EXPERIMENT 1

DETERMINATION OF GLUCOSE IN URINE (GLYCOSURIA)

Structure

1.1 INTRODUCTION

Glucose ($C_6H_{12}O_6$) is an essential monosaccharide for the human body since it is the primary respiratory substrate and thus the vital source of energy for all cells and tissues. Glucose is derived by breaking down fats, proteins, and carbohydrates obtained from the diet. It is also biosynthesized in liver through gluconeogenesis from non-carbohydrate substrates such as lactic acid, glycerol and glucogenic amino acids. Glucose is completely oxidized during cellular respiration to yield energy and generate the metabolic intermediates, which are further utilized in other metabolic processes. Thus, glucose metabolism plays a pivotal role in the entire physiology of a cell and is central to the general body metabolism. Excess glucose is stored in the form of glycogen, a branched polymer of d-glucose, majorly in the liver and in muscles and fat cells. It is readily mobilized from hepatocytes when required, depending upon glucose levels of blood and extra-hepatic cells.

Glucose homeostasis (Fig. 1.1) is tightly regulated with the help of pancreatic hormones insulin and glucagon, which maintain appropriate glucose levels in the blood. Disruption of glucose metabolism occurs in case of metabolic disorders, endocrine syndromes and certain other diseases. Glucose homeostasis disruption is manifested as conditions exhibiting abnormally low or high glucose levels, termed as hypoglycemia or hyperglycemia, respectively. Both these conditions can be assessed by determining the glucose levels in the blood.

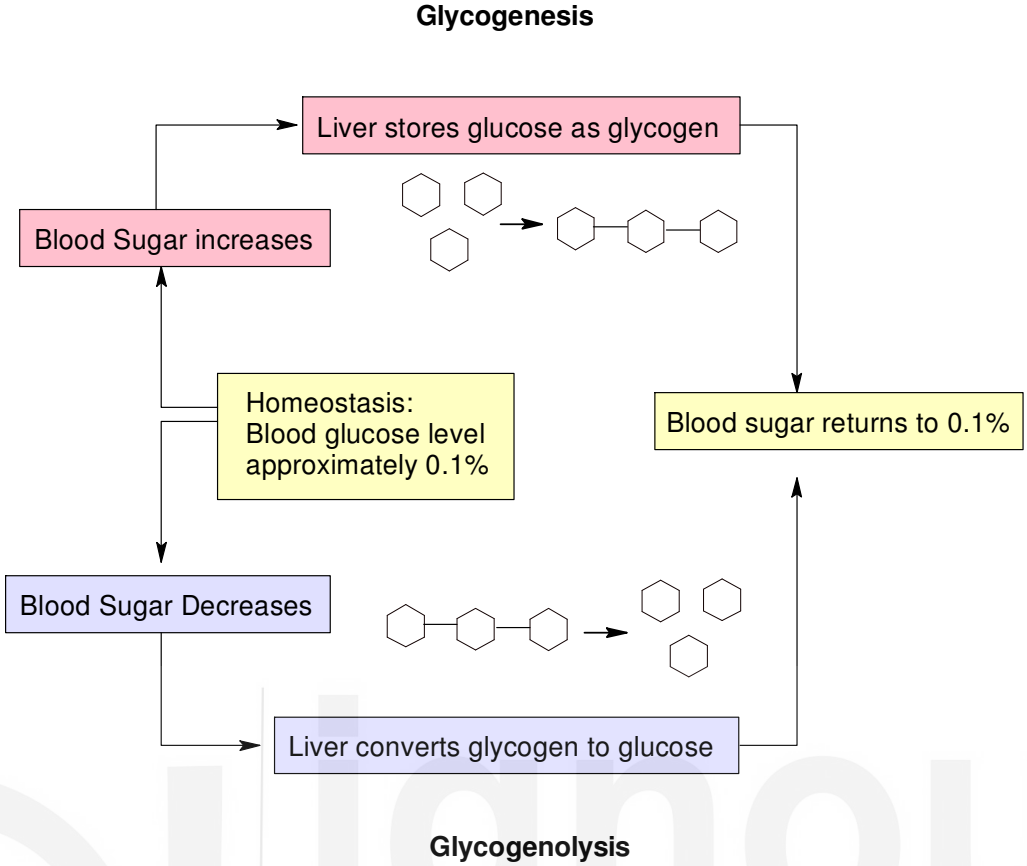

Fig. 1.1: Regulation of blood glucose through negative feedback.

Kidneys play a vital role in maintaining glucose homeostasis via three processes: gluconeogenesis in the renal cortex, glucose uptake from blood during filtration, and glucose reabsorption from the glomerular filtrate in proximal renal tubules. If the blood glucose level is maintained up to 180mg/dL, no glucose is excreted in the urine. Thus, the appearance of glucose in urine, also called glycosuria, is not normal and is indicative of conditions like Diabetes mellitus. During glycosuria, the blood sugar levels are also high, usually exceeding 180 mg/dl. Kidney malfunctioning can cause renal glycosuria in which the glucose is excreted in the urine, although the level of glucose is normal in the blood.

The biochemical measurement of glucose in the urine is an effective way of determining glycosuria, which is further helpful in the prognosis of several metabolic disorders and diseases.

