CHAPTER V

BODY FLUID, LYMPH, LYMPH NODE (LYMPH GLAND), SPLEEN AND RETICULO-ENDOTHELIAL (MACROPHAGE) SYSTEM

BODY FLUID

Water is the most vital and at the same time the most abundant component of the human body. It constitutes about 70 per cent. of the total body weight and within which the major cations like sodium, potassium, calcium, hydrogen, magnesium and anions like chloride, bicarbonate and protein of the body are dissolved. Without water there would be no form of life and it forms the intracellular medium within which metabolic reactions—characteristics of living substances take place. Water-deprivation brings about death more earlier than that of food-deprivation. If water is given instead of food, life may continue for several weeks by the loss of most of the body fat and 50 per cent. of tissue protein.

Total body water and its distribution. Total body water in an average human being, weighing about 70 Kg is 40 litres to 45 litres. In human being it is about 65 per cent. of the body weight in males and about 10 per cent. less in females. But the above values vary mostly with the relative degrees of leanness and fatness of the individual. In lean person, the value is higher than that of in obese person. In general, woman contains more fat than man. The total body water content can be determined most accurately by the **process of desiccation.**

In 1863 Bischoff determined the water content of an executed criminal by the method of desiccation. Mitchell and his associates (1945), Widdowson and his co-workers (1951) have also determined the water content of the human beings by direct method. The average water content in different tissues of the body has been presented in the following table:

Constituents	Weight in Kg	% of body weight	% of H_2O content	H_2O content in litres
Body	75	—	57·68	43·26
Skeleton	11	16	22	2·5
Muscles	30	42	76	23
Fatty tissues	13	18	30	4

It has been observed after studying thoroughly the water content of the body in man as well as in different animal species that the total water content in man is similar to that of in other animals. Besides this, the relative distribution of water in the various organs and tissues is mostly same in man as well as in other species.

% of water in organs in relation to their body weight

Constituents	Man	Dog	Rabbit	Rat
Skeleton	22	32	21	48
Muscles	76	74	75	76
Skin	72	59	71·5	77
Heart	79	78·5	79	78
Lungs	79	79	78	82
Kidneys	83	78	79	77
Brain	75	75	78	77·5
Liver	68	74	76	74
Blood	83	83	83	81
Entire body	63	66	69	65·5

The percentage of water in various tissues and the proportion of total weight of the body which each tissue represents, have been presented in the following table:

Constituents	% of water	% of body weight	Litres of water/70 Kg
Skeleton	22	16·0	2·5
Muscles	76	42·0	22·0
Skin	72	18·0	9·0
Heart	79	0·5	0·3
Lungs	79	0·7	0·4
Kidneys	83	0·4	0·3
Brain	75	2·0	1·1
Liver	68	2·3	1·0
Blood	83	8·0	5·0
Adipose tissue	16	13·0	0·9

The water of the body can be considered to be distributed within two main compartments—the *extracellular* and the *intracellular*. The distribution of body water in different compartments has been presented schematically in Fig. 105. The cell membrane actually provides the boundary in between the extracellular and the intracellular compartments.

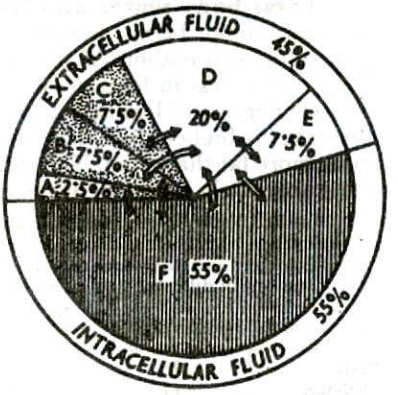


Fig. 105. Schematic representation of the distribution of body water in different compartments. A=transcellular water. B=dense connective tissue and cartilage water. C=plasma water. D=interstitial and lymph water. E=inaccessible bone water. F=intracellular water.

Extracellular compartment. The extracellular fluid compartment is a compartment containing a heterogeneous collections of fluids and not a continuous fluid phase. Edelman and Leibman (1959) have studied thoroughly the distribution pattern of body water by dilution technique and also by tissue analysis. It is postulated that 55 per cent. of water is present in the intracellular space and the rest in the extracellular space. The extracellular fluid phase can be divided into following subcompartments:

(*a*) Transcellular water—2·5 per cent.
(*b*) Dense connective tissue and cartilage water—7·5 per cent.
(*c*) Plasma water that is confined within the vascular system—7·5 per cent.
(*d*) Interstitial fluid and lymph—20 per cent.
(*e*) Inaccessible bone water—7·5 per cent.

The term **transcellular** was introduced by Edelman and associates (1952) in order to designate the extracellular fluid having been separated from the other extracellular fluid by an epithelial membrane. This transcellular fluid includes the (*i*) cerebrospinal fluid, (*ii*) joint or synovial fluid, (*iii*) intra-ocular fluid, (*iv*) fluids of the pleural, pericardial and peritoneal cavity, (*v*) fluids within the ducts of the digestive gland, (*vi*) mucous membranes of the nasorespiratory tract, gastro-intestinal tract and genitalia, and (*vii*) intraluminal fluid of gastro-intestinal system.

Intracellular fluid compartment. It is neither a continuous nor a homogeneous phase and represents the sum of the fluid contents of all the cells of the body. In a cell there are many anatomic subdivisions and for this reason there is a striking difference in water content and ionic composition in between the cytoplasm, nucleus, mitochondria and microsomes of various cell types. This intracellular fluid contains about 30–40% of the body weight and holds about 55% of the whole body water.

Measurement of body fluid compartments. Total body water and extracellular water can be measured by dilution technique with varying degree of precisions. Volume of water present in each compartment cannot be measured directly and thus indirect method—the dilution technique has been adopted for its determination. In this technique the amount of dye used, the final concentration of the dye in the solution is made, are considered for determining the volume of distribution. As for example, if a known quantity of dye —Q is taken and the final concentration is achieved as C, then the volume of distribution V will be: $V=(Q/C)$.

If a beaker of unknown capacity is taken, then its volume can be determined by mixing uniformly a known amount of dye in the volume of water present in the beaker. If the final concentration of the dye is determined by the colorimeter then the volume capacity of the beaker can be determined. Suppose 35 mg of dye has been added and the final concentration that has been achieved to be 0·07 mg per ml, then the volume of the beaker will be, volume $(V)=\frac{\text{quantity of dye}}{\text{concentration of dye}}=35/0{\cdot}07=500$ ml.

The result will be valid only when the drug will be mixed thoroughly.

