Accumulation of NH_3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of α-ketoglutarate. α-Ketoglutarate is a key intermediate in TCA cycle and its depleted levels impair the TCA cycle. The net result is that production of energy (ATP) by the brain is reduced. The ***toxic effects of NH_3*** on brain are, therefore, ***due to impairment in ATP formation***.

Trapping and elimination of ammonia : When the plasma level of ammonia is highly elevated, intravenous administration of sodium benzoate and phenyllactate is done. These compounds can respectively condense with glycine and glutamate to form water soluble products that can be easily excreted. By this way, ammonia can be trapped and removed from the body. In some instances of toxic hyperammonemia, hemodialysis may become necessary.

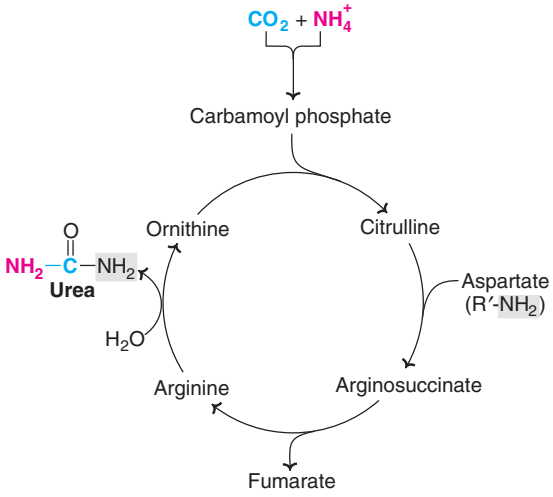

Fig. 15.9 : *Outline of urea cycle. (**Note :** In the synthesis of urea one amino group comes from ammonium ion while the other is from aspartate; carbon is derived from CO_2. This is represented in colours.)*

UREA CYCLE

Urea is the ***end product of protein metabolism*** (amino acid metabolism). The nitrogen of amino acids, converted to ammonia (as described above), is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine.

Urea is ***synthesized in liver*** and transported to kidneys for excretion in urine. Urea cycle is the ***first metabolic cycle*** that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as ***Krebs-Henseleit cycle***. The individual reactions, however, were described in more detail later on by Ratner and Cohen.

Urea has ***two amino ($-NH_2$) groups***, one derived ***from NH_3*** and the other ***from aspartate.*** Carbon atom is supplied by CO_2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first ***two enzymes*** are present in ***mitochondria*** while the ***rest*** are localized in ***cytosol***. The details of urea cycle are described (***Figs.15.9 and 15.10***).

1. **Synthesis of carbamoyl phosphate :** Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate. This step consumes two ATP and is ***irreversible***, and ***rate-limiting***. CPS I requires ***N-acetylglutamate*** for its activity. Another enzyme, carbamoyl phosphate synthase II (CPS II)—involved in pyrimidine synthesis—is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

2. **Formation of citrulline :** Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. (They are never found in protein structure due to lack of codons). Citrulline produced in this reaction is transported to cytosol by a transporter system.

3. **Synthesis of arginosuccinate :** Argino-succinate 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 and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

