Sem – II (PG)
Paper ZOO-202
Group B: Biochemistry

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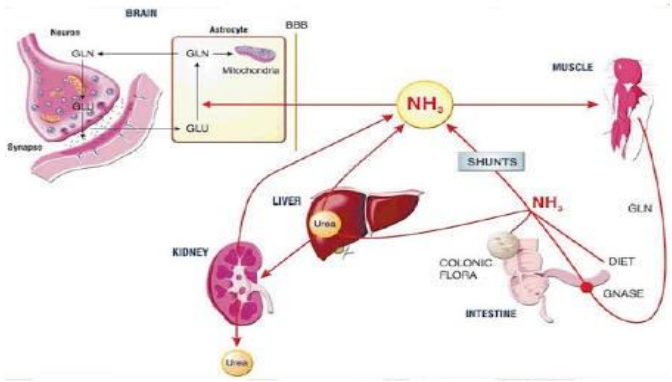
Protein metabolism

Ammonotelism, Ureotelism, Uricotelism

Nitrogen is a major constituent of amino acids and proteins. Generally animals receive excess of amino acids through their diet. The excess of amino acids is catabolized either for release of energy or is used for the synthesis of glycogen and fat. When proteins, amino acids or nucleic acids are catabolized, 3-N-containing predominant excretory end-products are formed – ammonia, urea and uric acid.

Ammonotelism:

Animals that excrete excess nitrogen in the form of ammonia (NH_3) as a result of deamination of the amino acids (the end product of protein metabolism), are called ammonotelic animals and the process is ammonotelism.



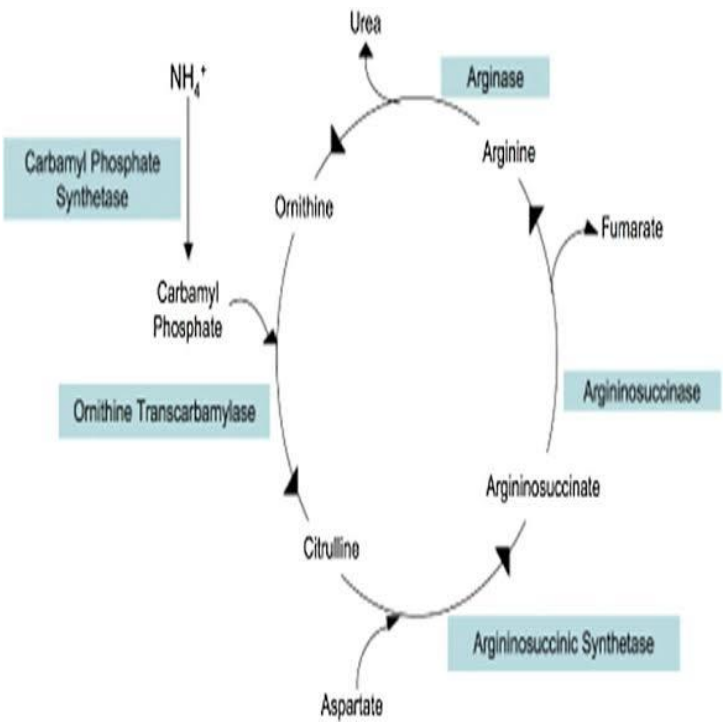
Examples: aquatic animals, Amphibians, Protozoans, Crustaceans, Platyhelminthes, Cnidarians, Poriferans, Echinoderms & other aquatic invertebrates.

Ammonia diffuses as ammonium ions through the cell membrane extremely fast because of its high water solubility and small molecular size. It is highly toxic to tissues. Hence, it can be excreted as such only when there is sufficient water for its rapid removal from the body in the form of a dilute solution. Therefore, excretion of ammonia occurs in aquatic animals. The route of ammonia diffusion in these animals is through skin, gills or kidneys.

Water loss is about 300-500 ml to remove one gram of ammonia.

