BLOOD

@
CBU SCHOOL OF MEDICINE

NGALA ELVIS MBIYDZENYUY
elvis.ngala@ kiu.ac.ug

BLOOD COMPOSITION, PROPERTIES, FUNCTIONS and VOLUME

COMPOSITION OF THE BLOOD

- Blood consists of cells suspended in a clear yellowish fluid called the plasma. The cells constitute 40-45% of the blood volume and include :

(I) Red blood cells or corpuscles (RB.Cs) or Erythrocytes: Normally there are about 5 million RBCs per mm^3. When they are decreased, the condition is called anemia, and when they are increased the condition is called polycythemia.

(2) White blood cells or corpuscles (W.B.Cs) or leukocytes : Normally, there are 4000 – 11,000 W.B.Cs per mm^3 . When they are decreased the condition is called leukopenia, and when they are increased, the condition is called leukocytosis.

COMPOSITION OF THE BLOOD

(3) Platelets or thrombocytes : Normally, there are about *300000 platelets per mm³*. When they are decreased the condition is called *thrombocytopenia*, and when they are increased the condition is called *thrombocytosis*

THE PLASMA

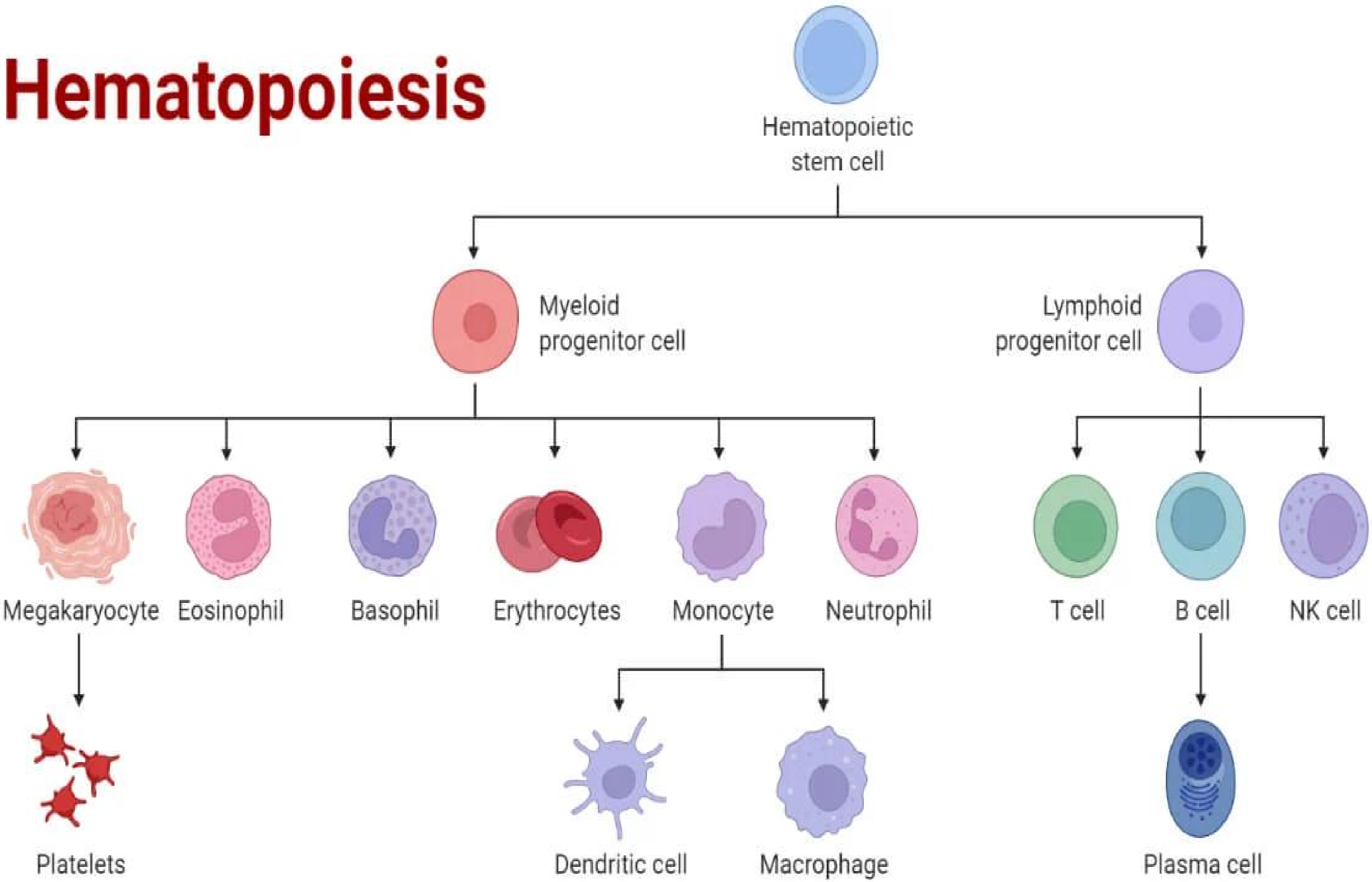
The plasma constitutes 55-60 % of the total blood volume, and it consists of water (90 %) and dissolved solutes (10 %).