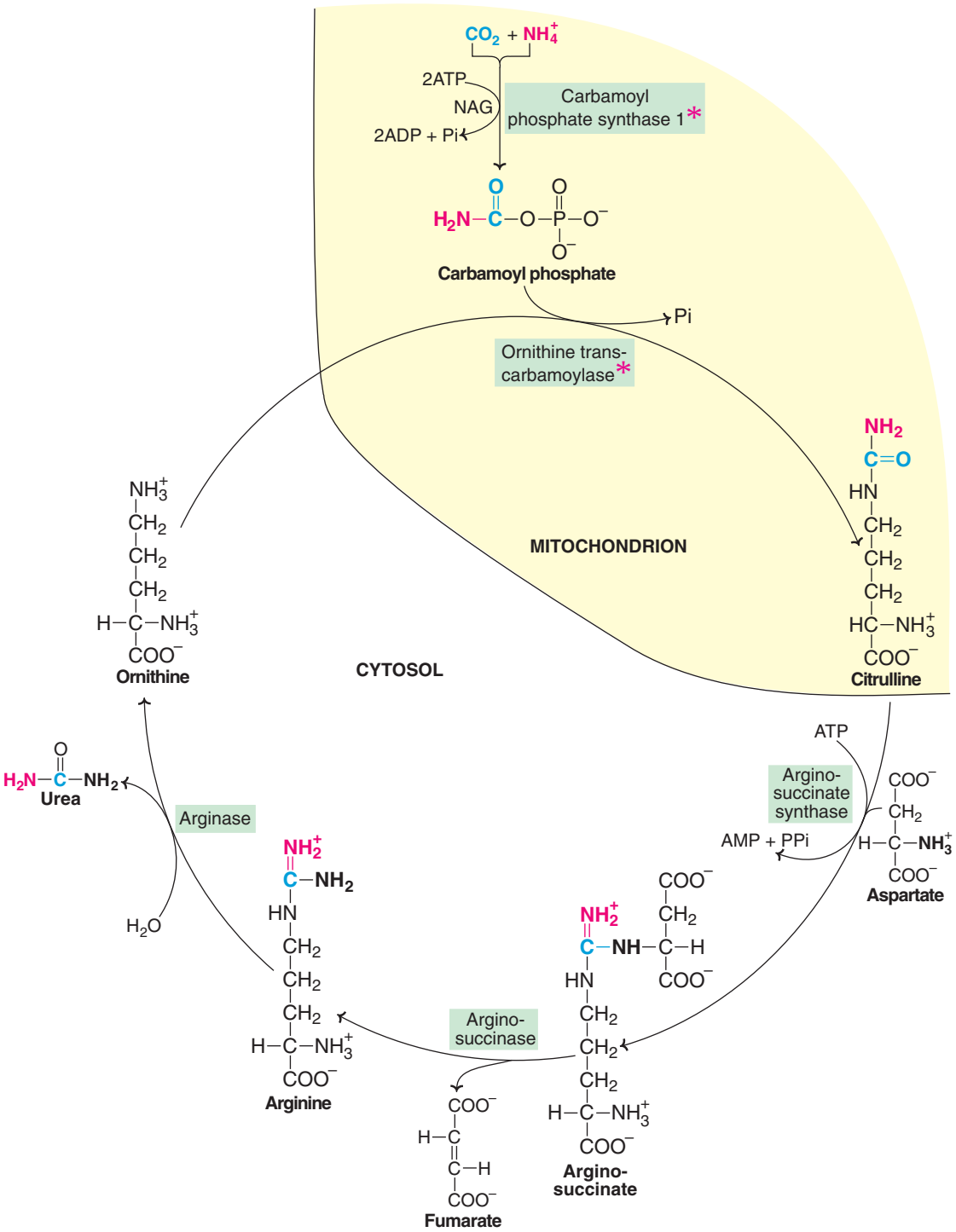

Fig. 15.10 : *Reactions of urea cycle (NAG—N-acetylglutamate; in the formation of urea, one amino group is derived from free ammonium ion while the other is from aspartate; carbon is obtained from CO_2;* * *mitochondrial enzymes, the rest of the enzymes are cytosomal).*

4. **Cleavage of arginosuccinate :** Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

5. **Formation of urea :** Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co^{2+} and Mn^{2+}. Ornithine and lysine compete with arginine (competitive inhibition). ***Arginase*** is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Overall reaction and energetics

The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP. Hence ***4 ATP*** are actually ***consumed***.

$NH_4^+ + CO_2$ + Aspartate + 3ATP $\longrightarrow$ Urea + Fumarate + 2 ADP + 2 Pi + AMP + PPi

Regulation of urea cycle

The first reaction catalysed by ***carbamoyl phosphate synthase I*** (CPS I) is ***rate-limiting*** reaction or committed step in urea synthesis. CPS I is allosterically activated by N-acetylglutamate (NAG). It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase (***Fig.15.11***).

The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. High concentrations of arginine increase NAG. The consumption of a protein-rich meal increases the level of NAG in liver, leading to enhanced urea synthesis.

Carbamoyl phosphate synthase I and glutamate dehydrogenase are localized in the mitochondria. They coordinate with each other in the formation of NH_3, and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.

