Urea cycle

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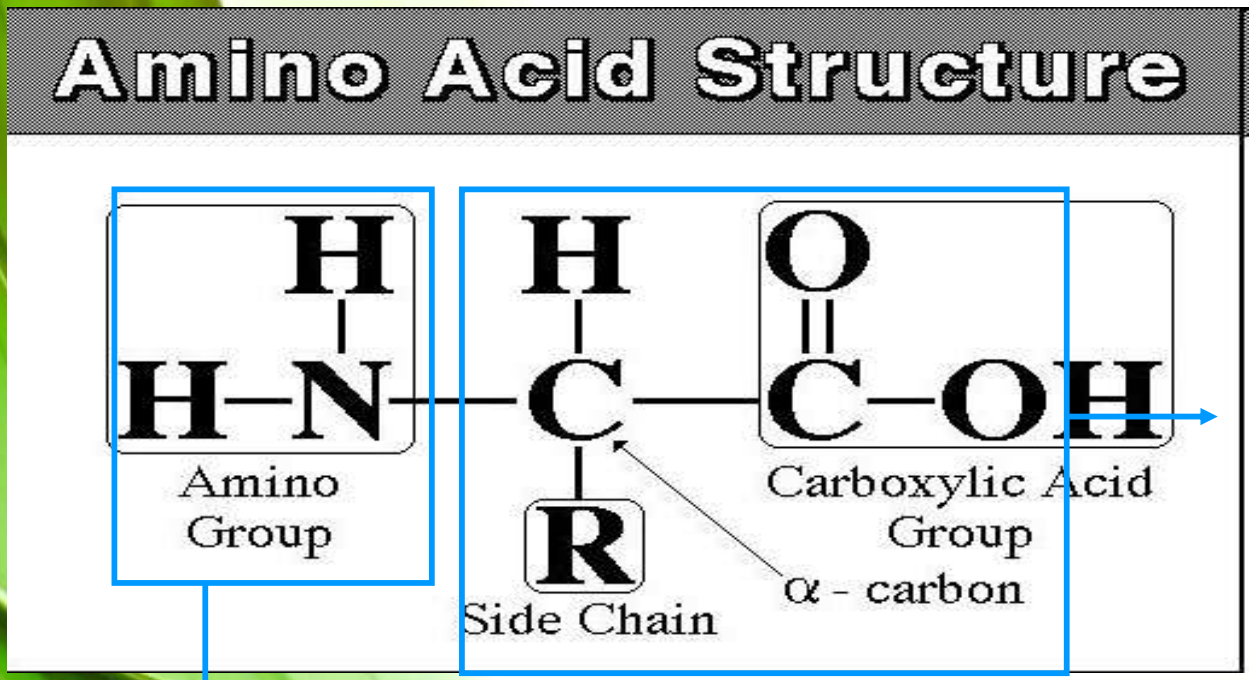
Amino acid oxidation and the production of urea



