Chapter-6
proteins & Amino acid Metabolism

(2M) **protein Turn over:-** The protein present in the body is in dynamic State. It is estimated that about 300-400g of protein per day is Constantly degraded and Synthesized which represents body protein turnover.

For instance, the plasma proteins & digestive enzymes are rapidly degraded, their half-lives being in hours or days the Structural proteins (Collagen have long half lives, often in month & years.

Control of protein turn over:- The turnover of proteins is influenced by many factors. A Small polypeptide called UBIQUITIN tags with protein & facilitates degradation. Certain proteins with Amino acid Sequence (PEST Sequence) proline, glutamine, Serine, Threonine are rapidly degraded.

(2M) **Nitrogen Balance:-**

It is determined by comparing the intake of nitrogen (Chiefly by proteins) & the Excretion of nitrogen (mostly undigested protein in feces; urea & Ammonia in urine)

Daily Dietary intake (I) = loss through (U) urine + Faeces (F) + Sweat (S).

Thus, an individual is said to be in a nitrogen balance if the intake & output of nitrogen are the Same.

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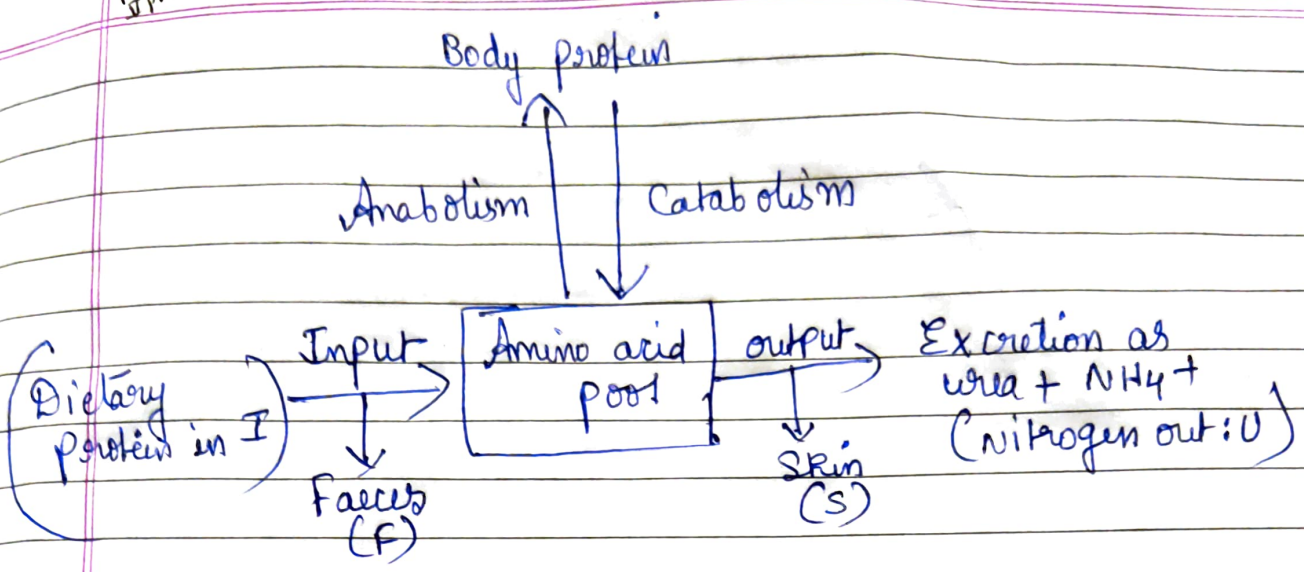


Positive nitrogen Balance:- This is a State in which the nitrogen intake & output of nitrogen are the ~~Same~~ not Same. Nitrogen intake is higher than output.

Eg:- Growing children, pregnant women, Recovery after Serious illness.

Negative nitrogen Balance:- This is a State in which the nitrogen output is higher than the input.

Eg:- children Suffering from kwashiorkor & Marasmus.

Prolonged Negative nitrogen Balance may lead to death

Transamination

The transfer of an Amino ($-NH_2$) group from an amino acid to a Keto acid is known as Transamination. This process involves the interconversion of a pair of amino acids and a pair of Keto acids, catalysed by a group of enzymes called Transaminases (aminotransferases)

