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M.Sc. Zoology (Semester II)
CC7- Biochemistry

Unit: 3.4

Biosynthesis of Urea

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Terrestrial organisms have evolved mechanisms to excrete nitrogenous wastes.

The animals must detoxify ammonia by converting it into a relatively nontoxic form such as urea or uric acid.

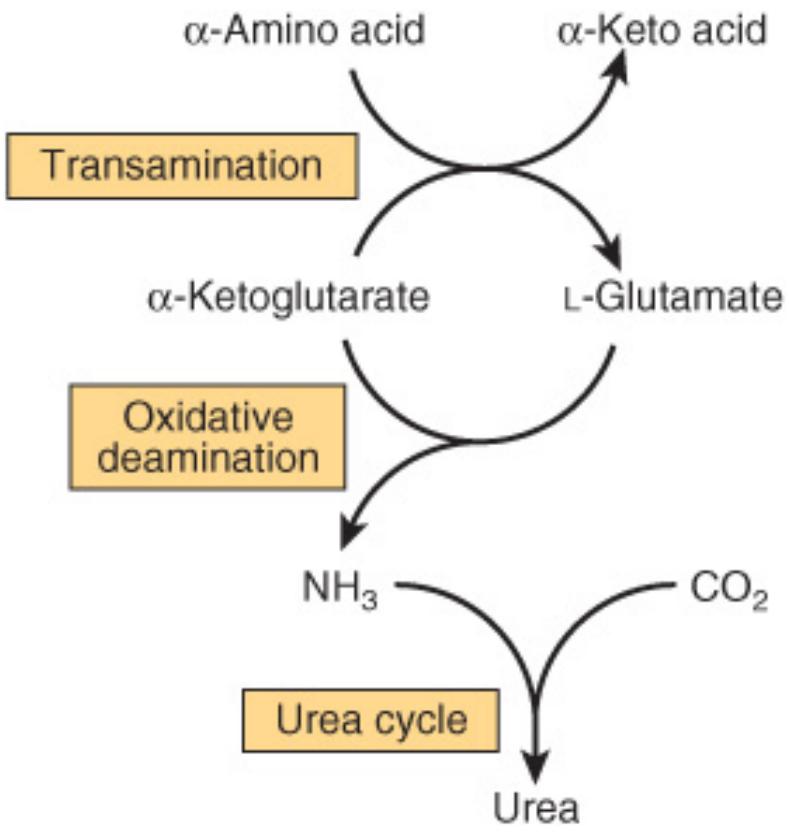
Mammals, including humans, produce urea, whereas reptiles and many terrestrial invertebrates produce uric acid.

The urea cycle, also called the ornithine cycle, was discovered by Hans Krebs at the University of Freiburg in Germany, in 1932.

Biosynthesis of Urea takes place in following four stages

1. Transamination
2. Oxidative deamination of glutamate
3. Ammonia transport
4. Reactions of the Urea cycle

Over all nitrogen flow in amino-acid catabolism



1. Transamination

It is a process of transferring amino groups from one molecule to another.

There is no formation and no excretion of ammonia, thus no net change in the nitrogen amount of body.

The raw materials for transmination are α-amino acid and α-ketoglutarate.

