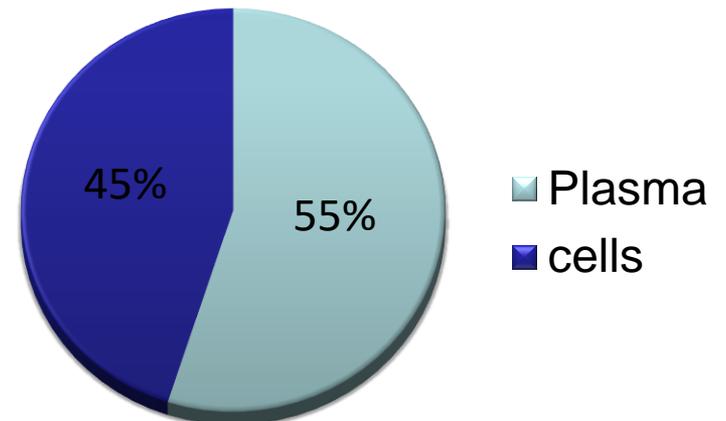


Blood

Mr. Bhartendu Vimal
Guest Faculty – Assistant Professor
College of Fisheries, Kishanganj
BASU, Patna

Introduction

- *Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients and oxygen and transports waste products away from those same cells.*
- *55% plasma*
- *45% blood cells*



Cont...

- *Blood is considered a specialized form of connective tissue.*
- *The volume of blood in adult person of 70kg is 5.5 liters.*

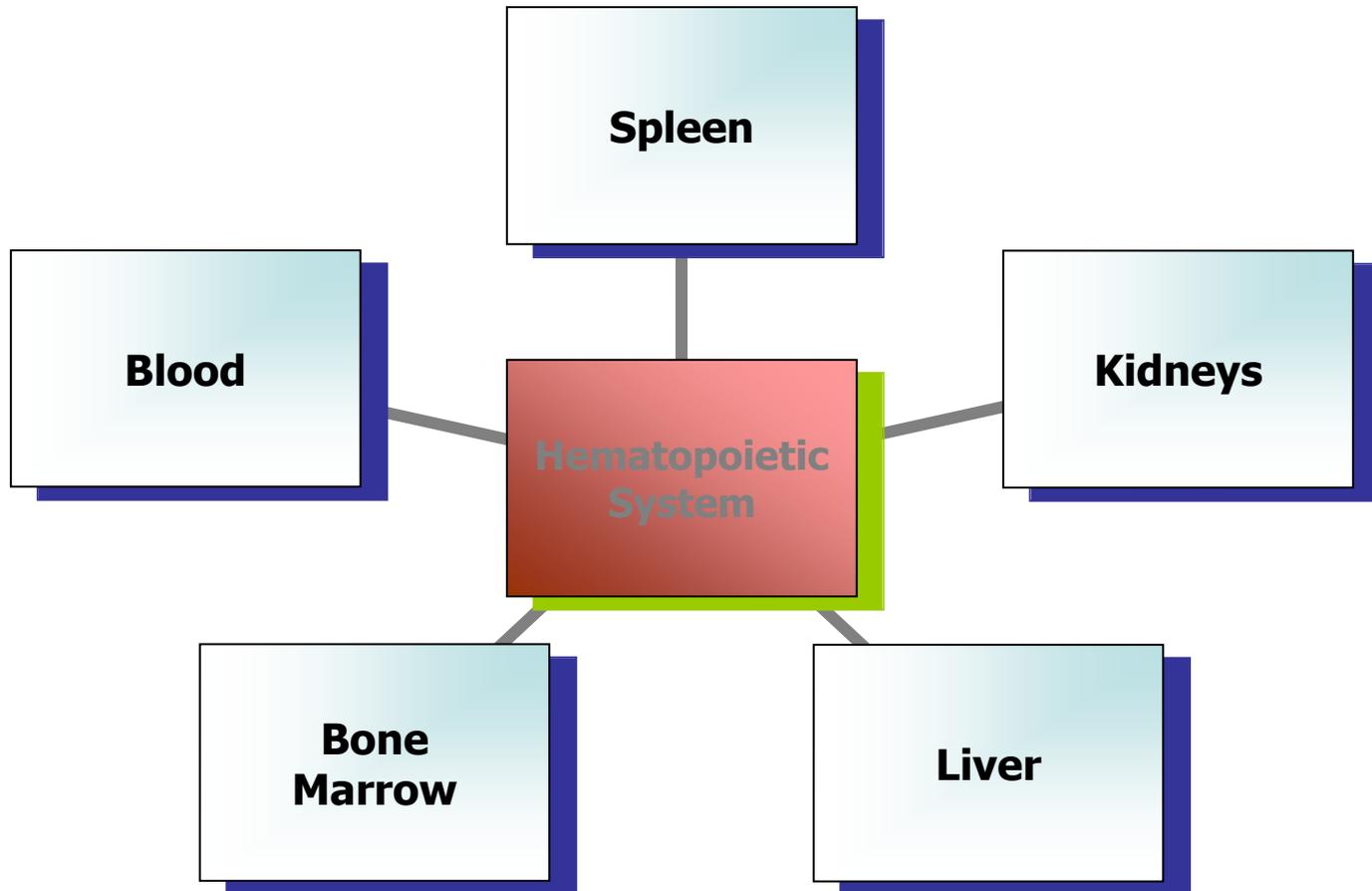
In fish blood volume is about 5% of the body weight.

