most persons with polycythemia. Because, the blood pressure regulating mechanisms can usually offset the tendency for increased blood viscosity to increase peripheral resistance and there by to increase arterial pressure.
2. *On skin colour :*
   a. In secondary polycythemia, cyanosis is almost always evident.
   b. In polycythemia vera , ordinarilly has a ruddy complexion but may at times have a bluish (cyanotic) tint to the skin.

(Ref. Guyton & Hall-11th edition; Page 428)

***Summary** : Effect of polycythemia*

1. Blood viscosity increased.
2. Blood volume increased.
3. Cardiac out put more or less normal.
4. The arterial pessure usually normal.
5. Cyanosis presenl.

Jaundice

Definition : When free or conjugated bilirubin accumulates in the blood, the skin, sclera and mucous membranes turn yellow. This yellowness is known as jaundice (icterus) and is usually detectable when the total plasma bilirubin is greater than 2 mg/dl (34 μmol/L).

(Ref. Ganong 22th edition, Page 503)

Normal serum bilirubin level :

a. Normal level : 0.2-0.8 mg/100 ml (dl) of blood.
b. In new born : 0.2-5.9 mg/100 ml (dl) of blood.

High level of serum bilirubin in infant is due to excessive break down of RBC and failure of immature liver to metabolise such.

Latent jaundice : Serum bilirubin level is 0.8-2 mg/100 ml.

Clinicl jaundice : Serum bilirubin level is more than 2 mg%

Types :

1. Haemolytic or pre hepatic
2. Hepatocellular or hepatic
3. Obstructive or cholestatic or post hepatic.

Pathogenesis : Jaundice may develope due to any one of the following conditions :

1. Excess production of bilirubin (haemolytic anaemia, etc).
2. Decrease uptake of bilirubin into hepatic cells.
3. Disturbed intracellular protein binding or conjugation.
4. Disturbed secretion of conjugated bilirubin into the bile canaliculi.
5. Intrahepatic or extrahepatic bile duct obstruction.

(Ref. Ganong 22th Edition; Page-503)

Causes of jaundice

A. *Haemolytic jaundice :*
   1. Haemolytic anaemia e.g. Malaria, Eryth roblastosis foetalis, Thalassemia etc.
   2. Increase production of shunt bilirubin in the bone marrow.
   3. Massive haemorrhage within the body.

B. *Hepatocellular jaundice :*
   1. Reduced uptake of bilirubin by hepatic cells : Gilbert's disease, Drug induced etc.
   2. Impaired conjugation of bilirubin : Neonatal jaundice, Crigler-Najji syndrome, Gilbert's disease.
   3. Impaired excretion of conjugated bilirubin :
      a. Intrahepatic causes : Dubin-Johnson syndrome, Rotor syndrome, Drug induced (Oestrogen, steroid) etc.
      b. Extra hepatic causes : It occurs due to the obstruction of biliary tract. The obstruction may be in the lumen, in the wall or out side the tract :
         i. Lumen : Gall stone, Worm etc.
         ii. Wall : Cholengitis, Tumour, Atresia, Stricture etc.
         iii. Out side the wall : Ca of the head of the pancreas, Sub-hepatic abscess, Enlarged lymph node etc.
      4. Combination of all : Viral hepatitis, Septicaemia, Cirrhosis of liver, Hepatic poisoning etc.

C. *Obstructive jaundice* : Same as the intra and extra hepatic causes of impaired excretion of bilirubin in hepatocellular jaundice.

Vanden Bergh's Reaction

It is a test by which prehepatic and posthepatic jaundice are detected . Here diazo reagents are used containing-

i. Sulphanilic acid
ii. Sodium nitrite
iii. Hydrochloric acid.

Types :

1. Direct
2. Indirect
3. Biphasic

Procedure : 0.5 ml of diazo reagent is added to 0.5 ml of serum and then mixed.

Result :

1. *Direct :* When red colour appears immediately, it is due to conjugated bilirubin.
2. *Indirect :* If red colour does not appear within 30 mins. then 0.5 ml 95% alcohol is added and mixed. If red colour then appears, it is an indirect reaction & due to unconjugated bilirubin.
3. *Biphasic :* The reaction is direct but the colour depends on standing. It is due to both conjugated and unconjugated bilirubin.

Interpretation :

1. *Direct reaction :* Found in obstructive jaundice where bilirubin is conjugated in serum. The conjugated bilirubin is water soluble & can easily mix with diazo reagent to give colour immediately.
2. *Indirect reaction :* Found in haemolytic jaundice where bilirubin is unconjugated & water insoluble. Here bilirubin cannot directly mix with diazo reagent. But when alcohol is added it helps unconjugated bilirubin to mix with diazo reagent & then colour appears.
3. *Biphasc reaction :* Found in hepatocelluar jaundice because here both conjugated & unconjugated bilirubin is present.

Differences between conjugated and unconjugated bilirubin

Unconjugated bilirubin	*Conjugated bilirubin*
1. Water insoluble.	1. Water soluble
2. Vanden Bergh's reaction— Indirect positive.	2. Vanden Bergh's reaction— Direct positive.
3. In normal person present in blood.	3. In normal person absent in blood.
4. Not passes in urine.	4. It passes in urine.
5. Lipid soluble.	5. Lipid insoluble.
6. May cause CNS damage.	6. Do not cause CNS damage.

Laboratory findings of different types of jaundice

Points	*Haemolytic J.*	*Hepatocellular J.*	*Obstructive J.*
Urine			
a. Bilirubin	Absent	Variable	Present
b. Urobilinogen	Increase	Variable	Decrease
c. Bile salt	Absent	Absent	Present
Stool			
a. Colour	Dark	Normal or pale	Pale
b. Stercobilinogen	Increase	Decrease	Absent
c. Bile salt	Normal	Normal or decrease	Absent
d. Fat	Normal	Normal or increase	Increase
Blood			
a. Serum bilirubin	Incrase (mostly unconjugated)	Increase (both conjugated & unconjugated)	Increase (mostly conjugated)
b. Alkaline phosphatase	Normal	Increase	Marked increase
c. Serum aminotransferase.	Normal	Increase	Normal
d. Serum protein	Normal	Decrease	Normal
e. Prothrombin time	Normal	Increase	Increase
f. LDH	Normal	Increase	Normal

White Blood Corpuscle (WBC)

Definition : White blood corpuscles are the nucleated colourless cells of blood and are the mobile units of the body's protective system.

Morphology

1. Normal size : 10-20 Micron
2. Normal counts : 4000-11000/μL of blood
3. Life span : Few hours to few days.
4. Production : In bone marrow, spleen, tonsil etc. When WBC enters in the circulation from bone marrow they never come back to bone marrow. Again, when they enters in the tissue from circulation they never come back to the circulation.

In the bone marrow- RBC : WBC = 1 : 50
In the circulation- RBC : WBC = 500 : 1

(Ref. Ganong 22th edition; page-516 & others)

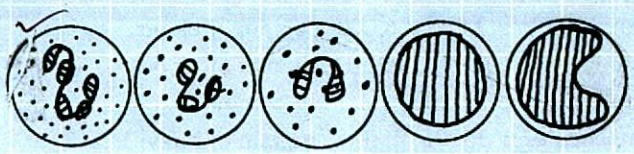
Life span of WBC :

The main reason white blood cells are present in the blood is to be transported from the bone marrow or lymphoid tissue to the areas of the body where they are needed. The life of the ***granulocytes*** once released from the bone marrow is normally 4 to 8 hours circulating in the blood and anthour 4 to 5 days in the tissues. In times of serious tissue infection, this total life span is often shortened to only a few hours becasue the granulocytes then proceed rapidly to the infected area, perform their functions, and in the process are themselves destroyed.

The ***monocytes*** also have a short transit time, 10 to 20 hours, in the blood before wandering through the capillary membranes into the tissues. Once in the tissues they swell to much larger sizes to become ***tissue macrophages***, and in this form, they can live for months or even years unless they are destroyed by performing phagocytic function. These tissue macrophages form the basis of the tissue macrophage system, that provides a continuing defense in the tissues against infection.

Lymphocytes enter the circulatory system continually along with the drainage of lymph from the lymph nodes and other lymphoid tissue. Then, after a few hours, they pass back into the tissues by diapedesis and then re-enter the lymph and return either to lymphoid tissue or to the blood again and again; thus, there is continual circulation of the lymphocytes through the body. The lymphocytes have life spans of weeks, months, or even years, but this debends on the body's need for these cells.

Ref. Guyton & Hall-11th edition; Page 431)



Neutrophil Eoinophil Basophil Lymphocyte Monocyte

Fig. 5-7. *Different types of WBC.*

Types of WBC :

On the basis of presence or absence of granules in the cytoplasm WBC are of two types

1. *Granulocytes :* Having granules in cytoplasm. Granulocytes on the basis of nucleus staining and size of the granules are of three types-
   a. Polymorphonuclear neutrophils
   b. Polymorphonuclear eosinophils
   c. Polymorphonuclear basophils.
2. *Agranulocytes :* Having no granules in cytoplasm. They again divided into two types -
   a. Lymphocytes
   b. Monocytes
3. Occationally, *plasma cells.*

N.B *The granulocytes and monocytes protect the body against invading organisms mainly by phagocytosis. The lymphocytes and plasma cells function mainly by in connection with immune system.*

(Ref. Guyton & Hall-11th edition; Page 430)

Normal values of WBC or *nomal count of WBC :*

1. Per microliter of blood : 9000 cells
   Approximate range : 4000-11000 cells.

(Ref. Ganong 22th Edition; Page 516)

1. Per microliter of blood : 7000 cells.

(Ref. Guyton & Hall-11th edition; Page 430)

Differential count of WBC

Neutrophils	:	50-70 %
Eosinophils	:	1-4 %
Basophils	:	0-0.4 %
Lymphocytes	:	20-40 %
Monocytes	:	2-8 %

(Ref. Ganong 22th Edition; Page 516)

Differential count of WBC (approximate average) :

Neutrophils	:	62.0 %
Eosinophils	:	2.3 %
Basophils	:	0.4 %
Lymphocytes	:	30.0 %
Monocytes	:	5.3 %

(Ref. Guyton & Hall-11th edition; Page 430)

Absolute count of WBC

Neutrophils	:	3,000 - 6,000 /cu. mm of blood
Eosinophils	:	150 - 300 /cu. mm of blood
Basophils	:	0 - 100 /cu. mmof blood
Lymphocytes	:	1500 - 4000 /cu. mm of blood
Monocytes	:	300 - 600 /cu. mm of blood.

(Ref. Ganong 22th Edition; Page-516)

Function of WBC

1. *Phagocytosis :* By this process WBC engulf bacteria, other foreign particles and micro-organism.
2. *Antibody formation :* Lymphocytes produce antibody and play an important role in defensive mechanism of the body.
3. *Secretion of heparin :* Basophil secretes heparin which prevents intravascular clotting.
4. *Formation of fibroblasts :* Lymphocytes may be converted into fibroblasts in the area of infection and thus help the process of repair .
5. *Anti-histamine function :* Eosinophil produces 5 HT.
6. *Chemotaxis :* Leucocytes have chemotactic properties by which they migrate out of the vessels to the site of injury by amoeboid movement.
7. *Act as scavanger :* By removing debris of dead and devitalized tissues they do the job of scavengers.

Properties of WBC

1. *Diapedesis :* It is the process by which WBC (Neutrophil and Monocytes) comes out through the pores of the blood vessels by squeezing. WBC comes to the vessel wall then small portion of the cell sque eze through the pores of the blood vessel then the squeezing continues and finally WBC comes out the blood ves sel.
2. *Ameboid movement :* It is a special type of movement by which WBC(Neutrophil and macrophages) moves towards the damaged tissue. The rate of movement is 40 micron per

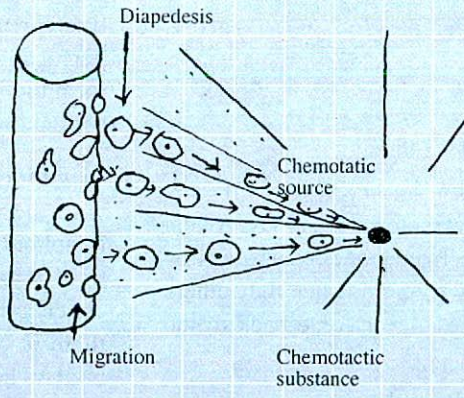


Fig. 5-9. *Diapedesis*

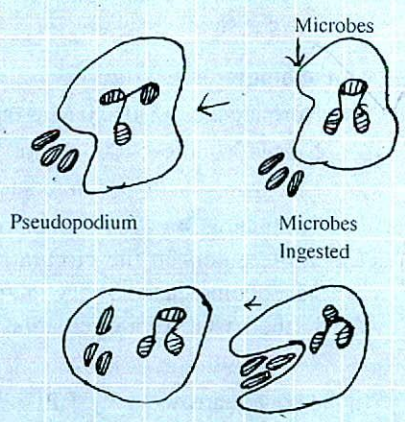


Fig. 5-10. *Phagocytosis*

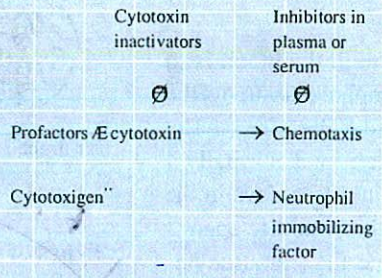


Fig. 5-11. *Chemotaxis*

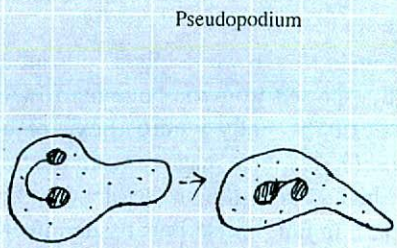


Fig. 5-12. *Ameboid Movement*

minute. Ameboid movement begins with protrution of a pseudopodium from one end of the cell. The pse- udopodium projects far out , away from the cell body and then the remainder of the cell moves toward the pseudopodium. This occurs due to continual changes of jel to sol within the cytoplasm of the cell.

3. *Chemotaxis :* A number of different chemical substances liberated by damaged tissue cause both neutrophil & macrophages to move toward the source of the chemical. This phenomenonis known as chemotaxi s. This chemical substances which either attract the WBC is called chemotaxic substance.

   These include :

   i. Bacterial toxins
   ii. Degenerated products of the inflamed tissues.
   iii. Components of complement complex
   iv. Several reaction products caused by plasma clotting in the inflamed area.

   Chemotaxis is effect upto 100 micrometers away from an inflammed tissue.

4. ***Phagocytosis :*** It is the process by which WBC(Neutrophil and macrophages) engulf foreign particles. It depends upon

   a. Rough surface of the foreign particles
   b. Surface charge of the foreign particles must be positive.
   c. Opsonin - It is globulin protein. It combines with dead tissue and foreign particles and help in phagocytosis.

   Mechanism : Phagocytosis involve 3 distinct steps :

   i. Recognition & attachment : Neutrophils & macrophages first rcognise the particles to be ingested and attach on their surface.
   ii. Engulfment : After attachment the phagocytic cells produce pseudopodes around the particle & completely enclose it. At this stage, the particle is enclosed in a membrane bound cytopasmic vesicle called phago-some.
   iii. Killing and/ or degradation: After engulfing most organisms destroyed with in the phagosome by the lysosomal enzyme.

Neutrophil

Its granules take either acidic or basic stain.

Character :

i.	Cell size	: 10 - 15 micron.
ii.	Nucleus	: Multilobed (3-6 lobed).
iii.	Granules	: Fine, uniformly distributed.
iv.	Cytoplasm	: Reddish-brown or violet in colour.
vii.	Life span	: Average 6 hours in the circulation
viii.	Production	: 100 billion neutrophils/day.

Functions :

1. They engulf bacteria by phagocytic activity.
2. They secrete proteolytic enzymes which digest the ingested particles causes degradation of tissue & thus hasten the process of recovery.
3. They collect at the site of infection by chemotactic properties.
4. It act as a first line defence aganist pyogenic infection.
5. They liberate chemotactic factor for macrophage.

Eosinophil

Its granules take acid stain.

Character :

i.	Cell size	: 10 - 12 micron.
ii.	Nucleus	: Bi or tri-lobed.
iii.	Granules	: Coarse, uniformly distributed.
iv.	Cytoplasm	: Red in colour.

Function :

1. Detoxify foreign particles.
2. Collect at the site of allergic reaction.
3. Inhibits antigen-antibody reaction.
4. Important for the dissolution of old clot .
5. Takes part in removing antigen-antibody complex after reaction.

Basophil

Its granules take basic stain .

Character :

i.	Cell size	: 8 - 10 micron
ii.	Nucleus	: Bi- lobed
iii.	Granules	: Coarse , not uniform, & overlap the nucleus.
iv.	Cytoplasm	: Purple blue or blue in colour.

Function :

i. It liberates heparin.
ii. It liberates 5 HT (hydroxytryptamine or sero tonin) and histamine.

Lymphocyte

Definition : Lymphocyte is one of the type of white blood cell. They have no granules.

Formerly, lymphocyte are classified only according to their size. i.e. small, medium or large However it has been shown that there are two main types of immunologically competent lymphocytes.

Character :

i. No granules present.
ii. More cytoplasm.

Cell size: 12-16 micron (*Large lymphocyte*). ***or***

Thin rim of bluish cytoplasm around the nucleus. Cell size : 9-12 micron (*Small lymphocyte*).

iii. Nucleus : Rounded .

iv. Stain : Nucleus - red , Cytoplasm- blue.

Function :

i. Carries antibody formed by reticulo - endothelial system.

ii. They give rise to monocytes, plasma cell, histocytes.

iii. They take part in immune reaction.

iv. They may be converted into firoblast & thus helps in repair of tissue.

v. Delayed hypersensitivity reaction.

Classification of lymphocyte :

A. *According to size :*

1. Small lymphocyte
2. Large lymphocyte

B. *Immunologically :*

1. T-lymphocyte: Responsible for cell- mediated immunity.
2. B-lymphocyte: Responsible for humoral immunity.

(Ref. Wright's 13th Page-57)

B - lymphocyte *(Bursa or bone-marrow processed) :*

Are chiefly involved in the production of humoral immunity in which the plasma cells secretes antibodies against encapsulated pyogenic bacteria (e.g pneumococcus and streptococcus).