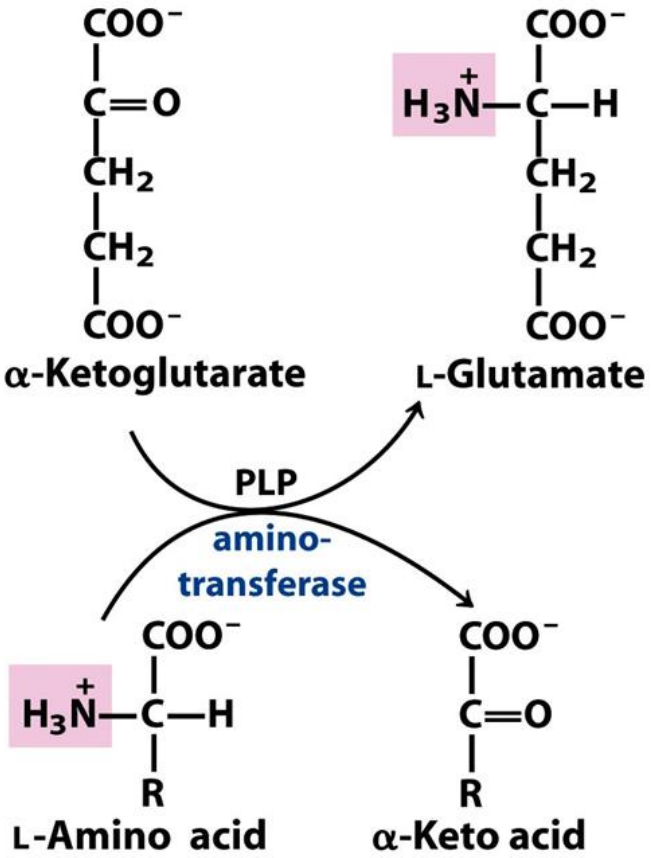
The reaction is catalysed by the enzyme aminotranferase (transaminase) which requires pyridoxal phosphate as a prosthetic group.

All transaminases contain this prosthetic group which derives from pyridoxine a water soluble vitamin also known as vitamin B_6.

The amino group from amino acids is temporarily uptaken by the pyridoxal phosphate as pyridoxamine phosphate prior to its donation to an α-ketoacid.

All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination process.

Transamination reaction



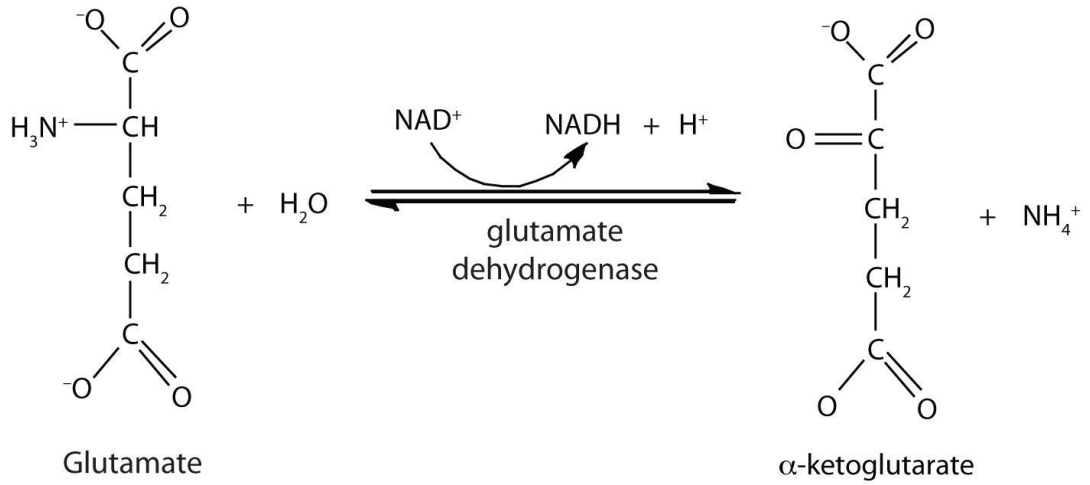
In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. Although the reaction is shown here in the direction of transfer of the amino group to α-ketoglutarate, it is readily reversible.

2. Oxidative Deamination

The amino group of glutamate is released as ammonia, regenerating α-ketoglutarate, by an enzyme glutamate dehydrogenase.

Glutamate is the only amino acid that undergoes oxidative deamination at a relatively high rate. The formation of ammonia from the amino group occurs mainly via the amino group of glutamate.

