1. What is urea? Describe the steps of urea synthesis. What is the significance of urea cycle? Name the disorders of urea cycle. (15M)

**Urea:** Urea is the end product of protein metabolism. It is toxic to the body in excess.

**UREA CYCLE:**

This cycle is first invented metabolic cycle in the body, also called Krebs Henslete cycle.

**Site:**

Urea is synthesized in liver. The enzymes for urea synthesis are present in both mitochondria and cytosol.

**Importance:**

- The ammonia released from transaminat reaction which is toxic to the body.
- Excess ammonia in the blood may deplete the α-ketoglutaric acid, which is an intermediate of TCA cycle.
- The depletion of α-ketoglutaric acid leads to depletion in the ATP. The major organs like brain, RBC etc. are dependent on continuous supply of ATP, and any condition which depletes ATP leads to adverse effects.
- Due to this reason ammonia is toxic to the body. Therefore our body convert toxic ammonia into less toxic urea. The urea is less toxic and soluble in aqueous medium, and is excreted through urine.

**Reactions:**

- The ammonia and carbon dioxide combines and converted to carbamoyl phosphate in the presence of carbomoyl phosphate synthase – I.
- The carbamoyl phosphate is combines with ornithine and converted to citrulline in the presence of ornithine transcarbamoylase.
- Citrulline combines with aspartate and converted to argininosuccinate in the presence of argininosuccinatesynthatase.
- Argininosuccinase is split into arginine and fumarate by the action of argininosuccinase.
- Arginine is again split into urea and ornithine by the action of arginase.
2. Write short notes on:
   a. **Structure and functions of human insulin (SN-Feb 09)**

Other name: Hypoglycemic hormone

The word insulin is derived from *Latin*, insula means islet. It is hormone with 2 polypeptide chains.

A chain carries 21 amino acids

B chain carries 30 amino acids. So, total 51 amino acids.

Inter chain disulphide bridge:

A7 – B7

A20 – B19

Intra chain disulphide bridge:

A6 – A11

Synthesis:

It is synthesized as prohormone “proinsulin “by beta cells of islet of langerhans of pancreas.

Actions of insulin:

- Enhance uptake of glucose by tissues
- Stimulates the glycolysis
- Promotes glycogenesis by activating glycogen synthase.
- Promotes fatty acid synthesis (lipogenesis) and inhibit lipolysis in adipose tissue by inhibiting hormone sensitive lipase
- Gluconeogenesis is inhibited by insulin
- It also inhibits glycogenolysis by inactivating glycogen phosphorylase.
- It depresses HMG Co A synthase and so ketogenesis is inactivated.

Biosynthesis:

7 DHC rich in malphigian layer of epidermis. The bond between 9 and 10 of 7DHC is cleaved and converted into cholecalciferol by the action of UV light

Activation of provitamin D (Cholecalciferol) into active vit D (calcitriol) is take place in two different sites

25 hydroxylation of Cholecalciferol in LIVER: Cholecalciferol through blood reaches the liver cells and undergoes hydroxylation at 25th carbon. This reaction is catalysed by calciol-25-hydroxylase

1 hydroxylation in KIDNEY: the active form of vit D is synthesized at kidney. 25 hydroxycalcidiol is hydroxylated at 1st C and converted into 1, 25 trihydroxycalciferol/calcitriol. It is the active form of vit D

Biochemical functions:

Vitamin D is act as steroid hormone. It binds to specific nuclear proteins and induces the synthesis of mRNA for specific proteins for instance Calmodulin (calbindin), which will leads to biological action

Effect on intestine:

It induces the synthesis of calmdulin from intestinal cells

Calmodulin helps in the absorption of calcium from intestine

Effects on bone:

Calcitriol stimulates the activity of osteoblasts
Osteoblasts are helps in mineralization of bone, briefly they secrete the ALP, and ALP in turn increases the ionic concentration of phosphate.