T - lymphocyte *(Thymus depended) :*

Are responsible for cell mediated immunity , which is dirrected against intra cellular pathogenic micro-organisms (mainly bacteria, viruses, fungi. and also involved in the rejection of foreign transplants.

Types of T- lymphocyte : There are many different types of T cells. These are classified into three major groups :

i. Helper T cell

ii. Cytotoxic T cell

iii. Suppressor T cell.

Helper T-cell :

The helper T-cells are by far the most numerous of the T cells, usually constituting more than three quarters of all of them. They are so called, as they help in the function of immune system, in many ways. In fact, they serve as the major regulator of virtually all immune functions. They do this by forming a series of protein mediators, called lymphokines, that act on other cells of the immune system as well as on bone marrow cells. Among the important lymphokines secreted by the helper T cells are the following : IL-2, IL-3, IL-4, IL-5, IL-6, GMCS factor, interferon-γ.

(Ref. Guyton & Hall-11th edition; Page 447)

Functions :

The helper T cells form a series of protein mediator called lymphokines that act on other cells of the immune system & on bone marrow. Some of the regulatory functions are-

1. Stimulation of growth & proliferation of Cytotoxic T cells and suppressor T cells.
2. Stimulation of B cell growth & differentiation to form plasma cells and antibodies.
3. Activation of macrophage system.
4. Feed back stimulatory effect on the helper cells themselves.

(Ref. Guyton & Hall-11th edition; Page 447)

Cytotoxic T cell

The cytotoxic T cell is a direct attack cell, capable of killing microorganisms and at times even some of the body's own cells.

The receptor proteins on the surfaces of the cytotoxic cells cause them to bind tightly to those organisms or cells that contain their binding specific antigen.

After binding, the cytotoxic cell sceretes hole-forming proteins, called perforins. Then the cytotoxic cell releases cytotoxic substances directly into the attached cell & kill them.

Functions :

1. They directly attacks the micro-organism & kill them.
2. They also play an important role in destroying cancer cell, heart transplant cells or other types of cells that are foreign to body.

(Ref. Guyton & Hall-11th edition; Page 447)

Suppressor T cells

It is so called because, this cell suppresses the functions of both cytotoxic and helper T cell. It is believed that these suppressor

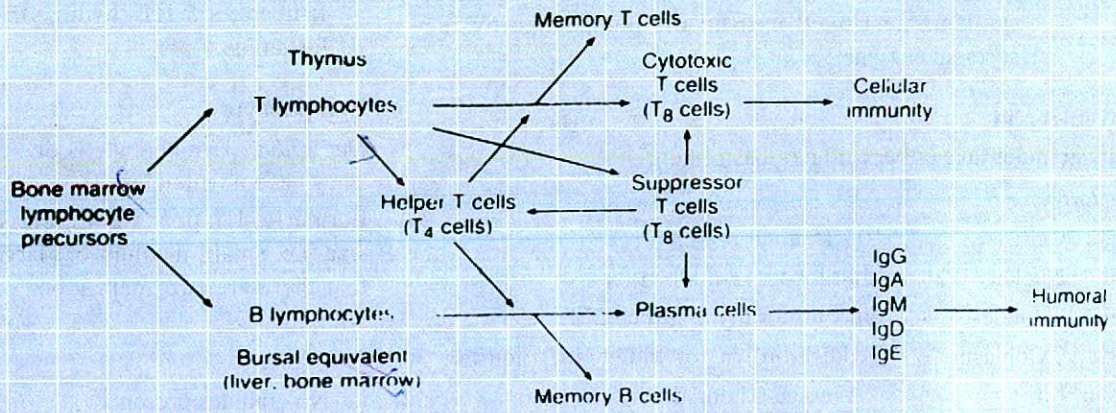


Fig. 5-13. *Development of the system mediating acquired immunity.*

functions serve the purpose of preventing the cytotoxic cells from causing excessive immune reactions that might be damaging to the bodies own response. They are also classified as *regulatory cells*. It is probable that the suppressor T system plays an important role in limiting the ability of the immune system to attack a person's own body tissues, called *immune tolerance*.

(Ref. Guyton & Hall-11th edition; Page 447)

Location of T and B-lymphocyte in adult :

i. *Peripheral blood :* Lymphocytes comprises 60-80 % T cells, 20-30 % B cells.
ii. *Thoracic duct :* Lymphocytes comprises 85-90 % T cells, 10-15 % B cells.
iii. *Lymph nodes :* Contain T cells in the paracortical region and B cells in the subcapsular region, in germinal centres and in medullary cords.
iv. *Spleen :* Contains T cells in periarteriolar sheaths and B cells in germinal centres, red pulp and around periarteriolar sheath.

(Ref. wright's 13th page 57)

Mast cell

Mast cells are heavily granulated wandering cells that are found in areas rich in connective tissue, and they are abundant beneath epithelial surfaces. Their granules contain heparin, histamine, and many proteases. The heparin appears to play a role in granule formation. They have IgE receptors on their cell membranes, and, like basophils, they degranulate when IgE-coated antigens bind to their surface. They are involved in inflammatory responses initiated by immunoglobulins IgE and IgG. The inflammation combats invading parasites. In addition to this involvement in acquired immunity, they release TNF-α in response to bacterial products by an antibody-independent mechanism, thus participating in the nonspecific ***natural immunity*** that combats infections. Marked mast cell degranulation produces clinical manifestations of allergy upto and including anaphylaxis.

(Ref. Ganong 22th Edition, Page-518)

Monocyte

Character :

i. Granules : Absent.
ii. Cell size : 15 - 20 micron.
iii. Nucleus : Kidney shaped .
iv. Cytoplasm : Pale blue in colour .

Function :

These cells are highly phagocytic and remove bacteria and cellular debris.

Monocytes and macrophages : *Monocyte* enter the blood from the bone marrow and circulate for about 72 hours. They then enter the tissues and become ***tissue macrophages***. Their life span in the tissues is unknown, but bone marrow transplantation data in humans suggest that they persist for about 3 months. It appears that they do not reenter the circulation. Some of them end up as the *multinucleated giant cells* seen in chronic inflammatory diseases such as tuberculosis. The tissue ,macrophages include the- *Kupffer cells* of the liver, pulmonary *alveolar macrophages*, and *microglia* in the brain, all of which come from the circulation, in the -past, they have been called the *reticuloendothelial system*, but the general term *tissue macrophage system* seems more appropriate.

The macrophages become activated by lymphokines from T lymphocytes. The activated macrophages migrate in response to chemotactic stimuli and engulf and kill bacteria by processes generally similar to those occurring in neutrophils. They play a key role in immunity. They also secrete up to 100 different substances, including factors that affect lymphocytes and other cells, prostaglandins of the F series, and clot-promoting factors.

(Ref. Ganong 22th Edition, Page-519)

Granulocyte & Macrophage Colony-Stimulating Factors

The production of red and white blood cells is regulated with great precision in healthy individuals, and the production of granulocytes is rapidly and dramatically increased in infections. The proliferation and self-renewal of the pluripotential cells depend on stem cell factor (SCF). Other factors are also involved. The proliferation and maturation of the cells that enter the blood from the marrow are regulated by glycoprotein growth factors or hormones that cause cells in one or more of the committed cell lines to proliferate and mature.

Three additional factors are called colony-stimulating factors (CSFs), because they cause appropriate single stem cells to proliferate in soft agar, forming colonies in this culture medium. The factors stimulating the production of committed stem cells include-

i. Granulocyte-macrophage CSF (GMCSF)
ii. Grarnilocyte CSF (G-CSF)
iii. Macrophage CSF (M-CSF).

Interleukins IL-I and IL-6 followed by IL-3 act in seqqence to convert pluripotential uncommitted stem cdlls to committed progenitor cells. IL-3 is also known as ***multi-CSF***. Each of the CSFs has a predominant action, but all the CSFs and interleukins also have other oveylapping actions. In addition, they activate and sustain mature blood cells. It is interesting in this regard that the genes for many of these factors are located together on the long arm of chromosome 5 and may have originated by duplication of an ancestral gene. It is also interesting that basal hematopoiesis is normal in mice in which the GM-CSF gene is knocked out, indicating that loss of one factor can be compensated for by others. On the other hand, the absence of GM-CSF causes accumulation of surfactant in the lungs.

(Ref. Ganong 22th Edition, Page-519)

Plasma cells

The activated B cells proliferate and transform into memory B cells and plasma cells. The plasma cells secrete large quantities of antibodies into the general circulation. The antibodies circulate in the globin fraction of the plasma and like anti bodies elsewhere, are called immuno globulins. The immuno globins are actually the secreted form of antigen-binding receptors on the B cell membrane.

Immuno globulin are :

a. IgM
b. IgG
c. IgA
d. IgD
e. IgE.

Ig stands for immu noglobulin.

(Ref. Ganong 21th edition)

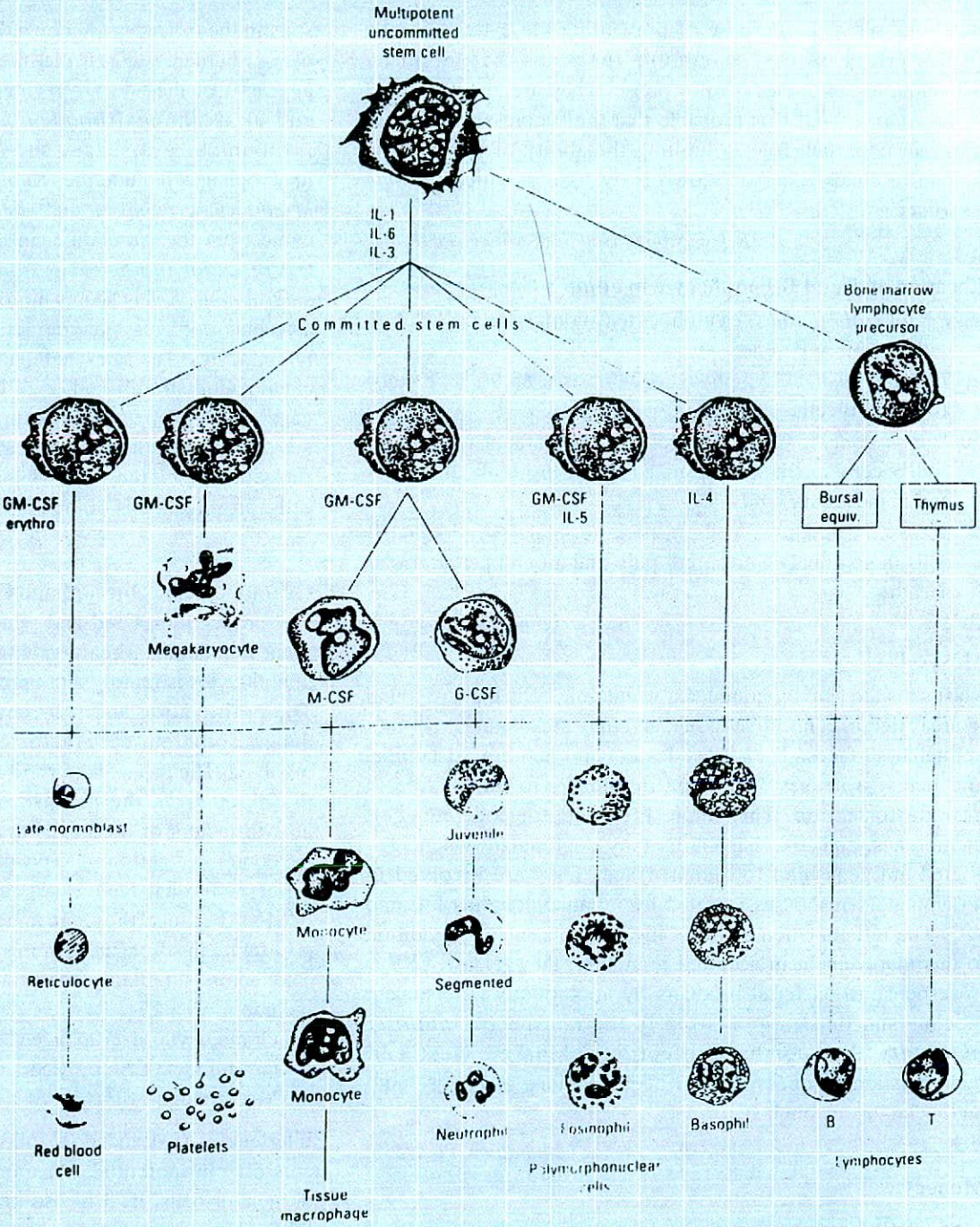


Fig. 5-14. *Development of various formed elements.*

Development of WBC

In the embryo the white corpuscles develop in the mesoderm and migrate secondarily into the blood vessels. In extrauterine life the granulocytes normally develop exclusively in the red marrow ; monocytes and a few lymphocytes also develop from stem cells in the bone marrow and partially in the lymph tissue(lymphocytes and plasma cells). T- lymphocyte matured in the thymus.

Granulopoiesis

The sequence of cells which give rise to the granulocytes in the marrow is as follows :

Pluripotent haemocytoblast

↓

Unipotent commited precursor stem cell

↓

Myeloblast

↓

Promyelocyte

↓

Myelocyte

(Neutrophil, Eosinophil, Basophil)

↓

Causes :

1. Infections :
   a. Bacterial : Typhoid fever
   b. Viral : Infective hepatitis
   c. Protozoal : Kala-azar, Malaria
2. Drug induced leucopcnia
3. Aplastic anaemia
4. Hyperspleenism etc.

Neutrophilia : Increased number of neutrophil above highest normal value of 2700/cu. mm of blood .

Causes:

1. Physiological :
   a. After cxercise
   b. Cold bath
   c. Pregnancy, Perpurium etc.
2. Pathological : Any acute infections. such as acute tonsilitis, pharyngitis, pleuritis, appen-dicitis, nephritis, salphingitis etc.

Neutropenia : Decreased number of neutrophil below lower normal limit of 1500/cu. mm of blood.

Eosinophilia : Increased number of eosinophil above the upper normal limit of 300/cu. mm of blood .

Causes :

1. Allergic disorders- Ashtma, Hay fever etc.
2. Trophical eosinophilia- Wucheriasis.
3. Parasitic infestation - Hook worm
4. Pulmonary eosinophilia-Ascaris lumbri-coides.
5. Malignancy.
6. Familial eosinophilia.

Eosinopenia : Decreased number of eosinophil below the lower normal limit of 150/ cu. mm of blood.

Causes :

1. Electric shock
2. Hormone - ACTH etc.

Basophilia

Increased number of basophil above the upper normal limit of 100/ cu. mm of blood .

Causes : Chronic myeloid leukaemia.

Leukaemia

Definition : The leukaemias are neoplastic disorders of haemopoietic tissue of unknown aetiology characterised by an uncontrolled, abnormal and wide spread proliferation of the leucocytic cells of the body infiltrating the bone marrow and other tissue of the body & usually but not invariably accompanied by the appearance of immature leucocytes in the peripheral blood.

Classification

A. *Depending on the clinical course of the disease :*
   1. Acute leukaemia
   2. Sub-acute leukaemia
   3. Chronic leukaemia.

B. *Depending on the predominant leukaemic cells :*

Acute leukaemia

1. Acute lymphocytic :
   a. Immunological :
      i. Null cell type
      ii. B-cell type
      iii. T-cell type.
   b. Morphological :
      i. Macroblastic
      ii. Microblastic
2. Acute myeloblastic :
   a. Acute granulocytic
   b. Acute pro-Myelocytic
   c. Acute myelomonocytic
3. Acute monocytic
4. Stem cell or undifferentiated type.

Chronic leukaemia :

1. Chronic lymphocytic
2. Chr. monocytic
3. Chr. granulocytic.

Rare subvarieties of acute & chr. leukaemia

1. Erythroleukaemia
2. Eosinophilic leukaemia
3. Megakaryocytic leukaemia
4. Plasma cell leukaemia.

C. *Depending on total WBC in periheral blood :*
   1. Leukaemic leukaemia
   2. Sub-leukaemic leukaemia.

D. ***French-American-Britis (FAB) classification of leukaemia :***
   1. Acute leukaemia :
      a. Acute lymphoblastic leukaemia (ALL) :
         L_1 : Small monomorphic
         L_2 : Large heterogenous
         L_3 : Large homogenous (Barkitt cell type)
      b. Acute myeloblastic leukaemia (AML) :
         M_1 : Myeloblastic (poorly differentiated)
         M_2 : Myeloblastic (well differentiated)
         M_3 : Hypergranular promyelocytic leukaemia
         M_4 : Myelomonocytic leukaemia
         M_5 : Monocytic leukaemia
         M_6 : Erythroleukaemia
   2. Chronic leukaemia :
      a. Chronic granulocytic

↓

Metamyelocyte

(Neutrophil, Eosinophil, Basophil)

↓

Neutrophil Eosinophil Basophil

(Granulocytes)

The cell from the myeloblast stage onwards can be identified in stained bone marrow smears in man. The characteristics of the myeloblast, myelocyte and mature granulocyte are described below :

Myeloblast (12-18 Micro meter diameter) : This cell develops from precursor stem cell; the nucleus is pale, purple blue and round with finely stippled chromatin and several nucleoli. The cytoplasm consists of a narrow blue rim without granules. Protein synthesis is very active as shown by a highly develop endoplasmic reticulum.

Myelocyte (10-15 micro meter diameter) : These cells are characterized by the presence of granules in the cytoplasm. Using Leishman's stain, the myelocyte may be classified according to the colour of the granules into neutrophil (most), eosinophil, and basophil (very scanty). The cytoplasm of the myelocytes as a whole is more extensive and less basophillic, the nucleoli have disappeared and the chromatin is coarser.

Leucocytes : Each type of metamyelocyte gives rise to the correspon ding leucocyte (neutro -phil, eosinophil, baso phil); the nucleus intended then becomes lobed, the granules, in fresh preparation (on a warm stage) instead of motionless, show dancing or streaming movements and the cytoplasm becomes more liquid, so that amoeboid movement occur. The more mature white cells are found lying just external to the sinusoids and pass actively through the intact endothelial lining of these vessels into the circulation.

