UNIT 2

NITROGEN EXCRETION AND UREA CYCLE

Structure

2.1 INTRODUCTION

The degradation of dietary and body protein yields amino acids. They are deaminated early in catabolism and the resulting nitrogen and carbon skeleton are processed independently. The α-amino group of most amino acids is removed by amino transferases and for other amino acids by specific enzymes such histidase and serine / threonine dehydratase. The ammonia produced from extra-hepatic tissues such as muscles and other tissues (except muscles) is transported to the liver in the form of alanine and glutamine, respectively. Free ammonia is toxic so it is detoxified to urea before excretion, in ureotelic organisms.

In this unit we shall learn about nitrogen balance, transamination reactions, ammonia production, Glc-Ala cycle, urea cycle and Krebs bicycle. Lastly we will discuss the disorders associated with deficiencies in enzymes of the urea cycle.

Expected Learning Outcomes

After studying this unit, you should be able to:

- explain nitrogen balance;
- indicate the role of transaminases and mechanism of transamination;
- describe the synthesis and transport of amino nitrogen from extrahepatic tissues to the liver for detoxification;
- understand the reactions and relevance of urea cycle;
- describe the aspartate-argininosuccinate shunt (Krebs bicycle); and
- enumerate urea cycle disorders & strategies for coping up with hyperammonemia.

2.2 NITROGEN BALANCE

Proteins constitute the major nitrogen containing macromolecules that perform wide range of roles. The intake nitrogen is primarily in the form of protein. Nitrogen balance is the difference between the amount of nitrogen ingested and nitrogen excreted in feces, urine (primarily as urea) and through perspiration. An individual may be in zero (N equilibrium), positive or negative nitrogen balance.

Zero nitrogen balance (N equilibrium)

When nitrogen intake matches nitrogen output it is known as zero nitrogen balance. In other words protein synthesis is equal to protein degradation. This type of situation is typically found in adults. Zero nitrogen balance may be represented as:

Nitrogen intake = Nitrogen output

Positive nitrogen balance

When intake of nitrogen is greater than the excretion of nitrogen, it is known as positive nitrogen balance. This type of situation is observed during pregnancy, childhood and adolescence. Positive nitrogen balance is given as:

Nitrogen intake > Nitrogen excretion

Negative nitrogen balance

When nitrogen output exceeds intake, it results in negative nitrogen balance. In other words protein synthesis is less than protein degradation. It is generally encountered in people who ingest inadequate amount or low quality protein; post surgical patients; infections and those in advanced stage of cancer. It is important to mention here that a deficiency of even one amino acid results in negative nitrogen balance (**all or none law**). The quality of a protein is directly related to its biological value (% of absorbed nitrogen retained by the body). Prolonged periods of negative nitrogen balance can be fatal if the loss of body protein reaches about one-third of the total protein. Negative nitrogen balance can be represented as:

Nitrogen intake < Nitrogen excretion

We shall now briefly elaborate why quality of proteins differs from one source to another. The quality of a protein is dependent on its essential amino acid content. Some proteins either have one or more essential amino acids in limited amounts or do not supply all essential amino acids. Such proteins are classified as **incomplete** and are generally derived from plant sources. Therefore vegetarians are advised to consume a combination diet, for instance of cereals and pulses to satisfy their daily requirement of essential amino acids. Animal proteins (milk, eggs) on the other hand are **complete proteins.**

SAQ 1

Explain why?

i) Vegetarians are advised to consume a combination diet such as cereals and pulses.

ii) Absence of even one essential amino acid leads to negative nitrogen balance.

2.3 TRANSAMINATION

The degradation of dietary and body protein yields amino acids. They are deaminated early in catabolism and then nitrogen and carbon skeleton are processed independently. The carbon skeleton of amino acids can have multiple fates depending on the requirements. Some of these fates include complete oxidation via TCA cycle to provide energy; glucose synthesis (gluconeogenesis); synthesis of lipids, fatty acid and ketone bodies and synthesis of non-essential amino acids.

The α-amino group of most amino acids is removed by **amino transferases** or transaminases. These enzymes mediate the transfer of amino group of an amino acid to a keto acid. During the process there is no net deamination. All transaminases are pyridoxal phosphate dependent enzymes which are covalently bound to the lysine residue of the enzyme through an aldimine linkage (Schiff's base). The keto acceptor of the amino group in majority of them is α-ketoglutarate thereby generating glutamate as one of the product. Some amino acids (histidine, serine, threonine, arginine, proline, amide nitrogen of glutamine and asparagine) do not undergo transamination. These amino acids are directly deaminated by specific enzymes about which you will learn in the next unit.

The formation of glutamate allows the cell to either utilise it as an amino donor in biosynthesis or for oxidative deamination of the amino groups collected from different amino acids primarily by glutamate dehydrogenase (GDH) and to some extent by L-amino acid oxidases. It goes without saying that cells do not need separate enzymes for deamination of each amino acid. The sequential action of a transaminase and GDH is termed **transdeamination.** Finally ammonia is detoxified into urea by the urea cycle and excreted (Fig.2.1).