In vivo determination of body fluid compartments by the dilution principle, certain points are generally considered. The dye injected in the body must be evenly distributed and confined to the body fluid compartment to be measured. If the dye is excreted or lodged in other compartments or metabolised then those amount should be determined and subtracted from the quantity administered. So the equation will be

$$\text{volume of distribution}=\frac{\text{quantity administered}-\text{quantity excreted}}{\text{equilibrium concentration}}.$$

Total body water. Total body water is generally determined by using **antipyrine.** The antipyrine is distributed evenly throughout all the body water compartments and thus diffuses readily across the cell membrane. It is not bound to any intracellular and extracellular compartments. It is also slowly excreted and slowly metabolised. Tritiated water (H_3O or HTO) and deuterium oxide (D_2O)—the two isotopes are often used for the determination of total body water. D_2O and H_3O are distributed in the body exactly like water. These are excreted in the urine, faeces and respiratory gases and also evaporated through the skin. As for example of using the D_2O or HTO for the measurement of total body water, suppose 100 ml of D_2O in isotonic saline solution is injected intravenously to a man of weighing about 75 Kg. After an equilibrium period of 2 hours, the plasma sample is analysed and D_2O concentration is found to be 0·0023 ml per ml. During the period of equilibrium it is found to have a loss (through respiratory, urinary and circulatory pathways) of average 0·5% of the quantity administered. So the volume of distribution will be

$$\text{volume of distribution} = \frac{\text{quantity administered} - \text{quantity excreted}}{\text{equilibrium concentration}}$$

$$= \frac{100 - 0{\cdot}5}{0{\cdot}0023} = \frac{99{\cdot}5}{0{\cdot}0023} = 43{,}260 \text{ ml}$$

or total body water is 43·26 litres.

So the body water will constitute $\frac{43{\cdot}26}{75} \times 100 = 57{\cdot}68\%$.

Extracellular fluid volume. The extracellular fluid volume is not determined so precisely only due to lack of substances that may diffuse to cross the capillary walls readily, enter the cell interstices easily but do not permeate through the cell membrane. Besides this, the substance must be non-toxic and the rate of excretion must be very low in comparison with the rate of distribution in extracellular compartment. There is no such ideal substances available but several substances that have been used are inulin, raffinose, sucrose, mannitol, thiosulphate, radiosulphate, thiocyanate, radiochloride and radiosodium.

Methods for the **measurement of plasma volume** has already been described (page 141).

Measurement of intracellular fluid. There is no direct method has yet been developed. It can be determined by subtracting the value of the extracellular compartment from the value of the total body water.

Tissue fluid and its formation

Tissue fluid is formed from the plasma by process of diffusion and filtration. This fluid occupies the intracellular spaces and forms the connecting link in the transport of nutrition, gases and the metabolic end products between blood capillaries, tissue cells and the lymph. It constitutes the internal environment of the body, which surrounds tissue cells.

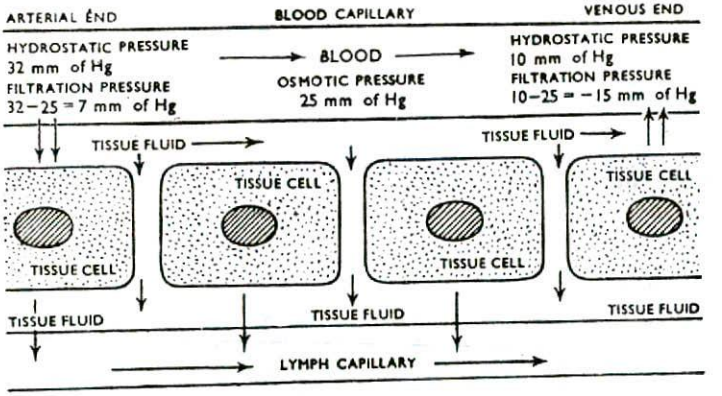


FIG. 106. Schematic representation of intracellular fluid formation.

Tissue fluid is derived from two sources: (1) **blood capillaries,** (2) **tissue activities.** The amount of tissue fluid formed from blood depends upon (*a*) *capillary permeability*, (*b*) the *difference of pressure* between the capillary and the tissue fluid, (*c*) the *difference of colloidal osmotic pressure* of blood and tissue fluid. It is obvious that anything that increases the capillary permeability will increase the amount of tissue fluid formed. Regarding

blood pressure and osmotic pressure, it is known that at the arterial (arteriolar) end of capillaries, the average blood pressure is about 32 mm of Hg and at the venous (venular) end, 10 mm of Hg. The colloidal osmotic pressure at both ends is same (25 mm of Hg on the average). At the arterial end, the net filtration pressure, which is the difference between the two, is 7 mm of Hg towards the tissue (interstitial) fluid. At the venous end, due to fall in blood or hydrostatic pressure, the filtration pressure is −15 mm of Hg to the opposite side, i.e., from tissue fluid to the capillary (FIG. 106).

The amount of tissue fluid formed from the tissue cells, depends upon the degree of metabolic activity of the cells. It should be noted that tissue cells produce water as an end product of metabolism. This metabolic water is added to the already existing tissue fluid. More the degree of activity, more will be the metabolic water formed and consequently the amount of tissue fluid will increase.

Two important exceptions to the capillary pressure are: (*a*) in capillaries of the lungs, hydrostatic pressure about 6 mm of Hg and (*b*) in capillaries of the kidneys, glomerular hydrostatic pressure about 60 to 70 mm of Hg.

If the hydrostatic pressure is increased within capillaries, then it will interfere the return of materials to the lymphatics or capillaries and will result in excess accumulation of tissue fluid (i.e., oedema).

Composition of tissue fluid. It is very difficult to obtain a pure sample of tissue fluid; hence, its exact composition is not known. It is believed that its composition is same as that of lymph, excepting that its protein content is negligible; and as such, its colloidal osmotic pressure is very low. The composition and volume of tissue fluid is regulated by constant interchange with blood and lymph. It has been mentioned above that filtration of tissue fluid takes place at the arterial end of the capillaries. At the venous end of the capillary the blood pressure is very low—about 10 mm of Hg and the colloidal osmotic pressure is much higher. These two factors help in drawing away just as much fluid comes out from the arterial side. But it has been already noted that water content of tissue fluid is derived from two sources—blood and tissue cells. The amount of water that goes out of blood is drawn in again at the venous side of the capillaries. But vascular capillaries cannot draw away the amount of metabolic water formed by the tissue cells. It is for the drainage of this excess water that the lymphatic system has developed. Thus it will be seen that blood and lymph remain as if on two sides of tissue fluid and try to keep it constant in volume and composition by continuous interchange.