(Ref. wright's)

Lymphopoiesis

The process of development and maturation of lymphocyte is called lymphopoiesis.

a. *In embryonic stage :* It develops at first from mesoderm. In later part of intrauterine life, the bone marrow also takes part in the lymphocyte formation.

b. *In adult :* The germinal centre of lymphoid tissue is the site of lymphocyte formation. The primitive stem cell is derived from the reticular cell of the lymphoid tissue & its gives rise to lymphoblasts. The mature lymphocytes are developed from the division of lymphoblasts into large lymphocyte and small lymphocyte. Some believe, there is a prolymphocytic stage in between the lymphoblast & large lymphocyte.

Stages of lymphopoiesis :

Reticular cell

↓

Primitive stem cell

↓

Lymphoblast

↓

Prolymphocyte

↓ ↓

Large lymphocyte small lymphocyte.

Leucopoiesis : The process of production of WBC under normal physiological condition is called leucopoiesis .

Granulopoiesis : The process of production of granulocytes under normal physiological condition is called granulopoiesis.

Difference between T & B lymphocyte

T-lymphocyte	*B-lymphocyte.*
1. Thymus dependent	1. Bursa dependent.
2. Concerned with cell mediated immunity.	2. concerned with humoral immunity.
3. Mainly deals with intra cellular bacteria & viruses.	3. Mainly deals with extra cellular bacteria.
4. Release soluble mediators : lymphokines.	4. Produce serum immuno globulins.
5. Constitute 70% of lympho-cytes in peripheral blood.	5. 20% of lymphoc;ytes in the peripheral blood.
6. Present in para-aortic area of a lymphnode.	6. Present in germinal centres of a lymph node.
7. Not inactivated by x-ray radiation.	7. Inactivated.
8. Inactivated by anti lympho-cytic serum.	8. Not inactivated.

Leucocytosis : Increased number of WBC above its upper normal of 11,000/cu mm of blood.

Causes :

A. Physiological :
   i. Emotion, Excercise
   ii. Pregnancy
   iii. Labour
   iv. Purpurium (few days after delivery).

B. Pathological : Any infection such as pyogenic infection, leukemia, malignancy, collagen diseases, tropical eosinophilia.

Leucopenia : Decreased number of WBC below normal limit of 4000/cu. mm of blood .

b. Chronic lymphocytic

3. Unusual type :
   i. Hairy cell
   ii. Polymorphocytic
   iii. Dismyelopoietic syndrome.

Monocyte-Macrophage System

(Reticuloendothelial System)

A large portion of monocytes, however, on entering the tissues and after becoming macrophages, become attached to the tissue and remain attached for month or even for years unless they are called upon to perform specific protective function. They have the same capabilities as the mobile macrophages to phagocytized large quantities of bacteria, viruses, necrotic tissue, or other foreign particles in the tissue and, when appropriately stimulated, they can break away from their attachments and once again become mobile macrophages that respond to chemotaxis and all the other stimuli related to the inflammatory process. Thus the body has a widespread 'monocyte-macrophage system' in virtually all tissue area.

The combination of monocytes, mobile macrophages, fixed tissue macrophages and a few specialized endothelial cells in the bone marrow, spleen and lymph node is collectively called *reticuloendothelial system*. However, all or almost all these cells originate from monocytic stem cells; therefore, the reticuloendothelial system is almost synonymous with the monocyte-macrophage system.

Yet because the term *reticuloendothelial system* is much better known in medical literature than the term *monocyte-macrophage system,* it should be remembered as a generalized phagocytic system located in all tissues but specially in those tissue areas where large quantities of particles, toxins, and unwanted substances must be destroyed.

Tissue macrophages in various tissues differ in appearances because of environmental differences, and they are known by different names ; Kuffer cells-in the liver , reticular cells-in lymph nodes, spleen and bone marrow , alveolar macrophages-in the alveoli of the lungs, tissue histocytes, clasmatocytes or fixed macrophages-in the subcutaneous tissues and microglia - in central nervous system.

(Ref. Guyton & Hall-11th edition; Page 432, 433)

Inflammation

Definition : Inflammation is the localized reaction of vascularized tissue in response to injury involving neurologic, vascular, humoral and cellular changes.

Cause :

Trauma, bacteria and other chemicals and foreign particles, heat or cold, surgery or any other phenomenon may cause inflammation of tissue.

Process of inflammation :

1. Release of vasodilator substances such as histamin, bradykinin, etc, from the injuried tissue.
2. Vasodilation and increase in blood flow to the inflammed area due to the vasodilators is called erythema.
3. Increased permeability of the capillaries with leakage of large quantities of fluid, proteins including fibrinogen into the intercellular spaces causes formation of nonpitting type of oedema.
4. Infiltration of the area by leukocytes.
5. And finally tissue healing by the ingrowth of fibrous tissue.

Purpose of inflammation :

i. To bring the phagocytic cell to the injuried area to engulf the invading agent .
ii. To bring some factors from serum to neutralise the infectious agents.

Cardinal signs of inflammation :

i. Calor : Increase temperature.
ii. Rubor : Redness.
iii. Dolar : Swelling.
iv. Functiolesia : Loss of function.

Principle cytokines

Cytokine	*Cell Lines Stimulated*	*Cytokine Source*
IL-1	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
IL-3	Erythrocyte Granulocyte Megakaryocyte Monocyte	T lymphocytes
IL-4	Basophil	T lymphocytes
IL-5	Eosinophil	T lymphocytes
IL-6	Erythrocyte Granulocyte Megakaryocyte Monocyte	Endothelial cells Fibroblasts Macrophages
IL-11	Erythrocyte Granulocyte Megakaryocyte	Fibroblasts Osteoblasts
Erythro-poietin	Erythrocyte	Kidney Kppffer cells of liver
SCF	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types

G-CSF	Granulocyte	Endothelial cells Fibroblasts Monocytes
GM-CSF	Erythrocyte Granulocyte Megakaryocyte	Endothelial cells Fibroblasts Monocytes
M-CSF	Monocyte	Endothelial cells Fibroblasts Monocytes
Thrombo-gdggsa	Megakaryocyte	Liver, kidney.q

L : Interleukin
CSF : Colony stimulating factor
SCF = Stem cell factor.

(Ref. Ganong 22th edition; page-520)

Macrophage and Nutrophil responses during inflammation

1. *Tissue macrophage is the First line of defense against infection* : Within minutes after inflammation those macrophages already present in the tissues, begin their phagocytic action are known as first line of defense.

   These are-

   i. Histocytes in the subcutaneous tissues
   ii. Alveolar macrophages in the lungs
   iii. Microglia in brain etc.

   When activated by the products of inflammation, the first effect is rapid enlargement of each of these cells. Next, break their attachments and become mobile, forming the first line of defense against infection during the first hour or so.

   (Ref. Guyton & Hall-11th edition; Page 434)

2. *Neutrophil invasion of the inflammed area is a second line of defense* : The neutrophils those are present in blood are called second line of defense, because they initiate their action next to tissue macrophages.

   Within the first hour or so after inflammation begins, large number of neutrophils begin to invade the inflamed area from blood and inititate the following reactions-

   i. They alter the inside surface of the capillary endothelium, causing neutrophils to stick to the capillary walls in the inflammed area.
   ii. They cause the endothelial cells of the capillaries and small venules to separate, allowing openings large enough for neutrophils to pass into tissue spaces.
   iii. Other products of the inflammation cause, chemotaxis of the neutrophils towards the injured tissues.

   Thus with in several hours after tissue damage begins, the area becomes well supplied with neutrophils and begin their scavenger functions for removal of foreign matter.

   (Ref. Guyton & Hall-11th edition; Page 435)

3. *A second macrophage invasion into the inflammed tissue is a third line of defense* : Reproduction of already present Macrophages, invasion of monocytes into inflamed area and rapid production & movement of neutrophils into inflamed area are third line of defense.

   The invading monocytes are immature cells requiring 8 hours or more to acquiring the full capacity for phagocytosis.

   (Ref. Guyton & Hall-11th edition; Page 435)

4. *Increased production of granulocytes and monocytes by the bone marrow is a fourth line of defense* : The fourth line of defense is greatly increased productuon of granulocytes and monocytes by the bone marrow. This results from stimulation of the committed granulocytic and monocytic stem cells. However it takes 3 to 4 days before newly formed granulocytes and monocytes reach the stage of leaving the bone marrow. If the stimulus from the inflamed area continues, the bone marrow can continue to produce these cells for months or even for years, sometimes at a rates of production as great as 50 times normal.

   (Ref. Guyton & Hall-11th edition; Page 435)

Immunity

Definition : Human body has the ability to resists almost all types of organism or toxins that tend to damage the tissues and organs. This capacity is called immunity.

Types :

1. *Acquired :* Immunity that develops against individual invading agents such as- bacteria, viruses, toxins and even foreign tissues from other animal. It divides into two types
   a. Humoral immunity or B cell immunity : Body develops circulating antibodies, which are globulin molecules that are capable of attacking the invading agents.
   b. Cell-mediated immunity or T cell immunity : Immunity is achieved throguh the formation of large number of activated lymphocytes that are specifically designed to destroy the foreign agents.
2. *Innate :* Immunity that develops from general processes rather than from process directed at specific disease organisms called innate immunity. It includes -
   a. Phagocytosis of bacteria and other invader by WBC and cells of tissue macrophage system.
   b. Destruction of organism by HCl in stomach and by the digestive enzymes of organism swallowed into the stomach.
   c. Resistance of the skin to invasion by organisms.
   d. Presence in the blood of certain chemical compounds that attatch to foreign organisms or toxins and destroy them such as -
      i. Lysozyme,

ii. Basic polypeptides,
iii. Compliment complex.
iv. Natural killer lymphocytes.

(Ref. Guyton & Hall-11th edition; Page 439)

Antigens

Definition : An antigen is a substance, protein or polysaccharide which when introduced into the system in a considerable dose and appropriate route is capable of inducing an immune response leadihg to formation of antibody with which it reacts specifically.

(Ref: Microbiology M. R. Chowdhury 3rd Page-94)

Haptens : These are the compounds which have lower molecular weights but still can act as antigens. These are called haptens.

After introduction haptens combine with a substance that is antigenic such as serum albumin and form a hapten-protein complex which inturn stimulates the production of an antibody specific aganist haptenic group only, Haptens are-Penicillin, Sulpha, picryl chloride etc.

(Ref. Microbiology M. R.chowdhury)

Criteria of an antigen :

1. It should be macro-molecule. Molecular weight more than $1x10^4$.
2. It shonld be protein or polysaccharide.
3. It should be foreign to the host.
4. It is specific in action.

(Ref. Microbiology M. R.chowdhury)

Antigenic determinants : In an antigenic compound, there are areas or sites which are antigenically active in contrast to other sites which are inactive. These active sites are called antigenic determinants.

(Ref. Microbiology M. R.chowdhury)

Antibody

Difinition : An antibody is a type of globulin (immuno-globulin) produced in response to an antigen with which it reacts specifically.

(Ref. Micro biology- M. R. chowdhury)

Classification of antibody : There are five general types of antibody respectively named-

a. IgM
b. IgG
c. IgA
d. IgD
e. IgE.

Ig stands for immunoglobulin. Of these ***Ig G*** comprises 75% of antibody of normal individuals. ***Ig E*** involved in allergic reaction .

(Ref. Guyton & Hall-11th edition; Page 444)

Structure of an antibody :

Antibody is a Y shaped molecule with a `yoke' and a `shaft'. It has four chains of polypeptide. Two are heavy chains (H) and are known as g, a, m and d in the molecules of IgG, IgA, IgM & IgD respectively. Two are short light chains (L) known as K (Kappa) or l (lamda)

Short light chains enclose heavy chains and all are linked together by disulphide bonds.

Heavy chains are bonded to each other and one short light chain is bound to each of the heavy chain.

There are two antigen combining sites in each molecule situated at the ends of the arms of `y'. There are two regions in each of heavy & short light chain, they are called "Variable portion" and "constant portion" The Variable portion are located near the antigen, combining site and constant portion determines the physical properties of antibody.

(Ref. Microbiology M. R.chowdhury)

Mechanism of action of antibody :

Antibodies act mainly in two different ways-

1. By direct attack on the invader.
2. By activation of the complement system.

Direct action of antibodies on invading agents : The antibodies can inactivate the invading agent in one of several ways, as follows :

1. *Agglutination:* In which multiple large particles with antigens on their surfaces, such as bacteria or red cells, are bound together into a clump.
2. *Precipitation :* In which the molecnlar complex of soluble antigen and antibody becomes so large that it is rendered insoluble and precipitates.
3. *Neutralization :* In which the antibodies cover the toxic sites of the antigenic agent.
4. *Lysis :* In which the antibody directly acts on the membranes of cellular agents and there by causing rupture of the cell.

(Ref. Guyton & Hall-11th edition; Page 444)

Platelets

Definition : Platelet or thrombocyte is one of the formed elements of blood.

Morphology :

i. Diameter : 1-4 µm (or 2-4 µm, *Ganong 21th edition*)
ii. Shape : Oval or rounded.
iii. Nucleus : Absent
(Some granules clamps at the centre of the cell and seems to be a nucleus called false nucleus).
iv. Cytoplasm : Divided into two parts -
   a. Hayalomere : Outer layer, containing granules material consists of microtubules and microfilaments

which contains thrombos thenin, responsible for change of the shape of platelets.

b. Chromatomere : Central deeply stained area, contains alpha granule, glycogen granule, mitochondria, ribosomes, serotonin, endo plasmic reticulum, phospholipid, ATP, ADP etc.

vi. Normal count : 1.5-3.0 lacs/cu. mm of blood. (about 30,000/μL of circulating blood. *(Ganong 22th edition)*

v. Life span : 8 to 12 days (Half life of about 4 days, Ganong).

The platelets in blood are replaced about once every 10 days; in other words, about 30,000 platelets are formed each day for each microliter of blood.

(Ref. Guyton 11th ed; page-457: Ganong 22th edition)

Function

1. *Haemostatic function (Role of platelet in haemostasis)* : It helps in the haemostasis in the following ways :
   a. Vasoconstriction : It liberates 5HT which is a vasoconstrictor.
   b. Formation of platelet plug : It adheres up with collagen tissue and each other and forms plug which prevent bleeding.
   c. Formation of prothrombin activator and help in coagulation : It secretes platelet thromboplastin, phospholipid, ADP which helps in the formation of prothrombin activator and help in coagulation.
   d. Clot retraction : Thrombosthenin of platelet in presence of ADP and Mg^{++} helps in clot retraction.
2. *Initiate blood clotting* : When blood is shed, the platelet disintegrate and liberate thromboplastin which activates prothrombin into thrombin.
3. *Repair capillary endothelium* : While in circulation, the platelet adhere to the damaged endothelial lining of the capillaries and thus bring about a speedy repair.
4. *Helps defensive mechanism* : Due to their increased agglutinating tendencies, they encircle the foreign bodies, thus aids in the defence mechanism of body.

Megakaryocyte

The parent cell of the platelet derived from the reticular cell. Megakaryocyte is a large cell of about 40-150 μm in diameter, polypoid in appearance and containing a large lobulated nucleus out of several nuclei found in the abundant cytoplasm. They are phagocytic and show amoeboid movement. These cell remain out side the vascular sinuses. They through out a part of their cytoplasm like the pseudopodium, through the vessel wall which is shed inside the sinus to form the platelet.

Development of platelets

The parent cell of the platelet is megakaryocyte, derived from reticular cell. They remain out side the vascular sinus. in bone morrow. the megakaryocytes through a part of their cytoplasm. like the pseudopodia, through the wall of the sinosoids. These pseudopodia are broken off in such a way that the individual fragment is surrounded by a unit membrane and are washed away by the blood stream. These fragments with unit membrane are platelet.

Destruction of platelets

Platelets are destroyed by reticuloendothelial cells of spleen & possibly of other places. Platelets contain serotonin, histamin, adrenaline & noradrenaline. They also contain thromboplastin and phosphatidyl-ethanolamine & phosphatidyl serine, needed for coagulation.

Thrombocytosis

Definition : Increased number of Platelet above the upper normal level of 50,000/cu. mm of blood.

Cause :

1. *Physiological :*
   a. Diurnal variation
   b. Exercise
   c. Meal
   d. Convalescence from infections.
2. *Pathological :*
   a. Haemorrhage.
   b. Allergic reaction
   c. Myeloid leukaemia.
   d. After surgical operation.
   e. Hodgkin's disease.

Thrombocytopenia

Decreased number of platelets below the lower normal limit of 2 lacs/ cu. mm of blood.

Haemorrhagic disorders

Classification :

A. ***Haemorrhagic disorders due to vascular defect***

Acquired :

1. Simple easy bruising
2. Senile purpura
3. The symptomatic vascular (non-thrombo cytopenic) purpuras
   a. Infections
   b. Drugs
   c. Uraemia
   d. Cushing's disease & adrenocorticoid administration
   e. Scurvy
   f. Dysproteinaemias
      i. Cryoglobulinaemia
      ii. Benign purpura hyperglobulinaemia
      iii. Macroglobulinaemia
      iv. Multiple myeloma

4. Miscellaneous disorders :
   a. Orthostatic purpuras
   b. Mechanical purpuras
   c. Fat metabolism
   d. Auto-erythrocyte sensitization
   e. Systemic disorders : Collagen diseases, specially polyarteritis nodosa, amyloidosis, allergy.

Congenital :

1. Hereditary haemorrhagic telangiectasia
2. Hereditary capillary fragility
3. Ehlers-Danlos disease.

B. ***Haemorrhagic disorders due to platelet defects*** *(Thrombocytopenia)*

Primary Thrombocytopenia :

1. Acute idiopathic thrombocytopenic purpura
2. Chronic idiopathic thrombocytopenic purpura
3. Cyclical (rare) idiopathic thrombocytopenic purpura.

Secondary thrombocytopenia :

1. Common causes :
   a. Drug & chemicals
   b. Leukaemias
   c. Aplastic anaemia
   d. Malignant lymphomas
   e. Bone marrow infiltration, secondary carcinoma, multiple myeloma, myelosclerosis.
   f. Hypersplenism
   g. D.L.E.
2. Less common causes :
   a. Infection
   b. Megaloblastic macrocytic ana-emia
   c. Liver disease.
   d. Alcoholism
   e. Massive blood transfusion
   f. Defibrination.
3. Rare causes :
   a. Thrombotic thrombocytopenic purpura
   b. Post-partum thrombocytopenia
   c. Post-transfusion thrombocytopenia
   d. Haemangiomas
   e. Food allergy
   f. Idiopathic cryoglobulinaemia

Neonatal and congenital thrombocytopenia :

1. Immune :
   a. Autoimmune : mothers with chronic ITP
   b. Isoimmune : platelet group incompatibility.
2. Infection : Congenital or neonatal
3. Drug adminstration to mother .
4. Congenital megakaryocytic hypoplasia :
   a. Isolated
   b. Associated with congenital abnormalities or pancytopenia.
5. Hereditary :
   a. Sex-linked :
      i. Pure form
      ii. Aldrich's syndrome.
   b. Autosomal dominant or recessive.
6. Congenital leukaemia
7. Giant haemangioma.

