# 5 CHAPTER Formation and Fate of Erythrocytes

## *Haemopoiesis*

It means formation of blood cells. This is necessary for replacement of the blood cells which are destroyed continuously in the body. The rate of destruction of the WBCs is higher, so their production rate is also higher than the RBCs. In normal adult human, blood cells are produced in bone marrow. More specifically, it can be said that the site of haemopoiesis is the red bone marrow.

## *Sites of Haemopoiesis*

Haemopoiesis starts from the third week of intra-uterine life. Initially it occurs in the area vasculosa of the **yolk sac**. Stem cells (see below) for haemopoiesis are derived from the mesoderm. The stem cells then migrate to **liver** and **spleen** by the third month of intra-uterine life. Haemopoiesis in these organs continue in full swing upto the fifth month of intra-uterine life and then gradually declines. From the fifth month onwards haemopoiesis gradually shifts to the **bone marrow** and after birth haemopoiesis is the function of bone marrow only (**medullary**). In case of emergency when the bone marrow fails, haemopoiesis may switch over to liver and spleen (**extramedullary haemopoiesis**).

## *Bone marrow*

It is the highly vascular tissue found in the medullary cavities of long bones and in the trabecular spaces of spongy bones. Bone marrow is of two types : red bone marrow and yellow bone marrow.

The **red marrow** is active and after birth it is found inside all the bones of the body. With advancement of age, in many places it is replaced by inactive yellow marrow and ultimately in adult life (after 20 years of age) the red marrow is found only in cranial bones, vertebrae, pelvic bones, ribs, sternum and in upper ends of femur and humerus. This is called adult pattern of marrow distribution. The red marrow is composed of large number of blood vessels which are called sinusoids (capillaries). These sinusoids form a network and between them are the cells which form the blood cells.

The **yellow marrow** contains less blood vessels and are mostly filled with fatty tissue. It is also called white marrow. It is inactive and blood cells are not produced here. If necessity arises, the yellow marrow becomes active and may be converted into red marrow, and start producing blood cells.

Extramedullary haemopoiesis (*i.e.*, in liver and spleen) can also occur in adult if the situation demands.

The cells in the red marow are mostly of the granulocyte series (60 to 70%), as the rate of formation of these cells, particularly of neutrophils is highest. The rest is composed of the cells of other groups, *i.e.*, RBC, lymphocyte, monocyte, platelet, etc. These cells are in various stages of development (*i.e.*, the precursors of the blood cells). These are found outside the sinusoids, *i.e.*, extravascular and normally enter in to the sinusoids only when they are converted into matured blood cells.

### Examination of Bone marrow

Bone marrow is examined to study haemopoiesis and also for diagnosis of different diseases like kala azar and those related to haemopoiesis, *e.g.*, anaemia, leukaemia, etc. To do this, bone marrow is collected by sternal puncture (tibial puncture in children). The marrow is aspirated by a special needle and is then spread on glass slides to make smears. These smears are then stained and examined under microscope (see back cover).

### Bone marrow transplantation

Bone marrow can be transplanted from one person to another after proper precaution (HLA matching p. 53). It is needed in the treatment of leukaemia. In the process the defective bone marrow is first destroyed and then normal marrow from a suitable donor is injected intravenously. These healthy marrow cells now grow inside the medullary cavities of the recipient and produce normal blood cells.

## *The Stem cells or haemopoetic stem cells*

These are the cells from which different types of blood cells are produced. Osteoclasts (p. 351), mast cell (p. 47), etc., are also produced from them. They are found in bone marrow and may also be found in peripheral blood *i.e.*, the blood circulating in the body. They look like lymphocytes.

There are two types of stem cells : (a) pluripotent stem cells, and (b) committed stem cells. The **pluripotent** stem cells can give rise to many varieties of cells but a **committed** stem cell can give rise to only one variety to which it is committed. Committed stem cells originate from the pluripotent stem cells.

## *Cell renewal*

It is known that the stem cells ultimately give rise to matured blood cells. But if all the stem cells are simply converted to matured blood cells, then the stem cells will be exhausted very soon. To prevent this, some stem cells remain as stem cell while others form blood cells. This is called cell renewal. It occurs as follows :

Stem cells multiply to many stem cells through mitosis. Some of the daughter stem cells are diverted to produce blood cells while others remain as stem cells (Fig. 2.17). These stem cells behave again in the same way. It is somewhat like the procedure practised by a farmer. After harvesting he keeps some grains for seed and the rest he consumes as food. Next season, the seeds are used for cultivation to produce more grains and part of which is again kept as seed. In the same way the stem cell number is maintained and side by side blood cell production continues.

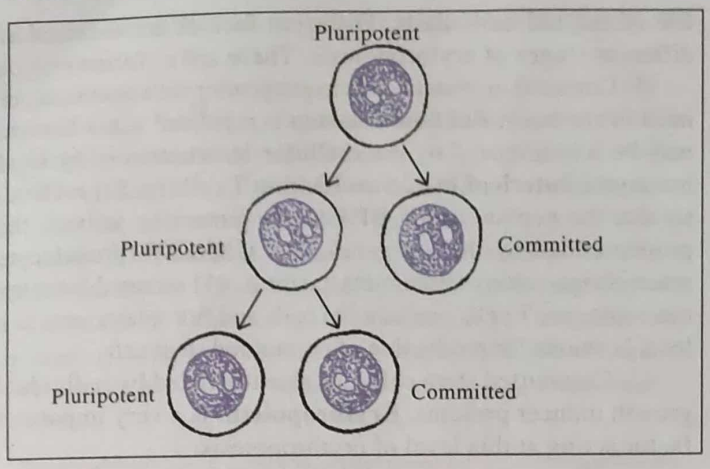
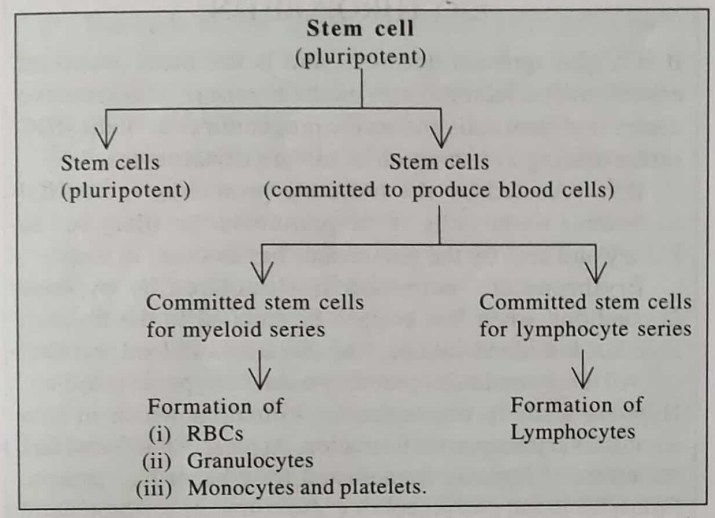



**Fig. 2.17.** *Stem cell renewal.*




**Note** : The committed stem cells are of different types and only the neutrophils and the monocytes have a common committed stem cell while others have separate committed stem cells for each of them. *e.g.*, erythrocyte, eosinophil, lymphocyte and megakaryocyte.