**Effects on kidney:**

Increases the reabsorption of calcium and phosphorous from renal tubules
c. Biological value of protein (SN-Feb 10)

It is the ratio between the amount of nitrogen retained and nitrogen absorbed during a specific interval

\[ BV = \frac{\text{retained nitrogen}}{\text{absorbed nitrogen}} \times 100 \]

Suppose 127 mg of protein ‘A’ was consumed by a rat in a day and 4 mg is recovered in faeces and 24 mg is seen in urine. Then

Amount ingested = 127 mg

Amount absorbed = 127 - 4 = 123 mg

Amount retained = 123 - 24 = 99 mg

Therefore BVP = 99/13X100 = 81%
d. Electrophoresis and its applications

It is one of the qualitative and quantitative techniques for separation of various biomolecules such as amino acids, proteins, carbohydrates, lipids, vitamins, drugs etc.

**Principle:** When the charged molecules placed in an electrical field they migrate to respective opposite charge/poles.

Requirements: electrophoresis apparatus, support medium (cellulose acetate, agarose etc.)

Types: basic gel electrophoresis, immune electrophoresis, SDS-PAGE

Serum protein electrophoresis:

It is a simple and more powerful tool for separation of various protein fractions as well as diagnostic purpose. It involves separation of serum proteins by an electric field at pH 8.6, using agarose as a support medium. It provides basic five-band pattern. Separation of these proteins is possible because each carries different charges and hence migrates at a differing rate when subjected to an electric potential.

Factors which affect electrophoresis: they are ionic strength of the buffer, voltage, temperature, application width, and staining.

Five major fractions and their normal serum values are serum protein electrophoresis are

- **Albumin** - 3.2-5.6 g/dL
- **α 1-globulin** - 0.1-0.4 (α 1- antitrypsin (which accounts for 70-90%), α 1-acid glycoprotein, or orosomucoid (which accounts for 10-20%), α 1-lipoprotein, prothrombin, transcortin, and thyroxine-binding globulin.
- **α2-globulin** - 0.4-0.9 (haptoglobin, α2-macroglobulin, α2-microglobulin, ceruloplasmin, erythropoietin) and cholinesterase.
- **β-globulin** - 0.5-1.1 (transferrin,β-Lipoproteins, complement C3, and hemopexin)
- **γ-globulin** - 0.5-1.6 ( includes IgG, IgA, IgM, IgD, and IgE)

**Interpretation of SPE:**

**Abnormal pattern of SPE:**

Hypo albumin: the conditions with lower levels of albumin are nephrotic syndrome, proteinlosingenteropathies, cystic fibrosis (hypoalbuminemia with edema may be an early abnormality in infants with this disease), glomerulonephritis, cirrhosis,
carcinomatosis, bacterial infections, viral hepatitis, congestive heart failure, rheumatoid arthritis, uncontrolled diabetes, intravenous feeding (the hypoalbuminemia is due to a deficiency of amino acids in portal blood), and dietary deficiency of proteins containing essential amino acids. Hypoalbuminemia also occurs in the acute stress reaction.

Elevated α 1 band: seen in acute phase reactions and also in chronic inflammatory and degenerative diseases and cancers, due to increases in α1-antitrypsin and α1-acid glycoprotein.

Falling of 2 band: seen in acute stress reactions (haptoglobin),

Elevation of 2 band with hypoalbuminemia: nephrotic syndrome (due to increase in VLDL, LDL)

Increased β globulin band: seen in anaemia, pregnancy due to increase in transferrin levels

Decreased β globulin band: liver, kidney diseases, neoplastic diseases

Elevated γ-band: seen in trauma and acute inflammatory processes
3. Discuss in detail glycogen metabolism. Mention glycogen storage disorders (SN-Feb 11)

Glycogen metabolism includes both glycogen synthesis and breakdown

**Glycogenesis**

Site: glycogen synthesis occurs mainly in liver (up to 6%) and muscle (up to 1%). Muscle contains about three to four times than liver because of greater mass

**Importance:**

Glycogen is the major storage carbohydrate in animals, it is a branched polymer of α-D-glucose. The excess carbohydrates are converted into glycogen for feature energy expenditure. Muscle glycogen is a readily available source of glucose for glycolysis within the muscle itself. Liver glycogen functions to store and export glucose to maintain **blood glucose** between meals. After 12–18 hours of fasting, the liver glycogen is almost totally depleted.

**Reactions proper:**

Glucose is phosphorylated to glucose-6-phosphate, catalyzed by **hexokinase** in muscle and **glucokinase** in liver.

Glucose 6-phosphate is isomerized to glucose 1-phosphate by **phosphoglucomutase**.