This reaction occurs primarily in liver mitochondria. Most of the NH4+ ion formed by oxidative deamination of glutamate is converted to urea and excreted in urine in a series of reactions known as the urea cycle.



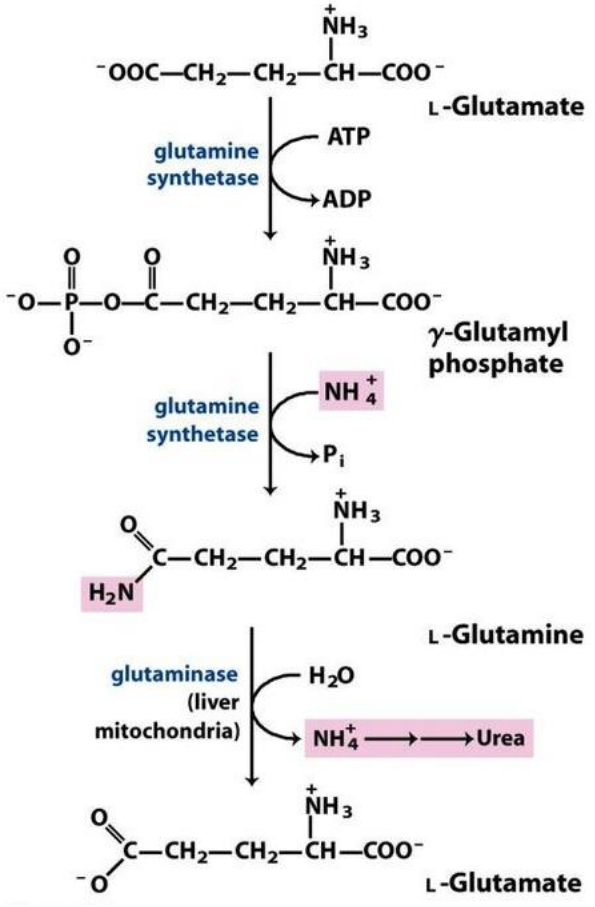
3. Transport of Ammonia

In tissues other than hepatocytes, NH4+ cannot be produced due to its toxicity.

In those tissues ammonium is transported to liver in other forms as discussed below

A) In the form of glutamine:

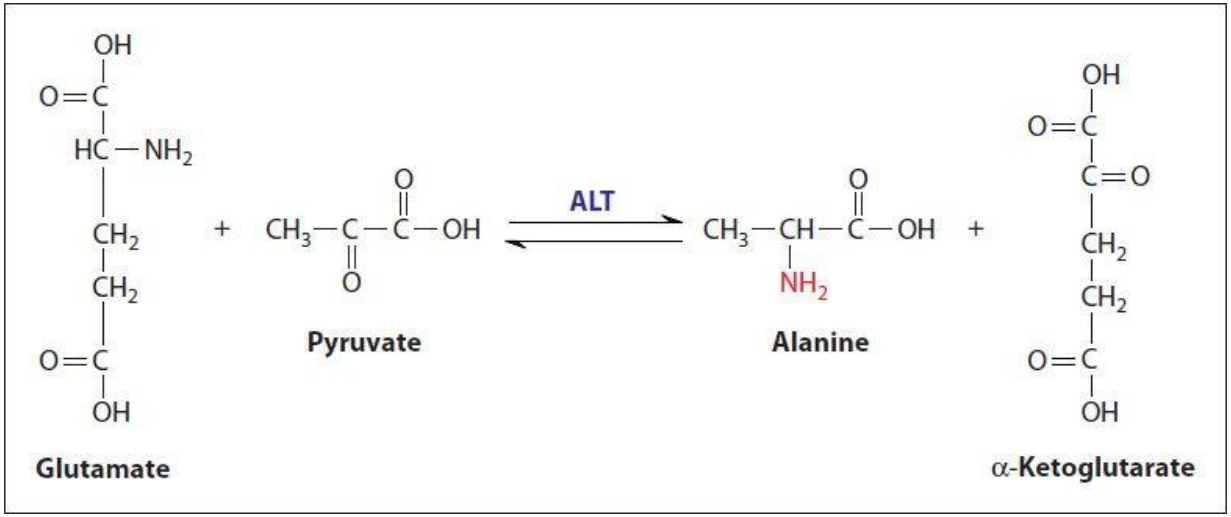
- l-glutamate produced as a result of transamination reaction is converted to glutamine in extra-hepatic tissues.
- Catalysed by glutamine synthetase.
- Ammonia is converted into non-toxic compound l-glutamine.
- L-glutamine is then transported to liver cell mitochondria.
- Liver cell mitochondrial matrix contains the enzyme glutaminase. Glutaminase converts l-glutamine back to l-glutamate and expels NH4+ for urea cycle.