Salient features of Transamination

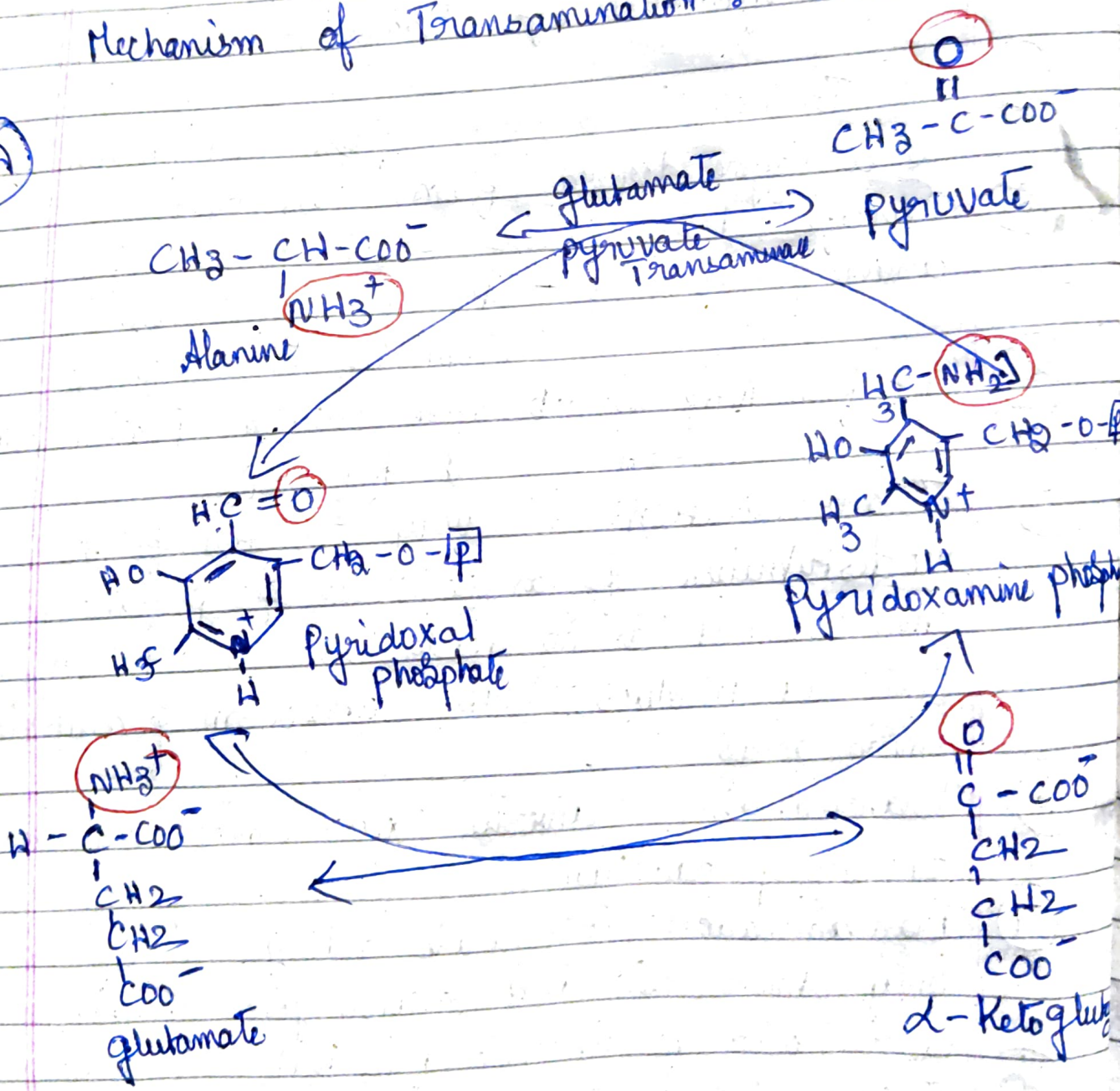
1) All Transaminases require Pyridoxal phosphate (PLP) a coenzyme derived from Vit B_6.
2) Specific transaminases exist for each pair of amino & Keto acids - Alanine Transaminase
   Aspartate Transaminase
3) No free NH_3 is liberated during Transamination
4) Transamination is Reversible.
5) Transamination is very essential for the redistribution of amino acids & production of non-essential amino acids as per the need of cell. It involves both Catabolism & anabolism of amino acids
6) Transamination diverts Excess amino acids towards Energy production
7) Transamination Concentrate nitrogen in glutamate. only Amino acid that undergoes oxidative deamination to liberate free NH_3 for urea Synthesis
8) Except Lysine, Threonine, proline & Hydroxy proline participate in Transamination.

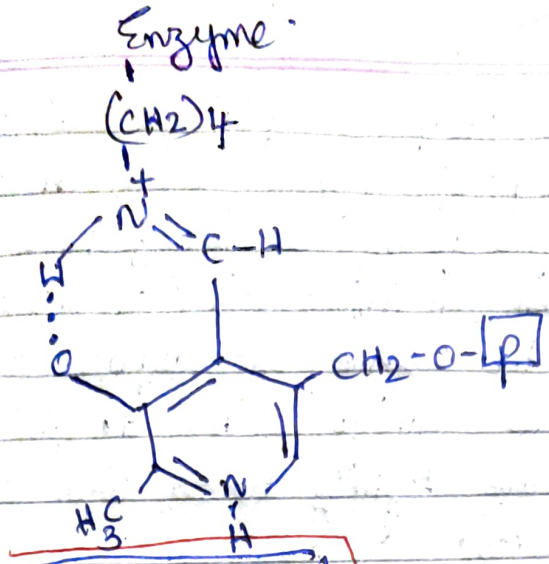
(9) Transamination ~~diverts~~ ~~The excess amino~~ is not restricted to α-amino groups. δ-amino group of Ornithine is Transaminated

(10) Serum Transaminases are important for diagnostic and prognostic Purposes.