Glucose 1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide uridinediphosphate glucose (UDPGlc)* and pyrophosphate, catalyzed by UDPGlcpyrophosphorylase.

**Glycogen synthase** catalyzes the formation of a glycoside bond between C1 of the activated glucose of UDPGlc and C4 of a terminal glucose residue of preexisting glycogen/glycogen, liberating uridinediphosphate (UDP).

Further glucose residues are attached in the 1→4 position to make a short chain that is a substrate for glycogen synthase.

**Branching**

When the chain has been lengthened to at least 11 glucose residues, **branching enzyme** transfers a part of the 1→4 chain (at least six glucose residues) to a neighboring chain to form a 1→6 linkage, establishing a **branch point**. The branches grow by further additions of 1→4-glucosyl units and further branching.

**Glycogenolysis**

**Glycogen phosphorylase** catalyzes the phosphorylytic cleavage by inorganic phosphate (phosphorylysis) of the 1→4 linkages of glycogen to yield glucose 1-
phosphate. The terminal glucosyl residues from the outermost chains of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a 1→6 branch.

Another enzyme glucan transferase transfers a trisaccharide unit from one branch to the other, exposing the 1→6 branch point. Hydrolysis of the 1→6 linkages requires the debranching enzyme. Hydrolysis of the 1→6 linkages requires the debranching enzyme. Further phosphorylase action can then proceed. The combined action of phosphorylase and these other enzymes leads to the complete breakdown of glycogen.

The reaction catalyzed by phosphoglucomutase is reversible, so that glucose 6-phosphate can be formed from glucose 1-phosphate. In liver and kidney, but not in muscle, there is a specific enzyme, glucose-6-phosphatase, that hydrolyzes glucose 6-phosphate, yielding glucose that is exported, leading to an increase in the blood glucose concentration.

Glycogen storage diseases:

"Glycogen storage disease are group of inherited disorders characterized by deposition of an abnormal type or quantity of glycogen in the tissues. The major glycogen storage disorders are tabulated.

<table>
<thead>
<tr>
<th>Glycogenosis</th>
<th>Name</th>
<th>Cause of Disorder</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Von Gierke’s disease</td>
<td>Deficiency of glucose-6-phosphatase</td>
<td>Liver cells and renal tubule cells loaded with glycogen. Hypoglycemia, lactic-acidemia, ketosis, hyperlipemia.</td>
</tr>
<tr>
<td>Type II</td>
<td>Pompe’s disease</td>
<td>Deficiency of lysosomal α-1→4- and 1→6-glucosidase (acid maltase)</td>
<td>Fatal, accumulation of glycogen in lysosomes, heart failure.</td>
</tr>
<tr>
<td>Type III</td>
<td>Limit dextrinosis, Forbes’ or Cori’s disease</td>
<td>Absence of debranching enzyme</td>
<td>Accumulation of a characteristic branched polysaccharide.</td>
</tr>
<tr>
<td>Type IV</td>
<td>Amylopectinosis, Andersen’s disease</td>
<td>Absence of branching enzyme</td>
<td>Accumulation of a polysaccharide having few branch points. Death due to cardiac or liver failure in first year of life.</td>
</tr>
<tr>
<td>Type V</td>
<td>Myophosphorylase deficiency, Mc Ardle’s syndrome</td>
<td>Absence of muscle phosphorylase</td>
<td>Diminished exercise tolerance; muscles have abnormally high glycogen content (2.5–4.1%). Little or no lactate in blood after exercise.</td>
</tr>
<tr>
<td>Type VI</td>
<td>Hers’ disease</td>
<td>Deficiency of liver phosphorylase</td>
<td>High glycogen content in liver, tendency toward hypoglycemia.</td>
</tr>
<tr>
<td>Type VII</td>
<td>Tarui’s disease</td>
<td>Deficiency of phosphofructokinase in muscle and erythrocytes</td>
<td>As for type V but also possibility of hemolytic anemia.</td>
</tr>
<tr>
<td>Type VIII</td>
<td></td>
<td>Deficiency of liver phosphorylase kinase</td>
<td>As for type VI.</td>
</tr>
</tbody>
</table>
4. Write short notes on
   a. Fatty liver and lipotrophic factors

Fatty liver refers to the accumulation of excess of TAG in liver cells. The ratio of factors causing fat deposition in liver to fat removal determines the severity of disease.