- *It is slightly alkaline fluid having pH 7.4.*

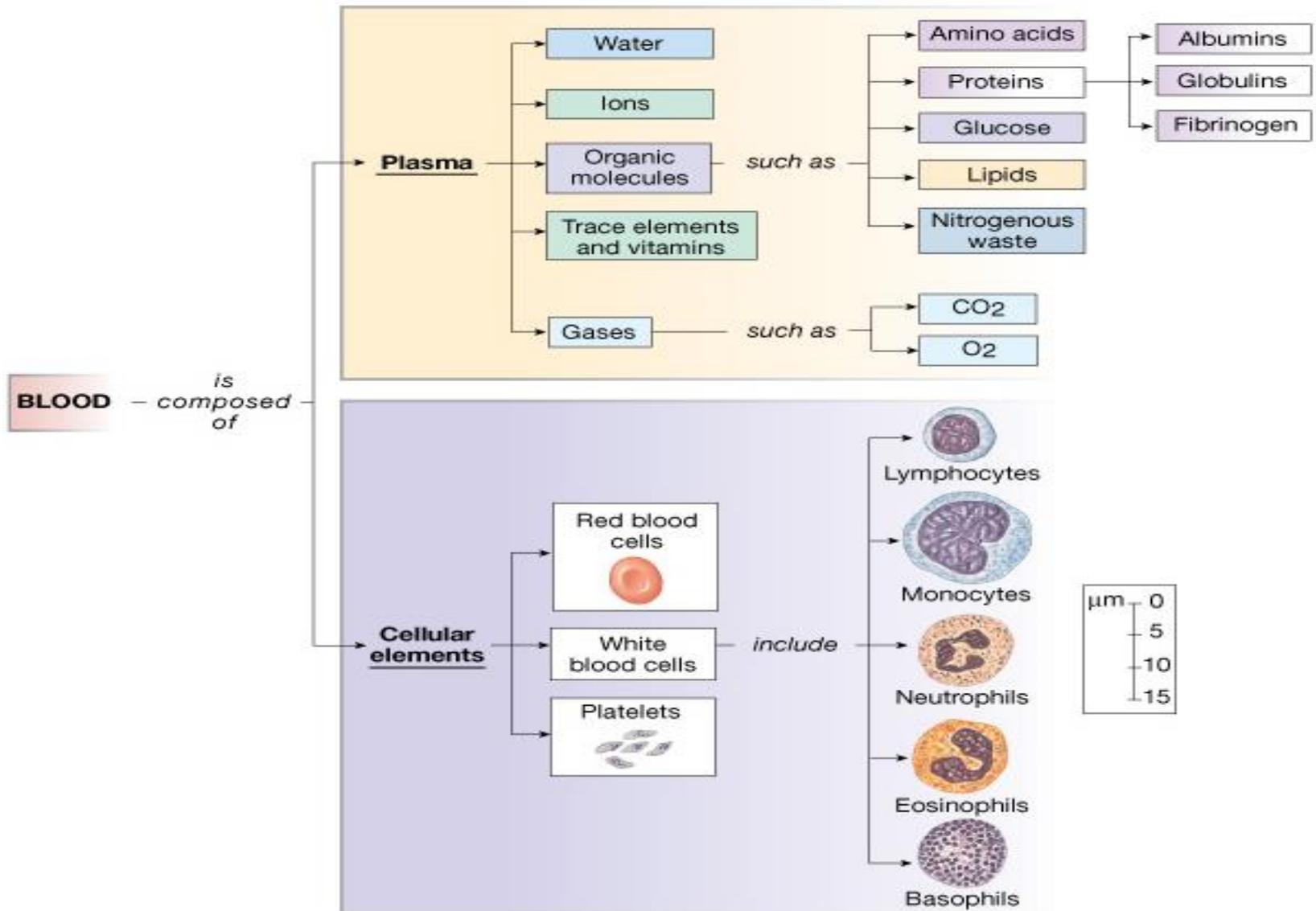
Functions:

- ❖ *Supply of oxygen to tissues.*
- ❖ *Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids)).*
- ❖ *Removal of waste such as carbon dioxide, urea, and lactic acid.*
- ❖ *Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies.*
- ❖ *Coagulation, which is one part of the body's self-repair mechanism (the act of blood clotting when you get cut to stop the bleeding.).*
- ❖ *Messenger functions, including the transport of hormones and the signaling of tissue damage.*
- ❖ *Regulation of body pH (the normal pH of blood is in the range of 7.35–7.45).*
- ❖ *Regulation of core body temperature.*