The latter includes:

(a)Organic substances : Plasma proteins (7.1 *%)*, lipids, hormones, enzymes, nutrients and waste products (2 %).

(b) Inorganic substances (0.9 %) which include the various electrolytes e.g. Na^+, K^+, Cl^-, HCO_3^-, Ca^{2+} and PO_4^{3-}

Hematopoiesis



HEMATOPOIESIS

- Hematopoiesis, arises from a single cell type known as a hematopoietic stem cell (HSC)
- Sites (embryonic, extramedullary and medullar)
- Multipotent or pluripotent HSC- self-maintaining, few, high proliferation capacity.
- Multipotent stem cells differentiate to either a common lymphoid progenitor cell or a common myeloid progenitor cell, which is dictated by its microenvironment
- During dev’t stem cells differentiate into progenitor cells, lose capacity for self-renewal, committed

HEMATOPOIESIS

- The progenitor productions are highly dependent on response to growth factors and cytokines, which help in the proliferation and differentiation of the progenitor cells

- Hematopoietic cells grow and mature on a **mesh of stromal cells** which are non-hematopoietic cells supporting the growth and differentiation of hematopoietic cells.

- Stromal cells include fat cells, endothelial cells, fibroblasts, and macrophages, and they influence the process of hematopoietic differentiation by providing a hematopoietic-inducing microenvironment

PROPERTIES OF THE BLOOD

1. The blood colour is red due to hemoglobin.
2. The pH of arterial blood is 7.4 (that of venous blood is 7.36).
3. Blood is opaque due to its cellular elements.
4. The blood specific gravity is about 1.060 (that of the plasma is 1.025- 1.030).
5. The blood viscosity is 5 times that of water due to its cellular elements and the plasma proteins (the plasma viscosity is 2 times that of water).
6. The *osmotic pressure (O.P.) of the plasma is about 5500 mmHg.* It is mostly due to *crystalloids* (electyrolytes, glucose. urea, etc.), since that of the *plasma proteins is only about 25 mmHg* (which is called the *plasma colloid osmotic pressure or oncotic pressure).* The total plasma osmolality is *290-300 mOsmllitre,* and the plasma proteins contribute by only 0.5 % (i.e. *less than 2 mOsmllitre)*

GENERAL FUNCTIONS OF THE BLOOD

- The blood links the various body systems together and greatly helps in homeostasis (= maintenance of the internal environment of the body constant as regards water, pH, electrolyte concentration, temperature. etc.). This is carried out through performing the following functions :

(I) It is the *major transport medium in the body.* This is essential for
a) *Nutrition* (by transporting food from the GIT to the tissues).
b) *Respiration* (by transporting 02 from the lungs to the tissues and C02 in the opposite direction).
c) *Excretion* (by transporting the waste products from the tissues to the excretory organs e.g. urea and creatinine to the kidneys).
d) *Regulation of metabolism* (by transporting hormones from various endocrine glands to the tissues and also by regulating their secretion},
e) *Regulation of the body temperature* by transporting heat to the skin (thus helping heat loss), and also by informing the hypothalamic thermoreceptors about the body core temperature .

GENERAL FUNCTIONS OF THE BLOOD

f) Distributes hormones, hence helps coordinate the activities of organs of the body

g) By transporting white cells and antibodies to tissues, the blood promotes defense against pathogenic organisms and foreign subst

(2) It plays an essential role in *maintaining the acid-base balance* and keeping the pH of the body fluids constant (by its buffer systems).

(3) It is important in *the regulation of water balance,* specially in cases of hydration (by removing excess fluids in the kidneys).

(4) The blood viscosity produces *peripheral resistance* to the blood flow which is essential for *maintenance of the arterial blood pressure*

(5) The property *of blood clotting* is a major *hemostatic mechanism* for prevention of excessive blood loss in cases of hemorrhage.