Expected Learning Outcomes

After studying this unit, you should be able to:

- detect and quantify the amount of glucose in urine samples;
- assess the occurrence of the hyperglycemic condition;
- understand the biochemical nature of glucose as reducing sugar; and
- interpret its role in redox reactions in metabolism.

1.2 PRINCIPLE

Glucose is a monosaccharide and a reducing sugar, consisting of an aldehyde group that can readily undergo a redox reaction. The glucose in the urine sample can reduce Cu(II) to Cu(I),and this reaction is utilized for its quantification through Quantitative Benedict's reagent method. This method is a slightly modified version of qualitative Benedict's test. Quantitative Benedict's reagent consists of copper sulphate, potassium thiocyanate, potassium ferricyanide and Sodium citrate, along with Sodium carbonate. The copper sulphate gives Cu(II) ions in the solution, which are reduced by the glucose present in the sample, under alkaline conditions created by the addition of a liberal amount of sodium carbonate. Citrate chelates Cu(II) ions and prevents the formation of copper carbonate precipitate. Potassium thiocyanate forms a white precipitate of cuprous thiocyanate (CuSCN) instead of the red precipitate of cupric oxide, while potassium ferricyanide prevents the pre-oxidation of copper. The change of blue color of Cu(II) ions to white precipitate of Cu(I) thiocyanate marks the endpoint of the titration. It enables the quantitative determination of glucose in the sample (Fig. 1.2).

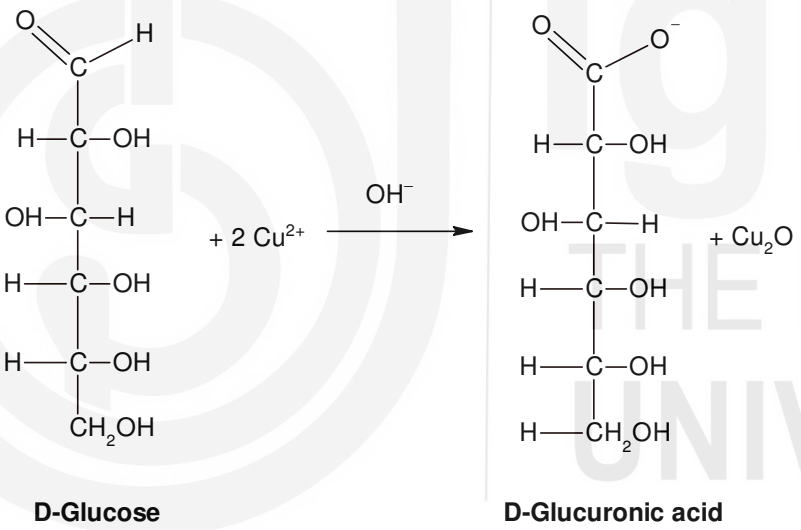

Fig. 1.2: The reaction of glucose with Benedict's solution.

1.3 MATERIALS REQUIRED

Chemicals

1. Sodium citrate 100g
2. Potassium thiocyanate 162.5g
3. Copper sulphate (18%) 9g
4. Potassium ferricyanide (5%) 0.5g
5. Sodium carbonate (anhydrous) 2g
6. Standard glucose solution 2 mg/ml
7. Glassware and other apparatus

1.4 PROTOCOL

Preparation of Quantitative Benedict's reagent:

1. Weigh 100 mg of sodium citrate and 62.5 g of potassium thiocyanate and dissolve in 300 ml of Distilled water (DW). The solution can be heated gently on a hot plate with constant stirring for complete dissolution. Filter the solution after cooling and mark it as 'A'.
2. Dissolve 9 g of CuSO4 in 50 ml of DW and mark it as 'B'.
3. Dissolve 0.5g potassium ferricyanide in 10 ml of DW and mark it as 'C'.
4. Mix 50 ml of 'B' and 2.5 ml of 'C' in 300 ml of 'A' and make the total volume up to 500 ml by adding DW. This solution is Quantitative Benedict's reagent.

Quantitative estimation of glucose in urine sample:

Standard titration:

1. Take 5ml of the Quantitative Benedict's reagent in a flask and add 2g of anhydrous sodium carbonate. Mix well and keep the mixture on a hot plate for continuous heating.
2. Add 0.5% glucose solution using a graduated pipette while stirring continuously until the blue color disappears. Note the volume of glucose utilized.

Sample titration

3. Again, take 5ml of the Quantitative Benedict's reagent in another flask and add 2 g of anhydrous sodium carbonate. Mix well and keep the mixture on a hotplate for continuous heating.
4. Add urine using a graduated pipette while stirring continuously until the blue color disappears. Note the volume of urine utilized.

1.5 OBSERVATIONS AND RESULTS

Observation Table:

Sample	Volume utilized (ml)	Concentration (mg/ml)
Glucose		
Urine		

Calculations:

The concentration of glucose in the urine can be calculated by the following formula-

$$C_1 = C_2V_2 / V_1$$

where

C_1 = Concentration of glucose in the urine

V_1 = Volume of urine utilized in the reaction

C_2 = Concentration of standard solution of glucose

V_2 = Volume of glucose solution utilized in the reaction

Results

The concentration of glucose in the given sample of urine is _____ mg/ml, as estimated through the Quantitative Benedict reaction.

1.6 PRECAUTIONS

1. Freshly prepared reagent solutions should be used.
2. Titration should be performed carefully while approaching the endpoint.