Specific gravity of the tissue fluid is about 1·015 to 1·023. It may contain a few erythrocytes. But regarding the white cells, the tissue fluid contains a good number of lymphocytes and a small number of granulocytes. Blood proteins and nutrient contents of it are very low. It does not contain platelets and may also clot but with a very slow process. It contains higher concentration of waste products but glucose, salt and water contents are more or less same as those are present in blood.

Functions of tissue fluid. (1) It constitutes the internal medium in which the tissue cells are bathed. The cells draw in oxygen and nutrition from the tissue fluid and excrete their metabolites into it. Hence, tissue fluid may be regarded as the medium which supplies all the immediate requirements of the cell.

(2) It acts as a great reservoir of water, salts, nutrition, etc. This function is very important. Under any condition, in which the blood volume is increased or diminished, physical forces are set up by which the blood volume is kept constant with the help of the tissue reserve. For example in haemorrhage, the capillary pressure becomes very low and goes below the colloidal osmotic pressure in the capillary which remains same. Due to this higher O. P. in the capillaries, water is drawn in from the tissue spaces, so that blood volume is restored. When water is drawn away from blood, such as due to diuresis, excessive sweating or diarrhoea, blood volume and blood pressure will be lowered, but the plasma proteins will be more concentrated. This will increase the colloidal O. P. of blood. This increased osmotic pressure of plasma and reduced blood pressure will increase the rate of absorption from the tissue fluid, and thus blood volume will be kept constant. On the other hand, when blood volume increases, as for instance, by intravenous injection of large quantities of isotonic saline, fluid will pass out into the tissue spaces due to two causes: (*i*) Saline will dilute the colloids and reduce the colloidal osmotic pressure and at the same time, (*ii*) by increasing the volume of blood will raise the blood pressure and cause more filtration. Both these factors will cause more fluid to run out into the tissue spaces, until blood volume comes back to the original level.

Aggregation of tissue fluid. Swelling or oedema observed sometimes in different parts of the body is due to the aggregation of the tissue fluid. This might result from several factors:

1. Increased capillary permeability resulting from dilated, damaged or inflammated capillary.
2. Increase in the capillary pressure which might be due to changes in posture (in lower extremities it is due to continued standing), obstruction to veins or rise in the venous pressure as observed in the cardiac failure.
3. Blockage of lymphatic nodes or vessels, as a result of inflammation of the node or blockage by very small worms like that of *Filaria*.
4. Loss of the plasma protein whether due to malnutrition or excessive loss resulting from the renal damage, causes decrease in plasma osmotic pressure and excessive aggregation of the tissue fluid.
5. Renal disease causes impairment of excretion of urine and the resulting water retention causes increase in the tissue fluid.
6. Unfamiliar exercise might cause swelling due to accumulation of metabolites.
7. Ingestion of a large amount of salts results in retention of water. Adrenal cortical extract also produces similar effects.

LYMPHATICS AND LYMPH

Descriptive outlines. The lymphatic vessels at the periphery are microscopic blind (closed) end vessels, spoken of **lymphatic capillaries.** These tiny vessels are situated in the intercellular spaces and their walls formed by endothelial cells supported by the fibrous connective tissue (Fig. 107). These capillaries repeatedly join together to form bigger lymphatic vessels, which pass through the lymph nodes, receive more tributaries and gradually increase in size. All the lymph from the body is finally collected into two big channels—the right lymphatic duct and the thoracic duct (or left lymphatic), which open respectively at the right and left subclavian veins. The right lymphatic duct, about 1·25 cm long, drains from the

right forelimb and the right side of the neck and chest (FIG. 108B). The thoracic duct, being about 38–45 cm long and about 4–6 mm in diameter, emerges from the **cisterna (receptaculum) chyli** and also receives the left cervical duct, which collects lymph from the left forelimb, left side of the neck and chest. The cisterna chyli, being situated on the front of the body of the second lumbar vertebra, receives all the lymph coming from two hindlimbs and alimentary canal (FIG. 108A). The lymphatic vessels are provided with valves which help the lymph stream to flow in the direction of the chest. The primary lymphatic vessels that remain in the centre of small intestinal villi are known as **lacteals** and during the course of digestion lacteals are filled with milk-white fluid, **chyle.** The chemical composition of chyle, except for its high fat content, is similar to that of the lymph in other parts of the body. In the central nervous system there are no lymphatics. Here, cerebrospinal fluid takes the place of lymph. Lymphatic capillaries are not also found in the cartilage, spleen, epidermis, internal ear and eyeball. The function of lymphatics is to carry tissue fluid from tissues to veins and the return of water and protein from the interstitial fluid to blood from which they come. And the *function of lacteals* is to help in the absorption of digested food materials generally fats from the intestine.



FIG. 107. Diagram represents the relationship of blood capillaries (containing R.B.C.) to lymph capillaries which are wider and thinner walled and blind (closed) at one end and return of materials to the blood stream. It is also seen that alveoli of a gland lying in areolar connective tissue (stippled area) which is filled with tissue fluid.

Properties of lymph. Lymph should be regarded as modified tissue fluid. Lymph is the clear watery-appearing fluid found in lymphatic vessels and is formed by the passage of substances from blood capillaries into tissue spaces. This process is spoken of **transudation** which involves the processes of diffusion and filtration. A pure sample of lymph can be obtained by inserting a *canula* in the thoracic duct of an animal. Lymph, as collected from thoracic duct during fasting, is transparent, yellowish in colour, faintly alkaline in reaction and clots slowly. Its colloidal osmotic pressure is lower than that of plasma but is believed to be higher than that of the tissue fluid. Its hydrostatic pressure is very low. After a fatty food, the lymph of the thoracic duct appears milky due to the presence of minute droplets of emulsified fat absorbed from the alimentary canal.

Composition of lymph. Microscopic examination of lymph depicts that it contains a large number of leucocytes (mostly lymphocytes) ranging from 500 to 75,000 per cu. mm. No blood platelets present. The composition of the non-cellular part of lymph (fasting) is as follows:

1. Water—94%.
2. Solids—6%

(*a*) **Proteins.** Total protein content is roughly half that of plasma and varies from 2·0–4·5%. It varies according to the part of the body from

which it is collected and also according to the degree of activity of the region. Lymph from the liver contains three times (6%) as much proteins as that coming from the limb (2%). Lymph from the intestine contains protein which is intermediate between these two (4%). Three varieties of