(6) The *white blood cells provide the main defense mechanism, of the body against* a wide variety of microorganisms

THE HEMATOCRIT (H)

- Hematocrit (packed cell volume) of a sample is the ratio of the volume of erythrocytes to that of the whole blood.
- It is expressed as a percentage or preferably, as a decimal fraction.
- Before, it was often referred to as volume percent erythrocytes or volume of packed red cells.
- It is calculated as follows :

$$(H) = \frac{\text{blood cell volume}}{\text{total blood volume}} \times 100$$

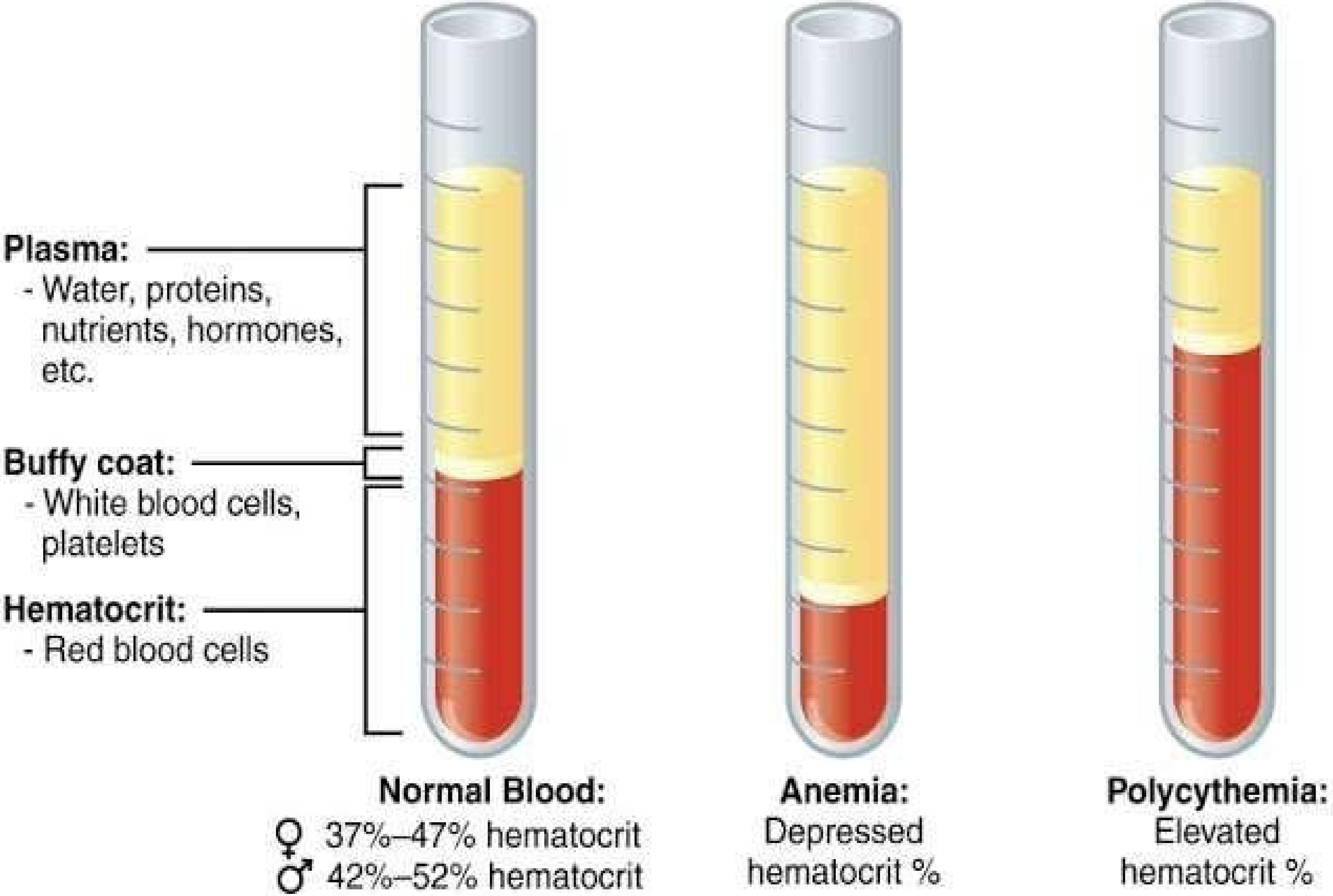
- The test measures the proportion of red blood cell to plasma in the peripheral blood, but not in the entire circulation. This is called then as the venous hematocrit.
- The ratio of the total erythrocyte mass to the total blood volume is the body hematocrit.
- Unless, otherwise specified, the term hematocrit often refers to venous hematocrit.

Determination of (H)

- A blood sample from the subject is placed in a special tube called *Wintrobe tube* that contains an anticoagulant and is then centrifuged

- The centrifugation is done for 30 minutes at 3,000 to 3,500 rpm.

- In the process, spontaneous sedimentation is accelerated by centrifugation.
- The erythrocytes will be packed at the bottom of the tube and above them is a layer of leukocytes, platelets and nucleated erythrocytes called the buffy layer (coat).
- Above the buffy coat is relatively cell–free plasma.