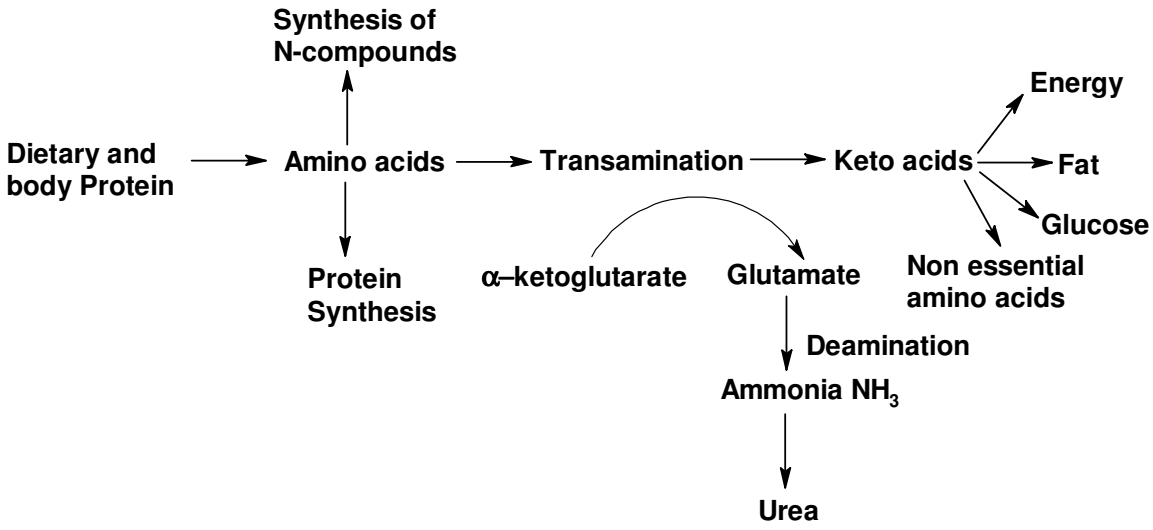


Fig. 2.1: An overview of the fate of amino acids derived from proteins.

In eukaryotes a number of transaminases are required for funneling nitrogen into glutamate. They differ in their specificity for the amino donor and are named accordingly such as aspartate amino transferase and alanine amino transferase. Almost every cell has amino transferases both in the cytosol and mitochondria. Some salient features of transaminases are:

- The coenzyme shuttles reversibly between the aldehyde (pyridoxal phosphate) and its reduced aminated form (pyridoxamine phosphate).
- Aspartate and alanine transaminase make significant contribution to overall transamination.
- Transamination reactions are freely reversible.
- Transamination reaction is involved in both catabolism and anabolism of amino acids.
- These reactions are involved in synthesis of non-essential amino acid.
- Transamination concentrates nitrogen from different amino acids into glutamate.
- Besides α-amino acids, some δ-amino acids such as ornithine also undergo transamination.
- Serum transaminases such as aspartate transaminase (AST) and alanine transaminase (ALT) are important prognostic and diagnostic biomarkers

The coenzyme pyridoxal phosphate is derived from the B_6 family of water soluble vitamins (Fig. 2.2).

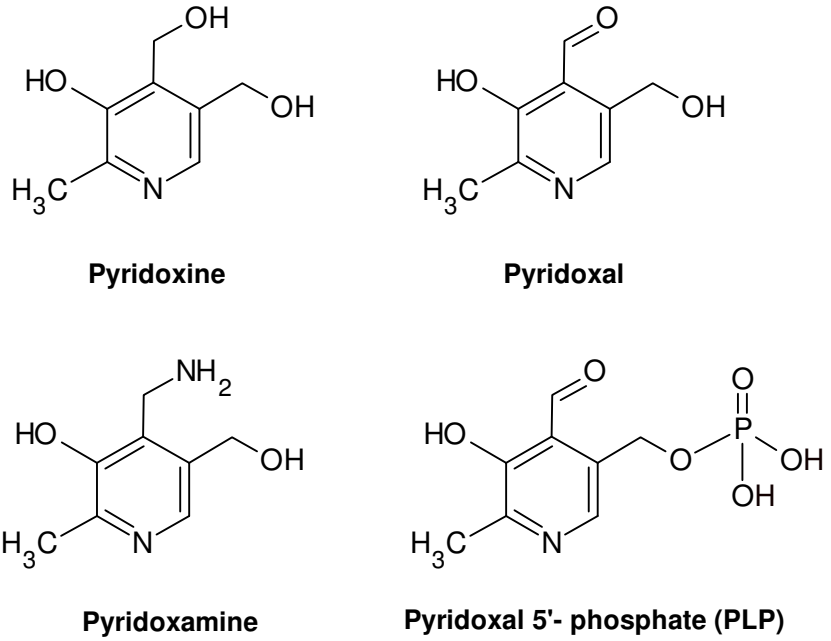


Fig. 2.2: Phosphorylated and non-phosphorylated forms of vitamin B6.

SAQ 2

Indicate whether the following statements are True or False:

i) The carbon skeleton of all amino acids is gluconeogenic in animals.

ii) Almost half of the amino acids are essential amino acids in animals.

iii) Glutamine is the primary amino donor for the synthesis of nitrogen containing biomolecules.