C. ***Haemorrhagic disorders due to Coagulation defects***

Congenital coagulation disorders :

1. The haemophilias :
   a. Haemophilia A
   b. Haemophilia B (Christmas disease)
2. Von Willebrand's disease
3. Other congenital deficiency disorders
   a. Fibrinogen deficiency
   b. Prothrombin deficiency
   c. Factor V deficiency
   d. Factor VII deficiency
   c. Factor X deficiency
   f. Factor XI deficiency
   g. Factor XII deficiency (Hageman factor)
   h. Factor XIII (Fibrin stabilizing factor) deficiency
   i. Fletcher factor deficiency.

Acquired coagulation disorders :

1. Vit-K deficiency
2. Liver disease
3. Anticoagulant drugs
4. Acute primary fibrinolysis
5. Disseminated intravascular coagulation (DIC)
6. Massive blood transfusion of stored blood
7. Increased circulating coagulation inihbitors.

Blood Group

Principal Blood Group

1. *ABO System* : Based upon the group specific substances, agglutinogens present in RBC membrane.
2. *Rh System* : Based upon the presence or absence of Rh antigen blood.
3. *M and N system* : Used in determination of paternity.

Classical blood group

ABO system of blood group are calledclassical blood group. Because-

i. It is the principal blood group.
ii. It maintain both 1st and 2nd part of Landstainer's law.
iii. The hazards of mismatched transfusion of ABO system is most effective and appears quickly than others.

Agglutinogens

It is a polysaccharide, present in the cell membrane of R.B.C less commonly in the salivary gland, pancreas, kidney, lungs etc. These are the blood group specific substances. There are thirty different agglutinogens. They appear in 6th week of foetal life and their concentration at birth is about 1/4th of adult. The concentration is maximum at puberty. There are two primary type of agglutinogen, A and B agglutiongen. Specific agglutinogen stimulate the production of specific agglutinin used in the production of immunity.

(Ref. wright's 13th page 47)

Genetic determination of the agglutinogen :

Two genes, one of each of two paired chromosomes, determined the ABO blood groups. These two genes are allelomorphic genes that can be any one of three types but only one type on each chromosome :

1. Type O
2. Type A
3. Type B.

The type O gene is either functionless or almost functionless, so that it causes no significant type O agglutinogen on the cells. On the other hand, type A and type B genes do cause strong agglutinogens on the cells.

The six possible combinations of genes, as shown in table, are OO, OA, OB, AA, BB, and AB. These combinations of genes are known as genotypes, and each person is one of the six genotypes.

Table. *Blood groups with their genotypes and their constituent Agglutinogens and Agglutinins.*

Genotype	*Blood types*	*Agglutinogens*	*Agglutinins*
OO	O	-	Anti-A and Anti-B
OA, AA	A	A	anti-B
OB, BB,	B	B	anti-A
AB	AB	A and B	-

We can observe from the table that a person with genotype OO produces no agglutinogens, and therefore, the blood type is O. A person with genotype OA or AA produces type A agglutinogens and therefore, has blood ype A. Genotypes OB or BB give type B blood and genotype AB gives type AB blood.

(Ref. Guyton & Hall-11th edition; Page 415, 416)

Agglutinin

The agglutinins are gamma globulins, most of them are IgM and IgG are produced in the blood in absence of corresponding agglutinogen in RBC cell membrane. They appears at 10 days after birth and the concentration is maximum at 10th years of age then decline. The two main agglutinins are alpha and beta. Each agglutinogen has its specific agglutinin.

α corresponding to agglutinogen A.

β corresponding to agglutinogen B.

(Ref. Guyton & Hall-11th edition & Wright's)

Q. ***Why agglutinins produced in persons who do not have the antigenic substance?***

Ans : A small quantities of group A and B antigens enter the body in food, in bacteria & in other ways and these substances initiate the development of ati-A or anti-B agglutinins in body.

Importance of blood group study :

i. To know blood group of individuals
ii. Blood transfusion
iii. Certain blood disease
iv. Paternity test (MN system)
v. In foreinsic medicine
vi. Various experimental studies in haematological laboratories.

Agglutination

It may be befined as clumping of red blood corpuscles due to antigen-antibody reaction.

Different group of ABO system

ABO system has 4 sub classes/group : A, B, AB and O.

Group	Agglutinogen (antigen)	Agglutinin (antibody)	Can receive blood from	Can donate to blood group	Percentage among caucasoids
A	A	Beta (β)	A,O	A,AB	41
B	B	Alpha (α)	B,O	B,AB	9
AB	AB	No	A,B,AB	AB	3
O	No	$\alpha\beta$	O	A,B,O	47

(Ref. Guyton & Hall 11th edition)

Group A is again divided into A_1 and A_2. So group AB is again divided into A_1B and A_2B. A_1 includes 75 persent of all group A, A_2 forms 25 percent.

(Ref. Wright's)

Tests done before blood transfusion

i. Group determination by slide method
ii. Cross matching

Determination of blood group *(Slide method)* : Blood is taken from the individual whose blood group is to be determined. It is

diluted 50 times with isotonic saline and thus a suspension of RBC is obtained.

Blood group	*α agglutinin*	*β agglutinin*
A	+	-
B	-	+
AB	+	+
O	-	-

Two seperate drops of suspension are placed on a microscopic slide and test serum containing agglutinin alpha (α) is mixed with one drop and test serum containing agglutinin beta (β) is mixed with other drop.

It is kept for some time and examined under microscope wheather agglutination occured or not. Blood group is obtained from the following data :

Blood group is A	If serum containing agglutinin α agglutinates.
Blood group is B	If serum containing agglutinin β agglutinates.
Blood group is AB	If both agglutinates.
Blood group is O	If none of them agglutinates.

Cross matching

Here the cells of donars blood are directly cross matched with plasma of recepient.

RBC suspension of donar's blood are prepared and mixed with defibrinogenated plasma of recipient. If there is no agglutination, donar and recepient are probably of compitable group and blood transfusion is possible between these two groups.

Difference between cross matching & blood grouping

Cross matching	*Blood grouping*
1. It is the only safe sure guard against transfusion.	1. It can not give such safe guard.
2. In cross matching recipient's serum is directly matched against the donor's RBC.	2. A Known serum (Anti A or anti B) is added to the persons blood.

Landstainer's law

If an agglutinogen is present in the cell membrane of RBC, the corresponding agglutinin must be absent from the plasma i.e in blood group A, agglutinogen A is present in the cell membrane of RBC and the corresponding agglutinin alpha is absent in the plasma.

If there is no agglutinogen in the cell membrane of RBC, the corresponding agglutinin must be present in the plasma i. e. blood group 'O' contain no agglutinogen in RBC membrane and corresponding agglutinin alpha and beta is present in the plasma.

Exception : Landstainer's law is not appreciated for Rh factor as the Rh negative blood contain no agglutinogen in cell membrane and no agglutinin in plasma; similarly absence of M or N substance is not accompanied by the presence of anti-M and anti-N in plasma.

(Ref. Wright's)

Universal Recepient

It is the blood group 'AB' as it can receive moderate amount (200-300 ml) of blood from all the other groups of ABO system.

Let us supose 'B' group is transfused to AB group. 'AB' group contains agglutinogen A and B & 'B' group contains agglutinogen B and agglutinin alpha. But agglutinin alpha present in the plasma of donar is negligible in respect to the agglutinogen A in recepients RBC. So when a moderate amount of blood (200-300ml) is given to AB group, no reaction takes place. But when a large amount of blood is transfused, then some side effects are observed.

Universal Donar

It is the blood group 'O' as it can donate moderate amount of blood (200 - 300ml) to all the other groups of ABO system.

Let us suppose that moderte amount of (200-300ml) group 'O' blood is given to group 'A'. 'A' group contains agglutinogen A in RBC membrane and agglutinin beta in plasma. "O" group contains agglutinin alpha and beta in plasma. But agglutinin alpha present in the donar's plasma is negligible in respect to agglutinogen A present in recepients RBC But when a large amount of blood is transfused, the some side effects are observed. So when a moderate amount of "O" group blood (200 - 300ml) is given to group 'A' no reaction takes place.

The classical terms universal donar and recipient, are however no longer valid as they ignore the complications produced by the existence of the Rh factors.

(Ref. Wright's)

Rh Blood group

It is one of the system of blood group due to the presence or absence of Rh antigen on the cell membrane of RBC. There are several Rh antigens - C, D, E, c, d, e of which D is common. In the Rh system, the agglutinins responsible for causing transfusion reaction almost never occur.

Rh positive blood : Blood with Rh antigen in the cell membrane and Rh antibody (anti-D) absent from theplasma is called Rh positive blood. It is about 85% of all Americans.

Importance : A person with Rh positive blood can receive both Rh positive and Rh negetive blood.

Rh negative blood : Blood group having no Rh antigen in the

RBC cell membrane and corresponding antibody (anti-D) in the plasma is called Rh negative blood. It is about 15% of all Caucasoids and 5% of all Americans.

Causes of development of Rh antibody in Rh negative person :

i. Transfusion of Rh positive blood to Rh negative individual.
ii. Presence of Rh positive foetus in Rh negative mother. It depends upon-
   a. Previous transfusion of Rh positive blood to the Rh negative mother.
   b. Number of pregnancy

Effects of Rh antibody (anti-D) on foetus :

1. Hydrops fetalis.
2. Erythroblastosis fetalis.

Hydrops fetalis : The patient is grossly oedematious; foetus either dies in uterus, or if born prematurely or at term, it dies within a few hours.

Erythroblastosis Fetalis

1. *Definition :* It is a clinical condition characterized by high erythroblast count in foetus.
2. *Cause* : Presence of Rh positive foetus in Rh negative mother. It depends upon-
   a. Previous transfusion of Rh positive blood to the Rh negative mother.
   b. Number of pregnancy.

   Explanation : When Rh positive fetus developed in Rh negative mother, the fetal erythrocyte enter into the maternal circulation during child birth. Then Rh antibody begins to appear in the maternal blood. In second pregnancy if the second fetus is also Rh positive, Rh antibodies (IgG) enter into the fetal circulation. Then agglutination and haemolysis occur (erythroblastosis fetalis).
   i. The infant is developed jaundice at the time of birth or become so within 24 hours.
   ii. There is no anaemia at the time of birth as RBC destruction is more or less compensated by hyperactivity of bone-marrow. But anaemia develops in first few days after birth as the destruction of RBC is maximum after birth.

   If the haemolysis is severe, the following conditions may developed :

   iii. *Kernicterus* : There may be severe neurological lesion involving the basal ganglion and secondarily yellow colouration of them by bile pigments (Kernicterus) due to undeveloped blood brain barrier.
   iv. *Hydrops fetalis* : The patient is grossly oedematious; foetus either dies in uterus, or if born prematurely or at term, it dies within a few hours.
3. Prevention : Anti-D injection is to be given to mother within 72 hours of delivery.
4. *Treament* : Replacement of the newborn infant's blood with Rh negative blood. Approximately 400ml of Rh negative blood is infused over a period of 1.5 or more hours while the baby's own Rh positive blood is being removed after a regular interval during the first weeks of life.

(Ref. Wright's)

Use of stored Blood

Blood transfusion is the ideal treatment for severe haemorrhage when given promptly and in adequate amounts. Modern medical and surgical procedures have greatly increased the number of transfusion; hence it has become necessary to institute blood banks, where blood stored at 4°c is always available.

Ordinarily such blood will be grouped and cross matched, intragroup transfusion being used. If time does not permit the grouping and cross matching of the recepient, Rh- blood should be used. In cases of extreme urgency (war casualties, train accidents) it may be necessary to give O Rh -ve blood, though a proportion of recepient will develop anti-D agglutinins.

(Ref. S. wright's)

Preservation of blood

Red cell undergo rapid changes during storage in simple citrate solution even at 4°C. They are preserved much longer in the presence of glucose.

This acts partly by liberating lactic acid, the resulting fall of pH favouring survival both in vivo and vitro, this effect is usually reinforced by using disodium hydrogen citrate instead of trisodium citrate as an anticoagulant. The cheif effect of glucose, however is to provide substrate for the metabolism, which even at 4°C is still important and contributes to cell survival. During cold storage, reduction of metabolism decreased active transport and cations move with concentration gradient so that cell K^+ fall and plasma K rises from the normal 4-5 mEq/L to 20 or 30 mEq/L in 2 weeks, while cell Na^+ normally about 10 mEq/L rises to 30 or 40 mEq/L.

The change may be sumerized as follows :

1. Increase in cell Na^+, decrease in cell K^+ with a net increase in cell total base and water.
2. As a result the cells swell and become shorter and fatter i.e more spherocytic in consequence they undergo haemolysis more rapidly in hypotonic solution and may rupture in vitro in salt concentrations as high as 0.8 percent.
3. Spontaneous haemolysis of the cells take place to an increasing degree while in contact with their own plasma in the blood bank.
4. The balance of phosphorylation and dephosphorylation is disturbed, phosphoric esters breaking down to liberate inorganic phosphate, which may rise in the cells from 2 to 30 mg per 100 ml, while adenosine triphosphate (ATP)

decreases, the fall expressed as P being from 10 to 20 mg per 100 ml cells. It is probable that the immediate link between glucose and glucolysis on the one hand and cell nutrition and active cation transport on the other is ATP. .

Q. ***Antibody of classical blood group is congenital but antibody of Rh blood group is acquired—Explain.***

Ans. Antibody or agglutinins of classical (ABO) blood group present in the blood in response to the corresponding agglutinogen. That is, blood group A contains- β antibody, group B contains antibody α; group AB contains no antibody and group O contain α, β antibody.

But in the case of individuals whose red cells contain no D agglutinogen, anti-D agglutinins are not naturally present in the plasma, but the production of anti-D is evoked by-

i. Transfusion of a Rh^- individual with Rh^+ blood (experimentally, 0.5ml may sufficient).

ii. By the presence of a Rh^+ fetus in a Rh- mother; in this case the titre of anti-D is not likely to be high, in less the woman has undergone one or more pregnancy.

(Ref. S wright's)

Hazards of mismatched Blood transfusion

The collective changes of the body observed after a mismatched transfusion is called hazards of mismatched transfusion. These are-

A. *Immediate Effect :* Soon after begining the transfusion, perhaps after a few ml of blood have been introduced, the patient complain of violent pain in the back & elsewhere & tightness in the chest. These symptoms are attributed to the agglutinated red cells forming clumps which block capillaries.

B *Delayed Effect :*

1. *Haemolysis of red blood cells :* Occasionally, antibodies are potent enough and composed of the appropriate class of immunoglobulins to cause immediate haemolysis but most frequently the cells agglutinate first and then are mainly entrapped in the peripheral vessels, which then haemolysed.
2. *Post-transfusion jaundice :* Due to the haemolysis of red blood cells concentration of haemoglobin in plasma and tissue spaces rises which is gradually ingested by phagocytic cells and converted into bilirubin. The concentration of bilirubin in the body fluids some times raised high enough to cause jaundice. But if liver function is normal, jaundice usually does not appear unless more than 300 to 500 ml of blood is haemolyzed in less than a day.
3. *Acute kidney shutdown :* It results from three different causes :
   i. The antigen-antibody reaction of transfusion reaction releases toxic substances from the haemolysing blood that cause powerful vasoconstriction.
   ii. The loss of circulating red cells along with production of toxic substances from cells from immune reaction often causes circulatory shock; the aterial blood pressure falls very slow and renal blood flow and urinary output decrease.
   iii. If the total amount of free haemoglobin in the circulating blood is greater than that of quantity which can bind with hepatoglobin; much of the excess leaks through the glomerular membranes into the kidney tubules. If this amount is still slight, it can be reabsorbed through the tubular epithelium into the blood and will cause no harm. But when excess, then the tubular Hb concentration rises and Hb precipitates and blocks many of the tubules; this is specially true if the urine is acidic.

   Thus renal vasoconstriction, circulatory shock and tubular blockage all add together to cause acute renal shut down. If renal shut down is complete, the patient dies within a week to 12 days.
4. Haemoglobinuria: Due to excessive bilirubin production it appears in urine.
5. Reaction due to anti-coagulant.

(Ref. Wright's & Guyton)

Q. *What are the hazards of blood transfusion?*

Ans. Hazards of blood transfusion may be -

a. Febrile reaction.
b. Allergic reaction.
c. Haemolysis.
d. Jaundice.
e. Circulatory overload.
f. Renal failure.
g. Transmission of diseases.
   1. Viral hepatitis
   2. AIDS
   3. Syphilis
   4. Malaria
   5. Other protozoal and bacterial infections.
h. Reaction due to infected blood.
i. Thrombophlebitis.
j. Transfusion haemosiderosis.
k. Complications of massive transfusion.
l. Post transfusion purpura etc.

Coagulation of Blood

Hemostasis

Definition : Hemostasis means prevention of blood loss or arrest of bleeding.

(Ref. Guyton & Hall-11th edition; Page 457)

Mechanism of Hemostasis

Whenever a blood vessel is severed or ruptured, hemostasis is achieved by several different mechanisms, including-

1. Vascular constriction
2. Formation of platelet plug
3. Formation of blood clot as a result of blood coagulation
4. Eventual growth of fibrous tissue into the blood clot to close the hole in the vessel permanently.

(Ref. Guyton & Hall-11th edition; Page 457)

Vascular Constriction : Immediately after a blood vessel is cut or ruptured, the stimulus of the traumatized vessel causes the wall of the vessel to contract; this instantaneously reduces the flow of blood from the vessel rupturd. The contraction results from both nervous reflexes initiated by pain impulses and local myogenic contraction of blood vessel-initiated by direct damage to vascular wall, local humoral factors from the traumatized tissues and blood platelets. The more the vessel that is traumatized, the greater the degree of spasm; this means that a sharply cut blood vessel usually bleeds much more than a blood vessel ruptured by crushing. Vascular spasm last for many minutes or even hours.