Oxidation

Waste or reuse

Ammonia has to be eliminated

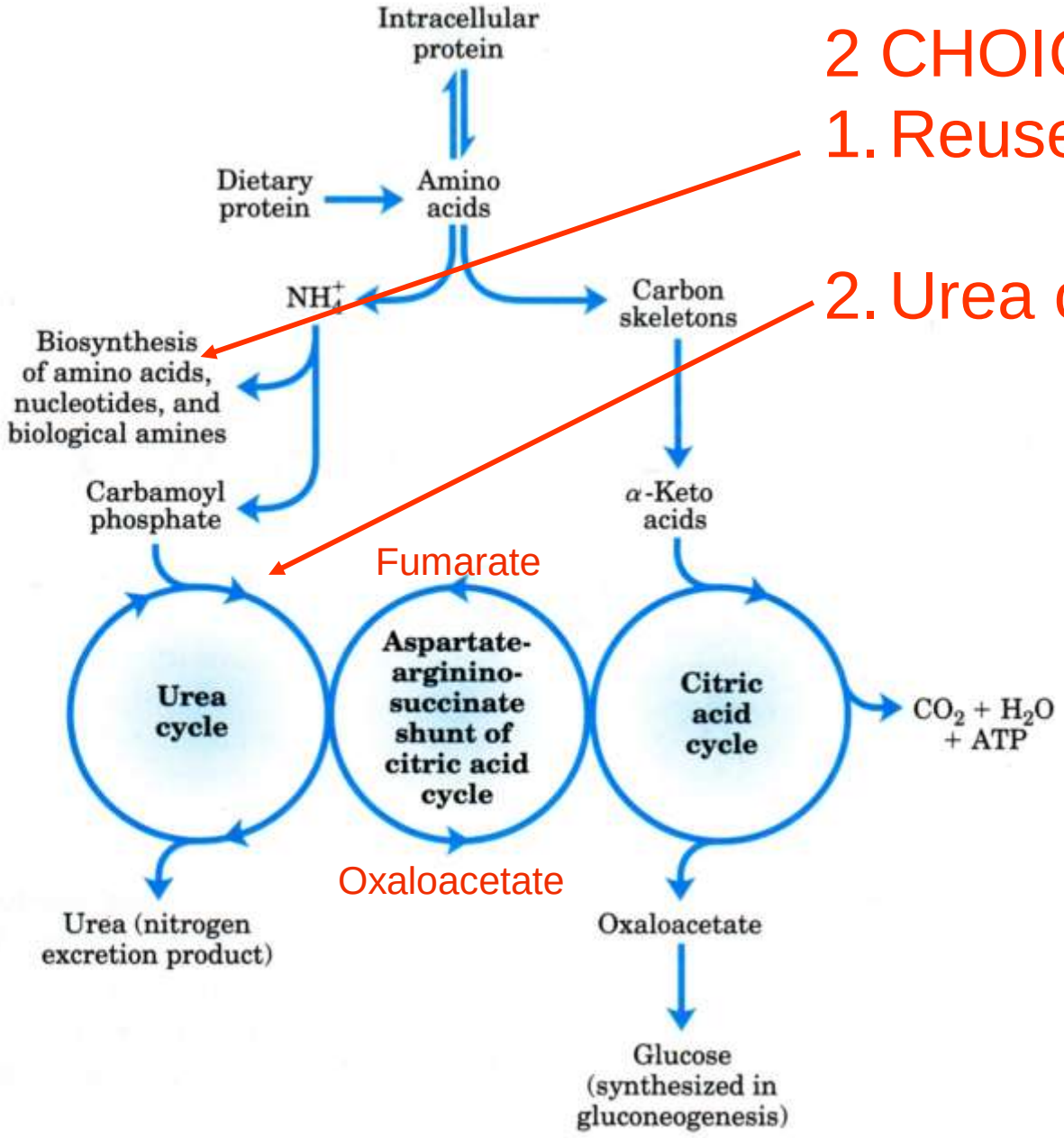
- Ammonia originates in the catabolism of amino acids that are primarily produced by the degradation of proteins – dietary as well as existing within the cell:
  - digestive enzymes
  - proteins released by digestion of cells sloughed-off the walls of the GIT
  - muscle proteins
  - hemoglobin
  - intracellular proteins (damaged, unnecessary)

Ammonia has to be eliminated

- Ammonia is toxic, especially for the CNS, because it reacts with α-ketoglutarate, thus making it limiting for the TCA cycle ⇒ decrease in the ATP level

- Liver damage or metabolic disorders associated with elevated ammonia can lead to tremor, slurred speech, blurred vision, coma, and death

