

## ***AMINO ACIDS METABOLISM***

- Single amino acids and bi peptides are absorbed by the mucosal cells.
- Bi peptides are hydrolysed by the mucosal cells and only free amino acids enter the portal circulation.
- Amino acids are actively taken up by all cells and use 7 types of ATP dependent Active pumps to do that.
- *Cystinuria* is a disease where cysteine is excreted in urine as its uptake mechanism is defective. This forms stones in the kidneys by impaction in the tubules.

## ***REMOVAL OF NITROGEN.***

- It is done by removing alpha amino group. Nitrogen is put into other substances for removal. Urea is the main compound that removes it from the body.

## ***TRANSAMINATION.***

- Here the amino groups are transferred to alpha ketoglutarate which then becomes glutamate. The aa is changed to an alpha keto acid.
- This glutamate goes into the formation of non-essential aa.
- The transfer is done by aminotransferases which may do this for only one or maybe a few aa only.
- Amino group is always accepted by alpha ketoglutarate.
- Alanine aminotransferase ( ALT , also GPT) and aspartate aminotransferase (AST , also GOT) *ARE TWO MOST IMPORTANT SUCH ENZYMES.*
- These enzymes are present in all cells.
- All transaminases require pyridoxal phosphate which transfers the amino group and transiently becomes pyridoxamine phosphate.

- These enzymes are especially valuable in liver injury such as acute or chronic hepatitis, trauma. Their serum levels increase greatly thus signifying injury.

### ***OXIDATIVE DEAMINATION.***

- Here the nitrogen is released as ammonia.
- These reactions take place in the liver and kidneys.
- The alpha keto acids can take part in energy producing reactions and the ammonia is used as a nitrogen source in urea synthesis.
- The enzyme glutamate dehydrogenase causes formation of alpha ketoglutarate from glutamate and releases  $\text{NH}_3$  as ammonia. NADP works here as co enzyme.
- The body maintains a balance in the amounts of these substances according to their availability. Although more protein intake (as after a meal) shall give more glutamate and thus more ammonia.
- ATP and GTP allosterically inhibit the enzyme and ADP and GDP activate it. So in low energy conditions, amino acid breakdown provides alpha ketoglutarate for TCA cycle.

### ***NITROGEN CARRIERS***

#### 1. Glutamate

Transfers one amino group WITHIN cells:

Aminotransferase → makes glutamate from alpha-ketoglutarate

Glutamate dehydrogenase → opposite

#### 2. Glutamine

Transfers two amino group BETWEEN cells → releases its amino group in the liver

#### 3. Alanine

Transfers amino group from tissue (muscle) into the liver

## ***FATE OF AMMONIA.***

### **Glutamate Releases Its Amino Group As Ammonia in the Liver**

The amino groups from many of the amino acids are collected in the liver in the form of the amino group of L-glutamate molecules. These amino groups must next be removed from glutamate to prepare them for excretion.

In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes oxidative deamination catalyzed by glutamate dehydrogenase. It is the only enzyme that can use either  $\text{NAD}^+$  or  $\text{NADP}^+$  as the acceptor of reducing equivalents. The combined action of an amino transferase and glutamate dehydrogenase is referred to as trans-deamination. The  $\alpha$ -ketoglutarate formed from glutamate deamination can be used in the citric acid cycle and for glucose synthesis.

### ***Glutamine Transports Ammonia in the Bloodstream***

- Ammonia is toxic to tissues, including the brain; some processes such as nucleotide degradation generate free ammonia.
- Much of the free ammonia is converted to a nontoxic compound before export from the extrahepatic tissues into the blood and transport to the liver or kidneys.
- glutamate, critical to *intracellular* amino group metabolism, so it is supplanted by L-glutamine.
- The free ammonia produced in tissues is combined with glutamate to yield glutamine by the action of glutamine synthetase. This reaction requires ATP
- Glutamine is a nontoxic transport form of ammonia; it is normally present in blood in much higher concentrations than other amino acids. Glutamine also serves as a source of amino groups in a variety of biosynthetic reactions
- the enzyme glutaminase converts glutamine to glutamate and  $\text{NH}_4^+$