Ureotelism:

Animals that excrete excess nitrogen in the form of urea, are ureotelic animals and the process is ureotelism.



Examples: mammals, semi-terrestrial adult amphibians, turtles.

Terrestrial animals can't use water freely for excretion (because of less availability in the environment), so, ammonia is converted into a less toxic and easily soluble product, urea in liver and released into blood which is filtered and excreted out by the kidneys. Water loss is about 50 ml to eliminate one gram of urea.

When the urea is synthesized in the liver by a metabolic pathway (detoxification of ammonia) from CO_2 and NH_4, then the whole process is called ornithine-urea cycle (discovered by Kreb and Hensleit, 1932).

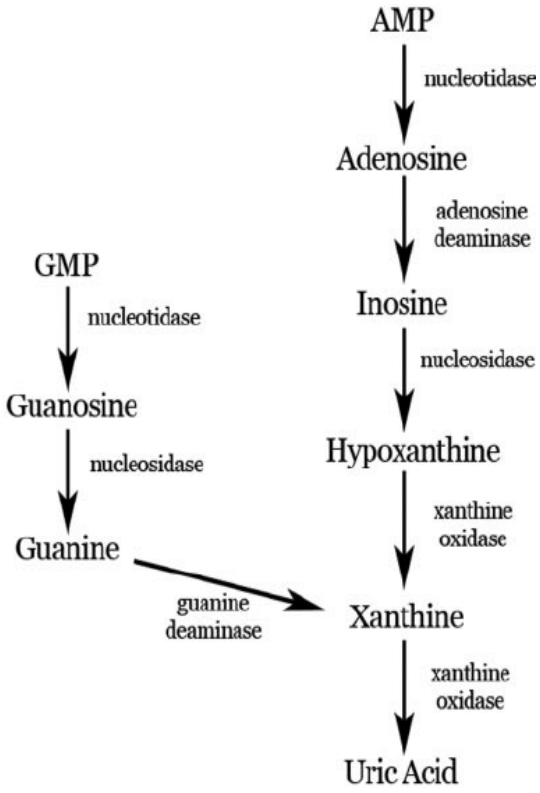
This metabolic pathway involves enzyme-catalyzed reactions:

1. $CO_2 + NH_4^+ + 2ATP \xrightarrow{carbamoylphosphate\ synthetase}$ Carbamoyl phosphate
2. Carbamoyl phosphate + ornithine $\xrightarrow{Ornithine\ carbamoyl-transferase}$ citrulline
3. Citrulline + Asp + ATP $\xrightarrow{argininosuccinate\ synthetase}$ Argininosuccinic acid $\xrightarrow{argininosuccinate\ lyase}$ Arg + fumaric acid
4. Arg $\xrightarrow{arginase}$ Urea + Ornithine

In this process of detoxification of ammonia, three molecules of ATP are consumed. This ornithine-urea cycle is associated with TCA cycle and glutamate dehydrogenase reaction.

Uricotelism:

Animals that excrete a mojor portion of nitrogenous waste in the form of semi-solid or solid uric acid, are uricotelic animals and the process is uricotelism.



Examples: Birds, terrestrial reptiles (lizards, snakes), terrestrial insects (formed in malphigian tubules), snails.

In these animals, ammonia is converted (from purines) into least toxic, relatively insoluble uric acid in liver cells, which can be excreted with a relatively small amount of water. Inosinic acid metabolic pathway is responsible for the synthesis of uric acid from ammonia. Water loss is about 10 ml to excrete out one gram of uric acid.

Many insects store excretory products in their body instead of eliminating them. The phenomenon is called storage excretion. It saves the expenditure of water during excretion. Since uric acid is non-toxic & highly insoluble, it can be retained in the body for a long time without any ill-effect. Cockroaches are adapted to this technique and they store uric acid in fat body and cuticle. In turn, the stored uric acid provides a nitrogen deposits for the mobilization at the times of nitrogen deficiency.

