Biochemistry- Paper II Aug 2009

I. Essay questions: (2 x 15 = 30)

1. Name the liver function tests with diagnostic significance of each. Write in detail the biochemical tests of any three done in your laboratory.

Ans.

Liver has around 1800 functions. The tests used to diagnose liver disease are called liver function tests. They are:

1. Tests of hepatic excretory function:

a. Serum Bilirubin:

b. Urine Bile pigments, Bile Salts, Urobilinogen**.**

2. Markers of liver injury

a. Alanine aminotransferase (ALT)

b. Aspartate aminotransferase (AST)

c. Alkaline phosphatase (ALP)

d. Gamma glutamyl transferase (GGT)

3. Tests for synthetic function:

a. Total proteins

b. Serum albumin, globulin, A/G ratio

c. Prothrombin time

4. **Special tests:**

a. Ceruloplasmin

b. Ferritin

c. Alpha -1 antitrypsin

d. Alpha fetoprotein

Explanation of three tests in detail:

1. **Serum albumin:**

Almost all plasma proteins with exception of immunoglobulins are synthesized by liver. Serum albumin is the quantitatively the most important protein synthesized by the liver, and reflects the extent of functioning liver cell mass.

Since albumin has a half life of 20 days, in all chronic diseases of liver, the albumin level is decreased. A reversal of A/G ratio is seen in cirrhosis of liver.

Normal albumin level is 2.5-3.5 g/dl.

2. **Prothrombin Time:**

Since Prothrombin is synthesized by the liver, it is a useful indicator of liver function. The half life of Prothrombin is 6 hours only. Therefore PT indicates the present function of liver.

PT is prolonged only when more than 80% of liver function is lost. In Vit K deficiency PT is prolonged. To differentiate from that Vit K is given to the patient and PT is measured. Elevated of PT after this indicates liver dysfunction.

3. Van Den Bergh test:

The serum bilirubin estimated by Van den Bergh reaction, where diazotised sulfanilic acid reacts with bilirubin to form a purple colored complex, azobilirubin. Normal serum does not give a positive van den Bergh test.

When bilirubin is conjugated, the purple color is produced immediately on mixing with the reagent, the response is said to be van den Bergh direct positive.

When the bilirubin is unconjugated, the color appears only after addition of alcohol, so it is said to be van den Bergh indirect positive.

When both conjugated and unconjugated bilirubins are present, it produces an immediate color, which intensifies on adding alcohol. It is then said to be biphasic.

<u>In hemolytic jaundice</u>- unconjugated bilirubin elevated- so indirect positive

<u>In obstructive jaundice</u>-conjugated bilirubin elevated- so direct positive

<u>In hepatic jaundice</u>- both conjugated and unconjugated bilirubin elevated- so biphasic

2. Describe the pathway for synthesis of urea from ammonia. What is the normal blood urea level? Name the conditions in which blood urea level is increased and give the biochemical basis.

Ans.

The urea cycle is the first metabolic pathway to be elucidated in 1932.

This cycle is also called as KREBS- HESELIT cycle.

The two nitrogen atoms of urea derived from two different sources, one from ammonia and the directly from aspartic acid (aminogroup).

STEP 1: **Formation of carbomyl phosphate**:

One molecule of ammonia condenses with CO2in the presence of two molecules of ATP to form carbomyl phosphate by the enzyme carbomyl phosphate synthase I.

STEP II: Formation of citrulline:

The carbomyl group is transferred to the NH2 group of ornithine by ornithine transcarbomylase.

STEP III: Formation of argino succinate:

One molecule of aspartic acid adds to citrulline forming a carbon to nitrogen which provides second nitrogen of urea by arginosuccinate synthetase.

Two high energy phosphate bonds utilized.

STEP IV: Formation of arginine:

Arginosuccinate cleaved by argino succinate lyase to arginine and fumarate.

STEP V: Formation of urea:

Hydrolysis of arginine to urea and ornithine by arginase.

Ornithine return to the mitochondria to react with other molecule of carbomyl phosphate for proceeds of cycle.