2.3.1 Mechanism of Transamination

A transamination reaction occurs by ping pong Bi Bi mechanism in which the first product leaves the active site before the second substrate binds (for details, refer to BBCCT-107). In Ping-pong (double displacement) mechanism the enzyme changes into an intermediate (temporary) form when the first substrate to product reaction occurs (Fig. 2.3). This mechanism was proposed by E. Snell, A. Braunstein and D. Metzler.

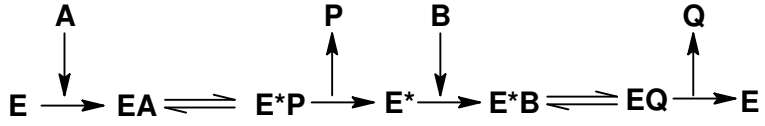
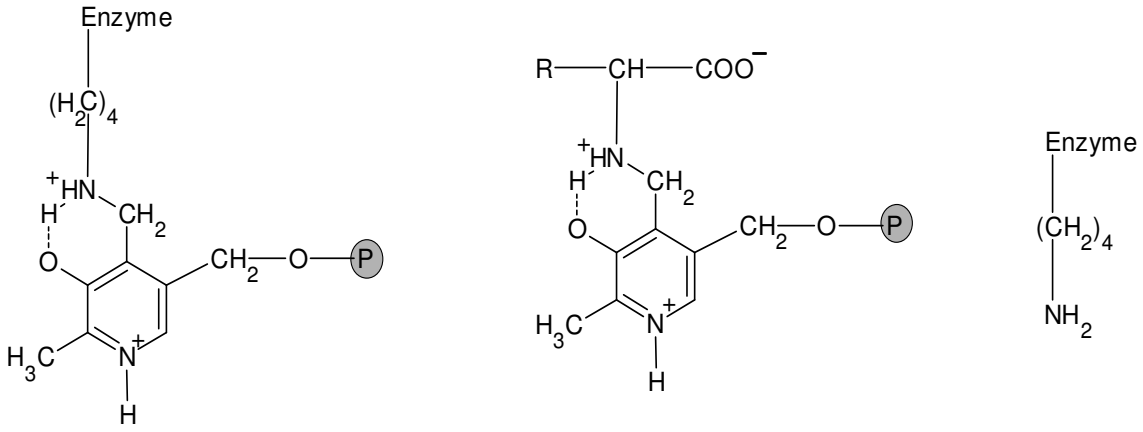


Fig. 2.3: A hypothetical representation of ping pong bimolecular mechanism.

The basic reaction proceeds in two stages. In the first stage (Fig. 2.4) the amino acid binds to the active site and forms a new aldimine linkage between the substrate and pyridoxal phosphate (external aldimine). This is followed by proton abstraction (from donor amino acid), protonation (of PLP) and hydrolysis of the resultant ketimine producing pyridoxamine phosphate and the first product leaves as α-ketoacid.

The second substrate now binds to the enzyme and forms a ketimine with the amino group of pyridoxamine phosphate. These steps are a reversal of stage 1. There is removal of proton, protonation of ketoacid and hydrolysis to release the amino acid with regeneration of PLP.



Enzyme-PLP Schiff base **Amino acid-PLP Schiff base**

Fig. 2.4: Aldimine formation between enzyme –PLP and substrate –PLP.

The steps of stage 1 can be summarised as follows:

Enzyme –PLP → Substrate –PLP → Quinonoid intermediate

↓

Rearrangement → hydrolysis

At this stage we have only considered the transamination of amino acids. There are many kinds of reactions in amino acid metabolism at C-2 to C-4 that are assisted by PLP. At the α-carbon; in addition to transaminations, decarboxylations and racemisations can also occur depending on the enzyme. It is a very versatile coenzyme. The conjugated structure of PLP is an electron sink that permits delocalization of electrons. You shall come across many of these in the subsequent units. Fig. 2.5 depicts a transamination reaction catalysed by glutamate-pyruvate transaminase and reversible transformation between two states of the coenzyme.

