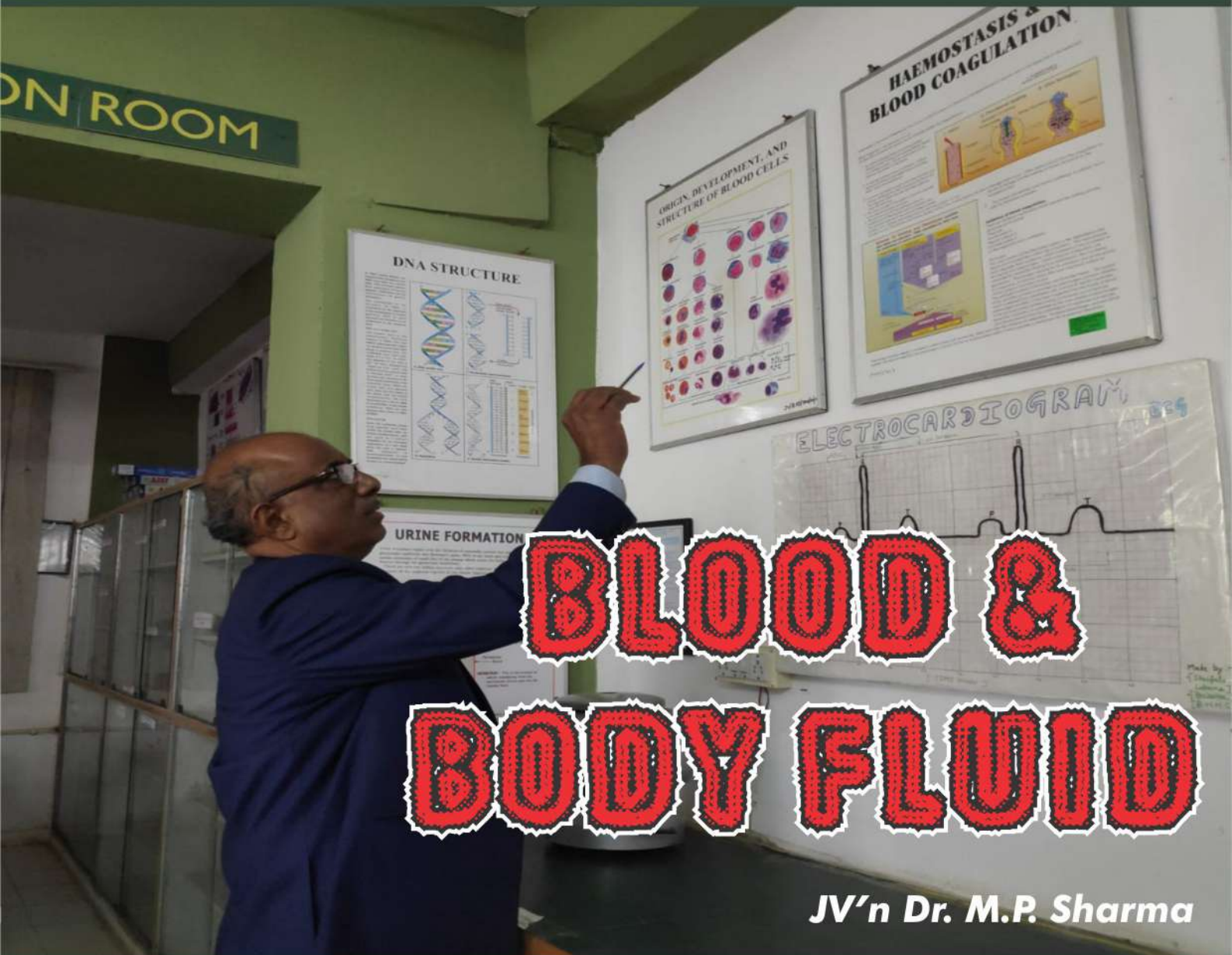




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BLOOD & BODY FLUID

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INTRODUCTION

Human beings are creatures that are primarily composed of water. It is the essence of life and the aqueous base solution in which all essential biochemical processes occur that produce life. Humans are approximately 75% water by mass as infants and 50% to 60% water by mass as adults. Furthermore, fluid is always in flux through a variety of regulatory mechanisms to maintain appropriate concentrations throughout the various compartments of the body. Fluid is largely regulated through passive diffusion following the concentration gradients of osmotically active solutes; however, hydrostatic pressures can influence fluid movement between spaces. Water is the major constituent of all body fluid compartments. In an adult human, average of 70-kilogram. Total body water is about 60 per cent of the body weight, or about 42liters.

PHYSIOLOGICAL VARIATION OF BODY FLUID VOLUME

Most of the variation in body fluid between individuals is as of a result of variation in amount of body fat or adipose tissue (fat is only about 10% water).

- Infant:73-80%
- Male adult:60%
- Female adult:40-50%
- Effects ofobesity
- Old age45%

BODY FLUIDS

Body is formed by solids and fluids. Fluid part is more than two third of the whole body. Water forms most of the fluid part of the body.

In human beings, the total body water varies from 45% to 75% of body weight. In a normal young adult male, body contains 60% to 65% of water and 35% to 40% of solids. In a normal young adult female, the water is 50% to 55% and solids are 45% to 50%. In females, water is less because of more amount of Subcutaneous adipose tissue.

In a normal young adult female, the water is 50% to 55% and solids are 45% to 50%. In females, water is less because of more amount of Subcutaneous adipose tissue. In thin persons, water content is more than that in obese persons. In old age, water content is decreased due to increase in adipose tissue.