Differences between ammonotelism, ureotelism and uricotelism:

Topic	Ammonotelism	Ureotelism	Uricotelism
What is:	Excretion of nitrogenous waste mainly as ammonia.	Excretion of nitrogenous waste mainly as urea.	Excretion of nitrogenous waste mainly as uric acid.
Use of energy:	Uses very little energy in forming ammonia.	Uses more energy in producing urea.	Uses far more energy in producing uric acid.
Loss of body water:	Causes considerable loss of body's water (300-500 ml for one gram of ammonia).	Causes less loss of body's water (50 ml for one gram of urea).	Causes least loss of body's water (10 ml for one gram of uric acid).
Toxicity:	Its product is very toxic.	Its product is less toxic.	Its product is least toxic.
Occurrence:	Occurs in aquatic animals	Occurs in aquatic and land animals	Occurs in land animals
Examples:	Amoeba, Scypha, Hydra, Prawn, Salamander, tadpole of frog, bony fishes.	Earthworm, cartilaginous fishes, frog, turtles, alligators, mammamls.	Insects, land crustaceans, land snails, land reptiles, birds.

Formation of Urea

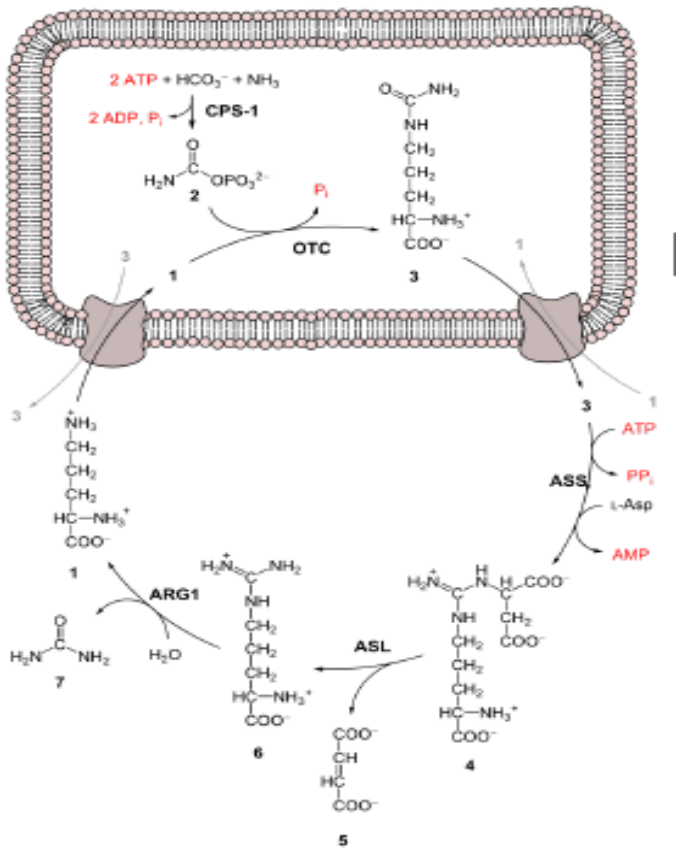
Amino acid catabolism results in waste ammonia. Most aquatic organisms, or ammonotelic organisms, excrete ammonia without converting it. Organisms that cannot easily and safely remove nitrogen as ammonia convert it to a less toxic substance such as urea or uric acid via the urea cycle, which occurs mainly in the liver. Urea produced by the liver is then released into the bloodstream where it travels to the kidneys and is ultimately excreted in urine. The urea cycle is essential to these organisms, because if the nitrogen or ammonia are not eliminated from the organism it can be very detrimental. In species including birds and most insects, the ammonia is converted into uric acid or its urate salt, which is excreted in solid form.