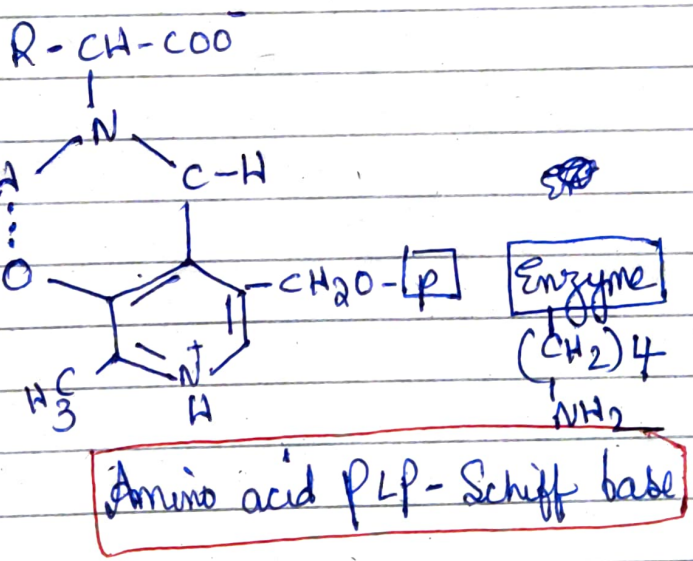
Mechanism of Transamination :-

(A)





Enzyme PLP Schiff base.



Amino acid PLP - Schiff base.

Transamination occurs in two stages:-

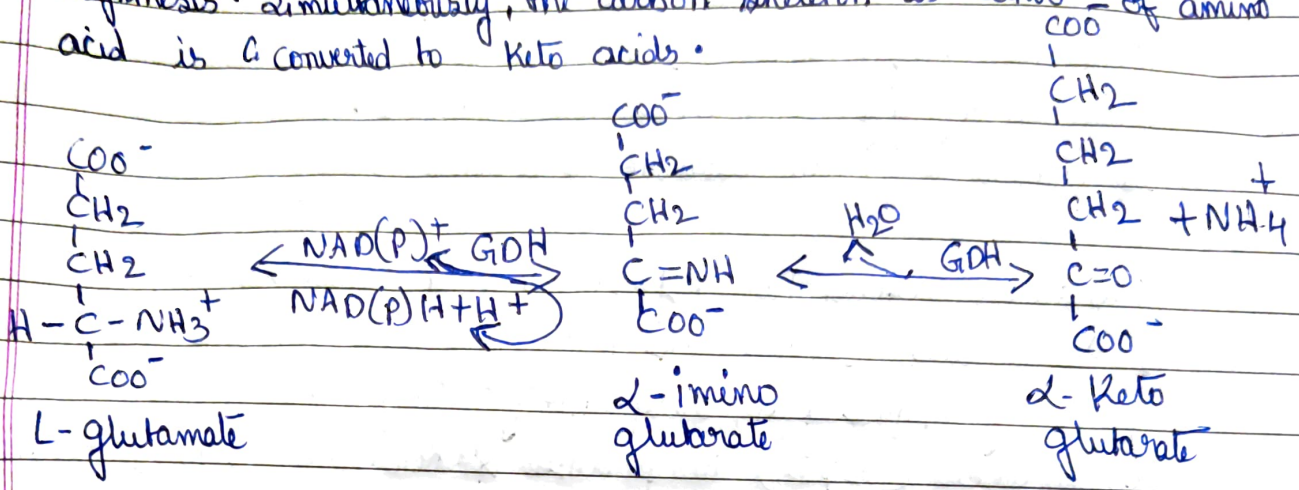
(1) Transfer of the amino group to the coenzyme Pyridoxal phosphate (bound to the coenzyme) to form Pyridoxamine phosphate.

(2) The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated

the aldehyde group of PLP is linked with ε-amino group of lysine residue at the active site of enzyme forming as Schiff base (imine linkage). when an amino acid (substrate) comes in contact with the enzyme, it displaces lysine & a new Schiff base linkage is formed. the amino acid PLP-Schiff base tightly binds with the enzyme by covalent forces.

Deamination

The removal of amino group from the amino acid as NH_3 is deamination. Deamination results in the liberation of NH_3 for urea synthesis. Simultaneously, the carbon skeleton of amino acid is converted to keto acids.



It might be oxidative (or) non-oxidative.
Transamination & deamination occurs simultaneously mostly involving glutamate as central molecule. For this reason, this process is also called as Transdeamination.

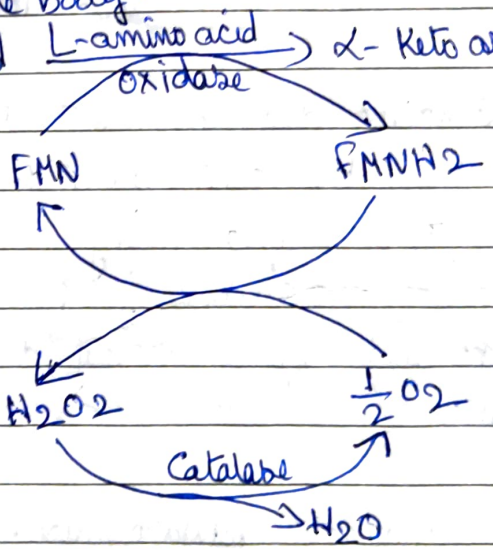
I. Oxidative deamination :- It is the liberation of free ammonia from the amino group of amino acids coupled with oxidation. This occurs mostly in liver & kidney. The purpose of oxidative deamination is to provide NH_3 for urea synthesis & α-keto acids for a variety of reactions, including energy generation.