### Progenitor cells

These are in between the committed stem cells and the blast cells. These are specific for each type of blood cells, *e.g.*, progenitor cells of erythrocytic series give rise to erythrocytes only.

### Blast cells

These are immature cells found in the early stages of development of all blood cells. These are also named accordingly, *e.g.*, erythroblast, myeloblast, megakaryoblast, lymphoblast and monoblast. These are normally not found in peripheral blood but only in bone marrow. However, these can be found in peripheral blood in diseases as in leukaemia. Blast cells are characterised by large nuclei with less cytoplasm. For the erythroblasts, two names are used : (a) normoblast, which will ultimately form normal cells (normocytes), and (b) megaloblast, which form larger RBCs (macrocytes).

## ERYTHROPOIESIS

It is the process by which erythrocytes are produced in the body (haemopoiesis includes the formation of all types of blood cells). In the later half of foetal life the site gradually changes to bone marrow and after birth erythropoiesis takes place only in bone marrow.

### *The stages of development of Erythrocytes*

#### Stem cell

These are pluripotent and are not for any particular type of blood cell, *i.e.*, common ancestor to all types of blood cells.

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#### Committed stem cell

These cells develop from the pluripotent stem cells. Committed stem cells give rise to progenitor cells.

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#### Progenitor cells

These are of two types : BFU-E and CFU-E which give rise to RBCs only.

⇩

**BFU-E** or burst forming unit of erythrocyte series : a single progenitor cell of this type gives rise to a large number of cells. These are taken as the most primitive progenitor cells.

⇩

**CFU-E** or colony forming unit of erythrocyte series : a single cell which gives rise to a smaller number of cells than BFU-E cells. CFU-E cells are most differentiated progenitor cells.

It is presumed that the BFU-E cells give rise to CFU-E cells and CFU-E cells give rise to the blast cells (the nucleated cells of the erythrocyte series). The blast cells are of two types : pronormoblasts and normoblasts.

The normoblasts, the nucleated precursors of the normal erythrocytes are of three types as stated below. Pronormoblasts are the precursors of the normoblasts.

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#### Proerythroblast (Pronormoblast)

These are the first blast cells of the erythrocyte series and develop from CFU-E. The cells are of 15 to 20 μ in diameter (Fig. 2.18); the nucleus containing many nucleoli is of 12 μ in diameter and strongly basophilic. Cytoplasm is also basophilic, show mitosis and contain no Hb. These are the earliest identifiable erythrocyte precursors.

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#### Early normoblast

Cells (10 to 17 μ) are smaller than the previous stage and show active mitosis. Nucleus is large and the cytoplasm is basophilic due to plenty of RNA, hence also called basophilic normoblast. Haemoglobin appears in small amount in the late part of this stage.

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#### Intermediate normoblast

Cells are of 10 to 14 μ in diameter, smaller than the previous stage and show mitosis. Nucleus becomes smaller. Sufficient Hb appears to make the cell partly eosinophilic and the cells are also called polychromatophilic nomoblasts (polychromatia). In the later part of this stage, when plenty of Hb appears, mitosis stops.

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**Late normoblast**

Cells are of 7 to 10 μ in diameter and mitosis is not seen. Cytoplasm is mostly eosinophilic due to more haemoglobin. Nucleus is very small and pyknotic (pyknotic = thickened and shrunken nucleus). These are also called orthochromatic normoblasts.

⇩

**Reticulocyte**

This stage is reached when the late normoblasts lose their nuclei. The cells are eosinophilic due to more Hb but show basophilic reticulum which gives the name. These are slightly larger than the matured RBCs. Hb synthesis continues in this stage (see also P. 25).

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**Erythrocyte**

The cells are of 7.2 μ in diameter, fully eosinophilic, non-nucleated and biconvcave discs, *i.e.*, matured RBC.

This process of erythropoiesis takes about 7 days to form a matured RBC. Time taken for conversion of proerythroblast to reticulocyte is 3 days and 4 days are required for reticulocytes to become matured RBCs. Of these 4 days, the last one day is spent in peripheral circulation by the reticulocytes. In the early stages, the rate of cell division is very high and other characteristics of a cell in general are also present, *i.e.*, less differentiated. As the cells move towards the matured RBC, these properties of general cells are lost and gradually they gain the properties of erythrocytes. This is called **differentiation**. In the process the nucleus gradually decreases in size and is lost ultimately. The cytoplasm which was initially basophilic due to plenty of RNA becomes eosinophilic due to accumulation of Hb.

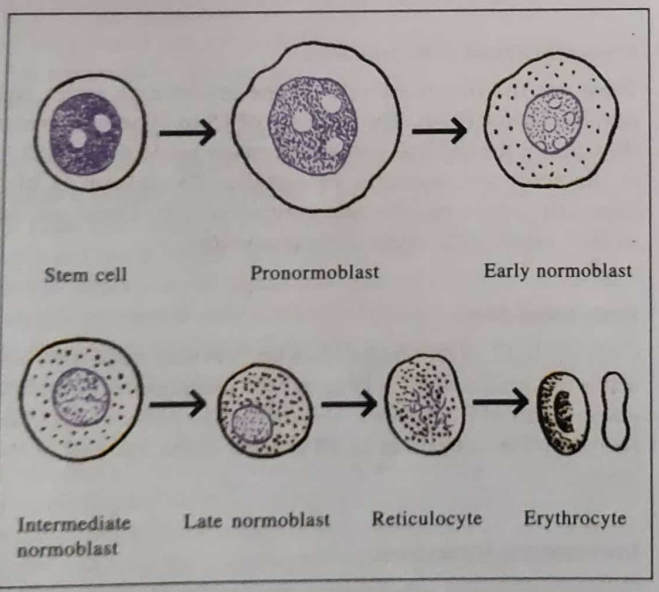



**Fig. 2.18.** *Stages of erythropoiesis.*

## *Factors influencing Erythropoiesis*

There are various factors involved in erythropoiesis, which maintain a steady rate of red cell production to replace about 6% of the old cells daily. Different factors are required at different stages of erythropoiesis. These are as follows :