(Ref. Guyton & Hall-11th edition; Page 457)

Mechenism of formation of platelet plug : When platelets come in contact with a damaged vascular surface, such as the collagen fibres in the vscular wall or even damaged endothelial cells, they immediately change their characteristics dramastically. They begin to swell; they assume irregular forms with numerous irradiating pseudopods protruding from their surfaces; they become sticky so that they adhere to the collagen in the tissue and to a protein called Von Willebrand factor that spreads throughout the plasma; they secrete large quantities of ADP, and their enzymes form thromboxane A_2. The ADP and thromboxane A, inturn, act on the nearby platelets to activate them as well, and the stickiness of these additional platelets causes them to adhere to the originally activated platelets. Therefore, at the site of any rent in a blood vessel wall, the damaged vascular wall or extravascular tissues elicit activation of successively increased numbers of platelets, thus forming the platelet plug. This is at first a loose plug, but it is usually successful in blocking the blood loss if the vascular opening is small. Then, during the subsequent process of blood coagulation, fibrin threads form. These attach tightly to the platelets, thus constructing an unyielding plug.

(Ref. Guyton & Hall-11th edition; Page 458)

Blood Coagulation

Coagulation : When blood is shed it losses its mobility and converts into semisolid mass due to the conversion of soluble fibrinogen into insoluble fibrin by the action of thrombin. *The process by which the soluble fibrinogen is converted into insoluble fibrin and forms a clot is known as coagulation.*

Causes of coagulation :

i. Trauma to the blood.
ii. Trauma to the tissue.
iii. Contact of blood with collagen fibres in the vascular wall or even damaged endothelial cells.

(Ref. Guyton 11th edition)

Blood coagulation Factors

Cloting factor	*Synonyms*
Fibrinogen	Factor I
Prothrombin	Factor II
Factor III	Tissue thromboplastin (Tissue factor)
Factor IV	Calcium
Factor V	Proacelerin, *labile factor;* Ac- globulin; Ac-G
FactorVII	Serum prothrombin conversion accelerator (SPCA); proconvertin; *stable factor.*
Factor VIII	Antihemophillic factor; AHF; *antihemophillic globulin* (AHG); antihemophillic factor A.
Factor IX	Plasma thromboplastin component; (PTC); *christmas factor*; antihemophil lic factor B
Factor x	Stuart factor; *Stuart-power factor*
Factor xi	*Plasma thromboplastin antecedent ;* (PTA); antihemophillic factor C
Factor xii	Hageman factor
Factor xiii	Fibrin stabilizing factor
Prekallikrein	Fletcher factor
HMWK	High molecular weight kininogen Fitzgerald factor;
Ka	Kallikrein
PL	Platelet phospholipid

(Ref. Ganong 22th edition & Guyton 10th ed; Page-459)

Q. What are the Essential factors for coagulation ? Why they are called essential?

Ans. Essential factors are :

1. Fibrinogen : Factor I
2. Prothrombin : Factor II
3. Tissue factor : Factor III, Tissue thromboplastin
4. Calcium : Factor IV.

They are called essential because due to absence of any one of them coagulation does not occur.

Basic theory of blood coagulation

Over 50 important substances that affect blood coagulation have been found in the blood and tissues, some promoting coagulation, called *procoagulants*, and other inhibiting coagulation, called *anticoagulants*. Whether or not the blood will coagulate depends on the degree of balance between these two groups of substances.

Normally the anticoagulants predominate and the blood does not coagulate; but when a vessel is ruptured, procoagulants in the area of damage become activated and over ride the anti-coagulant, and then a clot does develop.

(Ref. Guyton & Hall-11th edition; Page 459)

General mechanism of blood coagulation

(Stages of blood coagulation):

1. First : A complex of substances called prothrombin activator is formed in response to rupture of the vessel or damage to the blood itself.
2. Second : The prothrombin activator catalyzes the conversion of prothrombin into thrombin.
3. Third : The thrombin acts as an enzyme to convert fibrinogen into fibrin threads that enmesh platelets, blood cells, and plasma to form the clot itself.

(Ref. Guyton & Hall-11th edition; Page 459)

Formation of Prothrombin Activator

Prothrombin activator is formed in two basic ways-

a. By the extrinsic pathway that begin with trauma to the vascular wall and surrounding tissues.

b. By the intrinsic pathway that begin in the blood itself.

Extrinsic mechanism : The extrinsic mechanism for initiating the formation of prothrombin activator begin with blood coming in contact with traumatized vascular wall or extravacular tissues and occurs according to the following three basic steps.

1. *Release of tissue factor (or tissue thromboplastin) :* Traumatized tissue releases a complex of several factors called tissue factor or tissue thromboplastin. This includes specially *phospholipids* from the membranes of the tissue plus a *lipoprotein complex*, that functions mainly as a *proteolytic enzyme*.
2. *Activation of factor X - role of factor VII and tissue factor :* The lipoprotein complexe of tissue factor further complexes with blood coagulation factor VII, and in the presence of calcium ions, acts enzymatically on factor X to form activated factor X (Xa).
3. *Effect of activated factor X (Xa) to form prothrombin activator- role of factor V :* The activated factor X combines immediately with tissue phospholipids that are part of tissue factor or with additional phospholipids released from platelets as well as with factor V to form the complex called prothrombin activator.

(Within a few seconds, in the presence of calcium ions, this splits prothrombin to form thrombin. Then thrombin acts as an enzyme to convert fibrinogen into fibrin threads that enmesh platelets, blood cells, and plasma to form the clot itself.)

(Ref. Guyton & Hall-11th edition; Page 461, 462)

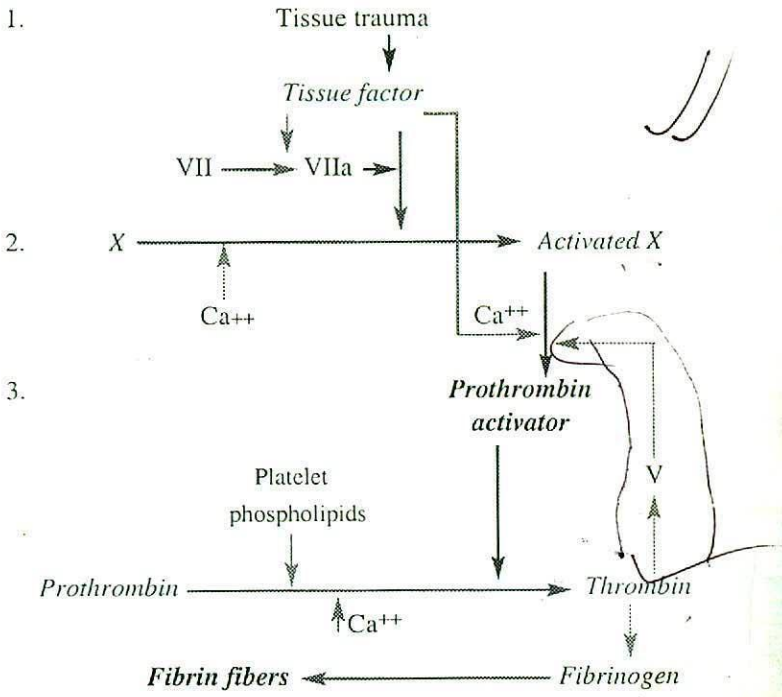


Fig. 5-16. *The extrinsic pathway for initiating blood coagulation.*

(Ref. Guyton & Hall-11th edition; Page 461)

Intrinsic mechanism : It begins with trauma to the blood itself or exposure of the blood to collagen from a traumatized blood vessel wall and continues through the following series of cascading reactions.

1. *Activation of factor XII and release of platelet phospholipids by blood trauma :* Trauma to the blood or exposure of the blood to collagen from a traumatized blood vessel wall alters two important clotting factors in the blood: factor XII and the platelets. When factor XII coming into contact with collagen or with a wetable surface such as glass (particularly a negatively charged surfac), it takes on a new configuration that converts it into a proteolytic enzyme called *activated factor XII*. Blood trauma also damages the platelets and this releases plateles phospholipid which also plays a role in subsequent clotting reactions.
2. *Activation of factor XI :* The activated factor XII acts enzymatically on factors XI to activates this factor as well. This reaction also requires *HMW kininogen*, and is accelerated by *prekallikrein*.

3. *Activation of factor IX by activated factor XI :* The activated factor XI acts enzymatically on factor IX in presence of Ca^{++} and converts it into activated factor IX.

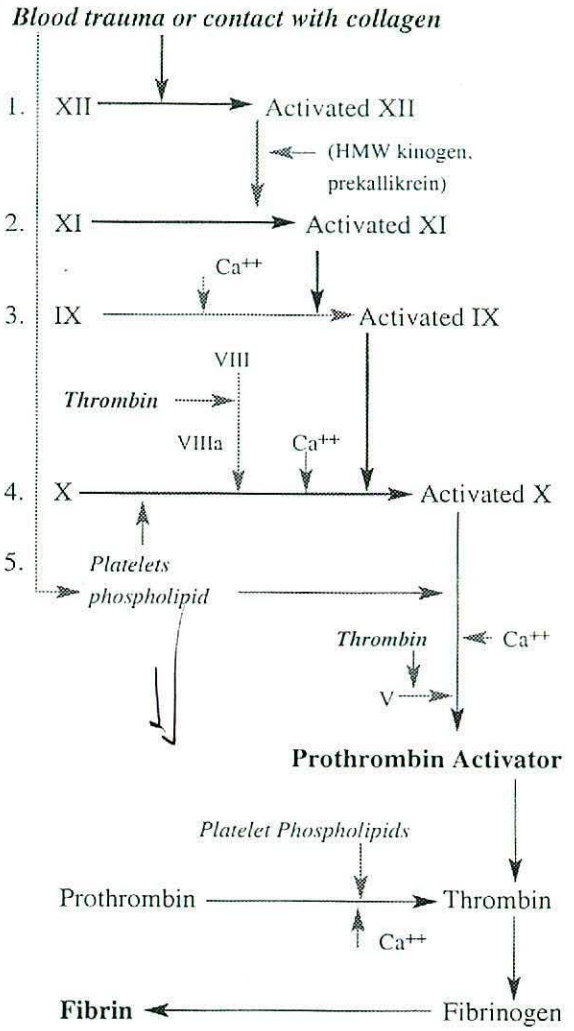


Fig. 5 - 17. *The intrinsic pathway for initiating blood coagulation.*

4. *Activation of factor X -role of factor VIII :* The activated factor IX, acting in concert with factor VIII and with platelets phospholipids and factor 3 from traumatized platelets, activates factor X.

5. *Activation of activated factor X to form prothrombin activator- role of factor V :* Activated factor X combines with factor V and platelet or tissue phospholipids to form the complex called prothrombin activator.

(Within a few seconds, in the presence of calcium ions, this splits prothrombin to form thrombin. Then thrombin acts as an enzyme to convert fibrinogen into fibrin threads that enmesh platelets, blood cells, and plasma to form the clot itself.)

(Ref. Guyton & Hall-11th edition; Page 462, 463)

Convertion of prothrombin to thrombin

Prothrombin is converted into thrombin under the influence of prothrombin activator and calcium ions.

The rate of formation of thrombin from prothrombin is almost directly proportional to the quantity of prothrombin activator available, which itself is approximately prortional to the degree of trauma to the vessel wall or to the blood.

(Ref. Guyton & Hall-11th edition; Page 459)

Conversion of Fibrinogen to Fibrin *(Formation of clot)*

Thrombin acts on fibrinogen to remove four low molecular weight peptides from each molecule of fibrinogen, forming a molecule of fibrin monomer that has the automatic capability of polymerizing with other fibrin monomer molecules. Therefore many fibrin monomer molecules polymerize within second into long fibrin threads that form the reticulum of the clot. In early stages of polymerization, the fibrin monomer molecules are held together by weak non-covalent bonds and the threads also are not cross linked with each other. But when fibrin stabilizing factor is activated, operates as an enzyme to cause covalent bonds between fibrin monomer molecules as well as multiple cross-linkages between the adjacent fibrin threads, thus adding tremendously to the three dimentional strength of the fibrin mesh work (clot).

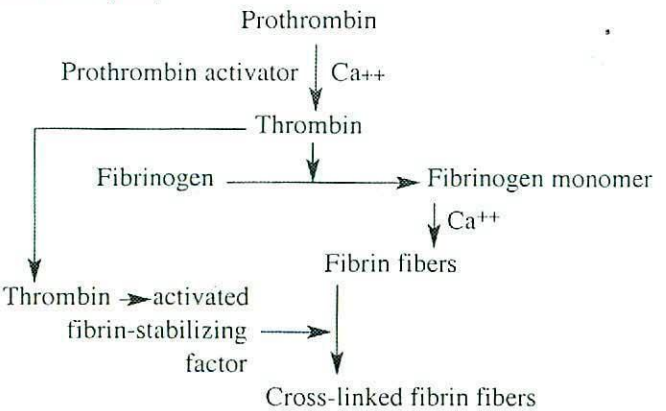


Fig. 5-18. *Schema for conversion of prothrombin to thrombin and polymerization of fibrinogen to form fibrin fibers.*

(Ref. Guyton & Hall-11th edition; Page 460)

Seeger's concept of blood coagulation :

Prothrombin is converted into prethrombin and auto-prothrombin III. Autoprothrombin III then converted into autoprothrombin C & peptides in presence of calcium ions, tissue extract & platelet co-factor 1. Autoprothrombin C then acts upon prethrombin and form thrombin. This process is

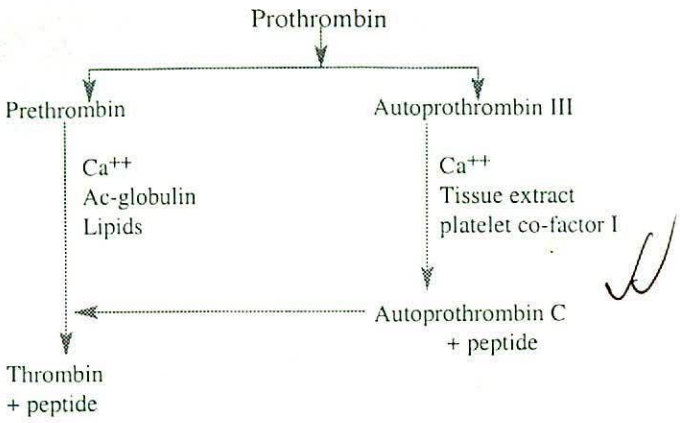


Table. *Seeger's concept of blood coagulation* *(Ref. S. Wright's)*

known as Seeger's concept of blood coagulation.

(Ref. S. Wright's)

Defference between extrinsic And intrinsic mechanism of coagulation.

Extrinsic mechanism	*Intrinsic mechanism*
1. It occurs due to trauma to tissue.	1. It occurs due to trauma to blood itself or contact with collagen.
2. It is explosive in nature to produce prothrombin activator.	2. It is much slower.
3. Tissue phospholipid acts in this pathway.	3. Platelets phospholipid acts in this pathway.
4. It begins with the activation of factor X and requires factor VII, V & Ca^{++}	4. It begins with the activation of factor XII & requires factor XI, IX, VIII, X & V and Ca^{++}.
5. Thrombin is not required in this pathway.	5. Thrombin is required.
6. In it, the composition of prothrombin activator is tissue phospholipid, factor X, V, and Ca++.	6. In it, the composition of prothrombin activator is platelet phospholipid, factor X, Vand Ca++.

Clot

Definition : When blood is shed, it converts into a semisolid mass due to conversion of soluble fibrinogen into insoluble fibrin thread. This solid mass is called clot. The clot is composed of a meshwark of fibrin threads running in all dirrection & entrapping blood cells, platelets and plasma.

Blood does not clot inside the blood vessel due to-

1. *Endothelial surface factor :*
   a. Smoothness of the endothelium prevents contact activation of the intrinsic clotting system i.e. prevents the initiation of the formation of the prothrombin activator.
   b. Presence of monomolecular layer of protein adsorbed to the inner surface of the endothelium that repel the platelets and clotting factors, thereby preventing activation of clotting.
2. *Speed of the blood flow :* It prevents the intravascular coagulation.
3. *Presence of natural anti-coagulant :*
   a. *Heparin :* Secreted by mast cells or basophil, prevents coagulation.
   b. *Antithrombin :* It removes the thrombin from blood as a result fibringogen can not be converted into fibrin.
   c. *Fibrin :* Fibrin thread itself absorbs thrombin.
   d. Absence of coagulation factor III or thromboplastin.

Q. ***What is clot retraction?***

Ans.

Within a few minutes after a clot is formed, it begins to contract and losses most of its fluid within 30 to 60 minutes and reduced in size. This is called clot retraction. The fluid that comes out after clot retraction is called serum which is straw in colour.

Q. ***Name the different anti-coagulant and their mode of action.***

Ans.

1. *Natural anti-coagulant :*
   a. Heparin: Blood level .01 mg/100ml of blood.
      i. By itself, heparin has little or no anticoagulant property, but it combines with antithrombin III and removes thrombin from blood and thus to acts as an anti-coagulant.
      ii. The complex of heparin and anti-thrombin III also removes several other activated factor from blood such as factor XII, XI, IX and X. Thus enhancing the efectiveness of anti-coagulant.
   b. Antithrombin : It removes the thrombin form blood.
   c. Fibrin : Fibrin threads itself absorbs thrombin.
   d. Anti-thromboplastin.
2. *Artificial anti-coagulant :*
   a. Heparin : It also can be prepared artificially.
   b. Sodium citrate or oxalate: By precipitating Ca^{++} from blood as calcium citrate or oxalate.
   c. EDTA (Ethylenediamine-tetraacetate) : Acts by removing ionic Ca^{++} from blood.
   d. Phenindione.

(Ref. Guyton & Hall-11th edition & others)

Q *What is haemorrhage? what are the causes of haemorrhagic state ?*

Ans. Haemorrhage is the abnormal internal or external discharge of blood. May be arterial, venous or capillary from blood vesssels into tissues, into or from the body.

Effect of haemorrhage : Decrease Blood volume → Decrease of venous return → Decrease of cardiac output → Decrease of blood pressure.