Reactions:

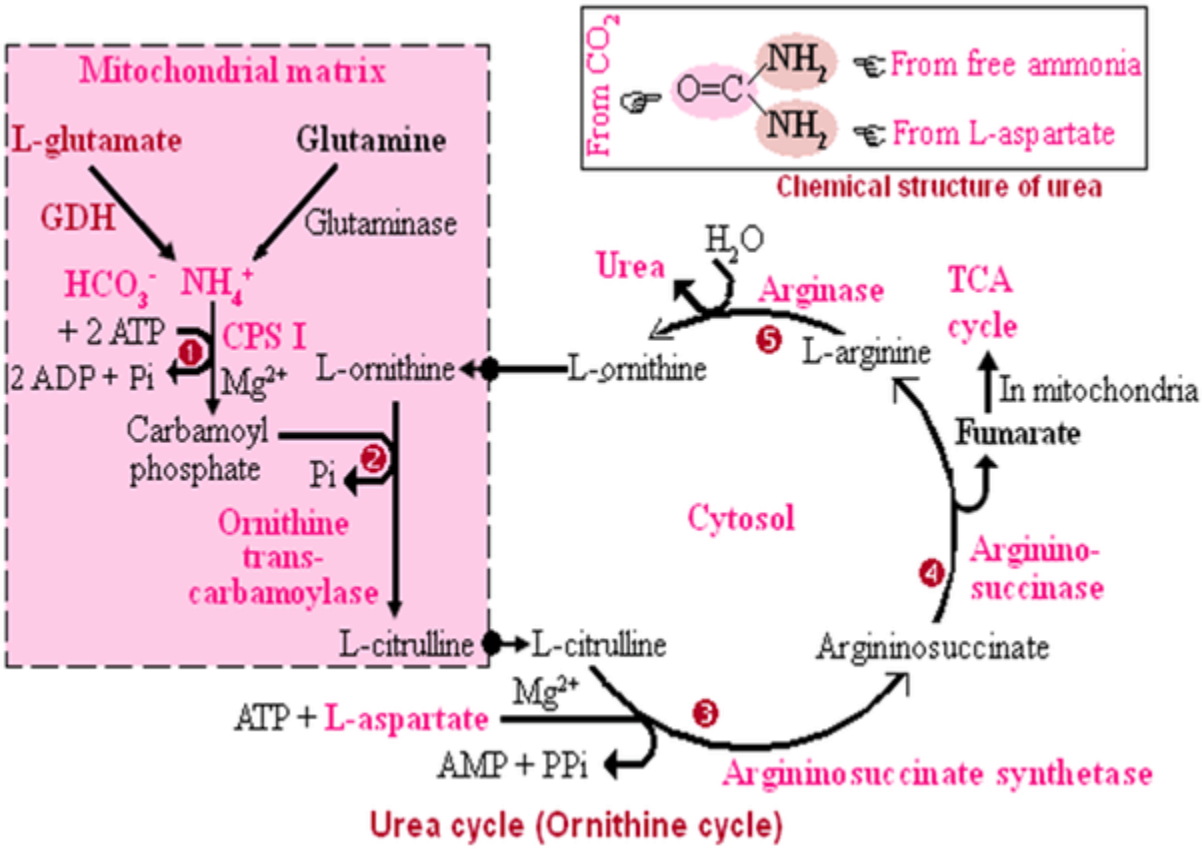
Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. The entire process converts two amino groups, one from NH_4^+ and one from Aspartate, and a carbon atom from HCO_3^-, to the relatively nontoxic excretion product urea at the cost of four "high-energy" phosphate bonds (3 ATP hydrolyzed to 2 ADP and one AMP). The conversion from ammonia to urea happens in five main steps.

The first is needed for ammonia to enter the cycle and the following four are all a part of the cycle itself. To enter the cycle, ammonia is converted to carbamoyl phosphate. The urea cycle consists of four enzymatic reactions: one mitochondrial and three cytosolic.

