CHAPTER IV

BLOOD

Blood may be described as a specialised connective tissue in which there is liquid intercellular substance known as plasma and formed elements, the red blood cells, the white blood cells and the platelets suspended in the plasma. The specific gravity of whole blood varies from 1·055 to 1·060. When freshly shed, blood is a red, thick, opaque and slightly alkaline. In order to understand the full significance of blood, it is imperative that one should know something about its genesis.

Life evolved first in the sea water. The primitive unicellular animal took its oxygen and nutrition from the ocean and excreted its waste products into it. But with the development of the multicellular forms, this simple arrangement of drainage and supply could no longer hold. The cells in the deeper parts of the body could not come into free contact with surrounding sea water, and would therefore suffer. To overcome this difficulty, a system of intercommunicating channels developed, pervading the whole animal body and opening upon the exterior. Through these channels the sea water could freely flow in and out, and in this way, the deeper cells could satisfy their needs. This channel system represents the primitive circulatory system and, it is obvious that, in its original form, this system was an open one.

But this open circulatory system had many disadvantages and consequently, with the birth of the higher forms, the open system became a closed one. This change represents a very important landmark in the history of animal evolution. The sea water, which used to remain outside the body, became entrapped within the body. In the course of evolution, that enclosed marine water has undergone profound modifications and has been transformed into what we call now *blood*. Although blood has departed a long way from its primitive ancestor, yet in its inorganic composition, it still maintains a close resemblance with sea water. This, in short, is the history of evolution of blood. Not only blood, but other body fluids also, should be regarded as the modifications of the same primitive marine environment.

From the above discussion, it will be evident that, in the multicellular animals, blood serves those purposes, which, for the unicellular forms, are carried out by the surrounding aquatic medium. But in addition to those, blood has to serve many other important functions, because it has to meet the complex requirements of the more specialised cells, in the higher forms. Functions of blood may be summarised as follows:

Functions of Blood

1. **Transport of respiratory gases.** It carries oxygen from the lungs to the tissues and CO_2 from the tissues to the lungs.
2. **Transport of nutrition.** It carries digested food material absorbed from the intestine to the tissue cells for utilisation. It also carries nutritive material from one place of the body to the other. For instance, from the storage depots to the tissue cells.
3. **It acts as a vehicle** through which the hormones, the vitamins and other essential chemicals are brought to their places of activity.

4. **Drainage of waste products.** It carries the waste products of cellular activity and brings them to the organ of excretion, viz., kidney, lungs, intestine, etc.

5. **Maintenance of water balance** (*vide* 'Water Balance').

6. **Maintenance of acid-base equilibrium.** By its efficient buffering power (e.g., plasma proteins, reduced and oxidised haemoglobin, etc.) and with the help of kidney, skin and lungs it helps to maintain a constant reaction of the body.

7. **Maintenance of ion balance** between the cells and the surrounding fluid.

8. **Regulation of body temperature.** The water content of blood possesses three qualities which make it very suitable for this purpose. (*a*) Due to its high *specific heat** it can absorb a large amount of heat and thereby prevent sudden change of body temperature. (*b*) High conductivity—the thermal conductivity of water is higher than that of any other ordinary liquid. This helps quick distribution of heat. (*c*) High latent heat of evaporation—latent heat of evaporation of water is very high and since water is constantly evaporating from skin and lungs a large amount of heat is lost in this way.

9. **Defensive action.** Blood acts as a great defensive mechanism in two ways. (*a*) The white cells due to their phagocytic properties engulf bacteria and foreign particles. (*b*) It develops antibodies which combat toxic agents.

10. **By the property of coagulation it guards against haemorrhage.**

11. **The plasma proteins of blood** have various functions (*vide* functions of plasma proteins).

12. **Regulation of blood pressure,** by changes in volume and viscosity (haematocrit value) of blood.

Composition of Blood

Blood is a highly complex fluid which is composed of two parts—a liquid, called the **plasma** and different types of **cells** which remain suspended in the plasma. The cells are called the blood corpuscles. The plasma constitutes about 55 per cent., and the cells about 45 per cent., of the total volume of human blood. The general composition of the whole blood is as follows:

Whole blood:

A. Cells—(1) Red blood corpuscles or erythrocytes (R.B.C.). (2) White blood corpuscles or leucocytes (W.B.C.). (3) Platelets or thrombocytes.

B. Plasma—(1) Water, 91 to 92 per cent.

(2) Solids, 8 to 9 per cent.

(*a*) Inorganic constituents, 0·9%. Sodium, potassium, calcium, magnesium, phosphorus, 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.

* Specific heat of a substance is the number of calories required to raise the temperature of one gram of the substance to one degree Centigrade.

(*iii*) Fats. Neutral fat, phospholipid, cholesterol, cholesterides, etc.
(*iv*) Carbohydrate. Glucose, etc.
(*v*) Other substances. Internal secretions, antibodies and various enzymes (amylases, proteases, lipases, phosphatases, etc.).
(*vi*) Colouring matter. The yellow colour of plasma is due to small amounts of bilirubin, carotene and xanthophyllin.

NORMAL VALUES OF BLOOD CONSTITUENTS COMMONLY OF CLINICAL IMPORTANCE

BLOOD CONSTITUENTS	PER 100 ml	WHOLE BLOOD	PLASMA	SERUM
Haemoglobin	gm/100 ml	12–17 (in male) 11–15 (in female)		
Proteins	gm/100 ml		6·5–7·5	6·0–6·9
Albumin	gm/100 ml			4·7–5·7
Globulin	gm/100 ml			1·3–2·5
Fibrinogen	gm/100 ml		0·2–0·4	
A/G ratio				1·2–1·8
True glucose	mg/100 ml	65–90		
Total lipids	mg/100 ml	360–820		
Fats	mg/100 ml			150–250
Total fatty acids	mg/100 ml		100–500	
Pyruvic acid	mg/100 ml	0·8–1·2		
Lactic acid	mg/100 ml	6–16		
Non-Protein nitrogen ..	mg/100 ml	28–39		
Urea	mg/100 ml	19–33		
Urea N	mg/100 ml	9–15		
Creatinine	mg/100 ml	1·2–1·5		
Uric acid	mg/100 ml	1·0–3·0		
Total cholesterol	mg/100 ml			150–250
Free cholesterol	mg/100 ml			40–70
Esterified cholesterol ..	mg/100 ml			100–180
Calcium	mg/100 ml			9–11
Sodium	mg/100 ml			310–350
Potassium	mg/100 ml			14·1–24·2
Sulphur as sulphates ..	mg/100 ml			2·0–4·0
Chlorides as NaCl ..	mg/100 ml			576–612
Phosphorus as inorganic phosphate	mg/100 ml			3·2–4·3
Ascorbic acid	mg/100 ml		0·7–1·5	
Iodine	μg/100 ml		0·2–0·8	
Bilirubin	mg/100 ml		60–180	
CO_2 capacity at 40 mm CO_2	vol/100 ml			55–75
O_2 capacity (exposed to air) ..	vol/100 ml	16–24		

Average figures in healthy adult Indians

Sugar, 85 mgm per 100 ml of whole blood; Urea, 25 mgm%; NPN, 23·4 mgm%; Uric acid, 4·0 mgm%; Creatinine 1·5 mgm%; Cholesterol, 154 mgm%; Chloride (as NaCl), 396 mgm per 100 ml of whole blood; Calcium, 10·2 mgm per 100 ml of serum; Inorganic phosphorus, 3·6 mgm%; and Lipid phosphorus, 9·7 mgm per 100 ml of whole blood.

Relative volume of corpuscles and plasma

In normal human blood (FIG. 94), plasma volume is proportionally more than total corpuscular volume. The plasma volume varies between 52% and 55% and the packed corpuscular volume varies from 45% to 48%. The normal packed cell volume for males is about 45% while that

in case of females is a bit lower about 40% of whole blood. The ratio of red blood corpuscles to plasma is expressed as the haematocrit value. This can be estimated by an instrument called **Haematocrit.** It consists of a specially prepared graduated capillary (Wintrobe's) tube of uniform bore in which a specimen of blood, treated with an anticoagulant, is taken. It is centrifuged at a speed of 3,000 revolution per minute for 30 minutes, until plasma and corpuscles are completely separated, and the sedimented corpuscles show no further shrinkage in volume. From the graduations on the tube the proportion of plasma and corpuscles can be known.

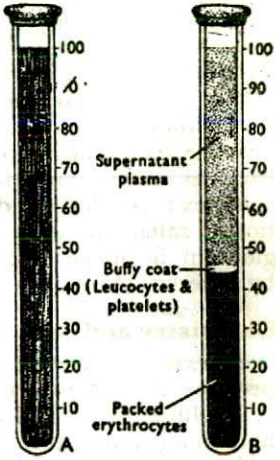


FIG. 94. A=blood sample before centrifuging in the haematocrit. B=the same after centrifuging in the haematocrit.

SPECIFIC GRAVITY OF BLOOD

Specific gravity of venous blood at 15°C. (59°F.) as determined by the pychometric method is given as 1·048 to 1·066 with averages of 1·052 to 1·063. The *clinical average* is 1·057 in males and 1·053 in females. There is a normal diurnal variation of about 0·003, it being generally lower in the afternoon and after meals and higher after exercise and during the night. The specific gravity of *blood serum* varies from 1·026 to 1·031 and that of *erythrocytes* is from 1·092 to 1·095. The specific gravity of the foetal blood in full term is highest and that of the mother at the same time is lowest (about 1·050). The specific gravity rapidly falls after birth due to the destruction of the red cells.

Specific gravity of blood rises (1) when water is lost from the body, such as in excessive sweating, diarrhoea, cholera, etc.; (2) by exudation of fluid into tissues or serous cavities due to inflammation or surgical operation; (3) when water intake is inadequate. It **falls** (1) when large quantity of water is taken; (2) in severe haemorrhage after which fluid is drawn in from the tissue spaces and the blood is diluted; (3) injection of saline into the veins causing dilution of blood.

Method of determination. The principle adopted, is to add drops of blood to a series of copper sulphate ($CuSO_4$) solutions of known specific gravity and to note in which particular solution the blood drop neither floats nor sinks. The specific gravity of that particular solution indicates the specific gravity of the blood sample. A series of mixtures of benzene and chloroform and mixtures of glycerine and distilled water of known specific gravity are also used.

PLASMA PROTEINS

Varieties. In normal individuals, total amount of plasma protein varies from 6·5 to 7·5% and average is about 7·0%. It is made up of following varieties: (1) **Serum albumin**—4·7–5·7%; (2) **Serum globulin**—1·3–2·5%; (3) **Fibrinogen**—0·2–0·4%. In addition to this about 0·1% of other proteins, such as (4) **Prothrombin,** seromucoid, etc., are also present. *Serum* is the fluid part of blood after clotting. It contains only serum

albumin and serum globulin. The albumin/globulin (A/G) ratio is ordinarily 1·5 : 1·0. Different methods of separation indicate slightly different A/G ratio. Electrophoretic method of separation gives a ratio 1·2 : 1·0. This ratio varies in different species but in the same species it remains almost constant in blood, lymph and serous transudations. In liver disease however due to diminished formation of albumin, the ratio may be reversed. Chemical analysis of the total serum proteins reveals that *arginine/lysine ratio* is 10 : 18. *This ratio remains more constant than the albumin/globulin ratio.*

Although it is customary to state that plasma contains several types of proteins yet it is highly probable that in the living animal all these different varieties remain combined together forming a single protein complex. This complex is very loose and is easily broken down into different parts by addition of salts, alteration of pH, etc. The so-called serum albumin, serum globulin, fibrinogen, etc., are the parts of the same parent complex, isolated by different techniques of separation.

Chemistry and separation of plasma proteins

Serum Albumin. It constitutes the major part of the total plasma proteins. It is albumin in nature having a molecular weight, about 69,000. It is soluble in distilled water. Recent studies show that serum albumin is not a single pure entity. It is a mixture of several albumins. It is precipitated by full saturation with ammonium sulphate. Isoelectric pH is 4·7. The albumin molecule is an *ellipsoid*, made up of a single polypeptide chain. It is smaller and more compact than other plasma proteins. It is heat-coagulable.

Serum Globulin. It is globulin in nature having a molecular weight, varying from 90,000 to 1,300,000. It is insoluble in distilled water, but soluble in salt solutions. It is coagulated at about 70°C. Globulin, like albumin, is also a mixture of several globulins.

By means of *Electrophoresis* serum globulin has been separated into three fractions: (1) α_1- and α_2-**globulins**—mol. wt. ranges from 41,000 to 200,000, isoelectric pH 5·1. α_1-globulin consists of two fractions: (*a*) one fraction combines with bilirubin, (*b*) another fraction helps in the carriage of lipids, steroids and glycoproteins. α_2-globulin consists of α_2-macroglobulins, mucoproteins, ceruloplasmin, hepatoglobulins—the latter combine with free haemoglobin in the plasma. (2) β-**globulin**—mol. wt. ranging from 90,000 to 1,300,000, isoelectric pH 5·6. β-globulins are (*a*) β-lipoprotein which helps in the carriage of lipid, steroid and carotene, (*b*) globulin which helps in the transport of iron, e.g., transferrin (siderophilin) which can also combine loosely with cupric copper. Prothrombin is a β-globulin. (3) γ-**globulin**—mol. wt. ranging from 150,000 to 190,000, isoelectric pH 6·0. *Antibodies belong to this class.*

By careful electrophoresis, several varieties of functionally different proteins have been isolated more or less in pure form from the globulin fraction. They are immune globulins, hypertensinogen, isohaemoagglutinins (α, β, $\alpha\beta$, etc., *vide* BLOOD GROUPS), prothrombin, plasma thromboplastins, certain anterior pituitary hormones, etc.

Fibrinogen. It is globulin in nature, mol. wt. 341,000, isoelectric pH 5·8. It is coagulated at about 56°C. and precipitated by one-fifth saturation with ammonium sulphate and half saturation with NaCl. It is insoluble in distilled water. It is distinguished from other plasma proteins by its property of clotting, during which fibrinogen is converted into **fibrin.**

The three proteins can be separated and isolated in the following way. Plasma is taken and its total protein is estimated by Kjeldal's process. This includes all the three proteins. Then the total protein of serum from same sample is estimated. Since, serum contains only albumin and globulin, the difference between the two total protein estimations will give the amount of fibrinogen. Another sample of serum is half saturated with ammonium sulphate. This will cause precipitation of all globulins. It is filtered. The filtrate which contains albumin is fully saturated with ammonium sulphate. Albumin will be precipitated. In this way the three fractions can be separated. [Other methods of separation are Electrophoresis, Isoelectric precipitation, Ultracentrifugation, Fractional separation by alcohol, etc.]

Origin of Plasma Proteins. In the embryo, the primitive plasma and the plasma proteins are produced either by secretion or actual solution of the mesenchymal cells. The albumin fraction is the first to be formed. The other varieties appear later. *In the adults all the four fractions are produced by the liver.* This is supported by isotopic experiments. *Fibrinogen, prothrombin and albumin are manufactured only in the liver.* Regarding globulin, several other sources have been suggested, such as (*a*) from the disintegrated blood cells, (*b*) from the reticulo-endothelial system (specially the γ-globulin), (*c*) from the tissue cells in general, (*d*) from lymphoid nodules. The plasma proteins are not static entities. Isotopic experiments indicate that they are completely used up and replaced every fourteen days. Albumin synthesis is stimulated by osmotic pressure changes and by hypoproteinemia; globulin, by a depressed blood protein pool and fibrinogen by systemic inflammation.

Rate of regeneration of Plasma Proteins. After depletion of plasma proteins, such as by severe haemorrhage or **after** blood donation, the plasma proteins come to the normal level in about **fourteen** days. Fibrinogen is regenerated first, then comes globulin and last of all serum albumin.

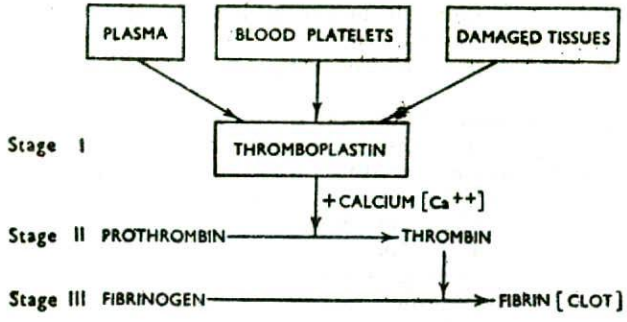
Relation of Diet to Plasma Proteins. Whipple performed the experiment of plasmapheresis upon dogs. The dog was bled, and the cells were separated from plasma. The plasma was rejected and the cells were re-injected being suspended in *Ringer-Locke's solution.* This is continued for several weeks after a protein concentration of 4% has been attained in order to exhaust the protein reserves. Then it is found that on a standard diet, the rate of plasma protein regeneration is constant. It is seen that during fasting only 2 to 8 gm of plasma proteins are formed weekly by the body tissues. Regarding the efficacy of food protein, the more a particular protein resembles the plasma proteins in the quality and quantity of its amino acid content the more effective it will be in this respect. Obviously, plasma itself will be the best. If whole plasma is given by mouth, for every 3 gm of plasma protein ingested, 1 gm of plasma protein is formed. The potency ratio is therefore 3 : 1. The potency ratio of the proteins in grains, potatoes, kidney or liver is 5 : 1. That of red cells, heart or spleen is 10 : 1. *From this, it is seen that excepting plasma proteins, a diet containing grains, potatoes, kidney or liver will regenerate the lost plasma proteins in the earliest time. Moreover, it is found that plant and grain proteins favour globulin formation, whereas animal proteins favour albumin formation.* **Role of essential amino acids** is noteworthy. Plasma proteins can be synthesised from amino acids if all the essential amino acids are freely supplied. Methionine is essential for long

continued production. Cystine is less effective and can replace methionine for short periods only.

Vitamin K helps in the formation of prothrombin in the liver.

Functions of Plasma Proteins

1. **Essential for blood clotting.** Fibrinogen and prothrombin are essential for coagulation of blood and take part in this process in the following way:



2. **Maintain colloidal osmotic pressure of blood and aid in regulating the distribution of fluid between blood and tissues.** All the three proteins take part. Albumin having the smallest and the most symmetrical molecule exerts the maximum osmotic pressure. Osmotic pressure depends upon a number of molecules in the solution. Albumin has a considerably smaller molecular wt. than globulin and comprise 52 per cent. of plasma protein. One gram of albumin in 100 ml will exert a pressure of 5·5 mm of Hg. Under the same conditions globulin exerts only 1·5 mm of Hg. The total colloidal O.P. varies from 25 to 30 mm of Hg and albumin is responsible for 80% of it.

3. **Maintain viscosity and blood pressure.** The proteins of plasma, mainly globulins due to larger molecules and asymmetry of their structure are responsible to some extent for the viscosity of blood, and viscosity is an important factor in maintaining blood pressure which is essential for efficient heart action. Whole blood is *isoviscous* with 25 per cent. albumin, 15 per cent. γ-globulin and 2 per cent. fibrinogen. The relative viscosity of blood is 4·7 for men, 4·4 for women, 4·2 for children; that of plasma as 1·8 and that of serum as 1·5 relative to distilled water at 37°C. (98·6°F.).

4. **Concerned with erythrocyte sedimentation rate (ESR).** The plasma proteins exert a great influence upon the suspension stability of blood. This is chiefly dependent on fibrinogen, less on globulin and least on albumin. An increase in fibrinogen rises the sedimentation rate of red blood corpuscles by increasing the speed of rouleaux formation. Since, the plasma proteins alter in various diseases, determination of sedimentation rate is of considerable clinical importance and constitutes a guide to the progress of disease. It is determined by the methods of Wintrobe, Westergren and Cutler.

5. **Act as buffers.** They act as buffers in maintaining acid-base balance.

6. **Act as a protein reserve.** The plasma proteins serve as a storehouse of proteins from which the tissue can draw during starvation or inadequate protein diet.

7. **Help CO_2 carriage by forming carbamino proteins** (*vide* CO_2 carriage).

8. **Form trephones.** The leucocytes prepare substances from the plasma proteins, called trephones, which are necessary for the nourishment of the tissue cells grown in culture.

9. **Antibodies.** The antibodies being γ-globulin in nature make up a small fraction of the globulins of plasma for defence against infection.

10. **Help transport of certain substances in blood.** Plasma proteins combine with certain substances and help to carry them in the blood stream. (*a*) Some hormones, enzymes, and clotting factors are part of globulin fraction of plasma proteins. (*b*) Iron (transferrin) and copper (ceruloplasmin) are bound to globulin fractions.

VISCOSITY OF BLOOD

Viscosity of blood is an important factor, since, it determines the peripheral resistance of the blood flow through the blood vessels and thus helps to maintain blood pressure. Human blood is five times more viscous than distilled water. The viscosity of the whole blood is mainly due to cells and that of plasma is due to plasma proteins. The viscosity of plasma is less than that of whole blood. The relative viscosities of water, plasma and whole blood are roughly 1, 1·8 and 4·7 respectively. Viscosity is measured by noting the time of flow of a given volume of blood through a specially prepared tube (*Viscosimeter*) and comparing the time taken by the same volume of distilled water to pass through the same tube. Viscosity is affected by various factors. It depends on the amount of plasma proteins, the number and volume of corpuscles and CO_2 tension. Viscosity rises in acidosis, hyperglycaemia, hypercalcaemia, polycythemia, cyanosis, icterus, diabetes mellitus, etc., and is reduced in anaemia, fever, exercise, edema, lymphatic leukaemia and malaria. Rise of temperature reduces the viscosity of blood.

SUSPENSION STABILITY OF BLOOD (ERYTHROCYTE SEDIMENTATION RATE OR ESR)

While in circulation, the red cells remain uniformly suspended in the plasma. If an anticoagulant is added to a specimen of blood and let stand it in a glass tube, the corpuscles (being heavier than plasma) are found to sediment gradually at the bottom of the tube, while the plasma remains as a clear supernatant fluid. This process is called *sedimentation* and the *rate of settling down* or *sedimentation* is known as the *erythrocyte sedimentation rate* (ESR).

Sedimentation continues in three phases—(1) a short stage of aggregation with a little fall, (2) true sedimentation following maximum velocity of fall and (3) finally slowing until packing is complete leaving an upper layer of clear plasma. It depends (*a*) on the differences in densities between red blood corpuscles and plasma, (*b*) on the degree of adherence of red blood corpuscles to one another (rouleaux formation) related to the plasma protein content and (*c*) on the resistance that plasma exerts on the red cell surface.

Red cells carry a negative charge, and hence any condition which increases the positive charge in plasma accelerates ESR. ESR is increased with (1) rise in O_2, (2) rise in cholesterol, (3) rise in fibrinogen and (4) rise in α-globulin. It is decreased with (1) rise in CO_2, (2) rise in albumin, (3) rise in nucleoprotein and (4) rise in lecithin.

ESR constitutes a guide to the progress of a disease. It is measured either in relation to a fixed time or distance by the methods of Wintrobe, Westergren or Cutler. The tube is put in a vertical position, the lower end of which is placed on a rubber cap and the upper end remains open. Height of supernatant plasma in mm separated out at the top of the vertical column of blood after an hour is noted. This is expressed as the erythrocyte sedimentation rate.

Normal ESR values by Wintrobe's method is 0·0–6·5 mm per hour for males, average being 3·7 mm per hour and 0·0–15·0 mm per hour for females, average being 9·6 mm per hour.

Physiologic variations are as follows: (1) Lowest in new-born, 0·0–2·0 mm per hour. In children it is from 3·0 to 13·0 mm/hour, average 9·0 mm/hour. (2) In pregnancy there is a definite acceleration beginning from the tenth to twelfth week. The rate gradually increases and reaches normal level only at third or fourth week post-partum. (3) In old age the ESR increases.

It is a non-specific reaction, and gives information of a general character, but is useful supplement when temperature, pulse, etc., show no clinical disorder. ESR is increased in all acute general infections. One of the most important uses of the test is in calling attention to the presence of more or less occult disease. It is also useful as an aid in differential diagnosis. Other things being equal, an accelerated rate suggests organic disease rather than functional disorder. It acts as a guide to the progress of a disease.

REACTION OF BLOOD

The pH of blood varies between 7·36 and 7·45, the average is about 7·4. The pH of venous blood during rest is lower by 0·02 than that of the arterial blood. The red cells are less alkaline than plasma with a pH of about 7·1. As ordinarily used, the term blood pH really means the plasma pH. The pH of blood is maintained at a constant level under physiological conditions. Even under extreme conditions of acidosis and alkalosis, very little alteration of pH is seen. The limits of pH compatible with life, are usually not higher than 7·8 and not lower than 6·8. Except in the terminal stages, blood reaction does not go beyond this range. The mechanism by which blood reaction is kept constant has been discussed elsewhere.

