BLOOD UREA

LEARNING OBJECTIVE :

- Clinical importance of blood urea nitrogen
- Clinical significance of blood urea nitrogen.
- Normal range of blood urea nitrogen
- Procedure of BUN
- Test principle and calculation of the BUN

INTRODUCTION:

Blood urea nitrogen (BUN) is a medical test that measures the amount of urea nitrogen found in blood. The liver produces urea in the urea cycle as a waste product of the digestion of protein. Normal human adult blood should contain between 6 and 20 mg of urea nitrogen per 100 ml (6–20 mg/dL) of blood. Individual laboratories will have different reference ranges as the assay used can vary between laboratories.

INTERPRETATION:

BUN is an indication of renal (kidney) health. The normal range is 1.8–7.1 mmol/L or 6–20 mg/dL. The main causes of an increase in BUN are: high protein diet, decrease in glomerular filtration rate (GFR) (suggestive of renal failure) and in blood volume (hypovolemia), congestive heart failure, gastrointestinal haemorrhage, fever, and increased catabolism. Hypothyroidism can cause both decreased GFR and hypovolemia, but BUN-to-creatinine ratio has been found to be lowered in hypothyroidism and raised in hyperthyroidism. The main causes of a decrease in BUN are severe liver disease, anabolic state, and syndrome of inappropriate antidiuretic hormone.

METHOD :

Bethelot -reaction method

CLINICAL SIGNIFICANCE :

Elevated levels of urea are observed in prerenal , renal, and postrenal conditions.

Prerenal condition : Diabetes mellitus , dehydration , cardiac failure , hematemesis, severe burns , high fever etc.

Renal condition: disease of kidneys

Post renal condition: Enlargement of prostate , stones in urinary tract, tumor of the bladder

Decreased values have been reported in severe liver disease , protein malnutrition & pregnancy.

NORMAL RANGE :

Birth to 1 yr : 4-16 mg/dl

1 to 40 yr: 7-21 mg / dl

TEST PRINCIPLE :

The procedure is based on the Berthelot reaction . urease splites urea into ammonia and carbon dioxide. The ammonia reacts with phenol in the presence of hypochlorite to form indophenols, which with alkali gives a blue coloured compound. The intensity of the colored compound can be measured a 546 nm. The color of the reaction is stable for 12 hrs.

SAMPLE MATERIAL:

Serum or heparinized plasma

Requirement:

Test tube

Push button pipette

Stop watch

Water bath

PROCEDURE:

	TEST	STD	BLANK
1. UREASE BUFFER REAGENT	0.5	0.5	0.5
2. SERUM /PLASMA,ML	0.02		
3. STANDARD UREA		0.02	
4. PHENOL REAGENT	1.0	1.0	1.0
5. HYPOCHLORITE REAGENT	1.0	1.0	1.0
6. DISTILLED WATER	5.0	5.0	5.0

CALCULATION:

$$\text{Serum urea nitrogen , mg/dl} = \frac{\text{O.D of test}}{\text{O.D of std}} \times \text{conc. Of std solution}$$

QUESTION BANK :

1. What are the clinical significance of blood urea .
2. Explain the procedure of blood urea nitrogen
3. Explain the purpose of doing blood urea estimation
4. Explain the material and method of blood urea nitrogen
5. What are the normal range of blood urea nitrogen.
6. Write the calculation formula of blood urea nitrogen.
7. Write name of the method of blood urea nitrogen.

BIOLOGICAL FLUID

LEARNING OBJECTIVE:

- **Characteristics of all the body fluids**
- **Cerebrospinal fluid**
- **Pleural fluid**
- **Peritoneal fluid**
- **Synovial fluid**
- **Pericardial fluid**
- **Storage of the fluid in laboratory.**

CEREBROSPINAL FLUID:

CSF is a clear, colourless, body fluid found in the brain & spin. It is present in the ventricles of brain and central canal of spinal cord. It is produced in the choroid plexuses of the ventricles of the brain.