- The  $\text{NH}_4^+$  from intestine and kidney is transported in the blood to the liver. In the liver, the ammonia from all sources is disposed of by urea synthesis.
- The kidneys form ammonia using glutaminase. The excretion is as ammonium ion  $\text{NH}_4^+$ . Acid base balance is achieved this way.
- Bacterial action in the intestine and from amines (such as neurotransmitters) and purines/pyrimidines.
- Ammonia level in the blood is always low. It is taken up by the liver. Glutamine forms a safe form of travel for ammonia. Muscles release their nitrogen in this form and as alanine. It is also important in the nervous system.

### ***Ammonemia toxicity on CNS***

The toxicity is due to the reason that increased concentration of ammonia in the blood and other biological fluids → ammonia diffuses into cells, across blood/brain barrier → increased synthesis of glutamate from  $\alpha$ -ketoglutarate by glutamate dehydrogenase, increased synthesis of glutamine.  $\alpha$ -ketoglutarate is consumed and not available to reach other processes such as TCA cycle. This causes decreased ATP production. Brain is more affected as it depends highly on the Krebs's cycle for energy production. Glutamate is excitatory. Neurotransmitters may contribute to the CNS effects – bizarre behaviour.

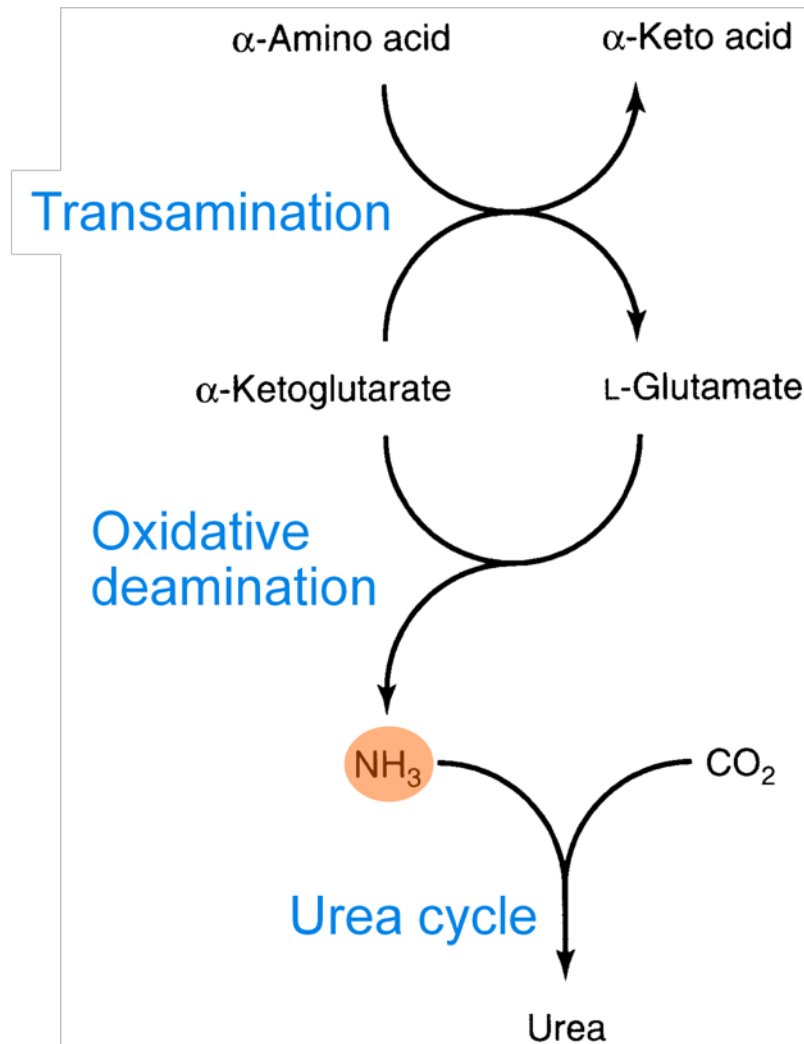
**Hyperammonemia** causes tremors and slurred speech. It may cause disturbed vision and coma if it continues leading to death.