Total quantity of body water in an average human being weighing about 70 kg is about 40 L.

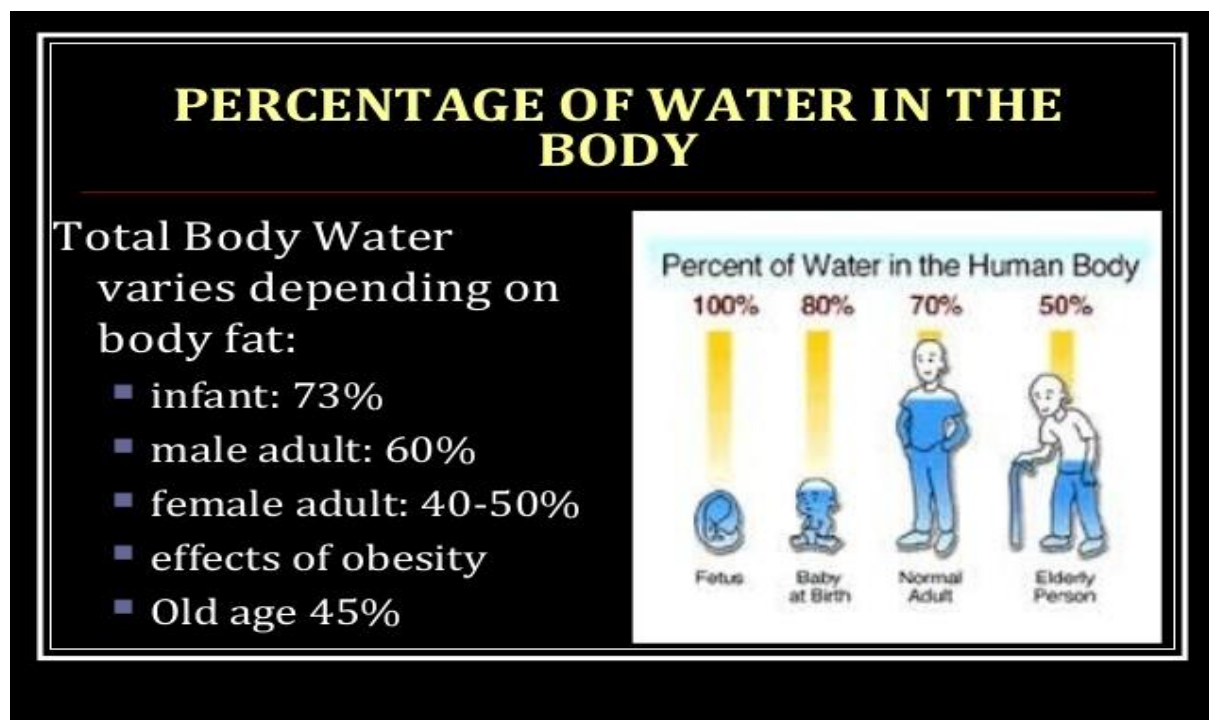


FIG. – 1 PERCENTAGE OF WATER IN HUMAN BODY

SIGNIFICANCE OF BODY FLUIDS

IN HOMEOSTASIS

It means keeping things constant and comes from two Greek words: 'homeo,' meaning 'similar,' and 'stasis,' meaning 'stable.')(Body cells survive in the fluid medium called **internalenvironment or 'milieu interieur'**

Internalenvironmentcontains substances such as glucose, amino acids, lipids, vitamins, ions, oxygen . which are essential for growth and functioning of the cell. Water not only forms the major constituent of internal environment but also plays an important role in homeostasis.

IN TRANSPORT MECHANISM

Body water forms the transport medium by which nutrients and other essential substances enter the cells; and unwanted substances come out of the cells.

Water forms an important medium by which various enzymes, hormones, vitamins, electrolytes, and other substances are carried from one part to another part of the body.

IN METABOLIC REACTIONS

Water inside the cells forms the medium for various metabolic reactions, which are necessary for growth and functional activities of the cells.

IN TEXTURE OF TISSUES

Water inside the cells is necessary for characteristic form and texture of various tissues

IN TEMPERATURE REGULATION

Water plays a vital role in the maintenance of normal body temperature.

DISTRIBUTION OF WATER / BODY FLUIDS IN HUMAN BODY

Total water in the body is about 40 L. It is distributed into two major compartments

1. **Intracellular fluid (ICF):** Its volume is 22 L and it forms 55% of the total body water.

2. **Extracellular fluid (ECF):** Its volume is 18 L and it forms 45% of the total body water.

ECF is divided into 5 subunits:

i. Interstitial fluid and lymph (20%)

ii. Plasma (7.5%)

iii. Fluid in bones (7.5%)

iv. Fluid in dense connective tissues like cartilage (7.5%) .

Transcellular fluid (2.5%) that includes:

a. Cerebrospinal fluid

b. Intraocular fluid –

c. Digestive juices

d. Serous fluid –intrapleural fluid, spaces of the lungs. pericardial fluidand peritoneal fluid.
an abnormal accumulation of protein and electrolyte rich fluid in the peritoneal cavity

f. Fluid in urinary tract.

Volume of interstitial fluid is about 12 L.

Volume of plasma is about 2.75 L. Volume of other subunits of ECF is about 3.25 L. Water moves between different compartments.