EXPERIMENT 2

ESTIMATION OF SERUM Ca^{2+}

Structure

2.1 INTRODUCTION

Calcium (Ca) is the most abundantly occurring mineral element in the human body. It is essential for many physiological functions of the body, such as muscle contraction, nerve impulse conduction, vascular dilation and contraction, blood clotting, hormone secretion, cell signaling, and cell membrane stability.

More than 99% of calcium is present in bones and teeth as the mineral Hydroxyapatite. It is in the form of hexagonal crystals made of calcium, phosphorus, and hydroxide with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. The crystal lattice structure of hydroxyapatite contributes to its rigidity and maintains the structural integrity of the skeletal system. The rest of the 1% of body Ca is found in cells, tissues, and extracellular and intracellular body fluids, including blood. This portion of Ca exists in three forms- cations (Ca^{2+}), complexed with anions or chelated, and protein-bound form. Approximately 50% of Ca in body fluids is in a cationic form which is free calcium and plays essential role in most physiological functions. About 10% of calcium is chelated or complexed with anions like phosphates, bicarbonates, oxalates and citrates, and is readily available to be absorbed by cells and tissues. Only 10% of the calcium is bound to proteins and is not freely available to cells and tissues. Majorly this fraction is bound with albumin and globulins.

All the three forms- ionic, chelated and protein-bound are present in serum. The normal serum calcium concentration in the body is maintained in a very narrow range,i.e., from 8.5 to 10.4 mg/dL, which is regulated and kept constant through Ca homeostasis with the help of parathyroid hormone (PTH),

Vitamin D3 and calcitonin. Derangements in calcium levels can be caused by several pathophysiological conditions and could lead to disturbance in Ca homeostasis, clinically manifested as hypocalcemia or hypercalcemia. These conditions can be diagnosed through the measurement of serum calcium in the laboratory (Fig. 2.1).

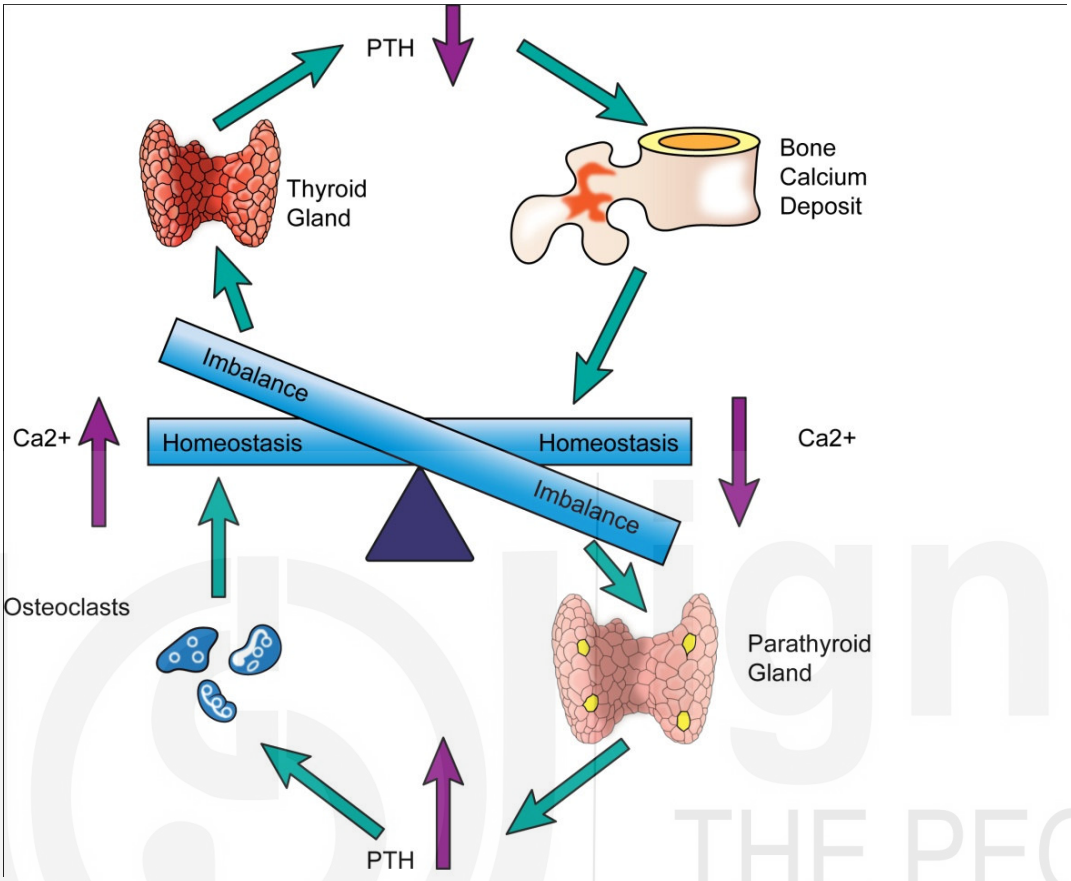

Fig. 2.1: Calcium homeostasis by Parathyroid Hormone.

Expected Learning Outcomes

After studying this unit, you should be able to:

- quantitatively determine the calcium levels in the serum sample;
- diagnose the occurrence of hypocalcemia or hypercalcemia; and
- assess the pathophysiological condition associated with hypocalcemia or hypercalcemia.