(1) Conversion of stem cells to progenitor cells occurs as per need of the body. But how this step is regulated is not known, may be it is achieved by **intercellular interaction** or by local hormones. **Interleukin-1, 6 and 3** from T cells (p. 51) act here, so also the peptide called **BPA** (burst promoting activity, the protein coded by the Scl gene). The **GM-CSF** (granulocyte macrophage-colony stimulating factor p. 41) secreted from the macrophages, T cells, endothelial cells and fibroblasts, acts as a local hormone for production of committed stem cells.

(2) Committed stem cells are also regulated by individual growth inducer proteins. **Erythropoietin** is a very important factor acting at this level of erythropoiesis.

### ERYTHROPOIETIN

It is a glycoprotein hormone and is the most important erythropoietic factor. It acts on the erythropoietin sensitive committed stem cells and on the progenitor cells of the RBC series leading to formation of mature erythrocytes.

It is secreted by the hormone producing interstitial connective tissue cells of the peritubular capillary bed in kidney and also by the perivenous hepatocytes in liver.

Erythropoietin secretion is stimulated by hypoxia (a condition when less oxygen is supplied to the tissues), anaemia, low blood volume, lung disease, low blood pressure, etc. All these conditions probably produce hypoxia in kidney. Hypoxia leads to prostaglandin formation which in turn stimulates erythropoietin formation. At present it is found that the effect of hypoxia is mediated by a haem like protein. Epinephrine and norepinephrine also stimulate erythropoietin secretion. Testosterone stimulates erythropoietin secretion and accounts atleast partly for higher RBC count in male. Though some erythropoietin is produced by the liver, the kideneys are vital for erythropoiesis. In diseases of kidney or after its removal, anaemia is produced due to lack of erythropoietin. In some kidney disease (*e.g.*, renal artery stenosis) there is increased RBC production (polycythaemia) due to continuous hypoxia in kidney leading to increased erythropoietin formation. Increased erythropoietin formation resulting in increased red cell count is also seen in different lung diseases with chronic hypoxia. So, it can be said.

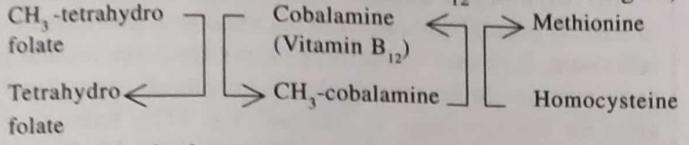
Hypoxia →↑ erythropoietin →↑ erythropoiesis. (↑ = increased)

Erythropoietin acts from the stage of committed stem cell (erythropoietin sensitive committed stem cells) upto the stage of intermediate normoblast. It is believed to prevent apoptosis (a type of cell death, see P.) in committed stem cells. Erythropoietin increases production of red blood cells by shortening the life cycle of the developing cells, helps them to skip division and also acts by accelerating synthesis of Hb (the **last** effect is due to induction of the enzyme δ ALA synthetase). Erythropoietin (recombinant) can be used in the treatment of anaemia of renal failure.

(3) **Iron** is another important factor. It is necessary for haemoglobin synthesis. Iron from transferrin is endocytosed by the normoblasts. In iron deficiency, Hb synthesis becomes

less and the RBCs show hypochromia, microcytosis and anisocytosis (p. 25, 38).

(4) **Vitamin $B_{12}$** and **Folic acid** (see also p. 76, 320) : These are necessary for DNA synthesis. Rapidly dividing cells like erythroblasts need them very much before every mitosis when the DNA material is to be doubled. For DNA synthesis thymine is essential and tetrahydrofolate is necessary for thymine synthesis. This tetrahydrofolate is formed from plasma folic acid (5-methyl tetrahydrofolate) after losing one methyl group to vitamin B12. (The methyl group from $B_{12}$ is later taken up by homocysteine and the same vitamin $B_{12}$ can be used again).




(5) **Intrinsic factor** is also important as it helps in the absorption of extrinsic factor, *i.e.*, vitamin $B_{12}$. In pernicious anaemia there is lack of this intrinsic factor due to atrophy of gastric mucous membrane.

This gastric atrophy leading to pernicious anaemia (p. 76) is caused due to an autoimmune disease where antibodies against gastric mucous membrane is formed. (Antibodies against IF is also found in many cases). Vitamin $B_{12}$ and folic acid deficiency lead to megaloblastic anaemia.

(6) **Nutritious diet** including protein, minerals and vitamins, is necessary for new cell formation so also for erythropoiesis. Vitamin C has its importance due to its role in iron absorption in gut. Role of vitamin $B_{12}$ and folic acid are discussed above.

(7) **General hormones** like thyroxine, growth hormone, corticosteroid, etc. are also necessary. Testosterone increases and oestrogen inhibitis erythropoiesis.