Causes of fatty liver

Mobilization of non-essential fatty acids form adipose tissue to liver: this condition seen in diabetes mellitus, the capacity of FA uptake exceeds its capacity to secrete as VLDL

Increase in synthesis of FA form glucose: excess intake of carbohydrates or fat may leads to accumulation of fat in liver

Reduced removal of fat form liver due to liver failure or infection:
b. Creatinine clearance test

Clearance is defined as the quantity of blood or plasma completely cleared of a substance per unit time and is expressed in millilitre per minute; it is the ml of plasma which contains the amount of that substance excreted by the kidney within a minute.

Formula for clearance \( C_0 = \frac{U \times V}{P} \)

Where \( U \) – concentration of the substance in urine; \( P \) - concentration of the substance in plasma or serum and \( V \) = the ml of urine excreted per minute. The value is expressed as L/min.

Ideal substance to measure clearance: if the substance is freely filtered across the capillary wall, and neither secreted nor reabsorbed, then its clearance is equal to glomerular filtration rate.

Eg: Creatinine

**Creatinine clearance test:**

It is gold standard test to measure kidney function, since creatinine is meets the ideal marker. Creatinine is a waste product, formed from creatinine phosphate. Concentration of creatinine is dependent on muscle mass not on diet, age, exercise. Women and children secrete less creatinine because they have less muscle than males.

Creatinine is ideal marker, since it production is continuous without any fluctuations.

**Test procedure:**

Give 500 ml of water to the patient, to promote good urine flow. After about 30 minutes ask to empty the bladder and discard the urine. Exactly after 1 hr, again void the urine and collect and note the volume. Then collect the blood sample and estimate creatinine in both the samples.

Uncorrected clearance = \( U \times V / P \)

It is useful to correct the clearance value with body surface area; this is important in children and persons with short or tall.

**Corrected creatinine clearance** = \( U \times V \times 1.73 / P \times A \)

Reference ranges for Creatinine clearance= males – 85-125 ml/min, females – 80-115 ml/min

**Interpretation of creatinine clearance**

Creatinine clearance decreased parallel with reduced GFR

Creatinine clearance is important in early assessment of kidney function

Creatinine clearance up to 75% is indicative of adequate kidney function.
c. Explain the chemiosmotic theory of oxidative phosphorylation (SN,Apr 2001)

The coupling of oxidation with phosphorylation is termed oxidative phosphorylation and is called as oxidative phosphorylation. Peter Mitchell in 1961 proposed a theory to explain oxidative phosphorylation.

Generation of proton gradient and synthesis of ATP: the complexes I, III and IV expel protons from inside to outside of the mitochondria membrane. This causes the electrochemical potential difference across the membrane, once established it inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back-translocation of protons across the membrane through the ATP synthase. This in turn depends on availability of ADP and P_i.
d. Discuss the manifestations, molecular bases and laboratory
diagnosis of sickle cell disease (SN,Mar-202)

Sickle cell disease: it is also called as HbS disease

Prevalence: 1 in 1 lack birth

Molecular basis of Disease: the genes for alpha chain and beta chains of Hb reside at 16 and 11 positions respectively. Mutation in these genes gives different forms of Hbs. In HbS case, due to mutations in 11th gene there is displacement of glutamate with valine at 6th position of beta chain. The substitution of hydrophilic glutamic acid by hydrophobic valine cause a localized sticking on the surface of the molecule. The deoxygenated HbS may be with protrusion on one side and cavity on the other side, so that many molecules can adhere and polymerise. This leads to the structural changes in RBC into sickle shape

Symptoms: sickled cells form small plugs in capillaries. Occlusion of major vessels can lead to infarction of affected organs. Death will occur in second decade of life. HbS give protection against malaria.

Laboratory diagnostic procedures:

Electrophoresis: electrophoresis at alkaline pH shows a slower moving band than Hba. At this pH carboxyl group of glutamic acid is negatively charged. But lack of this charge on HbS makes it less negatively charged, and decreases the mobility towards positive pole. In acidic pH HbS moves faster than HbA.

Sickling test

Blood smear is prepared and add reducing agent such as sodium dithionate. And observe RBC for sickling under light microscope

Management: blood transfusion (overload the iron), anti-sickling agents like urea, cyanate and aspirin which interfere with polymerisation