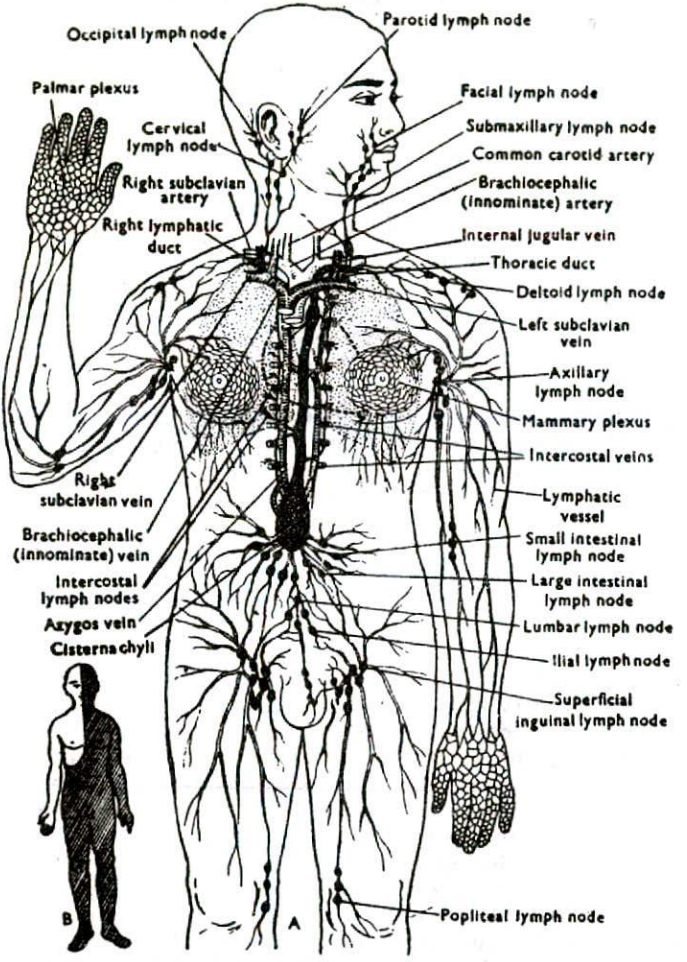


FIG. 108. (A) Anatomical organisation of lymph circulation showing the cisterna (*receptaculum*) chyli, the thoracic duct and other principal lymph vessels along with nodes in the body. (B) In inset the white portion showing the regions of the body from which lymph flows into the right lymphatic duct and the other area (cross-hatched) representing lymph, from all the rest of the body, which enters the general circulation by way of the thoracic duct.

proteins are found—albumin, globulin and fibrinogen. In addition to this, traces of prothrombin are also found. Fibrinogen content is very low. Probably it is due to this, that lymph coagulates very slowly. Albumin is proportionally much more than globulin, as compared with plasma. The

albumin/globulin ratio, which is about 1·5 : 1·0 in plasma, is much higher in the lymph. The protein content of lymph is higher than that of tissue fluid. But since lymph is derived from tissue fluid, this difference is not easily understood. It has been suggested that water is possibly removed from the lymph as it flows along the lymphatics; in this way, the proteins become concentrated. The higher amount of albumin is supposed to be due to its lesser molecular weight and size, and consequently higher rate of diffusion.

(*b*) **Fats.** In fasting condition fat content is low but after a fatty diet it may be 5·0–15%.

(*c*) **Carbohydrates.** Sugar, 132·2 mgm per 100 ml (Dog's plasma contains 123·0 mgm per 100 ml on the average).

(*d*) **Other constituents.** (Expressed in mgm per 100 ml) Urea, 23·5 mgm (plasma, 21·7 mgm); Non-protein nitrogenous substance, 34·8 mgm (plasma, 32·6 mgm); Creatinine, 1·4 mgm (plasma, 1·37 mgm); Chlorides, 711 mgm (plasma, 678 mgm); Total phosphorus, 11·8 mgm (plasma, 22 mgm); Inorganic phosphorus, 5·9 mgm (plasma, 5·6 mgm); Calcium, 9·84 mgm (plasma, 11·7 mgm). Enzymes and antibodies present.

From the above, the difference between plasma and lymph may be noted. Protien, calcium and total phosphorus are lower than in plasma. Chlorides and glucose are distinctly higher. Other constituents are also higher than in plasma to some extent.

Rate of flow. Rate of flow of lymph along the human thoracic duct is from 1·0–1·5 ml per minute. In dogs it is much higher. **Lymphatogogue** is the substance that increases the rate of lymph flow. **Regulation of the rate of lymph flow** depends upon the (*a*) interstitial pressure, (*b*) arterial pulsation, (*c*) intrathoracic pressure and (*d*) muscular massage.

Formation of lymph. Since lymph is formed from tissue fluid, anything that increases the amount of tissue fluid, will increase the rate of lymph formation. Lymph formation depends upon physical factors. There is no vital secretory process involved in it. The following factors are responsible for lymph formation:

1. **Capillary pressure.** If the capillary pressure is raised, the rate of lymph formation increases. This is seen in venous obstruction. [But after some time, the rate slows down due to increased accumulation of fluid in the tissue spaces and the consequent rise of hydrostatic pressure of the tissue fluid.]

2. **Permeability of the capillary wall.** Under any condition, where the permeability of the capillary wall is increased, more tissue fluid will be formed and consequently more lymph. The following factors increase capillary permeability:

(*a*) **Rise in temperature.** Increased temperature of a particular locality increases capillary permeability.

(*b*) **Substances acting directly on the capillary wall.** Peptone, foreign proteins, histamine and extracts of strawberries, crayfish, mussels, leech, etc., exert an injurious effect upon the capillaries and thereby increase their permeability.

(*c*) **Reduced oxygen supply.** Under conditions of oxygen lack lymph flow increases due to higher permeability of the vessels. It acts probably by damaging the capillary endothelium. Anoxia, anaemia, stasis of blood due to vascular congestion, produce such results.

3. **Substances that alter the osmotic pressure.** Anything that reduces the colloidal osmotic pressure of blood will increase the formation of tissue fluid and lymph. Normal or hypotonic saline, when given intravenously, will dilute the plasma colloids and reduce the osmotic pressure. Moreover, blood pressure will be raised. Both these factors will favour formation of tissue fluid and lymph. Hypertonic solutions will exert the same effect in a better way. Hypertonic solutions, introduced in the blood, will draw in more fluid from the tissue spaces at first and will increase the blood volume further. Blood pressure will be raised to a great extent, and plasma colloids will be further diluted. In this way hypertonic solutions will increase the formation of lymph much more than the normal or hypotonic solutions. Solutions of NaCl, glucose, Na_2SO_4, etc., may be used for this purpose.

4. **Increased metabolic activity of an organ.** Increased activity of a particular area increases the flow of lymph in the locality. It is due to (*a*) formation of more metabolites which increase the osmotic pressure of the tissue fluid; (*b*) local vasodilatation and increased capillary pressure and permeability; (*c*) relative anoxia; and (*d*) increased temperature of the locality. The last two also act by increasing the capillary permeability.