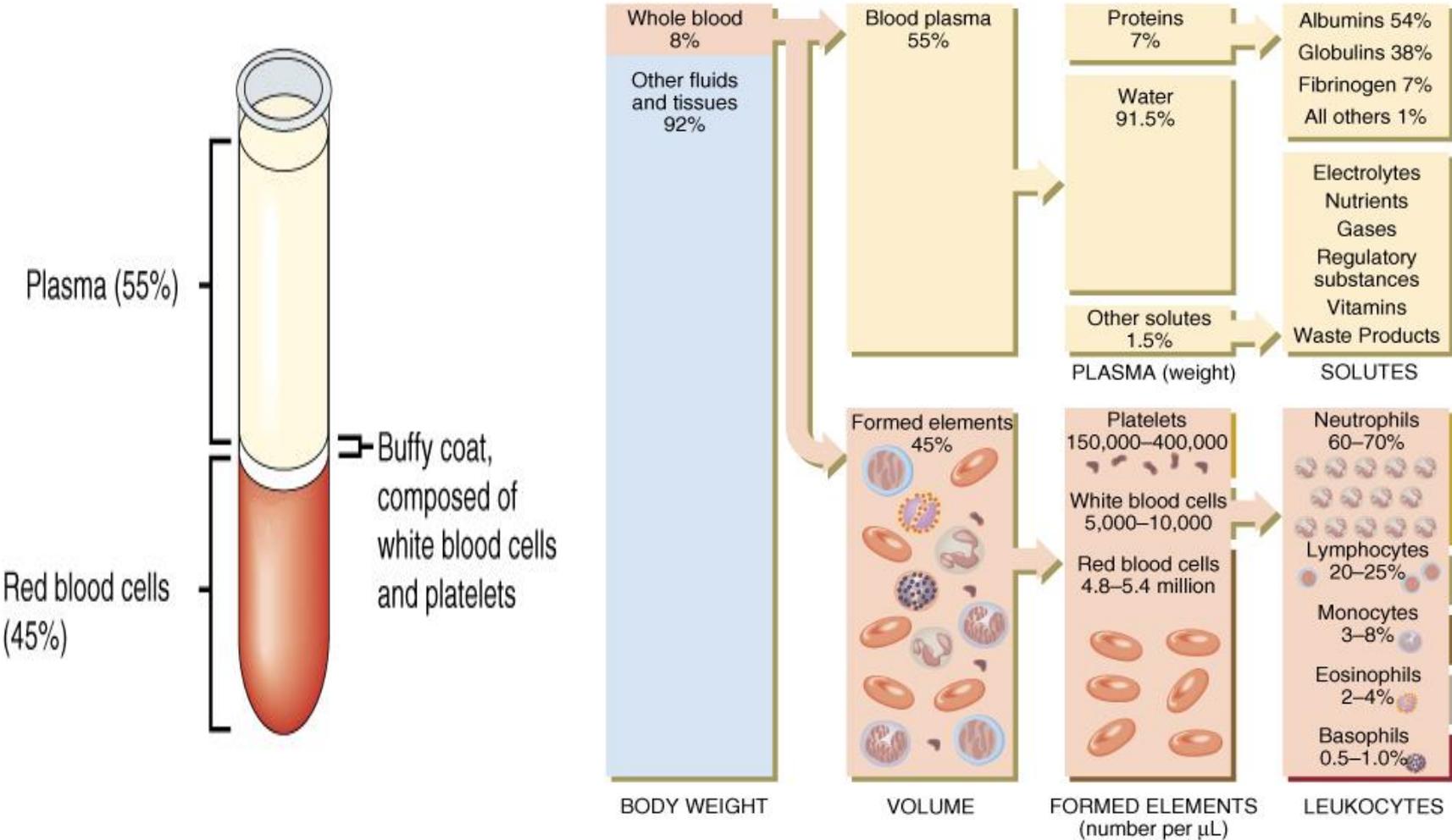
Hemotopoeietic system



Cellular Components



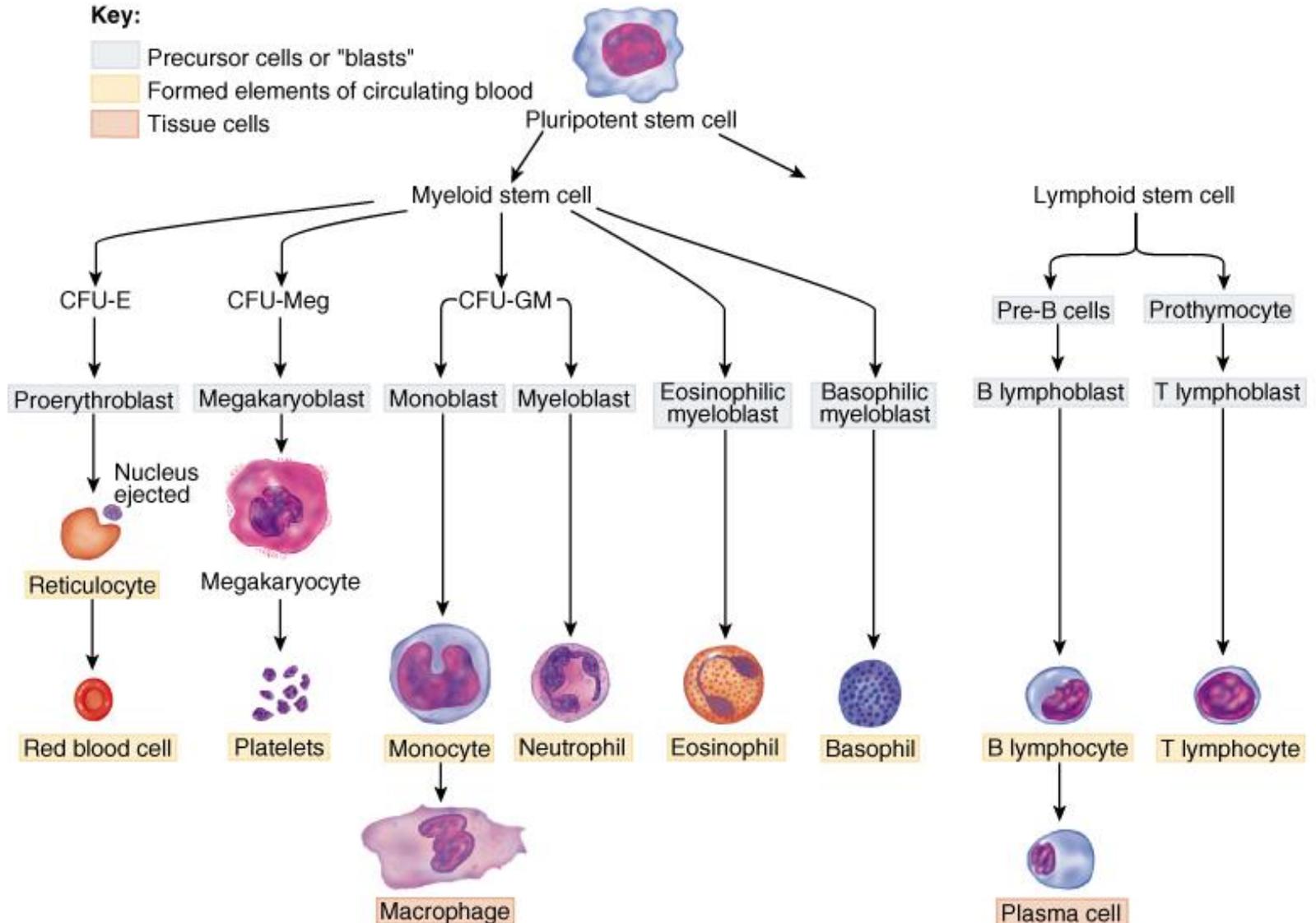
Components of Blood



Hematopoiesis

Key:

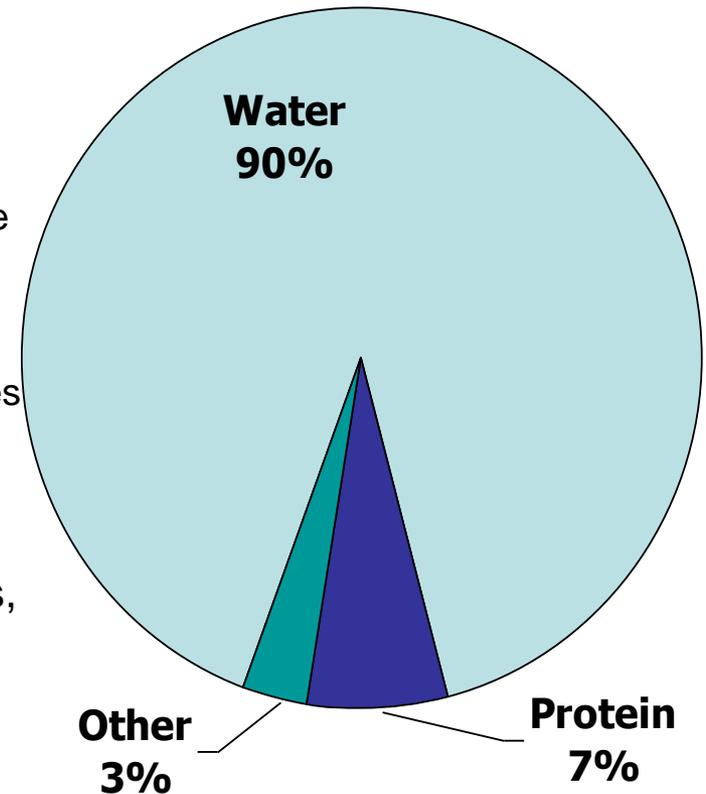
- Precursor cells or "blasts"
- Formed elements of circulating blood
- Tissue cells



Components of Blood

- Over 90% water
- 7% plasma proteins
 - created in liver
 - confined to bloodstream
 - albumin
 - maintain blood osmotic pressure
 - globulins (immunoglobulins)
 - antibodies bind to foreign substances called antigens
 - form antigen-antibody complexes
 - fibrinogen
 - for clotting
- 3% other substances
 - electrolytes, nutrients, hormones, gases, waste products