Plasma:
- Water, proteins, nutrients, hormones, etc.
Buffy coat:
- White blood cells, platelets
Hematocrit:
- Red blood cells
Normal Blood:
♀ 37%–47% hematocrit
♂ 42%–52% hematocrit
Anemia:
Depressed hematocrit %
Polycythemia:
Elevated hematocrit %

Clinical importance of hematocrit determination

1. Gives a rough estimate of the size of erythrocytes and the concentration of erythrocytes.
2. It is used in the calculation of mean corpuscular values and blood indices (Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC).
3. Hematocrit is a good simple screening test for anemia.
4. Estimation of the *blood volume*
5. Measurement of the *renal blood flow* RBF = RPF / 1 - hematocrit
6. The buffy coat obtained from the hematocrit tube has numerous uses

Normal values

Men	0.42 – 0.48
Women	0.37 – 0.42
Newborn	0.44 – 0.64

Factors that affect (H)

(H) is affected by *changes in the red cell volume relative to the plasma volume.* Accordingly, it is changed as follows :

1. It is *increased* in polycythemia (erythrocytosis)and cases of hemoconcentration (e.g. when the plasma volume is decreased due to dehydration).
2. It is *decreased* in anemia and cases of hemodilution (e.g. when the plasma volume is increased due to hydration).
3. Its value in small blood vessels is less than its value in large vessels due to *plasma skimming* and is also greater in venous blood than in arterial blood due to *chloride shift.*

- An increase in hematocrit is usually a relative increase (does not involve an actual increase in the red cell mass of the body) due to dehydration, endotoxemia or splenic contraction occurring in association with excitement or exercise.
- The anemia could be as a result of loss of erythrocytes, increased destruction of erythrocytes or low production of them.

THE BLOOD VOLUME

NORMAL VALUES OF THE BLOOD VOLUME

- The blood volume is normally about 8 % of the body weight, 80 ml/Kg of body weight and 3.2 litre/m^2 of body surface area.
- The total blood volume is about *5.6 litres in young adult males weighing 70 Kg.*
- These values are *less in* females (and also in obese persons) due to their greater body fat content (which is relatively avascular)

THE BLOOD VOLUME

VARIATIONS OF THE BLOOD VOLUME

- The blood volume may increase either physiologically (e.g. in pregnancy, high altitudes and following muscular exercise), or pathologically (e.g. in polycythemia vera).
- On other hand, its decrease is almost always pathological (e.g. in dehydration and after acute hemorrhage)

MEASUREMENT OF THE BLOOD VOLUME

- The blood volume approximately equals the *sum of the* plasma *volume and red cell volume* (since the volume or other blood cells is negligible)
- These can be measured by the *dilution principle* but it is unnecessary to measure both. *The blood volume can be estimated if only one of them is measured and the hematocrit (H) is known*

(A) MEASUREMENT OF THE PLASMA VOLUME

- The plasma volume can be measured by 2 methods:

(1) DYE METHOD :

- A known amount of a dye e.g. *Evans blue* is i.v. injected into the subject, and after 5-10 minutes (which allows uniform dispersion of the dye in the plasma), the concentration of the dye in the plasma is determined.
- From the latter, the plasma volume can be determined, and the total blood volume can also be calculated if (H) is known as shown in the following example :
  - If the amount dye injected is 30 mg, and its plasma concentration after mixing was 0.0 I mg/ml, then the *volume of distribution (i.e. the plasma volume) = 30/10.01 = 3000 ml.*
  - If (H) was 40 %, this means that the determined plasma volume constitutes 100-H i.e. 60 % of the total blood volume.
  - Accordingly, the total blood volume = *plasma volume* x *100/(100-H)* = 3000 x I00/60 = 5000 ml.

Characteristics of Evans blue

a) It is *non- toxic and is not metabolized in the body.*
b) It combines with the plasma proteins so it does not rapidly escape into the tissue spaces (and is also *not rapidly excreted by the kidneys).*
c) It is *not adsorbed to the red cells* and does not enter or hemolyze them.
d) Its plasma level is easily measured by the spectrophotometer

(2) ISOTOPE METHOD :

- This is more accurate than the dye method.
- A sample of serum albumin is incubated with ^{131}I (= radioactive iodine) in vitro.
- An amount of this labeled albumin with known radioactivity is i.v. injected into the subject, and after l0- 15 minutes, a blood sample is withdrawn, and the plasma radioactivity is measured.
- From the degree of dilution the plasma volume can be determined , and the total blood volume can also be calculated if the (H) is known as described above

REGULATION OF THE BLOOD VOLUME

REGULATION OF THE BLOOD CELL VOLUME

- The regulation of the red blood cell volume is particularly important in maintaining the total blood volume constant because of their much greater number than other blood cells

- This is normally achieved by the nearly equal rates of their formation and destruction. In cases of sudden blood loss e.g. due to severe hemorrhage their volume is restored by 2 mechanisms :

(l) A rapid compensatory mechanism: Red cells are rapidly shifted from the blood stores (e.g. the spleen) into the general circulation.