The clinical haemorrhagic states are due to

1. Defect in blood clotting or coagulation disorder :
   a. Fibrinogenopenia: Due to lack of fibringogen, coagulation time quick and prothrombin time prolongs.
   b. Hypoprothrombinaemia: Dimination of prothrombin formation due to lack of Vitamin K which helps in the synthesis of it. Prothrombin time is prolonged.
   c. Haemophilia : Due to lack of factor VIII, coagulation time abnormally prolongs here.
2. Defect due to vascular system or capillary contraction.
3. Defect due to deficiency of platelets.
4. The combined defect.

Examples of diseases due to deficiency of clotting factors.

Deficiency of Factor :	*Clinical Syndrome*	*Cause*
Factor i.	Afibrinogenesis	Depletion during pregnancy with mature separation of placenta; also congenital (rare).
Factor ii.	Hypoprothrom-binemia (hemorr-hagic tendency in liver disease	Decreased hepatic synthesis, usually secondary to vitamin K deficiency.
Factor v.	Parahemophilia	Congenital
Factor vii.	Hypoconvertivemia	Congenital
Factor viii.	Hemophilia A (classic hemophilia)	Congenital defect due to various abnormalities of the gene of X chromosome that codes for factor VIII; diseases is therefore inherited as sex linked characteristic.
Factor ix.	Hemophilia B (Christmas disease)	Congenital
Factor x.	Sturt-Prower factor deficiency	Congenital
Factor xi.	PTA deficiency	Congenital
Factor xii.	Hageman trait	Congenital

Difference between haemostasis and coagulation

Haemostasis	*Coagulation*
1. Haemostasis means prevention of blood loss or arrest of bleeding.	1. Coagulation is the process by which fibrinogon is converted into fibrin thread and forms a clot.
2. Haemostasis is achieved by the vascular spasm, platelet plug formation.	2. Coagulations begins with the formations of prothrombin activator.
3. Haemostasis occurs through 4 steps.	3. Coagulation is one of the steps of haemostasis.
4. Platelet is essential for haemostasis.	4. Platelet is not essential for coagulation (e.g extrinsisc pathway).

Q. *What is fibrinloysis? What is its singnificance?*

Definition : It is the process by which fibrin threads are liquified or dissolved by profibrinolysin. The profibrinolysin is converted into fibrinolysin by means of an activating enzyme present in the tissue serum, urine and some bacteria. The fibrinolysin then disintegrates the fibrin network and dissolves the clot into soluble peptide. But it also acts upon prothrombin and factor VIII, V and XII etc.

Significance :

i. Allows clearing of blood vessels.
ii. Re-open the cloted blood vessel
iii. Remove very minute clots from peripheral vessels.

Haemophilia

Definition : It is a sex linked disease transmitted by females who themselves show no symptom, to male who manifests signs of the disease. It is characterized by abnormally problonged coagulation time but the bleeding time is normal.

Types :

1. Haemophilia A : Due to deficiency of factor VIII (85%).
2. Haemophilia B : Due to deficiency of factor IX (15%).

Effects : Joint become severely damaged because of repeated haemorrhage during exercise or after injury.

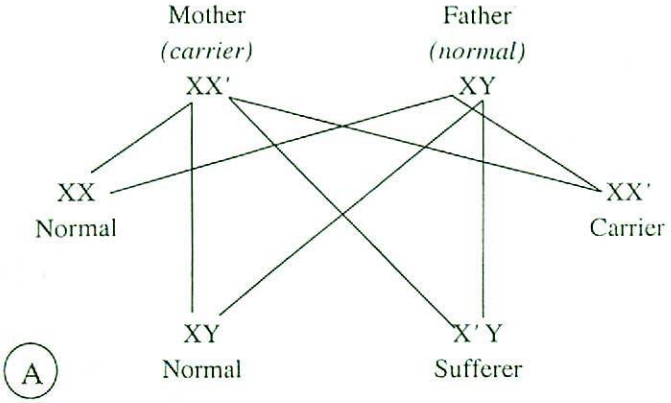
Q. *Why factor VIII is called anti-hemophillic factor?*

Ans. When either factor VIII or platelets are short in supply, activation of factor X into active factor X is deficient. Factor VIII is the factor that is missing in the person who has classic hemophilia, for which reason factor VIII is called anti hemophillic factor.

Q. *Female is carrier but not sufferer-why?*

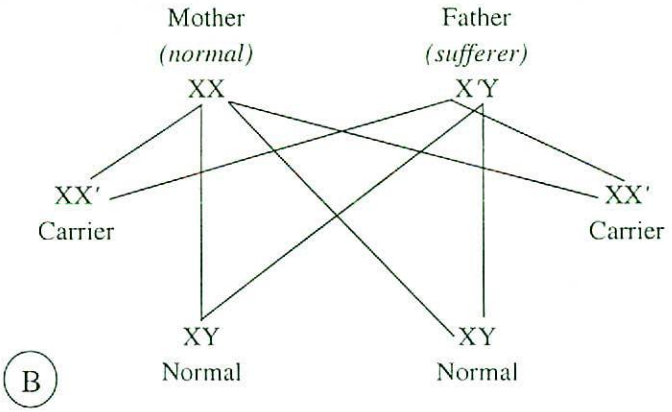
Ans. Haemophilia occurs due to deficiency of factor viii and ix. Both these factors are transmitted genetically by way of the

female chromosome as a recessive trait.



(A)

Therefore, almost never will a woman have hemophilia because at least one of her two X chromosomes will have the



(B)

appropriate genes. However, if one of her X chromosomes is deficient, she will be a haemophilia carrier, transmitting the disease to half her male offspring and transmitting the carrier state to half her female offspring.

(Ref. Guyton & Hall-11th edition; Page 465)

Purpura

It is a clinical condition characterized by a tendency of spontaneous haemorhage beneath the skin and mucous membrane from the various internal organs. It is due to platelet deficiency or vascular defects. Here coagulation time is normal but bleeding time is prolonged.

It is of two types :

1. *Thrombocytopenic purpura :* Due to low platelet count. Skin displays small purplish pteeches.
2. *Thrombosthenic purpura :* Platelet count normal but capillary are tortous and abnormal.

Difference Between Hemophilia and purpura

Hemophilia	*Purpura*
1. Inherited sex linked anomaly, transmitted by female to male who manifestes the sign.	1. It is a clinical condition characterised by a tendency of spontaneous haemorrhage beneath the skin and mucous membrane from the various internal organs.
2. Here coagulation time is prolonged, bleeding time is normal.	2. Here coagulation time is normal but bleeding time prolonged.
3. It is due to deficiency of factor VIII, IX and XI	3. It is due to platelet deficiency or capillary factor.
4. It is inherited, only shown in male.	4. It is not inherited, manifested by both male and female.

Bleeding time

Definition : Bleeding means extravastion of blood i.e. a process of coming out of blood from the vasculature. Bleeding time is the time required for the stopage of bleeding.

Normal bleeding time is 1 to 6 minutes. However, the time depends largely on the depth of the wound and other degree of hyperemia in the finger at the time of the test. Lack of several of the clotting factors can prolong the bleeding time but this is specially prolonged by lack of platelets.

Method of determination of bleeding time :

There are two methods :

1. *Duke's method :*
   i. After all antiseptic precautions prick the finger tip. Start the stop-watch when blood starts oozing out.
   ii. With a round blotting paper, touch the bleeding point at an 15 seconds interval, until bleeding stops. The time difference will give the bleeding time.
2. *Ivy's method :*
   i. Inflate the cuff of the sphygmomanometer over the elbow joint and keep at the preseure of 10 mm of Hg till the end of the test.
   ii. Taking all the antiseptic precautions, prick the skin with a needle at two places, 5-10 cm apart on the anterior surface of the forearm.
   iii. Repeat the second step of Duke's method.

Normal value :

i. Ivy's method : upto 11 min.
ii. Duke's method : upto 8 min.

Clotting time

i. *Definition :* Time required for coagulation of blood.
ii. *Normal clotting time :* It is about 6 to 10 minutes.
iii. *Normal clotting time is prlonged due to-*
   a. Haemophilia
   b. Deficiency of any of the factors in the intrinsie pathway for clotting

Measurement : *Methods of determination of clotting time :*
Following two methods are commonly used :

1. ***Lee and white method*** (Whole blood coagulation time) :
   i. Arrange four small test tubes which are previously warmed to 37°C.
   ii. Draw 5 ml. blood from antecubital vein. Start the stop-watch as soon as the blood enters the syringe.
   iii. Transfer one ml. of blood into each of the four test tubes.
   iv. Tilt each test tube after an interval of 30 seconds.
   v. Stop the stop-watch immediately when the blood does not spil out, even when the tube is tilted upto 90° angle.
   vi. Note the time period for the four tubes and the average of them will give the coagulation time.
2. ***Capillary tube method*** :
   i. After taking all antiseptic precautions, prick the finger or the ear lobe. Start the stop-watch when blood starts oozing out.
   ii. Wipe out the first drop of blood. By touching the tip to the blood drop, fill the capillary tube.
   iii. Break the capillary tube upto half an inch length, each time at an interval of thirty seconds.
   iv. Stop the watch at the point when fibrin thread appears from the side of the broken tubes. Note the time which will give the coagulation time.

Normal value :
i. 5 to 11 min in lee and white method.
ii. 2 to 4 min in capillary tube method.

Prthrombin time
i. *Definition* : The time required for coagulation to take place is known as the prothrombin time.
ii. *Normal value:* Approximately 12 seconds.
iii. *Importance* : rothrombin time gives an indication of the total quantity of prothormbin in the blood & also the quantities of other clotting factors in the body.

(Ref. Guyton 11th edition)

Bone Marrow

In the adult, red blood cells, many white blood cells, and platelets are formed in the bone marrow. In the fetus, blood cells are also formed in the liver and spleen, and in adults such extramedullary hematopoiesis may occur in diseases in which the bone marrow becomes destroyed or fibrosed. In children, blood cells are actively produced in the marrow cavities of the bones. By age 20, the marrow in the cavites of the long bones, except for the upper humerus and femur, has become inactive. Active cellular marrow is called red marrow; inactive marrow that is infiltrated with fat is called yellow marrow.

The bone marrow is actually one of the largest organs in the body, approaching the size and weight of the liver. It is also one of the most active. Normally 75% of the cells in the marrow belong ot the white blood cell-producing myeloid series and only 25% are maturing red cells, even though there are over 500 times as many red cells in the circulation as there are white cells. The difference in the marrow reflects the fact that average life span of white cells is short whereas that of red cells is long.

The bone marrow contains mulipotent uncommited stem cells (pluripotential stem cells) that differentiate into one or another type of committed stem cells (progenitor cells). These in turn differentiate into the various differentiated types of blood cells. There is now evidence that there are pluripotential stem cells and progenitor cells in the blood as well. The pluripotential cells few in number but are capable of completely replacing the bone marrow when injected into a host whose own bone marrow has been completely destroyed.

There are separate pools of progenitor cells for megakaryocytes, lymphocytes, erythrocytes, eosinophils, and basophils, whereas neutrophils and monocytes arise from a common precursor. The bone marrow stem cells are also the source of osteoclasts, Kupffer cells, mast cells, dentritic cells, and Langerhans cells.

Some important biochemical & haematological normal values

Venous Blood : Approx. Adult Reference values

Analysis	*Reference values*	
	S.I. Units	*Others units*
Acid phosphatase		0.1-0.4 u/1
Alanine aminotransferase (ALT)		10-40 u/1
(glutamic-pyruvic transaminase -GPT)		
Alkaline phosphatase		40-100 u/1
Asparatate Aminotransferase (AST)		10-35 u/1
(glutamic-oxaloacetic transaminase (GOT)		
Bilirubin (toal)	2–17 micro mol/1	0.3-1.0 mg/dl
Calcium (total)	2.12–2.62 mmol/1	8.5-10.5 mg/dl
Carbon dioxide (Total)	24–30 mmol/1	
Cholesterol (fasting)	3.6–6.7 mmol/1	145-270 mg/dl
Creatinine	55–150 micro mol/1	0.6-1.7 mg/dl
a–Fetoprotein	2–6 units/ml	< 30 mg/ml
Gulcose (fasting)	< 5.5 mmol/1	100 mg/dl
D–Glutamyl transferase		male— 10-55 unit/L
		female— 5-35 unit/L
Immunoglobulins (Ig) IgA	0.5–4.0 g/1 (40–300 i.u./1)	
IgG	5.0–13.0 g/1 (60–160 i.u./1)	
IgM-males	0.3–2.2 g/1 (40–270 i.u./1)	
females—	0.4–2.5 g/1 (50-300 i.u./1)	
Iron—males	14–32 micro mol/1	77-178 micro g/dl
females	10–28 micro mol/1	56-156 micro g/dl
Iron binding capacity (total)	45–72 micro mol/1	250-400 micro g/dl
Iron binding capacity (saturation)		14-47%
Lactate	0.4–1.4 mmol/1	3.6-13.0 mg/dl
Lactate dehydrogenase (LD)		100-300 u/l
Potassium	3.3-4.7 mmol/1	
Sodium	132-144 mmol/1	
Proteins—total	62-82 g/1	6.2-8.2 g/dl
Albumin	36-52 g/1	3.6-5.2 g/dl
Globulins	24-37 g/1	2.4-3.7 g/dl
Urea	2.5-6.6 mmol/1	15-40 mg/dl
Vitamin A	0.7-17 micro mol/1	20-50 micro g/dl

Arterial blood analysis :

Analysis	*Reference values*	
	S.I. units	*Other units*
Ammonia	11-35 micro mol per 1	20-63 micro g per dl
Hydrogen ion	36-44 n mol per 1	PH 7.37-7.45
Carbon dioxide ($PaCO^2$)	4.8-6.0 kPa	36-45 mm Hg
Plasma bicarbonate	21-27.5 mmol per 1	21-27.5 m Eq per L
Oxygen (PaO^2)	11-13 kPa	83-98 mm Hg
Cerebrospinal fluid		Gastrointestinal
(Lumber puncture)		Faecal fat : < 18 mmol per 24 h
Cells : 5/mm3 (all lymphocytes)		(< 5g per 24 h)
Glucose :	2.5-4.0 mmol per L (45-72 mg/dl)	Stool wt : < 200 g per 24 h
	50 to 70% of plasma concentration.	Liver
		Copper : < 50 micro g per g dry weight
Immunoglobulin G :	20-50 mg per 1	
Protein (total) :	100-400 mg per 1	(Wilson's disease > 250 micro g per g dry weight).
Pressure :	5-15 cm C.S.F.	

Haematological values

	S.I. Units	Other Units
Bleeding time	Up to II min	
Body fluid (total)		50% (obese) — 70% (lean) of body wt.
Intracellular		30-40% of body wt.
Extracellular		20-30 of body wt.
Blood volume		
Red cell mass—men	30 ± 5 ml per kg	
Women	25 ± 5 ml per kg	
Plasma volume (both sexes)	45 ± 5 ml per kg	
Total blood vol, men	75 ± 10 ml per kg	
women	70 ± 10 ml per kg	
E.S.R. (westergren)	0—6 mm in 1 h-normal	
(Figures given are for pts	7-20 mm in 1 h-doubtful	
under 60 yrs of age)	> 20 mm in 1 h-abnormal	
Fibrinogen	1.5-4.0 g per 1	150–400 mg per dl
Folate— Serum	2-20 micro g per 1	2-20 ng per ml
— red cell	> 100 micro g per 1	> 100 ng per ml.
Haemoglobin— men	13-18 g per dl	13-18 g per 100 ml
— women	11.5-16.5 g per dl	11.5-16.5 g per 100 ml
Haptoglobin	0.3-2.0 g per 1	30-200 mg per dl
Leucocytes—adults	4.0-11.0 x 10^9 per 1	4000-11000 per micro 1
D.C. (W.B.C.)		4.0-11.0 x 10^3 per m3
Neutrophil	2.5-7.5X10^9 per 1	40-75%
Lymphocytes	1.0-3.5 x 10^9 per 1	20-40%
Monocytes	0.2-0.8 x 10^9 per 1	2-10%
Eosinophil	0.04-0.4 x 10^9 per 1	1-6%
Basophil	0.01-0.1 x 10^9 per 1	0-1%
MCH	27-32 pg	27-32 micro micro g
MCHC	30-35g per dl	30-35%
MCV	78-98 fl	78-98 micro3 or micro m3
PVC or haematocrit—men	0.40-0.54	40-54%
women	0.35-0.47	35-47%
Platelets	150-400 x 10^9 per 1	15000-400000 per micro 1 or mm3
Prothromibin time	11-15s	
Red cell count—men	4.5-6.5 x 10^{12} per 1	4.5-6.5 x 10^6 per micro 1 or mm.
—women	3.8-5.8 x 10^{12} per 1	3.8-5.8 x 10^6 per micro 1 or mm.
Red cell life span (mean)	120 d	0.2-2%
Reticulocytes (adults)	10-100 x 10^9 per 1	160-925 pg per ml or micro micro g per ml.
Vit B12 (serum cyanocobalamin)	160-925 ng per 1	

(From Davidson's Medicine, 16 th edition)

Directions : *Write T for true & F for false against each of the following statement.*

Introduction

Q.01. **Blood**
- T a. is responsible for 8% of the total body weight.
- T b. acts as a buffer.
- T c. is a modified connective tissue.
- F d. pH ranges from 7.2 to 7.6.
- F e. volume decrease in polyeythemia.

Q.02. **Blood volume is**
- T a. 70 ml/kg body weight.
- T b. about 8% of the total body weight.
- T c. calculated by dye dilution method.
- T d. decreased after haemorrhage.
- F e. increased in severe dehydration.

Q.03. **Specific gravity of blood**
- T a. rapidly falls after birth.
- T b. is 1056
- T c. depends on its plasma protein contents
- F d. of foetal blood at full term is lowest.
- F e. is decreased in dehydration.

Q.04. **Packed cell volume is**
- T a. increased in bone marrow hyperplasia.
- T b. measured by centrifugation of blood.
- T c. determined by wintrobe method.
- T d. increased after fluid loss.
- F e. decreased in polycythemia.

Q.05. **Nitrogenous waste products in plasma are**
- T a. creatinine.
- T b. urea.
- F c. albumin.
- F d. amino acid.
- F e. cholesterol.

Q.06. **Viscocity of blood is**
- T a. related to haematocrit value
- T b. decreased in people with iron deficiency
- F c. related to temperature.
- F d. not increased in acclimatized mountaineer.
- F e. dependent on plasma protein only.