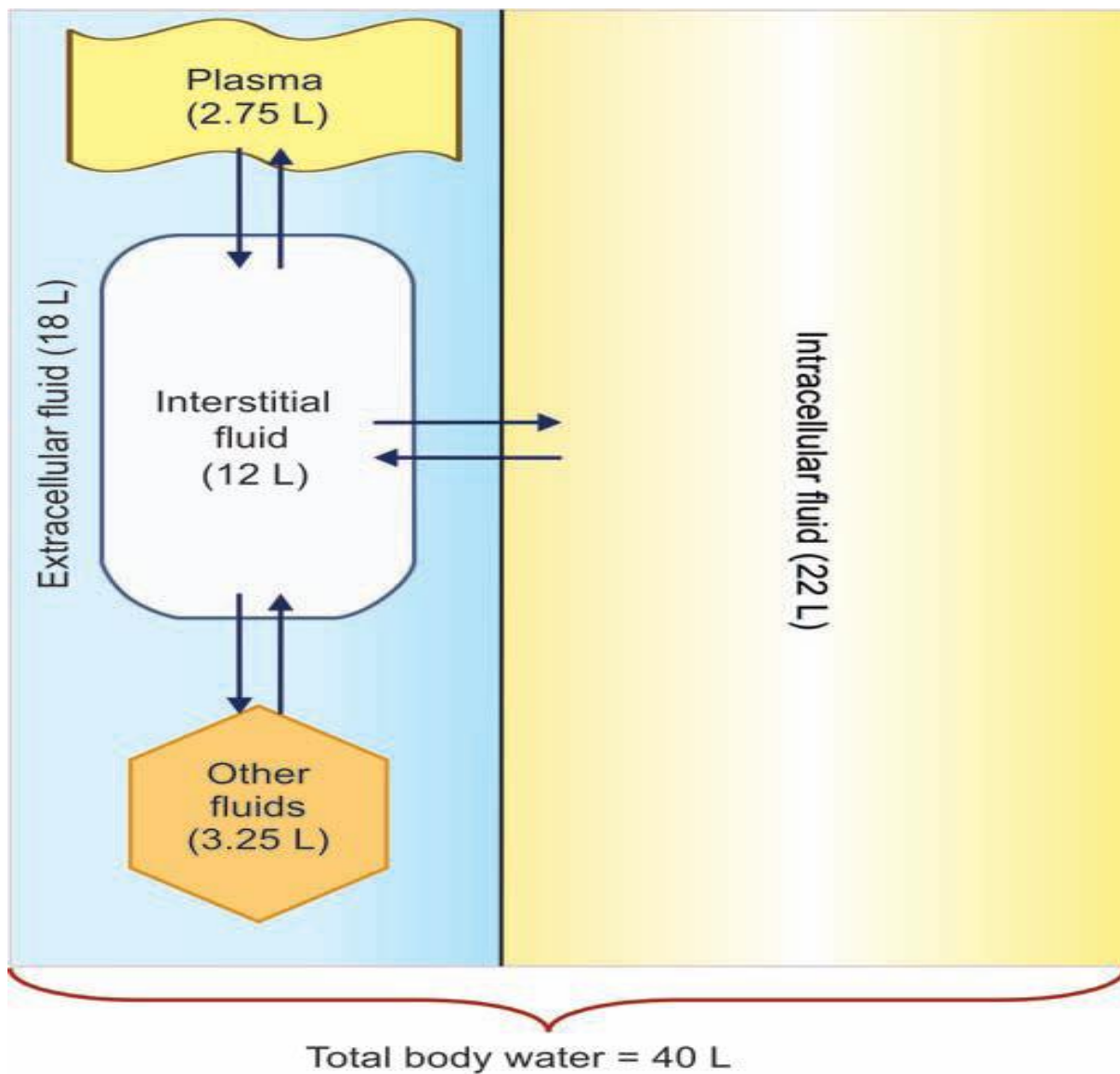


FIG -2 VOLUME OF FLUID

COMPOSITION OF BODY FLUIDS

Body fluids contain water and solids. Solids are organic and inorganic substances.

ORGANIC SUBSTANCES

Organic substances are glucose, amino acids and other proteins, fatty acids and other lipids, hormones and enzymes.

INORGANIC SUBSTANCES

Inorganic substances present in body fluids are sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate and sulfate. ECF contains large quantity of sodium, chloride, bicarbonate.

ECF contains large quantity of sodium, chloride, bicarbonate, glucose, fatty acids and oxygen. a carboxylic acid consisting of a hydrocarbon chain and a terminal carboxyl group, especially any of those occurring as esters in fats and oils ICF contains large quantities of potassium, magnesium, phosphates, sulphates and proteins. The pH of ECF is 7.4.

The pH of ICF is 7.0

Total body water and the volume of different compartments of the body fluid are measured by indicator dilution method or dye dilution method . In 1824 Hering introduced an **indicator-dilution method** for measuring blood velocity.

INDICATOR DILUTION METHOD

Principle

A known quantity of a substance such as a dye is administered into a specific body fluid compartment. These substances are called the marker substances or indicators.

After administration into the fluid compartment, the substance is allowed to mix thoroughly with the fluid. Then, a sample of fluid is drawn and the concentration of the marker substance

is determined. Radioactive substances or other substances whose concentration can be determined by using colorimeter are generally used as marker substances.

Formula to Measure the Volume of Fluid by Indicator Dilution

Method

Quantity of fluid in the compartment is measured using the formula

$$V = \frac{M}{C}$$

V = Volume of fluid in the compartment.