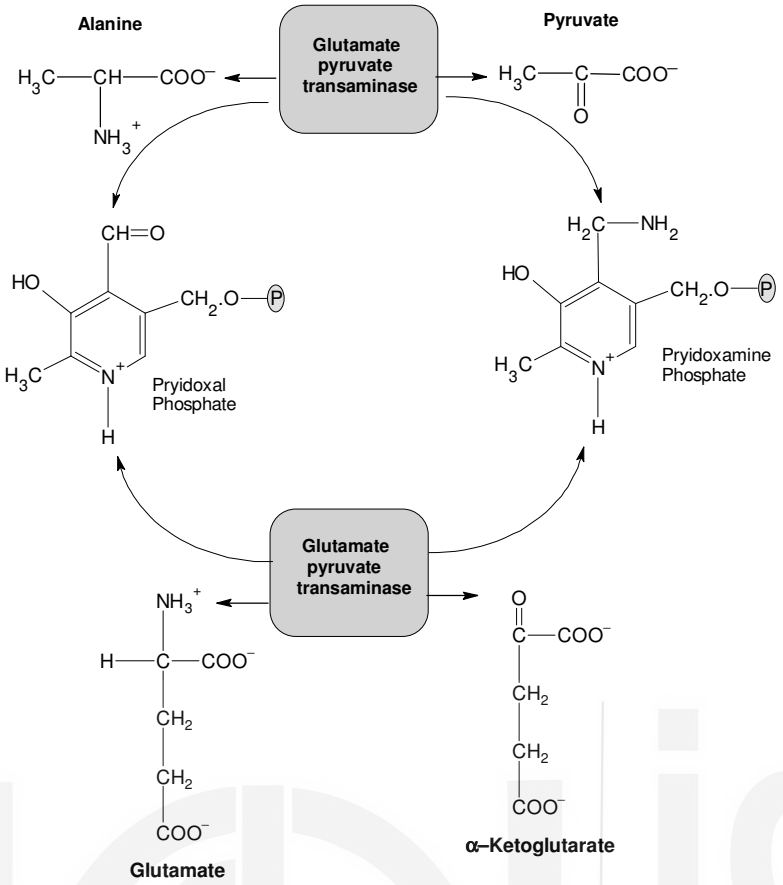


Fig. 2.5: Transamination reaction catalysed by glutamate-pyruvate transaminase.

2.4 GLUCOSE-ALANINE CYCLE

We have learned in the preceding section how the amino group of most amino acids are transferred by amino transferases to α-ketoglutarate generating glutamate. The oxidative deamination of glutamate yields ammonia which in blood /cells is toxic to cellular components. .At physiological pH, ammonia exists as ammonium (NH^+_4) ion. Ammonia produced from extra-hepatic tissues such as muscles and other tissues (except muscles) is transported to the liver in the form of alanine and glutamine, respectively (Fig. 2.6).

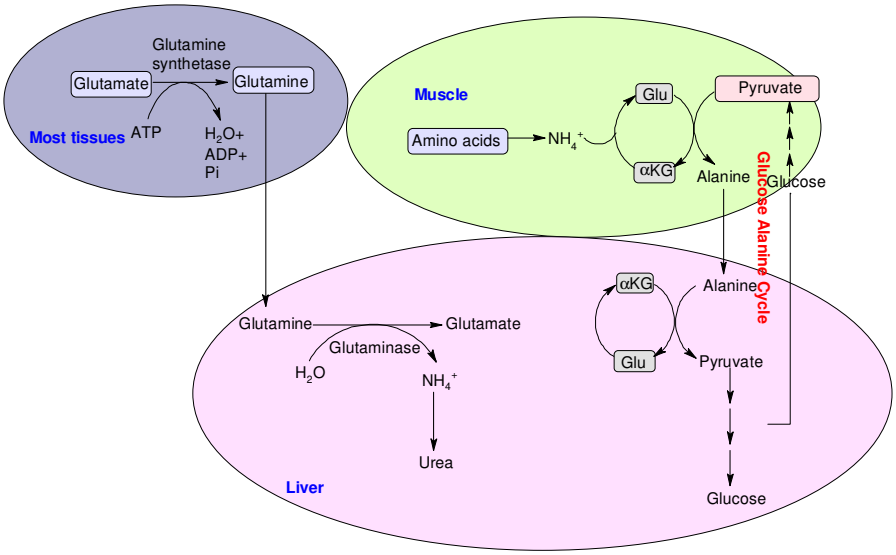


Fig. 2.6: Glucose-alanine cycle between muscle (& other tissues) and liver.

In most tissues (except muscle and liver), glutamate is converted into glutamine by glutamine synthetase ("synthetase" are enzymes that utilize ATP for synthesis of new bonds). Glutamine is a non toxic, neutral compound which can readily cross the lipid membrane. It is carried through blood circulation to the liver.

Glutamic acid + NH_3 + ATP ⟶ Glutamine + ADP +Pi

In muscle, NH_4^+ produced by amino acid degradation (transamination and deamination) is converted into glutamate by muscle glutamate dehydrogenase enzyme (this enzyme is different from the hepatic glutamate dehydrogenase, which removes ammonia from glutamate). Next pyruvate (from glycolysis) is transaminated to alanine by alanine amino transaminase. Alanine released into blood is picked up by the liver where pyruvate is reformed by reversal of alanine amino transferase reaction there by forming glutamate as the other product. The two products will now be processed independently. The entire process of converting glucose into alanine (in muscle) and then back to glucose (in liver cells) is known as **glucose-alanine cycle.**

In the liver, pyruvate is converted to glucose and released into circulation for use by muscle and other tissues. The nitrogen coming from different amino acids is deaminated by hepatic glutamate dehydrogenase (GDH). Similarly glutamine transported from other extrahepatic tissues (except muscle) is first acted upon by mitochondrial glutaminase producing glutamate and ammonia; the former is oxidatively deaminated. Ammonia released in the liver mitochondria is fed to the urea cycle for detoxification.

SAQ 3

Fill in the blanks:

i) Transamination and deamination are collectively known as _____.

ii) The cofactor for transaminases is_____.

iii) The two amino acids that carry ammonia through blood circulation are _____ and _____.

iv) Two examples of amino acids that do not remove their amino group by transamination are _____ and _____.