In cirrhosis or severe liver disease increased  $\text{NH}_3$  levels are seen and can cause a condition known as *hepatic coma* or *hepatic encephalopathy*.

### ***UREA CYCLE.***

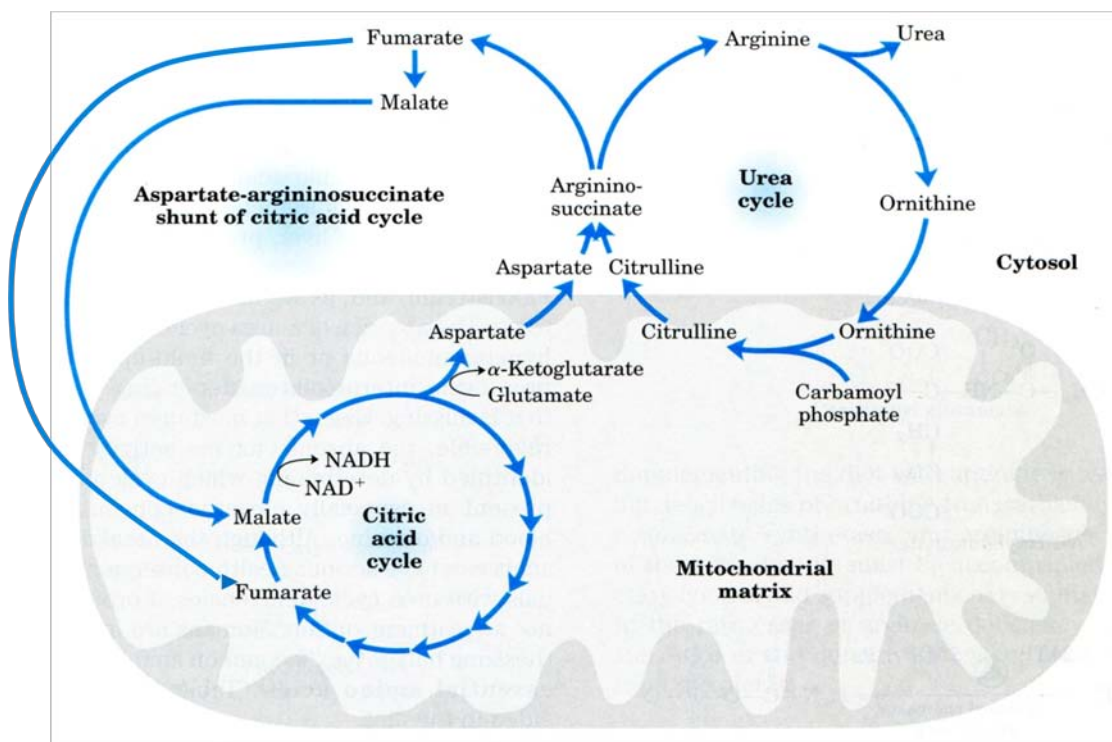
- The major disposal form of nitrogen, it constitutes 90% of nitrogen in urine. Aspartate and free ammonia each gives it one nitrogen. Glutamate is the precursor of both the nitrogens through glutamate dehydrogenase and AST.  $\text{CO}_2$  gives the carbon and oxygen.
- Urea is produced in the liver and kidneys excrete it.

- Ammonia is necessary to be removed from the blood stream as it is very toxic, especially for the nervous system.
- It starts in the mitochondria and then goes on into the cytosol.