Q.07. **Hemopoietic stem cells are found in**
- T a. peripheral blood.
- T b. bone marrow.
- F c. kidney.
- F d. liver.
- F e. serum.

Q.08. **Growth and production of all stem cells are stimulated by**
- T a. interleukin-3.
- F b. interleukin-1.
- F c. growth hormone.
- F d. erythropoietin.
- F e. hypoxia.

Q.09. **Increased blood viscosity and slow circulation causes**
- T a. RBC rouleux formation
- F b. Increased plasma skimming
- F c. Increased number of RBC in capillaries
- F d. None
- F e. All of the above.

Q. 10. **D_2O (Deuterium oxide) is used to measure volume of**
- T a. Total body water
- F b. Blood
- F c. Extracellular fluid
- F d. Intracellular fluid
- F e. Plasma.

Q. 11. **Normal blood pH is**
- T a. 7.40
- F b. 7.20
- F c. 7.30
- F d. 7.70
- F e. 7.10

Q. 12. **Decreased MCHC is found in**
- T a. Microcytic hypochromic anemia
- F b. Megaloblastic anaemia
- F c. Sideroblastic anemia

F d. Vit B_{12} deficiency
F e. All of the above.

Serum/Plasma

Q.13. **Serum is**
T a. used for biochemical estimation
T b. a body fluid.
T c. associated with clot retraction
F d. plasma minus plasma protein
F e. is enriched with fibrin.

Q.14. **Plasma is**
T a. a clear yellowish fluid
T b. a colloid solution.
T c. freely inter changable with the intersitial fluid.
F d. one litre/square meter of body surface area.
F e. 45% of the volume.

Q.15. **Serum globulin**
T a. is synthesized in the liver.
T b. is increased in inflammatory condition.
T c. contributes to viscosity.
T d. acts as carrier protein.
F e. acts as hormone.

Q.16. **Plasma albumin**
T a. is negatively charged at normal pH of blood.
T b. contributes 80% of plasma colloidal osmotic pressure.
F c. is filtered freely at the renal glomerulus.
F d. carries oxygen in blood.
F e. lacks in essential amino acids.

Q.17. **Plasma bilirubin is**
T a. sensitive to light
T b. not ideal to cross *blood brain banier*
F c. a steroid pigment
F d. converted to biliverdin in liver
F e. freely filtered in the renal glomerulus.

Q. 18. **Colloidal osmotic pressure of plasma is due to**
T a. Albumin
T b. Globulin
F c. Fibrinogen
F d. Na^+ ion
F e. Potassium ion.

Q. 19. **Osmotic pressure is mainly due to**
T a. Albumin
F b. Glucose
F c. Clorides
F d. All
F e. None

Q. 20. **Oncotic pressure of plasma is due to**
T a. Albumin
F b. Prealbumin
F c. Electrolytes
F d. Fibrinogen
F e. Prothombin.

Q. 21. **Osmolatily of plasma in a normal adult (in m Osm/L) is :**
T a. 270 - 290
F b. 250 - 270
F c. 300 - 310
F d. 310 - 330
F e. 170 - 190

Q. 22. **The normal albumin/globulin (A/G) ratio in blood is**
T a. 2 : 1
F b. 5 : 1
F c. 1 : 2
F d. 1 : 1
F e. 3 : 1

Q. 23. **Which is true about serum albumin?**
T a. Contributes maximally to plasma oncotic pressure
T b. It is anionic
F c. Freely permeable in kidney
F d. Not synthesized in liver
F e. acts as hormone.

Q. 24. **Hemotocrit of venous blood is :**
T a. 3% greater than arterial blood
F b. 3 times greater than arterial blood
F c. 3% less than arterial blood
F d. 3 times less than arterial blood
F d. 4 times less than arterial blood

Red Blood Cell

Q.25. **Functions of RBC include**
T a. maintainance of viscosity of blood.
T b. acid base balance.
T c. supply of oxygen to tissues.
F d. maintainance of blood volume.
F e. carriage of nutrients.

Q.26. **The biconcave shape of RBC**
T a. helps it to squeeze through capillary.
T b. provides greater surface area than volume.
T c. can resist limited hypotonicity.
F d. allows it to float freely.
F e. provides increased fragility.

Q.27. **The total count of RBC depends upon**
T a. age and sex
T b. physcial activity

T c. hormonal factor
F d. nervous factor
F e. cytokines.

Q.28. **Total count of RBC is increased in**
T a. hypoxia.
T b. high altitude.
T c. increased testosterone level.
F d. anaemia.
F e. erythroblastosis foetalis.

Q.29. **Red blood cells in the peripheral blood**
T a. include about 1% reticulocytes.
F b. include about 1% nucleated cells.
F c. are distributed randomly in the stream of blood.
F d. travel at a slower speed in venules than capillaries
F e. are lower in hypoxic state.

Q.30. **Packed cell volume is**
T a. increased in bone marrow hyperplasia.
T b. measured by centrifugation of blood.
T c. determined by wintrobe method.
T d. increased after fluid loss.
F e. decreased in polycythemia.

Q.31. **Sites of RBC production in adult include**
T a. bone marrow.
F b. lymph node.
F c. kidney.
F d. lungs.
F e. blood vessels.

Q.32. **In adult, RBC is produced by the red bone marrow of**
T a. skull bone
T b. ribs
T c. sternum
F d. all long bones
F e. liver.

Q.33. **Erythropoetic factors are**
T a. hypoxia
T b. vitamin B_{12}
T c. vitamin C
T d. ACTH
F e. protein.

Q.34. **Maturation factor for erythropoiesis include**
T a. copper
F b. dietary protein
F c. cholesterol
F d. erythropoietin
F e. androgen.

Q.35. **Maturation of Red Blood cells are**
T a. due to diminished DNA.
T b. affected greatly by a persons nutritional status.
T c. mainly by Vitamin B12 and folic acid.
T d. failed due to lack of intrinsic factor.
F e. not failed due to lack of intrinsic factor.

Q.36. **Maturation of RBC means**
T a. synthesis of Hb
T b. cytoplasm transforming from basic to acidic stain
F c. cells are old
F d. cells are larger in size
F e. presence of mitochondria in the cytoplasm.

Q.37. **Erythropoiesis is regulated by**
T a. rate of erythrocyte destruction
T b. erythropoietin
T c. vitamin-B_{12}
T d. androgen
F e. blood pressure.

Q.38. **Erythropoietin**
T a. stimulates RBC production.
T b. is secreted in response to hypoxia.
T c. is secreted from kidney.
F d. is activated in lung.
F e. is a chromoprotein.

Q. 39. **Biconcave shape of RBC's, is helpful because**
T a. Increased surface area for a given diameter
F b. Easily passage through capillaries
F c. Rouleax formation
F d. None
F e. None.

Q. 40. **Mean life (days) of RBCs in transfused blood is**
T a. 60
F b. 120
F c. 100
F d. 90
F e. 60.

Q. 41. **Life span of RBC is**
T a. 120 days
F b. 90 days
F c. 60 days
F d. 30 days
F e. 60 days

Hemoglobin

Q.42. **Hemoglobin is**
T a. a chromoprotein.
F b. a glycoprotein.
F c. present in myofibril.
F d. synthesized in endoplasmic reticulum.
F e. lipid soluble.

Q.43. **Hb synthesis requires**
- T a. iron
- T b. vit-C
- T c. glycine
- T d. protoporphyrin IX
- F e. vit-D.

Q.44. **Hemoglobin chain are normal in structurein**
- T a. thalassemia
- F b. sickle cell anaemia
- F c. pernicious anaemia
- F d. haemolytic anaemia
- F e. chronic blood loss.

Q. 45. **Most of the Hb synthesized during the developmental stage is**
- T a. normoblast
- F b. pronormoblast
- F c. reticulocyte
- F d. proerythroblast
- F e. erythrocyte.

Q.46. **Abnormal hemoglobins are**
- T a. HbC
- F b. Hb A
- F c. Hb F
- F d. methemoglobin
- F e. reduced hemoglobin.

Q.47. **Foetal hemoglobin**
- T a. binds easily with 2,3-DPG
- T b. is alkali resistant
- T c. has higher P50
- T d. can be found after birth
- F e. has low affinity for oxygen.

Q.48. **Foetal hemoglobin**
- T a. can bind 2,3-DPG more avidly.
- F b. is present in blood up to adolescent age.
- F c. has high affinity for CO_2.
- F d. can release oxygen very easily.
- F e. consists of 2α and 2β chain.

Q.49. **When fully saturated, 1gm pure Hb can combine with**
- T a. 1.39 ml of oxygen.
- F b. 1.33 ml of oxygen.
- F c. 1.34 ml of oxygen.
- F d. 1.32 ml of oxygen.
- F e. 1.35 ml of oxygen.

Q.50. **Oxygenation of hemoglobin means**
- T a. reversible binding of oxygen with hemoglobin.
- T b. loose combination with Hb.
- T c. formation of noncovalent bond.
- T d. combination with molecular oxygen.
- F e. loss of electron from haem.

Q. 51. **Carbon monoxide combines with**
- T a. Heam part of hemoglobin
- F d. Plasma
- F c. Epididymis
- F b. Globin part of hemoglobin
- F e. Albumin.

Q. 52. **Haemoglobin unlike myoglobin shows**
- T a. Parabolic curve of oxygen association
- F b. Positive cooperativity
- F c. Cooperative index of 81
- F d. Hill's coefficient of 1
- F e. None.

Q. 53. **Myoglobin binds with**
- T a. 1 mol of oxygen per mol
- F b. 2 mol of oxygen per mol
- F c. 3 mol of oxygen per mol
- F d. 4 mol of oxygen per mol
- F e. 2.5 mol of oxygen per mol

Q. 54. **The best method for estimation of Hb concentration in blood is**
- T a. Cyanmethaemoglobin method
- F b. Acid haemalion method
- F c. Alkali haematin method
- F d. All of the above
- F d. None of the above

ESR

Q.55. **ESR**
- T a. is increased in tuberculosis.
- T b. can be measured by Westergren method.
- F c. is normal in pregnancy.
- F d. has more diagnostic value than prognostic value.
- F e. is decreased in malignancy.

Q. 56. **Buffy coat contains**
- T a. leukocytes and platelets.
- F b. myelin.
- F c. actin myosin.
- F d. sarcolemma.
- F e. glycocalyx.

Q.57. **ESR is increased**
- T a. during muscular exercise.
- T b. during pregnancy.
- T c. during manstruation.
- F d. in afibrinogenemia.
- F e. in blood coagulation.

Iron & Fate of RBC

Q. 58. **Old RBC is removed from circulation by**

T a. liver and spleen
F b. liver only
F c. spleen only
F d. bone marrow only
F e. spleen, liver and bone marrow

Q.59. **Hemolysis of normal RBC is caused by**
T a. hypotonic solution.
T b. toxin.
T c. malaria.
T d. antigen antibody reaction.
F e. spherocytosis.

Q.60. **Iron containing compounds in our body are**
T a. catalase.
T b. cytochrome oxidase.
T c. myoglobin.
F d. immunoglobin.
F e. thyroglobulin.

Q.61. **Transferrin**
T a. is a plasma protein.
T b. carries iron in the blood.
T c. is synthesized in the liver.
F d. is an iron contianing enzyme.
F e. is an intracellualr protein.

Q.62. **Ferritin content is increased in**
T a. excess iron intake.
F b. iron deficiency.
F c. high protein diet.
F d. hemorrhagic condition.
F e. prolonged illness.

Q.63. **Rate of iron absorption depends on**
T a. storage of iron in the body.
T b. chemical convrsion of dietary iron.
T c. loss of body iron.
F d. availability of transferrin only.
F e. state of circulation.

Q. 64. **Iron is stored in the body in the following**
T a. RE system
T b. Spleen
T c. Bone marrow
F d. Gall bladder
F e. None.

Q. 65. **Iron is store in**
T a. Reticuloendothelial system
F b. RBC
F c. Plasma
F d. Gall bladder
F e. All.

Anaemia

Q.66. **Iron deficiency anaemia**
T a. is characterized by reduced Hb concentration.
T b. is produced by malnutrition.
T c. occurs due to vitamin C defiency.
F d. is more common in male than female.
F e. may be associated with increased size of RBC.

Q.67. **Pernicious anaemia**
T a. is caused by poor vitamin B_{12} absorption.
T b. may follow partial gastrectomy.
T c. is characterized by megaloblastic erythropoiesis.
F d. occurs in prolonged illness.
F e. is caused by chronic hemorrhage.

Q.68. **Functions of intrinsic factor are**
T a. carriage of vitamin B_{12} in the gut.
T b. protection of vitamin B_{12} from digestion.
F c. increasing the solubility of vitamin B_{12}.
F d. promotion of excretion of vitamin B_{12}.
F e. neutralization of acid.

Q.69. **Sickle cell anaemia is produced**
T a. due to abnormal erythropoiesis.
T b. in hypoxic condition.
T c. due to genetic defect.
T d. by excessive hemolysis.
F e. due to formation of normal amino acid chain.

Q.70. **Hemoytic anaemia occurs in**
T a. erythroblastosis foetalis.
T b. vitamin B_{12} deficiency.
T c. hereditary spherocytosis.
T d. hemoglobinopathy.
F e. thalassemia.

Q.71. **Sickle cell anemia**
T a. has abundant HbS
T b. has abundant crystals of Hb in RBCs.
T c. contains abnormal hemoglobin
F d. is less prevalent in African-Americans
F e. is not a kind of hemo]ytic anemia

Q.72. **Hemorrhagic anaemia may be characterized by**
T a. microcytic hypochromic anaemia.
T b. macrocytic anaemia.
T c. normocytic hypochromic anaemia.
T d. normocytic normochromic.
F e. reticulocytosis.

Q.73. **Laboratory classification of anaemia is done on the basis of**
T a. MCV.
T b. TC of RBC.
T c. Hb%.

T d. PCV.
F e. ESR.

Q.74. **Polycythemia**
T a. produces increased blood volume.
T b. is associated with reddish colour with bluish tint.
T c. causes increased viscosity of blood.
F d. causes increased blood flow.
F e. causes increased cardiac output.

Q. 75. **In anemia, the concentration of 2, 3-DPG is:**
T a. Increased
F b. Decreased
F c. A or b
F d. Not changed
F e. None.

Q. 76. **Patient with anaemia tends to have**
T a. Compensatory increase in cardiac output
T b. Increased incidence of heart murmurs
T c. Pallor of mucous membranes
F d. A low PO_2 in arterial blood
F e. None.

Jaundice

Q. 77. **In haemolytic jaundice**
T a. urinary bilirubin is absent.
T b. Vadenbergh test is indirect.
T c. faecal stercobilinogen is increased.
F d. urinary urobilinogen is decreased.
F e. liver function is impaired.

Q. 78. **In hepatic jaundice**
T a. Vandenbergh test is biphasic.
T b. faecal stercobilinogen is decreased.
F c. urinary urobilinogen is increased.
F d. bilirubin in urine is absent.
F e. liver function is normal.

Q. 79. **In obstructive jaundice**
T a. urinary bilirubin is present.
T b. Vendenbergh test is direct.
F c. faecal stercobilinogen is present.
F d. urinary urobilinogen is present.
F e. liver function is impaired.

Q. 80. **A premature infant is more likely than a full term infant to**
T a. Excrete urine with a uniform specific gravity
T b. Suffer from anaemia.
T c. Suffer from jaundice of hepatic origin
F d. Maintain a normal body temperature in a cold environment
F e. None.

White Blood Cell

Q. 81. **Blood cells that are not formed in bone marrow include**
T a. plasma cell.
F b. erythrocytes.
F c. monocytes.
F d. thromobocytes.
F e. granulocytes.

Q. 82. **Stem cells giving rise to granular leukocytes are**
T a. myeloblast.
F b. erythroblast.
F c. lymphoblast.
F d. megakaryoblast.
F e. normoblast.

Q.83. **Granules of granulocyte contains**
T a. anticoagulant.
F b. enzymes.
F c. toxins.
F d. vasoactive substances.
F e. local hormones.

Q.84. **Neutrophils**
T a. move freely to site of inflammation
T b. are phagocytic cells
T c. are chemotactic cells
F d. are less motile
F e. are fewer in lysosome.

Q.85. **Neutrophil count rises**
T a. during infection.
T b. during exercise.
F c. in allergic raction.
F d. in dehydration.
F e. during haemorrhage.

Q.86. **Basophil**
T a. secretes substances that increase capillary penneability
T b. releases histamine when they degenerate.
T c. is stimulated by antigen bound to IgE
F d. is the most abundant granulocyte in peripheral blood
F e. is suppressed by complement opsonin

Q.87. **Parasiticidal WBC is**
T a. esinophil.
F b. macrophage.
F c. neutrophil.
F d. monocyte.
F e. lymphocyte.

Q.88. **Monoeytes**
T a. are highly motile

T b. are phagocytie cells
F c. are uniformly spherical
F d. manufacture 1gM
F e. originate from precursor cells in lymph nodes.

Q.89. **Lymphocytes**
T a. are concerned with cell mediated immunity.
T b. produce interleukin.
T c. are produced in lymphoid tissue.
T d. are produced in bone marrow.
F e. induce passive immunity.

Q.90. **B lymphocytes are**
T a. converted into plasmoblast.
T b. activated by macrophage.
F c. processed after birth.
F d. are named after bone marrow.
F e. concerned with innate immunity.

Q.91. **Helper T cells**
T a. secrete interleukin
T b. stimulate growth of other T cells
T c. activate macrophages
T d. stimulate growth and differentiation of B cells
F e. are out numbered by cytotoxic T cells.

Q.92. **Suppressor T cells**
T a. never become plasma cells.
T b. play a role in tolerance.
F c. secrete lymphokines.
F d. prevent activation of complement cascade.
F e. more than 75% of the T cells are suppressor.

Q. 93. **B lymphocytes are associated with**
T a. CD 35
F b. CD 19
F c. CD 27
F d. CD 4
F d. CD 8

Q. 94. **Secondary granules of neutrophils contain**
T a. Lactoferin
F b. Catalase
F c. Myeloperoxidase
F d. Nucleotidase
F e. None.

Q. 95. **The function common to neutrophils, monocyte and macrophage is**
T a. Phagocytosis
F b. Immune response
F c. Liberation of histamine
F d. Destruction of old erythrocytes.
F e. None.