2.5 UREA CYCLE

Free ammonia is toxic to cells so it is detoxified prior to excretion. The normal concentration of ammonia in human plasma is 25-40 µmol /L. In ureotelic organisms, urea is the final product of protein/amino acid catabolism. About 90% of the nitrogen excreted in urine is in the form of urea. Urea cycle was first metabolic cycle to be elucidated by **Hans Adolf Krebs** and his medical student associate **Kurt Henseleit (1932).** It is also known as Krebs-Henseleit cycle.

Urea cycle operates in the liver. In this cycle two out of total five enzymes are present in mitochondria and rest are in the cytoplasm (Fig 2.7). In addition to these enzymes, specific transporters assist in the mitochondrial uptake of

ornithine and release of L-citrulline and transport of aspartate and glutamate. The enzymes that catalyse the first four reactions of the urea cycle are also present in kidney and intestinal mucosa where they are involved in arginine biosynthesis. The detoxification of ammonia to urea is energy consuming (4 high energy phosphate bonds utilized / urea synthesized) and entry to the cycle is regulated at step I. The net reaction of urea synthesis is:

$NH_4^+ + HCO_3 + 2H_2O + 3ATP + Asp \longrightarrow$ **Urea** $+ 2ADP + AMP + 2Pi + PPi + fumarate$

$PPi + H_2O \longrightarrow 2Pi$

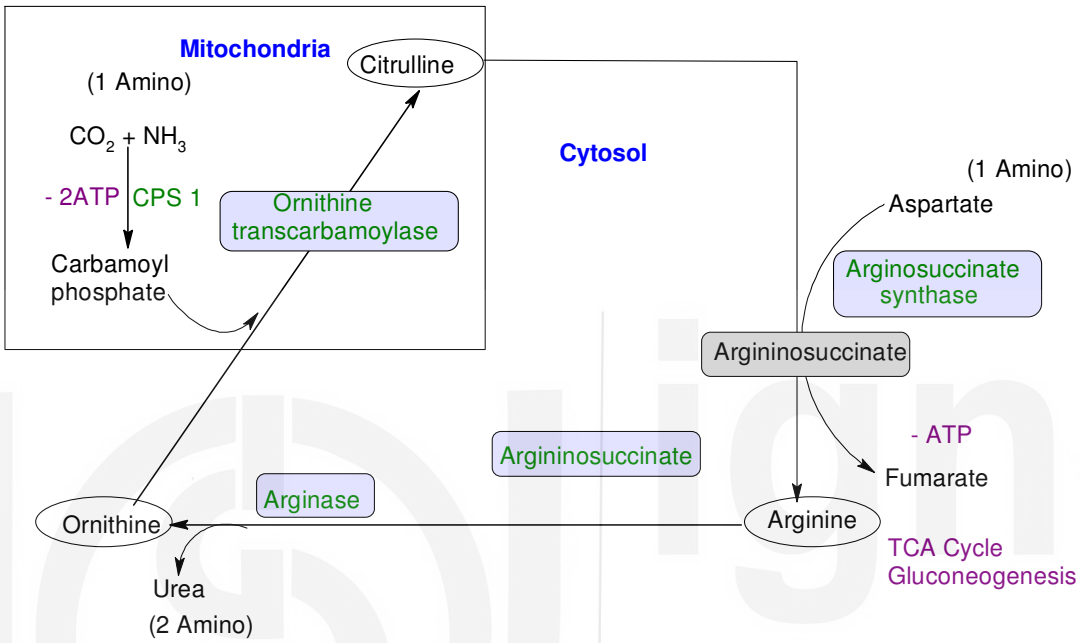


Fig. 2.7: Urea cycle.

The first step of urea cycle is catalysed by carbamoyl phosphate synthetase I (CPS-1). It is the rate limiting step of the cycle and brings about the nucleophilic addition of ammonia to activated bicarbonate forming an energy rich mixed anhydride, carbamoyl phosphate at the expense of two ATP molecules. The ammonia for this reaction is generated in the mitochondria predominantly by oxidative deamination of glutamate or hydrolysis of amide nitrogen of glutamine. Most amino acids catabolised in the liver or other tissues ultimately produce glutamate. Some ammonia also arrives in the liver via the portal vein from the intestine. Mitochondrial respiration produces CO_2 which is the source of bicarbonate.

CPS-I is allosterically regulated by a positive modulator, **N-acetyl glutamate**. The modulator is synthesized from glutamic acid and acetylCoA by N-acetyl glutamate synthase. An increase in glutamate (substrate) results in more N-acetyl glutamate. The enzyme is activated by arginine.

The activity of urea cycle enzymes goes up when an individual consumes protein rich diet (as excess proteins are not stored) or during prolonged starvation when breakdown of muscle proteins provides most of the energy. The urea cycle enzymes are also subject to long term regulation. The level of all enzymes is increased during starvation and on consumption of protein rich diets.

In the second reaction, ornithine transcarbamoylase (OTC) catalyses the nucleophilic addition of ornithine to the carboxyl group of carbamoyl phosphate to produce citrulline with release of inorganic phosphate. In this reaction, the δ-amino group of ornithine attaches to the carbonyl group of carbamoyl phosphate. Both ornithine and citrulline are non protein amino acids. Ornithine is produced in the cytosol and needs to be transported into the mitochondrial matrix while citrulline produced has to be moved into the cytosol. The movement of both metabolites across the mitochondrial inner membrane is assisted by ornithine translocase I (ORNTI).The next three steps are catalyzed by cytosolic enzymes.