Role of glutamate dehydrogenase :- During transamination, the amino groups of most amino acids are transferred to α-keto glutarate to produce glutamate. Thus, glutamate serves as a collection center for amino groups in the biological system. Glutamate rapidly undergoes oxidative deamination catalysed by glutamate dehydrogenase (GDH) to liberate NH_3. This enzyme can also utilize either NAD^+ (or) $NADP^+$ as co-enzyme.

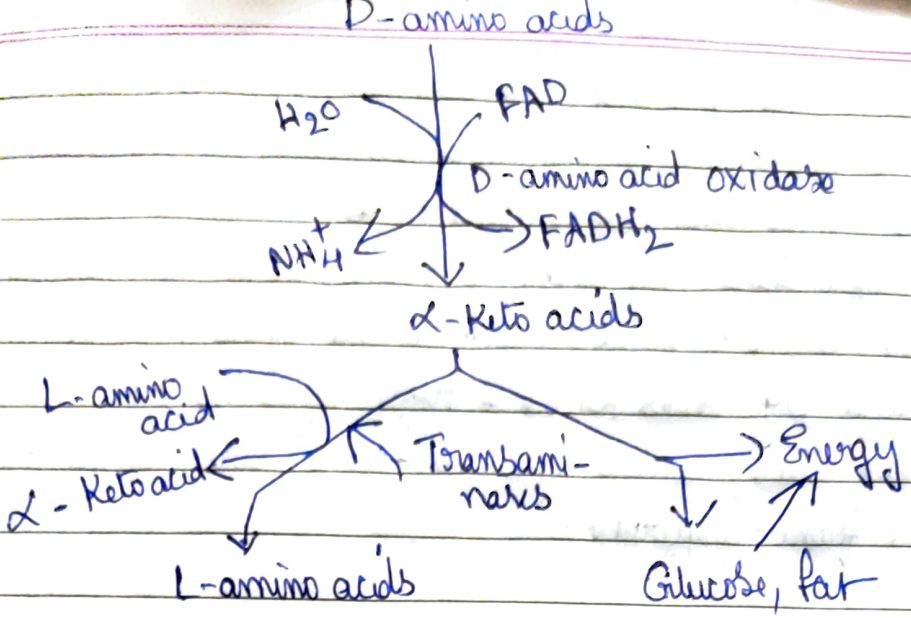
Conversion of glutamate to α-Ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate.

Glutamate dehydrogenase reversibly links up glutamate metabolism with TCA cycle through α-Keto glutarate. Glutamate dehydrogenase is involved in both catabolic & anabolic reactions.

Oxidative deamination by Amino acid oxidases.
L-Amino acid Oxidase and D-amino acid oxidase are flavoproteins possessing FMN & FAD, respectively. They act on the corresponding amino acids (L & D) to produce α-Keto acids & NH_3. In this reaction O_2 is reduced to H_2O_2, which is later decomposed by Catalase.
The ~~activity~~ of L-amino acid oxidase do not act on glycine & dicarboxylic acids. D-amino acids are regularly taken in diet & are metabolized by the body.



The α-Keto acids produced by the action of D-amino acid oxidase from D-amino acids undergo transamination to L-amino acid Keto acids may be oxidized to generate energy (ATP) act as precursors for glucose & fat Synthesis. D-amino acid oxidase is important for its conversion of unnatural D-amino acids to L-amino acids.



Non-oxidative deamination

Some of the amino acids are deaminated to liberate NH_3 without undergoing oxidation.

a) Amino acid dehydrases :- Serine, threonine & homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases.

Serine, Threonine, Homoserine $\xrightarrow{\text{Dehydratase}}$ Respective α-Keto acids. + NH_3

b) Amino acid desulfhydrases :- The Sulfur amino acids, namely Cysteine & homocysteine, undergo deamination Coupled with desulfhydration to give Keto acids

Cysteine $\xrightarrow{\text{Desulfhydrase}}$ Pyruvate. + $NH_3 + H_2S$

c) Deamination of Histidine :- The enzyme histidase acts on histidine to liberate NH_3 by a non-oxidative deamination process.

Histidine $\xrightarrow{\text{Histidase}}$ urocanate + NH_3

urea cycle

urea is the end product of protein metabolism. The nitrogen of amino acids, Converted to ammonia is toxic to the body. It is Converted to urea and detoxified.

urea is Synthesized in liver & transported to Kidneys for excretion in urine. Kreb's - Henseleit cycle (first metabolic cycle elucidated by Hans Krebs & Kurt Henseleit).

urea has two amino groups ($-NH_2$) one derived from NH_3 & the other from aspartate.