Methods of Determination of Blood Reaction

1. **Colorimetric Method** (Dale and Evans). A collodion sac holding about 2–3 ml of oxalated blood is made to dialyse for 15 minutes into a solution of 1 ml of normal saline. Contact with air is prevented to stop CO_2 loss. A few drops of an **indicator** (0·02% of neutral red) is added to the clear dialysate. The colour developed is matched against that of a phosphate solution of known pH and containing neutral red in the same proportion as the dialysate.

2. **Electrometric Method.** (*a*) By means of **hydrogen electrode** —A hydrogen electrode is composed of platinum black saturated with hydrogen gas. One of a pair of hydrogen electrodes is placed in a solution

of known pH and the other in the unknown solution. When the two electrodes are connected, a potential difference is set up between the two electrodes and from its magnitude the pH of the unknown solution can be calculated. (*b*) By means of **glass electrode**—Instead of hydrogen electrode glass electrode can be used in the same way. The glass electrode has the great advantage that it is not affected by oxygen, protein content and other substances in the test material.

COAGULATION OF BLOOD

When blood is shed it loses its fluidity in a few minutes and sets into a semisolid jelly. This phenomenon is called **coagulation** or *clotting*. On further keeping, the clot retracts to a smaller volume and presses out a clear straw-coloured fluid, called the **serum.** Serum will not clot any more.

When the process of coagulation is studied under the ultramicroscope, it is seen that, minute granules appear at first, often near a clump of disintegrating platelets. These granules join together to form needles, which again unite with one another to form long threads passing across the whole bulk of blood. These threads cross one another and form a sort of network, into the meshes of which the red and white cells become entangled. The clot gradually retracts and serum separates out.

It is to be noted that coagulation is the property of plasma alone. The red and white cells do not take part in it. They only become caught up in the meshes of the clot and are thereby removed. It is due to this fact that the clot has a red colour, and the serum is a clear non-cellular fluid. Blood platelets take some part in the process.

Normal Coagulation Time. Measured according to the method of Lee and White it is 6 to 17 minutes in glass tube and 19 to 60 minutes in siliconised tube.

Methods of Determination of Coagulation of Blood

1. **Capillary Glass Tube Method.** This method is usually adopted as a bedside procedure. The finger is pricked and the blood is made to flow into a capillary glass tube about 15 cm (6 inches) long. A small bit of the glass tube is carefully broken off every fifteen seconds until a fine thread of clotted blood appears while the tube is being broken. The period between the appearance of blood in the finger and the formation of this thread is taken as the *coagulation time*. The average time, by this method, is 3–4 minutes.

2. **Wright's Coagulometer.** The principle is same as above. Blood is allowed to flow into a dozen capillary tubes of equal calibre. The tubes are sealed on both sides and placed in water bath at 37°C. After 4 minutes, the first tube (the tube which was first filled with blood) is removed from the water bath, the ends are broken and the blood inside is expelled into water. The same procedure is repeated with all the other tubes at intervals of 30 seconds. When the blood expelled from a particular tube has the form of a worm-like clot, the end point is reached.

3. **Method of Lee and White.** 1 ml of blood is drawn from a vein by a dry syringe and placed in two clean test-tubes, 8 mm in diameter. The tubes are closed by a rubber cork. At 5 minutes after withdrawal of the blood in the manner described, the first tube is gently tilted 45 degrees at one minute intervals until it can be inverted 180 degrees without blood flowing. This time is recorded and the same procedure is repeated with the second tube. As handling favours coagulation, the time for the second tube

is taken as the true coagulation time since it was tilted less than first tube.

Bleeding Time. Normal average is 3·25 minutes, the range being 2–5 minutes. It is usually determined by Duke's method. The lobule of the ear is punctured and the time is noted. The blood oozing out is mopped up with a piece of filter paper every half a minute until bleeding stops. This indicates the end point.

Prothrombin Time (Quick). An approximate prothrombin time is generally 11 to 16 seconds. When tissue extract (thromboplastin) and calcium chloride are mixed (added) in an optimum amount to blood of normal fibrinogen content, the only factor which has an inadequate concentration of prothrombin, can vary the coagulation time. If the prothrombin is diminished the coagulation time increases. This test is a quantitative one for prothrombin in blood based on the coagulation time of oxalated blood plasma in the presence of tissue extract (thromboplastin) and calcium chloride. In each laboratory a curve of prothrombin concentration in the blood to the prothrombin time is usually drawn for the evaluation of the prothrombin time. The only precaution is that blood removed from the patient is immediately oxalated so that none of the prothrombin can be converted into thrombin. The method is as follows: In a test-tube 0·2 ml of commercial thromboplastin containing calcium is kept at 37°C. After 30 seconds, 0·1 ml plasma is quickly added from a pipette, and a stop-watch is started simultaneously. The tube is kept in the water bath and is shaken constantly but gently for 10 seconds. Then in bright direct illumination the tube is tilted continually from vertical to almost horizontal position once per second until a gel appears. This is the end point. A value of 11 to 16 seconds is satisfactory, but the test should always be performed in duplicate.

Importance of Coagulation of Blood. The phenomenon of coagulation is of enormous physiological importance. *Its purpose is to stop further haemorrhage.* When bleeding occurs, the shed blood coagulates and the bleeding vessels become plugged off by the clot. The retraction of the clot compresses the ruptured vessels further and in this way bleeding is stopped.

Mechanism of Coagulation or Clotting of Blood. As early as 1904 Morawitz described the basic facts about the mechanism of blood clotting in the following manner. When blood is shed, the platelets (by coming in contact with rough water-wettable surface), disintegrate and liberate thromboplastin. Certain amount of thromboplastin is also derived from the damaged tissues of the injured locality. Thromboplastin converts prothrombin into thrombin with the help of calcium ions and thrombin interacts with fibrinogen forming fibrin. This is the *clot.* This theory can be summarised in the following steps:

Normally: Prothrombin + Calcium ion + Fibrinogen ⟶ Nil.

After shedding of blood:

1. Thromboplastin + Prothrombin + Calcium ion ⟶ Thrombin.
2. Thrombin + Fibrinogen ⟶ Fibrin (Clot).

Since 1940, research work has indicated that the clotting mechanism is a complex process. In 1954 an International Committee was established. The committee suggested an international system of nomenclature time to time with the appearance of new factors.

NUMERICAL SYSTEM FOR INTERNATIONAL NOMENCLATURE OF BLOOD COAGULATION FACTORS

Clotting Factor	Synonym
I	Fibrinogen.
II	Prothrombin.
III	Tissue thromboplastin *or* Extrinsic thromboplastin *or* Platelet factor 3.
IV	Calcium.
V	Labile factor *or* Accelerator globulin *or* Ac-globulin *or* Ac-G *or* Thrombogene *or* Proaccelerin.
VI	Accelerin.
VII	Stable factor *or* Proconvertin *or* Precursor of serum prothrombin conversion accelerator (pro-SPCA) *or* Cothromboplastin *or* Autoprothrombin I.
VIII	Antihaemophilic factor (AHF) *or* Antihaemophilic globulin (AHG) *or* Platelet cofactor I *or* Thromboplastinogen.
IX	Plasma thromboplastin component (PTC) *or* Christmas factor (CF) *or* Platelet cofactor II *or* Autoprothrombin II *or* antihaemophilic factor B.
X	Stuart factor *or* Prower factor *or* Stuart-Prower factor *or* Venom substrate *or* Autoprothrombin III (I_o).
XI	Plasma thromboplastin antecedent (PTA) *or* Antihaemophilic factor C.
XII	Hageman factor *or* Surface factor *or* Contact factor *or* Clot-promoting factor.
XIII	Laki-Lorand factor (LLF) *or* Fibrin-stabilising factor *or* Fibrinase.
Prothrombin activator	Thrombokinase *or* Complete thromboplastin.

Blood Coagulation factors

Factor I or Fibrinogen. It is globulin in nature but has a much bigger molecule than serum globulin. The molecular weight is about 330,000. It is coagulated at about 56°C. and precipitated by one-fifth saturation with ammonium sulphate and half saturation with NaCl. It is distinguished from other plasma proteins by its property of clotting, during which fibrinogen is converted into fibrin.

Factor II or Prothrombin. It is protein in nature and present in normal plasma. It has a molecular weight of about 62,700. It is very labile in aqueous solution and is inactivated by acids at pH 4·8, by alkali at pH 10·0 and by heat at 60°C.; but is stable indefinitely when dried from the frozen state. In oxalated plasma, two forms of prothrombin are found 'A' and 'B'. The 'A' form is destroyed by oxygen and is heat-labile. The 'B' form is removable by aluminium hydroxide. In normal plasma the two forms remain united as calcium compound. When oxalate is added, calcium is removed and the two components become separate. It can be

isolated as follows: If the pH of plasma is adjusted at 5·3, both prothrombin and globulin are precipitated. Prothrombin is dissolved by treating the precipitate with dilute calcium bicarbonate. After filtration the pH of the filtrate is again adjusted to 5·3 when prothrombin separates out. In this form it is a white powder, insoluble in water and remains combined with a protein. 100 ml of plasma contains 40 mgm of this substance. The prothrombin activity of blood is measured by the clotting time of recalcified oxalated plasma, to which *tissue emulsion* has been added. *In human subject, the average 'prothrombin time' is* 12 *seconds. Prothrombin time* will be longer in deficiency of factor V or factor VII or Stuart factor. *Prothrombin is manufactured in the liver. Vitamin K is essential for the formation of prothrombin. During clotting, prothrombin is converted into thrombin.*

Factor III or Thromboplastin. It is derived from two sources: (1) Intrinsic in the plasma, (2) Extrinsic in the tissues. *Intrinsic thromboplastin* is formed in the plasma due to interaction between different plasma factors, e.g., Hageman factor or factor XII, PTA or factor XI, Christmas factor or factor IX, antihaemophilic globulin or factor VIII, calcium ions, factor V and factor X. Prothrombin is converted into thrombin with the help of intrinsic thromboplastin in presence of calcium ions. It should be noted that blood flowing normally through the circulatory system will not clot. But if the surface of the blood vessel becomes rough due to any reason, blood will clot even without the addition of tissue extract (extrinsic thromboplastin). *Extrinsic or tissue thromboplastin* is formed from different tissues, e.g., extracts of brain, lungs, etc., as a result of injury. So long it was known that prothrombin was converted into thrombin with the help of calcium ions and thromboplastin liberated from damaged tissues. But recently it has been found that various plasma factors, e.g., factor VII or proconvertin, are required for such conversion and the process is called *extrinsic thromboplastin formation.*

Factor IV or Calcium. Ionic calcium greatly helps in blood clotting, by acting as a cofactor in the coagulation process. It is essential for the formation of both intrinsic and extrinsic thromboplastins and also in the conversion of prothrombin into thrombin.

Factor V or Labile factor or Accelerator globulin or Proaccelerin. This factor is necessary for the complete conversion of prothrombin into thrombin by the extrinsic or intrinsic thromboplastin. It is a protein, heat-labile and is inactivated within half an hour at 56°C. or by increasing the pH to 10·5. It is present in plasma but is used up during clotting.

Factor VI or Accelerin. This factor is a hypothetical activation product of proaccelerin (factor V).

Factor VII or Stable factor or Proconvertin. This factor is present in plasma and is not used up during clotting. It is heat-stable and can withstand temperature up to 56°C. It is a protein and remains associated with prothrombin. It accelerates extrinsic or tissue thromboplastin formation, being activated by extract released from damaged tissue. Its formation is retarded after administration of *Dicoumarin* and in deficiency of vitamin K. During blood clotting proconvertin is changed into convertin.

Factor VIII or Antihaemophilic factor (AHF) or Antihaemophilic globulin (AHG) or Platelet cofactor I. This factor helps in the formation of intrinsic thromboplastin and intrinsic prothrombin conversion. It is present in the plasma and disappears when the blood clots. It is

protein in nature and remains in close association with fibrinogen. This factor is antihaemophilic. In *Haemophilia* (*bleeder's disease*) the defect is not in the platelets but it is due to the absence of this factor which helps in the breakdown of platelets and liberation of platelet cofactor I or thromboplastin factor. The deficiency of AHG resulting in *classical haemophilia* in the male is transmitted as a sex-linked recessive trait. The body fails to synthesise this essential globulin due to the absence of the specific enzyme which is controlled by the mutant gene. It is adsorbed on barium sulphate and has a molecular weight greater than 200,000.

Factor IX or Christmas factor or Plasma thromboplastin component (PTC) or Platelet cofactor II. This factor is necessary for intrinsic thromboplastin formation. Absence of this factor leads to a disease simulating Haemophilia known as **Haemophilia C** and is transmitted as a sex-linked recessive in the male. It is adsorbed by aluminium hydroxide, is labile to heat but is relatively stable on storage. It is precipitated by 50 per cent. ammonium sulphate. This type of disease was first found in a patient named *Christmas* and hence the name Christmas factor. This factor is not used up during clotting.

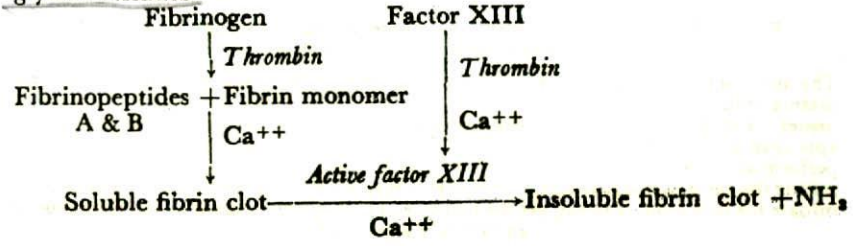
Factor X or Stuart factor. In 1959 the international nomenclature has been given to this factor. *Chemically* it has many of the properties similar to that of factor VII. Its synthesis is also retarded after administration of Dicoumarin. Absence of this factor leads to mild haemorrhagic diathesis. It is stable in room temperature, but destroyed rapidly at 56°C. in serum.

Factor XI or Plasma thromboplastin antecedent (PTA). This is activated by active Hageman factor, and ultimately leads to formation of thrombin. Deficiency of this causes mild bleeding tendencies of **haemophiloid D** type and is transmitted as a sex-linked dominant to both sexes.

Factor XII or Hageman or Surface factor. This is protein in nature. Inactive form is activated on surface contact. This in turn activates the protein-splitting enzyme kallikrein to produce plasma kinins. The resulting effects are increased vascular permeability and dilatation of blood vessels.

Factor XIII or Fibrin-stabilising or Laki-Lorand factor (LLF). The active form along with Ca^{++} converts soft fibrin clot to a solid, fibrous one. Its action also decreases the solubility of the clot in urea sol. Persons having congenital malformation of LLF suffer from poor wound healing.

Role of Thrombin. Thrombin is a homogeneous glycoprotein of molecular weight 40,000 acting as a proteinase. It autocatalyses its own formation by converting plasma Ac-globulin into the active serum Ac-globulin and by labilising platelets to accelerate thromboplastin generation and liberates vasoconstrictors. It splits only four peptide bonds in the conversion of fibrinogen to fibrin and these are all bonds between arginine and glycine residues.



Role of Phospholipid. Phospholipid kephalin (cephalin) helps in the formation of *prothrombinase*. In the intrinsic system it is in the platelet factor 3 and in the extrinsic one in tissue thromboplastin.

Role of Protein. Blood clotting factors, from V to XII, are plasma proteins mostly β-globulins. A few of them are however either α-globulin or γ-globulin.

The needs for contribution of many clotting factors are involved in the coagulation process. But the plasma protein clotting factors usually interact in pairs. Due to this interaction each of the clotting factors is in turn converted from an inactive form to an active one. Since all the clotting factors are

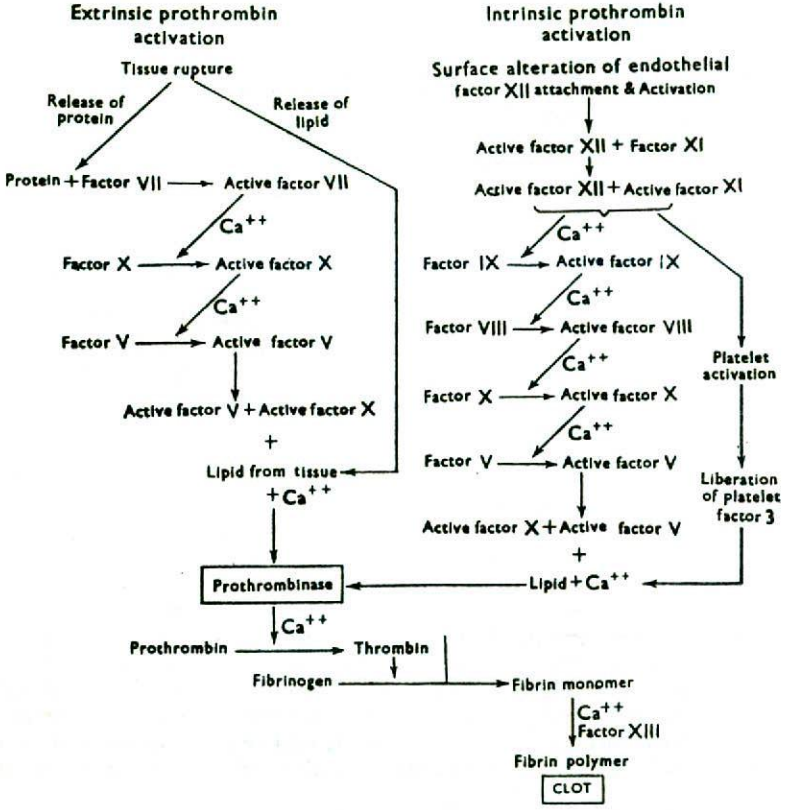


FIG. 95. Diagrammatic representation of the process of extrinsic and intrinsic factors in the coagulation of blood.

The above schematic diagram shows that the intrinsic system being wholly present in the plasma, initiates the formation of fibrin without provision of blood clotting factors from the tissues. On the other hand, the extrinsic system varies from the intrinsic system, by the appearance of factors derived from outside the plasma. These extrinsic factors interact to perform active Stuart factor (factor X). From this point the intrinsic and extrinsic systems pursue the same pathway to fibrin. Although either the extrinsic or the intrinsic system can initiate the formation of fibrin, yet the presence of both systems results in the formation of an extensive amount of it.

not supposed to possess enzyme actions, yet this conversion of enzymes from an inactive form to an active one is initiated in the sequence of action of clotting factors. The process of intrinsic and extrinsic systems has been shown schematically in Fig. 95.

Davie and Ratnoff (1965) have proposed what has been termed a 'waterfall' sequence hypothesis to explain the succession of events taking place in the coagulation of blood. Each protein coagulation factor exists in the plasma in an inactive (proenzyme) form and is activated sequentially until finally thrombin is formed which then converts fibrinogen into fibrin.

Macfarlane has suggested a scheme of coagulation called an **enzyme cascade** which is very similar to the 'waterfall' scheme (Fig. 96) of Davie and Ratnoff.

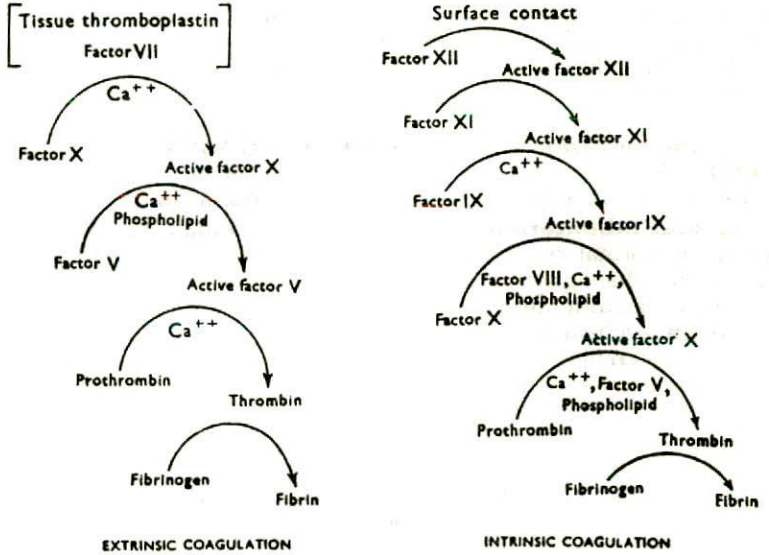


Fig. 96. 'Waterfall' schemes of extrinsic and intrinsic blood coagulation. (From Davie, E.W. & Ratnoff, O.D.).

Clot Retraction. Usually blood clot retracts to about half its initial volume within 20 to 24 hours. When blood is shed fibrins form a network-like structure. The platelets adhere to these fibrin networks and form knots. The fibrin framework then becomes twisted and shortened, and clot retraction occurs.

Diseases that occur due to defects in clotting mechanism

1. **Lack of Fibrinogen or Factor I.** Afibrinogenaemia or fibrinogenopenia is a rare congenital disease due to lack of fibrinogen. Sometimes it is found during abnormal pregnancy.

2. **Due to Diminution of Prothrombin or Factor II.** Vitamin K helps in the formation of prothrombin in the liver. Vitamin K is a naphthoquinone derivative. It is absorbed from the small intestine in the presence of bile salts. In the liver it helps in the synthesis of prothrombin

and factor VII or stable factor or proconvertin. In the liver disease, e.g., Cirrhosis of liver, Malignant disease of the liver, etc., there is diminution of synthesis of prothrombin in the liver. In *Obstructive jaundice* due to absence of bile salts, vitamin K is not absorbed. Due to lack of vitamin K, synthesis of prothrombin and factor VII is decreased. Prothrombin time is prolonged and haemorrhages often occur.

3. **Due to lack of AHG or Factor VIII—Haemophilia.** It is a disease which occurs in males but is transmitted through females. The coagulation time is abnormally prolonged. There is a tendency to bleed severely after trivial injuries. The knee or elbow joint may be distended with blood. The platelet count remains normal. There is lack of factor VIII or antihaemophilic globulin (AHG). Blood transfusion temporarily supplies AHG and stops bleeding. Sometimes it has been observed that if blood taken from two subjects are mixed together, coagulation time is normal although the coagulation time of each individual subject has got prolonged coagulation time. From this it has been assumed that there are two types of haemophilic subjects, one lacking in AHG and another lacking factor IX or Christmas factor or PTC.

4. **Due to Diminution of Factors V, VII and IX—Pseudohaemophilia.** In this disease there is congenital deficiency of factors V, VII and IX. The haemorrhagic condition simulates haemophilia.

Natural inhibitors of coagulation. To maintain blood in a fluid state in the normal condition, retarding influences coexist with positive coagulation-inducing factors in the circulating blood. Some of the ingrained safeguards against intravascular clotting are: (*a*) the relative slowness of thrombin production, (*b*) the unbroken continuity of the vascular endothelium and (*c*) removal of clotting intermediates by the R. E. cells. Besides these, other definite inhibitors of coagulation are present.

Antithrombin activities remove thrombin from blood. **Antithrombin I** is the thrombin-adsorbing effect of fibrin but whether it plays a role in normal coagulation is unknown.

Antithrombin II is a factor which acts jointly with heparin.

Antithrombin III is the so-called physiological antithrombin because it is present naturally and inactivates thrombin progressively.

Heparin is described separately below.

Antithromboplastins are present in normal blood, and one or more circulating antithromboplastins are claimed to be present.

Intravascular Clotting or Thrombosis

Thrombus is a clot formed inside the blood vessels. Thrombus is formed due to slowing of circulation and damage of the vascular endothelium. Atheromatous patches occur in blood vessels and the vascular endothelium is damaged in some abnormal conditions. Masses of platelets are deposited on the damaged endothelium. Filaments of fibrin form also a network in this region. The platelets liberate thromboplastin. The fibrin, entangled in the lamellae of platelets, forms the thrombus or clot. Intravascular thrombosis sometimes occurs in coronary and cerebral vessels which are called *coronary thrombosis* and *cerebral thrombosis* respectively. After surgical operations, etc., thrombosis may occur in big veins.