B) In form of Alanine

L-glutamate produced as a result of transamination reaction is converted to Alanine in muscle cells.

The pathway involved is called glucose-alanine cycle. The reaction is accomplished by Alanine-aminotransferase (ALT).



Alanine is then transported to liver through blood.

In liver cytosol, Alanine aminotranferase perform the reverse reaction to yield pyruvate and l-glutamate.

Finally, glutamate dehydrogenase acts on l-glutamate to release NH4+. Now NH4+ can further enter into Urea cycle

4. The Urea Cycle

The urea cycle is the metabolic pathway that transforms nitrogen to urea for excretion from the body.

Most terrestrial animals convert excess nitrogen to urea, prior to excreting it. Nitrogenous excretory products are removed from the body mainly in the urine.

Ammonia, which is very toxic in humans, is converted to urea, which is nontoxic, very soluble, and readily excreted by the kidneys.

The urea excreted each day by a healthy adult (about 30 g) accounts for about 90% of the nitrogenous excretory products.

Urea is formed in the urea cycle that occurs mainly in the liver.

The Urea cycle occurs in two stages (five steps)

A) The mitochondrial stage (two steps)
B) The cytosolic stage (three steps)

Steps of Urea Cycle

Step	Reactants	Products	Catalyzed by	Location
1	$NH_4^+ + HCO_3^- +$ 2ATP	Carbamoyl phosphate + 2ADP + P_i	Carbamoyl phosphate synthetase I	mitochondria
2	Carbamoyl phosphate + Ornithine	Citrulline + P_i	Ornithine transcarbamoylase	mitochondria
3	Citrulline + Aspartate + ATP	Argininosuccinate + AMP + pyrophosphate	Argininosuccinate synthetase	cytosol
4	Argininosuccinate	Arginine + Fumarate	Argininosuccinase	cytosol
5	Arginine + H_2O	Ornithine + Urea	Arginase	cytosol

A) The Mitochondrial Stage

The first two steps of the urea cycle occur in the mitochondria of the cell.

Step 1: The enzyme carbamoyl phosphate synthetase (CPS) takes ammonia and bicarbonate, and forms carbamoyl phosphate with the use of ATP.

This is the step in the cycle which determines how fast the cycle progresses.

Step 2: Ornithine transcarbamoylase (OTC) then condenses carbamoyl phosphate and ornithine, which forms citrulline.

This citrulline is then moved out of the mitochondria into the cytosol of the cell by the transporter.

B) The Cytosolic Stage

Step 3: Argininosuccinate synthetase (AS) takes the citrulline formed in the mitochondrial stage, and condenses it with aspartate to form argininosuccinate.

Step 4: Argininosuccinate is then broken into arginine and fumarate by argininosuccinase.

Step 5: Arginine is then further broken down into urea and ornithine by arginase.