5. **Massage and passive movements.** These increase lymphatic flow to some extent just like active muscular contraction.

Circulation of lymph. In the frogs circulation is maintained by rhythmically contracting lymph hearts. But in higher animals no such pump exists and the flow is maintained by a number of physical factors only. They are as follows: (1) **Pressure gradient**—In the tissues, the pressure of lymph (8 to 10 mm of Hg) is higher than that in the thoracic duct (0 to 4 mm of Hg). (2) **Presence of valves** in the lymphatic channels helps to maintain the flow in one direction. (3) **Muscular action**—Muscular (skeletal) contraction, active or passive, compresses the lymphatic vessels and carries the lymph onwards, because the valves within lymphatics prevent backflow. Contraction of the villi helps to pump the chyle from the central lacteals into the basal lymphatics. This lymph is carried up to the cisterna chyli by the help of the intestinal movements. (4) **Respiratory movements**—During inspiration, due to the descent of the diaphragm intrathoracic pressure falls, which thereby sucks in lymph into the thorax. Moreover, intra-abdominal pressure rises. This compresses the cisterna chyli, so that lymph flow through the thoracic duct is increased. These pressure changes during inspiration are very important factors in maintaining lymphatic circulation.

Functions of lymph. (1) **Nutritive**—It supplies nutrition and oxygen to those parts where blood cannot reach. (2) **Drainage**—It drains away excess tissue fluid and the metabolites and in this way tries to maintain the volume and composition of tissue fluid constant. (3) **Transmission of proteins**—Lymph returns proteins to the blood from the tissue spaces. (4) **Absorption of fats**—Fats from the intestine are also absorbed through the lymphatics. (5) **Defensive**—The lymphocytes and monocytes of lymph act as defensive cells of the body. The lymphatics also remove bacteria from tissues.

LYMPH NODE (LYMPH GLAND)

Lymph node is small, oval or bean-shaped body. It is a collection of lymphoid tissue enclosed in a connective tissue *capsule* and lying along

the lymphatic stream. From the capsule, strands of tissue pass into the substance of the node and are known as the connective tissue *trabeculae*. In the human lymphatic nodes the trabeculae are ill-defined. They undergo repeated branching and along with the reticular tissue of the node, compose of ill-defined sinuses, the meshes of which are filled up with lymphoid cells in various stages of development. *Histologically*, the node can be divided into an outer part—the *cortex*, and an inner part—the *medulla* (Fig. 109).

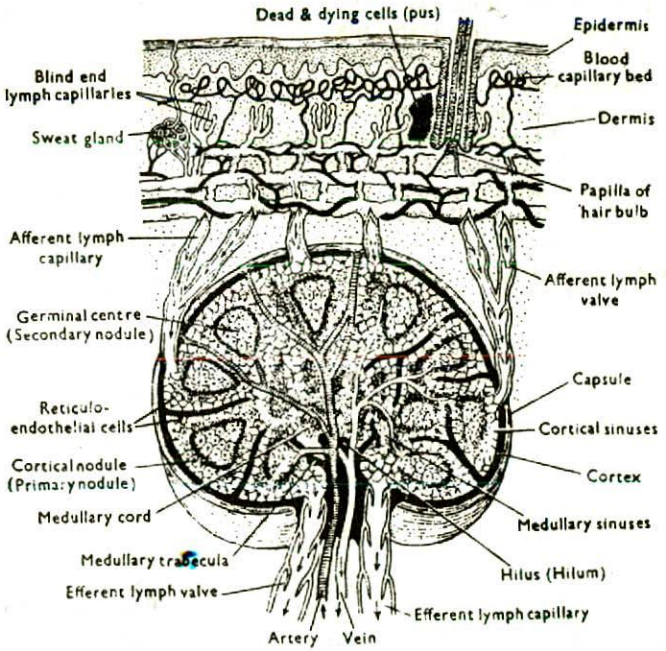


Fig. 109. Diagrammatic representation of the structure of a lymph node with a section of skin. Efferent lymph capillaries leave the lymph node at the hilus (hilum). The artery and the vein enter and leave at the hilus. Afferent valved lymphatic vessels bring lymph to the lymph node. Near a hair follicle, dead and dying cells (pus) surrounded by bacteria which are engulfed and carried by leucocytes through afferent lymphatic vessels to the lymph node where most of the bacteria are filtered and destroyed by enzymatic action.

Cortex. The lymphoid tissues remain scattered throughout the node but in the cortex they are found in specially collected islands—the *lymphoid nodules* or *follicles* which vary from 0·35 to 1·0 mm in diameter. These nodules remain arranged parallel to the surface, sometimes two or three layers deep. Each lymph nodule is pierced by a small blood vessel and shows a less dense area at its centre, which takes a lighter stain. Surrounding this lighter zone there is a wider area packed with lymphocytes. The central zone (lighter) is called the *germinal centre* or *secondary nodule*, whereas the peripheral area is called the *cortical nodule* or *primary nodule*. These lymph nodules are separated from the trabeculae and capsule by blood sinuses. The germinal centre is regarded as the area in which new lymphocytes are

being formed by active cell division. Numerous macrophages are found in the germinal centre under certain pathological conditions and for this reason this central zone is called as **reaction centre.**

Medulla. The medulla of the lymph node is much less dense than the cortex and is devoid of lymphatic nodules. It consists of scattered lymph cells, different varieties of reticulo-endothelial cells and sometimes a few multinucleated giant cells (Fig. 110). The cytological arrangement of the trabeculae in the medulla is irregular and frequently anastomosing *lymph cords* of it run through the communicating divisions surrounded by the meshes of the trabeculae. *Lymph sinuses* of the medulla separate the trabeculae and lymph cords. In some animals, such as ox, large pigmented branching cells are found in the medulla, which give it a darker colour.

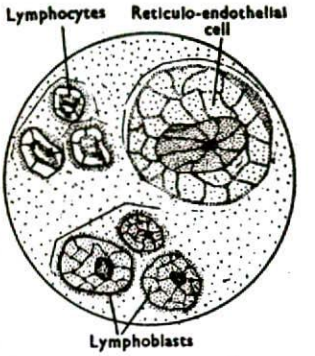


Fig. 110. Diagrammatic representation of the cells which are found in a lymph node.