Step	Reactants	Products	Catalysed by	Location
1.	$NH_3 + HCO_3^- + 2ATP$	Carbamoyl phosphate + 2ADP + P_i	Carbamoyl phosthate synthetase (CPS)	Mitochondria
2.	Carbamoyl phosphate + ornithine	Citrulline + P_i	Ornithine transcarbamoylase (OTC), zinc, biotin	Mitochondria
3.	Citrulline + aspartate + ATP	Argininosuccinate + AMP + PP_i	Argininosuccinate synthetase (ASS)	Cytosol
4.	Argininosuccinate	Arginine + Fumarate	Argininosuccinate lyase (ASL)	Cytosol
5.	Arginine + H_2O	Ornithine + Urea	Arginase (ARG), manganese	Cytosol



1. L – Ornithine
2. Carbamoyl phosphate
3. L – ctrulline
4. Argininosuccinate
5. Fumarate
6. L – arginine
7. Urea



Overall reaction equation:

In the first reaction, $NH_4^+ + HCO_3^-$ is equivalent to $NH_3 + CO_2 + H_2O$

Thus, the overall equation of the urea cycle is:

$NH_3 + CO_2 + Aspartate + 3ATP + 2H_2O \rightarrow Urea + Fumarate + 2ADP + 2P_i + AMP + PP_i$

Since Fumarate is obtained by removing NH_3 from aspartate (by reaction 3 & 4) and $PP_i + H_2O \rightarrow 2P_i$, the equation can be simplified as –

$2NH_3 + CO_2 + 3ATP + H_2O \rightarrow Urea + 2ADP + 4P_i + AMP$

Note: reactions of urea cycle also produce 2 NADH so the overall reaction releases slightly more energy than it consumes. The NADH is produced in two ways: i) One NADH molecule is produced by the enzyme glutamate dehydrogenase (GDH) in the conversion of glutamate to ammonium and α-ketoglutarate. Glutamate is the non-toxic carrier of amine groups. This provides the ammonium ion used in the initial synthesis of carbamoyl phosphate. ii) The fumarate released in the cytosol is hydrated to malate and this malate is then oxidized to oxaloacetate by cytosolic malate dehydrogenase, generating a reduced NADH in the cytosol. Oxaloacetate is one of the keto acids preferred by transaminases, and so will be recycled to aspartate, maintaining the flow of nitrogen into the urea cycle.

We can summarize this by combining the reactions:

$Glutamate + CO_2 + Aspartate + 3ATP + 2NAD^+ + 3H_2O \rightarrow Urea + \alpha\text{-ketoglutarate} + oxaloacetate + 2ADP + 2P_i + AMP + PPi + 2NADH$

The two NADH produced can provide energy for the formation of 5 ATP (cytosolic NADH provides 2.5 ATP with the malate-aspartate shuttle in human liver cell), a net production of two high-energy phosphate bond for the urea cycle.

Physiology in human:

The cycling of and excretion of urea by the kidneys is a vital part of mammalian metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons, which allows for re-absorption of water and critical ions from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons, thus raising the osmolarity in the medullary interstitium surrounding the thin descending limb of the loop of Henle, which makes the water reabsorb.

By action of the urea transporter 2, some of this reabsorbed urea eventually flows back into the thin descending limb of the tubule, through the collecting ducts, and into the excreted urine. The body uses this mechanism, which is controlled by the antidiuretic hormone (ADH), to create hyperosmotic urine—i.e., urine with a higher concentration of dissolved substances than the blood plasma. This mechanism is important to prevent the loss of water, maintain blood pressure, and maintain a suitable concentration of sodium ions in the blood plasma.

Urea cycle disorder:

Mutations lead to deficiencies of the various enzymes and transporters involved in the urea cycle and cause urea cycle disorders. If individuals with a defect in any of the six enzymes used in the cycle ingest amino acids beyond what is necessary for the minimum daily requirements, then the ammonia that is produced will not be able to be converted to urea. These individuals can experience hyperammonemia. All urea cycle defects (associated with CPS, ASS, ASL, ARG deficiencies), except OTC deficiency, are inherited in an autosomal recessive manner. OTC deficiency is inherited as an X-linked recessive disorder.