(2) A slow compensatory mechanism: The rate of erythropoiesis in the bone marrow is increased as a result of 02 lack

REGULATION OF THE BLOOD VOLUME

REGULATION OF THE PLASMA VOLUME

- Normally, the plasma volume is kept constant by 2 mechanisms:

(1)Water balance: This depends on the balance between water gain and water loss. Although it is a slow mechanism. It keeps the plasma volume constant over long periods.

(2) Fluid exchange between the plasma & tissue (interstitial) fluid : This is a rapid but limited mechanism.

- It occurs in the *capillaries* where the hydrostatic blood pressure acts as a *filtering force* and the oncotic pressure (or osmotic pressure) of the plasma proteins as a *reabsorbing force*

REGULATION OF THE BLOOD VOLUME

- The hydrostatic pressure is *30-40 (average 32) mmHg at the arterial ends* of the capillaries and *10-15 (average 12) mmHg at their venous ends,* while the oncotic pressure averages *25 mmHg allover*

- Fluids are *filtered at the arterial ends* (where the hydrostatic pressure exceeds the oncotic pressure) and *reabsorbed at the venous ends* (where the oncotic pressure exceeds the hydrostatic pressure).

- Normally, the rate *of fluid filtration slightly exceeds the rate of fluid reabsorption,* and the excess fluid is drained back to the bloodstream by *the* lymphatics *(lymph vessles)*

- If the plasma volume increases (e.g. in cases of hydration), the excess fluid is filtered in the tissue spaces due to rise of the hydrostatic pressure and decrease of the oncotic pressure.

- On the other hand, if the plasma volume decreases (e.g. in cases or dehydration), fluids are withdrawn from the tissue spaces into the bloodstream due to rise of the oncotic pressure and decrease of the hydrostatic pressure.

- The *ICF is similarly affected as the tissue fluid* i.e it increases in hydration and decreases in dehydration.

- *However if such changes in the ICF are excessive, they would lead to damage of the cells (and this is the cause why this mechanism is limited).*