Heparin. At first it was isolated from liver by McLean in Howell's laboratory, hence the name. Subsequently, it has been extracted from

many tissues in the body. It is anticoagulant, both *in vivo* and *in vitro. One unit of heparin is defined as the quantity of material which will prevent the clotting of* 1 *ml of cat's blood for* 24 *hours when kept in cold.* Chemically it is mucoitin-polysulphuric acid—Mucoitin is a polysaccharide, composed of glucosamine, glucuronic acid and esterified sulphuric acid forming an ester with molecular weight of about 17,000. It has been shown that any substance with a high molecular weight, and being composed of polysaccharides and several SO_4 groups, can act as an anticoagulant. *Hirudin,* found in cervical glands of the common medicinal leech (*Hirudo*), is a compound of this nature. *Heparin* is normally secreted by the mast cells. These cells are found in blood to about 1%. They remain scattered throughout the reticulo-endothelial system and found abundantly along the course of many blood vessels, such as those of liver. Sometimes they replace the intima of the blood vessels. These cells are found to contain granules which are supposed to be the precursors of heparin. It is doubtful whether heparin is present in normal blood in any appreciable amount and as such it probably takes no part in preventing intravascular clotting normally. Heparin helps to maintain the normal fluidity of the blood within the vascular bed. It inhibits the transformation of prothrombin to thrombin when accompanied by a plasma cofactor albumin X and neutralises the action of thrombin on fibrinogen.

Fibrinolysis. Clotted blood if kept sterile remains intact for several weeks. But if it is not kept sterile the clot breaks up. This fibrin breakdown in the clot is known as fibrinolysis and is brought about by a proteolytic enzyme in the plasma known as *plasmin* or *fibrinolysin.* The precursor of the enzyme *plasminogen* (also known as *profibrinolysin*) is activated to plasmin by activators present in tissues, serum, urine and some bacteria. Normally fibrinolysis is prevented by the presence of another substance in the blood known as *antiplasmin* which remains attached to the plasma albumin.

Factors preventing coagulation

1. By lowering temperature, coagulation can be prevented.
2. By avoiding contact with water-wettable surface and injured tissues. This prevents thrombokinase action. When blood is collected in a tube coated with paraffin, the surface not being water-wettable, the platelets will not break down and coagulation will not take place.
3. Removal of calcium ions. (*a*) By precipitation—This is the commonest practice in clinical laboratories. This is done by adding citrates or oxalates of Na or K. Sodium fluoride (0·3% solution) is also used. (*b*) By formation of a complex compound—The substances used are di- and trisodium citrate and ethylene diamine tetra acetate (EDTA).
4. Precipitation of fibrinogen. By adding various salt solutions in adequate amounts. When blood is mixed with one quarter of its volume of magnesium sulphate or with an equal volume of half-saturated sodium sulphate solution, clotting is prevented.
5. By the addition of substances of biological origin. (*a*) Protamines —simple proteins found in some fish. (*b*) Peptone—when it is injected into the veins, the coagulability of blood is reduced. [But peptone does not prevent the coagulation of a sample of blood *in vitro.*] Extracts of cray fish and mussels act in somewhat similar manner. They act by increasing secretion of heparin by the mast cells. (*c*) Heparin—mucoitin-polysulphuric acid produced by mast cells. (*d*) Hirudin (Leech extract) and the venom of certain

snakes—*Heparin, hirudin* and *venom* inhibit coagulation of blood by inhibiting activation of prothrombin and thrombin fibrinogen reaction. (*e*) Cysteine—same as heparin and hirudin. (*f*) **Dicoumarin** or **Dicoumarol**—It is chemically related to the naphthoquinone derivative. It is antagonist to vitamin K. It inhibits the synthesis of prothrombin in the liver by preventing the action of vitamin K. Dicoumarol lowers the plasma prothrombin level and depresses the activity of factor VII. (*g*) **Phenindione**—Action similar to that of dicoumarol. Its action is quick and depresses the activity of factor VII more than prothrombin.

6. By adding azo dyes and synthetic products—Chicago blue, trypan red, trypan blue act as anticoagulants both *in vivo* and *in vitro*.

Factors hastening coagulation

1. Warmth.
2. Contact with water-wettable surface and contact with rough surface.
3. Additions of foreign bodies into a sample of blood (*vide* 'Defibrinated blood'*).
4. Addition of thrombin.
5. Addition of thromboplastin.
6. Vitamin K injection or oral administration in high doses increases the prothrombin content of blood and increases the coagulability.
7. Addition of calcium chloride, both *in vivo* and *in vitro*.
8. Adrenaline injection produces constriction of blood vessels and helps in haemostatic mechanism.

Lipid and coagulation. Recently a good number of evidences have been put forward to suggest that elevation of both neutral and phospholipid concentration of blood leads to aggregation of fibrin deposit, possibly due to retardation of the fibrin-dissolving system. This leads to augmentation in thrombus formation, and might result in increased incidence of thrombosis and atherosclerosis. Saturated fats are more agile in decreasing clotting time than unsaturated ones.

BLOOD VOLUME

Definition. The term **blood volume** means the total amount of blood in circulation, as well as in the blood stores.

Normal blood volume. It can be expressed in two ways: (*a*) **In relation to body weight**—78 to 97 ml (average 90 ml) per Kg of body weight; or about 1/11th (9%) of the total body weight. That of plasma is 50 ml per Kg body wt. or 1/20th (5%) of the total body weight. (*b*) **In relation to body surface**—2·5 to 4 litres (average 3·3) per sq. metre of body surface. A man, weighing 70 Kg, has about 5 litres of blood in his circulation.

Variations under different physiological conditions. (1) **Age**—In infants the blood volume is greater in proportion to the body weight but is lesser in proportion to the body surface. [The body surface of infants is

* **Defibrinated blood.** When a sample of blood is constantly stirred or shaken with fine twigs or pieces of wire or glass beads, the blood clots and the fibrin gradually collects upon the surface of the foreign bodies. In this way all the fibrinogen becomes converted into fibrin in a short time and is quickly removed from the blood sample. This blood will not clot any further and remains fluid. It is composed of serum in which red and white cells remain suspended. Defibrinated blood is frequently used in physiological experiments, instead of using any anticoagulant.

proportionally larger than their body weight.] This larger volume is due to greater number of corpuscles as well as larger amount of plasma. (2) **Sex**—In males the blood volume is 7·5% higher (per sq. metre of body surface) than in females. This is due to greater number of red cells in the males. The plasma volume is same in both. (3) **Body weight** and **surface area** (*vide* above). (4) **Pregnancy**—Blood volume rises due to increase of both cells and plasma, but the rise in plasma volume is much greater than the rise in cell volume. It falls after delivery. (5) **Muscular exercise**—Raises blood volume probably due to contraction of spleen. (6) **Posture**—In erect posture there is about 15% diminution of total plasma. It passes out into the tissue spaces. (7) **Blood pressure**—Rise of blood pressure lowers blood volume by pressing out more fluid into the tissue spaces. Lowered blood pressure draws in more fluid from the tissue spaces and raises the blood volume. (8) **Altitude**—At higher altitude the blood volume rises, due to anoxia produced the number of red cells increase in such condition. (9) **Anoxia** due to any other cause will raise blood volume. (10) **Adrenaline injection**—Raises blood volume probably by splenic contraction.

Methods of determination of Blood Volume. (A) **Direct Method.** (1) (Welcker)—An animal is bled to death and the blood is collected. Then its blood vessels are washed out by pumping saline solution into the vessels. The saline washings are added to the already collected blood. The colour of this mixture is matched against the sample of normal blood of the same animal. From these data the volume may be calculated. In dogs the total blood volume is 7·7% of body weight. (2) (Bischoff)—The above method was applied upon decapitated criminals. It is obvious that the direct methods have got no place in clinical medicine, for which one of the following indirect methods are used. (B) **Indirect Method**—In this method a known amount of a particular substance is introduced into the blood stream. After some time a sample of blood is drawn out and the concentration of the injected substance is determined in it. From this, the degree of dilution is calculated. Total blood volume can be found out from these data. The substance to be used must have certain special qualities. It must not be toxic, must not alter blood volume, must not easily pass into the tissue spaces or be excreted, and must not be taken up by the phagocytic cells of the blood. Also it must not change its colour and chemical composition while in the body. Usually, two classes of substances are used for this purpose. (1) Dye-stuff like Congo red, Evans' blue, etc. (2) Radioactive substances.

1. **Dye method.** For clinical purposes the blood volume can be determined by dye method. The dye Evans' blue (T-1824) which is non-toxic and escapes slowly from the blood vessels, is used mostly in present times. 10 ml of venous blood from the subject is taken in a heparinised tube. This serves as the control sample. 5 ml of a 5% solution of Evans' blue in distilled water is then injected intravenously. 10 minutes after beginning of the injection another 10 ml sample is withdrawn from the vein of the opposite side into another heparinised tube. The Haematocrit of both samples 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 1 : 500) with control plasma and its optical density is determined.

$$\textit{Plasma volume (ml)} = \text{dye solution injected (ml)} \times \text{dilution of standard} \times \frac{\text{density of standard}}{\text{density of unknown}}.$$

$$\textit{Blood volume (ml)} = \frac{\text{plasma volume}}{1-(0{\cdot}96 \times \text{Haematocrit})} \quad (0{\cdot}96 \text{ is the correction factor}).$$

$$\textit{Red cell volume (ml)} = \text{blood volume} - \text{plasma volume}.$$

2. **Radioactive methods.** (*a*) *Radio-iodine Plasma Albumin Method.* To a sample of plasma, iodine-containing radioactive isotope ^{131}I or ^{132}I are added, and allowed to incubate for some time. This plasma is slowly injected intravenously. The degree of dilution of its radioactivity is determined, which is a measure of plasma volume. The advantage of this method is that the albumin, cannot permeate through the capillary endothelium and is not affected by lipaemia and haemolysis. The plasma and blood volume are measured in a similar way as the dye dilution method. The plasma volume determined by this method is about 2·5 litres in women and 3 litres in men.

(*b*) *Radioactive Iron Method:* Radioactive iron (^{55}Fe or ^{59}Fe) is incorporated into ferric ammonium citrate which is given by intravenous injection to a person (donor) belonging to group O (*vide* Blood Group). The radioactive iron is taken up by the newly formed blood cells which appear in the circulation within twenty-four hours. 75 ml of blood having a radioactivity of about 3,000 counts per minute per ml (determined by Geiger) is injected intravenously to recipient, whose blood volume is to be determined. After about 20 minutes of the injection, 15 ml of blood is withdrawn from the recipient. After interval of 20 minutes, another two samples of blood of the same quantity are withdrawn. The blood samples after proper dilution are centrifuged for half an hour. The samples taken out both from the donor and the recipient are wet-ashed and the iron is deposited electrolytically on copper. The radioactive iron present in the two samples is determined with the help of **Geiger counter.** The red cell volume is calculated from the number of millilitres of *donor's cell* injected, the radioactivity of the donor's cells and the radioactivity of the recipient's cells. The blood volume is determined by measuring the plasma volume by the dye method and the result is added to the value of the red cell volume. Similarly red cell volume can be determined either with labelled radioactive chromium (^{51}Cr) or radioactive phosphorus (^{32}P).

So from all the above methods it is clear that the dye Evans' blue or plasma labelled with radioactive iodine help in the measurement of plasma volume whereas radioactive iron, chromium or phosphorus is used to determine the red cell volume. No substance has yet been found which helps in measurement of the total blood volume directly. For an accurate measurement of blood volume, plasma and cell volumes must be determined simultaneously.

Regulation of Blood Volume. Although it is customary to talk about a constant blood volume yet it has been already shown that total blood volume does not remain constant and varies widely under different physiological conditions. This variation is mostly due to alteration of the cells and not due to that of plasma. The plasma volume remains fairly constant under normal conditions. The problem of regulation of blood volume is intimately linked up with that of water balance. The maintenance of *blood volume depends upon a balance between water intake and water loss and also upon the adjustment of fluid interchange between plasma and tissue spaces through the capillary walls.* A number of factors are involved: (1) **Physical factors.** Blood pressure, osmotic pressure, diffusion, the state of permeability of the capillaries, etc., are the important factors concerned in the regulation of the blood volume. (2) The **tissue spaces,** due to their enormous capacity, act as a ready reservoir. Any increase in the blood volume will lead to passage of more fluid from the plasma to the tissue spaces. While any decrease will draw in more fluid from the tissue spaces and maintain the blood volume. (3) **Vitamins.** Some vitamins, specially C, by controlling the permeability of the capillaries—take part in the process. (4) **Endocrines.** A number of endocrine factors are also involved here. (*a*) The **antidiuretic factor** of the posterior pituitary controls excretion of water through the kidneys. When blood is diluted the secretion of the factor is inhibited and thus more water is lost. When blood becomes concentrated, reverse changes occur. (*b*) **Parathyroids,** by their effect on calcium metabolism, control the permeability of the blood vessels and thereby the rate of interchange between blood and tissues. (*c*) **Adrenal cortex** is believed to exert important influence upon salt balance, kidney

function and excretion of water. (5) **Thirst.** Another important mechanism for replenishing the reduced blood volume is the phenomenon of thirst. When the water content of the body becomes low, 'thirst' is felt. The subject takes water and thus the blood volume is kept up. [Effects of saline injection, fluid loss and such others have been discussed elsewhere.]

Causes of decrease of Blood Volume. Blood volume is reduced in the following conditions:

1. Loss of whole blood, e.g., haemorrhage.
2. Reduction in number of R.B.C., e.g., anaemia.
3. Loss of plasma alone.
4. Loss of blood water or anhydraemia.
5. Acute exposure to cold causes moderate loss.
6. Posture—Blood volume is low in the erect position than in the recumbent state.

Causes of increase of Blood Volume. Blood volume is increased due to

1. High temperature.
2. Muscular exercise.
3. Emotional excitement.
4. Pregnancy.
5. Congestive heart failure.
6. Administration of mineralocorticoids (deoxycorticosterone and aldosterone).

BONE MARROW (MYELOID TISSUE)

The terms 'bone marrow' and 'myeloid tissue' are often used synonymously. *Myelos* meaning marrow is confined in the cavities of the bone in the postnatal life.

Bone marrow is the cellulovascular tissue occupying the medullary cavities and the cancellous spaces of the bone. Active marrow in the adult is estimated at from 3·5–6·0 per cent. of body weight. The volume of the marrow is 70 ml at birth and about 4000 ml in the adult. In the adult only about half the marrow is in an active state, known as **red bone marrow,** the remainder being inactive—**yellow bone marrow.** Though the yellow bone marrow is inactive, yet it has the capacity of resuming its activity to produce blood cells during urgent need. Under such condition a certain portion of the yellow bone marrow is converted into the red bone marrow. Although only half the marrow is active in the adult, yet it has enormous functional capacity and considerable room for expansion.

1. **Red Bone Marrow.** Red cells are actively manufactured here, hence the colour. In foetal stage most of the bones contain red bone marrow. But with the advancement of age and in postnatal life the red bone marrow is only located in the upper ends of humerus and femur, the bones of skull and thorax, the vertebrae and the innominate bones of the pelvis.

2. **Yellow Bone Marrow.** It is made up of fat and a little reticular tissue with blood vessels. Here, red cells are not manufactured. In the adult life they occupy the spaces where red marrow is absent.

(1) *From birth up to the fourth year* all the bones contain *red marrow.* (2) *By seven years* the marrow becomes less active and is pale red in colour. (3) *Between ten to fourteen years* a patch of yellow marrow appears in the distal ends of the shafts of the long bones and gradually extends on both sides.

(4) *At the age of twenty*, the entire red marrow of the long bones is replaced by yellow marrow except the upper ends of femur and humerus. Throughout adult life this distribution persists. As age advances yellow marrow proportionally increases. (5) *By seventy years* more than half the ribs and half of sternum contains yellow marrow.

Vascular Arrangement in the Bone Marrow. The nutrient artery breaks up into smaller branches which widens out and becomes blood sinuses. These sinuses are lined by single layer of endothelium, the cells of which divide and give rise to red cells. These sinuses, where active erythrogenesis is going on, remain collapsed, thus creating the anoxic condition favourable for red cell formation. When the red cells are sufficiently mature, these collapsed sinuses open up, blood stream enters and the newly born cells are washed away into the circulation. There is some evidence that marrow activity depends to some extent upon the sympathetic system. Turnbull (1936) and later Gilmour (1942) demonstrated that erythropoiesis is also extravascular.

Method of Examining Bone Marrow. *In animals*, the bone is taken out and the marrow is collected after breaking the bone. A smear is prepared in the same way as drawing a blood film on a glass slide. *In the human beings* the sternum is punctured with a special needle, the marrow is drawn out and the slides are prepared just like blood smears. **Staining**—(1) For staining the smear, *Jenner's stain* or *Leishman's stain* may be used in the same way as in staining blood film. For staining the reticulocytes cresyl blue should be used. (2) *Vital staining* of the living cells of the bone marrow can be carried out by injecting suitable preparation of *Janus green* and *Neutral red* in the circulation of living animal.

It is to be noted that in the circulation the average ratio between white and red cells is 1 : 700. In other words, the red cells are much more in proportion to the white cells. But in the red bone marrow, the relation is reversed. Myeloid cells are more in number than the erythroid cells. The proportion between the cells of the myeloid series and the erythroid series varies from 8 : 1 to 2 : 1. This reversed relation is due to the fact that the life of the white cells is much shorter in the circulation than that of the red cells. Consequently, the white cells should be more speedily manufactured than the red cells. Due to this reason, myeloid cell count is much higher than the erythroid cells in the bone marrow. The red bone marrow also contains giant cells known as *megakaryocytes* having a diameter of about 40μ. Each cell contains a ring of lobed nuclei. From these megakaryocytes platelets are formed.

Functions of Bone Marrow

1. **Haematopoietic (haemopoietic) function (Production and release of blood cells).** Production of myeloid elements is the important function of bone marrow. It has been described that red bone marrow is active and has the capacity of forming red cells as well as other blood cells. In the embryo and even in the new-born, only red bone marrow is formed, but in the adult stage nearly 50% of the red bone marrow is converted into yellow bone marrow. This ratio is not constant and is changed with the advancement of age and also with the degree of the need of haemopoietic elements. All the blood cells like erythrocytes, granulocytes, platelets, monocytes and lymphocytes are formed in the red bone marrow. It has

been studied that the marrow contains about $5{\cdot}6 \times 10^9$ erythroid precursors per Kg body wt. and $11{\cdot}4 \times 10^9$ neutrophilic precursors per Kg body wt.

Mechanisms by which the blood cells are released in the blood are not clear. Under certain urgent need and in case of anaemia, mature and even immature cells may be released in the circulation.

2. **Erythroclasia or destruction of R.B.C.** In the bone marrow not only the blood cells are formed but also the abnormal, imperfect, damaged and aged R.B.C. are destroyed. These cells are sequestered or trapped and phagocytised in the macrophages of the bone marrow. Iron portion is stored as **haemosiderin** and **ferritin** in the liver, spleen, R. E. cells and bone marrow and the rest of heam is ultimately converted into bile pigments (*vide* p. 153).

3. **Storage functions.** Bone marrow is an important site for storage of iron in the form of ferritin and of haemosiderin coming from food sources as **transferrin** (*vide* p. 160) and also from destruction of R.B.C. through phagocytosis. These stored irons are easily utilised for the synthesis of haemoglobin.

4. **Reticulo-endothelial function.** Bone marrow plays an important role in the inactivations of toxins or other toxic substances of the body. The free macrophages of the bone marrow are increased during the invasion of toxins or during rapid haemolysis.

5. **Immunological function.** Regarding its immunological function, the marrow is not so competent as it is found in spleen and lymph nodules. Presence of lymph nodules in the bone marrow has been reported by many.

6. **Osteogenic function.** The cellular elements which take part in the formation of bone are formed in the marrow. The osteoclast, osteoblast, osteocyte, endosteum blood vessels are found within the marrow.

7. **Connective tissue functions.** Due to its different connective tissue contents, the bone marrow performs several functions associated with the connective tissues.

FORMED ELEMENTS OF BLOOD

There are three types of cellular elements in blood, i.e., (1) **red blood corpuscles (R.B.C.),** (2) **white blood corpuscles (W.B.C.),** and (3) **platelets.** These three are collectively known as 'formed elements of blood'.

Theories of formation. There are two theories regarding their origin: (1) **Monophyletic theory,** (2) **Polyphyletic theory** (*vide* p. 171).

RED BLOOD CORPUSCLES (ERYTHROCYTES)

The mature human erythrocyte is a circular, biconcave, non-nucleated disc. The edges are rounded and thicker than the centre. Hence, the central portion appears to have a lighter shade. When viewed from the side it looks like a dumb-bell.

In all mammals, excepting camels, the red cells are of this type. In the camels the shape is oval, otherwise they are same as in others. In the non-mammalian vertebrates the red cells are oval, biconvex and nucleated. During the early part of foetal life even the mammalian red cells are all nucleated. But in the later part the nucleated cells disappear from the circulation.

The mature red cells is soft and flexible and can readily squeeze through narrow capillaries. Inside the corpuscles there is a framework, chiefly composed of proteins and lipids. The meshes of this framework

remain filled up with haemoglobin. Under the microscope a single red cell seems to have a light brown or yellowish colour. But when seen in bulk, the red cells appear to be red. Histologically, no definite cell membrane has been demonstrated, but still, there seems to be a delicate outer envelope formed by the condensation of surface molecules. It is believed that this membrane is composed of proteins, phosphatides and cholesterol. The inner and outer layers are made up of proteins and the middle layer of lipids. The permeability of this membrane is highly selective. The bigger colloidal molecules as well as the cations (K, Na, etc.) are not allowed to pass. But certain crystalloids (urea, etc.) and the anions (Cl, $-HCO_3$, etc.) are freely permeable.

Composition of the Red Cells. Each cell is composed of a colourless envelope enclosing semiliquid material, 65% water and 35% solids of which 33% is haemoglobin bound to 2% stromal meshwork of protein, phospholipid, cholesterol, cholesterol esters and neutral fat. Other organic substances, such as urea, amino acids, creatinine, adenyl pyrophosphates, diphosphoglycerates, etc., are also present, but in very small amounts. Of the total lipids 60% is phospholipid (half of this is cephalin), 30% free cholesterol and 10% fats and cholesterol esters. Of the salts in the corpuscles, potassium phosphate is the chief. [It should be noted that the chief salt of plasma is sodium chloride.]

Normal Red Cell Count. The normal average count in **adult male** is taken as **5 million** and in **female 4·5 million** per cubic millimetre. But most observers agree that the actual figures are a little higher, 5·4 million in males and 4·8 million in females. In infants the count is 6 to 7 million, whereas in foetus 7·8 million. In the first ten days of the postnatal life large number of red cells are destroyed. [This is one cause of Jaundice in the new-born.]

Variations of Red Cell Count under various physiological conditions

1. **Diurnal variation.** Variations amounting to about 5 per cent. occur in twenty-four hours. The count is lowest during sleep, then gradually rises and becomes maximum in the evening.
2. **Muscular exercise.** Exercise raises the count temporarily.
3. **Altitude.** At higher altitude the count rises, whereas at lower altitude (i.e., high barometric pressure) the count falls.
4. **High external temperature**—increases the red cell count.
5. **Any condition which lowers the oxygen tension** of arterial blood increases the red cell count.
6. **Injection of adrenaline** and **excitement** increase the count.

Size, Volume, Thickness, etc., of Red Blood Corpuscles. The diameter of the red cells when in the body varies from 5·5 μ to 8·8 μ and about one-third of this in thickness. But in the dried and fixed films (as done for clinical purposes) the cells shrink to some extent and has a **mean diameter 7·2 μ**

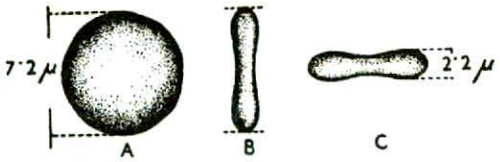


FIG. 97. The schematic diagram of Red Blood Cell (Erythrocyte) from a film preparation.

(FIG. 97–A & –B). The average size of the red cells can be determined from a film preparation with the help of an instrument known as *Halometer*. It can also be measured directly under the microscope by an instrument known as *Micrometer*. The **average thickness** of a red cell is about 2·2 μ (FIG. 97–C), the **average surface area** is about 120 square μ and the **average volume** is about 87 cubic μ.

