

MODULE 2 : Body fluids

Biochemistry – Undergraduate Programme

Faculty of Medicine and Allied Sciences

Rajarata University of Sri Lanka

Broad Objectives

At the end of this course, a student is expected to know the,

- relationship between the structure and functions of water.
- constituents and some of the functions of blood and plasma & the cellular elements suspended in it.
- important cellular components in the RBC and their functions.
- structures and functions of normal haemoglobins and changes in the functions following changes in the structure of globin chains.
- plasma proteins, their functions and separation.
- lipoproteins, their structure and their functions.
- biochemical pathways and the mechanisms used in the regulation of blood glucose.
- blood analytes used in clinical diagnosis.

1. Structure and Functions of Water

- 1.1 Recall the molecular structure of water and its distribution in liquid and solid phases.
- 1.2 Be aware of the dielectric constant and specific heat of water and explain their function in dissolution of ionic and polar molecules and heat distribution in the body.
- 1.3 Recall that water has a high heat of vaporization and that this can be used for the purpose of body cooling.
- 1.4 Recall the function of water as a medium for enzymatic reactions and the inhibition of their activity following dehydration.
- 1.5 Explain how water acts as a medium of transport for both water and lipid soluble substances.
- 1.6 Recall that water has the highest density at 4 °C and that biomolecules are more stable at this temperature than at 0 °C.

2. Blood

- 2.1 Recall that blood is a tissue comprising of plasma and cellular elements suspended in it.
- 2.2 Recall the average composition of the different types of cellular elements in the blood and their functions.
- 2.3 Recall the composition and the function of the constituents present in the blood plasma.
- 2.4 Explain the protective function of myeloperoxidase in the white blood cells.
- 2.5 Recall that prostaglandins derived from arachidonic acid comprises of thromboxanes, leukotrienes and prostacyclins and knows that they differ in

structure due to the differences in the configuration of their cyclopentane ring and their side chains.

- 2.6 Recall that neutrophils and monocytes release various types of prostaglandins.
- 2.7 Know the opposing action of thromboxanes and prostacyclins on platelet aggregation and the effect of aspirin and indomethacin.
- 2.8 Describe the role played by prostaglandins in atherosclerosis.

3. Red Blood Cells

- 3.1 Describe the unique structure of the red blood cells and the mechanisms that support to perform its function.
- 3.2 Recall the pathways used by glucose in providing energy and their importance in cell function.
- 3.3 Recall the importance of red cell acid phosphatase in diagnosis of red cell damage.
- 3.4 Recall the impairment of red cell function caused by structural variants of haemoglobin.
- 3.5 Recall the different types of haemoglobins present in foetal and adult red blood cells and how these can be identified in the laboratory.
- 3.6 Describe how HbA and HbF efficiently function at high and low pO₂ respectively, and the factors that regulate them.
- 3.7 Recall the structure of the red blood cell membrane and explain the biochemical basis of blood groupings A, B, AB & O.
- 3.8 Recall the biochemical pathways and the nutrients that contribute towards the maintenance of the integrity of the membrane and explain the biochemical basis of their actions.

4. Haemoglobin

- 4.1 Draw and describe the structural features of the haemoglobin molecule that depict its function.
- 4.2 List the haemoglobins in a neonate, one year old child and an adult, indicating the polypeptide chains present in each.
- 4.3 Recall that haem is synthesised using glycine and succinate in the mitochondria and that it is regulated at the ALA synthase step.
- 4.4 Recall that different non-allelic genes control the synthesis of the different polypeptide chains and that for each polypeptide chain there are two allelic genes, one from each parent.
- 4.5 Recall that haem binds to free alpha-beta dimers to form tetrameric haemoglobin.
- 4.6 Recall that haem stimulates the aggregation of polysomes and the synthesis of the globin chains.
- 4.7 Recall that alpha and beta globin chain synthesis is under feed back inhibition by free monomeric and dimeric chains.
- 4.8 Outline the steps involved in the catabolism of Hb listing the sites at which the degradations occur.
- 4.9 Diagrammatically illustrate how O₂ is reversibly bound to free Fe⁺⁺ in haem indicating the molecular forces that enable the iron to exist in the ferrous state.
- 4.10 Explain the difference between oxygenation and oxidation of haem.