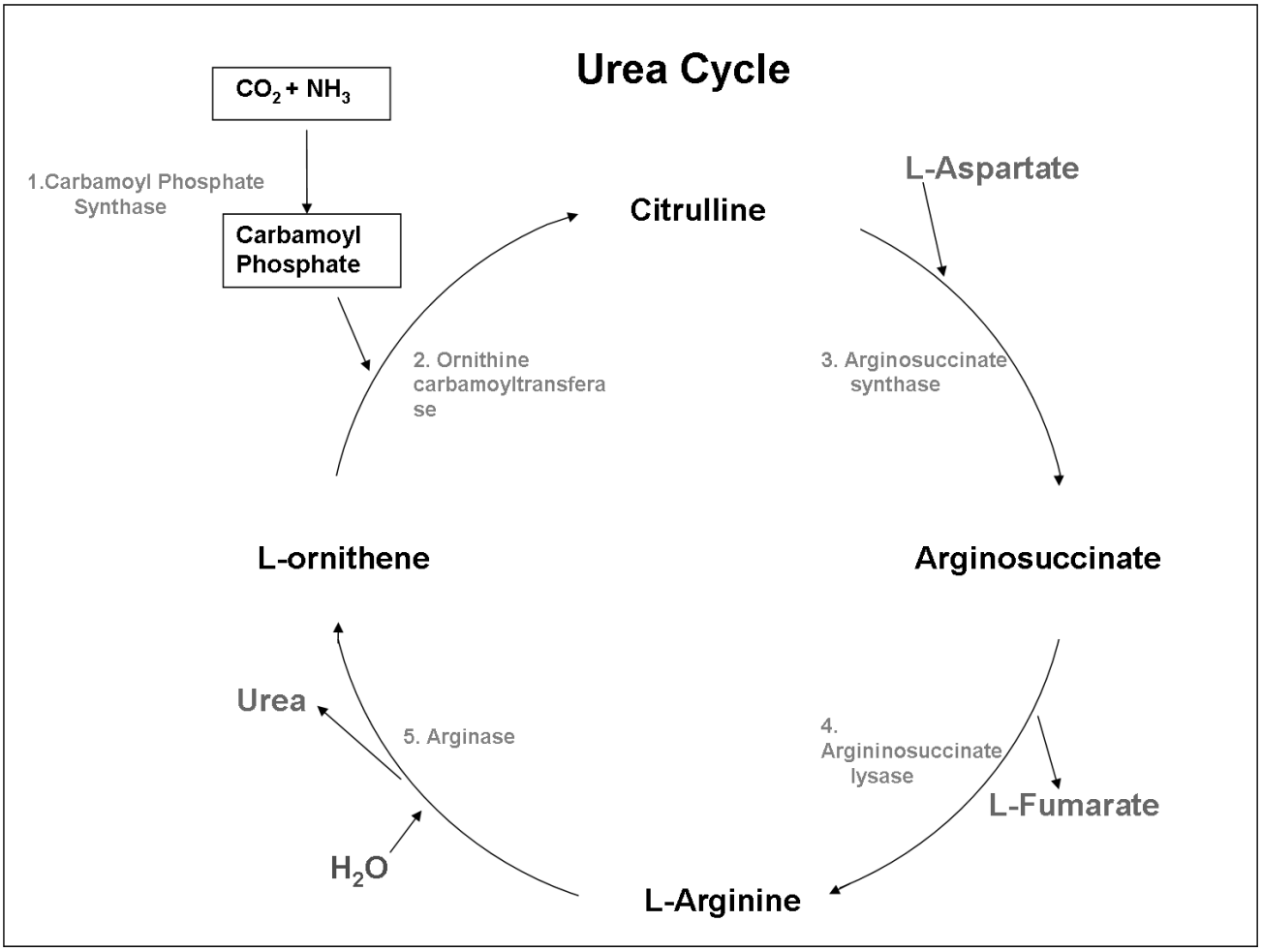


Fig. Urea cycle

Urea level in blood:

Normal urea level is 20-40mg/dl. Urinary excretion of urea is 15-30gm/day.