## *Life span of RBC*

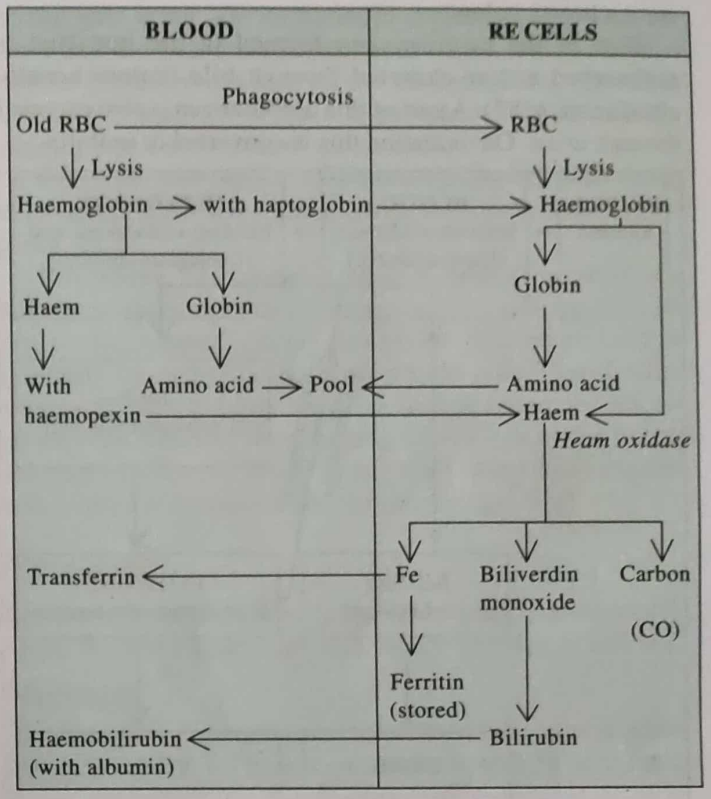
A matured RBC lives upto 120 days in the body, then it is destroyed and replaced. This life span of RBC can be measured as follows : RBC from a person is first tagged with radioactive chromium ($Cr^{51}$) by incubating with $NaCr^{51}O_4$ solution. These tagged RBCs are re-injected in the individual. Now it is to be noted, how long the tagged RBCs are found in the circulation of the individual. The longest duration gives the life span of the RBC. Older RBCs will be destroyed early but youngest one will survive for the longest time and will give the life span.

## FATE OF HAEMOGLOBIN/FATE OF RBC

When the RBCs become old, their walls become weak and the shape is changed due to slowing of enzyme activities. Some of these RBCs are then broken down by the force of the circulation. The haemoglobin released combines with haptoglobin (a plasma protein) and is carried to the RE cells. But most of the old RBCs are phagocytosed by the RE cells and are broken down. The Hb is released inside the RE cells. These RE cells are situated mainly in the spleen and also in the liver, bone marrow, etc. and these cells do not phagocytose the normal RBCs as the normal RBCs are flexible and can pass easily through the splenic filter. The old RBCs are deformed and stiff hence fail to pass through the filter and are phagocytosed by the RE cells situated near the filter.

There may be other mechanism for detection of the senile RBCs by the RE cells.

[Some Hb in plasma is split into haem and globin. This haem is carried to the RE cells by another plasma protein knwon as haemopexin].




The Hb is then processed within the RE cells as follows :

First step of the process is opening up of the tetrapyrrole ring. This is catalysed by the enzyme haem oxidase with liberation of one molecule of carbon monoxide (only reaction in the body producing CO). Then the globin is separated and added to the amino acid pool. The Fe liberated is either stored in the RE cells as ferritin or used again. The other portion, i.e., the opened up tetrapyrrole structure, forms the biliverdin which is reduced to bilirubin and is released from the RE cells.

$$\text{Haem} \xrightarrow[\text{oxidase}]{\text{Haem}} \text{Fe} + \text{CO} + \text{Biliverdin} \xrightarrow{\text{Reduction}} \text{Bilirubin}$$

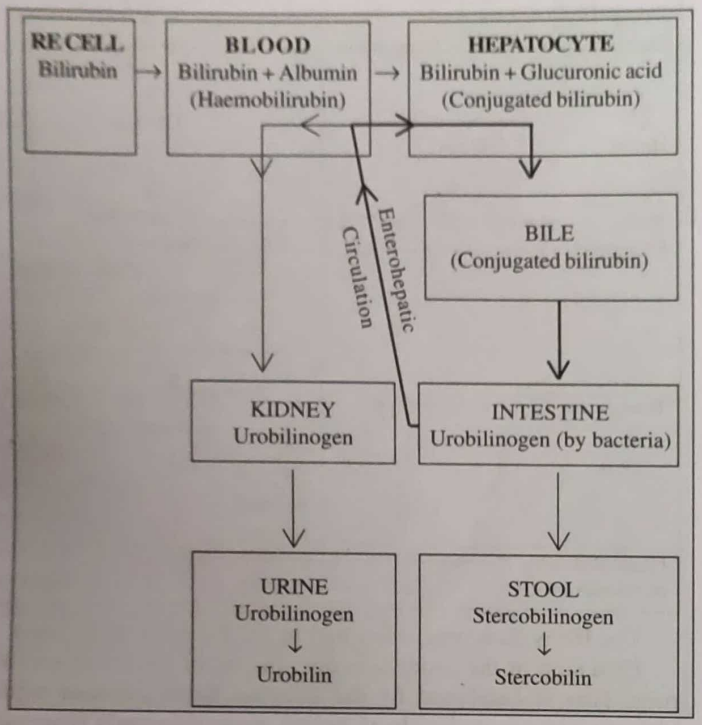
This bilirubin is then transported in the plasma as albumin-bilirubin complex (as this bilirubin is insoluble in plasma). It is also called free bilirubin or haemobilirubin and is carried to the hepatocytes (liver cells).

Inside the hepatocytes the bilirubin is bound to the cytoplasmic proteins called ligandins. There, with the help of the enzyme glucuronyl transferase it is conjugated with glucuronic acid to form bilirubin mono or diglucuronides in the smooth endoplasmic reliculum. Deficiency of this enzyme in neonates leads to physiological jaundice (p. 88). Now this is called conjugated bilirubin (soluble) and is excreted through the bile into the intestine. (A small amount of bilirubin sulphate, etc. are also formed). If bilirubin accumulates insides the body, jaundice occurs and **jaundice** (p. 88) may occur due to increased destruction of the RBCs, due to decreased

glucuronyl transferase activity or other problems in the liver, or due to obstruction of the bile flow to the intestine.

In the intestine the bilirubin is acted upon by bacteria to form urobilinogen. Some of the urobilinogen which passes through stool is called sterocobilinogen which gives yellow colour to the stool. This stercobilinogen on oxidation, outside the body, is converted into stercobilin which then gives the stool a brown colour.

Part of the urobilinogen formed in the intestine is reabsorbed and re-excreted through bile (entero-hepatic circulation, p. 87). A part of this urobilinogen is also excreted through urine. On oxidation this is converted to urobilin.




It is clear from the above discussion that bilirubin is formed from the dead RBCs and is excreted with bile, so bilirubin is purely an excretory product. But as stated before, the bilirubin level in the body increases either due to increased haemolysis or due to decreased excretion by the liver, hence its plasma level is a good indicator of liver disease and also of haemolysis.