- 4.11 Describe how oxygenated Hb may be differentiated from non-oxygenated (reduced) Hb using the spectroscope.
- 4.12 Draw the O₂-Hb equilibrium curve in an adult and explain the advantage of having a sigmoidal curve.
- 4.13 Explain the differences in the O₂- Hb equilibrium curves of Mb, HbA & HbF.
- 4.14 Describe the conditions and compounds that (a) decrease, (b) increase, the affinity of Hb for oxygen.
- 4.15 Explain the buffering action of haemoglobin and the effect of oxygenation on buffering.
- 4.16 List the factors that affect the concentration of 2,3 - DPG in the erythrocyte & explain the role of 2,3 DPG towards Hb-O₂ affinity.
- 4.17 Explain how HbF is designed to function better in a hypoxic environment in comparison to HbA.
- 4.18 State how carboxyhaemoglobin and methaemoglobin interfere with tissue respiration.
- 4.19 State how carboxyhaemoglobin may be identified.
- 4.20 Recall the polypeptide composition of the normal haemoglobins HbA, HbA₂, HbF, and embryonic Hb.
- 4.21 Recall that there could be variants of any of these haemoglobins depending on the amino acid sequences of the affected polypeptide chain.
- 4.22 Explain 'point mutation' using HbS and HbC as examples.
- 4.23 Describe the likely changes expected in the solubility & the oxygen carriage of Hb and the shape & mobility of erythrocytes, in the presence of abnormal haemoglobins.
- 4.24 Explain the differences in the globin genes expressed, the type and proportion of haemoglobins present in the sickle cell trait and sickle cell anaemia.
- 4.25 Explain the morphological changes seen in erythrocytes containing HbS based on the structure of the haemoglobin present.
- 4.26 Explain the shortening of red cell life span in HbS and HbC disease
- 4.27 Explain the occurrence of inclusion bodies in erythrocytes containing some abnormal haemoglobin.
- 4.28 Recall that thalassaemia is due to an imbalance in the rate of synthesis of the two chains in normal haemoglobin and know how such changes arise.
- 4.29 Describe the likely changes seen in the blood film of a thalassaemic. Explain the differences in the incidence and the survival of alpha and beta thalassaemics.
- 4.30 State the difference in the blood methaemoglobin level in those having abnormal haemoglobins, giving the likely reason for such a difference.
- 4.31 State the normal concentration of methaemoglobin in blood and how methaemoglobin may be identified in the blood.
- 4.32 State the causes of acquired methaemoglobinaemia and describe the mechanisms available to overcome such a disorder.

5. Plasma Proteins

5.1 General

- 5.1.1 Draw and label the electrophoresis separation of plasma proteins into
 - 5.1.1.1 albumin and globulins
 - 5.1.1.2 lipoproteins

and know the basis of their separation and identification.

- 5.1.2 Recall that plasma proteins are mostly synthesized in the liver and contributions are also made by the intestine and the plasma cells.
- 5.1.3 Recall that in pregnancy a decrease in serum albumin and increases in alpha1, alpha2 and β globulins are associated with increased demand.

5.2 Albumin

- 5.2.1 Explain why plasma albumin contributes nearly 80% towards intravascular osmotic pressure.
- 5.2.2 Explain the transport of certain nutrients, metabolites and drugs by albumin.
- 5.2.3 State the site of metabolism and the $t_{0.5}$ of albumin.
- 5.2.4 State the level of (a) total plasma proteins (b) albumin, at which oedema appears.
- 5.2.5 Recall what is meant by analbuminaemia and explain how a satisfactory osmotic pressure is maintained in a person with hypoalbuminaemia.
- 5.2.6 Recall that pre-albumin is a minor fraction of plasma protein and that it carries part of the circulating T4.

5.3 Alpha-1-Globulins

5.3.1 *Alpha antitrypsin*

- 5.3.1.1. Recall that it is the major fraction of alpha-globulins.
- 5.3.1.2. Explain its functions in the lungs and how a reduction will affect it.
- 5.3.1.3. Explain how it is structurally and functionally affected by smoking.

5.3.2 *Alpha lipoprotein*

- 5.3.2.1. Recall that high density lipoproteins belong to alpha – 1-globulins and at least two types, HDL₂ and HDL₃ are present.
- 5.3.2.2. Explain the function of HDL.

5.3.3 *Transcortin*

- 5.3.3.1 Recall that it transports cortisol.

5.3.4 *Thyroxine Binding Globulin (TBG)*

- 5.3.4.1 Recall that its concentration is low and that it transports most of the circulating T₃ and T₄.

5.3.5 *Alpha1 acid glycoprotein*

- 5.3.5.1 Recall that it is an acute phase protein and that it is elevated in inflammatory diseases.