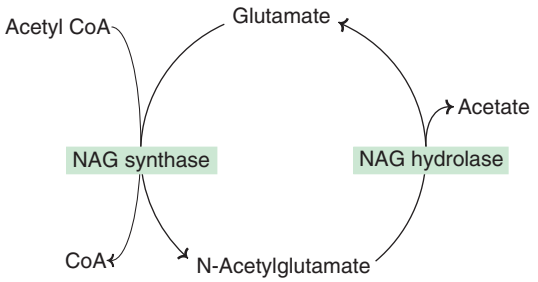

Fig. 15.11 : *Formation and degradation of N-acetylglutamate.*

Disposal of urea

Urea produced in the liver freely diffuses and is transported in blood to ***kidneys***, and excreted. A small amount of urea enters the intestine where it is broken down to CO_2 and NH_3 by the bacterial enzyme urease. This ammonia is either lost in the feces or absorbed into the blood. In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NH_3. Hyperammonemia (increased blood NH_3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

Integration between urea cycle and TCA cycle

Urea cycle is linked with TCA cycle in three different ways (***Fig.15.12***). This is regarded as ***bicyclic integration*** between the two cycles.

1. The production of ***fumarate*** in urea cycle is the most important integrating point with TCA cycle. Fumarate is converted to malate and then to oxaloacetate in TCA cycle. Oxaloacetate undergoes transamination to produce aspartate which enters urea cycle. Here, it combines with citrulline to produce arginosuccinate. Oxaloacetate is an important metabolite which can combine with acetyl CoA to form citrate and get

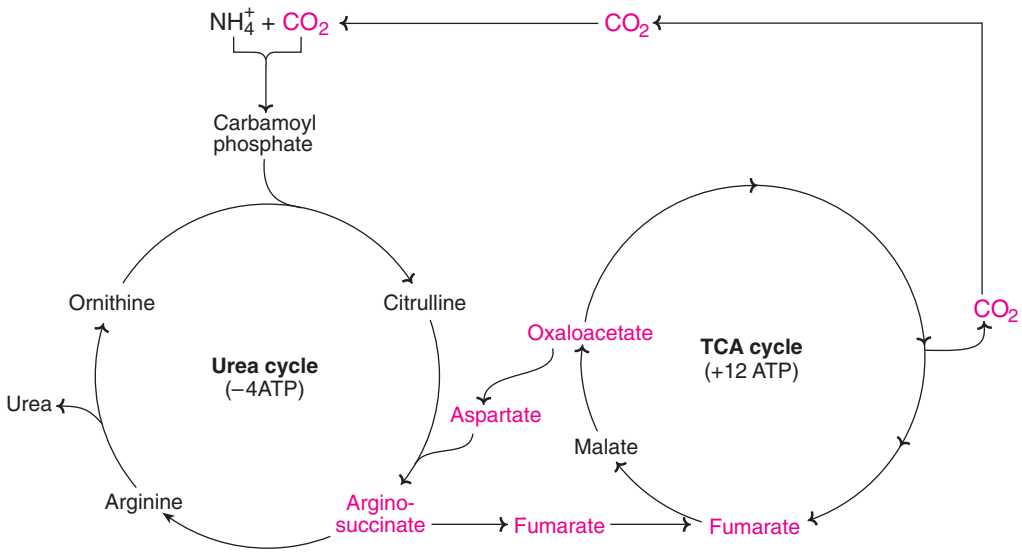

Fig. 15.12 : *Interrelation between urea and tricarboxylic acid (TCA) cycle (Depicted in blue colour).*

finally oxidized. Oxaloacetate can also serve as a precursor for the synthesis of glucose (gluconeogenesis).

2. ATP (12) are generated in the TCA cycle while ***ATP*** (4) are utilized for urea synthesis.

3. Citric acid cycle is an important metabolic pathway for the complete oxidation of various metabolites to CO_2 and H_2O. The ***CO_2*** liberated in TCA cycle (in the mitochondria) can be utilized in urea cycle.

Metabolic disorders of urea cycle

Metabolic defects associated with each of the five enzymes of urea cycle have been reported (***Table 15.1***). All the disorders invariably lead to a build-up in blood ammonia ***(hyperammonemia)***, leading to toxicity. Other metabolites of urea cycle also accumulate which, however, depends on the specific enzyme defect. The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental retardation.

Blood urea—clinical importance

In healthy people, the normal blood urea concentration is 10-40 mg/dl. Higher protein intake marginally increases blood urea level, however this is well within normal range. About 15-30 g of urea (7-15 g nitrogen) is excreted in urine per day.