M = Mass or total quantity of marker substance injected.

C = Concentration of the marker substance in the sample fluid.

Correction factor

Some amount of marker substance is lost through urine during distribution.

So, the formula is corrected as follows:

M - Amount of substance excreted

$$\text{Volume} = \frac{\text{M} - \text{Amount of substance excreted}}{C}$$

Uses of Indicator Dilution Method

Indicator dilution or dye dilution method is used to measure ECF volume, plasma volume and the volume of total body water.

Characteristics of Marker Substances

Dye or any substance used as a marker substance should have the following qualities:

1. Must be nontoxic
2. Must mix with the fluid compartment thoroughly within reasonable time.
3. Should not be excreted rapidly.
4. Should be excreted from the body completely within reasonable time.
5. Should not change the color of the body fluid.
6. Should not alter the volume of the body fluid.

Marker Substances Used to Measure Fluid Compartments

Marker substances used to measure different fluid.

MEASUREMENT OF TOTAL BODY WATER

Volume of total body water (fluid) is measured by using a marker substance which is distributed through all the compartments of body fluid.

Deuterium oxide and tritium oxide mix with fluids Triturated water is a radioactive form of water where the usual hydrogen atoms are replaced with tritium. In its pure form it may be called tritium oxide of all the compartments within few hours after injection.

Since plasma is part of total body fluid, the concentration of marker substances can be obtained from sample of plasma. The formula for indicator dilution method is applied to calculate total body water. Antipyrine is also used to measure total body water.

But as it takes longer time to penetrate various fluid compartments, the value obtained is slightly low.

MEASUREMENT OF EXTRACELLULAR FLUID VOLUME

Substances which pass through the capillary membrane but do not enter the cells, are used to measure ECF volume. Such marker substances are listed in Table 6.2.

These substances remain only in ECF and do not enter the cell (ICF). When any of these substances is injected into blood, it mixes with the fluid of all sub compartments of ECF within 30 minutes to 1 hour. Indicator dilution method is applied to calculate ECF volume. Since ECF includes plasma, the concentration of marker substance can be obtained in the sample of plasma.

Some of the marker substances like sodium, chloride, insulin and sucrose diffuse more evenly throughout all sub compartments of ECF. So, the measured volume of ECF by using these substances is referred as **Sodium space, chloride space, inulin space and sucrose space.**

MEASUREMENT OF INTRACELLULAR FLUID VOLUME

Volume of ICF cannot be measured directly. It is calculated from the values of total body water and ECF.

ICF volume = Total fluid volume – ECF volume.

Concentration of body fluids is expressed in three ways:

1. Osmolality **effects this distribution of water through the generation of osmotic** For example, a solution of 1 mol/L NaCl corresponds to an osmolarity of 2
2. Osmolarity the concentration of a solution expressed as the total number of solute particles per litre.

Tonicity, which is directly related to the osmolarity of a solution, affects osmosis by determining the direction

Body fluid compartments and movement of fluid between different compartments. Other fluids = Transcellular fluid, fluid in bones and fluid in connective tissue.

OSMOLALITY

Measure of a fluid's capability to create osmotic pressure is called osmolality or osmotic (osmolar) concentration of a solution.

In simple words, it is the concentration of osmotically active substance in the solution.

Osmolality is expressed as the number of particles (osmoles)

Per kilogram of solution (osmoles/kg H₂O). the concentration of a solution in terms of osmoles of solutes per kilogram of solvent. The normal value for serum osmolality is 270–300 **mOsm/kg water**.

Osmolarity is another term to express the osmotic concentration. It is the number of particles (osmoles) per liter of solution (osmoles/L). Osmotic pressure in solutions depends upon osmolality. However, in practice, the osmolarity and not osmolality is considered to determine the osmotic pressure because of the following reasons:

- i. Measurement of weight (kilogram) of water in solution is a difficult process.
- ii. Difference between osmolality and osmolarity is very much negligible and it is less than 1%. Often, these two terms are used interchangeably. Change in osmolality of ECF affects the volume of both ECF and ICF. When osmolality of ECF increases, water moves from ICF to ECF. When the osmolality decreases in ECF, water moves from ECF to ICF.

Water movement continues until the osmolality of these two fluid compartments becomes equal.

Mole and Osmole

A mole (mol) is the molecular weight of a substance in gram. Millimole (mMol) is 1/1000 of a mole. One osmole (Osm) is the expression of amount of osmotically Active particles. It is the molecular weight of a substance in grams divided by number of freely moving particles liberated in solution of each molecule. One milliosmole (mOsm) is 1/1000 of an osmole.

TONICITY

Usually, movement of water between the fluid compartments is not influenced by small molecules like urea and alcohol, which cross the cell membrane very rapidly. These small molecules are called ineffective osmoles. On the contrary, the larger molecules like sodium and glucose, which cross the cell membrane slowly, can influence the movement of water. Therefore, such molecules are called effective osmoles. Osmolality that causes the movement of water from one compartment to another is called effective osmolality and the effective osmoles are responsible for this. Tonicity is the measure of effective osmolality.