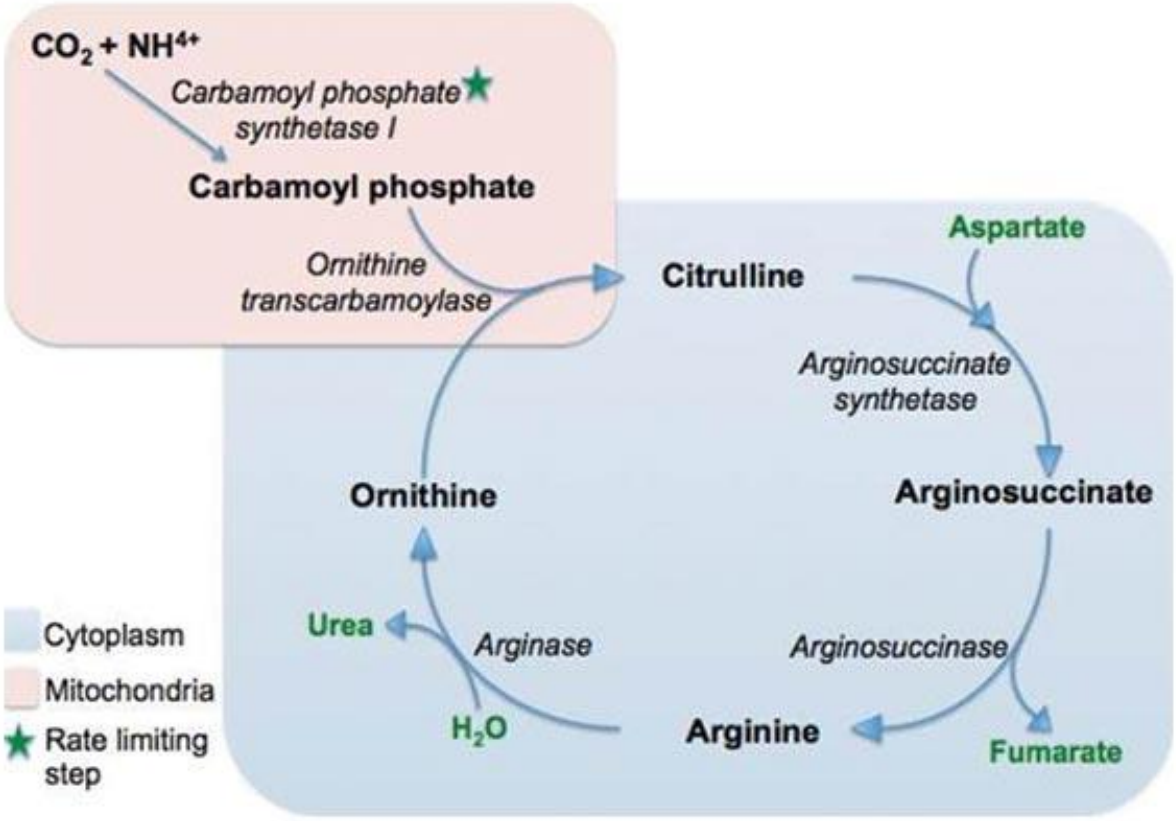
Arginine can also be acquired from the diet, and this can also be taken in by the liver cells and broken down into urea and ornithine by arginase.

The ornithine is then transported into the mitochondria by ornithine translocase. There, it is used by OTC again, to form citrulline.

The citrulline is then processed to form urea and ornithine again, and the cycle continues.