Synthesis of urea:-

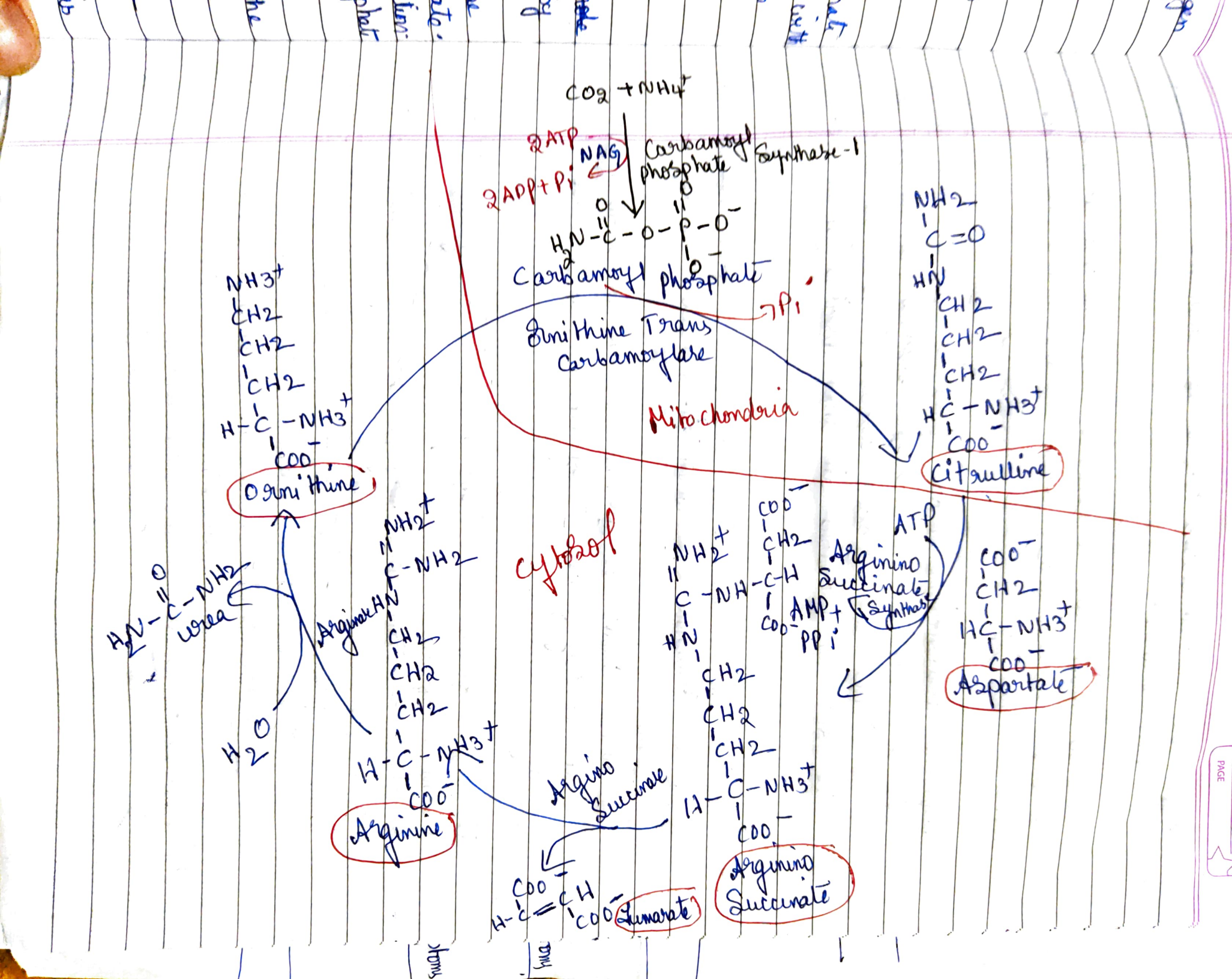
1) Synthesis of Carbamoyl phosphate:- Carbamoyl phosphate Synthase-I of mitochondria Catalyses the Condensation of NH_4^+ ions with CO_2 to form Carbamoyl phosphate. This step Consumes 2 ATP & is irreversible & rate-limiting. Carbamoyl phosphate-I requires N-acetylglutamate for its activity.

2) Formation of citrulline:- Citrulline is Synthesized from Carbonyl phosphate & Ornithine by Ornithine transCarbamoylase Ornithine is regenerated and used in urea cycle. Therefore, ~~its role~~ Citrulline produced in this reaction is transported to cytosol by a transporter System.

3) Synthesis of ArginoSuccinate:- ArginoSuccinate Synthase Condenses citrulline with Aspartate to produce arginoSuccinate the Second amino group of urea is incorporated in this reaction This step requires ATP which is cleaved to AMP & pyrophosphate (PPi).

4) Cleavage of ArginoSuccinate:- ArginoSuccinase Cleaves arginoSuccinate to give arginine & fumarate. Arginine is the immediate precursor for urea. Fumarate Connects with TCA cycle & gluconeogenesis.

5) Formation of urea:- Arginase is the fifth & final enzyme that cleaves arginine to yield urea & Ornithine. Ornithine enters mitochondria & is reused in the urea cycle. Arginase is activated by Co_2^+ & Mn^{2+}.