Hilus (Hilum). At one side of the node there is a depression, known as the *hilus* where the capsule is thickened. Here the cortical part becomes very much thin and the medulla comes to the surface. At the hilus the node is pierced by three vessels—an artery, a vein, and the efferent lymph channel. The artery after entering the node breaks up into numerous arterioles which proceed at first along the trabeculae and then each arteriole breaks up into a bunch of capillaries which become surrounded by masses of lymphatic cells. The capillaries are then collected into venules, which join repeatedly to form the main vein that leaves the node through the hilus. Thus the blood vessels of the lymph node enter and leave the node at the hilus. But this is not the case with the lymphatics. The afferent lymphatic vessels enter the node through the whole of the outer surface by piercing the fibrous capsule, and have valves opening toward the node. These afferent vessels are generally thinner and more numerous than the efferent vessels. After entering the node the lymph vessels undergo repeated branching, the branches proceed along the trabecular framework and after traversing and draining the whole of the lymph node they converge to form the main efferent lymph channel, which passes out of the node at the hilus, and possess valves opening outward from the node. *Thus the afferent lymph vessels enter the capsule in a scattered distribution and penetrate the subscapular sinuses. Efferent vessels leave the node through the hilus* (Fig. 109).

The nerves supplying the lymph nodes are non-medullated autonomic fibres, which are distributed to the plain muscles in the blood vessels, the capsule and the trabeculae.

Haemal lymph node. In many animals some lymph nodes are found to possess red colour, due to the presence of blood in them. In man they are found in the retroperitoneal tissues and also in the mediastinum. In these nodes some of the so-called lymphatic channels contain blood, while the rest of the nodes possesses the same structure as the typical lymph node. Spleen may be regarded as the modified haemal lymph (haemolymph) node.

Function of lymph nodes. 1. They produce and supply lymphocytes to the blood and as a supportive function the trabeculae carry blood vessels which supply the node.

2. They make screening of the lymph by means of phagocytic activity.
3. They serve a great defensive role against bacterial infections (FIG. 109).
4. They temporarily stop the spread of cancer cells as those cells have to penetrate through the lymph vessels to the lymph nodes from where they spread in the body.
5. They act as mechanical filters to resist the entrance of poisonous substances into circulation.
6. They carry out immunological responses. They help in elaboration of antibodies and in the development of immunity.
7. Lymph nodes produce γ-globulin.

SPLEEN

Spleen (*lien*) is the largest lymphoid tissue in the body and specialised, bean-shaped organ for filtering blood. It is a highly vascular haemopoietic organ situated in the left hypochondrium directly beneath the diaphragm, above the left kidney and descending colon, behind the fundus of the stomach and weighing about 150 gms in adult. It also plays an important role in the metabolism and defense mechanism of the body. There is no afferent lymphatic vessels.

Structure of spleen. Histological structure (FIG. 111) of a spleen shows (*a*) capsule with its outer covering the peritoneum, (*b*) trabeculae with blood vessels or without blood vessels, (*c*) hilus (hilum), (*d*) white pulp scattered throughout the red pulp, (*e*) red pulp, (*f*) reticular meshwork, (*g*) blood vessels.

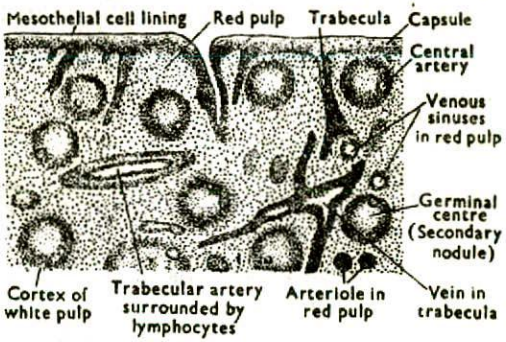


FIG. 111. Histological structure of spleen (diagrammatic).

Spleen is covered by a connective tissue capsule which is again enveloped by a serous membrane, the peritoneum. The peritoneum is closely adherent to the outside of the capsule. The capsule is deeply indented at the medial aspect of the organ and this indentation is known as **hilus** (*hilum*) of the spleen. Blood vessels, lymphatics and nerves pass through the hilus. From the inner surface of the capsule and from the hilus many trabeculae radiate into the substance of the spleen and subdivide or delineate the organ into many communicating compartments or *lobules*. Each lobule is supplied with blood vessels that run along with the trabeculae. The lobules are not distinct because these are not completely surrounded by trabeculae.

Splenic pulp. The parenchymal tissue which is enclosed within the capsule is the splenic pulp. The splenic pulp is of two distinct types: (*a*) *white pulp* and (*b*) *red pulp*. The white pulp is composed of typical lymphatic tissue whereas the red pulp is composed of an atypical lymphatic tissue.

(*a*) **White pulp.** In a freshly sectioned spleen the white pulps are seen scattered all throughout the red pulp as *grey patches*. These grey patches at

early periods were described as *Malpighian bodies*. But for confusion these terminology has been avoided in the literature in case of spleen and instead, white pulp has been used.

The white pulp is the accumulation of lymphatic tissue surrounding a major arterial vessels of the spleen. This lymphatic tissue is comprised of *lymphocytes, plasma cells, macrophages* or other free cells lying in the meshwork of reticular fibres.

In the white pulp there are two distinct components are seen. As the arteries leave the trabeculae and enter the splenic parenchyma, the vessels lose its adventitia and are replaced by reticular tissue followed by invasion with lymphocyte. This constitutes the **periarterial lymphatic sheath** of the white pulp. At various points along the course of the vessels where the infiltration of lymphocytes is greater, it forms spherical or ovoid nodules which are called as **splenic nodules** of white pulp. The splenic nodules may have *typical germinal centres*.

(*b*) **Red pulp.** It is a modified lymphatic tissue and is mostly infiltrated with cells of the circulating blood. It consists of two components: (*i*) *splenic sinuses* or *sinusoids* and (*ii*) *splenic cords*.

Splenic sinuses are long vascular channels having 35 to 40 μ in diameter. They may have an irregular course and may vary in diameter. They extend throughout the red pulp.

Splenic (or **Billroth**) **cords** appear as continuous partitions in between the sinuses. These cellular cords ultimately form a spongy network of modified lymphatic tissue that gradually merges into the white pulp. In *mammalian embryos* the red pulp contains myelocytes, erythroblasts and also megakaryocytes. These types of cells are not present in adult spleen except in certain pathological conditions.

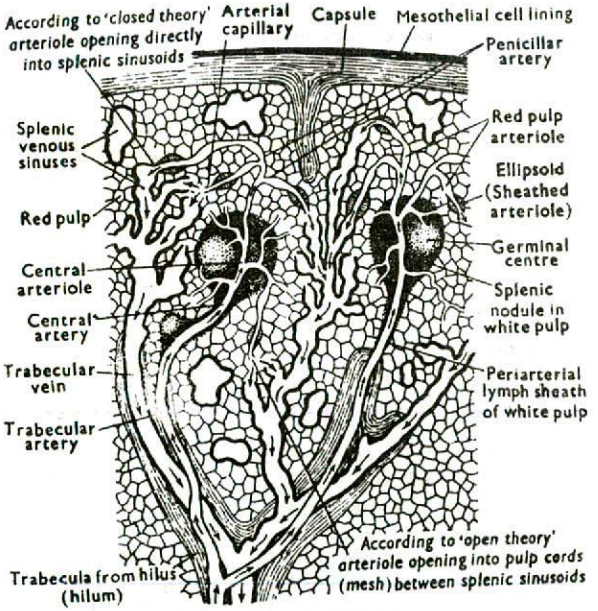


FIG. 112. Anatomy of circulation in a splenic lobule showing both *closed* circulation and *open* circulation (diagrammatic).