In terms of tonicity, the solutions are classified into three categories:

i. Isotonic fluid a solution having the same osmotic pressure as blood.

isotonic solutions are 0.9% sodium chloride, commonly called normal saline (NS),

ii. Hypertonic fluid having a higher osmotic pressure than a particular fluid, typically a body fluid or intracellular fluid. or in a state of abnormally high muscle tone.

iii. Hypotonic fluid. A solution having a higher osmotic pressure than a comparison solution .

i. *Isotonic Fluid*

ii. Fluid which has the same effective osmolality (tonicity) as body fluids is called isotonic fluid.

iii. Examples are 0.9% sodium chloride solution (normal saline) and 5% glucose solution.

iv. Red blood cells or other cells placed in isotonic fluid

v. (normal saline) neither gain nor lose water by osmosis .

- vi. This is because of the **osmotic equilibrium** between inside and outside the cell across the cell membrane.

ii. Hypertonic Fluid

Fluid which has greater effective osmolality than the body fluids is called hypertonic fluid. Example is 2% sodium chloride solution. When red blood cells or other cells are placed in hypertonic fluid, water moves out of the cells (exosmosis) resulting in shrinkage of the cells (crenation).

iii. Hypotonic Fluid

Fluid which has less effective osmolality than the body fluids is called hypotonic fluid. Example is 0.3% sodium chloride solution. When red blood cells or other cells are placed in hypotonic fluid, water moves into the cells (endosmosis) and causes swelling of the cells .

Now the red blood cells become globular (spherocytic) and get ruptured (haemolysis).

MAINTENANCE OF WATER BALANCE

Body has several mechanisms which work together to maintain the water balance. The important mechanisms involve hypothalamus and kidneys.

Effect of isotonic, hypertonic and hypotonic solutions on red blood cells-

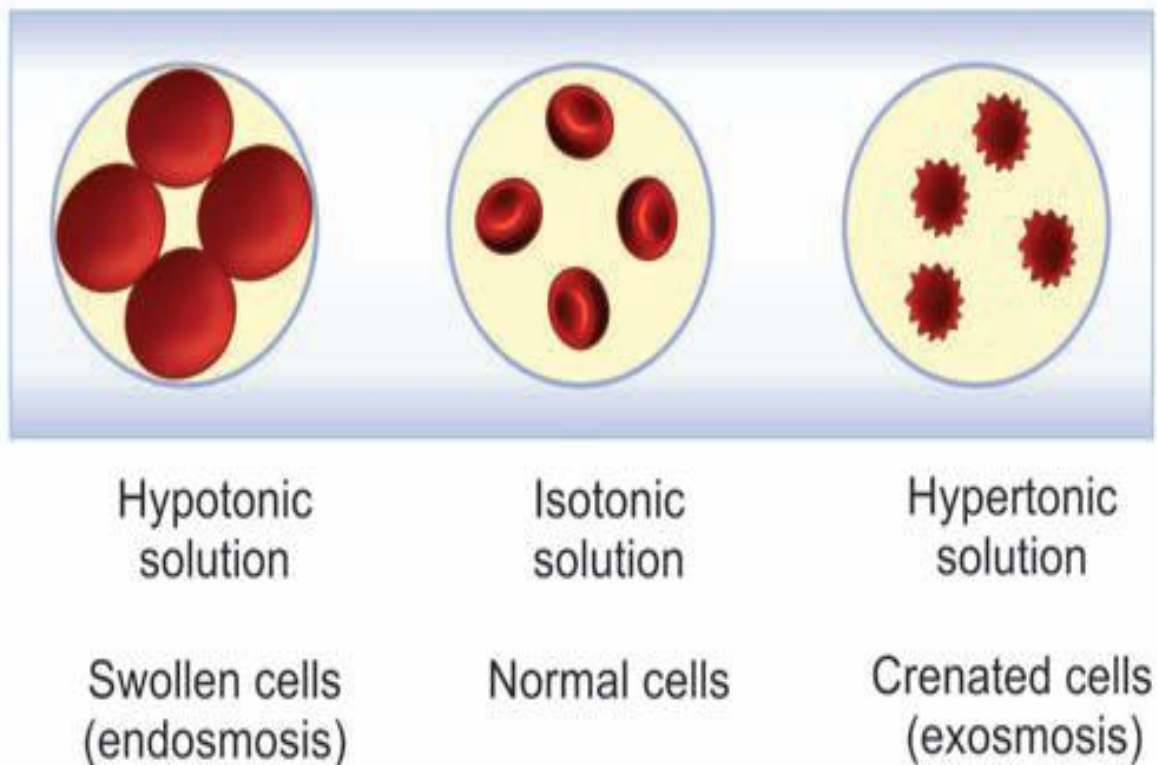


FIG -3 EFFECTS OF TONICITY ON RED BLOOD CELLS

APPLIED PHYSIOLOGY

DEHYDRATION

Dehydration is defined as excessive loss of water from the body. Body requires certain amount of fluid intake daily for normal functions. Minimum daily requirement of water intake is about 1 L. This varies with the age and activity of the individual. The most active individuals need 2 to 3 L of water intake daily. Dehydration occurs when fluid loss is more than what is consumed.

Basically, dehydration is of three types:

1. ***Mild dehydration:*** *It occurs when fluid loss is about 5% of total body fluids.*

Dehydration is not very serious and can be treated easily by rehydration.