Urea level may be increased due to protein intake and inborn errors of urea cycle. Blood urea level increased leads to uremia.

UREA CYCLE DISORDERS:

Hyper ammonemia type I

Enzyme deficient is carbomyl phosphate synthase I.

It is an autosomal recessive disease, mental retardation

Hyper ammonemia type II

Enzyme deficient is ornithine transcarbomylase.

Ammonia, glutamine increased in blood. Orotic aciduria.

Citrullinemia:

Enzyme deficient is argino succinate synthetase .

It is autosomal recessive disorder.

High blood levels of ammonia and citrulline.

Argininosuccinic aciduria:

Enzyme deficient is Argininosuccinate lyase

Argininosuccinate in blood and urine. Friable brittle tufted hair

(Trichorrhexis nodosa)

Hyperargininemia:

Enzyme deficient is Arginase

Arginine increased in blood and CSF. Instead of Arginine, cystein and lysine are lost in urine.

II. write short notes on (10 x 5 = 50)

1. Denaturation

- Ans. The loss of secondary, tertiary and quaternary structure of proteins without alteration of the primary structure(aminoacids connected by peptide bonds) is called denaturation
- Mild heating, treating with urea, X-rays, UV radiation, high pressure, vigorous shaking may lead to denaturation
- The biological activity of the protein is lost due to loss of higher structures
- These proteins are vulnerable to Proteolytic enzymes, so cooked food is easily digestible than uncooked food.
- Denaturation is many times reversible if the agent causing the denaturation is removed. Eg. IG treated with urea

2. Reverse transcription

Ans.

Many living organisms' genetic material is in DNA. DNA dependent RNA polymerase is involved in transcription (production of mRNA from DNA).

But some viruses and other organisms have only RNA as their genetic material. Retro viruses are RNA viruses. Eg. HIV. The enzyme reverse transcriptase will make DNA strand from RNA. Usually transcription is production of mRNA from DNA. So this process of making DNA from RNA is named as reverse transcription.

Once the virus infects human cells, the viral RNA is converted into DNA by RT and the RNA is destroyed by an RNAse. The single stranded DNA acts as template to produce ds DNA. This DNA is incorporated into human genome and it leads to production of viral proteins and RNA. Some of the tumor causing viruses eg. Human papilloma virus also infects using RT.

3. Sphingolipidosis

Ans.

Sphingolipidoses are lysosomal storage disorders arising in degradation of Sphingolipids. They are

- **Tay-Sachs disease**- Hexosaminidase deficiency. Accumulation of GM2 Ganglioside. It is characterized by Mental retardation, blindness, muscular weakness.
- **Fabry's disease** – α-Galactosidase. deficiency. Accumulation of Globotriaosylceramide. It is characterized by Skin rash, kidney failure (full symptoms only in males; X-linked recessive).
- **Metachromatic leukodystrophy**- Arylsulfatase deficiency. Accumulation of 3-Sulfogalactosylceramide. It is characterized by Mental retardation and psychologic disturbances in adults; demyelination.
- **Krabbe's disease -**β-Galactosidase deficiency. Accumulation of Galactosylceramide. It is characterized by Mental retardation; myelin almost absent.
- **Gaucher's disea**se- β Glucosidase deficiency. Accumulation of Glucosylceramide. It is characterized by Enlarged liver and spleen, erosion of long bones, mental retardation in infants.
- **Niemann-Pick disease** - Sphingomyelinase deficiency. Accumulation of Sphingomyelin. It is characterized by Enlarged liver and spleen, mental retardation; fatal in early life.
- **Farber's disease** Ceramidase deficiency. Accumulation of Sphingosine. It is characterized by Hoarseness, dermatitis, skeletal deformation, and mental retardation; fatal in early life.

4. GOUT

Ans.