2.2 PRINCIPLE

The laboratory estimation of Serum calcium can be done through the o-cresolphthaleincomplexone (oCPC) method. The calcium ions (Ca^{2+}) in the serum samples react with o-cresolphthalein at alkaline pH to form a Ca-oCPC complex. This complex gives intense violet-red color and shows absorption maxima at 577nm. The absorbance of the reaction mixture is thus measured spectrophotometrically at 570 nm, which is directly proportional to the calcium concentration in the serum sample.

Ca^{2+} + oCPC —(8-Hydroxyquinoline, OH^-)→ Calcium-CPC Complex (Violet-red)

2.3 MATERIALS REQUIRED

Chemicals-

1. Buffer- 2-amino-2-methyl-1-propanol (AMP) or Diethanolamine buffer (2M), pH= 10-11
2. Chromogenic reagent-

   o-Cresolphthalein 0.60mmol/l

   8-Hydroxyquinoline 70 mmol/l
3. Calcium Standard: 10 mg/dl

Equipment: colorimeter or spectrophotometer

2.4 PROTOCOL

1. Prepare chromogenic reagent by mixing 2.0 ml of a buffer solution with 2.0 ml of Chromogenic reagent.
2. Take three test tubes and label each as 'A', 'B' and 'C' for Blank, Standard and Sample, respectively.
3. To each of these test tubes, pipette 0.5 ml of buffer and 0.5 ml of Chromogenic reagent.
4. Then pipette 0.02 ml each of Blank, Standard and Sample in respective test tubes.
5. Mix well and incubate the reaction mixtures at room temperature for 5-7 minutes.
6. Measure the optical density (OD) against the blank in a colorimeter or spectrophotometer at 570 nm.

2.5 OBSERVATIONS AND RESULTS

Observation table:

Test Tube	Contents	OD @ 570 nm
A	Blank	
B	Standard	
C	Sample	

Calculation

Serum Calcium (mg/dl) = (OD of sample / OD of Standard) X Concentration of Standard

Results:

The concentration of Calcium in the given serum sample estimated by o-CPC colorimetric method is _____ mg/dl.

2.6 PRECAUTIONS

1. All reagents should be freshly prepared for the accuracy of results. However, the chemicals mentioned in this experiment are stable up to 24 hours at room temperature and up to 7 (Seven) days under refrigeration (4°C).
2. Reagents should be stored in dark.
3. Serum sample should be freshly collected and hemolysis free.
4. Chelating agents such as EDTA can interfere with calcium estimation.
5. All reagents and samples should be handled following biosafety rules.

EXPERIMENT 3

ESTIMATION OF SERUM T_4

Structure

3.1 INTRODUCTION

Thyroxine hormones are tyrosine-based, iodine-containing and lipophilic hormones produced by thyroid glands. They control and regulate the basal metabolism, growth and development of the body. Thyroid glands are located in the front lower portion of the neck below the larynx and secrete two Thyroxine hormones- L-thyroxine or tetraiodothyronine (T4) and L-triiodothyronine (T3). The secretion of these hormones is regulated by the pituitary gland and the hypothalamus. Hypothalamus secretes Thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary gland to secrete thyroid-stimulating hormone (TSH). TSH further regulates the secretion of thyroxine hormones from thyroid glands. The trio Thyroid-hypothalamus-pituitary functions through a well-coordinated and tightly regulated feedback mechanism (Fig. 3.1).

T4 is secreted in much higher amounts in blood than T3 and is comparatively inactive than T3. About 99.9 % of T4 is bound to proteins, majorly thyroxine-binding globulin (TBG), while only 0.1% T4 is free and active. Therefore, the sum amount of bound and unbound T4 found in the blood is clinically measured as Total T4, while the unbound fraction of T4 in the blood is called Free T4. Several factors affect Total T4; thus a measurement of this value can give a fair idea about certain medical conditions such as hypothyroidism and hyperthyroidism. The normal range of Total T4 level in adults is 5.0 to 12.0µg/dL.