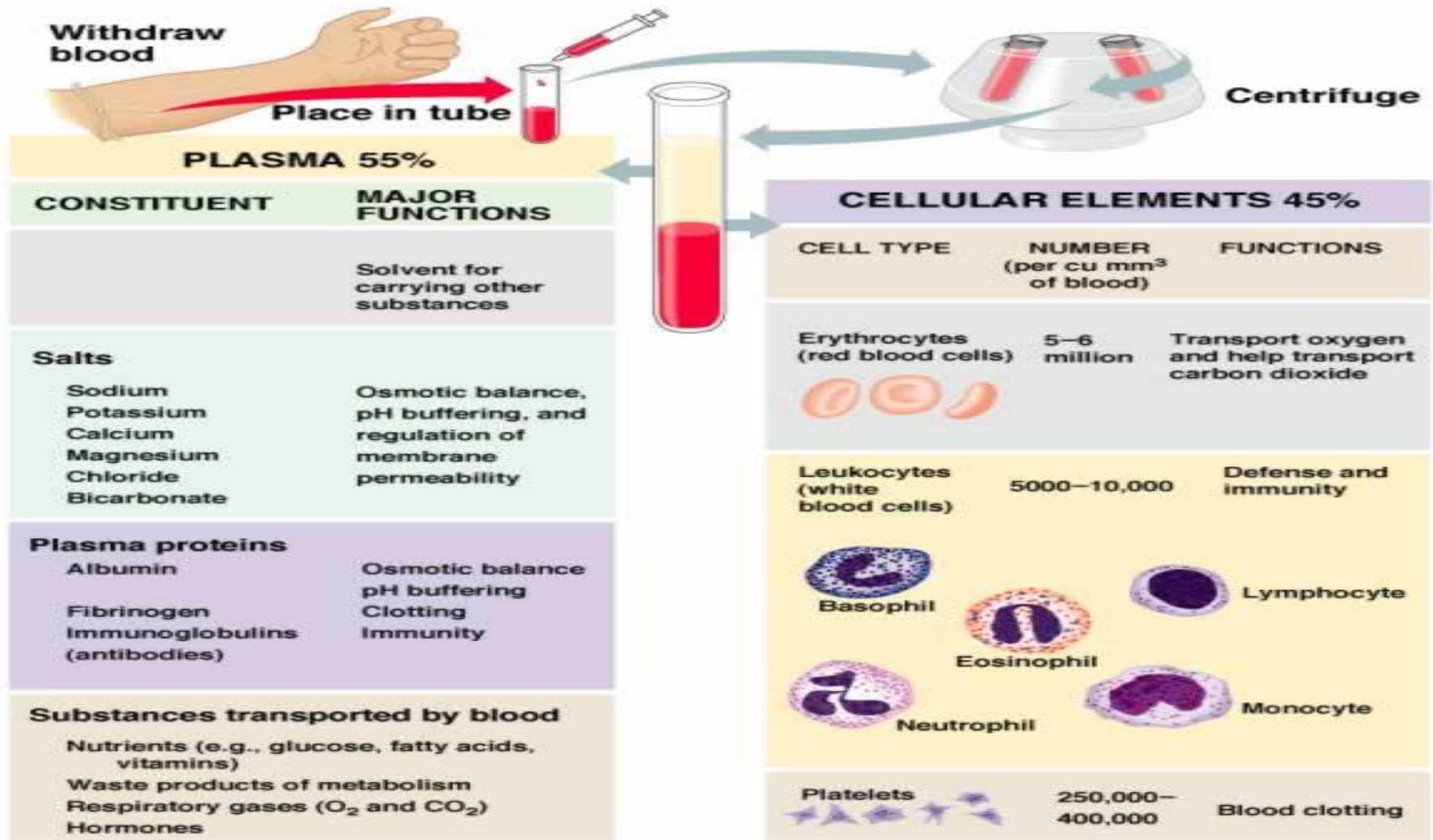
Plasma Components



Components of Blood

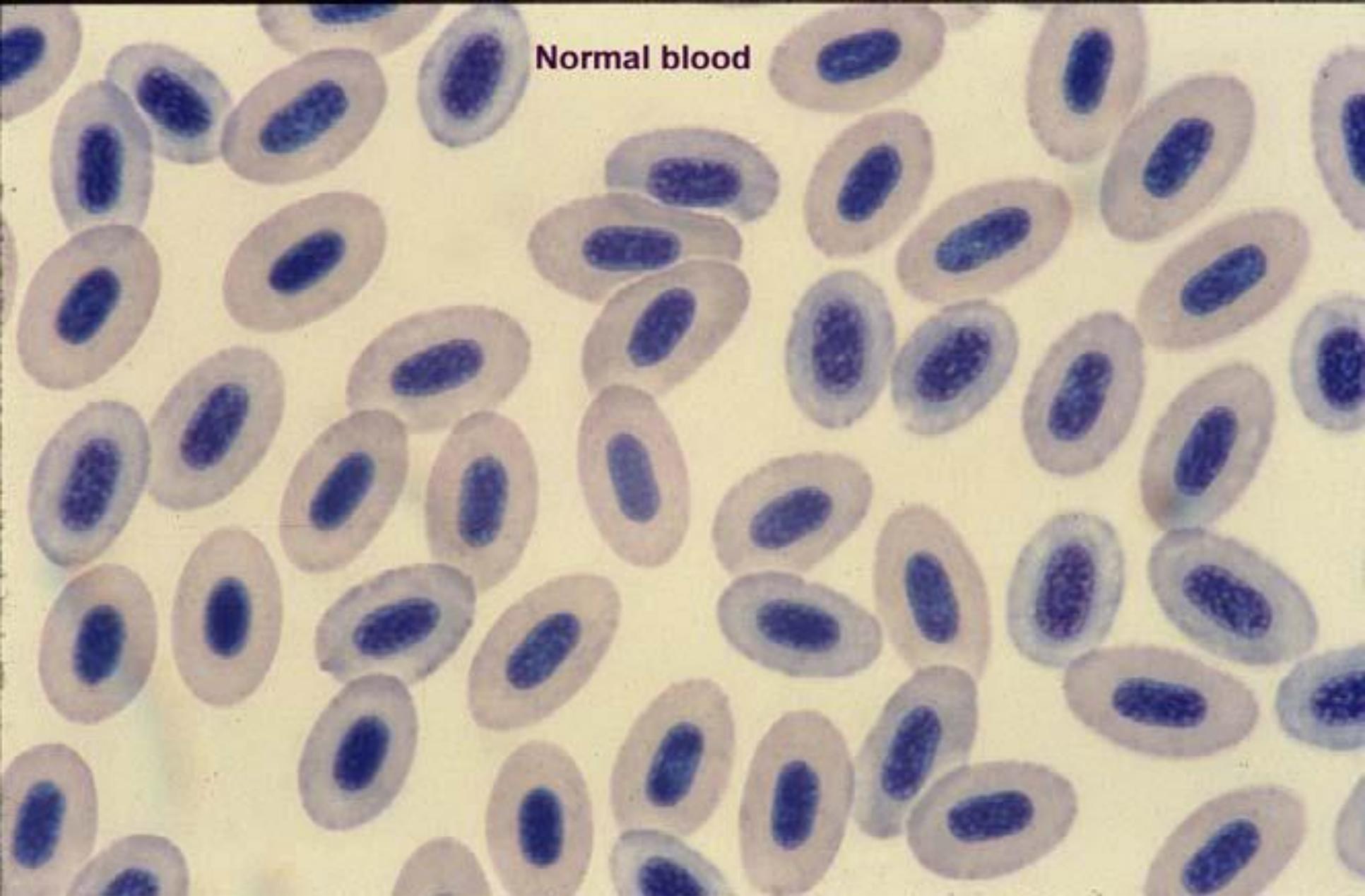
- Red blood cells (erythrocytes)
- White blood cells (leukocytes)
 - granular leukocytes
 - neutrophils
 - eosinophils
 - basophils
 - agranular leukocytes
 - lymphocytes = T cells, B cells, and natural killer cells
 - monocytes
- Platelets (special cell fragments)

Components of Blood



Erythrocytes

- Function to *transport oxygen* in the blood.
- Erythrocytes contain *tremendous amounts of hemoglobin*, the protein that binds oxygen.
- In order to make room for more hemoglobin to carry more oxygen, erythrocytes lose their nucleus and other organelles as they develop in the bone marrow.
- Because they lack a nucleus and other cellular machinery, erythrocytes *cannot repair themselves when damaged*



Normal blood

Source : Sonia Mumford et al.,

Cont....