When uric acid levels increase in the blood it tends to get deposited as crystals in synovial fluid of joints leading to inflammation and acute arthritis. This disease is called Gout

Etiology:

- **primary gout**
    - 5-phosphoribosyl amidotransferase- there will be increased production of purines due to absence of regulation on this enzyme. It's a genetic defect
    - Abnormal PRPP synthase- there will be increased production of PRPP due to absence of regulation on PRPP synthase. It's a genetic defect.
    - Salvage pathway enzyme deficiencies-there would be more availability of PRPP leading to production of purines→ uric acid
    - Von Gierke's Disease- due to G-6-Pas deficiency, G-6-P is not converted to glucose. So it goes through HMP shunt resulting in more nucleotide bases, increasing urate production.
- **Secondary Gout:**
    - Increased production of uric acid- malignancy- lymphomas, leukemias; after treatment of cancer, cancer cells breakdown, leading to hyperuricemia; trauma-tissue damage; starvation-where catabolism is increased
    - Reduced excretion- renal failure, thiazide diuretics- which inhibits urate secretion, lactic acidosis and Ketoacidosis- interferes with urate secretion

Clinical features:

Uric acid gets deposited in the cooler areas of body like distal joints to form tophi. Hypeuricemia leads to increased excretion of uric acid through the kidneys, so uric acid crystals gets deposited in the urinary tract leading to renal calculi.

Treatment:

1. dietary purine intake should be reduced, alcohol should be restricted

2. Uricosuric drugs which increases the excretion of uric acid like probenecid should be used

3. For calculi Allopurinol can be used. It inhibits xanthine oxidase and reduces the formation of uric acid. It's a type of suicide inhibition like aspirin, where the enzyme becomes completely functionless.

4. Colchicine, an anti-inflammatory drug used in RA can be used to reduce inflammation in joints.

Lysch Nyhan syndrome

- it is a X-linked inborn error of purine metabolism, incidence 1:10,000
- deficiency of HGPRTase which acts in salvage pathway
- so the salvage pathway is stopped and PRPP accumulates which will go for catabolism to uric acid
- hyperuricemia leads to nephrolithiasis and gout
- it is also characterized by self mutilation, mental retardation

5. Metabolic acidosis

Ans.

Acidosis is reduction of pH less than 7.38. it is classified into metabolic and respiratory acidosis.

Metabolic acidosis is primarily due to base deficit. The bicarbonate deficit may occur due to excess acid production or depletion of bicarbonate.

Anion gap: it is difference between measured cations and measured anions. Usually it shows the unmeasured anions. The normal value is 12 mmol/L.

Metabolic acidosis is classified into:

1. High anion gap metabolic acidosis- accumulation of acid

- Renal failure- H^+ excretion is less.
- DKA- ketoacid production is more
- Lactic acidosis- hypoxia, circulatory failure, many drugs, and bacterial metabolism increases lactic acid.
- Methanol, ethanol also causes lactic acidosis

2. Normal anion gap metabolic acidosis- both anions and cations lost but acidosis present

- Diarrhea- loss of bicarbonate from intestinal secretions
- Hyperchloremic metabolic acidosis- in renal tubular acidosis- which may be due to either failure to secrete acid or conserve bicarbonate; acetazolamide treatment

Compensation:

Metabolic acidosis is compensated by hyperventilation so that pCO_2 comes down.

Features:

pH will be low, Bicarbonate will be low. PCO_2 starts decreasing due to respiratory compensation

6. Tumor markers

Ans.

Definition: Tumor markers are substances whose presence or elevation in body is used to identify or confirm the presence or tell valuable other information about the cancer in the body.

Some examples of tumor markers are:

- Alpha feto protein -Hepatoma, Germ cell tumors,
- Carcinoembryogenic antigen- Colorectal and GI tumors
- CA-125 - Ovarian cancer
- Alkaline phosphatase - Bone scondaries, Liver secondaries
- Prostatic acid phosphatase - Prostate cancer
- Prostate specific antigen - Prostate cancer
- Neuron specific enolase - Nervous system tumors
- Beta hCG - Choriocarcinoma
- Calcitonin - Medullary Ca of Thyroid
- ACTH - Lung oat cell cancer
- VIP - APUDomas
- VMA - Pheochromocytoma, Neuroblastoma
- Bence Jones proteins - Multiple Myeloma

7. Colorimeter

Ans.