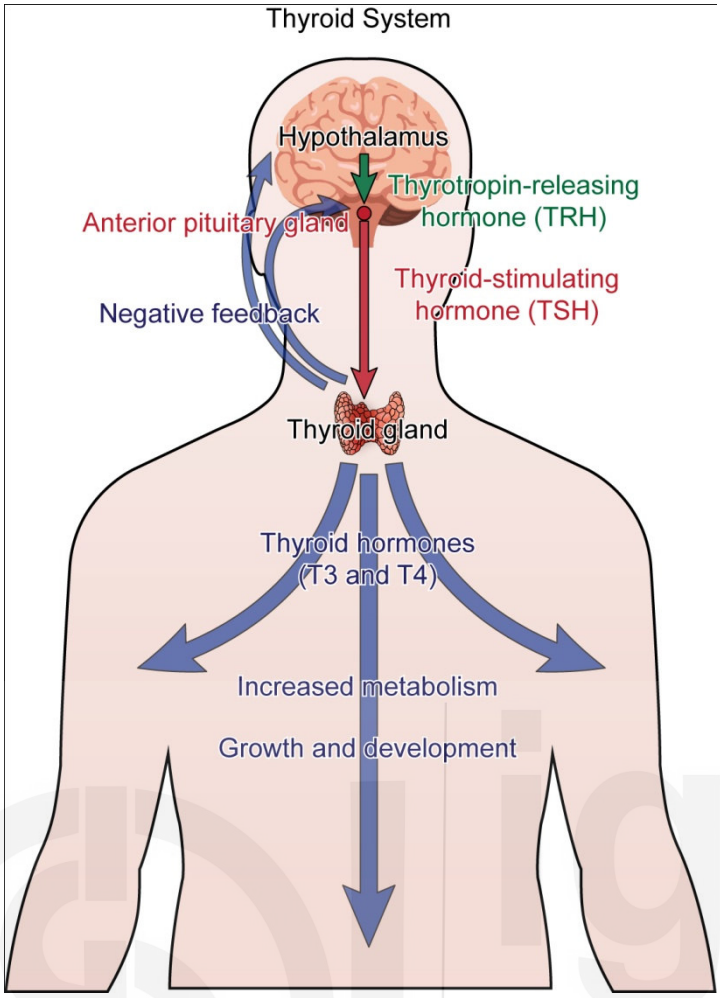

Fig. 3.1: Thyroid-hypothalamus-pituitary Functioning.

Expected Learning Outcomes

After studying this unit, you should be able to:

- Quantitatively measure the amount of T4 in serum samples through Enzyme immunoassay;
- Understand the significance of conjugation of HRP with T4;
- appraise the sensitivity of enzyme immunoassay;
- relate the serum T4 levels to medical conditions like hypothyroidism and hyperthyroidism.

3.2 PRINCIPLE

Total T4 is measured through Enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA). In this method, microtiter wells are coated with a known amount of anti-T4 antibody, and a specific amount of serum is added along with a known amount of enzyme-conjugated T4 or labeled T4. The most common enzyme used for conjugation in EIA is horse radish peroxidase (HRP). The mixture is incubated at RT for 60 minutes, and the wells are washed few times by distilled water to remove unbound labeled T4. The serum T4 competes with enzyme-conjugated T4 for a fixed number of

binding sites on the immobilizedanti-T4 antibody. Then, a substrate specific to the conjugated enzyme is added, and the mixture is further incubated for 20-30 minutes. Color is developed due to the enzyme-substrate enzyme-substrate reaction, which can be measured spectrophotometrically. The substrate TMB (3,3',5,5'-Tetramethylbenzidine) is used for the HRP enzyme and absorbance is measured at 450nm. The concentration of T4 in the serum sample is determinedbased on absorbance, using T4 standards as reference (Fig. 3.2).

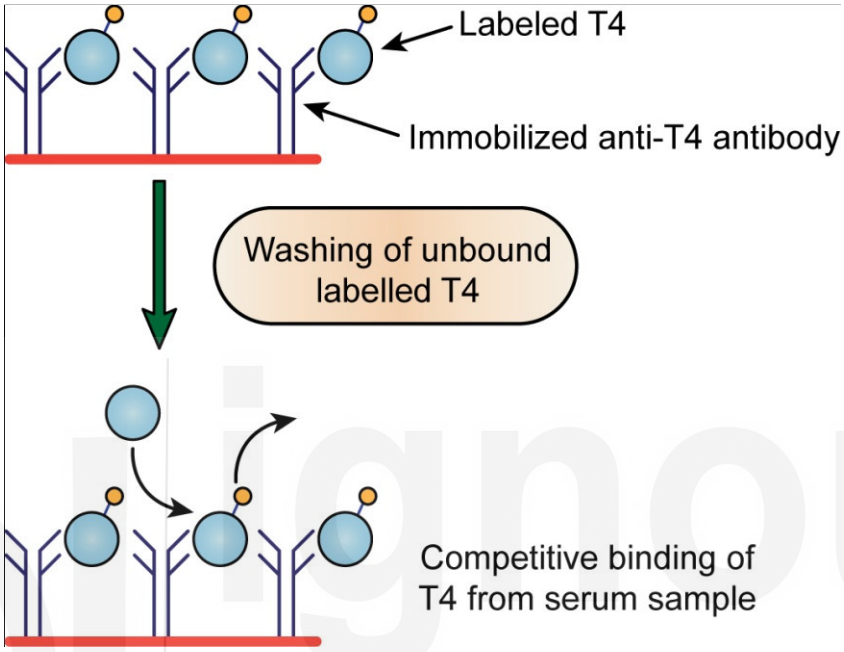

Fig. 3.2: Enzyme immunoassay (EIA) for determination of T4 in serum sample.

3.3 MATERIALS REQUIRED

Chemicals and Reagents

1. Microtiter well plate with 96 wells coated with sheep anti-T4.
2. T4-HRP Conjugate concentrate (10X)
3. A diluent solution consisting of TRIS buffer (pH=7.6) and 8-anilino-1-napthalene sulfonic acid (ANS).
4. T4 reference standards of concentration range 2 to 25 µg/dl.
5. 3, 3', 5, 5' tetramethylbenzidine (TMB) reagent
6. 1N HCl
7. Serum sample

Apparatus and equipment:

Autopipettes volume 20µL, 100 µL, 200 µL, and 1000 µL

Vortex mixer

ELISA reader

3.4 PROTOCOL

1. Take the microtitre plate having wells pre-coated with sheep anti-T4 and mark for standard, sample and control.
2. Pipette 20 µL of control, standard and sample in respective wells of the microtitre plate.
3. Prepare working solution of T4-HRP Conjugate by mixing 0.1 ml of T4-HRP Conjugate concentrate with 1 ml of diluent solution. This can be stored at 2-4^{o} C for 24 hours.
4. Pipette 100 µL of working solution of dilute T4-HRP Conjugate into each assay well and mix gently.
5. Incubate the mixture at room temperature for 60 minutes.
6. After incubation, empty the microtitre wells and rinse with distilled water 4-5 times to wash all the unbound contents.
7. Tap the wells on an absorbent paper to remove the water completely from the wells.
8. Pipette 100 µL of TMB reagent in each assay well and mix properly.
9. Incubate the mixture at room temperature for 30 minutes.
10. Pipette 100 µLof 1N HCl as stop solution in each assay well, mix well. This will stop the reaction.
11. Measure the absorbance at 450nm with an ELISA reader.