Marginal zone. It is the junctional region in between the white pulp and the red pulp, and consists of a meshwork of branched reticular cells in association with extracellular reticulum, into which many arterial vessels open.

Blood vessels and nature of blood circulation. At the hilus of the

spleen, arteries enter and divide into several trabecular branches. The trabecular (interlobular) branches pass along the trabeculae and after coursing for a certain distance the arteries may enter the splenic parenchyma. After entering the splenic parenchyma the artery loses its adventitia and takes the character of reticular tissue and afterwards become infiltrated with lymphocyte. In this way the vessels are ensheathed with lymphocyte constituting the periarterial lymphatic sheath. Along the blood vessels and at various points there are greater infiltration of lymphocyte forming the so-called splenic nodule of the white pulp.

Thus after leaving the trabeculae as central artery or arteriole passes through the white pulp where it gives off several branches. From here arterioles enter the red pulp. Here the arteriole is subdivided into several branches and as these vessels lie close like a brush, are called **penicillar vessels.** These penicillar vessels have three distinct successive components: The first long portion having thin smooth muscle is known as **pulp arteriole;** the middle one having thick sheath is known as **sheathed arteriole** or **ellipsoid** or **Schweigger-Seidel sheath,** the terminal one is **arterial capillaries** one to two in number. The arterial capillary ultimately ends in the splenic venous sinuses.

There are two theories regarding the opening of the arterial capillaries into sinuses: According to **open theory** the arterial capillary opens into the pulp reticulum or pulp cord and then blood gradually filters into the splenic venous sinuses. According to **closed theory** the arterial capillary directly opens into the splenic venous sinuses.

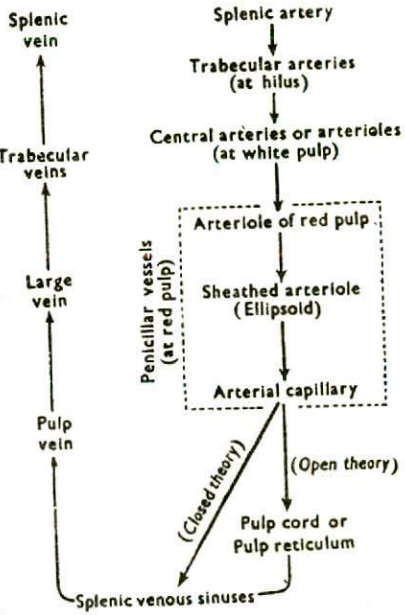


FIG. 113. Schematic representation of the anatomy of circulation in spleen.

The splenic venous sinuses have got irregular anastomosing tunnels throughout the red pulp. The wall of the sinus is composed of specialised reticular cells of phagocytic type and belongs to reticulo-endothelial (macrophage) system.

The blood from the splenic venous sinuses empty into the *pulp vein* which combines to form the large veins and ultimately blood return in the *trabecular vein.* In the hilus many trabecular veins join to form *splenic veins* and as such leave the spleen (FIGS. 112 & 113).

Functions of spleen

I. **Formation of blood.** In the embryo, the spleen functions as a haemopoietic organ of some importance but in *normal* adult life it never functions in the formation of R.B.C. In some pathological conditions, the spleen may undergo myeloid metaplasia and a large number of erythroblasts, megakaryocytes and myelocytes appear in the tissue.

II. **Blood destruction.** The old red cells and white cells are destroyed by the R. E. cells of the spleen. Spleen has got some influence on the formation of platelets. In thrombocytopenic purpura, splenectomy causes increase in the number of platelets in the blood. Haemoglobin breaks down and formation of bilirubin also takes place in the spleen (Biligenic function). Thus, in the post-natal life, spleen acts as a filter, which removes the old useless cells and allows only the young active cells to pass into the circulation.

III. **Reservoir of blood.** Spleen acts as a great reservoir of blood. It is observed that the spleen may release about 150 ml of blood (mainly erythrocytes) to circulation. In cat, as much as one-sixth of the total blood volume or one-third of the total red cells, may remain in the spleen. In anoxic conditions (such as in haemorrhage, asphyxia, severe muscular exercise, high altitude, etc.), spleen contracts and sends out the stored blood into the general circulation. Adrenaline injections also have the same effect. All these conditions stimulate the sympathetic system, which causes contraction of spleen. In carbon monoxide (CO) poisoning this is a great help. Due to the presence of slow circulation in the spleen, CO cannot saturate all the splenic red cells. When spleen contracts these healthy red cells are turned out into the circulation and are used for carrying oxygen.

IV. **Relation to storage and metabolism.** 1. *Pigment metabolism.* Haemoglobin is broken down into haem and globin, the haem is further split into iron and pigment **haematoidin,** which becomes bilirubin of the plasma.

2. *Iron metabolism.* The iron that is liberated from haemoglobin is at first stored in the splenic pulp cells. Then it is gradually transferred to other places, being carried by the monocytes and the detached R. E. cells. It is specially taken to the liver for storage and to the bone marrow for further haemoglobin synthesis. After splenectomy this storage function suffers and more iron is lost. In abnormal condition when there is marked red cell destruction, iron and iron-containing pigment **haemosiderin** (storage form) are deposited in the spleen.

V. **Defensive action.** (*a*) Many **pyroninophil cells** (probably plasma cells) are found in the splenic red pulp and hence the spleen is a chief site of immune body formation. Splenectomised animals cannot be immunised against tetanus toxin. Such animals easily succumb under intercurrent infection.

(*b*) The R. E. cells engulf bacteria, parasites like those of Leishman-Donovan (L.-D.) bodies in *Kala-azar* and foreign particles.

(*c*) The pulp cells unite with certain toxins, specially of diphtheria and remove them from general circulation.

(*d*) The lymphoid cells of spleen also react against infections.

VI. **Manufactures haemolysin.** 1. When red cells of one species be repeatedly injected in another, a specific haemolysin is formed in the spleen.

2. Clinical cases have been reported, where spleen was found to elaborate a haemolysin—causing severe haemolytic anaemia in the patients. After splenectomy such cases were cured.