- Normal conc. of ammonia in blood: 30-60 μM

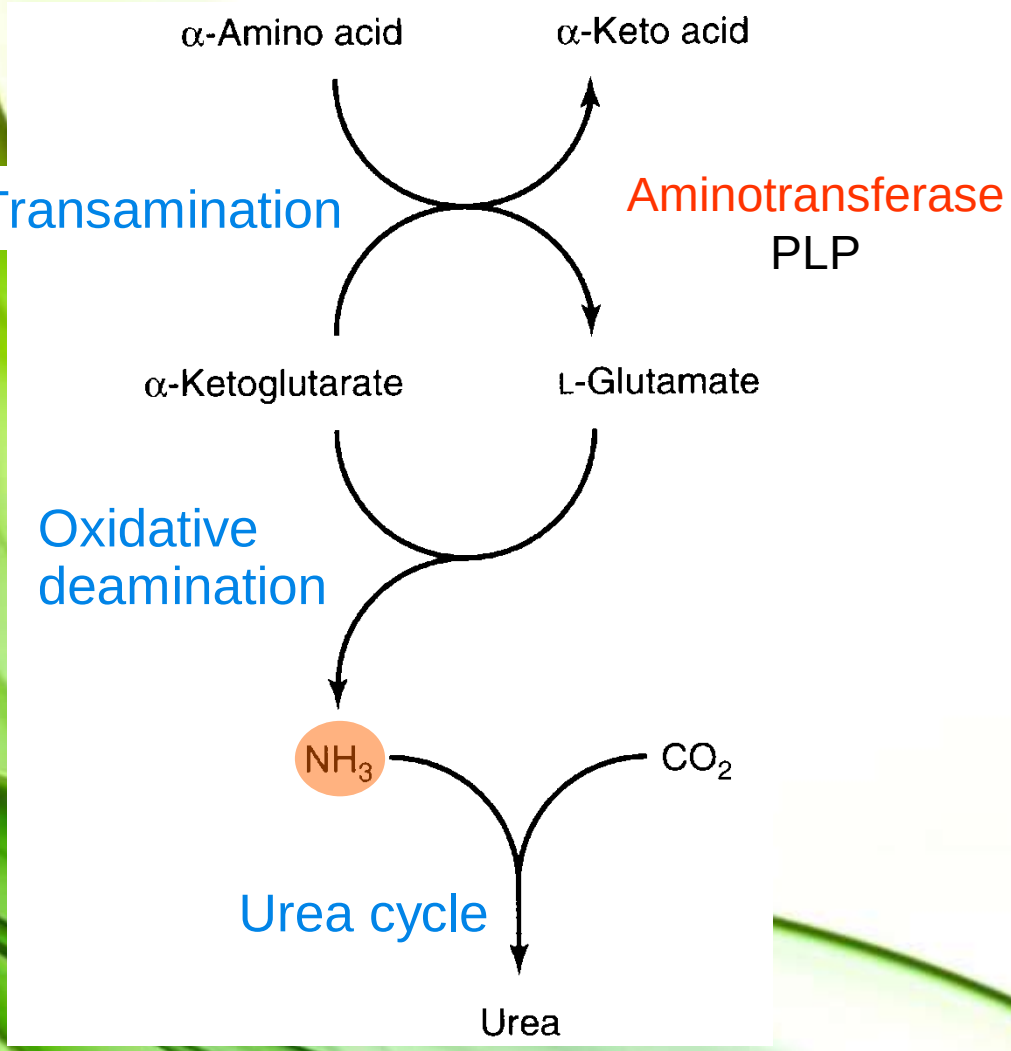


2 CHOICES

1. Reuse
2. Urea cycle

Overview of amino acid catabolism in mammals

Nitrogen removal from amino acids



Nitrogen removal from amino acids

Step 1: Remove amino group

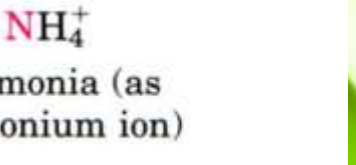
Step 2: Take amino group to liver for nitrogen excretion

Step 3: Entry into mitochondria

Step 4: Prepare nitrogen to enter urea cycle

Step 5: Urea cycle

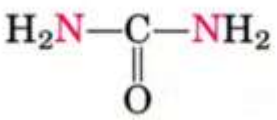
Excretory forms of nitrogen



NH_4^+

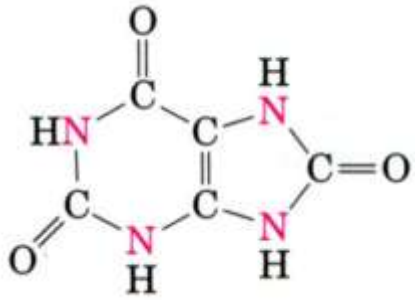
Ammonia (as ammonium ion)

Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia



Urea

Ureotelic animals: many terrestrial vertebrates; also sharks



Uric acid

Uricotelic animals: birds, reptiles

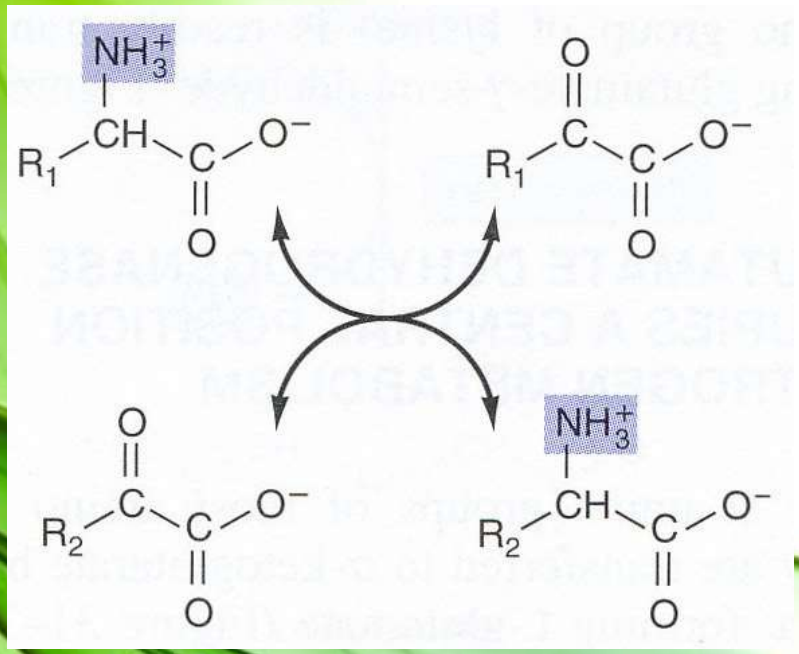
a) Excess NH_4^+ is excreted as ammonia (microbes, aquatic vertebrates or larvae of amphibia),

b) Urea (many terrestrial vertebrates)