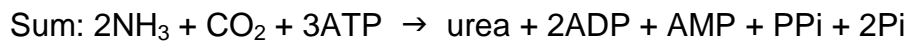
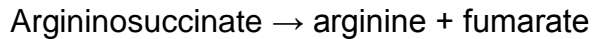
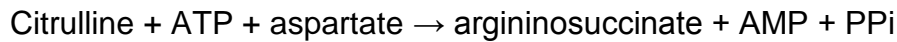
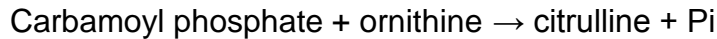


- Carbamoyl phosphate is the first step. The enzyme is *carbamoyl phosphate synthase I*. Two molecules of ATP are used and this drives the reaction in the forward direction.
- The enzyme requires N-acetylglutamate .
- Next is formation of citrulline.
- Ornithine is changed to citrulline by ornithine transcarbamoylase.

- Synthesis of arginosuccinate is next. Citrulline and aspartate combine to form this. A molecule of ATP is used here. And PPi is released.
- Next arginosuccinate is cleaved into fumarate and arginine.
- Fumarate is a link to other metabolic processes when it is hydrated to malate and enters TCA cycle in the mitochondria. It may be oxidised to oxaloacetate and then to aspartate or glucose.
- Arginine is cleaved to ornithine and urea. But arginase is present in liver only.
- Part of the urea in the blood is absorbed in the intestinal mucosa and cleaved into ammonia and CO<sub>2</sub> by the bacteria through their *urease*.
- Part of it is absorbed back and part is excreted in the faeces.
- In renal failure the amount of urea is more in the blood, more is split in the gut and more ammonia comes back in the blood causing hyperammonemia.
- N-acetylglutamate activates carbamoyl phosphate synthase I. it needs acetyl co A and glutamate to form. After a meal these compounds increase in amount and more urea is produced.



## ***The overall chemical balance of the biosynthesis of urea***



## ***Regulation of urea cycle***

The activity of urea cycle is regulated at two levels:

- Dietary intake is primarily proteins → much urea (amino acids are used for fuel)

Prolonged starvation → breaks down of muscle proteins → much urea also

The rate of synthesis of four urea cycle enzymes and carbamoyl phosphate synthetase I (CPS-I) in the liver is regulated by changes in demand for urea cycle activity.

Enzymes are synthesized at higher rates in animals during:

- starvation
- in very-high-protein diet

Enzymes are synthesized at lower rates in

well-fed animals with carbohydrate and fat diet

animals with protein-free diets

N-acetylglutamic acid – *allosteric* activator of CPS-I

High concentration of *Arg* → stimulation of N-acetylation of glutamate by acetyl-CoA

## ***Deficiencies of urea cycle enzymes***

- Infant born with total deficiency of one or more enzymes survive at least several days.
- Many enzymes deficiencies are partial → enzymes have altered  $K_m$  values.
- Case are known of deficiencies of each enzymes.
- Interruption of the cycle at each point affected nitrogen metabolism differently
  - some of the intermediates can diffuse from hepatocytes → accumulate in the blood → pass into the urine.
- If symptoms are not detected early enough → severe mental retardation → **brain damage is irreversible.**
- Treatment of urea cycle disorders is principally aimed at controlling the level of free ammonia. This includes the reduction of protein in the diet, removal of excess ammonia, and replacement of intermediates missing from the urea cycle

## ***CARBON SKELETON METABOLISM***

- Amino acids are also classified as ketogenic or glucogenic depending on the end product of their metabolism.
- Also there are essential and non essential amino acids.
- Amino acids producing acetoacetate, acetyl co A or aceto acetyl co A (precursors of the former) are ketogenic as they can take part in formation of fats.
- But only two are exclusively included here. Lysine and leucine.
- Amino acids producing pyruvate, oxaloacetate , alpha ketoglutarate , succinyl co A , fumarate are termed glucogenic as they can take part in gluconeogenesis. These cause storage of glycogen by virtue of this characteristic.