## *Applied Physiology*

Abnormalities either in the rate of formation or of destruction of RBCs lead to problems. Too much RBCs are produced to result in **polycythaemia** (p. 31). Less production leads to **anaemia**. Similarly too much destruction of the RBCs leads to anaemia. Normal rate of production with defective RBCs or abnormal haemoglobin, also leads to anaemia. Too much destruction leads to jaundice due to more bilirubin formation. **Jaundice** is discussed with bile (p. 88) and a short discussion on anaemia is given below.

### ANAEMIA

It is the qualitative and or quantitative diminution of haemoglobin or RBC or both in respect to the age and sex of the individual. The net effect is failure in transport of adequate amount of $O_2$ to the tissues. Generally anaemia is said to occur when Hb is less than 10 g% but symptoms depend not only on the reduction but also on the rate of reduction of Hb.

Anaemia may be due to various reasons and are of various types but a simple classification suitable at this stage is as follows :

(a) **Normocytic anaemia** : In this type the morphology of RBC is normal and anaemia occurs due to decreased red cell count. It occurs when there is less erythropoiesis as in bone marrow depression (aplastic anaemia), immediately after an haemorrhage or haemolysis. (The haemolytic anaemias due to changed morphology of the RBCs, e.g., sickle cell anaemia, cannot be included in this group).

In this type of anaemia, if all the RBC indices (p. 32) are normal except the cell count then it is called **normocytic normochromic anaemia,** but in **normocytic hypochromic anaemia** MCH, MCHC, CI; all will be less than normal.

(b) **Macrocytic anaemia :** Here the erythrocytes are larger than normal. This type occurs in vitamin $B_{12}$ and folic acid deficiency.

These two vitamins are needed for DNA synthesis. Due to their lack, DNA material cannot be doubled, so cell division does not occur properly. The cells become larger and MCH becomes higher, so also the colour index but the cell count is low. The erythroblasts in the bone marrow are of large size (megaloblasts), hence this anaemia is also called **megaloblastic anaemia**.

(c) **Microcytic anaemia** : Here the RBCs are smaller than normal. This type of anaemia is typically found in chronic iron deficiency states. There may be anisocytosis. Cells are hypochromic, *i.e.*, contain less Hb (low colour index). MCH, MCHC, MCV, Hb% are low.

(d) **Other anaemias** : There are some other varieties like **sickle cell anaemia**. Here anaemia occurs due to increased destruction of RBC due to sickling. So also the case of hereditary spherocytosis leading to **congenital haemolytic anaemia**. **Pernicious anaemia** (p. 76) is a special type of macrocytic anaemia produced due to lack of absorption of vitamin $B_{12}$ in atrophic gastritis. This condition also shows neural complications.

The above is a morphologic classification but the aetiopathological classification, more popular among the haematologists, is as follows :

(A) Due to decreased production of RBCs; as in bone marrow failure (aplastic anaemia), factor deficiency (iron deficiency, vitamin $B_{12}$ deficiency, etc.).

(B) Due to increased destruction of RBCs, as in all haemolytic conditions and in hypersplenism.

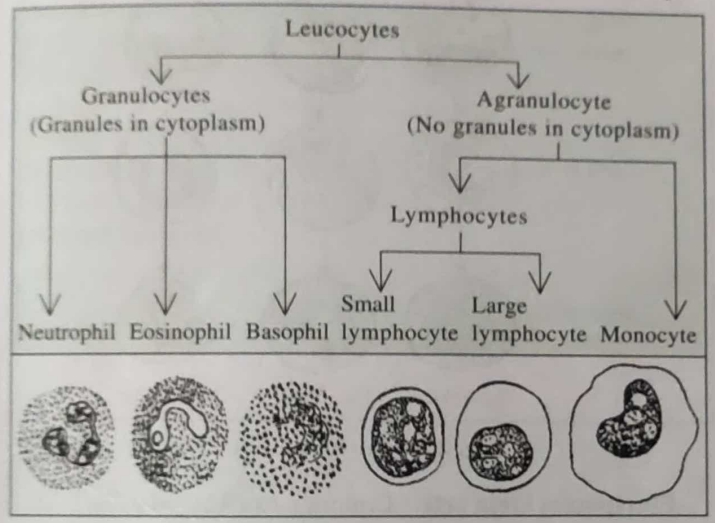
(C) Miscellaneous, *e.g.*, sideroplastic anaemia (which responds to vitamin $B_6$ though there is iron deficiency), etc.

# 6 CHAPTER The Leucocytes

The leucocytes are also called **white blood corpuscles** (WBC) or simply the white cells of the blood but these are not white, these are colourless. The white cells defend the body against diseases by fighting infections (bacterial, viral, parasitic, etc.), antigens and also against malignancy.

## *Types*

The leucocytes are usually divided into granulocytes (with granules in their cytoplasm) and **agranulocytes** (without granules). The granulocytes are further subdivided into three types : **neutrophils, eosinophils** and **basophils**. The agranulocytes are the **lymphocytes** and the **monocytes**. All these cells can be studied under microscope after staining.




## *Staining*

It means colouring of a tissue or cells with a suitable dye so that it can be studied properly under microscope. As the leucocytes are transparent, these cannot be seen properly under microscope unless stained. Ordinary stains like methylene blue colours only the acidic materials and the stains like eosin colours the basic materials of a cell. None of these can stain the various types of granules of the leucocytes properly. This can be achieved by Leishman's stain which is a modification of the Romanowsky stain like Wright's stain, Jenner's stain, Giemsa stain, etc. Romanowsky stain is the solution of a compound formed by interaction between watery solutions of methylene blue and eosin on long standing. This compound is nothing but the maturation products of methylene blue (Azure B) along with eosin. This is a polychrome dye which stains both acid and basic materials simultaneously, hence it alone can stain the granules of the leucocytes (Eosin stains the basic and azure B stains the acidic materials).

**Leishman s stain,** which is usually used to stain blood films, is prepared by treating methylene blue with $Na_2CO_3$ to produce oxidation products (methylene azure) which are then mixed with eosin. The resultant precipitate is then washed, dried and the Leishman powder is obtained. This Leishman powder is dissolved in acetone free methyl alcohol to produce Leishman's stain. If acetone is present it will disturb the staining particularly that of the nucleus.

Supravital staining is used to study a living cell (only living cells accept these dyes like methylene blue, brilliant crysyl green, etc.)

**Procedure** : Leishman's stain is poured in sufficient amount to cover a dry blood film on a glass slide and left for one to two minutes. During this period fixation of the film occurs by the methyl alcohol present in the stain. Then distilled water sufficient to cover the glass slide is added and left for another ten minutes. Now staining takes place by the watery solution of the dye. The film is then washed with distilled water, dried and examined under microscope.