Abnormal forms of erythrocytes. A variation in size is known as **anisocytosis.** Red cells that are larger than normal are **macrocytes,** those smaller microcytes. Deviation from normal shape is **poikilocytosis.**

The small size and the great number of the red cells are of considerable importance. This makes the available surface area very large and thereby facilitates rapid exchange of gases and other materials between the cells and the plasma. It is estimated that the total surface area of the red cells is about 1,500 times greater than the surface area of the whole body. Per litre of blood, the total surface area of the red cells is about 600 sq. metres. The diameter and the volume of red cell increase when blood tends to become acid. Hence, increased CO_2 tension, anoxia, acidosis, etc., increase the volume and diameter of the red cells. Due to these reasons the red cells in the venous blood are slightly larger than those in the arterial blood. Alkalosis produces opposite effects.

The absence of nucleus is of great benefit. It gives the red cells their biconcave shape and also makes room for more haemoglobin. The biconcave shape of the red cells is also of great advantage for many reasons. For instance, (1) it allows considerable alteration of the cell volume without increasing the tension on the cell membrane. The concave part freely moves out and in as the volume increases or diminishes and in this way, can withstand considerable change of osmotic pressure and resist haemolysis. In the venous blood about 7·5% increase of the cell volume occurs. This is due to the shift of the Cl ions (*vide* under *Chloride Shift*) into the cells, increasing the osmotic pressure and consequently drawing more water into the cells. (2) It allows easy 'folding' of the red cell when the latter passes through capillaries whose diameter is often narrower than its own. (3) Due to biconcave shape the haemoglobin remains distributed in a very thin layer. This facilitates quick saturation or desaturation with the gases.

Development of Red Blood Corpuscles

Theories of origin. There are two theories: **intravascular** and **extravascular.**

Formerly, it was believed that the red cells were formed only intravascularly from the capillary endothelium. But Turnbull and Gilmour (1941) have shown that they are undoubtedly produced from the extravascular sources. They have shown that the parent cell is an extravascular cell, known as **haemocytoblast,** which by active amoeboid movement burrows into the blood sinuses, multiply there and mature into normal erythrocytes. The general trend of opinion seems to be in favour of the extravascular theory now.

Site of development. In the **embryo,** the red cells develop from the area vasculosa of the yolk sac. The mesodermal cells in this area remain as a mass of protoplasm with scattered nuclei. Fluid droplets appear in it, and run together to form channels. This fluid is the primitive plasma and those cells which line these channels become the vascular endothelium,

from which the early red cells develop. At first the cells are all nucleated. From the middle of foetal life the nucleated cells disappear from the peripheral circulation.

Stages of blood formation in the embryo and foetus. There are three successive stages of blood formation in the embryo and foetus: (1) **Mesoblastic haemopoiesis** is first demonstrable in the first two months of embryonic life. Throughout this period, no blood forming organ is present and most of the cells are formed outside the embryo. This stage is markedly diminished in a human embryo of nine weeks. (2) **Hepatic haemopoiesis** constitutes the second stage and includes the splenic and thymic blood formation. This stage occurs from the second to the fifth month. The **final or myeloid period of haemopoiesis** begins approximately at the fifth month, with the establishment of the placental circulation. At first, the liver is chiefly occupied with erythropoiesis and the bone marrow leucopoiesis, but the bone marrow soon takes overall haemopoietic activity. The other sites however retain their haemopoietic potentialities throughout life.

After birth the bone marrow is the main site of erythrogenesis. During early years all bones are filled up with blood forming red marrow, but by twentieth year almost all the long bones are replaced with inactive yellow marrow, and R.B.C. formation in this location stops. Only the upper ends of femur and humerus contain red marrow and continue to form red cells throughout life. In addition to this, the vertebrae, the ribs and the flat bones produce red cells continuously.

Arterial O_2 content, Tissue O_2 tension and Erythropoiesis. The most important factor controlling the rate of red cell production is the oxygen content of the arterial blood, a decrease in oxygen content stimulates erythropoiesis. The oxygen content of the blood may fall either due to diminution of the amount of haemoglobin content of blood or due to inadequate oxygenation of haemoglobin. During haemorrhage there is fall in circulating haemoglobin which leads to increased production of reticulocytes. In high altitude also there is increased red cell production. Decrease in oxygen content in the arterial blood leads to decrease of oxygen tension in the tissue. It has been suggested that lowering of oxygen tension in the tissues has got no direct stimulating effect but acts through humoral mechanism. It is the erythrocyte-stimulating factor or *erythropoietin* or *haemopoietin* which stimulates erythropoiesis. Erythropoietin is a glycoprotein of low molecular weight. It is formed in the renal tissue probably due to the effect of adrenocorticotrophic hormone (ACTH) or some other hormones of the anterior lobe of the pituitary.

Maturation and Multiplication. It should be noted that the phenomenon of development involves two distinct processes—one is *multiplication* and the other is *maturation.* By the latter process, the cell becomes specialised to perform that particular work for which it is meant. It should also be noted that, these two faculties—multiplication and maturation—are antagonistic attributes. They cannot go hand in hand in the same proportion. Maturation cannot take place when multiplication is actively proceeding and multiplication will cease in the same ratio as maturation is in progress. In the case of red cells the process of maturation involves three different changes: *First,* a gradual reduction of cell size; *secondly,* the

acquirement of haemoglobin; and *thirdly*, the disappearance of the nucleus. Of these three, again, haemoglobin formation seems to be the most important. For this reason it will be seen that as soon as haemoglobin begins to appear, cell division gradually ceases.

Stages of development. The different stages of development, as suggested by both intravascular and extravascular theories,—are summarised below. The names used by the intravascular school are given in brackets. The intravascular theory suggests that the first stage starts with the *endothelial cell*, while the extravascular theory holds that it starts with *haemocytoblast*. Histologically these cells differ (described below). In all the other stages the histology of the corresponding cells is closely similar. The stages are as follows:

Haemocytoblast. A big cell, 18–23 μ in diameter, with a large nucleus and a thin rim of deep basophilic cytoplasm. [According to intravascular theory, this stage starts with **endothelial cells.** They are large, undifferentiated reticulo-endothelial cells, lining the sinusoids of bone marrow. They proliferate and give rise to megaloblast.]

(Endothelial cells) ↓

Proerythroblast. 14–19 μ in diameter, basophilic cytoplasm, large nucleus with distinct nucleoli and a reticulum of fine chromatin threads. *Haemoglobin absent.* Actively multiplies into the next form, only in states of stress.

(Megaloblast) ↓

Early normoblast. Smaller in size, 11–17 μ, nucleus and chromatin more dense. Nucleoli absent or rudimentary. Actively divides and passes into the next stage.

(Early erythroblast) ↓

Intermediate normoblast. Size still smaller, 10–14 μ, with fewer mitochondria. Nucleus more condensed, often eccentric. No nucleolus. Haemoglobin appears at this stage and consequently the cytoplasm becomes polychromatic. The later forms do not divide, they mature to form *late normoblasts*. The process of maturation involves the acquisition of more *haemoglobin* and a condensation of the *nuclear chromatin*.

(Late erythroblast) ↓

Late normoblast. The size is more reduced; so that it is just a little larger (7–10 μ) than a mature red cell. The nucleus is very dense and takes a deep stain (*pyknotic*), looking like a drop of ink (**ink-spot nucleus**). The amount of haemoglobin has increased. The further maturation of the normoblast involves the complete loss of the nucleus. TWO VIEWS: **One view** is that the nucleus undergoes fragmentation (*karyolysis*). The amount of haemoglobin increases at the expense of the nucleus. **Another view** is that the nucleus is extruded from the cell as a whole. The factors that cause dissolution of the nucleus are not at all understood. The normoblast, after the loss of the nucleus, passes into the next stage.

(Normoblast) ↓

Reticulocyte. When stained with vital stain (such as cresyl blue), these cells show a net-like structure (*reticulum*) in the cytoplasm. From the reticular appearance its name has been derived. In normal blood they are present to about 1%; in the new-born baby, about 30–50%. In the first week of life the number

Reticulocyte drops to 1%. It is from this stage that the red cells begin to appear in the peripheral circulation. Their number increases when active regeneration of red cells takes place, for instance, during recovery from anaemia. Under this condition even normoblasts may be found in the peripheral circulation. Regarding the *origin of the reticulum*, it is held that they are the remnants of the original basophilic cytoplasm of the immature non-haemoglobinised red cells. Instead of using vital stains, if ordinary Leishman's stain is used, the reticulocytes may appear in two other forms. They may either take a diffuse blue stain (**polychromatophilia**) or may display a number of discrete blue particles in the cytoplasm (**punctate basophilia**). The latter variety is specially prominent in cases of lead poisoning for no obvious reasons. The reticulocyte matures into erythrocyte.

Normal erythrocyte. The normal red cell.

It takes about 7 days time to pass from the stage of proerythroblast (megaloblast) to that of reticulocyte and another two days from reticulocyte to mature erythrocyte.

The stages of cells used by **British** *school:*	*The stages of cells used by* **American** *school:*
Proerythroblast	**Megaloblast**
↓	↓
Early normoblast	**Early erythroblast**
↓	↓
Intermediate normoblast	**Late erythroblast**
↓	↓
Late normoblast	**Normoblast**
↓	↓
Reticulocyte	**Reticulocyte**
↓	↓
Erythrocyte	**Erythrocyte**

Factors controlling erythropoiesis. The red cells are constantly being destroyed and are regenerated. The rate of destruction and regeneration are same, otherwise, a constant red cell count would not be possible. These facts signify that some stimulus, exactly proportional to the number of red cells destroyed, is constantly acting upon bone marrow in order to replace the lost cells. The exact nature of the stimulus is not known.

Certain factors are necessary for the formation and maturation of red cells. They are as follows:

1. **Diet.** Food, rich in first class proteins (or proteins of high biological value), is important. First class proteins supply essential amino acids for the synthesis of globin of haemoglobin. It is also necessary for the formation of stromaproteins and the nucleoproteins of the red cells.

2. **Anoxia and Erythropoietin or Erythrocyte-stimulating factor (ESF).** The exact nature, as already mentioned, is not known. But the stimulus becomes more effective or is supplemented when there is low O_2 tension in the tissues. When air with low oxygen tension (as in high altitude) is breathed for some length of time, the red cell count rises due to liberation of erythropoietin or haemopoietin or erythrocyte-stimulating

factor (ESF). It stimulates the bone marrow and increases the rate and maturation of red cell formation.

3. **Stimulus for maturation.** It is now generally agreed that as the red cells mature, various factors influence the passage of the maturing red cell from stage to stage. Our knowledge in this respect—though far from complete,—may be briefly summarised as follows:

Haemocytoblast. Nothing definite is known as to the factors tha come into operation in this stage. In certain diseases this stage fails to occur. Red cell formation stops and the result is known as *Aplastic anaemia.*

(Endothelial cells)

Proerythroblast. *Haematinic principle of Castle* (haemopoietic principle or PA factor)—vitamin B_{12} (extrinsic factor) and *folic acid* are required for the conversion of proerythroblast (megaloblast) into early normoblast (erythroblast). For the proper absorption of extrinsic factor, intrinsic factor present in gastric mucosa is essential. For details *vide* anaemia (page 165).

(Megaloblast)

Early normoblast. A number of factors influence this process:

(Early erythroblast)

1. **Metals.**
   - (*a*) Iron—essential for haemoglobin formation especially for synthesis of haem. Dietary intake of iron is required for the formation of haemoglobin. Deficiency of iron in the diet leads to *Hypochromic* or *Iron-deficiency anaemia.*
   - (*b*) Copper and Manganese—help in the conversion of iron into haemoglobin by catalytic action.
   - (*c*) Cobalt—as a component of vitamin B_{12}. It is of proved value in man and lower animals. The nature of action probably same as Mn and Cu.
   - (*d*) Calcium—helps indirectly by conserving more iron and its subsequent assimilation.
2. **Bile salts.** Presence of bile salts in the intestine is essential for the proper absorption of these metals.
3. **Endocrine glands.**
   - (*a*) Thyroxine is of undoubted value. Hypothyroidism is associated with hypochromic, macrocytic anaemia due to lowered metabolic activity in bone marrow.
   - (*b*) Adrenal cortex—Adrenocortical insufficiency is often associated with anaemia; *polycythemia* might be present in Cushing's syndrome. The changes are possibly due to general metabolic alterations and not any direct effect on bone marrow.
4. **Vitamins.** Vitamins C, B_6 and B_{12}, folic acid, riboflavin, pantothenic acid and nicotinic acid are all important.
5. **Pigments.**
   - (*a*) Bile pigments.
   - (*b*) Chlorophyll and other porphyrins. Deficiency of these factors will give rise to less haemoglobin formation and therefore Hypochromic anaemia. Their mode of action is unknown.

Late normoblast. The same factors that operate in the previous stage, are also acting here. But the exact nature of the forces that lead to the disappearance of the nucleus is unknown.

(Normoblast) ↓

Erythrocyte. The normal mature red cell.

Energy metabolism of R.B.C. Mature red cells lack nucleus, DNA, RNA and mitochondria. These cells are not capable of synthesising haemoglobin. Krebs cycle is absent. But nucleated R.B.C. of bone marrow can be compared with other nucleated tissue cells of the body in respect of its metabolic processes. Mature red cells contain no glycogen and for metabolic processes it has to depend upon plasma glucose that has constant access through erythrocyte membrane. The exact mechanism of transport of glucose through the membrane is not clearly known. But most of the investigators are of opinion that this is happened mostly through active transport mechanism. As Krebs cycle (TCA cycle) is absent in mature R.B.C. (non-nucleated), the metabolic breakdown of glucose takes place through (*i*) **Embden-Meyerhof glycolytic pathway** (*vide* Metabolism) and (*ii*) **Pentose-phosphate pathway** or **Hexose monophosphate shunt** (*vide* Metabolism). Thus the energy requirement of the muscle is obtained from the above two metabolic processes. The longivity of red cells depends upon the maintenance of these energy-producing metabolic processes. The energy is required for the active transport mechanisms of the R.B.C. During active transport, Na is pumped out of the R.B.C. and K is pumped in. Besides this, structural integrity and the transport of glucose depends upon the availability of ATP.

Normal life span of R.B.C. is about 120 days. In young R.B.C. the enzymes concerned with the metabolic pathway of glucose breakdown are present in a large amount. With the aging processes of the R.B.C., level of certain enzymes is decreased. Hexokinase, which acts in the first step of glucose metabolism, is decreased in amount during aging process. Glucose-6-phosphate dehydrogenase, which catalyses the first step of hexose monophosphate shunt is decreased in amount considerably during aging. Other enzymes which take part in different metabolic processes of R.B.C. are also decreased considerably. With the alteration of glucose metabolism, the ATP source of the R.B.C. is decreased. Due to reduction of available ATP, the structural integrity of the R.B.C. is lost, transport mechanisms are disrupted and ultimately destruction of the cells occurs.

Span of life. The **average span** of life of a mature red blood corpuscles was formerly believed to be about 3–4 weeks. But recent experiments, (using radioactive Fe, or glycine labelled with isotopic ^{15}N, which enters into the composition of haemoglobin) indicate that it is about **120 days** in man.

Fate of the Red Blood Corpuscles. As the cells grow senile they change their shape and size and become more brittle. At first the cells throw out processes like pseudopodia and become flask-shaped. These are called *poikilocytes*. These processes are broken off and in this way the R.B.C. disintegrates. This fragmentation takes place in the circulation and the fragments are swallowed up by the R. E. cells. The R. E. cells of the spleen, liver, etc., can also engulf the senile red cells as a whole and break them down intracellularly. Haemoglobin is released and by degradation opening

of the *porphyrin ring* system occurs. The degraded compound is known as *verdohaemoglobin* or *choleglobin* where the four *pyrrole nuclei* form a chain instead of a ring. In the next stage it is broken down into protein and haem. Protein is broken down into amino acids. The iron present in the haem is stored in the body as **ferritin** and **haemosiderin** which help in the formation of new haemoglobin. The rest of the haem molecule is converted into a yellow pigment *bilirubin* which is oxidised into a green pigment *biliverdin* or according to some, biliverdin is first formed and which by reduction forms bilirubin. Bilirubin and biliverdin probably combine with plasma α_1-globulin and circulate through the blood stream and enter the liver. In the liver cells bilirubin and biliverdin are separated from globulin and conjugate with uridine diphosphate glucuronate to produce monobilirubin and dibilirubin glucuronide (*cholebilirubin*), the uridine diphosphate is set free. These compounds enter the duodenum through the bile duct and then into the intestine. In the large intestine by bacterial action they are changed into stercobilinogen (*urobilinogen*). Some urobilinogen is reabsorbed and excreted in the urine as urobilinogen. The rest is excreted in the faeces as stercobilinogen and stercobilin which are responsible for the brown colour of the stool (FIG. 98).

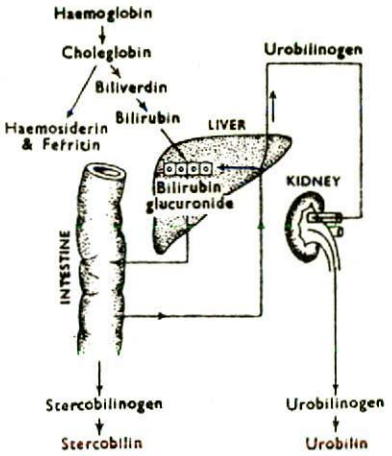


FIG. 98. Diagrammatic representation of the fate of Red Blood Corpuscles and Haemoglobin.

Functions of Red Blood Corpuscles

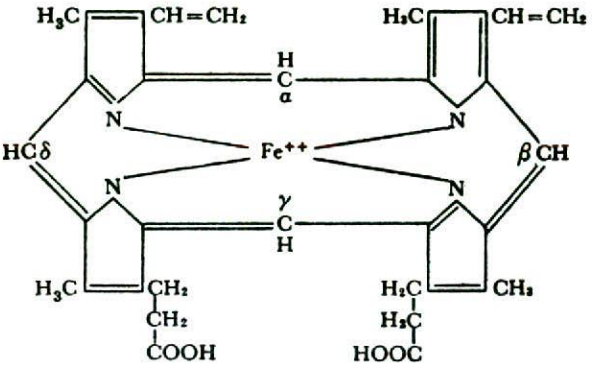
1. Respiratory—Red cells carry oxygen and carbon dioxide.
2. Acid-base balance—They help to maintain acid-base balance. It is carried out by the buffering action of haemoglobin and other intracellular buffers.
3. Red cells maintain ion balance—By the special permeability of the cell membrane, the red cells help to maintain balance of positive and negative ions in the blood.
4. Viscosity of blood—Red cells help to maintain the viscosity of blood.
5. Various pigments are derived from haemoglobin after the disintegration of the red cells, e.g., bilirubin, biliverdin, etc.

HAEMOGLOBIN

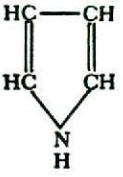
Chemistry. *Haemoglobin is the red pigment of blood.* It is a chromoprotein consisting of two parts: One part (96%) is a specific simple protein known as **globin** (histone) and the other (4%) is a non-specific prosthetic group—an iron-containing pigment called **haem.** Haem is a protoporphyrin compound and consists essentially of four pyrrole groups joined together. The porphyrin molecule can combine with metals forming metalloporphyrin compounds. *Haem is a metalloporphyrin where the metal is iron. The iron content of*

haemoglobin is about 0·34% *and about* 3 *gm of iron is present as haemoglobin in the total amount of blood of an adult. Iron remains in ferrous* (Fe^{++}) *form. Globin* helps *haem* to keep the iron in ferrous state and to combine loosely and reversibly with molecular oxygen.

Haemoglobin { **Globin**, **Haem** } { **Ferrous iron**, **Protoporphyrin** }



HEAM—METALLOPORPHYRIN



PYRROLE

The **molecular weight** of haemoglobin is about **68,000.** It contains 4 atoms of iron and 8 atoms of sulphur. The haemoglobin molecule is probably an aggregate of four unitary molecules, each having one atom of iron and two atoms of sulphur and with a molecular weight of 17,000. The approximate chemical formula is $(C_{712}H_{1130}O_{245}N_{214}S_2Fe)_4$.

Properties. (*i*) The most characteristic property of haemoglobin is the ease with which it combines with oxygen and dissociates from it. 100 ml of water will absorb one-third ml of oxygen at body temperature, under atmospheric pressure. But 100 ml of blood, under the same conditions, will take up 20 ml of oxygen (60 times), due to the presence of haemoglobin. [About 1,200 ml of oxygen can be carried by the total amount of blood in an adult man.] One gram of haemoglobin combines at normal (standard) temperature pressure (NTP) with 1·34 ml of oxygen. This corresponds to two atoms of oxygen for each atom of iron. The compound oxyhaemoglobin gives off its full oxygen content when placed in vacuum. (*ii*) Oxyhaemoglobin holds its oxygen loosely which can be easily displaced by many other gases forming more stable compounds, e.g., CO, NO, H_2S will form carboxyhaemoglobin (carbonmonoxy-haemoglobin), nitric oxide haemoglobin, sulphaemoglobin respectively. (*iii*) The globin part of haemoglobin directly combines with CO_2 and forms **carbamino compounds.** (*iv*) *Crystallisation*—Haemoglobin can be easily crystallised. The form of the crystals, their solubility and the ease of crystallisation are characteristic of the species from which haemoglobin is obtained. Most bloods, including human blood, form rhombic prisms or needles. Haemoglobins of different species are said to be immunologically distinct. The distinction lies in the globin part of the molecule and not in the haem part. It is known that the

amino acid composition of the various globins (derived from haemoglobin of different species) varies considerably specially in respect to their *cystine* content. The haemoglobin of different species also shows different affinity for oxygen. (*v*) Isoelectric pH of haemoglobin (reduced Hb) is 6·8, that of oxyhaemoglobin is 6·6. (*vi*) Spectroscopic appearance—Haemoglobin (reduced Hb) gives one broad band between the Fraunhofer's lines D and E (corresponding to the wave-length—λ 559). That of oxyhaemoglobin consists of two bands between D and E. The band nearer D is called α-band (corresponding to the wave-length—λ 579). The band nearer E is broader and is called the β-band (with a corresponding wave-length of λ 542).

Varieties. In man probably there are at least two varieties of haemoglobin, the foetal haemoglobin (HbF) and the adult haemoglobin (HbA). *Foetal haemoglobin* differs chemically and spectroscopically from the adult haemoglobin. It has a greater affinity for oxygen and releases CO_2 more readily. This is due to some difference in the globin fraction. This property helps to compensate the relative anoxia of foetal blood. At low O_2 pressure foetal haemoglobin can take up larger volumes of O_2 than adult haemoglobin. Foetal haemoglobin is 70 per cent. saturated at 20 mm of O_2 pressure whereas adult haemoglobin is only 20 per cent. saturated at this pressure. A small quantity of foetal haemoglobin persists for some weeks or months after birth.

The form in which haemoglobin exists in cells. The actual state in which haemoglobin exists in the red cells is not yet fully understood. The quantity of haemoglobin in the red cells is too great for it to be in simple solution and it is also known that it is not present in crystalline form. From these observations it has been suggested that haemoglobin remains in some combined form. It is believed that haemoglobin remains *adsorbed* to the lipid material of the stroma and the envelope of red cells. In certain lower invertebrates, haemoglobin remains free in the plasma. Its inclusion in the red cells has taken place gradually in the course of evolution.

Causes of inclusion of the haemoglobin in red cells. (1) Though haemoglobin is protein having its molecular weight 68,000 yet it passes through the endothelial lining of the blood vascular system as well as through the normal glomerular membrane. **Haemoglobinuria** is the condition when free haemoglobin is excreted through the urine. If Hb is free in the plasma then it is excreted through the urine. In plasma, Hb is present normally bound to protein at a concentration of about 5 mg/100 ml of whole blood. In plasma Hb remains normally as **haemoglobin-hepatoglobin complex** which cannot pass through the urine. Under normal condition about 100 to 150 mg of free Hb is released from red cells due to haemolysis. Hb can be bound as haemoglobin-hepatoglobin complex by the hepatoglobin present in the plasma. If released Hb exceeds the capacity of the hepatoglobin to bind the haemoglobin, then it is excreted through the urine. The free Hb passes readily through the glomerulus and of which certain amount is reabsorbed and rest is excreted through the urine. During reabsorption of Hb through the tubular epithelium, certain amount of it, is converted into **haemosiderin** and excreted in the urine. So plasma Hb concentration is always associated with **haemosiderinuria** though the condition of haemoglobinuria may or may not be happened. Thus, if the Hb was not enclosed by the cell membrane

of erythrocytes then this Hb would have been passed quickly through glomerular membrane and excreted through the urine. Because R.B.C. cannot pass through the glomerular membrane in normal condition.