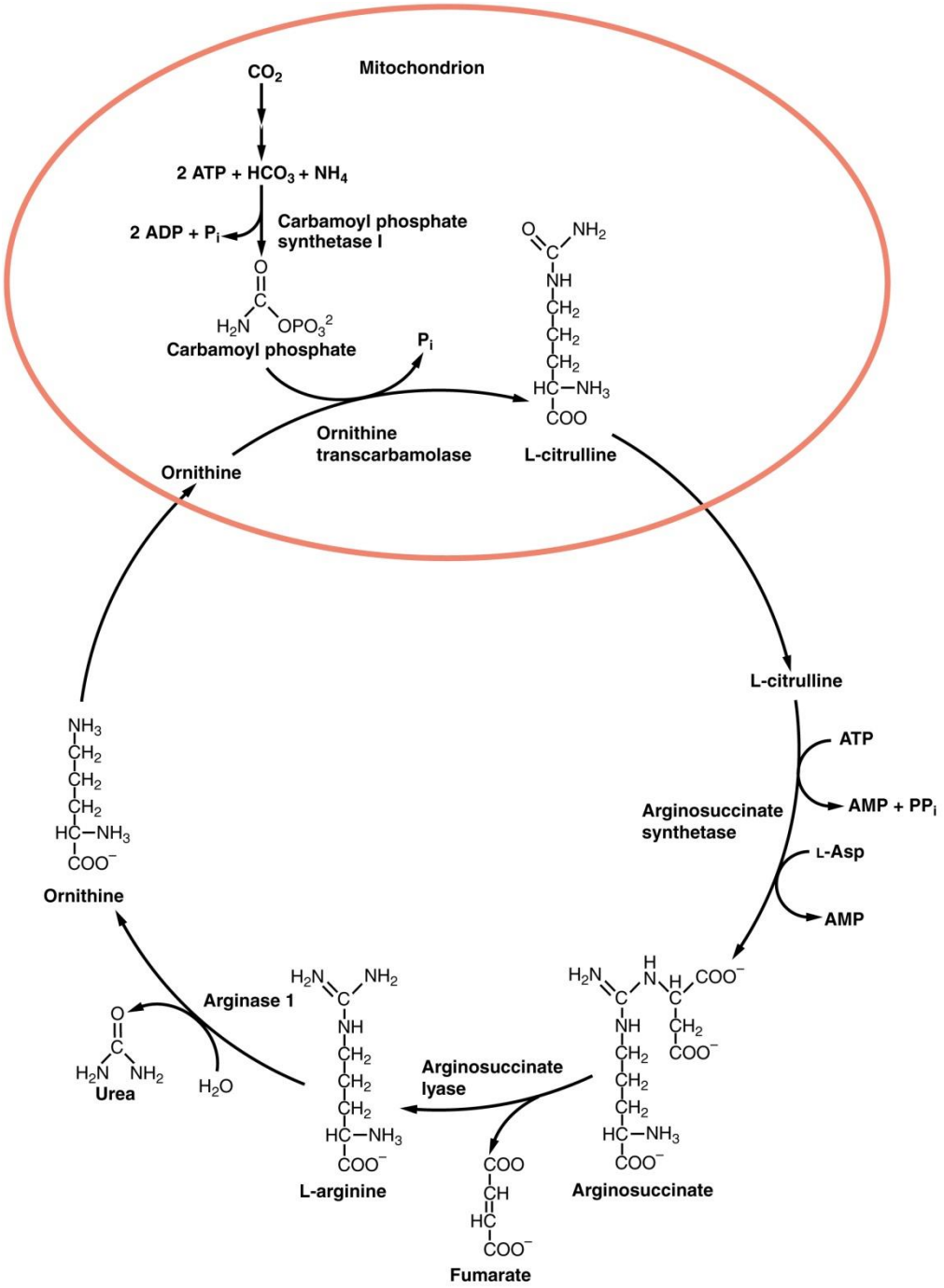
During the cycle, urea is the only new product which is formed, while all other molecules used in the cycle are recycled.