Q. 96. **Eutropenia is seen in all except :**
T a. Trauma
F b. Pernicious anaemia
F c. Severe bacterial infection
F d. Bone marrow depression
F e. None.

Q. 97. **Macrophages are the mature form of**
T a. Monocytes
F b. Neutrophils
F c. Eosinophils
F d. Basophils
F e. Lymphocytes

Q. 98. **Which one of the following statements about lymphocytes is incorrect?**
T a. Concentration falls in the blood abruptly and immune reaction is disturbed after removal of thymus in adult
F b. Produced by thymus, red bone marrow, spleen and lymph nodes
F c. Probably change into plasma cells
F d. Constitute 20-40% of leukocytes
F e. Do not perform an important phagocytic function

Q. 99. **Which one of the following statements concerning the monocyte is incorrect?**
T a. Unlike neutrophil does not accumulate outside circulation in area of inflammation
F b. More common in blood than eosinophil and basophil
F c. Produced in the adult by the bone marrow and lymph nodes
F d. Not classified as a granulocyte
F e. None.

Q. 100. **Arneth count is**
T a. Counting the lobes in the neutrophil
F b. Counting the lymphocytes
F c. Counting the granules in the eosinophil.
F d. WBC counting in bone marrow.
F e. None.

Immunity

Q.101. **Immunity**
T a. cause damage to body tissue in some disease.
T b. is responsible for transplantation reaction
F c. produces generalised weakness.
F d. helps in invasion by bacteria.
F e. helps in hemostasis.

Q. 102. **Mode of actions of complement are**
T a. opsonization.
T b. agglutination.

T c. lysis.
T d. phagocytosis.
F e. enzyme activation.

Q.103. **Cell mediated immunity is mediated by**
T a. T lymphocyte.
F b. macrophage.
F c. neutrophil.
F d. B lymphocyte.
F e. plasma cell.

Q. 104. **Complement protein**
T a. activates mast cells and basophlis.
T b. activates phagocytosis
T c. activates chemotaxis
T d. causes cellular lysis (cytolytic)
F e. is weakly activated by antigen-antibody complex

Q.105. **Principle of vaccination is**
T a. activation of clone of lymphocyte.
T b. memory cell production.
F c. antigen antibody complex formation.
F d. antibody production only.
F e. proliferation of lymphocytes.

Q.106. **Immunoglobulin that crosse placenta are**
T a. IgG
F b. IgA
F c. IgD
F d. IgM
F e. IgE.

Q.107. **Antibody attacks foreign invader by**
T a. preciptation.
T b. lysis.
F c. chemotaxis.
F d. clotting
F e. toxin.

Q.108. **Most powerful mode of action of antibody is**
T a. activated complement system.
F b. supressor T cells.
F c. cytotoxic T cells.
F d. helper T cell.
F e. macrophage.

Q.109. **Chief regulatory cell of immunity are**
T a. helper T cell.
F b. suppressing T cells.
F c. cytotoxic T cells.
F d. activated B cell.
F e. macrophage.

Q.110. **Immune tolerance is responsible for**
T a. absence of antibody against antigen.
F b. autoimmune disease.
F c. all inflammatory condition.
F d. rejection of organ transplantation.
F e. allergic condition.

Q. 111. **Antigen presenting cells are specialised cells present on the followings**
T a. Skin
T b. Lymph node
T c. Spleen
F d. Kidney
F e. Liver.

Q. 112. **MHC class II antigens are located on which of the following cells?**
T a. Dentritic cells of the spleen
T b. Macrophages
T c. Langerhans cells of skin.
T d. B cells
F e. Platelets

Q. 113. **Immune complexes are removed from blood by**
T a. Kupffer cell
F b. B cell
F c. Basophil
F d. Plasma cell
F e. T cell.

Q. 114. **phagocyttosis in the CNS is done by**
T a. Microglia
F b. Astrocytes
F c. Schwann cells
F d. Oligocytes
F e. Macrophage.

Q. 115. **Immunity is most suppressed in**
T a. Patients on ACTH therapy
T b. In AIDs
F c. Liver failure
F d. Anemia
F e. Renal failure

Q. 116. **T-Lymphocyte**
T a. Mature in lymphnodes
F b. Produce antibodies
F c. Comprise 10 to 15% lymphocytes
F d. Principal cells in lymphnode cortical centre.
F e. None.

Inflammation

Q.117. **Tissue inflammation produces**
T a. change in vascular diameter.
T b. change in vascular permeability.
T c. migration of WBC.
T d. chemotactic agents.
T e. oedema.

Q.118. **Inflammatory agents are**
- T a. histamine.
- T b. serotonin.
- F c. adrenaline.
- F d. fibrinogen.
- F e. albumin.

Q.119. **Host response to inflammation includes**
- T a. coagulation of extracellular fluid.
- T b. acute neutrophilia.
- F c. acute polycythemia.
- F d. inactivation of macrophage.
- F e. endothelial proliferation.

Q.120. **The phagocytic cells are**
- T a. macrophage.
- T b. neutrophil.
- T c. eosinophil.
- F d. endothelial cell.
- F e. epithelial cell.

Q. 121. **Nitric oxide is produced in**
- T a. Endothelium
- F b. Plasma
- F c. Platelets
- F d. Serum
- F e. Blood.

Q. 122. **Thromboxane A_2 is mainly produced by**
- T a. Platelets
- F b. Vascular endothelium
- F c. Liver
- F d. Damaged tissue.
- F e. None.

Q. 123. **SRS-A is a**
- T a. Leukotriene
- F b. Seratonin
- F c. Histamine
- F d. Prostaglandin
- F e. None.

Q.124. **Leukemia**
- T a. produces increased hemorrhagic tendency.
- T b. causes decreased body defense.
- T c. causes anaemia.
- F d. causes increased coagulation time.
- F e. may cause depletion of normal tissue protein.

Q. 125. **Pyrogens raise body temperature by**
- T a. Releasing interleukins
- F b. Setting the the thermostat to higher level
- F c. Decreasing peripheral heat liberating mechanism
- F d. Causing peripheral vasoconstriction
- F e. None.

Platelet

Q.126. **Platelets**
- T a. form plug by sticking to each other.
- T b. release vasoconstrictor agents.
- T c. store Ca+ in granules.
- T d. provide phospholipid for intrinsic pathway.
- F e. are destroyed by osmotic force.

Q.127. **Platelets**
- T a. have role in blood coagulation.
- T b. liberate 5-HT.
- T c. help in phagocytosis.
- T d. secrete ADP.
- F e. liberate immunoglobulins.

Q.128. **Proteins produced by platelets include**
- T a. thromboplastin.
- F b. plasminogen.
- F c. fibrinogen.
- F d. prothrombin.
- F e. casinogen.

Q. 129. **Aggregins are**
- T a. Meant for platelet aggregation
- F b. For adhesion of cells to basement membrane
- F c. For adhesion of fibrinogen receptors to platelets
- F d. For platelet adhesion to endothelium.
- F e. None.

Q. 130. **A reliable screening test for platelet function is**
- T a. Clot retraction time
- F b. CT
- F c. PT
- F d. Thrombin time
- F e. None.

Q. 131. **Thromboxane A2 is released mainly by the**
- T a. Platelets
- F b. Vascular endothelium
- F c. Liver
- F d. Muscles
- F e. Kidney.

Q. 132. **Half life of transfused platelets is**
- T a. 8 days
- F b. 4 Hours
- F c. 12 Hours
- F d. 15 days
- F d. 10 days.

Blood group

Q.133. **ABO blood group antigens are**
- T a. present since birth.

T b. agglutinogen in prsence of corresponding antibody.
F c. found in the plasma.
F d. protein in nature.
F e. determined for tissue typing.

Q. 134. **Blood group antigens are**
T a. present in fetal blood
T b. present in red cells membrane.
F c. carried on the Hb molecules
F d. immunoglobins
F e. regularly immunogenic

Q.135. **All agglutinins are**
T a. present in O blood group.
T b. formed after birth.
F c. present in all blood groups.
F d. present in AB blood group.
F e. present since birth.

Q. 136. **Antibodies against both group A and group B antigens are found in the blood plasma of a person who possesses**
T a. group O.
F b. group A.
F c. group B.
F d. group AB.
F e. Rh positive blood.

Q.137. **A person with group A (+ve) blood**
T a. has A agglutinogen in the red blood cell membrane.
T b. has anti-A antibody in the plasma.
T c. has 'D' agglutinogen in the RBC membrane.
F d. may have the genotype AB.
F e. can donate blood to a person with group B +ve blood.

Q.138. **Rh+ve blood group is safer than Rh-ve because it**
T a. can receive both Rh-positive and Rh negative blood
T b. has no antibody.
T c. does not produce antibody against Rh negative.
T d. can not receive Rh-negative blood.
F e. can be given to Rh-negative person.

Q.139. **Erythroblastosis fetalis**
T a. is due to destruction of RB Cs.
T b. leads to neonatal jaundice.
T c. leads to hepatomegaly.
T d. leads to splenomegaly.
F e. is common in Rh+ve mother.

Q. 140. **Stored blood as compared to fresh blood has**
T a. High extracellular K^+
F b. More 2,3 DPG
F c. High extracellular Hb
F d. Increased platelets.
F e. Less 2,3 DPG

Q. 141. **True about Rh factor**
T a. Has no naturally occuring antibody
F b. Seen only in humans
F c. Not important for blood transfusion
F d. None
F e. All.

Q. 142. **Addition of glucose to stored blood is to**
T a. Increase 2,3 DPG
F b. Prevent hemolysis
F c. Provide nutrition
F d. In crease Hb content
F e. All.

Q. 143. **Blood group antigens are**
T a. Found in saliva
F b. Carried by sex chromosomes
F c. Attached to plasma proteins
F d. Attached to Hemoglobin molecule
F e. All.

Q. 144. **AB blood group antigen are known as factors**
T a. Landsteiner
F b. Duff
F c. Rhesus
F d. Lutheran
F e. Kidd.

Coagulation of blood

Q.145. **Granules of granulocyte contains**
T a. anticoagulant.
F b. enzymes.
F c. toxins.
F d. vasoactive substances.
F e. local hormones.

Q. 146. **The activation of factor X is**
T a. essential for prothombin activation.
T b. part of both extrinsic and intrinsic pathway.
F c. part of the intrinsic pathway only.
F d. part of the extrinsic pathway only.
F e. essential for activation of Christmas factor.

Q.147. **Thrombin**
T a. combines with antithrombin III.
T b. is removed by antithrombin III.
T c. is accelerated by factor V.
F d. removes clot from circulation.
F e. converts plasminogen to plasmin.

Q.148. **Chemical factors related to hemostasis are**
T a. serotonin.
T b. thrombaxane A_2.

F c. adrenalin.
F d. prostaglandin.
F e. leukotrein.

Q.149. **Coagulation of blood requires**
T a. platelet.
T b. calcium ion.
T c. Vitamin K.
F d. Vitamin C.
F e. inactivation of heparin.

Q.150. **Essential clotting factors are**
T a. Calcium ion.
T b. Fibrinogen.
T c. Prothrombin.
T d. Thrombin.
F e. Hageman factors.

Q.151. **Vitamin K is necessary for synthesis of**
T a. Factor IX.
T b. Factor X.
T c. Prothrombin.
F d. Factor V.
F e. fibrinogen.

Q.152. **Platelet plug**
T a. is composed of swollen and aggregated platelets.
T b. forms platform for deposition of fibrin thread.
T c. stops bleeding from injured tiny vessels.
T d. formation is facilitated by ADP.
F e. is induced by Ca^{++}

Q. 153. **Prothrombin activator is formed by**
T a. activated factor X
T b. tissue phospholipids
T c. factor V
F d. activated factor XII
F e. vitamin K.

Q.154. **Extrinsic mechansim of clot formation is**
T a. initiated by release of tissue factor from injured cell membrane.
T b. a vicious process.
F c. responsible for blood clot in test tube.
F d. involved with activated factors VIII.
F e. a slow process.

Q.155. **Intrinsic pathway of coagulation begins with tmuma to the**
T a. blood itself
F b. vassel wall
F c. damaged tissues
F c. collagen fibers
F d. skin.

Q.156. **Convesrsion of fibrinogen to fibrin is**
T a. followed by polymerization of fibrin monomers.
T b. effected by thrombin.
F c. effectd by prothrombin.
F d. activated by Heparin.
F e. reversed by plasma.

Q.157. **Clotting time is**
T a. the time required for activation of clotting factors.
T b. a test for bleeding disorder.
F c. increased in thrombocytopenia.
F d. decreased in hemophilia.
F e. 15 minutes in a normal person.

Q.158. **Bleeding time is**
T a. prolonged in low platelet count.
T b. an indicator of natural hemostatic mechansim.
T c. normal in hemophilia.
T d. 1-6 minutes in normal person.
F e. the time required for coagulation of blood in a wound after injury.

Q.159. **Fate of a clot is**
T a. calcification.
T b. fibrous organization.
T c. lysis.
T d. resolution.
F e. not enzymatic destruction.

Q.160. **Clot retraction**
T a. is responsible for recanaliation of vessel.
T b. leads to serum formation.
T c. is contributed by platelet.
T d. gives an idea about platelet count.
F e. is related to Ca^{++} concentration.

Q. 161. **In the blood in a test tube, after clot retraction there is formation of**
T a. serum.
F b. tissue fluid.
F c. lymph.
F d. plasma.
F e. transcellualr fluid.

Q.162. **Serum is**
T a. used for biochemical estimation.
T b. associated with clot retraction.
T c. a body fluid.
F d. is enriched with fibrin.
F e. plasma minus plasma protein.

Q.163. **Factors maintaining fluidity of blood in the circulation are**
T a. thrombomodulin complex.
F b. rough endothelium.
F c. reynold's number.

F d. antibody.
F e. plasmin.

Q.164. **Plasmin is**
T a. causing fibrinolysis.
F b. involved in intrinsic clotting system.
F c. involved in extrinsic clotting system.
F d. promoting formation of emboli.
F e. found in serum.

Q.165. **Plasminogen is**
T a. activated by tissue plasminogen activator (TPA)
T b. associated with clot lysis.
F c. produced in plasma cell.
F d. a vasodilator.
F e. concerned with platelet activation.

Q.166. **Profibrinolysin**
T a. is synthesized by hepatic cell.
T b. remains inhibited in circulation.
F c. converts fibrinogen into fibrin.
F d. is an active proteolytic enzyme.
F e. helps in platelet plug formation.

Q.167. **Followings are anticoagulants**
T a. double oxalate.
T b. heparin.
T c. 3.8% sodium citrate.
F d. n/10 HCI.
F e. normal saline.

Q.168. **Heparin is**
T a. produced by mast cell.
F b. a procoagulant.
F c. an enzyme.
F d. protein in nature.
F e. synthesized by palsma cell.

Q.169. **Heparin prevents blood coagulation primarily because it**
T a. blocks the action of thrombin.
F b. dissolves fibrinogen.
F c. blocks conversion of prothrombin to thrombin.
F d. activates thromboplasin.
F e. chelates calcium.

Q.170. **Heparin prevents clotting primarily because it**
T a. blocks the action of thrombin.
F b. dissolves fibrinogen.
F c. blocks conversion of prothombin to thrombin.
F d. activates thromboplstin.
F e. chelate Ca^{++}.

Q.171. **Warfarrin is**
T a. an anticoagulatn.
T b. vitamin K antagonist.
F c. an antibiotic.
F d. used in hemorrhagic condition.
F e. protein in nature and binds with calcium.

Q.172. **Tests for bleeding disorder are**
T a. coagulation time.
T b. bleeding time.
T c. thromboplastin generation time.
T d. platelet count.
F e. RBC count.

Q.173. **Causes of bleeding are**
T a. vitamin -K deficiency
T b. hemophilia
T c. thrombocytopenia
F d. anaemia
F e. cyanosis.

Q.174. **In purpura there are**
T a. bleeding under skin.
T b. prolonged bleeding time.
T c. indication of weak hemostatic mechanism.
F d. skin pigmentation.
F e. leucopenia.

Q. 175. **In Christmas disease**
T a. blood fails to coagulate properly
F b. hematocrit is low
F c. few red cells are produced
F d. ESR is raised
F e. factor VIII is deficient

Q.176. **Hemophilia is**
T a. a genetic disorder.
T b. lack of factor VIII.
F c. a dvelopmental abnormality.
F d. caused by defective autosome.
F e. more common in female.

Q. 177. ***Haemophilia A is associated with all except**
T a. Increase PT
F b. Soft tissue haematoma
F c. Pseudotumor
F d. Increase PTT
F e. None.

Q. 178. **In Hemophilia**
T a. Factor VIII is decreased
T b. Cloting time is increased
F c. Factor VII is decreased
F d. Bleeding time is increased
F e. Prothrombin time is increased

Q. 179. **Hemophilia is due to deficiency of factor**
T a. VIII
F b. II

F c. V
F d. XIII
F e. IX.

Q. 180. **The best screening test for hemophilia is :**
T a. PTT
F b. BT
F c. PT
F d. CT
F e. CRT

Q. 181. **To prolong Lee White clotting time in a patient for the next eleven hours what would be the procedure of choice**
T a. IV heparin
F b. IV sodium citrate
F c. IV vitamin K
F d. Dicumarol therapy
F e. CRT

Q. 182. **The normal non-fasting blood ketone level is**
T a. 2-10 mg%
F b. 0.1-0.5 mg%
F c. 0.5-2 mg%
F d. 100-500 mg%
F e. 0.2-1.00 mg%

Q. 183. **A procoagulant not normally circulating in the plasma is**
F a. Prothrombin
F b. Fibrinogen
F c. Antihemophilic factor
F d. Factor V
T e. None of the above

Q. 184. **Which one of the following is released by blood platelets during haemorrhage to produce vasoconstriction?**
T a. Serotonin
F b. Histamine
F c. Thrombosthenin
F d. Accelerator globulin
F e. Bradykinin

Q. 185. **Endothelial cells synthesize**
T a. Factor VII
F b. Fibrinogen
F c. Factor X
F d. Factor XII
F e. Factor IX

Q. 186. **In vitro coagulation is initiated by factor**
T a. XII
F b. XI
F c. X
F d. VII
F e. IX

Q. 187. **Anaphylaxis is mediated by all except**
T a. Serotonin
F b. Bradykinin
F c. Anaphylotoxin
F d. Prostaglandin.
F e. Leukotrin.