- *But fishes have nucleated rbc*
- *Consequently they have a limited life span of about 120 days.*
- *The removal of old and dying erythrocytes is carried out by the spleen.*
- *Die at a rapid rate, 2-3 million erythrocytes die every second.*
- *Erythrocyte production must equal erythrocyte death or the cell population would decline.*

Cont....

Number :

- **Human blood - 4.4 to 5 million/mm³**
- **Fish blood-1.05 to 3 million/mm³**
- *Low total count of RBS leads to **anemia**.*
- *Abnormal rise in the number of RBC is known as **polycythemia**.*
- *Decrease in the number of RBC is called **erythrocytopenia** which causes O₂ shortage in blood and tissue.*
- *It is important to note that oxygen shortage stimulates the kidney to secrete a hormone called **erythropoietin** into the blood.*
- *Erythropoietin stimulates the bone marrow to increase the production of RBCs.*

Shape

Human – spherical

Fish – elliptical

Size

Erythrocytes have a 7 μm diameter and a width of 1-2 μm .

Quantity of hemoglobin in RBCs

100ml of blood of a normal man contains about 15g of hemoglobin and normal woman 13g of hemoglobin.

A RBC has about 280 million hemoglobin molecules

Formation

- Formation of erythrocytes is called as *erythropoiesis*.
- In the early few weeks of embryonic life, primitive nucleated RBCs are produced in the yolk sac.
 - Human – Bone marrow, liver and spleen.
 - Fish – Anterior kidney, liver and spleen.
- Iron and protein are the necessary raw materials for the synthesis of hemoglobin.
- However vitamin B12 and folic acid - maturation of RBCs.
- Deficiency causes anemia.
- Excess RBCs are stored in spleen.

Leukocytes

- *White blood cells (WBCs), or **leukocytes**, are a part of the **immune system** and help our bodies fight infection.*
- *They **circulate in the blood** so that they can be transported to an area where an infection has developed.*

Human - 5,000 to 10,000/mm³

Fish - 90,000-1,50,000/mm³

Cont...

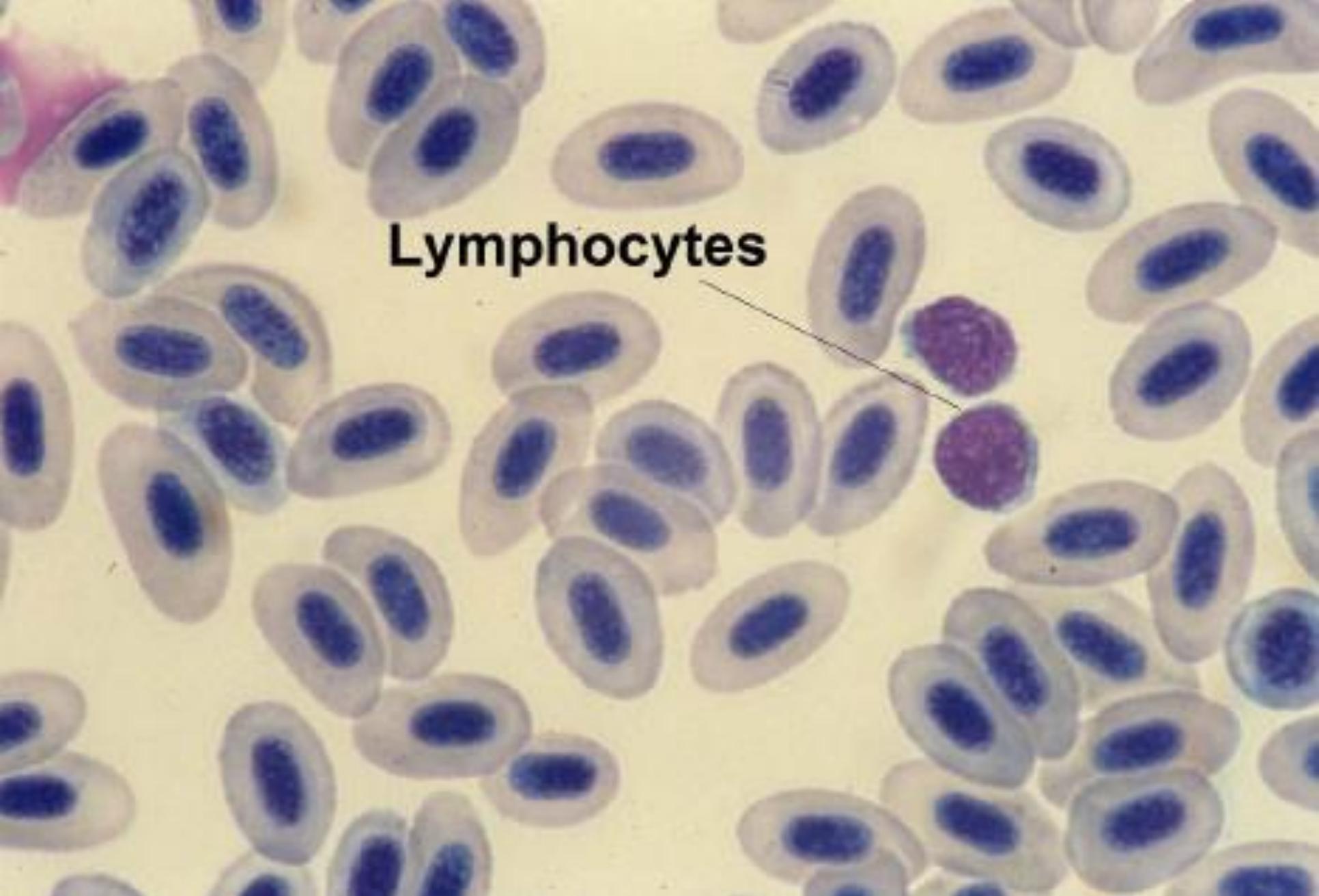
- *When the number of WBCs in your blood **increases**, this is a sign of an infection somewhere in your body.*
- *They are broadly classified in to two types and they are*
 1. *Agranulocytes*
 2. *Granulocytes*

Agranulocytes

- *The granules are not present in the cytoplasm of these cells. They are of two types*
 1. *Lymphocytes*
 2. *Monocytes*

Lymphocytes

- Contains scant cytoplasm with *large rounded nucleus*.
- *Non-motile and non-phagocytic*.
- They *produce antibodies* to destroy microbes and their toxins reject grafts and kill tumour cell.
- They also help in *healing of injuries*.
- Lymphocytes exist in two major groups in circulation. These are *B and T lymphocytes*.
- The number of lymphocytes in the blood is noticeably greater in fish than in mammals.
 - Plaice is $48 \times 10^3 / \text{mm}^3$
 - Man about $2 \times 10^3 / \text{mm}^3$.