5.4 Alpha-2-Globulins

5.4.1 *Haptoglobin*

- 5.4.2.1 Recall that there are genetic variations
- 5.4.2.2. Recall that Hb is made less toxic and removed from circulation rapidly by hepatocytes.
- 5.4.2.3. Recall that its level is reduced in haemolytic anaemia.

5.4.2. *Macroglobulin*

- 5.4.2.1. Recall that it acts as a proteinase inhibitor and has antithrombin activity.

5.4.3. *Caeruloplasmin*

- 5.4.3.1. Recall its importance in Cu metabolism.
- 5.4.3.2. Explain why it is referred to as a ferro-oxidase.

5.4.4. *Pre-beta lipoprotein (VLDL)*

- 5.4.4.1. List the constituents of this fraction and state the relative proportion of triglyceride and cholesterol in it.
- 5.4.5.2. State the function of VLDL and the significance of an increased concentration.

5.5 β Globulin

5.5.1. *Transferrin*

- 5.5.1.1. Recall that its t_{0.5} is 7-10 days.
- 5.5.1.2. State its function.
- 5.5.1.3. Explain how the level of transferrin and its saturation with iron changes in iron deficiency anaemia, pregnancy, haemochromatosis etc.
- 5.5.1.4. Explain how transferrin level can be used as an index of protein status.

5.5.2. *C-Reactive protein*

- 5.5.2.1. Know that it promotes phagocytosis.

5.5.3. *β Lipoprotein (LDL)*

- 5.5.3.1. State the composition of LDL.
- 5.5.3.2. Recall that it carries most of the circulating cholesterol.
- 5.5.3.3. Explain the role of LDL cholesterol in atherosclerosis and its prevention by antioxidants.
- 5.5.3.4. Explain the functions of LDL.

5.5.4 *Complement Factors*

- 5.5.4.1. Recall the factors and their functions.
- 5.5.4.2. Explain how they bring out cytolytic activity.

5.6 Fibrinogen

- 5.6.1. Describe the structure and the function of fibrinogen.
- 5.6.2. Describe the changes that occur in the fibrinogen molecule in clot formation.

- 5.6.3. Recall that three structural genes are involved in the synthesis of fibrinogen and that these are on the same chromosome and are coordinately regulated.

5.7 Immunoglobulins (Gamma globulins)

5.7.1. *Immune System*

- 5.7.1.1 State the two major components of the immune system and explain why they are called by these names. (See 5.5.4)
- 5.7.1.2. Recall that plasma cells are responsible for the synthesis of immunoglobulins.
- 5.7.1.3. Explain the general structure of an immunoglobulin.
- 5.7.1.4. Recall that it is the nature of the H-chain that determines the class of the immunoglobulin.
- 5.7.1.5. Recall that myelomas are tumours of plasma cells which produce excessive amount of a specific immunoglobulin.
- 5.7.1.6. Explain why only some myeloma proteins appear in urine and how these can be detected.
- 5.7.1.7. Explain how malnutrition affects immunity.

5.7.2 *IgG*

- 5.7.2.1. Recall that it is the most abundant (80%) immunoglobulin and the concentration is about 1g/dl.
- 5.7.2.2. Describe the structure, showing the constant & variable regions and the antigen binding sites.
- 5.7.2.3. Recall that L and H chains are synthesised as separate molecules and assembled within the plasma cell in to a mature tetrameric-glycoprotein.

5.7.3 *IgA*

- 5.7.3.1. State its location and function.
- 5.7.3.2. Recall that the IgA dimer secreted combines with another secreting protein of the adjacent epithelial cells and that this complex is resistant to proteolytic enzymes.
- 5.7.3.3 Explain why IgA synthesis is reduced in vitamin A deficiency.

5.7.4 *IgM*

- 5.7.4.1. Recall that it is a pentamer formed in association with a J-chain.
- 5.7.4.2. Recall that it is the type of antibody formed in the early stages of immunization and infection.
- 5.7.4.3. Explain why it is very effective as an agglutinating antibody.

5.7.5 *IgD*

- 5.7.5.1. Recall that it is expressed in human cord blood lymphocytes.

5.7.6 *IgE*

- 5.7.6.1. Recall that antigen-antibody reaction for this class of immunoglobulin results in the release of vasoactive amines and hence elicit symptoms of allergies.

- 5.7.6.2. Recall that histamine released helps in the ejection of parasites.
- 5.7.6.3. Recall that plasma IgE concentration is increased in parasitic infections.