In step 3, citrulline condenses with aspartate in an ATP requiring reaction catalysed by argininosuccinate synthetase (ASS 1). Prior to condensation, ATP activates citrulline by transfer of an adenylyl group (AMP) with release of pyrophosphate. In essence two high energy phosphate bonds are used in this reaction as pyrophosphate is invariably hydrolysed to inorganic phosphate. The activated intermediate is attacked by the α-amino group of aspartate forming argininosuccinate and AMP. At the end of this step, both nitrogen atoms of urea have been acquired.

The fourth reaction is catalysed by arginine succinate lyase (ASL); also called argininosuccinase. It non-hydrolytically cleaves argininosuccinate to arginine and fumarate. The latter product links urea cycle to the Krebs cycle.

The other transporter required for the smooth operation of the urea cycle is citrin (aspartate/ glutamate carrier). It carries glutamate from the cytosol to the mitochondria and aspartate in the reverse direction. Aspartate is resynthesised from fumarate by TCA cycle enzymes to oxaloacetate followed by transamination to be utilised again in the urea cycle.

In the final step, the guanidine group of arginine is hydrolysed by arginase into urea and ornithine. Arginase is activated by Co^{2+} and Mn^{2+}. The regeneration of ornithine completes the cycle. Urea produced in liver diffuses into the blood and is sequestered by the kidneys for excretion. Some amount of urea enters the intestine where it is degraded into $CO_2$and NH_3 by intestinal bacteria. The NH_3 produced is either absorbed into the blood again or excreted.

2.6 KREBS BICYCLE

The integration of Urea cycle to Krebs (tricarboxylic acid; TCA) cycle is known as Krebs bicycle. Hans Krebs was involved in the discovery of both these cycles. It is also known as the aspartate-argininosuccinate shunt. Fumarate produced in the urea cycle is an integrating point to the Krebs cycle (Fig. 2.8). In hepatic cytosol, fumarate is converted sequentially to malate and oxaloacetate by cytosolic isozymes of fumarase and malate dehydrogenase, respectively. Malate is either transported via the malate-aspartate shuttle or utilised in the cytosol itself. In the mitochondria malate is converted back to oxaloacetate (a keto acid) .Finally a transamination reaction in the mitochondria between oxaloacetate and glutamate forms aspartate which is transported to cytosol to be used again as nitrogen donor in the urea cycle. These reactions together constitute aspartate-argininosuccinate shunt. The shunt provides metabolic links between separate pathways by which the amino groups and carbon skeleton of amino acids are processed.

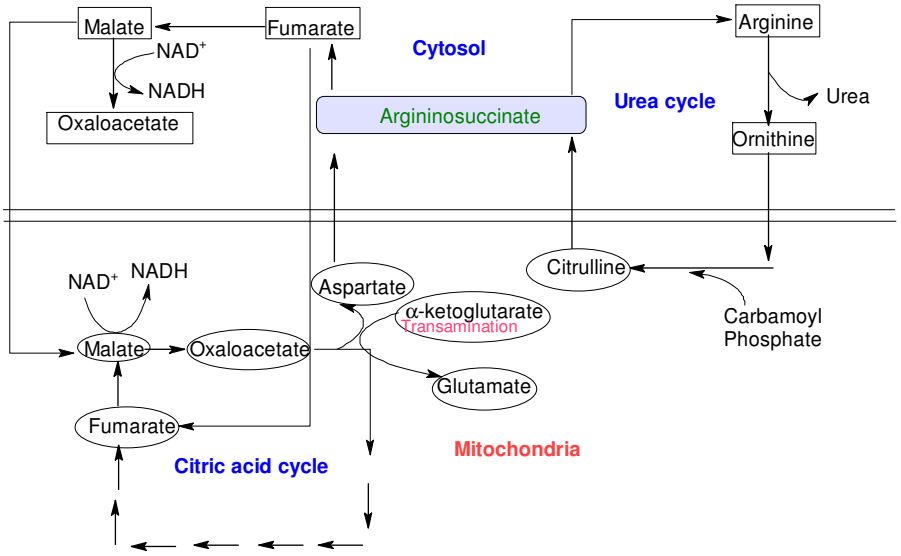


Fig. 2.8: Krebs bicycle.

SAQ 4

Fill in the blanks:

i) The pathways linking the Krebs and Urea cycle are known as _____

ii) Urea cycle was elucidated by _____and _____

iii) The enzymes of the urea cycle are compartmentalised in ___ and____

iv) An incomplete urea cycle operates in kidney & intestinal mucosa to synthesize_____

2.7 INHERITED DEFECTS OF UREA CYCLE

A deficiency of any of the urea cycle enzyme would result in hyperammonemia. The condition is more severe when one of the earlier steps is blocked because ammonia itself gets accumulated and symptoms appear early in life. A deficiency of the later enzymes of the urea cycle pathway results in accumulation of other intermediates of the cycle (Table 2.1). As these intermediates are less toxic so is the severity of the condition. Hyperammonemia is less severe in those with a partial deficiency. With the exception of OTC (X-linked gene) all genes encoding enzymes and transporters of urea cycle are present on autosomes.