## *Morphology*

In a blood film, stained with Leishman stain, the leucocytes when examined under light microscope are seen as follows.

### Neutrophils

These are the most numerous white blood cells with diameter 10 to 15 μ (RBC 7.2 μ) and constitute 60 to 70% of the total leucocyte count. Nucleus shows variable number of lobes (2 to 7) and hence called **polymorophonuclear leucocytes** (Fig. 2.19A). The pink cytoplasm shows fine violet (redbrown) granules, which are amphophilic, *i.e.*, take both acidic and basic stain and the granules are not neutral. The granules of the neutrophils are of two types—primary and secondary. Only the secondary granules are seen in Leishman's stain. Nucleus stains purple blue and the chromatin is coarse and ropy.

### Eosinophils

These cells, seen not infrequently, are of diameter 10 to 15 μ. The nucleus is usually bilobed (Fig. 2.19B). Cytoplasm is packed with coarse brick red granules which take acidic stain, hence named eosinophil. Nucleus stains purple blue and chromatin is coarse and ropy.

### Basophils

Occasionally seen in blood slides from a healthy person (Fig. 2.19C). The cells are of 10 to 15 μ in diameter and contain bilobed or 'S' shaped nucleus which is obscured by coarse basophilic (blue) granules. Nucleus stains purple blue and chromatin is coarse and ropy.

On an average all the granulocytes are of equal size.

### Small lymphocytes

These cells are found in plenty and are of about 7.5 μ in diameter (Fig. 2.19D). Nucleus is like that of large lymphocyte and

cytoplasm is scanty, so the cell is almost occupied by the nucleus. Nucleus stains deep blue.

**Large lymphocytes**

These cells are also seen frequently in the film and have diameter of about 10 to 18 μ (Fig. 2.19E). They contain a round nucleus with coarse and lumpy chromatin. There is plenty of clear blue cytoplasm without any granule. Nucleus stains deep blue.

**Monocyte**

The largest of all leucocytes (10 to 20 μ) with a large kidney shaped nucleus which takes light stain (Fig. 2.19F). The cytoplasm is pale blue and contains fine purple granules.

RBCs and platelets also take stain and are seen in the blood film. The RBCs are eosinophilic.

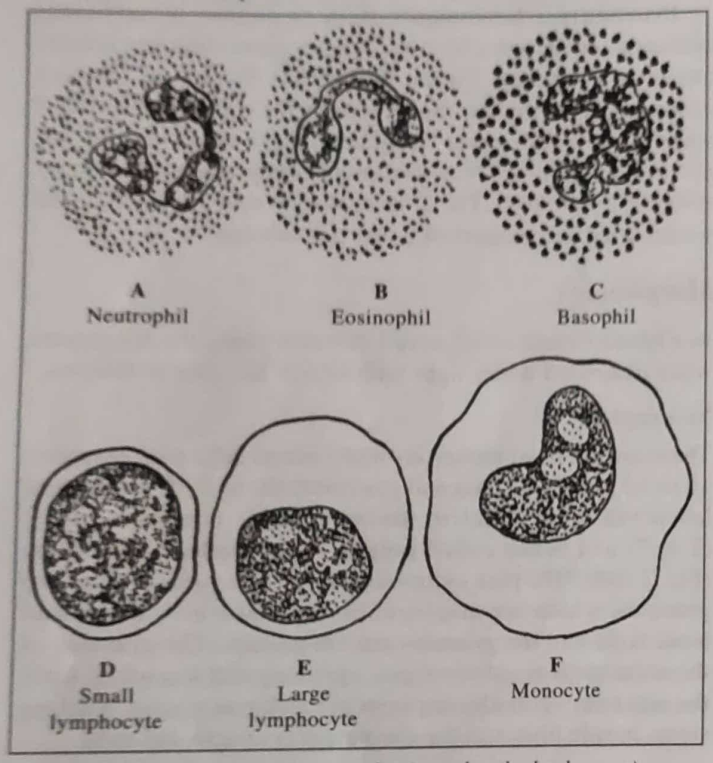



**Fig. 2.19.** *White blood corpuscles (see also the back cover).*

## Development of Leucocytes

Leucocytes develop from the same pluripotent stem cells which gives rise to all blood cells. But the committed stem cells are separate for each type of leucocyte except for the neutrophil and monocyte which develop from the same committed stem cell.

The neutrophils are most numerous and are of shortest life span, hence their rate of formation is also high. The bone marrow contains highest number of neutrophils in different stages of development and also the matured ones. The neutrophils are divided into different **pools** or collections. The **bone marrow pool**. (*i.e.*, the neutrophils present in bone marrow) contains the highest number (90%) and only 3% are in circulation (of them 1.5% are in actual circulation and 1.5% are attached to endothelium *i.e.*, **marginal pool**). The rest 7% are in the **tissue pool**. Monocytes are found in blood and in tissues as RE cells. The lymphocytes are distributed in bone marrow, blood, lymph, lymph nodes and other tissues.

**Neutrophils**

The neutrophils develop from the pluripotent stem cells in the bone marrow through different stages as follows (Fig. 2.20) :