Haemoglobinuria may be caused under the following conditions: (*a*) in strenuous exercise, (*b*) due to mismatched blood transfusion, (*c*) *black-water* fever due to virulent type of malaria and *red-water* fever due to another type of parasite which invades the erythrocyte causing release of Hb in the plasma, (*d*) paroxysmal nocturnal haemoglobinuria, (*e*) hypotonicity of plasma, (*f*) thermal or chemical injuries, (*g*) paroxysmal cold haemoglobinuria.

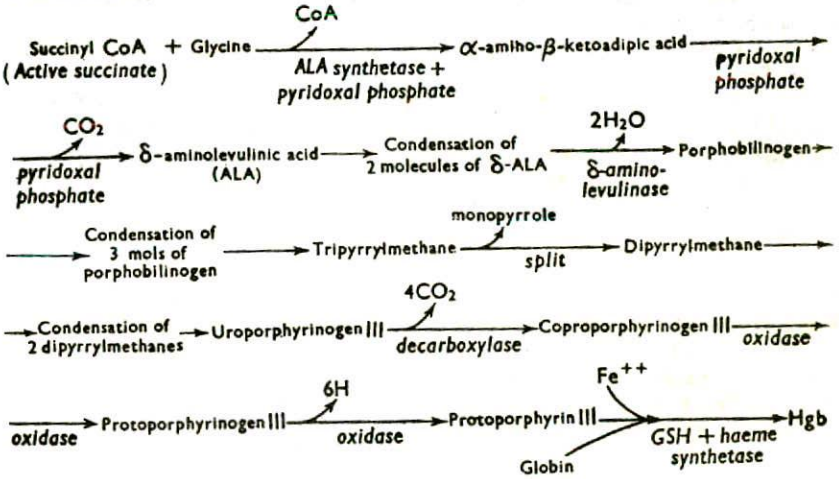
(2) In transport of CO_2 from the tissues to the lungs the erythrocyte with its haemoglobin plays an important part. Because the enzyme *carbonic anhydrase* is present within the erythrocyte.

(3) If haemoglobin instead of staying within the corpuscles, remains free in the plasma (as occurs in haemolysis), many injurious effects will be produced. The degree of the deleterious effects will depend upon the number of red cells haemolysed and the rapidity of haemolysis. Briefly the following ill effects will be produced: (*a*) Viscosity of blood will rise. (*b*) The colloidal osmotic pressure, normally about 30, will rise to 100 mm of Hg or more. This will seriously disturb interchange of various substances in the capillary area and will also disturb the formation of urine. (*c*) Loss of haemoglobin will reduce the amount of blood buffers and will cause acidosis. This is all the more enhanced by the disintegration of red cells, and the loss of available surface area of the erythrocytes, which plays a considerable role in maintaining the acid-base balance and the ion balance in the blood. (*d*) Loss of haemoglobin will reduce the oxygen-carrying capacity of blood, thus producing anoxia and acidosis. (*e*) Bile pigments will be produced in larger amounts by the R. E. cells from the released haemoglobin and in this way additional pressure will be put upon the liver to deal with them. (*f*) While being excreted through kidneys, haemoglobin will be precipitated in the acid urine and in this way the kidney tubules will be blocked. This will cause serious disturbance of the kidney function. (*g*) As a delayed result, hypochromic anaemia will be produced.

Synthesis of haemoglobin. Haemoglobin is synthesised inside the red cells in the bone marrow. *A number of factors are necessary* for the synthesis of haemoglobin. They are as follows: (1) **First class proteins** (or Proteins of high biological value)—It is necessary for the synthesis of the globin part of haemoglobin. Certain individual amino acids, such as histidine, phenyl alanine, leucine, etc., have been found to possess a special stimulating action on haemoglobin formation. A diet containing kidney, spleen, heart and certain fruits are very helpful. Four kinds of globin peptide chains—α, β, γ and δ have been isolated. Human haemoglobin contains 2α and 2β chains. The haem portions are attached to the α and β chains. (2) **Metals**—(*a*) Iron—It is an essential constituent of haemoglobin. Daily intake of 12 mgm is adequate. (*b*) Copper, Manganese and Cobalt—These metals, particularly copper, help in the incorporation of iron in the protoporphyrin molecules for the formation of metalloporphyrin. The ratio between Cu : Fe in the daily diet should be 1 : 100. Cobalt has a definite value as a constituent of vitamin B_{12}. They act as catalytic agents. (3) **Endocrine**—Of the endocrines, only thyroxine is of proved value. (4) **Vitamins**—Vitamins C and B_{12} are definitely helpful in this

respect. It is also believed that folic acid, riboflavin, nicotinic acid, pantothenic acid and pyridoxine also play some parts in the formation of haemoglobin. (5) **Porphyrins**—Of the two types of porphyrins, I and III that are found in Nature, the latter is utilised for haemoglobin formation. Studies with *radioactive carbon* show that protoporphyrin III, required for this purpose, is synthesised in the animal body from simpler substances like glycine, acetic acid, aceto-acetic acid, succinic acid or any amino acid that can give rise to the formation of succinic acid during metabolism or through tricarboxylic acid cycle. Glycine and succinate help in the synthesis of protoporphyrin. Aminolevulinic acid (ALA) is formed by the interaction of succinate and glycine. Two molecules of aminolevulinic acid after condensation forms porphobilinogen (PBG). From porphobilinogen ultimately uroporphyrinogen III is formed. On decarboxylation, the uroporphyrinogen III is converted into coproporphyrinogen III and which on oxidation ultimately gives rise to protoporphyrin III. Protoporphyrin III in presence of globin and Fe^{++} is converted into haemoglobin.

The steps involved in the **synthesis of Hb** (*Hgb*) can be presented schematically:



Amount of haemoglobin in normal blood. The average adult figure, irrespective of sex, is **14·5 gm per cent.** Different observers give different figures for haemoglobin. For instance, Sahli—17·3%, Haldane—14·8%, Gower—15%, and Hellige—14%. [The best method of expressing the amount of haemoglobin in blood is to mention it in absolute figure, viz., as so many grams in 100 ml of blood. If it is expressed in percentage, the scale used should be mentioned.]

Methods of Estimation of Haemoglobin

1. **Tallqvist's method.** A drop of blood from the patient's finger is soaked in a piece of filter paper and compared against a standard colour scale, before the blood on the filter paper dries up. This method does not give accurate results.

2. **Haldane's Haemoglobinometer.** (Haldane's modification of Gowers' method)—The instrument consists of two tubes, one of which

contains 20 cu. mm of blood haemolysed with distilled water and saturated with CO gas. The colour of this tube is used as standard. In the other tube a little distilled water is taken and 20 cu. mm of patient's blood, collected from the finger tip by a special pipette, is added. When blood becomes fully haemolysed, it is saturated with CO by passing coal gas through it. The colour developed is compared against that of the standard. If the colour of the unknown is stronger, it is diluted with distilled water until the tinge is same in both. The graduation up to which the blood has been diluted gives the percentage of haemoglobin.

3. **Gowers' Haemoglobinometer.** Here, the standard used is a solution of picrocarmine gelatin. Otherwise the method is same as Haldane's. The disadvantage of this method is that the colour of the standard gradually fades away.

4. **Sahli's method.** Here, instead of distilled water, (N/10) HCl is used. This converts haemoglobin into acid haematin. The colour developed is matched against that of standard and the result is obtained as in Haldane's method.

5. **Von Fleischl's Haemometer.** Here, the standard used consists of a set of coloured glasses.

6. **Colorimetric method.** Here, the comparison is done with the help of a colorimeter.

7. For very accurate work the **method of Van Slyke and Stadie** should be used. In this method iron of haemoglobin is estimated and from that the corresponding amount of haemoglobin is calculated. The photo-electric methods of Haliday, Kerridge and Smith (1935) may be adopted.

8. **Spectrophotometric method.** It is the modern method and is based as the measurement of absorption of light at certain wave-lengths of *cyanmethaemoglobin* formed by treating the haemoglobin with ferricyanide and then with KCN.

Variations of Hb under different physiological conditions

1. **Age.** In the foetus the concentration is highest. At birth the average concentration is about 23 gm per 100 ml. By the end of third month it falls below normal, probably, because of deficiency of iron in milk. After this gradual recovery takes place and at the end of the first year, the average amount is 12·5 gm. Then it rises gradually up to normal figures.

2. **Sex.** In females the amount of haemoglobin is slightly lower than in males. In adult females the average is 13·7%, in adult n[illegible] 15·8%.

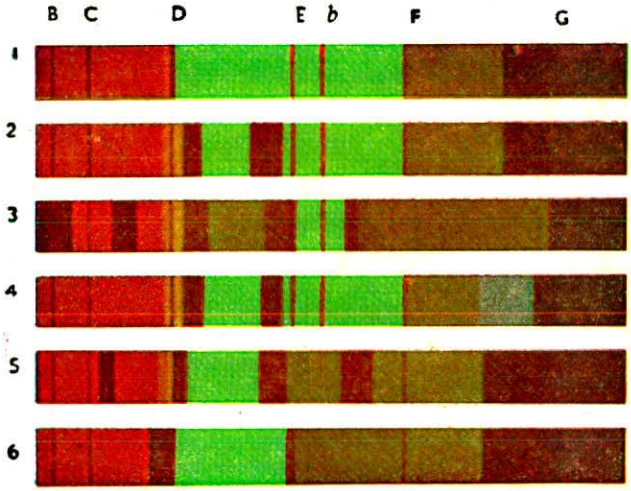
3. **Diurnal variation.** Variation of at least 10% o[illegible] roughout the day. In the morning it is lowest; in the evening highest.

4. **Altitude.** At higher altitude haemoglobin percenta[illegible]ses.

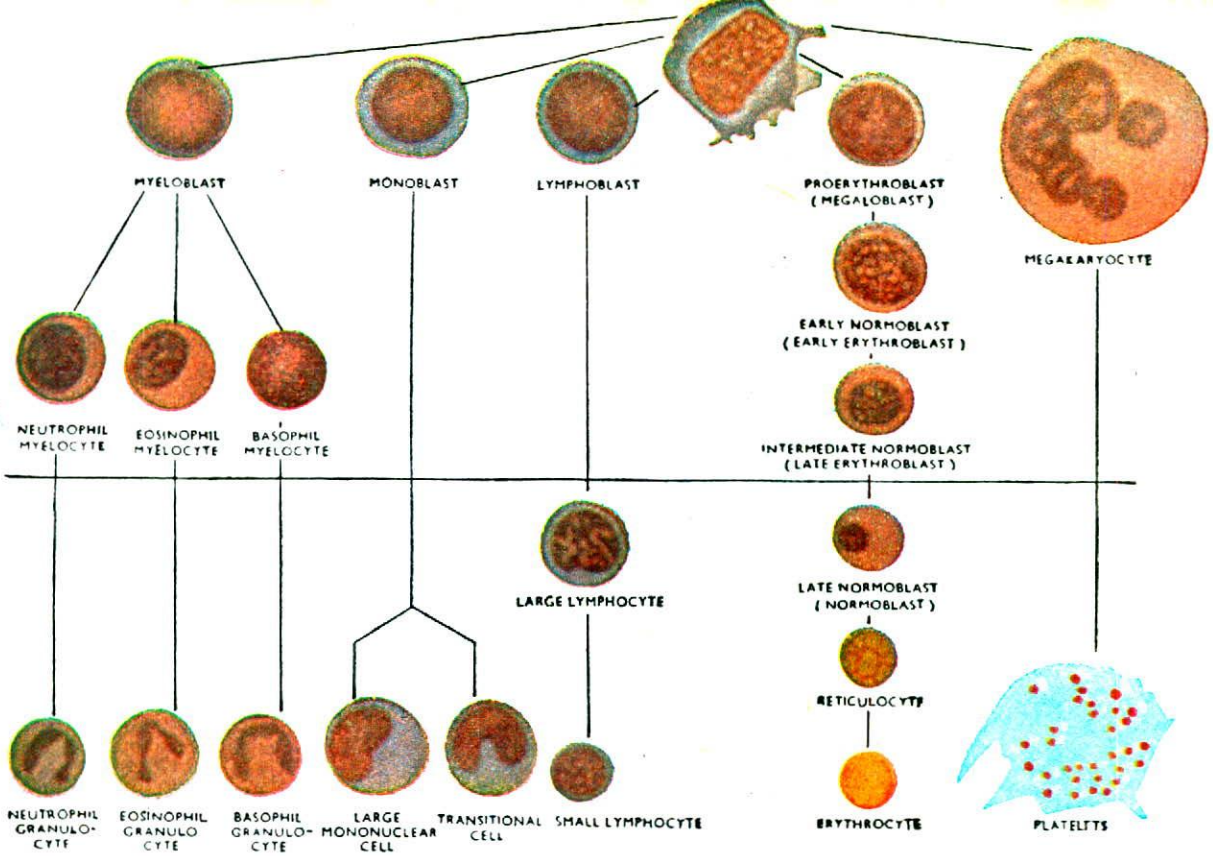
5. **Exercise, excitement, adrenaline injection,** e[illegible]ease the amount of haemoglobin.

It should be noted from the above that normal variation of haemoglobin is mostly due to alteration of number of red cells and not due to any change in the absolute quantity of haemoglobin in each cell. Anything that alters the red cell count will alter the percentage of Hb pro[illegible]ly.

Functions of Haemoglobin. (1) It is essential for oxygen carriage. (2) It plays an important part in CO_2 transport. (3) It constitutes one of the important buffers of blood and helps to maintain its acid-base balance. (4) Various pigments of bile, stool, urine, etc., are formed from it.



1. Solar Spectrum showing Seven Colours.
2. Spectrum of Oxyhaemoglobin—two absorption bands between D & E.
3. Spectrum of Methaemoglobin—one absorption band between C & D.
4. Spectrum of Carboxyhaemoglobin or Carbonmonoxy-haemoglobin—two absorption bands between D & E.
5. Spectrum of Acid Haematin—an absorption band between C & D of the Spectrum.
6. Spectrum of Alkaline Haematin—an absorption band near D line.



Normal blood cells and their precursors. The cells above the horizontal line and the late normoblast (normoblast) below the horizontal line are present in the red bone-marrow. The cells below the horizontal line except the late normoblast (normoblast) are present in the blood stream.

Derivatives of Haemoglobin

I. COMPOUNDS:

1. **Oxyhaemoglobin.** It is a compound of haemoglobin with oxygen. Iron remains in the ferrous (Fe^{++}) state in oxyhaemoglobin. It is not a stable compound. Oxygen may be removed when the blood is exposed to a vacuum. It has got two absorption bands between D and E.

2. **Methaemoglobin.** It is also a compound of haemoglobin with oxygen. It can be produced after treating the blood with potassium ferricyanide. It is chocolate-brown in colour. It is a stable compound. Oxygen cannot be removed by exposing the blood to a vacuum. Iron remains in the ferric (Fe^{+++}) state. It has got one absorption band between C and D.

3. **Carbohaemoglobin.** It is a compound of haemoglobin with CO_2. The compound is formed by union of CO_2 with the globin portion.

4. **Carboxyhaemoglobin or Carbonmonoxy-haemoglobin.** Haemoglobin combined with CO instead of oxygen. It is present in blood in coal gas poisoning. It has got two absorption bands between D and E. The affinity of human haemoglobin at 38°C. for CO is 210 times greater than O_2. The extremely poisonous nature of the gas can easily be understood from the above statement.

5. **Sulphaemoglobin.** It is formed by the combination of haemoglobin with H_2S. The compound is very stable and is sometimes found in the blood after certain kinds of drug poisoning.

6. **Nitric oxide haemoglobin.** Haemoglobin combined with NO instead of oxygen, found in nitric oxide poisoning.

II. DERIVED PRODUCTS:

A. **Iron-containing.** (*i*) **Haematin**—This derivative can exist in two forms—acid and alkaline and may be prepared from haemoglobin by the action of acid or alkali. This is sometimes found in the urine in old cases of haemorrhage. It is a ferric compound. Acid haematin has got an absorption band between C and D. The absorption band of alkaline haematin is near D line. (*ii*) **Haemin**—Haemin is haematin hydrochloride. It is prepared by boiling oxyhaemoglobin with NaCl and glacial acetic acid. It is a ferric compound. (*iii*) **Haemochromogen**—When alkaline haematin is reduced by ammonium sulphide, this derivative is obtained. It is a ferrous compound. Haem with ferrous iron is combined with denatured globin. Of all the haemoglobin derivatives, haemochromogen possesses the most characteristic spectrum. It has a very distinct band between D and E, as well as a fainter band between E and *b* lines. Due to this property this compound is often used to identify doubtful blood stains. (*iv*) **Cathaemoglobin**—It is a compound of haem containing ferric iron with denatured globin. (*v*) **Haem**—It is a ferrous compound produced by the reduction of haematin in alkaline solution.

B. **Iron-free.** (*i*) **Haematoporphyrin**—This derivative can exist in two forms—acid and alkaline. It is prepared by mixing blood with sulphonic acid. When mixed with alkali the alkaline variety is formed. Normal urine may contain traces. It is found in the blood and urine in sulphonal poisoning and in certain cases of liver disease. (*ii*) **Haemopyrrole**—When haematoporphyrin is reduced, this compound is formed. It is probably a mixture of several pyrrole compounds. (*iii*) **Haematoidin**—This compound is produced by the breakdown of haemoglobin in the body. It is found as yellowish-red crystals in the region of old blood extravasations.

Some authors believe that it is identical with bilirubin. (*iv*) **Bilirubin**—It is the chief pigment of bile and is produced from haemoglobin in the whole of the reticulo-endothelial system. From it are derived all the other bile pigments, the pigment of the stool, stercobilin; the pigments of the urine, such as urobilinogen, urobilin and urochrome.

IRON

Sources. *All animal food*, e.g., meat, liver, egg, etc., excepting milk and butter; *vegetables*, e.g. peas, lentils, *green leaves, fruits.*

Daily requirement. At least 12 mgm, better 15–20 mgm, per day. Generally enough in the normal diet. Pregnant and lactating females should have more. Milk being deficient in iron, the infant may develop anaemia. The foetal liver contains a large store of iron which is used up in the first three months. After third month, infants should have added amounts.

Distribution. Iron is distributed in the body (*i*) as **iron porphyrins** in haemoglobin, myoglobin, and also (*ii*) as **iron enzymes** in catalase, cytochrome and peroxidases. Besides these, the iron is also present as **non-iron porphyrins** in transferrin, ferritin and haemosiderin.

Absorption and Transport. Iron is absorbed mostly from the whole of the gastro-intestinal tract but a large amount is absorbed from the upper part of the small intestine particularly the deodenum. Dietary iron is absorbed through the mucosal cells as **ferrous** (Fe^{++}) form. Iron in diet is mostly present as **ferric** (Fe^{+++}) state which is reduced to ferrous form during absorption. Vitamin C, glutathione and amino acid-SH groups help in reduction of ferric to ferrous form. After entering the mucosal cell as ferrous form, the iron molecules are rapidly reconverted into ferric state. The ferric iron as ferric-hydroxide phosphate combines with a protein, **apoferritin** of the mucosal cells with the formation of iron-phosphorus protein complex, ferritin. This **ferritin** is one of the storage forms of iron in the tissue.

It is claimed that homeostasis of iron content in the body is maintained not through excretion but through controlling the absorption in the G. I. tract. It has been postulated by Granick (1954) that at the border of the mucosal cells, the saturated apoferritin acts as a **mucosal block** and further absorption of iron is prohibited. But at present it is believed that apoferritin mechanism does not tend to stabilise the iron absorption.

From the mucosal cell the ferritin iron passes into the blood. At first the ferritin iron is reduced into ferrous form and as such enters the blood stream. Here vitamin C also helps in transformation of ferric to ferrous form. However, the ferrous iron after entering the blood stream is re-oxidised into ferric form and combines with β_1-iron-binding globulin, **transferrin** or **siderophilin** of the plasma.

It has been described by Osaki and others (1966) that **ceruloplasmin** (copper-binding protein) of the plasma also exerts a catalytic activity in plasma to convert Fe^{++} to Fe^{+++} form and thus incorporation of iron in the plasma transferrin is hastened. Transferrin iron complex is the transport form of iron of the plasma and is carried to the myeloid tissue, liver, spleen, lymph node and other tissues of the body. Transferrin is thus the iron-binding protein of the plasma and it shuttles iron atoms between tissues without itself being utilised appreciably. The transferrin can bind two atoms of Fe^{+++} per molecule of protein to form the red-coloured ferric protein complex.

Normal protein-bound iron (PBI) in the plasma of males is approximately 120–140 μg per cent. and that of in females is 90–120 μg per cent. The total iron-binding capacity (TIBC) is about 300 to 360 μg per 100 ml in both sexes.

In the capillary blood vessels, the Fe^{+++} of transferrin passes through the peripheral capillary wall directly into the tissue spaces. But from the tissue spaces, the iron enters the tissues as ferrous (Fe^{++}) state and is stored as ferritin (Fe^{+++}) state (FIG. 99). If the parenteral administration of iron exceeds the capability of the tissue to store as ferritin, then the excess is stored as haemosiderin.

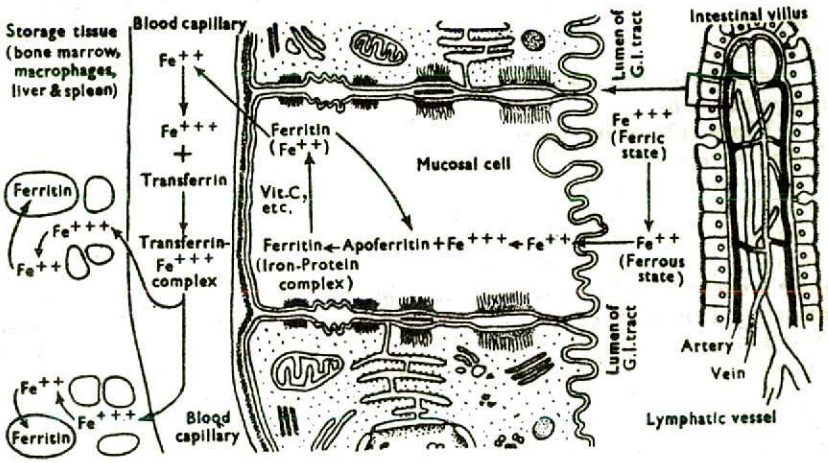


FIG. 99. Diagrammatic representation of the pathway of absorption and transport of iron.

Absorption of iron depends upon the following factors:

(*a*) **Iron requirement of the subject.** In some unknown way, the degree of immediate need of the body for iron determines the rate and amount of absorption from the small intestine. Miller (1954) suggests that iron absorption is under the control of a regulatory mechanism geared to the erythron need rather than to the reserve stores. Other workers suggest that reserve iron stores control iron absorption *via* the mucosal cell through a low oxygen supply induced by low Hb in the blood stream. The ferritin-Fe^{+++} stored in the mucosal cell, its shifting towards Fe^{++} for diffusion into blood and subsequent conversion to ferritin-Fe^{+++} prevent loss of iron into the intestinal tract. Ferritin with 17 to 23% of Fe is a ferric compound of the colourless apoferritin (protein) and colloidal ferric hydroxide phosphate by weight.

Iron absorption is increased during growth, menstruation, pregnancy and in blood disorders. Absorption is increased markedly when the iron needs are acute as in haemorrhage or in anaemias.

(*b*) **Form of the compound.** *It is said that iron is best absorbed in ferrous* (Fe^{++}) *form.* Most of the iron taken with food is in *ferric* (Fe^{+++}) *form.* They are at least partly converted into ferrous compounds before absorption. Organic iron of food is much less available for absorption than the inorganic. Insoluble forms are not absorbed.

(*c*) **Reaction of the gastro-intestinal contents.** The acidity of the gastric juice helps absorption. The gastric HCl helps the liberation of iron from the organic compounds in diet. Reduction from ferric form to ferrous one takes place in stomach with the help of gastric secretions. Partial gastrectomy often leads to iron-deficiency anaemia.

(*d*) **Pigments.** Absorption of iron is increased by *chlorophyll* and *bile pigments*. It is believed by certain workers that bile or gastric HCl is not needed for the absorption of inorganic iron salts.

(*e*) **Calcium** and **vitamin C.** A small amount of Ca decreases the formation of insoluble iron phosphates and thus helps absorption, but large amounts of Ca inhibit iron assimilation. Vitamin C increases the absorption of iron from foods, possibly by reducing the ferric iron to the ferrous state.

Absorption is retarded by excessive mucus, administration of *alkalies* or *low gastric acidity*.