Principles of colorimetry

Colorimeter is an instrument used to quantitate biological substances. The amount of light absorbed or transmitted by a colored solution is in accordance with the beer-lamberts law.

BEER'S LAW:

Intensity of the color is directly proportional to the concentration of the colored particles in the solution.

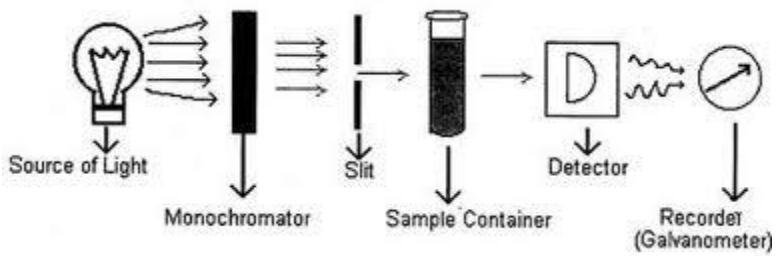
LAMBERT'S LAW:

Amount of light absorbed by a colored solution depends on the length of the column or the depth of the liquid through which light passes.

Colorimeter is based on the above said principles.

Instrument:

Colorimeter consists of a light source, usually a tungsten filament lamp, a monochromator to allow a particular wavelength of light, slit, Cuvette containing the sample solution and a photoelectric cell, which converts light energy to electrical energy.



8. Functions of adrenal cortical hormones

Ans.

Glucocorticoids:

- Carbohydrates- gluconeogenetic enzymes stimulated, glycolytic enzymes suppressed leading to hyperglycemia
- Lipids- increased lipolysis leading to mobilization of fats and depositing in unusual sites like neck
- Proteins- catabolism increased→ aminoacids go for gluconeogenesis
- Bones- inhibits osteoblast function causing osteoporosis
- Immune system- suppresses immune system by lysis of lymphocytes. Anti-inflammatory and antiallergic

Mineralocarticoids:

Aldosterone causes increased reabsorption of sodium and water in the distal tubules leading to

- Reduced urine formation
- Increasing the BP

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9. Plasmid

Ans.

Chimeric or hybrid DNA molecules can be constructed in **cloning vectors**—typically bacterial plasmids, phages, or cosmids—which then continue to replicate in a host cell under their own control systems. In this way, the chimeric DNA is amplified.

Bacterial **plasmids** are small, circular, duplex DNA molecules whose natural function is to confer antibiotic resistance to the host cell.

Plasmids have several properties that make them extremely useful as cloning vectors. They exist as single or multiple copies within the bacterium and replicate independently from the bacterial DNA. The complete DNA sequence of many plasmids is known; hence, the precise location of restriction enzyme cleavage sites for inserting the foreign DNA is available. Plasmids are smaller than the host chromosome and are therefore easily separated from the latter, and the desired plasmid-inserted DNA is readily removed by cutting the plasmid with the enzyme specific for the restriction site into which the original piece of DNA was inserted.