6. Plasma Lipoproteins

- 6.1 List the different lipoproteins present in the post absorptive and fed states.
- 6.2 State the approximate composition of the lipids and proteins present in different lipoproteins.
- 6.3 State the site/s of origin of the different lipoproteins.
- 6.4 Explain the catabolism of the different lipoproteins.
- 6.5 Describe the electrophoretic pattern of lipoproteins and interpret any abnormalities.
- 6.6 Describe a method for the quantitative estimation of cholesterol in serum.
- 6.7 Describe the preparation of a subject for serum lipid profile.
- 6.8 Explain why intravenous injection of heparin into a person with hyperlipidaemia results in clearing of the hyperlipidaemia.
- 6.9 State the properties of lipoprotein lipase and the conditions necessary for its action.
- 6.10 Describe the action of lecithin cholesterol acyl transferase (LCAT).
- 6.11 Describe the changes in blood lipids and lipoproteins in old age, heart disease, diabetes, exercise, and smoking, ingestion of nicotinic acid and consumption of alcohol.
- 6.12 Explain the effects of dietary polyunsaturated fat and fibre on the plasma cholesterol level.

7. Blood Glucose Homeostasis

7.1 Regulation of blood glucose

- 7.1.1 Describe the routes of entry and exit of blood glucose.
- 7.1.2 Explain how the blood glucose level is maintained during the post-prandial, post-absorptive and prolonged starvation phases.
- 7.1.3 Recall that an interplay of a number of hormones is responsible for maintaining normoglycaemia.
- 7.1.4 Describe the mechanisms used by hormones listed under 8.1.3 to regulate the blood glucose level.
- 7.1.5 Explain how stress affects glucose metabolism in the blood.
- 7.1.6 Explain the significance of measuring the post-prandial blood glucose level in comparison to the fasting blood glucose level.
- 7.1.7 Explain the effect of ethanol ingestion on the blood glucose level in the (a) fasting state and (b) fed state.

7.2 Starvation

- 7.2.1 Describe the changes in the level of (a) ketone bodies, (b) amino acids, (c) free fatty acids, in the blood during starvation.
- 7.2.2 Recall the function of the glucose-alanine cycle in the production of glucose in the post absorptive phase.
- 7.2.3 Describe the importance of the glucose-fatty acid cycle in glucose homeostasis in the prolonged fasting phase.
- 7.2.4 Describe the hormones that regulate blood glucose in the fasting state.

8. Blood Analytes

- 8.1 Explain how the concentration of Na^+ , K^+ , and HCO_3^- is altered in acidosis and recall that their relative levels is a reflection of the intensity of the disorder.
- 8.2 Recall that trace elements Fe^{3+} , Cu^{2+} , and Zn^{2+} are associated with energy production, growth and reproduction and a reduction results in their inhibition.
- 8.3 Recall that there is an inverse relationship between Ca^{2+} and PO_4^{3-} and that a lowering of the Ca^{2+} concentration is associated with tetany.
- 8.4 Recall that an abnormality in glucose homeostasis is associated with an imbalance in insulin and other hormones.
- 8.5 Explain the use of urea and creatinine levels in assessing kidney function.
- 8.6 Recall that an elevation of bilirubin above 2.5 mg/dl results in jaundice and that direct and indirect bilirubin levels are used in differential diagnosis.
- 8.7 List the common conditions that result in elevated urate levels and explain the biochemical basis of their occurrence.
- 8.8 Giving reasons, explain the conditions that result in a lowering of the haemoglobin level.
- 8.9 Recall the conditions that result in a lowering of albumin / globulin ratio.
- 8.10 List the conditions that result in a lowering of albumin level.
- 8.11 Recall that infection and allergies result in an increase in gamma globulins and that myeloma protein appear as a distinct band in serum electrophoresis.
- 8.12 Recall that specific proteins are used as markers in the diagnosis of myocardial infarction and cancers of different origin.
- 8.13 Explain the biochemical basis of the use of isoenzymes in clinical diagnosis.
- 8.14 Describe the use of amylase, creatine phosphokinase, aminotransferases, transpeptidase, acid and alkaline phosphatase and lactate dehydrogenase in identifying the site of clinical disorder and its intensity.
- 8.15 Recall the use of total serum cholesterol, HDL cholesterol, LDL cholesterol and serum triacylglycerol in diagnosing lipid disorders.
- 8.16 Explain the use of T3, T4 and TSH measurement in the diagnosis of thyroid dysfunction.
- 8.17 Recall the use of FSH, LH and prolactin in the diagnosis of abnormalities in reproduction.
- 8.18 Recall the diurnal variation of cortisol and its use in the diagnosis of Cushing's syndrome and abnormalities in glucose metabolism.

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