Lymphocytes

Source : Sonia Mumford et al.,

Monocytes

- They are the largest of all type of leucocytes and somewhat *amoeboid in shape*.
- They have *much cytoplasm*.
- The *nucleus is bean shaped*.
- They are motile phagocytic in nature and engulf bacteria and cellular debris.
- Generally *they changes into macrophages after entering tissue spaces*.
- The monocyte of fishes forms *about 0.1% of the circulating leukocyte population*.



thrombocyte

monocyte

Source : Sonia Mumford et al.,

Granulocytes

- *They contain granules in their cytoplasm. Their nucleus is irregular or lobed or subdivided.*
- *According to their staining property, the granulocytes are divided into three types.*
 1. *Eosinophils*
 2. *Basophils*
 3. *Neutrophils*

Eosinophils

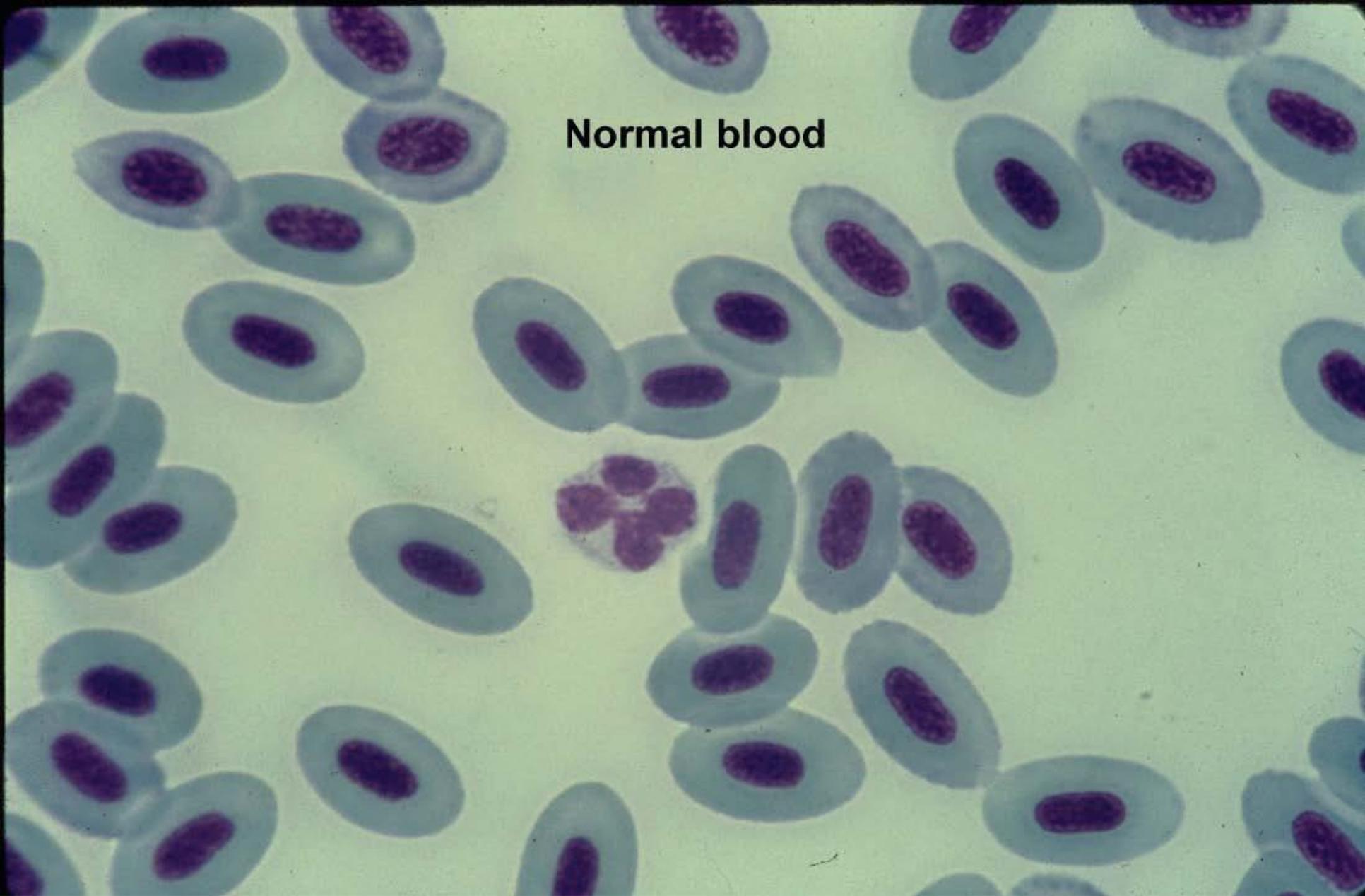
- The nucleus is *bilobed*.
- They have coarse granules.
- The granules take *acidic stains* (e.g., eosin).
- Their number increased in people with *allergic condition such as asthma or hay fever*.
- They also help in *dissolving blood clot*.
- They are non-phagocytic.
- Eosinophil's can *attach themselves to parasitic forms* and cause their destruction by *liberating liposomal enzymes* on their surface.

Basophil

- *The nucleus is usually **three lobed**.*
- *They have less number of coarse granules. Their granules take basic stain (e.g.: methylene blue) strongly.*
- *They release heparin, histamine and serotonin.*
- *They are probably like mast cells of connective tissue.*

Neutrophils

- *The nucleus is **many lobed**.*
- *They have five granules.*
- *They stain weakly with **both acidic and basic stains**.*
- *Neutrophils are the most numerous of all leukocytes.*
- *They eat harmful germs and are therefore phagocytic in nature.*