## ***OXALOACETATE forming amino acids.***

- ASPARAGINE is hydrolysed by asparaginase into ammonia and aspartate which forms oxaloacetate.

### ***ALPHA KETO GLUTARATE forming amino acids.***

- Glutamine converts to glutamate and ammonia through glutaminase.  
Glutamate dehydrogenase converts glutamate to alpha k.g through oxidative deamination or transamination.
- PROLINE converts to proline 5 carboxylate and then to glutamate and to alpha kg.
- ARGININE is converted to ornithine by arginase in liver in the urea cycle. Then it gets converted to alpha kg.
- HISTIDINE is converted by histidase to N formimino glutamate. (Figlu). It donates its formimino group to folate and glutamate is left behind.
- Figlu is increased in the urine if folic acid is deficient as there is deficiency of group acceptor. This has been used as a test for folic acid deficiency.

### ***PYRUVATE forming amino acids.***

- ALANINE forms pyruvate by through transamination by al. amino transferase.
- Glycine is converted to serine and then pyruvate or oxidised to  $\text{NH}_4^+$  and  $\text{CO}_2$ .
- Cystine is reduced to cysteine. This gives pyruvate.
- SERINE is converted to pyruvate by serine dehydratase. Serine can also be converted to glycine.

### ***FUMARATE forming amino acids.***

- PHENYLALANINE and TYROSINE form fumarate.
- Phenylalanine can be hydroxylated to tyrosine by ph.ala. Hydroxylase. Acetoacetate is also formed at the end.
- Deficiencies in this pathway lead to phenylketonuria ,alkaptonuria , albinism.

### ***SUCCINYL CO A forming amino acids.***

- METHIONINE is converted to S adenosyl methionine. (high energy compound). The methyl group is activated and can be transferred to many other compounds. Loss of energy makes this step irreversible.
- Adenosine and homocysteine is formed after loss of methyl group.
- Homocysteine may combine with serine giving cysteine and alpha kg. ultimately succinyl co A is formed from propionyl co A.
- METHIONINE CAN BE REFORMED FROM HOMOCYSTEINE by accepting a methyl group from N methyl THF. This requires vit. B<sub>12</sub>.
- VALINE , ISOLEUCINE , THREONINE are also converted to succinyl co A. threonine is also converted to pyruvate.

### ***ACETYL CO A and ACETOACETYL CO A forming amino acids.***

- These substances are formed directly by the amino acids without pyruvate coming in the line. Phenylalanine and tyrosine also form these.
- LEUCINE , ISOLEUCINE, LYSINE , TRYPTOPHAN all make acetyl co A or aceto acetyl co A.
- All of these are thus ketogenic.

### ***Catabolism of branched chain amino acids.***

- Isoleucine ,leucine and valine are branched as well as essential amino acids. These are catabolised in muscles rather than the liver.
- THE REACTIONS INVOLVE TRANSAMINATION (aminotransferase), OXIDATIVE DECARBOXYLATION (keto acid dehydrogenase which uses thiamine) AND DEHYDROGENATION. It is the oxidation of acyl co A products.
  - Deficiency of keto acid dehydrogenase causes the branched chain amino acids to accumulate and excrete in urine. It has a sweet odour. The disease is known as *maple syrup urine disease*.
  - ROLE OF FOLIC ACID.
    - THF (tetra hydro folic acid) is the active form produced by dihydrofolate reductase using NADPH. This acts as a one

carbon unit carrier. Examples of such substances are methane , methanol, formaldehyde , formic acid , carbonic acid.

### ***NON ESSENTIAL AMINO ACID SYNTHESIS.***

- These are produced either from metabolic intermediates or from other amino acids ( like cysteine, tyrosine).
- Different methods are employed for the purpose.
- Synthesis from alpha keto acids.
  - Alanine, aspartate and glutamate can be synthesized by transferring an amino group to keto acids pyruvate , oxaloacetate , alpha ketoglutarate in the same order.
- Synthesis by amidation.
  - Glutamine synthetase forms glutamine from glutamate. Ammonia is added here and this aa transports ammonia in a safe manner.
  - Asparagine synthetase forms asparagine from aspartate .glutamine is the amide donor here.
  - Both the reactions require ATP.
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### **METABOLIC DEFECTS**

Due to accumulation of metabolites, often they cause mental retardation and other defects.