From such evidence it is thought that spleen is either normally concerned with actual haemolysis of old red cells or prepares them for final haemolysis.

Effects of Splenectomy

1. Moderate degree of Hypochromic anaemia, being maximum after about one and a half months and recovering slowly during the following three to four months. The red cell count seldom falls below 3 million per cu. mm and the haemoglobin below 55%. Immature nucleated red cells appear in the circulation. Since spleen normally destroys red cells, anaemia after splenectomy is the exact opposite to one's expectation. This is one of the reasons which led to the suggestion that spleen manufactured something which normally stimulates the activity of the bone marrow. The recovery from this anaemia is due to the compensatory overgrowth of bone marrow.
2. The animals cannot withstand severe anoxia.
3. There is diminished resistance against infections.
4. The leucocyte count increases, and may go up to 20,000–40,000 per cu. mm. This rise is chiefly due to the increase in the number of polymorphs. Later on, lymphocytes and in some cases eosinophils may also rise. This may be explained from the fact that in the absence of the spleen these cells fail to be destroyed.

Regarding the defensive action of spleen much of it can be taken over by the hypergrowth and hyperactivity of cells of the R. E. system elsewhere. Enlargement of spleen occurs in certain pathological conditions (Gaucher's and Banti's diseases, certain anaemias). Furthermore enlargement also accompanies malaria, Hodgkin's disease, leukaemia. Splenectomy is adopted as a treatment for certain pathological conditions.

RETICULO-ENDOTHELIAL (MACROPHAGE) SYSTEM

It has been known for many years that if a *vital dye* (viz., Trypan blue, Lithium carmine, etc.) be injected into a living animal, certain cells engulf the dye particles, whereas others do not.* It is further seen that these *phagocytic cells* generally occupy the endothelial lining and the reticular spaces of the connective tissues. From this distribution, Aschoff and Landou (1913) have introduced the name of **reticulo-endothelial (R. E.) system** to these cells. These cells develop from the mesenchyme. It is to be noted that while most of the cell in our body have specialised for special functions, the cells of this system have resisted further differentiation and have retained to a very large extent, their primitive embryonic characteristics. For this reason, it will be seen that these cells are endowed with remarkable powers of altering their shape, size, mode of living and thereby undergo differentiation into other types of tissue cells (*mutation*).

Classification, varieties and distribution of R. E. cells (Fig. 114). The cells of the R. E. system may be divided into two large groups: (I) The fixed cells. (II) The wandering cells.

I. Fixed Reticulo-Endothelial Cells

They include the following varieties: (1) The **tissue histiocytes**—They are found in the connective tissues and loose areolar tissue of the serous membranes, viz., omentum, pleura, etc. Although they are generally fixed, yet, under suitable stimulus (such as inflammation), these cells may become actively motile. (2) The **reticulum cells** of the spleen, lymphatic

* This method is generally adopted for staining the cells of this system. The **vital dye** is intravenously injected, the animal is then killed and the various tissues and organs examined under microscope.

node and bone marrow. They remain in the reticular spaces of these organs. They are large cells and remain joined to one another by long branching processes. Under suitable stimulus they may be actively motile. (3) The **endothelial cells**—They are present in the lining membrane of the blood sinuses of spleen, bone marrow, adrenal cortex, pituitary, liver, etc. In the liver they appear as large, flat, stellate cells lining the blood capillaries and are known as the **Kupffer cells.** (4) **Microglia**—These are small cells found in the central nervous system.

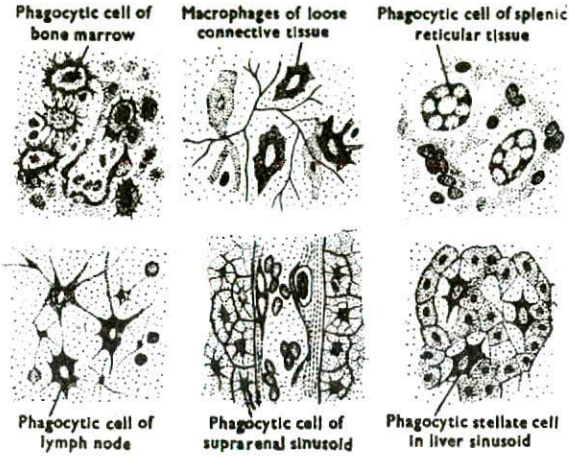


FIG. 114. Diagrammatic representation of different types of cells in reticulo-endothelial (macrophage) system.

II. WANDERING RETICULO-ENDOTHELIAL CELLS

They comprise the following varieties: (1) The **wandering histiocytes**—These are large wandering cells found in the omentum, splenic pulp, lymph nodes, bone marrow, etc. Due to their large size they are also called *macrophages*. Sometimes they may contain many nuclei, for instance, osteoclasts and megakaryocytes of the bone marrow. In addition to these the fixed tissue histiocytes, described above, may become wandering. (2) The **wandering R. E. cells of the blood stream**—They are of two varieties: (*a*) The *normal monocytes* of blood. (*b*) *Abnormal foreign cells* which generally remain in the tissues but enter the blood stream under the influence of some pathological stimulus. Such cells are found in Leukaemia, bacterial endocarditis, etc.

Functions of the Reticulo-endothelial system

1. **Phagocytosis.** This is the main characteristic property of this system of cells. They engulf foreign particles, bacteria and parasites and in this way, act as a great defensive mechanism.

2. **Formation of antibodies.** The antitoxic and antibacterial bodies are manufactured by this system. This is also a great protective mechanism.

3. **Formation of R. B. C.** Red blood cells develop from the R. E. cells. Turnbull holds that they develop from the extravascular R. E. cells (*haemocytoblasts*).

4. **Formation of leucocytes.** The granulocytes, lymphocytes and monocytes—are all derived from the R. E. system.

5. **Destruction of red cells and white cells.** The senile red cells and white cells are engulfed and destroyed by the R. E. cells. In adult life, it takes place chiefly in the spleen and liver.

6. **Scavengers of tissue debris and bacteria in infectious processes.** The R. E. cells help in the scavenging process.

7. **Formation of bile pigments.** The R. E. cells manufacture bilirubin from haemoglobin (*vide* Fate of the R.B.C.—pages 152-53).

8. **Storage function.** A large amount of lipids, cholesterol and iron are stored in the R. E. cells.

9. **Manufacture of plasma proteins.** Serum globulin and certain other plasma proteins are manufactured, to some extent, by the R. E. cells.

10. **Formation of tissue cells.** Since the cells of this system are undifferentiated, they can be converted into ordinary connective tissue cells, such as fibroblasts, under suitable stimulus. This is seen during repair stage of inflammatory process.