Schematic of Urea cycle

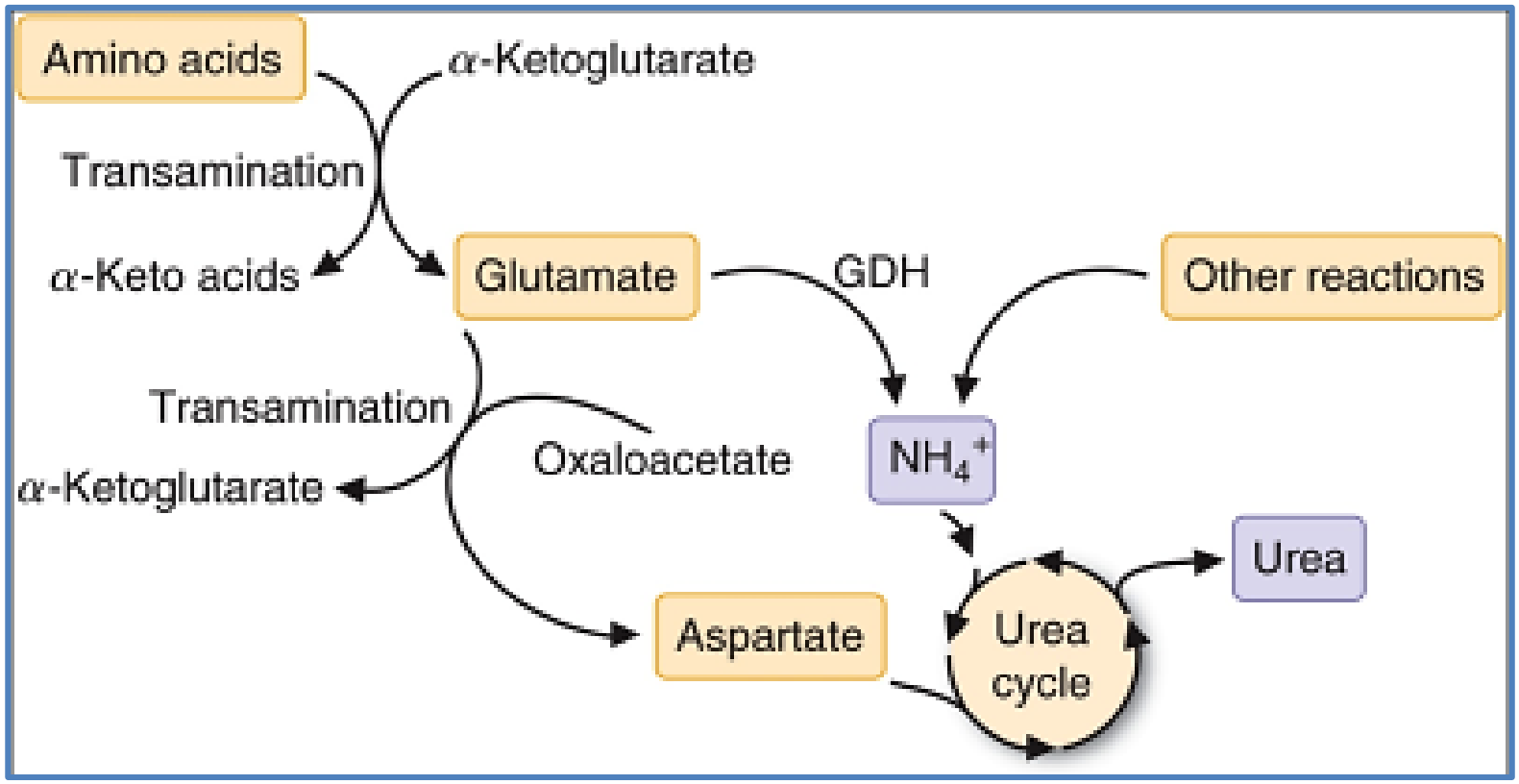


To memorise Urea cycle "OCAA" summarises all steps:
O- Ornithine binds carbamoyl phosphate to form
C- Citrulline, which accepts aspartate to form
A- Argininosuccinate, which is cleaved to fumarate and
A-Arginine, which on hydrolysis liberates urea and ornithine

Schematic of Urea cycle with detailed structure of all intermediate molecules



Summary of Nitrogenous waste (Urea) formation



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