$CO_2 + NH_4^+$
2ATP
2ADP + P_i
NAG
Carbamoyl phosphate Synthase-1
Carbamoyl phosphate
P_i
Ornithine Trans Carbamoylase
Mitochondria
Ornithine
Citrulline
cytosol
Urea
H_2O
Arginase
Arginine
Argino Succinate
ATP
AMP + PP$_i$
Argino Succinate Synthase
Aspartate
Fumarate
Argino Succinate

(2M) ① What are Transaminases? Write the diagnostic importance of liver transaminases?

Transaminases are a group of enzymes that catalyse transfer of amino group ($-NH_2$) from an amino acid to a keto acid (Transamination).

Aspartate Transaminase (AST) & Alanine Transaminase (ALT) are very important as diagnostic enzymes. Serum AST is mostly elevated in cardiac disorders (Myocardial infarction) while ALT is increased in liver diseases (viral hepatitis)

② What is the normal blood urea level? ~~Name two Conditions~~ Name two conditions in which blood urea level is elevated.

The normal blood urea level concentration is 10-40 mg/dl. About 15-30g of urea (7-15g nitrogen) is excreted in urine per day.

Blood urea estimation is used as a screening test for the evaluation of Kidney function.
Elevation of Blood urea may be broadly classified in 3 categories:

① pre renal - After major surgery (↑ed protein breakdown) Burns,
② Renal - acute glomerulonephritis, polycystic kidney
③ Post Renal - obstruction in urinary tract (Tumors, Stones)

"uremia" - is the term used to indicate increased blood urea levels due to renal failure.

(3) Name four Metabolic disorders of urea cycle with enzyme defect.

Defect	Enzyme Involved	Characteristics
1. Hyperammonemia Type-I	Carbamoyl phosphate Synthase-I	Highly elevated blood NH_3, Mental Retardation
2. Hyperammonemia Type-II	Ornithine Trans-Carbamoylase	Increased NH_3, & glutamine in blood & urine
3. Citrullinemia	Arginino Succinate Synthase	High blood Conc. of NH_3 & citrulline
4. Arginino Succinic aciduria	Arginino Succinase	elevated arginino Succinate in blood & urine.

Production of Bile pigments

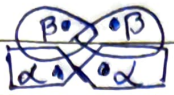
Erythrocytes have a life span of 120 days. Erythrocytes are taken up & degraded by the macrophages of Reticulo Endothelial System in Spleen & liver.

→ the Hemoglobin is cleaved to protein part globin & non-protein heme. the globin may be recutilized for the formation of Hb (or) degraded into individual amino acids

→ A Complex Microsomal enzyme Called heme oxygenase, utilizes NADPH & O_2 & cleaves the methenyl bridges between two Pyrrole rings (A & B) to form Biliverdin (green pigment)

Aged Erythrocytes

Macrophage

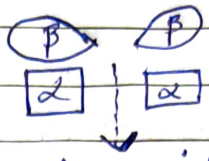


Hemoglobin

Other heme proteins

Apoproteins

Heme



Amino acids

Reutilized / degraded.

O_2 NADPH+H$^+$

NADP$^+$

Heme oxygenase

Fe^{3+}, CO

Biliverdin

NADPH+H$^+$

NADP$^+$

Biliverdin Reductase

Bilirubin

Blood

Bilirubin - albumin complex

LIVER

Bilirubin

2 UDP glucuronate

Bilirubin glucuronyl Transferase

Bilirubin diglucuronide (to bile)

Intestine | Kidney

Microbial enzymes

UROBILINOGEN

Microbial enzymes

Kidney

Urobilin

TO urine

Stercobilin

TO faeces

→ Biliverdin reductase reduces methenyl bridges (Between pyrrole rings C & D) of Biliverdin & converts it into Bilirubin (yellow pigment).

→ Bilirubin is lipophilic & is insoluble in aqueous solution Bilirubin is transported in the plasma in a bound form with albumin. This albumin - Bilirubin Complex enters liver.

→ Certain drugs & Antibiotics displace Bilirubin from albumin. Due to this, bilirubin can enter CNS & cause damage to neurons

→ The albumin - Bilirubin Complex enters liver, bilirubin dissociates & taken up by hepatocytes through active transport.

→ Bilirubin Conjugates with 2 molecules of glucuronate & this reaction is catalysed by bilirubin glucuronyl Transferase.

→ Now, the Bilirubin diglucuronide is in Conjugate form & is completely water soluble.

→ Conjugated Bilirubin enters into bile.

→ Bilirubin glucuronides are hydrolyzed in the intestine by bacterial enzymes namely β-glucuronidases & is converted to urobilinogen (colourless). A small part enters into circulation.

→ Urobilinogen is converted to urobilin (yellow coloured compound) in the kidneys & is excreted.

→ Major part of urobilinogen is converted by bacteria into Stercobilin.

→ The characteristic brown colour of faeces is due to Stercobilin.

Jaundice & its types

The normal Serum total bilirubin Concentration is the range of 0.2 to 1.0 mg/dl.

Jaundice is a clinical Condition characterized by yellowish discolouration of Sclera of eye & Skin.
It is caused by deposition of bilirubin due to its elevated levels in the Serum.