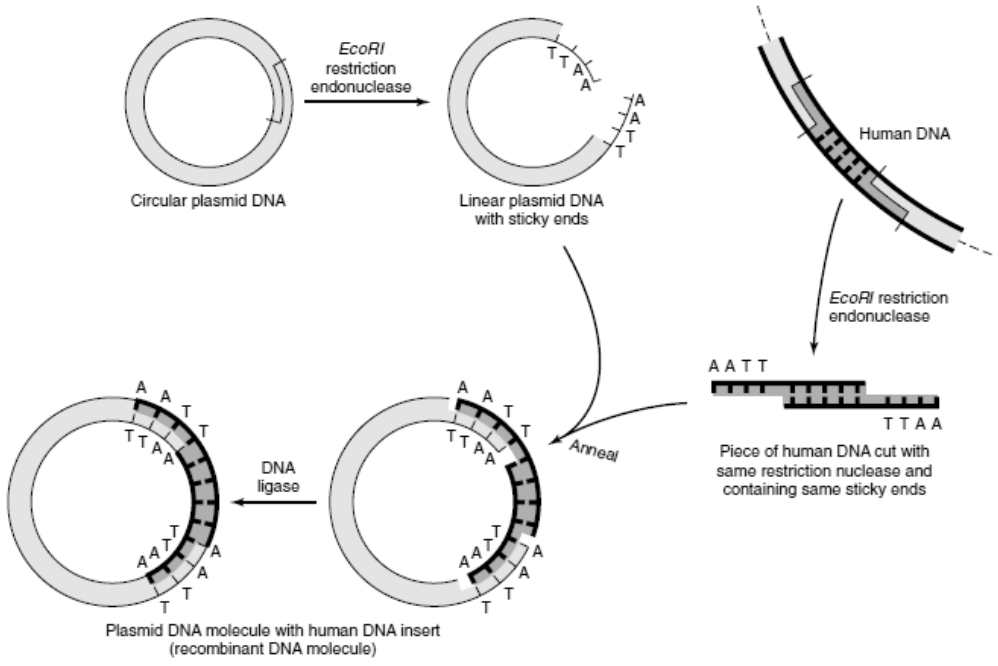

Fig. use of plasmids in making chimeric DNA in Recombinant DNA technology

10. Functions of albumin

Ans.

- Transport protein- it transports various substances like bilirubin, free fatty acid, drugs like aspirin, hormones like thyroxine, steroid hormones, minerals like calcium, copper, etc.,
- Colloid osmotic pressure- albumin cannot pass between intracellular and extracellular compartment. So exerts a net osmotic pressure. The osmotic pressure of plasma is about 278-305mosm/kg, which is necessary for movement of water from ECF to ICF in arteriolar end and reverse in venular end of capillaries
- Buffer-the 16 Histidine residues in albumin can bind to H^+ and can function as a buffer
- Nutrition- liver takes up aminoacids from diet and converts it into albumin. It is then released in blood and taken up by other tissues by pinocytosis. So albumin is a source of aminoacids for the cells. PEM is characterized by hypoalbuminemia which results in growth retardation and edema

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III. Short answer questions: (10 x 2 = 20)

1. Maple syrup urine disease

Ans.

Branched chain aminoacids are valine, leucine and isoleucine. The enzyme of their catabolism is branched chain keto acid decarboxylase. This enzyme is deficient in MSUD. The incidence is 1:100,000. The urine smells like burnt sugar or maple syrup. The disease is characterized by convulsions and mental retardation in early life. Urine contains branched chain keto acids. Diet deficient in branched chain aminoacids should be given. Thiamine is helpful in some patients.

2. Alkali reserve

Ans.

Bicarbonate represents the alkali reserve and it has to be sufficiently high to meet the acid load. If it was too low to give a ratio of 1, all the HCO_3^- would have been exhausted within a very short time; and buffering will not be effective. So under physiological circumstances, the ratio of 20 (a high alkali reserve) ensures high buffering efficiency against acids.

The normal bicarbonate level in serum 24mmol/L.

3. Biological value of proteins

Ans.

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

BV = retained nitrogen/absorbed nitrogen X 100

Suppose 127 mg of protein ‘A’ was consumed y 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 = 123mg

Amount retained =123-24 = 99mg

Therefore BVP = 99/13X100 = 81%

4. Carcinogenic virus

Ans.

Viruses cause cancers in humans and animals by activating oncogenes or damaging tumor suppressor genes or by other mechanisms.

a. Epstein Barr virus : Burkitt's Lymphoma is caused by EBV. It infects the B lymphocytes. The B cells are now immortalized. But they are dependent on B cell growth factor for proliferation. Next it causes transposition of oncogenes, so that they get BCGF independence. But the cells divide slowly. Then the virus activates the c-myc oncogene which causes cancer.

EBV is also linked with nasopharyngeal carcinoma.

b. Human papilloma virus- some strains causes cervical cancer.

c. Hepatitis B virus- Hepatoma

5. Electrophoretic technique and its importance

Ans.