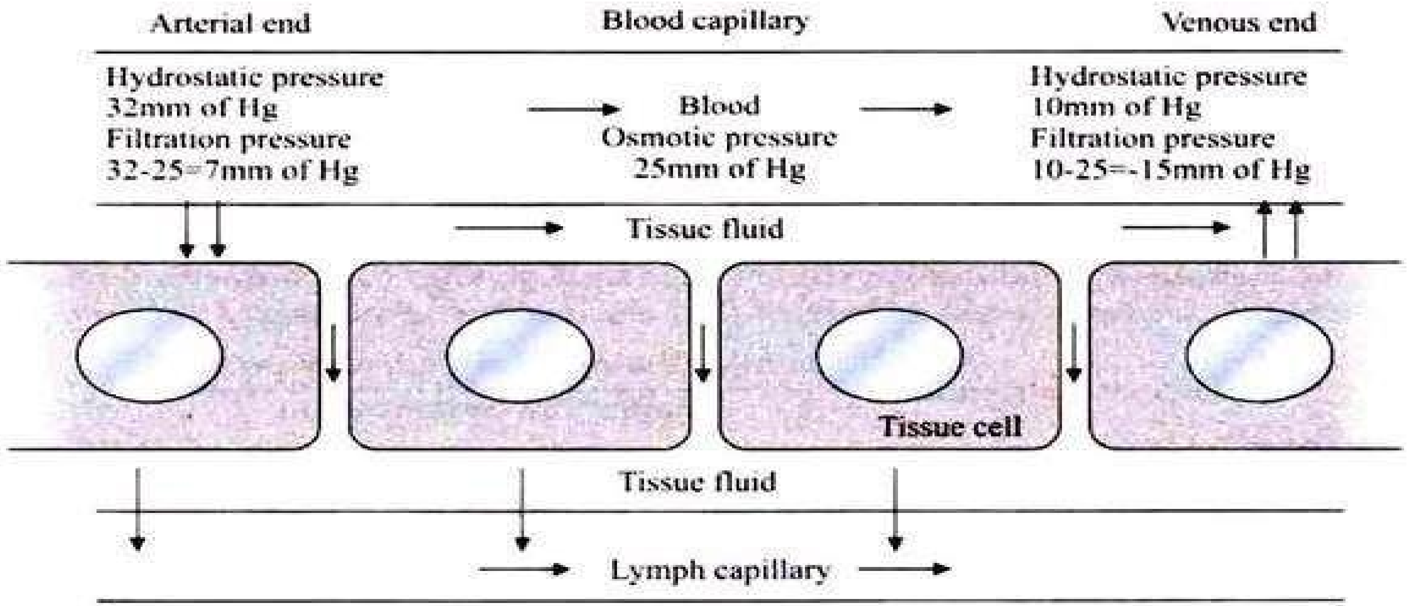
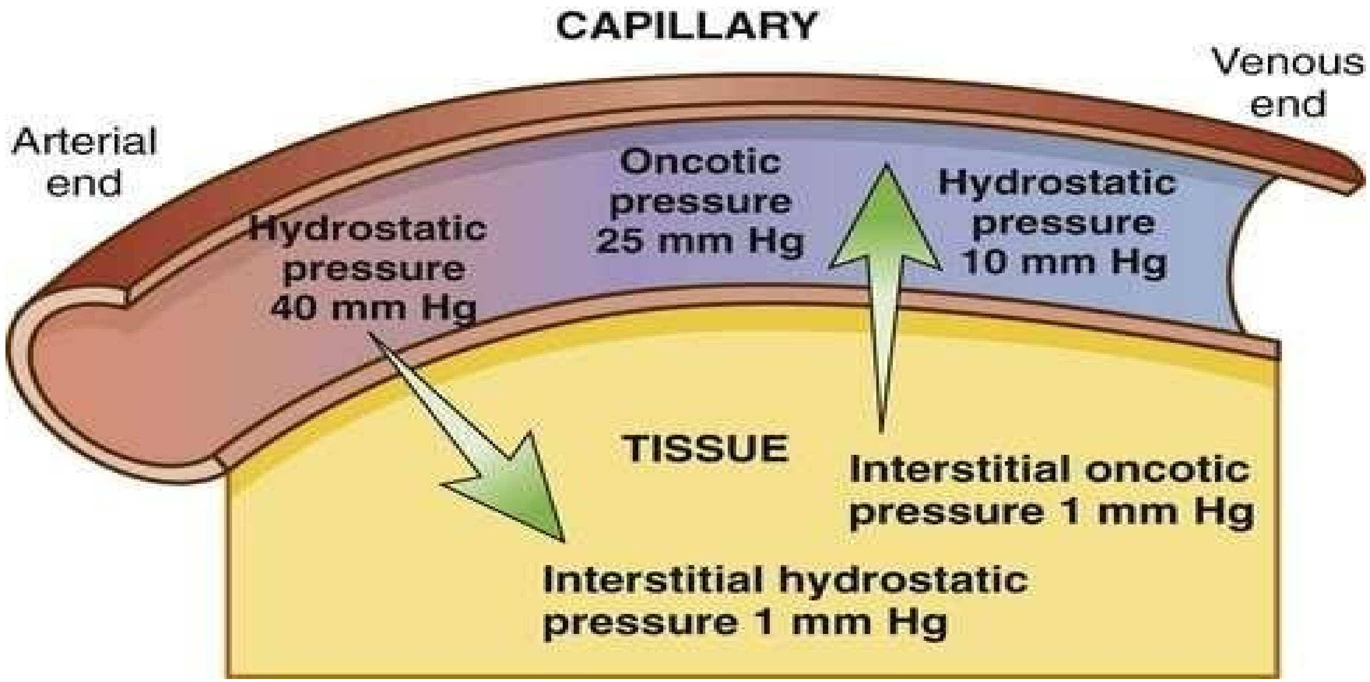


Fig. 5.2 Intracellular fluid formation (Schematic representation)



PLASMA PROTEINS

❑The proteins present in the plasma of human blood are a mixture of simple proteins, glycoproteins, lipoproteins, and other conjugated proteins are called “**Plasma Proteins**“.

❑The three major fractions of plasma proteins are known as Albumin, globulins, and Fibrinogen. Their average amount is 7gm % (6-8 gm %)

Albumin – This is the most abundant (55.2%) plasma protein. Its amount averages 4 gm% (3.5-5 gm %) and its molecular weight is 69000.
Globulins- These average 2.7 gm % (2.3 - 3.5 gm %), and their (m.w.) range from 90000 to 156000

- α1-Globulin – 5.3% (α1-Antitrypsin, TBG, Transcortin, etc)
- α2-Globulin – 8.6% (Haptoglobulin, ceruloplasmin, α2-macroglobulin, etc)
- β-Globulin – 13.4% (β1-transferin, β-lipoprotein, etc)
- ¥-Globulin – 11.0% (Antibodies, etc)

Fibrinogen – This is the least abundant (6.5%) plasma protein. Its amount averages 0.3 gm%, and its (m. w.) is 340000.