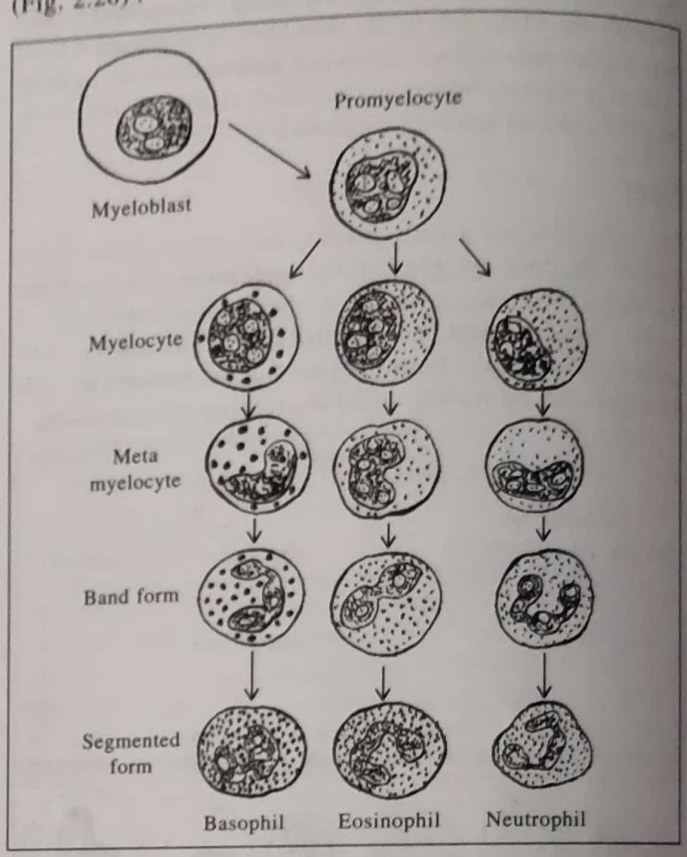



**Fig. 2.20.** *Granulopoiesis.*

**Pluripotent stem cells** : Common to all blood cells.

⇩

**Committed stem cells** : Separate for each type except for the monocyte and the neutrophil (p. 34).

⇩

**CFU-GM** (colony forming unit-granulocyte macrophage) : These are the progenitor cells for the granulocytes and the monocytes.

⇩

**Myeloblasts** : Cells are 10 to 18 μ in diameter, contain blue cytoplasm. The nuclei are large and round with 1 to 5 nucleoli containing fine stippled chromatin. These are actively mitotic and contain no granules in the cytoplasm. These cells can be identified separately in a properly stained bone marrow smear.

⇩

**Promyelocytes** : Cells are slightly larger with primary granules in the cytoplasm (azurophilic graule). Nucleoli decrease in number. Nucleus is round, chromatin has started to condense. Mitosis is seen in this stage.

⇩

**Myelocytes** : The cells are still larger (10 to 18 μ) with indentation in the nucleus which is oval or round, and eccentric.

The chromatin is condensed further. Primary granules are no longer visible. In this stage the secondary or specific granules (for three different granulocytes, *i.e.*, neutrophilic, eosinophilic and basophilic granules) appear. The cytoplasm is still basophilic. Nucleoli are absent. Mitosis is observed in this stage also.

⇩

**Metamyelocytes** : The cells are of 10 to 18 μ in diameter with deeply indented nuclei. The specific granules are plenty, and the cytoplasm is yellow-pink. Mitosis is not seen, chromatin is highly condensed.

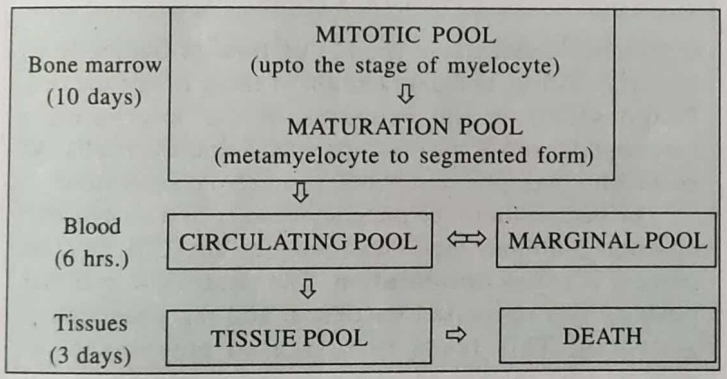
⇩

**Band form** or Juvenile neutrophils : The cells are slightly larger than the matured granulocytes (10 to 15 μ), nucleus becoming horse-shoe shaped or crescent shaped. Specific granules are in plenty. In case of increased production of neutrophils, this form is also released in circulation.

⇩

**Segmented form** or the matured neutrophils : It is the matured stage and morphology is like a matured neutrophil. The cells have acquired all the properties of a neutrophil, *i.e.*, phagocytosis, chemotaxis, etc. and also the surface receptors. The cells pass into the sinusoids to go into circulation.

From the stage of myeloblast to the stage of matured neutrophil it takes about 10 days, of which half is required for development upto the stage of myelocyte (**mitotic pool**) and rest half is spent from metamyelocyte to matured neutrophil (**maturation pool**).




This time period is decreased during an acute infection when more neutrophils are needed. The matured neutrophils stay in the circulation for a short time (half life 6 hours). Then the cells enter into the tissues and die after 3 to 4 days. The neutrophils are destroyed by the RE cells, eliminated via gastrointestinal tract and via respiratory tract secretions. Bone marrow contains 3 days reserve of matured neutrophils.

### Eosinophils and Basophils

Eosinophils and basophils develop with their specific granules in the same way as the neutrophils. Eosinophils, like the neutrophils, also pass to the tissues and are then destroyed, but the fate of the basophils after entering the tissues is not known.

### Monocytes

These are produced from the same committed stem cells from which the neutrophils develop. The stages of development are : stem cell→committed stem cell→progenitor cell→monoblast→promonocyte→monocyte. The monocytes, after a short stay in the circulation pass to the tissues and are then called macrophages. These tissue macrophages may be fixed or mobile. The monocytes acquire special functional characteristics within the tissues, *e.g.*, antigen processing, (p. 48). Genesis of the monocytes is stimulated by GM-CSF and M-CSF.

Monocytes are phagocytic cells, so also the tissue macrophages, hence these are included in the **mononuclear phagocytic system** (p. 53) and that includes the whole RE system. These cells are found in bone marrow, liver, blood, spleen, lymph node, lung, skin, etc.

### Lymphocytes

Like all other blood cells lymphocytes also have their stem cells in bone marrow from where the precursor cells originate. These cells then come to the thymus or other structures and mature further. After this the cells go in circulation and therefrom to the peripheral lymphoid tissues like lymph nodes, where further maturation and division takes place. These lymphocytes re-enter in the circulation through the thoracic duct. So, the genesis of the lymphocytes is totally different from other white cells and these cells recirculate, mature and can divide in the periphery. The life span of a lymphocyte is hence undetermined.

## *Factors regulating Granulopoiesis*

Number of the neutrophils can be increased in peripheral circulation by mobilisation from marrow pool and marginal pool, and due to increased production in bone marrow. Increased production from bone marrow is characterised by a rise in the band form. Granulopoiesis is stimulated by colony stimulating factors (CSF), prostaglandins of E series (PGE), ACTH, cortisol, interleukins, etc. (Most of these are tested in vitro studies and are presumed to be physiologically active). Previously the so called granulocyte inducing factor from the dead neutrophils was believed to play and important role. CSF, interleukins, cortisol, ACTH etc., increase production but epinephrine and exercise increase the number due to mobilisation from the marginal pool in particular. Isoferritin and some prostaglandins lead to inhibition of production. Whatever may be the factor, the neutrophil production is finely regulated and the number in circulation is remarkably fixed in health. The rise in need is also very prompt.