- Mostly enzymes are absent or deficient.
- More common include
- Hyperphenylalaninaemia.
- Phenylketonuria is the most common form of elevated phenylalanine levels. It is caused by a deficiency in phenylalanine hydroxylase.
- Deficiency in tetra hydro biopterin due to any cause will also cause elevated levels.

- The Cause is important as clinical management is different in both cases.
- In the case phenylalanine hydroxylase is deficient, the amino acid needs to be restricted in diet.
- If tetra hydro biopterin is deficient it needs to be replaced as it also works with tyrosine and tryptophan hydroxylase as coenzyme also.
- Clinical symptoms include mental retardation, non achievement of milestones, hyperactivity, growth failure and tremor.
- Pigmentation is decreased and the patient is fair skinned with light coloured eyes and hair.
- Neonatal diagnosis is done after 48 hrs of birth after protein diet and is done by Guthrie test.
- Treatment is diet restricted in phenylalanine and must be administered within first month of life along with tyrosine.
- If mother is affected, the diet must be controlled prior to pregnancy.
- Otherwise the child is affected during pregnancy and shows microcephaly, mental retardation and other physical defects.

### ***Some important terms***

1. **Aminotransferase:** any of a family of enzymes that catalyze the transfer of an amino group between a  $\alpha$ -amino acid and an  $\alpha$ -keto acid
2. **AST and ALT:** aspartate aminotransferase (AST; also called serum glutamateoxaloacetateaminotransferase, [SGOT]) and alanine transaminase (ALT; also called serum glutamate-pyruvate aminotransferase [SGPT]), the two prevalent liver enzymes whose elevations in the blood have been used as clinical markers of tissue damage
3. **Glucose-alanine cycle:** mechanism for skeletal muscle to eliminate nitrogen while replenishing its glucose supply. Glucose oxidation produces pyruvate which can undergo transamination to alanine; the alanine then enters the bloodstream and is transported to the liver where it is converted back to pyruvate, which is then a source of carbon atoms for gluconeogenesis

4. **Urea:** nitrogen compound composed of 2 amino groups ( $-\text{NH}_2$ ) joined by a carbonyl ( $-\text{C}=\text{O}$ ) functional group; is the main nitrogen-containing substance in human urine
5. **Glucogenic:** relating to amino acids whose catabolic products can be used for net glucose synthesis during gluconeogenesis
6. **Ketogenic:** relating to amino acids whose catabolic products enter the tricarboxylic acid (TCA) cycle but cannot be used for net glucose synthesis during gluconeogenesis
7. **Asparaginase:** enzyme catalyzing the deamination of asparagine to aspartate; enzyme is used clinically as a chemotherapeutic treatment for certain leukemias
8. **Cystathionine  $\beta$ -synthase:** enzyme involved in cysteine biosynthesis, deficiencies are most common cause of homocystinurias
9. **Glycine cleavage complex:** also called glycine decarboxylase, enzyme catabolizing glycine, defects in activity cause glycine encephalopathy
10. **Phenylketonuria (PKU):** a particular form of hyperphenylalaninemia resulting from defects in the phenylalanine hydroxylase gene
11. **Branched-chain amino acid:** consists of leucine, isoleucine, and lysine; defects in the catabolic enzyme branched-chain keto acid dehydrogenase (BCKD) result in Maple syrup urine disease (MSUD)
12. **Alcaptonuria:** first inherited error in metabolism to be characterized, benign disease caused by defects in the tyrosine-catabolizing enzyme homogentisate oxidase, urine of afflicted patients turns brown on exposure to air