SITE OF SYNTHESIS OF THE PLASMA PROTEINS

- In the embryonic stage, the mesenchymal cells are responsible for plasma cell production.

- The first protein to be synthesized is albumin, followed by globulin and the other plasma proteins

- In Adults -the reticuloendothelial cells of the liver, bone marrow, degenerating blood cells, general body tissue cells, and the spleen also contribute to the formation of plasma proteins.

- Gamma globulins originate from B lymphocytes, which in turn form immunoglobulins.

ORIGIN (SOURCE) OF THE PLASMA PROTEINS

- Plasma proteins are primarily formed from *food proteins*, but they can be formed from *tissue proteins* if the protein content in food is low

(A) Food proteins: These are the most effective for synthesis of plasma proteins when:

(I) Their structure is similar to that of the plasma proteins

(2) They are of a high biological value (i.e. rich in the essential amino acids).

Certain proteins favour albumin formation (e.g. those from muscle) while others favour globulin formation (e.g. plant proteins).

(B) Tissue proteins: These are either *fixed tissue proteins* that cannot be converted into plasma proteins or *reserved tissue proteins.* The latter can be converted into plasma proteins and are of *2 types:*

(a) Dispensable reserve proteins: These are mobilized from the tissues to the liver during starvation where they are used for energy production and plasma protein formation.

(b) Labile reserve proteins: These are structurally similar to the plasma proteins. They are mobilized from the tissues to the bloodstream directly if the amount of the plasma proteins is suddenly decreased

e.g. in case of hemorrhage (in the latter case, fibrinogen, globulins, then albumin are then gradually regenerated in that order).

Types of PPs	Function	Pattern in electrophoresis	Clinical significance
Prealbumin (Transthyretin)	A transport protein for: • Thyroid hormones. • Retinol (vitamin A).	• Migrates faster than albumin in electrophoresis (as it's smaller in size than albumin). • Separated by immunoelectrophoresis.	• Lower levels found in: -Liver disease -Nephrotic syndrome -Acute phase inflammatory response -malnutrition • Short half-life (2 days)

Immunoelectrophoresis is a biochemical method for separation of proteins based on electrophoresis & reaction with antibodies (immunoglobulins)

Types of PPs	Synthesis & Functions
Albumin	Synthesized in the liver as preproalbumin & secreted as albumin. **Functions:** 1- Maintains oncotic pressure: • The osmotic pressure exerted by plasma proteins that pulls water into the circulatory system. • Maintains fluid distribution in & outside cells & plasma volume. 2- **80% of plasma oncotic pressure is maintained by albumin**, as it's the most abundant plasma protein (~40 g/L) in normal adult. 3- A **non-specific** carrier of: hormones, calcium, free fatty acids, drugs, etc. 4-Tissue cells can take up albumin by pinocytosis where it is hydrolyzed to amino acids. 5-Useful in treatment of **liver diseases**, hemorrhage, shock & burns.

Clinical significance

- $T_{1/2}$ in plasma: 20 days
- Decreases rapidly in injury, infection & surgery.

Hypoalbuminemia

causes:

-Decreased albumin synthesis (liver cirrhosis, malnutrition)

-Increased losses of albumin

- Increased catabolism in infections
- Excessive excretion by the kidneys (nephrotic syndrome)
- Excessive loss in bowel (bleeding)
- Severe burns (plasma loss in the absence of skin barrier)

Effects:

1- Edema due to low oncotic pressure

- Albumin level drops in liver disease causing low oncotic pressure
- Fluid moves into the interstitial spaces causing edema

2-Reduced transport of drugs & other substances in plasma (may cause drug toxicity).

3-Reduced protein-bound calcium

- Total plasma calcium level drops
- Ionized calcium level may remain normal (because ionized Ca doesn't need albumin to be transported)

Hyperalbuminemia

- No clinical conditions are known that cause the liver to produce large amounts of albumin.
- **The only cause of hyperalbuminemia is dehydration.**

Types of PPs		Synthesis & Function
α1-Globulins	α_1-Antitrypsin	Synthesized by the liver & macrophages. Functions: -Is an acute-phase protein that inhibits proteases. -Proteases are produced endogenously & from leukocytes & bacteria: • Digestive enzymes (trypsin, chymotrypsin). • Other proteases (elastase, thrombin). -Infection leads to protease release from bacteria & leukocytes.