It acts as a cushion or buffer for the brain cortex by providing basic, mechanical, and immunological protection to the brain inside the skull. The brain produces roughly 500ml of cerebrospinal fluid per day. This fluid is constantly reabsorbed, so that approximately 100-150ml is present at any time. CSF perform following function:

1. It helps to protect the brain and spinal cord from injury.
2. It acts as a shock absorber for the brain and spinal cord.
3. It help to maintain a constant pressure inside the head and around the spinal cord.
4. It keeps the brain and spinal cord moist.
5. It also acts as a medium for the transport of substances from brain , tissue and spinal cord to blood.
6. It plays an important role in flushing metabolic toxins on wastes from he brains tissues.

Sample collection of CSF specimen:

1. The specimen should be collected by a physician, a specially trained technician or nurse.
2. You will lie your side with your knees pulled up towards the chest and chin tucked downward. Sometime the test is done sitting up , but bent forward.
3. After the back is cleaned , the health care provider will inject a local numbing medicine into the lower spine
4. A sterile lumber puncture needle is inserted between the 4^{th} and 5^{th} vertebra to a depth of 4-5cm.
5. Once the needle is in position , CSF pressure is measured and a sample of 1 to 10ml is collected.
6. The needle is in position, CSF pressure is measured and a sample of 1 to 10 ml is collected.
7. The needle is removed, the area is cleaned , and a bandage is placed over the needle site. You may be asked to remain lying down for a short time after the collection.

PLEURAL FLUID:

The pleura includes two thin layers of tissue that protect and cushion the lungs. The inner layer wraps around the lungs and is stick so tightly to the lungs that it can not be peeled off. The outer layer lines the inside of the chest wall. The very thin space between the layers is called pleural cavity. A liquid called pleural fluid, lubricants the pleural cavity so that the two layers of pleural tissue can slide against each other.

The excess fluid that accumulates in the pleural cavity the surrounds the lungs in known as pleural effusion. This excess can impair breathing by limiting the expansion of lungs. It can be classified into transudate and exudates.

PERICARDIAL FLUID:

Pericardial fluid is the serous fluid secreted by the serous layer of the pericardium into the pericardial cavity. The pericardial fluid reduces friction within the pericardium by lubricating the epicardial surface allowing the membranes to glide over each other with each heartbeat.

PERITONEAL FLUID:

Peritoneal fluid is a liquid made in the abdominal cavity. It cover most of the intra-abdominal organ. An increased volume of peritoneal fluid is called ascites. It is also known as ascetic fluid.

SYNOVIAL FLUID:

Synovial fluid is a viscous, non-Newtonian fluid in the cavities of synovial joint. It is present in each large joint such as knee, ankle ,hip, , wrist, elbow and shoulder. Composition of synovial fluid resembles with that of other body fluids such as serous fluid and spinal fluid. In addition, it contains a mucopoly saccharide. The principle role of synovial fluid is to reduce friction between the articular cartilage of synovial joints during movement. Synovial fluid may be classified into normal, non-inflammatory, inflammatory, specific and haemorrhagic.

STORAGE: The specimen of synovial fluid is stored with anticoagulant and without anticoagulant at 4°C before examination.

Seminal Fluid: - seminal fluid is an organic fluid that may contain spermatozoa. It is secreted by the sexual organ of male and can fertilize female ova. Seminal fluid is also known as semen.

Semen is produced and originates from the seminal vesicle which is located in the pelvis. The process that result in the discharge of semen is called ejaculation. Semen is also a form of genetic material. Semen has been collected for cryoconservation.

QUESTION BANK :

1) Full form of CSF.
2) Name the needle used for CSF collection.
3) What are the functions of CSF?

4) write down the procedure for collection and preservation of CSF specimen.
5) What is the composition of CSF?
6) Give at least four examples of body fluids.
7) write a short note on:
   a) pleural fluid
   b) synovial fluid
   c) seminal fluid
   d) peritoneal fluid
   e) pericardial fluid
8) what are the important precaution need to be taken for CSF collection.