Table 2.1: Urea cycle disorders (UCDs)

Enzyme /protein deficiency	Disorder	Accumulated product
Carbamoyl phosphate synthetase-I (CPS-I)	Hyperammonemia type-I	Ammonia
Ornithine transcarbamoylase	Hyperammonemia type-II	Ammonia
Argininoscuccinate synthase or citrin (transporter)	Citrullinemia	Citrulline
Argininoscuccinate lyase (ASL)	Argininoscuccinate aciduria	Argininoscuccinate
Arginase (ARG 1)	Argininemia	Arginine

The initial symptoms of hyperammonemia in a child are non specific such as failure to feed, frequent vomiting, loss of thermoregulation and later lethargy and behavioural abnormalities and irreversible brain damage. The terminal stages of ammonia toxicity leads to coma and eventually death.

The toxic effects of hyperammonemia may be due to the following reasons, although complete understanding of the molecular basis is still lacking.

(a) Excessive amount of α-ketoglutarate from citric acid cycle is used to form glutamate and then glutamine. Both these reactions consume ammonia. The synthesis of glutamate by GDH uses α-ketoglutarate. This results in depletion of α-ketoglutarate for Krebs cycle and thereby decreased production of ATP.

(b) Both glutamate and GABA are neurotransmitters. The level of these inhibitory neurotransmitters is decreased as more and more glutamate is converted to glutamine.

(c) An increase in glutamine produces osmotic effects leading to cerebral oedema (swelling) due to increased uptake of water.

A number of treatments are in use to handle hyperammonemia and specific urea cycle defects. Since most of the steps of urea cycle are physiologically irreversible, the deficient / missing enzyme can be generally identified by determining the intermediate whose level is significantly elevated in blood and urine facilitating specific treatment.

One of the strategies for coping up with hyperammonemia is to feed orally preparations of sodium benzoate and phenyl butyrate / acetate for long term management. In acute episodes they are infused intravenously. The treatment works by indirectly consuming ammonia. Sodium benzoate is activated to benzoyl CoA and then conjugated to glycine forming a non toxic product, hippuric acid which is excreted in urine. Glycine (a non essential amino acid) is then replenished by glycine synthase reaction that consumes ammonia. Similarly phenyl butyrate is converted to phenyl acetate by β-oxidation and phenyl acetylCoA is complexed with glutamine forming phenylacetylglutamine which is excreted. The loss of glutamine is overcome by its synthesis from glutamate and ammonia.

The other therapies are targeted to specific defects, for example an argininosuccinase deficiency is bypassed by providing surplus arginine and restricting total protein intake. This will fulfil both arginine requirements and generate ornithine for the cycle to continue.

2.7.1 Hyperammonemia Type-I and Type-II

A deficiency of the first two enzymes of the urea cycle, namely carbamoyl phosphate synthetase-I and ornithine transcarbamoylase are associated with an elevation of plasma ammonium concentration which is classified as type I and type II hyperammonemia, respectively. Type-I deficiency is the most severe UCD. It is a life threatening condition which may affect patients at any age. In neonatal type the symptoms appear early in life and may be lethal while the delayed-onset type is less severe and shows up at mid to late age. Type II hyperammonemia is the most common inherited urea cycle disorder.

The associated symptoms include lethargy, vomiting, coma and cerebral oedema. The symptoms are as severe as CPS-1 deficiency in case of males. Citrulline is useful in reducing ammonia levels.

2.7.2 Citrullinemia

The disease arises due to accumulation of ammonia and other toxic substances in blood. Hyperammonemia can be quite severe. It is characterised by marked elevation of plasma citrulline and excretion of large quantities of citrulline (citrullinuria) in urine.

Two type of citrullinemia is known. **Type I** or classic citrullinemia is usually associated with acute neonatal or early onset. It is due to a deficiency of argininosuccinate synthase. Within a few days the infant suffers from energy loss, poor feeding, seizures, convulsions and unconsciousness. These symptoms vary in severity. They can be managed on a low protein, ammonia scavenger drugs and arginine supplementation. **Type II** citrullinemia is due to a defect in the mitochondrial transporter, citrin. It is more common in people of Japanese descent. It mainly affects the nervous system which results in confusion, restlessness, memory loss, abnormal behaviours seizures, and coma. The symptoms generally show up in adulthood. These patients respond well on a high protein and low carbohydrate diet.

2.7.3 Argininosuccinic aciduria

It is also known as argininosuccinic acidemia. In this condition argininosuccinic acid is elevated in the blood and urine of the patient due to a deficiency of argininosuccinate lyase. Sometimes elevated levels of ammonia are also reported. This disease is mainly found in newly born babies. These children may be lethargic, unwilling to eat, and experience difficulty in breathing.

2.7.4 Argininemia

The affected individuals develop progressive spasticity, stiffness in the legs, developmental delays and difficulty with balance and coordination. The symptoms become evident by age 3. The plasma concentration of arginine is markedly elevated (hyperargininemia).

SAQ 5

The severity of the urea cycle defect is influenced by the position of the defective protein in the cycle and whether it leads to a partial deficiency or complete absence. Explain.