Types	Clinical significance & Lab diagnosis
-Over 30 types, the most common is **M type** -Genetic deficiency of α1-Antitrypsin (**commonly affects Z type not M type**) : • Synthesis of the defective 1-Antitrypsin occurs in the liver but it cannot secrete the protein • 1-Antitrypsin accumulates in hepatocytes & is deficient in plasma	**Consequences of α1-Antitrypsin Deficiency:** -**Neonatal jaundice** with evidence of cholestasis -**Childhood liver cirrhosis.** -Pulmonary emphysema in young adults. **Laboratory Diagnosis** Lack of α1-globulin band in protein electrophoresis **Quantitative measurement of α1-Antitrypsin by:** **Radial immunodiffusion, isoelectric focusing or nephelometry**

Types of PPs		Synthesis & Function
α1-Globulins	α-fetoprotein	• Synthesized in the developing embryo and fetus by the parenchymal cells of the liver • AFP levels decrease gradually during intra-uterine life and reach adult levels at birth Function: • is unknown but it may protect fetus from immunologic attack by the mother • No known physiological function in adults

Clinical significance

- Elevated maternal AFP levels are associated with:
- Neural tube defect, anencephaly

- Decreased maternal AFP levels are associated with:
- Increased risk of Down's syndrome

- AFP is a tumor marker for:
- Hepatoma and testicular cancer

Types of PPs		Function
α2-Globulins	Ceruloplasmin	▪ Synthesized by the liver ▪ Contains >90% of serum copper ▪ An oxidoreductase that inactivates ROS causing tissue damage in acute phase response (ROS =reactive oxygen species) ▪ Important for iron absorption from the intestine
	haptoglobin	• Synthesized by the liver • Binds to free hemoglobin to form complexes that are metabolized in the RES • Plasma level decreases during hemolysis • Limits iron losses by preventing Hb loss from kidneys

Clinical significance

- **Wilson's disease:**
- Due to low plasma levels of ceruloplasmin
- Copper is accumulated in the liver and brain

Types of plasma proteins		Function
β-Globulins	**CRP (C-Reactive Protein)**	• An acute-phase protein synthesized by the liver • Important for **phagocytosis**
	transferrin	• A major iron-transport protein in plasma • 30% saturated with iron • A negative acute phase protein
	β2-microglobulin	• A component of human leukocyte antigen (HLA) • Present on the surface of lymphocytes and most nucleated cells • Filtered by the renal glomeruli due to its small size but most (>99%) is reabsorbed

Clinical significance

- **High plasma levels** are found in many inflammatory conditions such as **rheumatoid arthritis**
- A marker **for ischemic heart disease**

- **Plasma level drops in:**
  Malnutrition, liver disease, inflammation, malignancy
- Iron deficiency results **in increased hepatic synthesis**

- **Elevated serum levels are found in:**
  Overproduction in disease
- **May be a tumor marker for:**
  Leukemia, lymphomas, multiple myeloma

FUNCTIONS OF THE PLASMA PROTEINS IN GENERAL

1.Hemostatic function: *Fibrinogen* is essential for blood coagulation.

2.They share in production of blood viscosity specially fibrinogen and globulins (due to their large m.w) which causes peripheral resistance in blood flow. This helps maintenance of the arterial blood pressure particularly the systolic pressure.

(3) The osmotic pressure (about 25 mmHg) is essential for reabsorption of fluids from the tissue spaces at the capillary venous ends, which maintains the blood volume. 70-80% of the osmotic pressure is produced by albumin (because of its greater amount and smaller m.w).

(4)Buffer action : They provide about 15% of the *buffering power of the blood* e.g. a strong acid such as lactic acid can be buffered as follows: lactic acid + Na proteinate > Na lactate + proteinic acid (a weak acid)

(5)Defence (immunity) : Gamma globulins are antibodies that attack bacteria, so they are called immunoglobulins

(6)Conservation function : The plasma proteins combine loosely with many substances such as hormones (e.g. thyroxine and cortisol) and minerals (e.g. iron and copper). This serves as *a reservoir for* these substances and *also prevent their rapid loss in the urine.*

(7)Control of capillary permeability: The plasma proteins close the pores in the capillary walls, thus limiting their permeability. This favors development of edema in cases of hypoproteinemia

(8)Carriage of CO2: C02 combines with the amino groups of the plasma proteins and is carried as a carbamino compound (NHCOOH)

(9)Nutritional function : The plasma proteins can be utilized for nutrition of the tissues in cases of *prolonged starvation.*

(10)Specific functions : Certain plasma proteins exert specific functions e.g. various hormones, clotting factors and angiotensinogen.

***Globulins and fibrinogen increase the erythrocyte sedimentation rate by favouring formation of rouleaux shapes of R.B.C.s

QUESTIONS?

Your success in life is highly dependent on who you have chosen to trust. The voice you permit to speak to you is the voice that molds your success