Jaundice (also called as Icterus) is a Symptom rather than a disease. Jaundice is classified into 3 types -
Haemolytic, Hepatic & obstructive

(1) Haemolytic Jaundice:- This Condition is associated with increased haemolysis of Erythrocytes (Eg:- incompatable blood transfusion, Malaria, Sickle cell anemia)
Due to increased destruction of Erythrocytes, there is overproduction of bilirubin & the liver fails to conjugate it & excrete the same.
→ Male bilirubin is excreted into bile which leads to increased formation of urobilinogen & Stercobilin

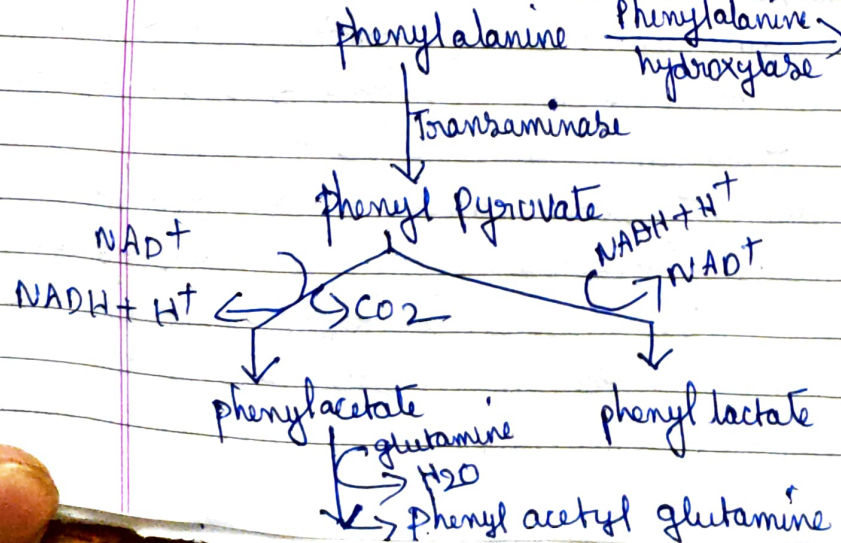
Haemolytic Jaundice is characterized by:-
1) Elevation in Serum unconjugated bilirubin.
2) Increased Excretion of urobilinogen in urine
3) Dark brown Colour of faeces due to high Stercobilin.

(2) Hepatic Jaundice:- This type of Jaundice is caused by dysfunction of the liver due to damage of parenchymal cells. This may be due to hepatitis, poisons & toxins ($CHCl_3$, CCl_4, phosphorous etc).
Damage to the liver affects the uptake of bilirubin & its Conjugation by liver cells.

Hepatic Jaundice is characterized by:-
1) Increased level of Conjugated & unconjugated bilirubin in Serum
2) Dark Coloured urine due to the excessive Excretion of bilirubin & urobilinogen.
3) Increased levels of ALT (Alanine Transaminase) & AST (Aspartate transaminase) in the blood.
4) Patients Pass Pale Coloured Stools due to the absence of Stercobilinogen.
5) Nausea & Anorexia (loss of appetite).

3) obstructive (Regurgitation Jaundice) :- This is due to an obstruction in the bile duct that prevents the passage of bile into the intestine. The obstruction may be Caused by gallstones & tumours etc. obstructive Jaundice is characterized by :-
1) Increased Concentration of Conjugated bilirubin in Serum
2) Serum alkaline phosphate (ALP) is elevated, as it is released from the damaged bile duct.
3) Dark Coloured urine due to elevated Excretion of bilirubin & clay Coloured faeces due to absence of Stercobilinogen.
4) Nausea & gastrointestinal pain.

Phenylketonuria



phenylKetonuria (PKU) is the most common metabolic disorder in amino acid metabolism. The incidence of this disorder is 1 in 10,000 births.

It is due to deficiency of hepatic enzyme, phenylalanine hydroxylase caused by a autosomal recessive gene.
In few cases, a variant of PKU was seen which is due to defect in dihydrobiopterin reductase. This enzyme deficiency impairs the Synthesis of Tetrahydrobiopterin required for the action of phenylalanine hydroxylase.
In phenylKetonuria, phenylalanine is not converted to Tyrosine.

Phenylalanine Metabolism in PKU :- Due to disturbances in metabolism phenylalanine is diverted to alternate pathways, resulting in the Excessive production of phenyl pyruvate, phenyllactate & phenylacetate & phenylacetyl glutamine.
All of these are excreted at higher ~~lives~~ levels in urine & gives it a mousy odour.

Clinical Manifestations of PKU :-
1) Effects on CNS :- Mental retardation, failure to walk & talk failure of growth, Seizures & Vomit
If untreated, the patients show very low IQ.
⇒ Accumulation of phenylalanine in brain impairs the transport & metabolism of other aromatic amino acids (Tryptophan & Tyrosine).
2) the Synthesis of Serotonin from Tryptophan is insufficient.
3) Defect in Myelin formation is observed.
4) Effect on pigmentation :- Accumulation of phenylalanine competetively inhibits Tyrosinase & impairs Synthesis of Melanin from Tyrosine. This results in Hypopigmentation that cause light skin colour, fair hair, blue eyes etc.