3.5 OBSERVATIONS AND RESULTS

Observation Table

Microtitre Plate Well Contents	T4 Concentration (µg/dl)	Absorbance @450 nm
Control	0	
Standard	5	
	10	
	15	
	20	
	25	
Sample	__	

Results

The concentration of T4 in the serum sample obtained from the calibration curve is ____ µg/dl.

3.6 PRECAUTIONS

1. Freshly prepared reagent solutions should be used.
2. Do not use reagents if they appear cloudy or a change in physical properties is observed.
3. Store reagents as per the manufacturer's directions.

EXPERIMENT 4

HCG BASED PREGNANCY TEST

Structure

4.1 INTRODUCTION

Human chorionic gonadotropin (HCG) is a glycoprotein hormone biosynthesized post-fertilization by syncytiotrophoblast tissue of the blastocyst. HCG is a heterodimer protein consisting of two subunits- α and β, each having 14.5 and 22.2 kD molecular weight, respectively. The α subunit is comprised of 93 amino acids while the β subunit is made up of 145 amino acids; thus HCG is altogether made up of a total of 238 amino acids. HCG subunits are also attached with 8 carbohydrate side chains. HCG stimulates the corpus luteum graviditatis to produce a high amount of progesterone along with some estradiol and estrone to maintain the pregnancy. HCG itself promotes angiogenesis in the endometrium and progesterone secreted by corpus luteum graviditatis primarily maintains the thick layer of endometrium in the uterus to support embryo development. HCG appears in the serum and urine within 9 days of conception, and thereafter its levels increase rapidly, reaching up to 100,000-200,000 mIU/mL in about 10-12 weeks of pregnancy. Since it is detectable in an early stage of pregnancy and also continues to appear in serum and urine during the entire gestational period, it is used as a biomarker for pregnancy tests (Fig. 4.1).

Expected Learning Outcomes

After studying this unit, you should be able to:

- detect the pregnancy by measuring HCG levels in the urine sample;
- understand the rapid chromatographic immunoassay;
- handle the HCG Pregnancy Test Kit; and
- appraise the sensitivity of enzyme immunoassay.

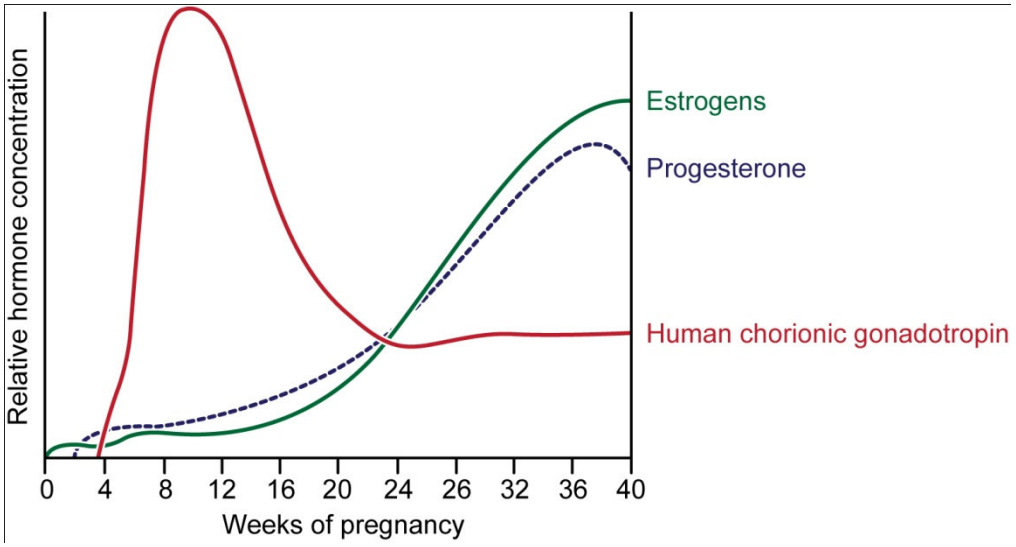

Fig. 4.1: HCG Levels during pregnancy.

3.2 PRINCIPLE

The clinical measurement of HCG levels in urine can be done through rapid chromatographic immunoassay using an HCG Pregnancy Test Kit. It comprises of a card or strip on which monoclonal and polyclonal antibodies (anti-HCG) are immobilized that selectively bind to the HCG. The urine specimen is loaded on the testing card, and it moves along the membrane through capillary action. As soon as the HCG hormone encounters the labeled antibodies, the two react to form antibody-HCG- conjugate, which appears in the form of a colored line on the card. The card is also provided with a control, that will always give the colored line on loading of the sample. This is to ensure that the test has been completed. The sensitivity of the HCG Pregnancy Test Card is 25 mIU/mL; therefore, it can detect the pregnancy as early as 9 days after conception (Fig. 4.2).