CARBOHYDRATE:

LEARNING OBJECTIVE:

- **Definition of carbohydrates**
- **Composition, sources, and its importance**
- **Classification of carbohydrates**
- **Important of monosaccharaides, disaccharides, and polysaccharides**

DEFINATION:

A carbohydrate is a biomolecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen–oxygen atom ratio of 2:1 (as in water) and thus with the empirical formula $C_m(H_2O)_n$ (where *m* may be different from *n*). This formula holds true for monosaccharides. Some exceptions exist; for example, deoxyribose, a sugar component of DNA, has the empirical formula $C_5H_{10}O_4$. The carbohydrates are technically hydrates of carbon; structurally it is more accurate to view them as aldoses and ketoses.

The term is most common in biochemistry, where it is a synonym of 'saccharide', a group that includes sugars, starch, and cellulose. The saccharides are divided into four chemical groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Monosaccharides and disaccharides, the smallest (lower molecular weight) carbohydrates, are commonly referred to as sugars. The word *saccharide* comes from the Greek word , meaning "sugar". While the scientific nomenclature of carbohydrates is complex, the names of the monosaccharides and disaccharides very often end in the suffix -ose, as in the monosaccharides fructose (fruit sugar) and glucose (starch sugar) and the disaccharides sucrose (cane or beet sugar) and lactose (milk sugar).

Carbohydrates perform numerous roles in living organisms. Polysaccharides serve for the storage of energy (e.g. starch and glycogen) and as structural components (e.g. cellulose in plants and chitin in arthropods). The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g. ATP, FAD and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development.

Starch and sugars are the most important carbohydrates in human diet. They are found in a wide variety of natural and processed foods. Starch is a polysaccharide. It is abundant in cereals (wheat, maize, rice), potatoes, and processed food based on cereal flour, such as bread, pizza or pasta. Sugars appear in human diet mainly as table sugar (sucrose, extracted from sugarcane or sugar beets), lactose (abundant in milk), glucose and fructose, both of which occur naturally in honey, many fruits, and some vegetables. Table sugar, milk, or honey are often added to drinks and many prepared foods such as jam, biscuits and cakes.

Cellulose, a polysaccharide found in the cell walls of all plants, is one of the main components of insoluble dietary fiber. Although it is not digestible, insoluble dietary fiber helps to maintain a healthy digestive system by easing defecation. Other polysaccharides contained in dietary fiber include resistant starch and inulin, which feed some bacteria in the microbiota of the large intestine, and are metabolized by these bacteria to yield short-chain fatty acids.

MONOSACCHARIDES :

Monosaccharides are classified according to three different characteristics: the placement of its carbonyl group, the number of carbon atoms it contains, and its chiral handedness. If the carbonyl group is an aldehyde, the monosaccharide is an aldose; if the carbonyl group is a ketone, the monosaccharide is a ketose. Monosaccharides with three carbon atoms are called trioses, those with four are called tetroses, five are called pentoses, six are hexoses, and so on. These two systems of classification are often combined. For example, glucose is an aldohexose (a six-carbon aldehyde), ribose is an aldopentose (a five-carbon aldehyde), and fructose is a ketohexose (a six-carbon ketone).

Each carbon atom bearing a hydroxyl group (-OH), with the exception of the first and last carbons, are asymmetric, making them stereo centers with two possible configurations each (R or S). Because of this asymmetry, a number of isomers may exist for any given monosaccharide formula. Using Le Bel-van't Hoff rule, the aldohexose D-glucose, for example, has the formula $(C\cdot H_2O)_6$, of which four of its six carbons atoms are stereogenic, making D-glucose one of $2^4=16$ possible stereoisomers. In the case of glyceraldehydes, an aldotriose, there is one pair of possible stereoisomers, which are enantiomers and epimers. 1, 3-dihydroxyacetone, the ketose corresponding to the aldose glyceraldehydes, is a symmetric molecule with no stereo centers. The assignment of D or L is made according to the orientation of the asymmetric carbon furthest from the carbonyl group: in a standard Fischer projection if the hydroxyl group is on the right the molecule is a D sugar, otherwise it is an L sugar. The "D-" and "L-" prefixes should not be confused with "d-" or "l-", which indicate the direction that the sugar rotates plane polarized light. This usage of "d-" and "l-" is no longer followed in carbohydrate chemistry.