Normal blood

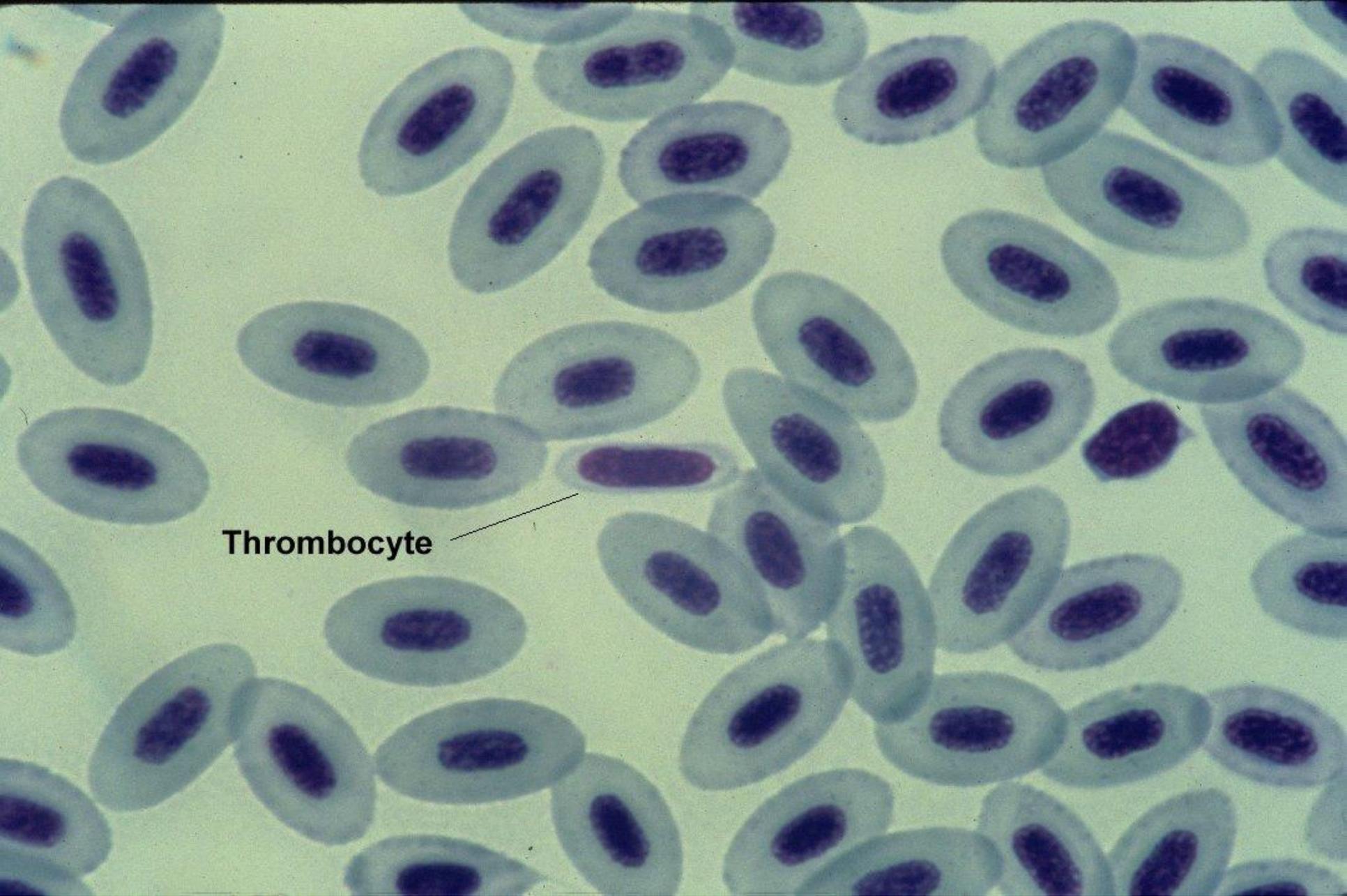
Source : Sonia Mumford et al.,

Life span

- *Granulocytes - 4 to 8 hour circulating in the blood and another 4 to 5 days in tissues.*
- *Monocytes - 10 to 20 hours.*
- *The lymphocytes - of few days to months or even years, but this depends on the body's need for these cells.*

Thrombocyte

- *Thrombocytes are responsible for blood clotting.*
- *Thrombocytes are found in all non-mammalian vertebrates.*
- *Typically they are elongated cells, often termed spindle cells.*
- *Densely staining nuclei, surrounded by a minute amount of cytoplasm.*
- *It is this spent thrombocyte which has been frequently confused with the lymphocyte.*
- *When observed in the living state by phase-contrast microscopy, a retractile vacuole can be seen at the base of the pointed end of the thrombocyte, just anterior to the nucleus.*



Thrombocyte

Source : Sonia Mumford et al.,

Blood cells	Human blood	Fish blood
Erythrocytes	4.4 to 5 million/mm ³	1.05 to 3 million/mm ³
Leucocytes	5000 – 10,000/mm ³	90,000-1,50,000/mm ³ *
1)Arganulocytes		
a)Lymphocytes	20-45%	70-90%
b)Monocytes	2-10%	0.1%
1)Granulocytes		
a)Eosinophils	1-6%	1-3%
b)Basophils	0-1%	0-1%
c)neutrophils	40-75%	6-8%
		(number of neutrophils is as same as mammals i.e. 3000-6000/mm ³ but the percentage varies)
Platelets (Thrombocytes)	2,50,000/mm ³	60,000 – 70,000/mm ³

COMPLETE BLOO COUNT (CBC)

RBC

- **Name:** Red Blood Cells (erythrocytes).
- **Normal Range:** 4.4 to 5 million/cu.mm for human and 1.05 to 3 million/cu.mm for fish.
- **Abnormality:** Low RBC count (anemia) diminishes the body's ability to carry oxygen to the tissues.
- **Function/Test Purpose:** Formed in the bone marrow, a typical red blood cell lives for about 120 days. These cells transport oxygen to all parts of the body. This test is done to support other tests in **diagnosis of anemia** and to supply figures for computing the erythrocyte indices, which reveal RBC size and hemoglobin content.
- **Test Mechanism:** Red Blood Cells are counted with an instrument called a **Coulter Counter**. The sample is diluted in an electrically charged solution and moves slowly through an aperture across which a specific voltage passes. As each cell passes through the voltage changes, creating a pulse. The voltage magnitude varies also with cell size. This way the cells are counted, and sized. Particles greater than 36 fL are counted as RBCs.

HCT

- **Name:** Hematocrit
- **Normal Range:** 42-51% (For human)
- **Abnormality:** Lowered hematocrit.
- **Function/Test Purpose:** The volume occupied by the packed red blood cells in a given volume of centrifuged blood. It is used in **determination of anemias**, and is usually expressed as a percentage of the volume of the whole blood sample.
- **Test Mechanism:** This is a calculated value from the values of the red cell count and the Mean Corpuscular

$$HCT = RBC \times MCV$$

HGB

- **Name:** Hemoglobin
- **Normal Range:** 14-18 g/100 ml. (For human)
- **Abnormality:** Lowered hemoglobin.
- **Test Purpose:** Hemoglobin is the pigment in red blood cells that contains iron and transports oxygen to the tissues. This is the main component of the red blood cell. The test coordinates with other red blood cell data.
- **Test Mechanism:** An instrumental method, using a **spectrophotometer**, measures the intensity of light which passes through the blood sample. Less light transmittance equates to more hemoglobin.

MCV

- **Name:** Mean Corpuscular Volume
- **Normal Range:** 80-96 femtolitres (For human)
- **Abnormality:** Varies; however, MCV may be elevated due to erythrocyte aggregation.
- **Function/ Test Purpose:** This is the average volume of the red blood cells, the ratio of hematocrit to the RBC count. The Mean Corpuscular Volume expresses the **average size of the erythrocytes, and indicates whether they are undersized, oversized or normal.**
- **Test Mechanism:** Measured with a **Coulter Counter**. The cell volume is derived from the amount of voltage Variance as each cell is counted.

$$MCV = \frac{HCT \times 10}{RBC \text{ count (millions/ l)}}$$

MCH

- **Name:** Mean Corpuscular Hemoglobin
- **Normal Range:** 20-33 pico grams (For human)
- **Abnormality:** Varies; hemoglobin will be depressed if RBC count is lowered.
- **Function/Test Purpose:** Mean Corpuscular Hemoglobin is the content of hemoglobin in the average red blood cell, the hemoglobin/RBC ratio. *It expresses the weight of hemoglobin in an average RBC.*
- **Test Mechanism:** A calculated value using *RBC count, hemoglobin and hematocrit.*

$$MCH = \frac{\text{Hemoglobin (g/L)} \times 10}{\text{RBC count (millions/l)}}$$