Time taken for absorption. *The rate of absorption of iron is determined by the iron requirement for Hb synthesis.* In anaemic cases, after a single dose of iron, a rise of serum iron takes place in 30 minutes, reaching its maximum in 3–5 hours (0·35 mgm%, compared to normal value, 0·10 mgm%) and returns to normal in about 12 hours. Maximum absorption is completed in 18 hours. [In normal animals little iron needs to be absorbed, because very little is excreted. The body observes a rigorous principle of economy in this respect.] Iron derived from the disintegration of the red cells remains stored, and is utilised for the synthesis of further haemoglobin. If a man be in iron balance—any excess of iron administered per mouth will not be absorbed, but passes out in the faeces. If iron salts are given by injections, only traces appear in the urine, the rest remains stored in the reticulo-endothelial cells as haemosiderin. Haemoglobin in the blood falls when *iron loss* exceeds that of iron absorption and anaemia develops.

Iron in blood. Whole blood contains about 45–50 mgm of iron per 100 ml. The total quantity present in all the red cells is about 3 gm. Another 1–3 gm is present in the rest of the body. Iron is present in blood in two forms: (*a*) **Plasma iron**—only traces of iron (average 0·10 mgm per 100 ml) is present in plasma. It represents the form in which iron is transported in blood, from place to place, the compound known as **transferrin** or **siderophilin.** It is increased when the red cell formation is diminished, e.g., in Aplastic anaemia, Pernicious anaemia, etc. It is diminished when there is rapid red cell formation. (*b*) **As haemoglobin**—this accounts for about 92–98% of the total blood iron. It corresponds to about 50 mgm of inorganic iron per 100 ml of blood.

Storage of iron. Iron is stored in two forms: **ferritin** and **haemosiderin.** The former is water-soluble while the latter is granular and insoluble in water. Reticulo-endothelial system in general, particularly, liver, spleen and bone marrow store iron. Liver iron is readily increased by iron administration. In condition of excess haemolysis iron is deposited in these places in large quantities. Normally the iron liberated from the breakdown of red blood cells is also stored in these places and is utilised for Hb synthesis.

Excretion. Only in traces in urine, bile and faeces. In an adult the urinary loss is on the average 0·2 mgm per day. It is believed that the *iron content of the body is controlled by regulation of its absorption and not by excretion.*

Iron loss occurs during pregnancy, during labour, in the menstrual period, due to loss of blood, etc. (Fig. 100).

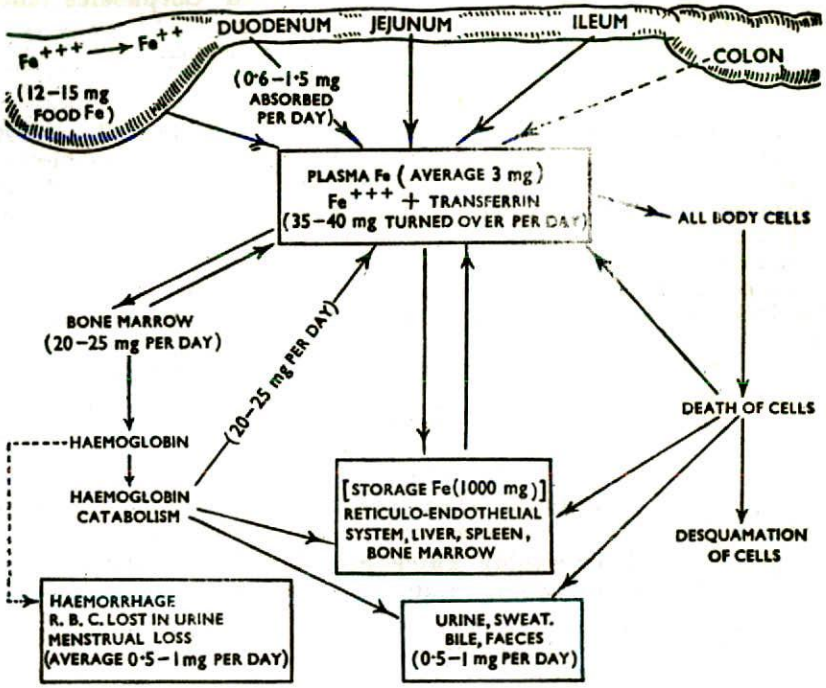


Fig. 100. Schematic representation of iron metabolism and its pathway.

Functions of Iron

(1) **Formation of Hb.** The primary function of iron is to form haemoglobin. (2) **Development of red cells.** Iron is not only necessary for Hb synthesis but also for the formation and maturation of red cells. (3) **Oxygen carriage in blood** in the form of Hb. One gm of Hb carries about 1·34 ml of oxygen. (4) **Related to tissue oxidation.** (*a*) Cytochrome is an iron-containing compound. It is concerned with the oxidation of metabolites in the cells. (*b*) Indophenol oxidase is also an iron compound. (5) **Supplies O_2 to the muscle.** Myoglobin of muscle is an iron-containing chromoprotein like haemoglobin. It combines with O_2 and acts as an oxygen store for muscle. (6) **Relation with the cell nucleus.** The chromatin of the nucleus contains iron. It is possible that this iron takes an essential part (may be oxidative) in the functions of nuclei. (7) **Relation with oxidation in nerve cells.** Nissl granules, present in the cytoplasm of the nerve cells, contain organically combined iron. Here, iron serves some essential roles probably in oxidation. These granules disappear during activity of the nerve cells, and reappear during rest.

Deficiency signs. Iron deficiency causes secondary anaemia (Microcytic, Hypochromic). The haemoglobin content of the red cells is diminished. The size and volume of the red cells are below average. There is

normoblastic hyperplasia in the red bone marrow. Iron-deficiency anaemia occurs in children and adults due to severe blood loss.

Some Important Indices about Red Blood Corpuscles and Haemoglobin

1. **Colour Index** (C.I.). It is calculated as follows:

$$\frac{\text{Percentage of Hb}}{\text{Percentage of Red Cells}}$$

The haemoglobin percentage is determined as well as the red cell count. A count of 5 million red cells per cu. mm is taken as 100%. If a subject is found to possess 60% haemoglobin and only 4 million red cells (i.e., 80% of the normal 5 million), then the colour index will be $\frac{60}{80}=0{\cdot}75$. The normal colour index is 1, but slightly lower index, i.e., 0·85, is more commonly found and is also not abnormal. *Colour index indicates the proportion of haemoglobin present in each red cell with respect to normal.* In Hypochromic anaemia the index is low, in Hyperchromic or Macrocytic anaemia the index is above 1.

2. **Mean Corpuscular Diameter** (M.C.D.). In film preparations the average diameter is 7·2–7·3 μ.

3. **Mean Corpuscular Volume** (M.C.V.)

$$=\frac{\text{Volume of Packed Red Cells in ml per litre}}{\text{Red Cells in million per cu. mm}}=87 \text{ cubic } \mu \pm 7.$$

Anaemia is **macrocytic** when the M.C.V. is over 96 cu. μ, **microcytic** if below 85 cu. μ and **normocytic** at normal level of it.

4. **Mean Corpuscular Thickness** (M.C.T.)$=2{\cdot}1$–$2{\cdot}2$ μ. Mean corpuscular thickness can be determined from the formula:

$$\text{M.C.T.}=\text{M.C.V.}\times\left\{\frac{1}{\pi\,(\text{mean diameter}/2)^2}\right\}.$$

5. **Volume Index**

$$=\frac{\text{\% of Corpuscular Volume (45 ml—100\%)}}{\text{\% of Red Cells (5 millions per cu. mm—100\%)}}$$
$$=\text{Normal Average 1 (Range 0·85–1·5).}$$

6. **Relative Volume of Packed Red Cells and Plasma.** This is determined by centrifuging oxalated blood in Haematocrit. The cells occupy 45% of the total volume and the rest is made up by plasma. The ratio, cell : plasma = 45 : 55 is a good normal.

7. **Mean Corpuscular Haemoglobin** (M.C.H.). It is calculated as follows: $\frac{\text{Haemoglobin in grams per litre of blood}}{\text{Red Cells in million per cu. mm}}$ The average figure is 29·5 $\gamma\gamma\pm2{\cdot}5$. [1 micro-microgram or $\gamma\gamma$ is 1 million-millionth of one gram.] This indicates the absolute amount of haemoglobin per cell, e.g., each red cell contains, on the average 29·5 $\gamma\gamma$ of Hb.

8. **Mean Corpuscular Haemoglobin Concentration** (M.C.H.C.). It is calculated in the following way:

$\frac{\text{Haemoglobin in grams per 100 ml}}{\text{Volume of Packed Red Cells in 100 ml}}\times100$. The average figure is $35\%\pm3$.

This figure indicates how much of the average volume of the corpuscle is filled up by haemoglobin. Normally 35% of the average volume of each red cell is filled up with Hb. If the M.C.H.C. is below 30% the anaemia resulting is **hypochromic** type. The value is never higher than above normal. Anaemia with normal M.C.H.C. is **normochromic.**

9. **Saturation Index.** It indicates the concentration of haemoglobin in the red cell and is obtained by $\frac{\%\text{ of wt. of Haemoglobin per 100 ml}}{\%\text{ of Corpuscular Volume}}$ = average 1. [Range 0·9–1·1].

ANAEMIA. Deficiency of R.B.C. in blood causes anaemia. Anaemia might be due to either excessive blood loss or increased destruction of R.B.C. (haemolysis). It might also result from either defective formation in bone marrow (Aplastic anaemia) or deficiency of maturating factor or nutritional defects.

I. **Blood Loss.** After severe haemorrhage the lost plasma is restored within a short time but not the R.B.C. The blood is thus diluted and there is a fall in Haematocrit. The anaemia is of the Normochromic, Normocytic type. After continued haemorrhage along with R.B.C. a considerable amount of iron is also lost which cannot be replaced by ordinary diet. Tissue anoxia stimulates bone marrow to form R.B.C. which is of the Hypochromic, Microcytic type. Administration of iron is an effective cure in such a case.

II. **Increased Destruction of R.B.C.** There are several types of anaemia. In all cases R.B.C. formation is abnormal making it very fragile. In (*a*) **Familial haemolytic anaemia,** the cells are small, spherical and can be easily broken down. (*b*) **Sickle cell anaemia** is due to cells having a sickle shape. The haemoglobin of such patients is of haemoglobin S type (instead of normal haemoglobin A). (*c*) **Mediterranean anaemia** (or Thalassemia or Cooley's anaemia) is due to very small fragile R.B.C. containing haemoglobin F.

Certain diseases like malaria and syphilis cause increased destruction of R.B.C. as also the haemolytic agents described under fragility of R.B.C. (*vide* page 166). The increased R.B.C. destruction, due to haemolysis, causes a rise in plasma bile pigment (bilirubin) and there might be *Jaundice* along with the anaemia.

III. **Failure of Functions of Bone Marrow (Aplasia).** The resulting anaemia is called **Aplastic anaemia.** This failure might be (*a*) primary failure of bone marrow itself or from (*b*) enormous exposure to X-ray or γ-ray, the latter resulting from atomic explosion, (*c*) cancer in bone marrow, (*d*) poisoning from aromatic organic chemicals or some toxins, (*e*) kidney disease, (*f*) substitution of normal marrow with fibrous tissue. The resulting anaemia is *Normochromic, Normocytic* type.

IV. **Defective Formation of R.B.C.** For the conversion of proerythroblast also called megaloblast to mature R.B.C., several maturating factors are essential. This includes 'extrinsic factor' present in certain foods which are essentially vitamin B_{12} and folic acid and a gastric 'intrinsic factor' which helps in the proper absorption of the extrinsic factor. The haematinic principle absorbed is stored in the liver and transported to bone marrow. In absence of any of them, Pernicious (Addison's) anaemia also called Megaloblastic anaemia results. In such a condition R.B.C. count is greatly reduced, the anaemia is of the Macrocytic type and is usually Normochromic. The anoxia produced stimulates the bone marrow which is filled up with nucleated R.B.C. Along with blood changes, achlorhydria, lowered pepsin content and subacute combined degeneration of tracts of the spinal cord (the demyelination of tracts of the spinal cord occupying the dorsal and lateral columns) also occur. Vitamin B_{12} which is essentially the extrinsic factor, if injected at this stage (but not fed orally) cures not

only the blood condition but also the neurological manifestations. Folic acid can cure the blood condition only. Possibly both the vitamins act as co-enzymes for the synthesis of DNA. Vitamin B_{12} alone is necessary for the formation of RNA, the nucleic acid required for the integrity of the central nervous system. The basic cause of Pernicious anaemia is atrophy of the stomach mucosa. *Gastrectomy* or stomach cancer also leads to similar blood changes. In all such cases there is loss of intrinsic factor. Absence of extrinsic factor, defect in liver storage or reduced response of bone marrow to maturating factor (as in Myxedema) might also be a contributory factor.

Atrophy of the intestinal mucous membrane, leading to the failure of absorption of vitamin B_{12}, leads to **non-tropical sprue.** Anaemia is of the Macrocytic, Hypochromic type. Diet deficient in essential amino acids or vitamins (vitamin C, pyridoxine, riboflavin, nicotinic acid, pantothenic acid), combined with or without diarrhoea, produces similar blood picture **(tropical nutritional anaemia).** Inadequate intake or increased requirement of iron, as in pregnancy, causes hypochromic, microcytic, **iron-deficiency anaemia.**

Polycythemia. Increase in number of R.B.C. is called polycythemia. This might result in persons living at high altitude usually 15,000 feet above sea level. In some animals a large dose of cobalt might produce polycythemia due possibly to increased production of erythropoietins. *Polycythemia vera* (erythremia) is a pathological condition having R.B.C. count well above normal.

FRAGILITY OF THE RED BLOOD CELLS (HAEMOLYSIS)

By the term *haemolysis* is meant the disruption of red cells with the escape of haemoglobin from the corpuscles to the plasma. When red cells are suspended in isotonic solution, no change occurs. When suspended in hypertonic solutions, water is drawn out of the cells, the cell volume shrinks and they become crenated. When placed in hypotonic solutions, water enters the cells, they gradually swell up, become spherical and ultimately burst. Haemoglobin comes out into the plasma and the phenomenon is called Haemolysis or *Laking of blood.* The fragility of the red cells depends upon the readiness with which the cells are haemolysed, when placed in hypotonic solution. It can be determined quantitatively as follows:

The blood sample to be examined is diluted two hundred times in a series of red cell-counting pipettes, using hypotonic salt solutions of gradually diminishing strength, as diluting fluids. After half an hour the cells in each pipette are counted and the number is compared with the count done in the same way by using normal saline. From this the percentage of ruptured cells are found. That particular concentration of the hypotonic salt solution, where 50% of the corpuscles are found to be haemolysed is determined and is used as the standard for comparing the fragility of other blood samples.

Normally, haemolysis starts at 0·48% and is completed at 0·33% of NaCl solution. In acidosis fragility increases. The red cells in the venous blood show increased fragility than in the arterial blood. This is due to the fact that in the venous blood the cell volume is already larger and consequently can withstand much less degree of further swelling. Hence, the cells become more easily haemolysed when placed in hypotonic solution.

Blood may be haemolysed in the following different ways: (1) **By adding fat solvents**—Ether, chloroform, benzene, etc., causes laking

of blood by dissolving away the fatty red cell membrane. (2) **By causing osmotic disturbance**—Addition of distilled water or hypotonic salt solution increases the cell volume and causes haemolysis. (3) **By disturbing the surface tension** of the red cells—Addition of bile salts or saponi. causes laking. (4) **Physical methods**—Alternate freezing and thawing of blood break down the R.B.C. (5) **Mechanical**—Vigorous stirring and shaking lead to haemolysis, but complete haemolysis of all the red cells in a sample is unusual by this process. (6) **Addition of incompatible blood**—This at first agglutinates the red cells and then causes haemolysis. (7) **Adding bacterial haemolysins.** (8) **Adding snake venom** (Viper). (9) **Drugs** like quinine, phenacetin, nitrites, chlorates, etc., cause haemolysis. In addition to above there are various pathological conditions in which the fragility of the red cells is seriously disturbed.

WHITE BLOOD CORPUSCLES (LEUCOCYTES)

White blood corpuscles (W.B.C.) are an important variety of cells in the blood. These cells differ from the red cells in many respects. For instance, (1) they do not contain any haemoglobin, (2) they are bigger in size, (3) they are nucleated, living cells, (4) they are actively amoeboid, (5) they are much less in number, (6) their span of life is shorter, (7) their origin is purely from extravascular tissue, (8) their functions are absolutely different from those of red cells, (9) there are several varieties of leucocytes, whereas red cells are only of one variety.

Chemistry. Leucocytes are rich in nucleoprotein and also contain lipids, glycogen, cholesterol, ascorbic acid and a variety of enzymes, specially proteolytic.

Total number. The average total number of white cells is 6,000 to 8,000 per cu. mm, the normal range being 4,000–11,000 per cu. mm. The average ratio of the total white cell count with the total red cell count is about 1 : 700; i.e., for one W.B.C. there are 700 R.B.C.

Variations in normal count of White Blood Corpuscles

1. **Diurnal variation.** The total count varies from day to day and even from hour to hour. Sometimes, such variations are found to be random and without any obvious cause. In the morning or after rest, the count is lowest. The count rises after midday and usually becomes highest in the evening.
2. **Muscular exercise** and any condition leading to asphyxia (O_2 lack and CO_2 excess) increase the total count.
3. **Relation with meal.** Formerly it was believed that there is leucocytosis after meals, which is now thought to be only a diurnal variation.
4. **Injection of adrenaline** increases the count.
5. **Emotional stress.** Fear, pain, etc., due to liberation of adrenaline.
6. **Age.** In the new-born, the count is very high, about 20,000 per cu. mm. After second week, the count starts falling, but throughout infancy and childhood, the count remains proportionally higher. During infancy and childhood, the lymphocytes constitute about 40–50%.
7. **Relation with pregnancy and labour.** In pregnancy at full term the count is higher, being highest (17,000) during labour. There is also an increase during menstruation.
8. **Increased cellular destruction** from infections, surgical operations, etc., produces derivatives of nucleic acids and causes a rise in neutrophil leucocytes.

9. **Asthma, hay fever, skin disease,** etc., **and also** after hostile invasion with parasites there is an increase in eosinophils (*eosinophilia*).

10. **Adrenal cortical steroids and ACTH** (adrenocorticotrophic hormone) cause an increase in neutrophil and decrease in lymphocytes and eosinophils. The fall in eosinophil is very specific for this hormone, and is used as an assay method.

11. **Starvation** and **administration** of *certain chemicals* like benzene, sulphonamide, etc., produce leucopenia (fall in leucocytes).

12. Leucopenia also occurs after bacterial, viral or protozoal **infection** and also in aplastic **bone marrow disease** or after exposure to **ionising radiation.**

It is generally agreed that stores of white blood corpuscles remain in various parts of the body and under different conditions they may rapidly appear and disappear from the blood.

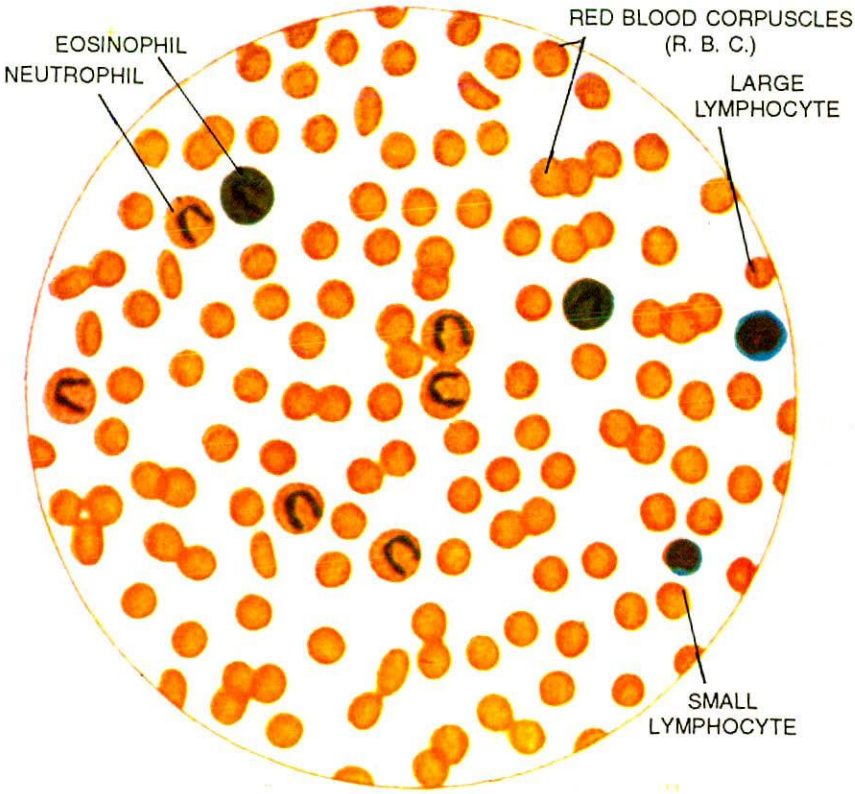
Classification and Differential Count of White Blood Corpuscles or Leucocytes

There are several varieties of white blood corpuscles, each type possessing characteristic morphology and staining property. Determination of the percentage of different varieties of leucocytes is known as the *differential count of white blood corpuscles.* The classification and the differential count, as generally accepted, are as follows:

I. Granular Leucocytes or Granulocytes. Cells with granular cytoplasm. They are formed in the bone marrow from the time of birth onwards. The granules may take either of the three different stains, neutral or acid or basic. Consequently this group includes the following three varieties:

1. **Neutrophil (Polymorphonuclear)** .. .. The nucleus is many-lobed (2–7 lobes) and the granules in the cytoplasm take neutral stain.	60–70%.	Absolute No. 3,000–6,000 per cu. mm.
2. **Eosinophil** .. .. The nucleus is commonly two- or three-lobed. The granules are coarse and stain with acid dyes (*eosin*).	1–4%.	Absolute No. 150–400 per cu. mm.
3. **Basophil** .. .. The nucleus is lobed, the cytoplasm contains granules of various sizes which take deep basic stain.	0–1%.	Absolute No. 0–100 per cu. mm.
II. Lymphocytes .. .. (*a*) Small Lymphocyte. (*b*) Large Lymphocyte.	25–30%.	Absolute No. 1,500–2,700 per cu. mm.
III. Monocytes (including transitionals)	5–10%.	Absolute No. 350–800 per cu. mm.

Both lymphocytes and monocytes are derived from the reticulo-endothelial system although a few lymphocytes may be formed in the bone marrow.



Normal blood picture under the microscope showing Red Blood Corpuscles (R. B. C.) and different types of White Blood Corpuscles (W. B. C.).

I. Granular Leucocytes or Granulocytes

1. **Neutrophil** is about 10–12 μ in diameter. Most numerous in the adult blood, about 60–70%. The nucleus is many-lobed; the number of lobes varies from 2 to 7 or more. The youngest cell has a single nucleus. The number of lobes increases with the degree of maturity of the cells. Due to presence of lobes in the nuclei these cells are called *polymorphs*. In the peripheral blood, the cells with three- to four-lobed nuclei are the most numerous. The cytoplasm contains fine neutrophilic granules which appear pale-violet with Leishman's or Giemsa's stain. They are actively amoeboid and phagocytic. The neutrophil leucocytes contain a variety of enzymes like phosphatase, nucleotidase, protease, amylase, lipase, etc. They also contain ascorbic acid, glutathione and glycogen. Metabolically these cells are much more active than erythrocytes. Some physiologists have described certain sex differences in the nuclei of neutrophil (polymorphonuclear). The sex chromatin body is a small mass, usually adjacent to the nuclear membrane which stains deeply with haematoxylin, and is attached to the nucleus by a slender thread, giving a drumstick-like appearance. This sex difference is found in polymorphs of about 17% of normal females and is absent in the males.