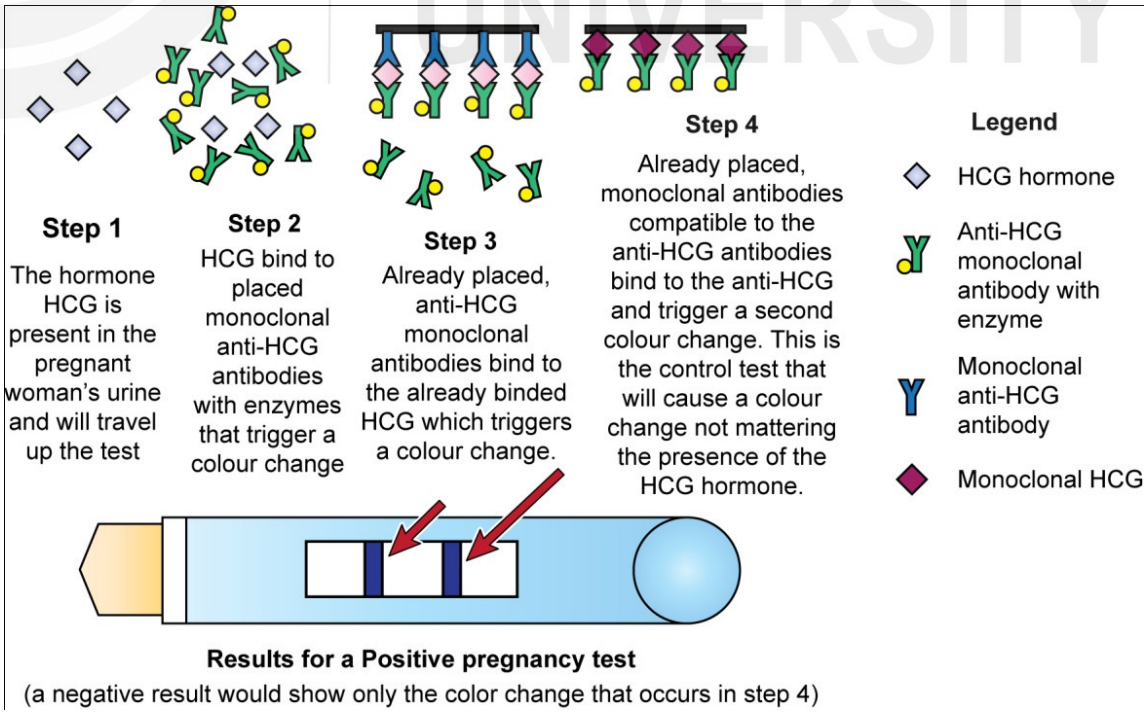

Fig. 4.2: Mechanism of HCG immunoassay.

4.3 MATERIALS REQUIRED

1. Testing card or device
2. Disposable pipette
3. Sample container
4. Sample- Urine

4.4 PROTOCOL

1. Urine is used as a sample to test the presence of HCG.
2. Sample can be collected any time; however, it should be preferably collected in the morning as a midstream urine specimen.
3. The sample can be stored at 2-6^{o}C for up to 24 hours.
4. The sample should be clear and should not contain any precipitate.
5. While testing, the sample and the testing card or device should be at room temperature.
6. Keep the testing card on a leveled surface.
7. Load the sample well gently with 2 drops of urine using a disposable dropper.
8. Wait up to 5 minutes for the result to appear.

4.5 OBSERVATIONS AND RESULTS

Observations:

Negative Positive

Observation table:

Appearance of Red Line	C (Y/N)	T (Y/N)	Test (Positive/ Negative)
Test-1			
Test-2			
Test-3			
Test-4			

Results:

The card displays the markings 'C' for control and 'T' for Test. The appearance of the red line against 'C' indicates that the test is completed successfully, while the red line against 'T' indicates the presence of HCG in the sample. Thus, a test is POSITIVE when red lines appear against both 'C' and 'T'. Red line appearing ONLY against 'C' and NOT against 'T' means that the test is NEGATIVE.

In other cases, if NO Red line appears or if it appears only against 'T', it means that the test has been performed incorrectly and is invalid. There can be several reasons for invalid testing, such as loading insufficient sample volume in the sample well, not allowing sufficient time for observation, using expired or damaged testing cards or handling the card inappropriately.

4.6 PRECAUTIONS

1. HCG Test card should not be expired or damaged.
2. Ensure successful completion of the test by observing the Control line.

   Wait until the appropriate time for the colored line to appear.

EXPERIMENT 5

ESTIMATION OF SERUM ELECTROLYTES

Structure

5.1 INTRODUCTION

Electrolytes maintain the water balance, osmotic balance, pH balance and electrical neutrality in the cells and tissues. Therefore, the presence of electrolytes in the physiologically desirable range is highly essential for all metabolic processes and general body functioning. They are derived through food and fluid intake; some of the major electrolytes are sodium, chloride, potassium, magnesium, calcium, phosphorus and bicarbonate. They are present in all body fluids, extracellular and intracellular, and play a regulatory role in muscle contraction, nerve activity, heart functioning, blood clotting, blood pressure and kidney functioning.