MCHC

- **Name:** Mean Corpuscular Hemoglobin Concentration
- **Normal Range:** 32-36% (For human)
- **Abnormality:** Varies; if hemoglobin is lowered, hematocrit is also lowered.
- **Function/Test Purpose:** The ratio of hemoglobin weight to hematocrit. This test defines the concentration of hemoglobin in 100 ml. of packed red blood cells. It helps distinguish the normally colored cells from paler cells to help classify different anemias and aid in determining cause.
- **Test Mechanism:** A calculated volume using RBC count, hemoglobin and hematocrit.

$$\text{MCHC} = \frac{\text{Hemoglobin (g/dl)} \times 100}{\text{HCT (\%)}}$$

ESR

- **Name:** *Erythrocyte Sedimentation Rate*
- **Normal Range:** *0-30 mm/hr in people over 50 (For human)*
- **Abnormality:** *Elevated rates are usually found in people with WM because of the presence of excessive amounts of the macroglobulin IgM.*
- **Function/Test Purpose:** *This is a determination of erythrocyte and/or plasma abnormalities. It aids in the ability to distinguish among diseases with similar symptoms.*
- **Test Mechanism:** *This test establishes the distance that erythrocytes have fallen in a vertical column of anticoagulated blood under the influence of gravity. The test procedure itself is easily influenced by a number of environmental factors, including blood cell size and shape, temperature, handling.*
This test does not stand alone as a good diagnostic tool.

PLT

- **Name:** Platelets (thrombocytes)
- **Normal Range:** 2,50,000/cu.mm for human and 60,000 to 70,000/cu.mm for fish
- **Abnormality:** Low platelet count (thrombocytopenia) and function. Platelets become coated with IgM molecules, decreasing their clotting capacity.
- **Function/Test Purpose:** Some of the same stem cells that produce the red blood cells in the bone marrow develop into large specialized cells called Megakaryocytes; each of these is packed full of as many as 4000 platelets (discs with a sticky surface substance); the large cells erupt and the separate platelets circulate throughout the bloodstream to assist in clotting, surviving for about ten days.
- **Test Mechanism:** During the **Coulter Counting** cycle, as the platelets and RBCs pass through the apertures, the particles which are between 2 & 20 fL in size are counted as platelets.

MPV

- **Name:** Mean Platelet Volume
- **Normal Range:** 7.2-11.1 % (For human)
- **Abnormality:** Varies; if platelets are depressed, platelet hematocrit will be lowered.
- **Function/Test Purpose:** The ratio of Platelet Hematocrit to Platelet Count; aids in determining platelet volume and size.
- **Test mechanism:** This is a calculated value.

$$MPV = \frac{\text{Platelet Hematocrit}}{\text{Platelet Count}}$$

WBC

- **Name:** *White Blood Cells* (Leukocytes) are composed of *Basophils, Eosinophils and Neutrophils*, which are collectively called *Granulocytes*. Other white blood cells are *Lymphocytes and Monocytes*.
- **Normal Range:** *4.8-10.8 thousand/cu.mm for human 90,000 to 1,50,000/cu.mm for fish.*
- **Abnormality:** *Lowered WBC count (leukopenia)*
- **Test Purpose:** *Primarily formed in the bone marrow; white blood cells may also be produced in organs of the lymphatic system such as spleen, thymus and lymph nodes. They serve to fight off infections and protect the body from disease. This test serves to monitor disease progression and/or response to chemotherapy.*
- **Test Mechanism:** *White Blood Cells are counted with an instrument called a **Coulter Counter**. The sample is diluted in an electrically charged solution which is passed slowly through an aperture across which a specific voltage passes. As each cell passes through, the voltage changes, creating a pulse. The voltage magnitude varies also with cell size. This way the cells are counted, and sized.*

Viscosity

Name: Serum Viscosity Test

Normal Range: 1.0-1.8(For human)

Abnormality: Increased blood viscosity (hyperviscosity)

Function/Test Purpose: Viscosity is the property of blood that resists a force causing it to flow. It is compared to the viscosity of distilled water at room temperature, and is a function of the concentration of protein in the blood. High serum viscosity is most often caused by an overabundance of IgM.

Test Mechanism: Blood is allowed to clot. Then the serum is separated by centrifuging and warmed. The serum is allowed to flow down through a narrow tube (viscometer), and its rate of flow is timed. It is compared to the rate of flow for distilled water.

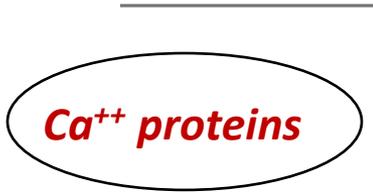
$$\text{Serum Viscosity} = \frac{\text{Flow time of blood serum}}{\text{Flow time of distilled water}}$$

Blood Clotting Mechanism

Injured Tissue



Thromboplastin



Blood Platelets



Platelet Factor 3 (Platelet Thromboplastin)



Prothrombinase



Prothrombin

Thrombin



Fibrinogen

Fibrin

Fibrin + Blood cells

Clot

Clotting time

- *The normal clotting time is about 6 to 10 minutes.*

Role of vitamin K in blood clotting

- *Vitamin K is essential for blood clotting because it is necessary for the synthesis of prothrombin in liver. In vitamin K is not sufficient in the body, blood clotting becomes insufficient.*

Clotting Factors

<i>Factor I</i>	<i>Fibrinogen</i>
<i>Factor II</i>	<i>Prothrombin</i>
<i>Factor III</i>	<i>Tissue Thromboplastin</i>
<i>Factor IV</i>	<i>Calcium Ions</i>
<i>Factor V</i>	<i>Labile Factor</i>
<i>Factor VII</i>	<i>Stable Factor</i>
<i>Factor VIII</i>	<i>Antihemophilic Factor</i>
<i>Factor IX</i>	<i>Christmas Factor or Plasma Thromboplastin</i>
<i>Component</i>	
<i>Factor X</i>	<i>Stuart-Prower Factor</i>
<i>Factor XI</i>	<i>Plasma Thromboplastin Antecedent (PTA)</i>
<i>Factor XII</i>	<i>Hageman Factor</i>
<i>Factor XIII</i>	<i>Fibrin Stabilizing Factor</i>

Anticoagulants

- *CPD (Citrate Phosphate Dextrose),*
 - *ACD (Acid Citrate Dextrose) and*
 - *EDTA (Ethylene Diamino Tetra Acetic Acid)*
- are used by blood banks to prevent blood samples from clotting.*
- *Blood clotting can be prevented in test tubes by adding a little oxalates or citrate.*
 - *Oxalate or citrate reacts with calcium to form insoluble compounds.*
 - *So free calcium ions necessary for clotting are unavailable.*

Difference between blood plasma & serum

<i>Sl No</i>	<i>Blood plasma</i>	<i>Blood serum</i>
<i>1</i>	<i>It is the fluid minus blood corpuscles</i>	<i>It is the liquid minus clotting elements</i>
<i>2</i>	<i>It is faint yellow in colour</i>	<i>It is pale yellow</i>
<i>3</i>	<i>It has fibrinogen and other clotting materials</i>	<i>It doesn't have fibrinogen and other clotting materials</i>
<i>4</i>	<i>It takes part in blood clotting</i>	<i>It doesn't take part in blood clotting</i>