The electrophoresis apparatus consists if a tank which contains electrodes connected to a power supply and buffer. The pH of buffer is selected so that it imparts maximum charge to the electrophoresed substances(eg. Proteins get separated well in a buffer pH of 8.6

Supporting medium is the surface on which electrophoresis is carried out. It may be agar gel, agarose gel, cellulose acetate, paper, etc.

After the run the bands are visualized using naked eye or if needed to be quantified a densitometer can be used

1. serum protein electrophoresis:

- in nephrotic syndrome – globulin is produced more by liver in compensation of renal loss of albumin. So alpha 2 band is prominent

- cirrhosis- albumin band is less prominent

- multiple myeloma- light chain immunoglobulins are produced more so there will be a prominence in gamma globulin region(M band)

2**. hemoglobin electrophoresis**

- S band is seen in sickle cell anemia

- various hemoglobinopathies and thalasemias can be diagnosed

6. Met hemoglobin

Ans.

It is a type of Hb variant. The iron in the heme is in ferrous form. When it is oxidized to ferric form then it is called met hemoglobin.

Small quantities of met Hb formed are reduced by metHb reductase systems using NADH and cytochrome b5. Remaining is reduced using NADPH dependent enzyme. Glutathione dependent met Hb reductase system is also present.

Normally the met Hb level in blood is less than 1%. It has decreased capacity of transporting oxygen. Methemoglobinemia is manifested as cyanosis.

Cytochrome b5 reductase deficiency is the cause for congenital Methemoglobinemia. Oral administration of Methylene blue reduces the symptoms.

Intake of water containing nitrates or absorption of aniline dyes can also cause Methemoglobinemia. Acetaminophen, sulphanilamides, amyl nitrate ingestion are other causes.

G6PD deficiency causes reduced availability of NADPH for the RBCs. At that time the NADPH dependent methemoglobin reductase will be inactive leading to Methemoglobinemia.

7. Importance of glucose six phosphate dehydrogenase deficiency

Ans.

It is the most common inborn error of metabolism. The prevalence is 400million worldwide. It is X linked recessive disease.

The deficiency is manifested only when exposed to certain oxidant drugs or toxins like pimaquine for malaria or ingestion of fava beans. Sulpha drugs may also precipitate the hemolysis. This disease offers protection from malaria since the parasite requires reduced glutathione which is not available in this enzyme deficiency.

8. G-proteins

Ans.

G proteins are signaling transducing molecules attached to the membrane receptors.

Receptors that couple to effectors through G proteins (GPCR) typically have seven membrane-spanning domains. In the absence of hormone, G-protein complex (α, β, γ) is in an inactive GDP bound form and is probably not associated with the receptor.

This complex is anchored to the plasma membrane through prenylated groups on the β, γ subunits. On binding of hormone (H) to the receptor, there is a presumed conformational change of the receptor and activation of the G-protein complex. This results from the exchange of GDP with guanosine triphosphate (GTP) on the α-subunit, after which α and βγ dissociate. The α subunit binds to and activates the effector (E).

9. Renal threshold substances

Ans.

Compounds whose excretion in urine are dependent on blood level are known as threshold substances. At normal or low plasma levels they are completely reabsorbed and are not excreted. But when the blood level is elevated the tubular reabsrptive capacity is saturated and the excess will be excreted in urine.

For glucose the renal threshold is 180mg/dl above which it will be seen in urine. The tubular reabsorptive capacity for glucose is 375mg/min (T_{max}).

In renal glycosuria the renal threshold for the substances are reduced and sen in urine even when they are in normal levels.

10. Carbon monoxide

Ans.

CO is a colorless, odorless gas that is produced in large quantities by vehicles and exhaust from industries. It has 200 times more affinity than oxygen towards hemoglobin. When one molecule of CO binds with Hb, it increases affinity of oxygen toward Hb, so the Hb wont release oxygen. This hemoglobin is unsuitable for oxygen transport. The hemoglobin becomes carboxy hemoglobin once it binds to CO. when the CO-Hb level in blood exceeds 20% breathlessness, headache, nausea, vomiting ensues. At 40-60% death can occur

Oxygen administration is effective.