2.8 SUMMARY

In this unit you have learnt that:

- Nitrogen balance is the difference between the amount of nitrogen ingested and nitrogen excreted in feces, urine (primarily as urea) and through perspiration. An individual may be in zero (N equilibrium), positive or negative nitrogen balance.

- The degradation of dietary and body protein yields amino acids. During catabolism amino acids are deaminated and then nitrogen and carbon skeleton are processed independently.
- The α-amino group of most amino acids is removed by PLP dependent amino transferases. The keto acceptor of the amino group in majority of them is α-ketoglutarate thereby generating glutamate as one of the product.
- The formation of glutamate allows the cell to either utilise it as an amino donor in biosynthesis or for oxidative deamination of the amino groups collected from different amino acids primarily by glutamate dehydrogenase (GDH). Finally ammonia is detoxified into urea by the urea cycle and excreted.
- Ammonia produced from extra-hepatic tissues such as muscles and other tissues (except muscles) is transported to the liver in the form of alanine and glutamine, respectively. The entire process of converting glucose into alanine (in muscle) and then back to glucose (in liver cells) is known as glucose-alanine cycle**.**
- In ureotelic organisms urea is the final product of protein/amino acid catabolism. Urea cycle operates in the liver and of the five enzymes two are present in mitochondria and rest in cytoplasm. The detoxification of ammonia to urea is energy consuming.
- The integration of Urea cycle to Krebs (tricarboxylic acid; TCA) cycle is known as Krebs bicycle or aspartate-argininosuccinate shunt. It provides metabolic links between separate pathways by which the amino groups and carbon skeleton of amino acids are processed.
- A deficiency of any of the urea cycle enzyme would result in hyperammonemia. With the exception of OTC (X-linked gene) all genes encoding enzymes and transporters of urea cycle are present on autosomes.
- A number of treatments are in use to handle hyperammonemia and specific urea cycle defect. One of the strategies for coping up with hyperammonemia is to feed orally preparations of sodium benzoate and phenyl butyrate / acetate for long term management. In acute episodes they are infused intravenously.

2.9 TERMINAL QUESTIONS

1. Explain negative and positive nitrogen balance.
2. Indicate the role of transaminases.
3. Describe the glucose-alanine cycle.
4. Illustrate urea cycle.
5. Write short notes on aspartate-argininosuccinate shunt
6. Compare transamination and oxidative deamination.

2.10 ANSWERS

Self-Assessment Questions

1. i) A combination diet is recommended for vegetarians because plant proteins from a given source are generally incomplete proteins. A diet for instance of cereals and pulses complements the deficiency of essential amino acids and allows vegetarians to avoid a state of negative N balance.

   ii) Deficiency of even one essential amino acid results in negative N balance as synthesis of proteins will pause when that amino acid has to be incorporated. At the same time there will be enhanced degradation of proteins to fulfil the deficiency.

2. i) False

   ii) True

   iii) False

3. i) Transdeamination

   ii) Pyridoxal phosphate (PLP)

   iii) Glutamine and alanine

   iv) Histidine; Threonine

4. i) Krebs bicycle

   ii) Krebs and Henseleit

   iii) Mitochondria and cytosol

   iv) Arginine

5. The severity of urea cycle defect is more pronounced when one of the earlier steps is blocked because ammonia itself gets accumulated and symptoms appear early in life. Logically a partial deficiency is better tolerated as some amount of enzyme is available to catalyse the reaction.

Terminal Questions

1. Positive balance is when nitrogen intake > nitrogen excretion. It is observed during pregnancy, childhood and adolescence.

   When nitrogen output exceeds intake it results in negative nitrogen balance. It is generally encountered in people who ingest inadequate amount or low quality protein; post surgical patients; infections, etc.

2. The α-amino group of most amino acids is removed by amino transferases. These enzymes mediate the transfer of amino group of an amino acid to a keto acid. The keto acceptor in majority of them is α-ketoglutarate thereby generating glutamate as one of the product. The formation of glutamate allows the cell to either utilise it as an amino donor in biosynthesis or for oxidative deamination of the amino groups collected from different amino acids.

3. The process of converting glucose into alanine (in muscle) and then back to glucose (in liver cells) is known as glucose-alanine cycle**.** The amino group of alanine is transaminated to glutamate and deaminated to ammonia which is then detoxified to urea prior to excretion. Refer to section 2.4.

4. Refer to Fig.2.7

5. The Krebs bicycle is also known as the aspartate-argininosuccinate shunt. The shunt provides metabolic links between separate pathways by which the amino groups and carbon skeleton of amino acids are processed. Refer to section 2.6.

6.

Transamination	**Oxidative deamination**
It is catalysed by transaminases that inter-convert – amino acid & - keto acids	It is primarily catalysed by GDH & to some extent by amino acid oxidases.
They are pyridoxal phosphate dependent enzymes.	They are NAD or FAD dependent enzymes, respectively.
They catalyse neither a net deamination nor a net oxidation.	They catalyse a net oxidative deamination of an amino acid**.**
Their role is to funnel amino into one or few amino acids (predominantly Glu) which can either be oxidatively deaminated or used as an amino donor.	Ammonia released is either used for biosynthesis or fed to the urea cycle for detoxification prior to excretion**.**

2.11 FURTHER READINGS

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