Diagnosis of PKU :- It is mostly detected by Screening the newborn babies for increased levels of phenylalanine (28-65 mg/dl) normal (1-2 mg/dl) using GUTHRIE Test. phenylpyruvate in urine can be detected by Ferric chloride Test.

Treatment of PKU :-
1) The plasma phenylalanine Concentration in plasma can be maintained at normal range by selecting foods with low phenylalanine
2) Tyrosine should be supplemented in the diet in sufficient quantity.
3) In serious cases, 5-hydroxy Tryptophan & DOPA are administered to restore the synthesis of serotonin & Catecholamines.

Alkaptonuria :-
It was first described by Lusitanus in 1649 & Charackerized in 1859.
The prevalence of this disease is 1 in 25,000.

The defective enzyme is homogentisate oxidase in Tyrosine metabolism. Because of the lack of this enzyme Tyrosine metabolism stops with accumulation of homogentisate in tissues & blood.

Homogentisate oxidizes to quinones, which polymerizes to give black (or) brown colour. The urine of alkaptonuric patients resembles coke in colour.

Biochemical Manifestations :- Homogentisate gets oxidized by polyphenol oxidase to benzoquinone acetate which undergoes polymerization to produce a pigment called Alkapton.

Alkapton deposition occurs in connective tissue, bones & various organs (nose, ear etc) resulting in a condition called ochronosis. The deposition of alkapton leads to arthritis.

Diagnosis :- The urine gives a positive test with $FeCl_3$ & $AgNO_3$. This is due to strong reducing activity of homogentisate. Benedict's test used for reducing sugars detection is also positive with homogentisate.

Treatment :- It is not a dangerous disorder & does not require any specific Treatment.

Homogentisate

↓ Polyphenol oxidase

Benzoquinone acetate

↓ Polymerization

Alkapton

↓

Binds to tissues

~~Albinism~~ :-

Porphyrias :-

Porphyrias are the metabolic disorders of heme Synthesis characterized by the increased excretion of porphyrins & porphyrin Precursors. They are broadly classified into two categories

1. Erythropoietic : enzyme deficiency occurs in the erythrocytes
2. Hepatic : Enzyme defect lies in the liver.

All the known porphyrias are inherited as autosomal dominant disorders Except Congenital Erythropoietic porphyria is an Exception, since it is an autosomal recessive.

Different types of porphyrias are described as follows :-

Type of porphyria	enzyme defect	characteristics
Hepatic :-		
Acute intermittent porphyria	uroporphyrinogen I Synthase	Abdominal pain, neuropsychiatric Symptoms.
porphyria cutanea tarda	uroporphyrinogen decarboxylase	photosensitivity.
Heriditary coproporphyria	coproporphyrinogen oxidase	Abdominal pain, photosensitivity, neuropsychiatric Symptoms
Variegate porphyria	protoporphyrinogen oxidase	Abdominal pain, photosensitivity, neuropsychiatric Symptoms
Erythropoietic		
Congenital Erythropoietic porphyria	uroporphyrinogen III co Synthase	photosensitivity, increased hemolysis
Protoporphyria	Ferrochelatase	photosensitivity.

(1) Albinism :-

Albinism (Greek: Albino - white) is an inborn error, due to lack of Synthesis of pigment melanin. It is an autosomal recessive disorder with a frequency of 1 in 20,000.

The melanin Synthesis Can be influenced by a variety of factors. The possible Causes for the albinism have been identified as :-

(1) Deficiency (80) lack of enzyme ~~Tyrosinase~~ Tyrosinase
(2) Decrease in melanosomes of melanocytes
(3) Impairment in melanin polymerization.
(4) Limitation of substrate (Tyrosine) availability.
(5) presence of inhibitors of Tyrosinase.

Melanin protects the body from Sun radiation. lack of melanin in albinos makes them sensitive to Sunlight. Increased Susceptibility to Skin Cancer. photophobia due to lack of pigment in eyes were observed.

(2M) 2. what is Homocystinuria? what are its characteristics?

Homocystinurias are a group of metabolic disorders characterized by accumulation & increased excretion of homocysteine & S-adenosyl methionine. plasma Concentration of methionine is increased. Accumulation of Homocysteine results in various complications:- thrombosis, osteoporosis, mental retardation, usually die with myocardial infarction, stroke & pulmonary embolism.

(2M) 3. what are Maple Syrup urine disease? what are its characteristics.

Maple Syrup urine disease is a metabolic disorder of branched chain amino acids. The urine of the affected person Smells like maple Syrup (80) burnt Sugar - hence the name.

This disease is due to the defect in the enzyme branched chain α-Keto acid dehydrogenase. This blocks the Conversion of α-Keto acids to the respective acyl CoA thioesters. It is also known as branched chain Ketonuria.

→ It leads to impairement in transport & function of other amino acids
→ Reduced protein biosyntheses.
→ Branched chain amino acids Competitively inhibit glutamate dehydrogenase
→ acidosis, lethargy, Convulsions, mental retardation, coma & death occurs with in one year after birth.