Blood urea estimation is widely used as a screening test for the evaluation of ***kidney*** (renal) ***function***. It is estimated in the laboratory either by urease method or diacetyl monoxime (DAM) procedure. Elevation in blood urea may be broadly classified into three categories.

1. **Pre-renal :** This is associated with ***increased protein breakdown***, leading to a negative nitrogen balance, as observed after major surgery, prolonged fever, diabetic coma, thyrotoxicosis etc. In leukemia and bleeding disorders also, blood urea is elevated.

Table 15.1 Metabolic defects in urea cycle

Defect	*Enzyme involved*
Hyperammonemia type I	Carbamoyl phosphate synthase I
Hyperammonemia type II	Ornithine transcarbamoylase
Citrullinemia	Arginosuccinate synthase
Arginosuccinic aciduria	Arginosuccinase
Hyperargininemia	Arginase

2. **Renal :** In renal disorders like ***acute glomerulonephritis***, chronic nephritis, nephrosclerosis, polycystic kidney, blood urea is increased.

3. **Post-renal :** Whenever there is an ***obstruction*** in the ***urinary tract*** (e.g. tumors, stones, enlargement of prostate gland etc.), blood urea is elevated. This is due to increased reabsorption of urea from the renal tubules.

The term '***uremia***' is used to indicate increased blood urea levels due to renal failure. ***Azotemia*** represents an elevation in blood urea/or other nitrogen metabolites which may or may not be associated with renal diseases.

Non-protein nitrogen (NPN)

As is obvious from the name, the term NPN refers to all the nitrogen-containing substances other than proteins. These include urea (most abundant), creatinine, creatine, uric acid, peptides, amino acids etc. In healthy persons, NPN concentration in blood is 20-40 mg/dl.

The molecular weight of urea is 60 and about half of it (28) is contributed by the two nitrogen atoms. Thus, if blood urea concentration is 60 mg, then about half of it—28 mg—is ***blood urea nitrogen (BUN)***. Therefore,

$$\text{BUN} = \frac{1}{2}\ \text{NPN}$$

$$\text{NPN} = 2\ \text{BUN}$$

In some countries, estimations of BUN or NPN are used rather than blood urea for assessing kidney function. The normal range for ***ratio*** of ***BUN*** to serum ***creatinine*** is 10:1 to 15:1.

METABOLISM OF INDIVIDUAL AMINO ACIDS

In the preceding pages, the general aspects of amino acid metabolism have been discussed. A summary of the biologically important or specialized products obtained from or contributed by the amino acids is given in the ***Table 15.2***. The metabolism of individual amino acids with special emphasis on the specialized products is described next.

Table 15.2 A summary of the specialized products formed/contributed by amino acids

Amino acid	*Specialized product(s)*
Glycine	Creatine, glutathione, heme, purines, conjugated bile acids.
Tyrosine	Thyroxine, triiodothyronine, epinephrine, norepinephrine, dopamine, melanin.
Tryptophan	NAD^+, $NADP^+$ (coenzymes of niacin), serotonin, melatonin.
Methionine	Active methionine, creatine, epinephrine, polyamines.
Cysteine	Glutathione, taurine, coenzyme A, active sulfate.
Histidine	Histamine
Arginine	Creatine, nitric oxide
Lysine	Carnitine
Glutamate	γ-Amino butyric acid, glutathione, γ-carboxyglutamate.
Glutamine	Purines, pyrimidines, amino sugars.
Aspartate	Purines, pyrimidines
Serine	Phosphatidylserine, sphingomyelins, choline.
β-Alanine	Coenzyme A

GLYCINE

Glycine (Gly, G) is a non-essential, optically inactive and ***glycogenic*** (precursor for glucose) amino acid. It is indispensable for chicks. The outline of glycine metabolism is depicted in ***Fig.15.13***. Glycine is actively involved in the synthesis of many specialized products (heme, purines, creatine etc.) in the body, besides its incorporation into proteins, synthesis of serine and glucose and participation in one-carbon metabolism. Glycine is the most abundant amino acid normally excreted into urine (0.5–1.0 g/g creatinine).

Glycine in proteins

Glycine is one among the commonest amino acids found in protein structure. Being small and non-polar, glycine is mostly present in the interior structure of protein. Collagen contains very high (about 30%) content of glycine.