Arneth count or Arneth index (modified by **Von Bonsdorff** and later by **Cooke**). Following the idea that the age of the neutrophils is proportional to the number of lobes in their nuclei, Arneth (1904) divided them into five groups. The Arneth index can be determined by counting the number of nuclear lobes in each of hundred neutrophils. Of 100 neutrophils counted the proportion of different groups are as follows: **Group I** (One-lobed nucleus—round, oval, indented or horseshoe-shaped)—**5-10%**. **Group II** (Two-lobed nucleus)—**25-30%**. **Group III** (Three-lobed nucleus)—**45-47%**. **Group IV** (Four lobed nucleus)—**16-18%**. **Group V** (Nucleus with five or more lobes)—2%. *Cells with three-lobed nuclei are maximum in number, fully mature and functionally most efficient* (FIG. 101). *Cells with*

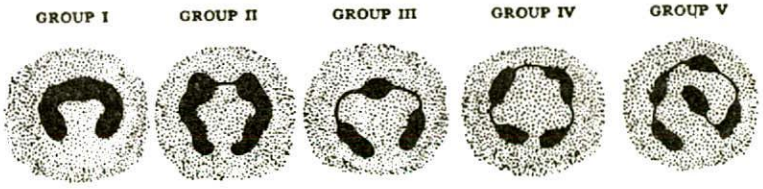


FIG. 101. Diagram shows the different numbers of lobes in polymorphs corresponding to different groups in the Arneth count.

less nuclear lobes are immature, while cells with more lobes are increasingly senile. A **shift to the left** ([Group I+Group II] > 45%) means a relative or absolute increase of immature cells and consequently a less efficient leucocyte defensive system. A **shift to the right** means diminished leucopoiesis with the increase of hypermature and senile cells.

Schilling index. It is simpler classification of neutrophils into four groups such as (*a*) *myelocyte* having only one lobe, (*b*) *juvenile metamyelocyte* in which the nucleus is indented, (*c*) *Staff cell* or *band cell* of Schilling having unsegmented but T-, V- or U-shaped nuclei. It is an older metamyelocyte and corresponds to the first stage of Arneth, and (*d*) older or segmented neutrophils.

In response to acute need certain younger forms of neutrophils such as myelocyte and juvenile type are used to come out from the bone marrow. This has been indicated by Schilling as *regenerative shift.* His another concept the *degenerative shift to the left* is referred to the failure of the neutrophils to mature as the result of the depression of bone marrow function. In this type of shifting, an increased number of immature forms is found in the blood. It is assumed that these cells can never mature.

2. **Eosinophil** is about 10–12 μ in diameter. The nucleus commonly possesses two lobes and stains less deeply than those in the polymorphonuclear cells. The cytoplasm contains coarse granules, oval or round in shape, taking acid stain and showing red colour with *eosin.* The cell membrane seems to be very delicate and is often found to be broken in a blood film. The eosinophils are amoeboid, but *not* phagocytic. They contain variable amount of histamine. By some unknown mechanism the cells are drawn towards the tissue concentration of histamine and inactivate it. They also counteract with 5-hydroxytryptamine (5-HT).

3. **Basophil.** Their size is somewhat smaller, with a diameter of about 8–10 μ. The nucleus is usually kidney-shaped or only slightly lobulated. The cytoplasm contains a large number of round granules which take deep basophilic stain. The granules are less numerous than in the eosinophils and vary much in size. Unlike the eosinophils, the granules are often found to overlap the nucleus and obscure the details of its morphology. They are actively amoeboid but are less so than the other varieties of granulocytes. They are believed to belong to cells of the reticulo-endothelial system. The basophils are similar to connective tissue mast cells and like them exhibit metachromasia, the granules containing both histamine and heparin and also 5-hydroxytryptamine (5-HT). They possibly has some role in local anticoagulation and formation of ground substance.

The increase of granulocytes in the blood is called as *granulocytosis.* Diminution of granulocytes in the blood is known as *granulocytopenia.* Complete disappearance of granulocytes is known as *agranulocytosis.*

II. Lymphocytes

1. **Small Lymphocyte.** It is slightly larger than a red cell, the diameter being about 7·5 μ. The nucleus is relatively large and occupies the major part of the cell. The cytoplasm is basophilic, showing no distinct granules and makes a thin rim around the nucleus. In early childhood they make up about 50% of the total white cell count. Their number diminishes with age. At the age of 10 years it makes about 35%.

2. **Large Lymphocyte.** About 12 μ in diameter, the nucleus may be round, oval or kidney-shaped. The cytoplasm is proportionally more and forms a wider zone around the nucleus. It is basophilic and shows no distinct granules. They are considered to be the younger forms of small lymphocytes. In the adults they are very few in number (4–8%) but are more frequent in children. In well-stained specimens a few reddish-purple granules (metachromatic granules) are found in the cytoplasm, generally collected at one pole of the cell.

III. Monocytes (*Synonyms*—**Large Mononuclear cell, Transitional cell, etc.**)

About 16 to 18 μ in diameter, the nucleus is round or oval when the cells are young. But as they grow older, the nucleus becomes convoluted,

kidney-shaped or horseshoe-shaped. [Following the original view of Ehrlich, this last variety was formerly called *transitional cells*. Because this type was supposed to represent the transitional stage in the formation of many-lobed polymorphonuclear cells from its mononuclear precursor. But this view is no more accepted and the transitional cells are now considered as the more mature and active forms of monocytes.] The nucleus is eccentric with a large amount of clear non-granular cytoplasm, may be, with vacuoles in it. Fine reddish-blue metachromatic granules may be found throughout the cytoplasm in well-stained specimens. When the nucleus is round it is difficult to distinguish them from the large lymphocytes. But the following points may be helpful. In the monocytes, the nucleus may be eccentric and the cytoplasm resembles 'frosted glass', whereas in the large lymphocytes the nucleus is central and the cytoplasm has a 'clear glass' appearance. In the warm stage the monocytes are found to be actively motile whereas the large lymphocytes show very little movement.

Development of Leucocytes

Monophyletic and Polyphyletic theories. Broadly, there are two views regarding the development of all blood cells in postnatal life: (*a*) the *monophyletic* theory, and (*b*) the *polyphyletic* theory. The **monophyletic theory** holds that all the blood cells are derived from a common primitive ancestor, which is called the 'stem cell' or **haemocytoblast.** The **polyphyletic school,** in its complete form, holds that for every variety of blood cell, there is a distinct type of 'blast cell', viz., erythroblast, myeloblast, lymphoblast, monoblast, etc. The **dualistic school** believes that there are two distinct types of primitive cells. One of them remains in the bone marrow and gives rise to the red cells, granulocytes and *megakaryocytes*, from which the platelets develop. The other cell remains in the lymphoid tissue from which the lymphocytes are derived. Similarly, there is a **trialistic school** believing in three primitive cells. All schools agree that in the early embryo all the blood cells are derived from a single primitive reticulo-endothelial cell.

It is generally conceded that, in postnatal life, the development of leucocytes is completely extravascular. The granulocytes are derived exclusively from the red marrow; while the lymphocytes and the monocytes come mainly from the spleen and lymphatic glands and to some extent, from the bone marrow.

Development of Granular Leucocytes or Granulocytes

Reticulum cell of the bone marrow. According to the monophyletic theory this stage starts with **haemocytoblast** (*vide* above).

↓

Primitive white blood cell. Cells are large with a large number of round nucleus and a thin rim of basophilic, non-granular cytoplasm.

↓

Myeloblast.* Smaller in size (12 to 18 μ), non-granular cytoplasm and a round or bean-shaped nucleus with several nucleoli. There are numerous mitochondria. They are non-motile or less motile. They divide and form the next stage. It should be noted that up to this stage the cytoplasm has no granules when stained with the ordinary Romanowsky methods. By the peroxidase method granules may be found in the more mature of the myeloblasts. The number of nuclei is an important differentiating point from

↓

* Ferrata recognises another cell, the **promyelocyte,** being the transitional stage from the myeloblast to the myelocyte.

Myeloblast ↓ the lymphoblasts. The latter have definite and distinct nucleoli whereas in the former, the nucleoli look like irregular gaps.

Myelocyte. This stage is characterised by certain remarkable peculiarities which are quite distinct from the previous stages. The peculiarities are as follows: (1) *Multiplicity*—although all the cells before this stage are multiplying, yet the multiplicity of the myelocytes is maximum. It is by the multiplication of the myelocytes that the normal supply of the white cells is kept up. (2) *Granules appear* in the cytoplasm. The granules may take neutral, acid or basic stains and accordingly the myelocytes are of three varieties: (*i*) **neutrophilic myelocyte,** (*ii*) **eosinophilic myelocyte,** and (*iii*) **basophilic myelocyte.** Recent observations show that each type of myelocyte passes through three stages of development—A, B, and C. Type A contains few granules and many mitochondria. Type C contains many granules but few mitochondria. Type B is intermediate. (3) Cytoplasm is less basophilic. (4) Nucleoli disappear and chromatin is coarser.

Metamyelocyte. At this stage, again, certain characteristic changes appear: (1) The nucleus becomes deeply indented and is almost bilobed. This shows that maturation is advancing. (2) The cells *no more multiply* but only mature henceforward. (3) *Amoeboid movement appears* and due to this, a few of these cells may burrow into the blood vessels, and (4) *appear in the peripheral circulation.*

Leucocyte. The nucleus contains many lobes which are produced by maturation of the previous stage. The maturation takes place in the bone marrow. Because, once in the peripheral circulation, no more maturation can take place. It is believed that the degree of maturation is proportional to the number of lobes of the nucleus.

Development of Lymphocytes

In the central part of lymph node, there is a small area about 1 mm in diameter and pierced by a small blood vessel. This is called the **germinal centre** or *secondary lymphoid follicle.* It takes a lighter stain than the surrounding area. Some of the reticulum cells in this area proliferate and give rise to the lymphoblasts. In recent years, evidence has been accrued and shows that the thymus is an important and probably the main source of production of lymphocytes. Apparently, there seem to be two types of lymphocytes in the peripheral blood, a longer-lived type derived from the thymus, and a shorter-lived type, probably arising from the lymphoid tissue. The stages of proliferation are the same. The *lymphoblast* is 15 to 20 μ in diameter, with a round or oval nucleus and a non-granular cytoplasm. These cells divide and give rise to the **large lymphocyte.** The normal number of lymphocytes in the blood is maintained chiefly by the division of the lymphoblast cells. The large lymphocytes are fairly mature cells and do not multiply any further. These cells are found in the circulation. The **small lymphocytes** are derived by further maturation of the large lymphocytes. These cells leave the gland through the efferent lymphatics, thoracic duct and right lymphatic duct, and reach the circulation.

Development of Monocytes

The monocytes are developed mainly from the reticulum cells of spleen and lymph nodes and to a lesser extent of the bone marrow. The stages

are—**Reticulum cells → Monoblasts → Monocytes.** Monocytes can be differentiated from the myeloblast by positive peroxidase reaction in the latter. The so-called 'transitional leucocytes' are one variety of monocytes.

Life and Fate of Leucocytes. The *life* of the different varieties of leucocytes differs. Sabin holds that granulocytes live from 1–2 days only, Osgood believes that neutrophils live from 2–4 days; eosinophils, 8–12 days; basophils, 12–15 days. The average life of lymphocyte seems to be more than a day, and may be 2 or 3 days.

Regarding the *fate* of leucocytes, it is known that all the varieties die, disintegrate and disappear. The neutrophils and other granular leucocytes undergo fragmentation in the blood stream and are also broken down by the reticulo-endothelial cells. The monocytes have the same fate. Regarding the fate of the lymphocytes, it is believed that most of the lymphocytes leave the body (and are destroyed) by passing out through the intestinal and other mucosa. But, some are certainly destroyed by the phagocytic cells of reticulo-endothelial system like the other varieties.

Functions of White Blood Corpuscles

1. **Phagocytosis.** The neutrophil polymorphonuclear leucocytes and the monocytes engulf foreign particles and bacteria, and generally digest them. This process is called phagocytosis. When the bacteria invade the body, the leucocytes pass out of the blood vessels and surround the threatened area and through their pseudopodial processes engulf the bacteria and destroy them. The eosinophils and lymphocytes have got slight phagocytic action. Migration of lymphocytes occur in chronic inflammation. The neutrophil polymorphonuclear leucocytes manufacture a trypsin-like enzyme with which they digest the bacteria and the dead tissue. Due to this, the dead tissue in an inflammatory area becomes liquefied and the so-called *pus* is formed. Lymphocytes and monocytes are supposed to elaborate a pepsin-like enzyme by which they partially liquefy the solid dead tissue, thus converting them suitable for phagocytosis and removal.

2. **Antibody formation.** Lymphocytes manufacture β- and γ-fractions of serum globulin. Immune bodies are associated with γ-globulin fraction. So the lymphocytes play an important role in the defensive mechanism of the body of an immunological nature. Adrenal cortical steroids are responsible for dissolution of lymphocytes and so increase in antibody concentration in blood.

3. **Formation of fibroblasts.** It is believed that the lymphocytes may be converted into fibroblasts in an area of inflammation and thus help the process of repair.

4. **Manufacture of trephones.** Leucocytes manufacture certain substances from plasma proteins which exert great influence on the nutrition, growth and repair of tissues. These substances are called trephones.

5. **Secretion of heparin.** The basophil leucocytes are supposed to secrete 'heparin', which prevents intravascular clotting.

6. **Antihistamine function.** The granulocytes, specially the eosinophil cells, are very rich in histamine. They are believed to defend against allergic conditions, in which histamine-like bodies are produced in excess.

Control of Leucopoiesis. The factors which control the formation of leucocytes under physiological conditions is rather complex. From the fact that these cells are constantly dying away, and in spite of it, a fairly

constant number is maintained in the circulation, it is evident that some stimulus must be constantly acting upon the leucopoietic tissues, so that the dead cells are replaced by new formation and a balance is kept up between production and loss. One thing is almost certain that for each variety of cells a specific stimulus is necessary and the stimulus is largely chemotactic.

It has been shown that injection of nucleic acid or its derivatives, increases the neutrophil cells in the blood. From such observations it has been suggested that the nucleic acid, derived from the dead neutrophils, may act as the normal stimulus for the formation of fresh neutrophils. In this way the normal neutrophil count is maintained. Regarding the normal stimulus, for the other varieties of leucocytes, nothing definite is yet known.

Some suggest that proteins are responsible for lymphocytic proliferation and lipids for monocytic proliferation.

Experimental and clinical evidences suggest that some control regulating the release of cells into the circulation is exercised by the actual number of circulating leucocytes. Menkin (1955) isolated two simple polypeptides, one thermolabile and the other thermostable as the specific leucocytosis-promoting factors in the pseudoglobulin fraction of exudates and two thermostable polypeptides as specific leucopenic factors. Others in 1961 obtained evidence of the presence of a neutropoietin and still others in 1965 isolated from serum fraction which stimulates production and another which depresses production and maturation of cells.

Total Count of Red Blood Corpuscles and White Blood Corpuscles

Enumeration of Red Corpuscles. Instrument used for this purpose is **Improved Neubauer Haemocytometer** (FIG. 102). The instrument consists of (1) a special glass slide (counting chamber), (2) one graduated pipette for R.B.C. count, (3) another pipette for W.B.C. count. In addition to this, diluting solutions and cover-slip are also required.

1. The **R.B.C. pipette** has got 3 graduations. Two graduations—0·5 and 1, are present on the stem of the pipette and the third mark—101, is placed just above the bulb. Blood is drawn, say, up to mark 1 and the rest of the bulb is filled by sucking up diluting solution up to the mark 101. The bulb of the pipette is so constructed that it holds exactly 100 times the volume of fluid contained in the stem of the pipette up to mark 1. Although fluid is drawn up to 101, the dilution of the blood will be 100, because the last part of the fluid remains locked up in the stem and is not available for dilution. If blood be drawn up to mark 0·5, the dilution will be 1 : 200.

2. The **W.B.C. pipette** is also constructed on similar lines but the third mark above the bulb is 11. This will give a dilution of 1 in 10.

3. **The Counting Chamber.** The ruling area consists of 9 square millimetres. The central square millimetre is ruled into 25 groups of 16 small squares, each group separated by triple lines as indicated in FIG. 102. The side of each small square is 1/20th mm.

Method of Counting R.B.C. 1 drop of diluted blood from the red cell-counting pipette is introduced in the counting chamber under the cover-slip. Since the under surface of the cover-slip is 1/10th mm high from the surface of the counting chamber, the volume of each small square is 1/4,000th cu. mm. Red cells are counted in the five groups of 16 small squares. To avoid counting a corpuscle twice, those on a line are counted only when on the top and left lines or on the bottom and right lines.

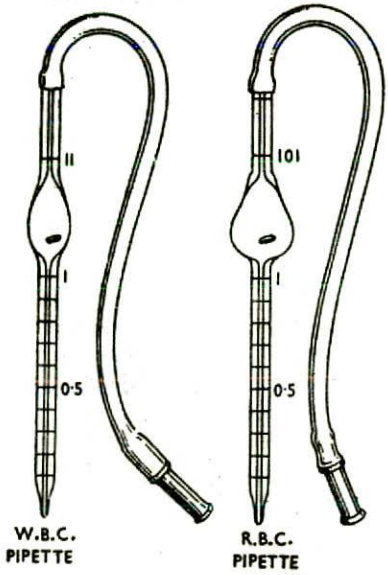
The number of red cells per cu. mm

$$= \frac{\text{Number of cells counted} \times \text{Dilution} \times 4{,}000}{\text{Number of small squares counted}}.$$

When the dilution is 1 : 200 the formula reduces to

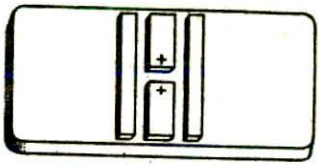
$$\frac{\text{Number of cells counted} \times 200 \times 4{,}000}{80}$$

$= 10{,}000 \times$ number of cells counted in 80 small squares.

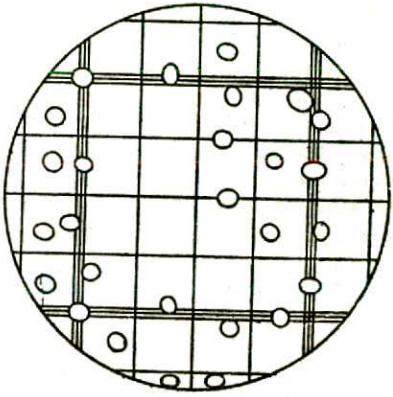


W.B.C. PIPETTE

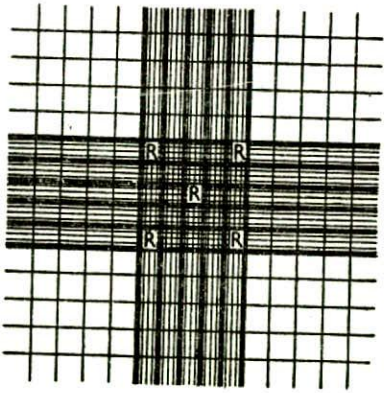
R.B.C. PIPETTE



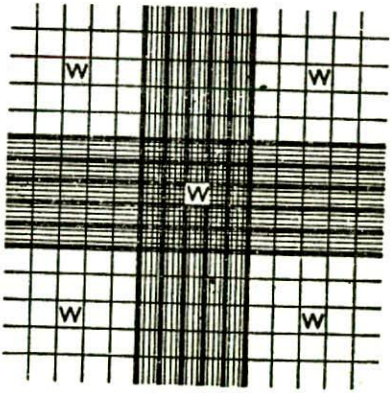
COUNTING CHAMBER. + DENOTES CENTRAL COUNTING AREA.



SMALL SQUARES IN THE COUNTING AREA (HIGH MAGNIFICATION) FOR RED CELL COUNT.



SMALL SQUARES IN THE CENTRAL COUNTING AREA. R DENOTES SMALL SQUARES FOR RED CELL COUNT.



BIG SQUARES IN THE COUNTING AREA. W DENOTES BIG SQUARES FOR WHITE CELL COUNT.

FIG. 102. Diagrammatic representation of Improved Neubauer Haemocytometer.

Method of Counting W.B.C. The white cells are counted in the four corners of 1 square millimetre and in the central ruled area on both sides of the counting chambers of the haemocytometer (10 square millimetres in all). The white cells are recognised by the refractile appearance and by the slight colour given to them by the *stain contained* in the diluting fluid. The cells touching the boundary lines are not counted. The number is multiplied by 10 or 20 (the dilution).

The number of leucocytes per cu. mm

$$= \frac{\text{Number of cells counted} \times \text{Dilution} \times 10}{\text{Number of 1 square millimetre counted}}.$$

Diluting Fluid for R.B.C. The following solutions are commonly used: (1) Sodium chloride—0·6 gm, Sodium citrate—1 gm, Formalin—1 gm, Distilled water up to 100 ml. (2) Hayem's solution—Sodium chloride—1 gm, Sodium sulphate—5 gm, Corrosive sublimate—0·5 gm, Distilled water—200 ml.

Diluting Fluid for W.B.C. Commonly the fluid is made up as follows: Glacial acetic acid—1·5 ml; 1% solution of gentian violet in water—1 ml; Distilled water—98 ml. The glacial acetic acid haemolyses the red cells, while the gentian violet slightly stains the nuclei of leucocytes. The leucocytes may, therefore, be easily recognised. A small quantity of thymol may be added to the diluting fluid to prevent the growth of moulds.

Abnormal Variation in White Blood Corpuscles Count

Leucopenia. Leucopenia is a condition in which there is a decrease in the W.B.C. count below 4,000 per cu. mm. Commonly this is due to a fall in the neutrophil cells.

Leucocytosis. A rise in the W.B.C. count above 11,000 per cu. mm is called leucocytosis. It might be due to an increase in neutrophil, eosinophil or basophil. There might also be lymphocytosis or monocytosis.

Number of inflammatory conditions like Pneumonia, etc., cause neutrophil leucocytosis in which this particular variety of the cell rises up to 90 per cent. of total W.B.C. In allergic conditions and also during infections there is eosinophilic leucocytosis.

Agranulocytosis. In such a condition there is a great fall in the number of circulating granulocytes, which usually result from the harmful effect of certain drugs.

Leukaemia. This is a malignant disease of one or other variety of the W.B.C., the number of which are greatly increased. Immature forms of the W.B.C. make their appearance in the circulating blood in such condition.

BLOOD PLATELETS (THROMBOCYTES)

Histology. Platelets are non-nucleated round or oval, biconvex discs having various sizes and covered by unit membrane. The average size is 2·5 μ. But bigger forms (4–5 μ) are also seen. In ordinary blood film the platelets are generally not seen separately but in clumps. If antiagglutinant (EDTA) is applied, in the blood prior to drawing of film then the same can be seen separately in light microscope. In light microscope generally two components of platelets are seen in the stained slide. One is the clear ground substance—the **hyalomere** (*hyalos*=glass, *meros*=part) that is stained very faintly and the other is the deeply stained central portion—the **chromatomere** or **granulomere.**

Electron microscopic studies of platelets stained with glutaraldehyde followed by osmium tetroxide reveal additional components other than two components mentioned above. It is also claimed that the surrounding membrane of each platelet is covered by a thin film of carbohydrate.

In electron microscope the **hyalomere** is seen to consist of homogeneous fine granular materials. These hyalomeres in the periphery of platelets were also found to contain **microtubules** and **microfilaments.** The microtubules probably give the ovoid structure of platelets and the microfilaments are presumably associated with microtubules. The microfilaments contain **thrombosthenin** which can contract like actin and myosin in muscle. This contractile element—the thrombosthenin is responsible for change of the shape of platelets.

Similarly, under electron microscope, the **chromatomere** is seen to contain numerous components. These components (Fig. 103) are as follows:

1. **Alpha granules.** These granules are oval or sometimes round in shape and having diameter, 0·2 μ and length, 0·3 μ to 0·4 μ. These granules are often seen enclosed in a membrane. A rounded **dense osmophilic area** is often present in these granular matrices of the organelle.

The function of α–granules is still uncertain. It is claimed that it has got lysosomal function which is important in platelet release reaction and aggregation, in clot resolution or in the platelet's phagocytic capacity. Whether these granules are the sources of the platelet factor-3 are not clear.

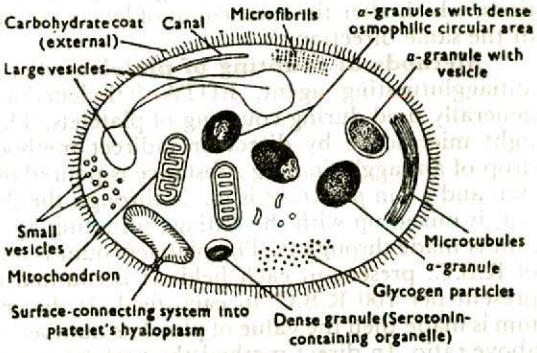


Fig. 103. Diagrammatic structure of a platelet showing its various components.

2. **Mitochondria.** These are 2–3 in number and are clearly seen in a thin section of platelets.

3. **Sydersomes.** These are irón-containing (ferritin) vesicles. These are not seen very frequently.