Electrolyte imbalances occur when the levels of these electrolytes are either low or high in the body fluids. For example, low and high sodium levels are termed Hyponatremia and Hypernatremia, respectively, while low and high levels of Potassium are termed Hypokalemia and Hyperkalemia, respectively. The normal ranges of some major electrolytes in serum of adults are mentioned in the Table 5.1.

Table 5.1: Normal level of major electrolytes in serum

Electrolyte	**Normal serum level**
Sodium (Na^+)	135 – 145 mmol/l
Chloride (Cl^-)	96-106 mmol/L
Potassium (K^+)	3.5- 5.5 mmol/L
Magnesium (Mg^{2+})	0.85 - 1.10 mmol/L
Bicarbonate (HCO_3^-)	23 - 29 mmol/L

Derangements of electrolyte levels from the normal range indicate physiological disorders or diseases and might potentially lead to morbidity. Thus, the measurement of serum electrolytes is often correlated to morbidities and disorders and is used to assess various clinical conditions.

Expected Learning Outcomes

After studying this unit, you should be able to:

- quantify electrolytes in serum sample potentiometrically;
- appraise the sensitivity of the potentiometric method;
- compare the electrolyte concentration of the given sample with normal range values; and
- identify the electrolyte imbalance and relate it with the disease or illness.

5.2 PRINCIPLE

Serum electrolytes like Na^+, K^+ and Cl^- are commonly measured through potentiometric methods using Ion Selective Electrodes (ISE). This method measures the potential difference between two electrodes- an ion-selective electrode and a reference electrode, by a Voltmeter connected to the electrochemical cell. This potential difference is directly proportional to the concentration of the selected ions in the sample or the solution.

The working of the electrodes is similar to those in a pH meter. The reference electrode is exposed to the reference or standard solution of known concentration, while the ion-selective electrode, also called indicator electrode, is exposed to the sample solution. The reference electrode is an Ag/AgCl electrode, made up of silver wire coated with silver chloride, immersed in an AgCl saturated Potassium chloride (KCl) solution also containing the ions to be measured. The ion-selective electrode consists of a selective membrane that specifically allows only particular ions to pass through, excluding the passage of other ions across the membrane. When both the electrodes are exposed simultaneously to the respective ion solutions, a liquid junction is formed and a junction potential is created in the bridge region between the reference solution and the sample solution, forming an electrochemical concentration cell.

When the electrodes are dipped in the solution, the selected ions move across the membrane of the ion-selective electrode, accumulate on both sides of the membrane and at equilibrium, an electrochemical potential is created, which is proportional to the logarithm of the ion concentration in the solution and is governed by Nernst equation-

$E=E^0+(2.030\ RT/nF)\log C$

where,

E = potential,

E^0= the standard cell potential, a constant for the ISE,

R = gas constant (8.314 J/K.mol),

T = temperature (in K),

n = charge of the selected ion

F = Faraday constant (96,500 coulombs/mol).

By using the internal reference solution and according to the Nernst equation, a linear plot can be obtained, and the ion concentration of the sample solution can be determined. The cell potential is proportional to the logarithmic molar concentration of ions to be determined and is represented in molar units. This relationship is linear for most ions over the physiological range such as Na^+, and K^+ and Cl^-, and therefore, the method can be used to correlate the ion concentration to various pathophysiological conditions clinically.

5.3 MATERIALS REQUIRED

Ion Selective Electrodes (ISE) Buffer,

Standard solutions for selected ions,

Reference Solution,

Ion Selectivity Check Solution.

Sample- Serum (hemolysis free)

Equipment: Ion Selective Electrodemeter

5.4 PROTOCOL

1. Standard solutions of known concentrations of selected ions are used for preparing the calibration curve.
2. Prepare a stock solution of the standard by dissolving weighed amount of salt in a known volume of distilled water. The standard solution used for ions Na^+ and Cl^- is of NaCl is, and for K^+ ions, it is KCl. The stock concentration of each of these is prepared for 1000 ppm.
3. Make serial dilutions to prepare the concentration range of 1, 10, 100 and 1000 ppm.
4. Add an equal volume of Ionic Strength Adjustment Buffer (ISAB) to each concentration of standard solution to adjust its ionic strength.
5. Dip the ion selective electrode and reference electrode in the solution of least concentration and measure the electrode potential in the ISE meter.
6. Rinse the electrodes thrice in distilled water.
7. Measure the electrode potential of all standard dilutions successively in the same manner.
8. Plot the calibration curve using the electrode potential values measured for the entire range of standard concentrations.

9. Mix the measured volume of the serum sample with an equal volume of Ionic Strength Adjustment Buffer (ISAB) for ionic strength adjustment.

10. Measure the electrode potential of the serum sample as done for the standard solutions.

5.5 OBSERVATIONS AND RESULTS

Observation Table

Sample	Concentration	Electrode Potential
Standard	1 ppm	5
	10 ppm	10
	100 ppm	15
	1000 ppm	20
Serum		

Results

The concentration of selected ions in the serum sample as displayed by the Ion Selective Electrode meter is _____ mEq/L.

5.6 PRECAUTIONS

1. Standard solutions should be freshly and accurately prepared.

2. Electrodes should be rinsed thoroughly before immersing in any solution.

   The ISE meter should be properly calibrated.