2. ***Moderate dehydration:*** *It occurs when fluid loss is about 10%. Dehydration becomes little serious and immediate treatment should be given by rehydration.*

3. ***Severe dehydration:*** *It occurs when fluid loss is about 15%. Dehydration becomes severe and requires hospitalization and emergency treatment. When fluid loss is more than 15%, dehydration becomes very severe and life threatening.*

On the basis of ratio between water loss and sodium loss, dehydration is classified into three types:

1. ***Isotonic dehydration:*** *Balanced loss of water and sodium as in the case of diarrhea or vomiting.*

2. ***Hypertonic dehydration:*** *Loss of more water than sodium as in the case of fever.*

3. ***Hypotonic dehydration:*** *Loss of more sodium than water as in the case of excess use of diuretics.*

Causes

1. Severe diarrhoea and vomiting due to gastrointestinal disorders
2. Excess urinary output due to renal disorders
3. Excess loss of water through urine due to endocrine disorders such as diabetes mellitus, diabetes insipidus and adrenal Insufficiency.
4. Insufficient intake of water
5. Prolonged physical activity without consuming adequate amount of water in hot environment.
6. Excess sweating leading to heat frustration (extreme loss of water, heat and energy). Severe sweating and dehydration occur while spending longer periods on regular basis in the saunas. Apply to hot rocks. Voila, wet **sauna**. That's it. A wet **sauna** experience is simply when you pour water over the heater rocks, thus producing steam. Thus, a **dry sauna** is the absence of any humidity.
7. Use of laxatives or diuretics in order to lose weight quickly. This is common in athletes.

Signs and Symptoms

Mild and moderate dehydration

1. Dryness of the mouth
2. Excess thirst
3. Decrease in sweating
4. Decrease in urine formation
5. Headache
6. Dizziness

7. Weakness
8. Cramps in legs and arms

Severe dehydration

1. Decrease in blood volume
2. Decrease in cardiac output
3. Low blood pressure
4. Hypovolemic cardiac shock
5. Fainting.

Very severe dehydration

1. Damage of organs like brain, liver and kidneys
2. Mental depression and confusion
3. Renal failure
4. Convulsions
5. Coma.

Dehydration in Infants

Infants suffering from severe diarrhoea and vomiting caused by bacterial or viral infection, develop dehydration. It becomes life threatening if the lost body fluids are not replaced. This happens when parents are unable to recognize the signs.

Aging Effects on Dehydration

Elders are at higher risk for dehydration even if they are healthy. It is because of increased fluid loss and decreased fluid intake. In some cases, severe dehydration in old age may be fatal.

TREATMENT

Treatment depends upon the severity of dehydration. In mild dehydration, the best treatment is drinking of water and stopping fluid loss. However, in severe dehydration drinking water alone is ineffective because it cannot compensate the salt loss. So the effective treatment for severe dehydration is oral rehydration therapy. Oral rehydration therapy (ORT) is the treatment for dehydration in which **oral rehydration solution (ORS)** is administered orally.

ORS was formulated by World Health Organization (WHO). This solution contains anhydrous glucose, sodium chloride, potassium Chloride and trisodium citrate. In case of very severe dehydration, proper treatment is the intravenous administration of necessary water and electrolytes.

WATER INTOXICATION OR OVERHYDRATION

Water intoxication is the condition characterized by great increase in the water content of the body. It is also called over hydration, hyper hydration, water excess or Water poisoning.

Causes

Water intoxication occurs when more fluid is taken than that can be excreted. Water intoxication due to drinking excess water is rare when the body's systems are functioning normally. But there are some conditions that can produce water intoxication.

1. Heart failure in which heart cannot pump blood properly .
2. Renal disorders in which kidney fails to excrete enough water in urine.

3. Hyper secretion of antidiuretic hormone as in the case of **syndrome of inappropriate hyper secretion of antidiuretic hormone (SIADH)**.

4. Intravenous administration of unduly large amount of medications and fluids than the person's body can excrete.

5. Infants have greater risk of developing water intoxication in the first month of life, when the filtration mechanism of the kidney is underdeveloped and cannot excrete the fluid rapidly .

6. Water intoxication is also common in children having swimming practice, since they are more prone to drink too much of water while swimming.

7. An adult (whose heart and kidneys are functioning normally) can develop water intoxication, if the person consumes about 8 L of water everyday regularly.

Signs and Symptoms

1. Since the brain is more vulnerable to the effects of water intoxication, behavioural changes appear first.

2. Person becomes drowsy and inattentive.

3. Nausea and vomiting occur.

4. There is sudden loss of weight, followed by weakness and blurred vision.

5. Anaemia, acidosis, cyanosis, haemorrhage and shock are also common.

6. Muscular symptoms such as weakness, cramps, twitching, poor coordination, and paralysis develop.

7. Severe conditions of water intoxication result in:

i. Delirium (extreme mental condition characterized by confused state and illusion)

ii. Seizures (sudden uncontrolled involuntary muscular contractions) **loss of consciousness**

ii. Coma (profound state of unconsciousness, in which the person fails to respond to external stimuli and cannot perform voluntary actions).

TREATMENT

Mild water intoxication requires only fluid restriction. In very severe cases, the treatment includes:

1. Diuretics to increase water loss through urine.
2. Antidiuretic hormone (ADH) receptor antagonists to prevent ADH-induced re absorption of water from renal tubules.

In general these drugs produce stimulant and convulsant effects,

1. Intravenous administration of saline to restore Sodium.

BLOOD

Blood is a connective tissue in fluid form. It is considered as the 'fluid of life' because it carries oxygen from lungs to all parts of the body and carbon dioxide from all parts of the body to the lungs. It is known as 'fluid of growth' because it carries nutritive substances from the digestive system and hormones from endocrine gland to all the tissues. The blood is also called the 'fluid of health' because it protects the body against the diseases and gets rid of the waste products and unwanted substances by transporting them to the excretory organs like kidneys.