DISACCHARIDES :

Two joined monosaccharides are called a disaccharide and these are the simplest polysaccharides. Examples include sucrose and lactose. They are composed of two monosaccharide units bound together by a covalent bond known as a glycosidic linkage formed via a dehydration reaction, resulting in the loss of a hydrogen atom from one monosaccharide and a hydroxyl group from the other. The formula of unmodified disaccharides is $C_{12}H_{22}O_{11}$. Although there are numerous kinds of disaccharides, a handful of disaccharides are particularly notable.

Sucrose, pictured to the right, is the most abundant disaccharide, and the main form in which carbohydrates are transported in plants. It is composed of one D-glucose molecule and one D-fructose molecule. The systematic name for sucrose, *O*-α-D-glucopyranosyl-(1→2)-D-fructofuranoside, indicates four things:

- Its monosaccharides: glucose and fructose
- Their ring types: glucose is a pyranose and fructose is a furanose
- How they are linked together: the oxygen on carbon number 1 (C1) of α-D-glucose is linked to the C2 of D-fructose.
- The *-oside* suffix indicates that the anomeric carbon of both monosaccharides participates in the glycosidic bond.

Lactose, a disaccharide composed of one D-galactose molecule and one D-glucose molecule, occurs naturally in mammalian milk. The systematic name for lactose is *O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose. Other notable disaccharides include maltose (two D-glucoses linked α-1,4) and cellulobiose (two D-glucoses linked β-1,4). Disaccharides can be classified into two types: reducing and non-reducing disaccharides. If the functional group is present in bonding with another sugar unit, it is called a reducing disaccharide or Boise.

METABOLISM :

Carbohydrate metabolism denotes the various biochemical processes responsible for the formation, breakdown and interconversion of carbohydrates in living organisms.

The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species: plants synthesize carbohydrates from carbon dioxide and water by photosynthesis storing the absorbed energy internally, often in the form of starch or lipids. Plant components are consumed by animals and fungi, and used as fuel for cellular respiration. Oxidation of one gram of carbohydrate yields approximately 9 kJ (4 kcal) of energy, while the oxidation of one gram of lipids yields about 38 kJ (9 kcal). The human body stores between 300 to 500 g of carbohydrates depending on body weight, with the skeletal muscle contributing to a large portion of the storage. Energy obtained from metabolism (e.g., oxidation of glucose) is usually stored temporarily within cells in the form of ATP. Organisms capable of anaerobic and aerobic respiration that metabolizes glucose and oxygen (aerobic) to release energy with carbon dioxide and water as by products.

CATABOLISM:

Catabolism is the metabolic reaction which cells undergo to break down larger molecules, extracting energy. There are two major metabolic pathways of monosaccharide catabolism: glycolysis and the citric acid cycle.

In glycolysis, oligo- and polysaccharides are cleaved first to smaller monosaccharides by enzymes called glycoside hydrolases. The monosaccharide units can then enter into monosaccharide catabolism. A 2 ATP investment is required in the early steps of glycolysis to phosphorylate Glucose to Glucose 6-Phosphate (G6P) and Fructose 6-Phosphate(F6P) to Fructose 1,6-biphosphate (FBP), thereby pushing the reaction forward irreversibly. In some cases, as with humans, not all carbohydrate types are usable as the digestive and metabolic enzymes necessary are not present.

QUESTION BANK:

1. Define carbohydrate
2. Explain the importance of carbohydrate
3. Explain he classification of Carbohydrate
4. Explain monosaccharides
5. Explain disaccharides
6. Explain catabolism of carbohydrates
7. Expalin metabolism of carbohydrates.
8. Write the formula of glucose.
9. How many metabolic pathways are generates at and of the metabolism of glucose or carbohydrate molecule
10. How many ATP generates at the and of the glycolysis.