4. **Very dense granules** (Serotonin-containing granules). These types of granules have been demonstrated by Silver & Gardner in rabbit's platelets. These granules have got diameter 0·05 μ to 0·13 μ and is surrounded by a unit membrane. These granules have been described to contain **serotonin.** Because these granules are osmophilic and as serotonin (5-HT) is also highly osmophilic. Furthermore following treatment with reserpine, the relative concentration of the osmophilic granules in these organelles is decreased.

5. **Glycogen granules.** These granules are also distributed in certain parts of the platelets.

6. **Ribosomes.** These are generally seen in newly formed platelets.

7. **Systems of tubules and vesicles.** These are of two types. One is the surface-connecting system and the other is the dense-connecting system. The surface-connecting system communicates with the surface of platelets and are concerned with phagocytosis. The dense-connecting system originates from the Golgi apparatus of the megakaryocyte. This system does not communicate with the surface of the platelets.

The *average life of platelets is about* 5 *to* 9 *days.* They are destroyed in the spleen and other reticulo-endothelial cells.

Chemistry. Not exactly known. Platelets possess protein and a considerable amount of phospholipid, much of which seems to be cephalin.

Properties. Characteristic properties are—(1) sticking to water-wettable surface or otherwise rough surface (injured or diseased endothelium, etc.), (2) easy clumping, and (3) easy disintegration and thus liberation of thrombokinase.

Total number and its variations. The average number of platelets present per cubic mm of blood is about 250,000 to 450,000. Fairly rapid changes in number take place from day to day and even from one part of the day to another. Roughly speaking, those physiological conditions, which alter the total count of leucocytes also alter the platelet count in the same direction.

Methods of counting of platelets. For avoidance of clumping, an antiagglutinating agent, EDTA (ethylenediamine tetra-acetic acid) is generally used during counting of platelets. This can be done in ordinary light microscope by direct or indirect method. In ordinary method, a drop of antiagglutinating substance is poured over the clear surface of the skin and a pin puncture is made through the drop. The blood that comes out, is mixed up with the antiagglutinating agent and an ordinary blood film is made through it. The platelet count is made along with the number of R.B.C. present in each field. It is counted as the number of platelets present per 100 R.B.C. in each field. If the erythrocyte count per cubic mm is made then the value of platelets number can be worked out from the above ratio. In direct method the platelet count is made by counting the same along with R.B.C. in counting chamber. In this method, measured amount of blood and measured amount of antiagglutinant along with a dye are taken in the pipette and are thoroughly mixed. The platelets and R.B.C. present are counted in a counting chamber.

Development of platelets. The giant cells (megakaryocytes) of the bone marrow introduce pseudopodia through the walls of the sinusoids. These processes 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 platelets.

Functions of Platelets

1. **Initiate blood clotting.** When blood is shed, the platelets disintegrate and liberate thromboplastin which activates prothrombin into thrombin.

2. **Repair capillary endothelium.** While in the circulation, the platelets adhere to the damaged endothelial lining of the capillaries and thus bring about a speedy repair. It is known that the capillary walls, being very delicate, are easily damaged and unless these weak spots are quickly mended, the vessels will break at these spots and capillary bleeding will

take place. When the platelet count falls (below 50,000 per cu. mm) such capillary bleeding occurs.

3. **Haemostatic mechanism.** This process seems to play by the dual functions of platelets such as agglutination and coagulation. The cessation of blood flow from ruptured blood vessels takes place through simultaneous coagulation and agglutination by platelets.

4. **Hasten clot retraction.** Speed of clot retraction (*syneresis*) is directly proportional to the number of platelets present and this retraction process is dependent upon the *thrombosthenin* (contractile protein of platelets) in presence of ATP and magnesium ions.

5. **When platelets disintegrate, 5-hydroxytryptamine and histamine are liberated.** 5-hydroxytryptamine (5-HT) has vasoconstrictor effect and helps in haemostatic mechanism.

6. **Contain some substances which are like ABO blood antigens.** Platelets may be agglutinated by specific antisera and in the presence of complement, lysis may occur.

PURPURA

In this disease there is diminution of platelets in the blood. Haemorrhage occurs beneath the skin and mucous membrane. The appearance of lesions varies with the type of purpura, the duration of lesions, and the acuteness of the onset. The colour is first red, becoming gradually darker, then purple, fading to a brownish yellow. It may result in permanent pigmentation or it may disappear in course of 2 or 3 weeks but the damaged capillary endothelium is not repaired. The *coagulation time* remains normal but the *bleeding time* is prolonged. The clot does not retract.

IMMUNITY

Bacterial or other foreign proteins, entering blood stream and tissues, are called *antigens*. Antigens are generally composed of protein nucleus and polysaccharides or mucopolysaccharides (*haptens*). The specific proteins formed in blood plasma as a result of these antigens are *antibodies* or immune bodies. They are members of the γ-globulin fraction of plasma proteins. The antibodies may be of four types:

1. *Agglutinins*—those which clump together the antigens.
2. *Cytolysins* or *Haemolysins*—antibodies those are responsible for haemolysis of the R.B.C. or cytolysis of cells.
3. *Precipitins*—those which cause the precipitation of foreign proteins.
4. *Antitoxin*—produced as a result of entry of certain toxins, to neutralise them.

Immunity is a defense mechanism against (1) micro-organisms, (2) toxins produced by micro-organisms or (3) foreign proteins.

An animal might be permanently resistant to any of the above three agents from birth in which case it is named as natural immunity. An active acquired immunity might be due to *resisted attack* from (*a*) particular micro-organism, (*b*) closely similar micro-organism, (*c*) micro-organism artificially made less hostile, (*d*) killed micro-organism, (*e*) modified toxin.

Serum of an animal containing antitoxin produced as a result of active immunity, if injected into human beings, produces *passive immunity* within a short time.

BLOOD TRANSFUSION : BLOOD GROUPS

Intravenous administration of blood to help replenish excess blood loss

due to haemorrhage or otherwise, is known as blood transfusion. It is a most effective therapeutic tool when judiciously applied.

Total blood transfusion allows the transfer of those elements that are required for the defence against infection, the transport of oxygen, and the formation of platelet plug (a white thrombus). It permits the circulating blood to be re-established and corrects condition of shock and vascular collapse. It also supplies those clotting factors which are required for haemostasis. Modern transfusion methods not only use transfusion of stored blood from bottles in acid citrate dextrose (ACD) solution, but it also uses cellular transfusions, plasma component transfusions, plasma transfusions, depending as the case may be, on the requirement of the patient. The blood may be stored for two or three weeks in a refrigerator at +4°C.

Blood bank. Maintenance of a constant volume of blood is essential for our life. If due to any reason, there is excessive loss of blood, it must be transfused from other sources. Due to this reason blood should always be kept handy for emergency and establishment of blood bank is essential in every hospital. So, blood bank is a specialised medical centre which collects and stores human blood for transfusion and for operation with the artificial heart-lung. The blood thus collected is separated into fractions, each of which has a particular use, serum, red cells, white cells, platelets, etc.

Whole blood in presence of acid citrate as anticoagulant can be preserved with dextrose solution at +4°C. for several weeks. Red cells kept in such a condition are destroyed in a similar way as the normal R. B. C. and so older samples contain lesser number of cells. Frozen separated plasma can be kept for years at −20°C. Lyophilised (frozen dried) plasma, from which the water has been removed, can even be stored at room temperature. Required amount of water is added to it during use.

Indications. (1) *Haemorrhage* either acute or chronic (specially if the haemoglobin falls below 40%). (2) *Shock* in order to increase the blood volume. (3) *Blood diseases*, e.g., in all varieties of severe anaemia where the haemoglobin is below 40%, aplastic anaemia, haemorrhagic diathesis of the new-born, haemophilia, purpura haemorrhagica, etc. In haemorrhagic diseases blood transfusion increases coagulability. (4) In *carbonmonoxide poisoning, coalgas poisoning*, etc., where haemoglobin has formed some other abnormal compounds, blood transfusion provides a fresh supply of oxyhaemoglobin.

When blood of one species of animal is mixed with that of another, it is often seen that the corpuscles are at first agglutinated and then haemolysed. Amongst human beings this phenomenon of haemoagglutination is also seen. The red corpuscles of one subject may be agglutinated by the serum or plasma of another, but may not be affected in any way by that of a third man. These facts are of extreme importance in connection with blood transfusion. In various diseases, it is sometimes necessary to inject a large quantity (even 500 ml) of blood, taken from a healthy man **(donor)**, directly into the vein of the patient **(recipient)**. If the two bloods be not compatible haemolysis of the donor's corpuscles will take place, leading to disastrous effects to the recipient. For this reason, before undertaking blood transfusion, compatibility between the donor's and the recipient's blood must be carefully tested.

After observing the interaction between the red cells and plasma of a large number of cases, Landsteiner in 1901 demonstrated that human

beings can be divided into several groups. The salient facts about blood grouping are summarised below.

BLOOD GROUPS

I. A, B and **O** groups. **II. Rh** factor. **III. M** and **N** factors.

Some other blood group characteristics (i.e., P, Lutheran, Lewis and others) excepting the above three are also present.

I. A, B and O groups

1. The phenomenon of haemoagglutination is due to the interaction between two factors—**agglutinogens,** present in the corpuscles and **agglutinins,** present in the plasma (or serum).*

2. There are two primary agglutinogens—**A** and **B;** and there are two corresponding agglutinins—α and β. The agglutinogens are inherited as *Mendelian dominants*, whereas agglutinins, as *recessives.*

3. The corpuscles of a particular subject may contain only A or only B or both A and B or no agglutinogens at all, i.e., O. Similarly, the serum may contain only α or only β or both α and β or no agglutinins, i.e., o.

Agglutinogens start appearing in the sixth week of foetal life. Concentration gradually rises and at birth it reaches one-fourth of the adult level. The adult level is reached at about puberty.

Agglutinogens, A and B, are polysaccharides. They are not only found in red cells but in the cells of many other organs, such as salivary glands, pancreas, liver, lungs, testes, etc. They are soluble in water and as such diffuse out into the body fluids.

4. Thus human beings may be put into four groups according to the nature of the agglutinogens possessed by their corpuscles. These groups are called **O, A, B,** and **AB.**

Group A is subdivided into A_1 and A_2. **Group AB** is also divisible into A_1B and A_2B. Approximately 75–80% of group A belong to the **subgroup A_1** and 60% of group AB, to the **subgroup A_1B.** The remainder belong to subgroups A_2 and A_2B.

Bernstein postulated that there are three allelic genes, A, B, O. Any-one of which can occupy a given position on a chromosome. The O gene is assumed to be recessive and consequently the genotypes AO and BO are expressed as A and B respectively. The finding of subgroups A_1 and A_2 suggests that there are four alleles, A_1, A_2, B and O, leading to six phenotypes:

Phenotype (Group)	Genotype (Group)
O	OO
A_1	A_1A_1 A_1O A_1A_2
A_2	A_2O A_2A_2
B	BO BB
A_1B	A_1B
A_2B	A_2B

The blood group substances are found in many body fluids, saliva and secretions, and Morgan (1948) suggests that this ability to secrete is a Mendelian dominant controlled by a pair of allelic secretor genes—*Se*. It is probable, that ABO antigens exist in two groups—(*a*) water-soluble,

* *Agglutinogens* are also called *Isoagglutinogens, Isohaemoagglutinogens* or *Group Specific Substance.* Similarly, *agglutinins* are also known as *Isoagglutinins* or *Isohaemoagglutinins.*

present in most body fluids under control of *Se* and (*b*) alcohol-soluble present in R.B.C. but not in secretions.

5. The agglutinin α is again subdivided into α_1 and α-proper. α_1 reacts with A_1, α-proper reacts with both A_1 and A_2, and β with B; hence α is also known as **Anti-A** and β as **Anti-B** respectively. Consequently, 'A' cells are not compatible with α-serum and 'B' cells are not compatible with β-serum.

6. From the above it follows that, in the same individual, the agglutinogens in the corpuscles and the agglutinins in the plasma can never be incompatible; otherwise, the red cells would be haemolysed and the subject could not be alive.

The four main ABO groups are as follows:

Group	Agglutinogens present in *R.B.C.*	Agglutinins present in *Serum*
O	O	α and β
A	A	β
B	B	α
AB	A and B	neither α nor β

7. While testing for compatibility the *reaction between the donor's corpuscles and recipient's plasma*, and the *donor's plasma* and *recipient's corpuscles should be taken into account.*

8. Taking into consideration all the above facts, the reactions between the red cells and plasma of the four blood groups are summarised in the following table:

Donor's Corpuscles	Recipient's Plasma			
	o	α	β	$\alpha\beta$
O	—	—	—	—
A	—	+	—	+
B	—	—	+	+
AB	—	+	+	+

+ means agglutination (i.e., incompatible); — means no agglutination (i.e., compatible).

9. From the above it is evident that group O can give blood to all but can take only from its own group. Hence, it is called **universal donor.** Group AB can take blood from all (**universal recipient**) but can give blood only to its own group. The terms universal donors and universal recipients are no longer applicable after the discovery of Rh factor. Groups A and B can give blood to their own groups and also to group AB and can take blood from their own groups and from group O. Other combinations are not compatible (Fig. 104).

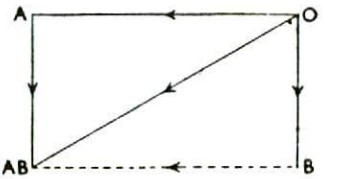


Fig. 104. The direction of the arrows indicates the type of donors who may give blood to a recipient of another group. It is self-evident that any patient is able to receive blood from a donor of the same type as himself.

Frequency of blood groups. A racial difference in the distribution of blood groups has been noted by some workers. Group O has been found to be more

common amongst Indian Tribes of America, a section of Australians and Africans, North-Western Europeans, etc. White people of America, West Europeans and West Asians have a higher frequency of group A whereas group B is more common in Central and South-East Asians. Amongst Indians group B is highest in North-East (25–30%) and lesser in South (20%). It should be noted that this statistical study is far from being complete.

Group H. Nearly all human red cells have blood group substance H. In some of them, serum has been found to contain **anti-H.** Among the ABO groups, group O has the most H material and A_1B, the least.

II. Rh factor

Another important agglutinogen has been demonstrated (1940) in human red corpuscles also by Landsteiner and Wiener. It is the agglutinogen of the **Rhesus** monkey and is present in 85% of White people. Although information is limited, yet it is found that amongst Indians and Ceylonese, the proportion is even larger, about 95% or more. *There is no corresponding agglutinin in the human plasma.*

Recent studies indicate the Rh factor is not a single entity. There are six Rh agglutinogens—**C, c; D, d; E, e.** Of these, D and d are the commonest. These two will provide three subgroups—**D, Dd** and **d.** D is *Mendelian dominant,* while d is *recessive.* Hence, groups D and Dd (collectively called D group) will be Rh *positive* (Rh+ve) and d will be rh *negative* (rh−ve). Practically all Rh positive people belong to D group and rh negative people to group d.

The **clinical importance** is as follows: (1) If Rh+ve blood be transfused to a rh−ve patient, an **Anti-Rh** factor will develop in the patient's blood in about 12 days. If a second transfusion of same blood be given to such a patient after this period, haemoagglutination of the donor's corpuscles will take place. In other words, blood which was compatible before, has become incompatible now. So that before transfusion the test for Rh factor should be carefully done. (2) During pregnancy the foetus may be Rh+ve whereas the mother rh−ve. The Rh agglutinogen (slightly present also in the plasma) from the foetus passes into the maternal blood and stimulates the formation of Anti-Rh factor. This antibody enters the foetal blood and destroys the red cells of the foetus. The foetus may die (causing miscarriage) or if born alive, suffers from severe anaemia (Erythroblastosis foetalis). (3) Such a mother becomes sensitised to Rh factor. In future if she gets a transfusion of otherwise compatible blood but containing Rh factor, agglutination will take place. (4) For the same reason, a rh negative woman, before menopause should not be given transfusion of Rh positive blood. Because, in case she becomes pregnant with Rh positive foetus, the problem as described under (2) above will become all the more acute.*

* 1. The specific agglutinins are not present in the foetal plasma. But maternal agglutinins, being filtered through the placenta, are found in the foetal plasma. Only 50% of new-born infants show an appreciable amount of this agglutinin.

2. Specific agglutinins start appearing from about the 10th day after birth and rises to the maximum at about the 10th year. Agglutinins, like other antibodies, are found in the globulin fraction of the serum. They are also present in low dilutions in body fluids which are rich in proteins, such as milk, lymph exudates and transudates. They are not found in urine and cerebrospinal fluid. Haemoagglutinins increase temporarily during serum sickness and are reduced in Leukaemia.

Details about Rh factors are briefly as follows:

1. **Rh agglutinogens.** There are six or three pairs of Rh agglutinogens—C, c; D, d; and E, e. C, D and E are Mendelian dominants, while c, d and e are recessives.

2. **Human Red cells** will always carry three agglutinogens—one from each pair, but they will never carry both the members of any pair. Thus CDE, CDe, cDE are possible but CcD and CDd are not.

3. **Rh groups** (Genotypes). It follows therefore that there will be eight possible combinations, any one of which may be carried by both the parents. Hence mathematically, there will be sixty-four possible combinations (genotypes). Of these twenty-eight being identical, thirty-six subgroups are biologically available. Of these again, only five are commonly found, viz., CDe/CDe, CDe/cDe, CDe/cde, cDe/cde and cde/cde. Others are rare.

4. **Rh+ve** and **rh—ve.** Groups containing the dominant agglutinogens, i.e., C.D.E. will be Rh+ve. But since, C and E seldom remain without D practically all Rh+ve cases contain D, i.e., belong to group D. The rh—ve cases will contain the recessive agglutinogens—c, d and e, and due to similar reasons stated above belong to group d. Every man carries some Rh agglutinogen. Majority have D and are Rh+ve. The rest carry d and are rh—ve. All Rh incompatible reactions are due to interactions between group D (*donor*) and group d (*recipient*).

5. **Rh antibody.** (*a*) Each of the six agglutinogens has antigenic property, that is, they can stimulate antibody formation. The corresponding antibodies are known as **Anti-C, Anti-D,** etc. *D is strongly antigenic.* Others are very feeble. (*b*) If D cells are repeatedly injected into a rh—ve subject Anti-D will develop. This antibody may be of two types *early* and *late.* The early Anti-D is formed first and is called **complete antibody.** It can agglutinate D cells *in vitro,* when they are suspended either in saline or albumin solution. Hence, it is also known as **saline agglutinin.** The late Anti-D is formed later and is called **incomplete antibody.** It can agglutinate D cells *in vitro,* when they are suspended in albumin solutions only and not in saline solutions. Hence, it is also called **albumin agglutinin.** But in the latter case, though the D cells are not agglutinated, yet they are somewhat modified. Because, these cells once treated in this way, will not be agglutinated by early Anti-D serum, even when they are suspended in albumin solution. Hence, the late Anti-D is also known as the **blocking antibody.** (*c*) As mentioned above D is very strongly antigenic. *It causes Anti-D formation even by intramuscular injection; so that repeated intramuscular injections of whole blood, as often done in medical practice—without matching the blood groups is not necessarily a safe procedure.* Hence, direct cross-matching before each such undertaking is the only surest safeguard.

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3. Like other antibodies, the concentration of specific agglutinin varies at all ages from man to man and even in the same individual under different conditions. They act best at a lower temperature.

4. *The blood group of a particular subject is a fixed character and does not vary with age, or disease.*

5. Non-specific agglutinins may sometimes appear in the blood which act in cold (at 0°–5°C.) and not at body temperature. These *cold agglutinins* may at times be sufficiently high to cause auto-agglutination at body temperature. For this reason there may be intravascular haemolysis leading to haemoglobinuria (Paroxysmal haemoglobinuria).

6. **Racial distribution.** White people—85% Rh+ve, of which D —35%, Dd—48% and the remaining 2% also contain D along with some other agglutinogen. Indians, Ceylonese—95% Rh+ve. Japanese about 100% Rh+ve. Hence, in the latter, Rh incompatibility reactions are extremely rare.

Haemolytic disease of the new-born

This disease is due to destruction of the Rh+ve R.B.C. in the foetus by an Anti-Rh agglutinin, present in the mother's serum, which has filtered through the placenta during pregnancy. The incompatibility between the blood of mother and child is caused by the inheritance of the Rh factor.

GENETIC COMBINATIONS OF THE RHESUS FACTOR

Father	Mother	Children
Rh+ve/Rh+ve	Rh+ve/Rh+ve	100% Rh+ve
Rh+ve/Rh+ve	Rh+ve/rh−ve	50% Rh+ve/Rh+ve
		50% Rh+ve/rh−ve
Rh+ve/rh−ve	Rh+ve/rh−ve	50% Rh+ve/rh−ve
		25% Rh+ve/Rh+ve
		25% rh−ve/rh−ve
Rh+ve/rh−ve	rh−ve/rh−ve	50% Rh+ve/rh−ve*
		50% rh−ve/rh−ve
Rh+ve/Rh+ve	rh−ve/rh−ve	100% Rh+ve/rh−ve*
rh−ve/rh−ve	rh−ve/rh−ve	100% rh−ve/rh−ve

Incompatibility of the blood might arise only in cases marked asterisk (*) as in these two groups the mother is capable of producing an Anti-Rh agglutinin to destroy the Rh+ve R.B.C. present in the foetus.

In this disease, destruction of the normal R. B. C. leads to the presence of abnormal nucleated R.B.C. in circulation. A few hours after birth there is anaemia, acute jaundice and related symptoms.

III. M and N factors

Besides the A, B, O system, other supplementary agglutinogens have been identified. They are known as M and N factors. This will provide three other independent groups M, N, and MN. These groups are of no importance for blood transfusion but have got medicolegal importance, e.g., **paternity test.**

There are two blood genes in each person, e.g., M+M, N+N or M+N. If a baby belongs to M group then the parents must have given M+M. If the baby has got N group then the parents must have given N+N. In the baby belongs to MN group then the parents must have given M+N. In the latter case if the mother's supplementary group is N then the father must belong to group M. These may be represented in a tabular form:

Child's group	Parents' contributory group	Mother's group	Group **absent** in father
M	M+M	Unrestricted	N
N	N+N	Unrestricted	M
MN	M+N	N	N
MN	M+N	M	M

This test can only show that the suspected person might or might not be the actual father.

In chemical composition, the blood group substances are nitrogenous, neutral, hetero-polysaccharide containing D-galactose, methylpentose fucose, D-glucosamine, D-galactosamine present as N-acetyl derivatives and other amino acids such as threonine, serine, protein, etc. The specificity of the blood group substances are dependent on the terminal components which may be non-reducing sugars discussed above.

P system

Landsteiner and Levine in 1927 demonstrated still another system, P^+ and P^- by immunisation experiments in rabbits. Subsequently the antigen T_j^a was found and was shown to be part of the P system. According to some, the P system is a strong system with regularly occurring antibody like Anti-A, Anti-B, etc. But others suggest that the P factor is unimportant in transfusion reactions on haemolytic disease but is important in obstetric cases. It is inherited as a Mendelian dominant.

Lutheran, Kell, Duffy, Kidd and other Blood groups. Lutheran, Kell, Duffy and Kidd blood groups, spoken of after their names in whose blood they first discovered and were recognised between 1946 and 1951. More have been discovered subsequently, and new blood groups continue to be reported as research in the line progresses.

Importance of Blood Group studies. This study is of great value for: (1) Blood transfusion, (2) Certain blood diseases, (3) Paternity test, (4) In forensic medicine, (5) Ethnological studies, (6) Anthropological studies, and (7) Various experimental purposes.

Hazards of Incompatible Blood Transfusion

When mismatched blood is injected intravenously to a recipient whose serum contains antibodies like α, β or *Anti-D*, serious symptoms even leading to death may occur. The red cells are agglutinated, which later on undergo haemolysis.

The results of incompatible blood transfusion may be summarised as follows:

1. *Agglutination of red cells* and *haemolysis.*
2. *Jaundice*—It is due to breakdown of red cells. Haemoglobin is released and is broken down to form bilirubin. When the concentration of bilirubin rises in blood it produces jaundice.
3. *Haemoglobinuria*—Red cells are agglutinated and then haemolysed. Large amount of haemoglobin is released and partly converted into bilirubin. Part of the released haemoglobin is excreted in urine as such.
4. *Renal failure*—There is fall in renal arterial blood pressure, and so reduced formation of glomerular filtrate. Haemoglobin filtered through the glomeruli is converted into acid haematin and blocks the renal tubules and lessens the flow of urine which may lead to *anuria.* The nitrogenous constituents of the blood accumulate in it and *uraemia* may develop.