1. Color: *Blood is red in color. Arterial blood is scarletred because it contains more oxygen and venous blood is purple red because of more carbon dioxide.*

2. Volume: *Average volume of blood in a normal adult is 5 L. In a newborn baby, the volume is 450 ml. It increases during growth and reaches 5 L at the time of puberty. In females, it is slightly less and is about 4.5 L. It is about 8% of the body weight in a normal young healthy adult, weighing about 70 kg.*

3. Reaction and pH: *Blood is slightly alkaline and its pH in normal conditions is 7.4.*

4. Specific gravity:

Specific gravity of total blood : 1.052 to 1.061

Specific gravity blood cells : 1.092 to 1.101

Specific gravity of plasma : 1.022 to 1.026

5. Viscosity: *Blood is five times more viscous than water. It is mainly due to red blood cells and plasma proteins.*

COMPOSITION OF BLOOD

Blood contains the blood cells which are called formed elements and the liquid portion known as plasma.

BLOOD CELLS

Three types of cells are present in the blood:

1. Red blood cells or erythrocytes
2. White blood cells or leukocytes
3. Platelets or thrombocytes.

COMPOSITION OF BLOOD

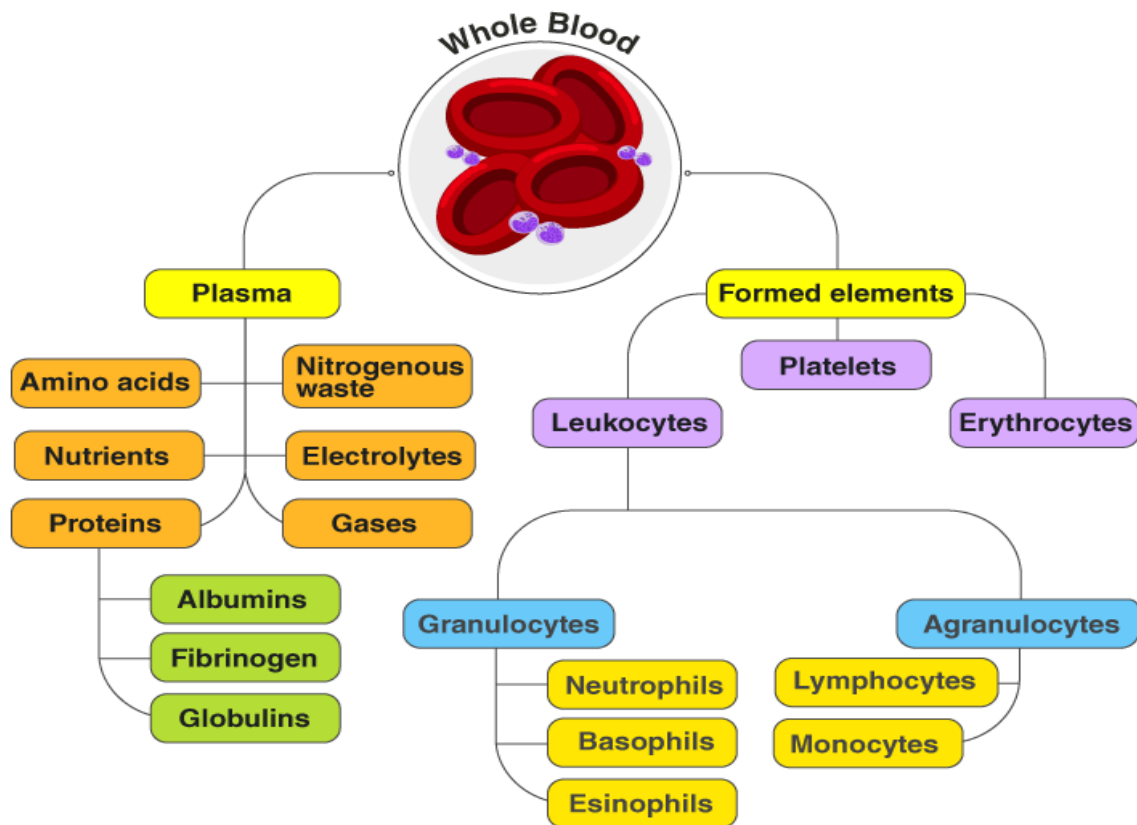


FIG -4 COMPOSITION OF BLOOD

HAEMATOCRIT VALUE

If blood is collected in a haematocrit tube along with a suitable anticoagulant and centrifuged for 30 minutes at a speed of 3000 revolutions per minute (rpm), the red blood cells settle down at the bottom having a clear plasma at the top. Plasma forms 55% and red blood cells form 45% of the total blood. Volume of red blood cells expressed in percentage is called the

haematocrit value or packed cell volume (PCV). In between the plasma and the red blood cells, there is a thin layer of **white buffy coat**. **This white buffy coat is formed by the aggregation of white blood cells and platelets.**

PLASMA

Plasma is a straw-coloured clear liquid part of blood. It contains 91% to 92% of water and 8% to 9% of solids. The solids are the organic and the inorganic substances (Fig. 7.1). Table

7.1 gives the normal values of some important substances in blood. Serum is the clear straw-coloured fluid that oozes from blood clot. When the blood is shed or collected in a container, it clots. In this process, the fibrinogen is converted into fibrin and the blood cells are trapped in this fibrin forming the blood clot. After about 45 minutes, serum oozes out of the blood clot.