Various factors controlling the development of the monocytes and the lymphocytes are mentioned above.

**COLONY STIMULATING FACTOR (CSF)**

**CSF** is protein in nature and is produced by the T-lymptocytes, macrophages, endothelial cells, monocytes, etc. It is of three different types—colony stimulating factor-granulocyte monocyte (GM-CSF), colony stimulating factor for granulocytes (G-CSF) and colony stimulating factor for monocyte (M-CSF). Each of these induces mitosis and formation of a colony of the respective committed stem cells. These CSFs are presently available in the market and are used to increase WBC production in suitable cases.

## *Functions of the Leucocytes*

(a) **Neutrophils** : These are also called microphages as these cells take up smaller particles than by the macrophages. These are called the first line of defence as these move first to fight the invading bacteria. Neutrophils with their enzymatic armoury are superb killers.

The neutrophils at first bind to the endothelium by neutrophil adhesion molecule after being attracted to it by **selectin** on the endothelium. With the help of the contractile proteins actin, myosin-I, etc., in the cytoskeleton; the neutrophils move by **diapedesis** and pass through the pores in between the endothelial cells. The neutrophils then move to the site invaded by bacteria. This movement is due to chemotactic substances like complement (C5a), leukotrienes and some polypeptides produced by the lymphocytes, mast cells and basophils. This directed movement of the neutrophils through a chemical gradient is called **chemotaxis**.

The opsonised bacteria, *i.e.*, bacteria coated with antibodies etc., then attach to the receptors on the neutrophils which are now activated. The activated neutrophils engulf the bacteria (phagocytosis) and release different enzymes into the phagocytic vesicle. The bacteria are then killed and digested.

The enzyme NADPH oxidase from the cell membrane leads to formation of $O_2^-$ (**super oxide**) and $H_2O_2$ is formed from $O_2^-$ by the action of super oxide dismutase. Both the chemicals are highly toxic and are used for killing the bacteria. The enzyme in the primary granules, *e.g.*, myeloperoxidase, also produce toxic substances like HOCl, HOBr, etc., with the help of $H_2O_2$. The phagocytosed material is digested by the hydrolysing enzymes from the primary granules. (The contents of the secondary or specific granules are also antimicrobial, e.g., lactoferrin, etc). The enzymes from the primary granules also cause inflammation and destroy body tissues when released from the cells (see also phagocytosis and inflammation).

(b) **Eosinophils** : Eosinophils contain a major basic protein (MBP), which damages the larvae of parasites. There is one eosinophilic cation protein which probably neutralises heparin. The aryl sulphatase-B present in eosinophil inactivates the slow reacting substance (SRS) released from mast cells and prevent anaphylaxis (anti-allergic action). Furthermore, histaminase, etc., from eosinophils destroy the substances released from mast cells. Eosinophils also contains peroxidase. These are motile and phagocytic (take up antigen-antibody complexes). Chemotaxis is also shown by the eosinophils.

**Note** : *Anaphylaxis = Exaggerated response to an antigen to which the body was sensitised previously (see p. 52).*

(c) **Basophils** : Basophils like the mast cells have histamine in the granules and when the histamine releasing factors (*e.g.*, IgE-antigen complex) bind, the histamine is released resulting in immediate hypersensitivity reaction like urticaria, rhinitis, anaphylactic reactions, etc., and also have role in inflammation. The basophils also contain heparin, proteases and some other mediators of inflammation. These are motile and phagocytic.

(d) **Lymphocytes** : These cells are involved in the very important defence mechanism, called specific immunity. From the functional view point the lymphocytes are of two types : the B lymphocytes and the T lymphocoytes. The B lymphocytes are responsible for humoral immunity and T lymphocytes are for cell mediated immunity. Lymphokines secreted by some T cells have many important functions. In addition, lymphocytes produce antibodies, interferons, lymphotoxin, and also control the immune mechanism by the helper and suppressor T-cells. The small lymphocytes are believed to be the resting phase and when active these cells become large (see immunity).

(e) **Monocytes** : Monocytes after a short stay in blood enter in the tissues to be converted into macrophages, where these cells take up specific positions and wait for the prey. In the tissues these cells are matured further which enable them to : process antigen for presentation to the lymphocytes, secretion of CSF and intrleukin-1; phagocytosis of micro-organism, dead tissue, etc.; formation of bilirubin and so on. The monokines (interleukin-1) secreted by monocytes stimulate T cells, take part in inflammation, act as pyrogen (fever producing), stimulate formation of acute phase proteins, etc. These cells phagocytose and kill like the neturophils but due to lack of proper enzymes the digesting ability is less than that of the neutrophils.

If necessity arises the monocytes fuse together to form multinuclated giantcell *e.g.*, in tuberculosis.

### PHAGOCYTOSIS

It includes endocytosis (p. 11) of foreign bodies (*e.g.*, bacteria), killing and degradation of these inside the cell. Phagocytosis is the property of the microphages (neutrophils) and of the macrophages. Some other cells like eosinophil, basophil and platelet can also phagocytose.

The bacterium to be phagocytosed is first coated with opsonins [antibody (IgG) and complement (C3), etc], the process is called **opsonisation**. The phagocytic cell then binds to this opsonised bacterium and the phagocyte is activated. This leads to increased movement and endocytosis, release of enzymes and increased oxygen consumption (**respiratory burst**) forming toxic substances like $O_2^-$, $H_2O_2$, HOCl, NO, etc. This kills the bacteria. Subsequently the endocytosed materials are digested.

Phagocytes also release these toxic materials into the interstitial fluid and can damage the microbes before phagocytosis, and also the normal cells of the body in the vicinity.

Phagocytosis of the damaged body tissues and of the old RBCs is also achieved by the macrophages. These cells are called **scavenger cells** as these cells keep the body clean of these debris. Sometimes they cannot digest the ingested material and the foreign body remains inside *e.g.*, carbon particles. Some bacteria like mycobacteria of leprosy escape digestion and grow inside the macrophages. Phagocytosis if fails due to defect in movement or in enzymes, disease results. However, phagocytosis of normal body tissues does not occur.