For clinical investigations, serum is separated from blood cells and clotting elements by centrifuging. Volume of the serum is almost the same as that of plasma (55%). It is different from plasma only by the absence of fibrinogen, i.e. serum contains all the other constituents of plasma except fibrinogen. Fibrinogen is absent in serum because it is converted into fibrin during blood clotting.

Thus, Serum = Plasma – Fibrinogen

FUNCTIONS OF BLOOD

1. NUTRITIVE FUNCTION

Nutritive substances like glucose, amino acids, lipids and vitamins derived from digested food are absorbed from gastrointestinal tract and carried by blood to different parts of the body for growth and production of energy.

2. RESPIRATORY FUNCTION

Transport of respiratory gases is done by the blood. It carries oxygen from alveoli of lungs to different tissues and carbon dioxide from tissues to alveoli.

3. EXCRETORY FUNCTION

Waste products formed in the tissues during various metabolic activities are removed by blood and carried to the excretory organs like kidney, skin, liver, etc. for excretion.

4. TRANSPORT OF HORMONES AND ENZYMES

Hormones which are secreted by ductless (endocrine) glands are released directly into the blood. The blood transports these hormones to their target organs/tissues.

Blood also transports enzymes.

5. REGULATION OF WATER BALANCE

Water content of the blood is freely interchangeable with interstitial fluid. This helps in the regulation of water content of the body.

6. REGULATION OF ACID-BASE BALANCE

Plasma proteins and haemoglobin act as buffers and help in the regulation of acid-base balance

7. REGULATION OF BODY TEMPERATURE

Because of the high specific heat of blood, it is responsible for maintaining the thermoregulatory mechanism in the body, i.e. the balance between heat loss and heat gain in the body.

8. STORAGE FUNCTION

Water and some important substances like proteins, glucose, sodium and potassium are constantly required by the tissues. Blood serves as a readymade source for these substances. And, these substances are taken from blood during the conditions like starvation, fluid loss, and electrolyte loss.

9. DEFENSIVE FUNCTION

Blood plays an important role in the defence of the body. The white blood cells are responsible for this function. Neutrophils and monocytes engulf the bacteria by phagocytosis. Lymphocytes are involved in development of immunity. Eosinophils are responsible for detoxification, disintegration, and removal of foreign proteins.

PLASMA PROTEINS

Plasma proteins are:

1. Serum albumin
2. Serum globulin
3. Fibrinogen. Serum contains only albumin and globulin. Fibrinogen is absent in serum because, it is converted into fibrin during blood clotting. Because of this, the albumin and globulin are usually called serum albumin and serum globulin.



Major Types:

- Albumin (60%)**
Major component of osmotic pressure of plasma
- Globulins (35%)**
Antibodies (immunoglobulin) and transport proteins
- Fibrinogens (4%)**
Functions in blood clotting
- Other (<1%)**
Various roles (α -1-antitrypsin, coagulation factors, etc.)

FIG -5 TYPES OF PLASMA

NORMAL VALUES OF PLASMA

Normal values of the plasma proteins are:

- Total proteins : 7.3 g/dL (6.4 to 8.3 g/dL)
- Serum albumin : 4.7 g/dL
- Serum globulin : 2.3 g/dL
- Fibrinogen : 0.3 g/dL

ALBUMIN/GLOBULIN RATIO

Ratio between plasma level of albumin and globulin is called albumin/globulin (A/G) ratio. It is an important indicator of some diseases involving liver or kidney.

Normal A/G ratio is 2 : 1.

Plasma proteins are separated by the following methods.

1. PRECIPITATION METHOD

Proteins in the serum are separated into albumin and globulin. This is done by precipitating globulin with 22% sodium sulphate solution. Albumin remains in solution.

2. SALTING-OUT METHOD

Serum globulin is separated into two fractions called **euglobulin** and **pseudo globulin** by **salting out with different** solutions. Euglobulin is salted out by full saturation with sodium chloride solution; half saturation with magnesium sulphate solution and one third saturation with ammonium sulphate solution. It is insoluble in water. Pseudo globulin is salted out by full saturation with magnesium sulphate and, half saturation with ammonium sulphate. It is soluble in water, but it cannot be salted out by sodium chloride solution

3. ELECTROPHORETIC METHOD

In this, the plasma proteins are separated depending on their differences in electrical charge and the rate of migration. It is done in a **Tiselius apparatus** by using paper or cellulose or

starch block. By this method, the proteins are separated into albumin (55%), alpha globulin (13%), beta globulin (14%), gamma globulin (11%) and fibrinogen (7%).

4. COHN'S FRACTIONAL

PRECIPITATION METHOD

By this method, plasma proteins are separated into albumin and different fractions of globulin, depending upon their solubility.

5. ULTRACENTRIFUGATION METHOD

In this method, albumin, globulin and fibrinogen are separated depending upon their density. This method is also useful in determining the molecular weight of these proteins.

6. GEL FILTRATION CHROMATOGRAPHY

Gel filtration chromatography is a column chromatographic method by which the proteins are separated on the basis of size. Protein molecules are separated by passing through a bed of porous beads. The diffusion of different proteins into the beads depends upon their size.

7. IMMUNOELECTROPHORETIC METHOD

By this method, the proteins are separated on the basis of electrophoretic patterns formed by precipitation at the site of antigen antibody reactions. This technique provides valuable quantitative measurement of different proteins

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