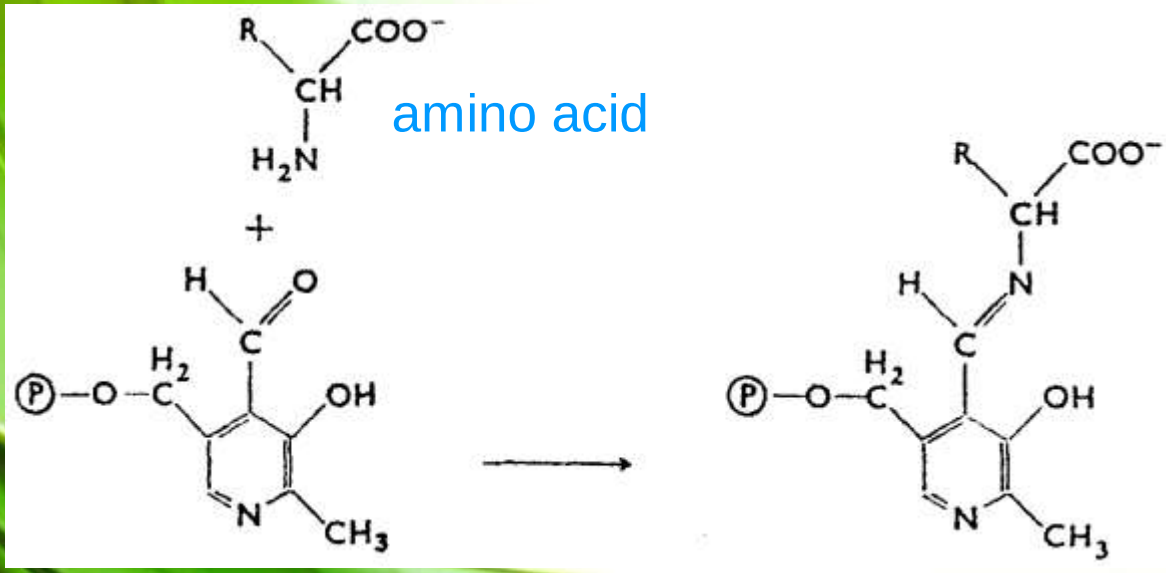
c) or uric acid (birds and terrestrial reptiles)

Step 1. Remove amino group

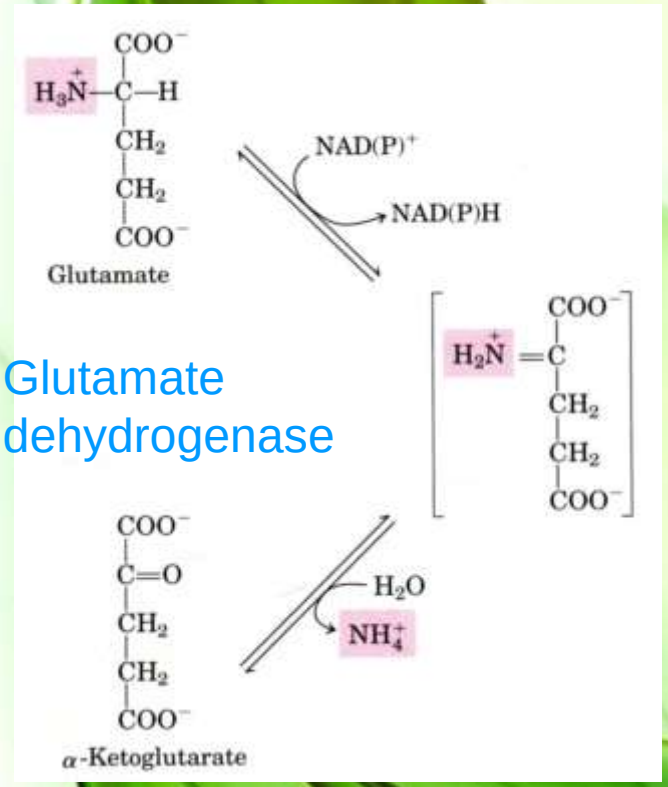
- Transfer of the amino group of an amino acid to an α-keto acid $\Rightarrow$ the original AA is converted to the corresponding α-keto acid and vice versa:



- Transamination is catalyzed by transaminases (aminotransferases) that require participation of pyridoxalphosphate:



Step 2: Take amino group to liver for nitrogen excretion



Glutamate releases its amino group as ammonia in the liver.

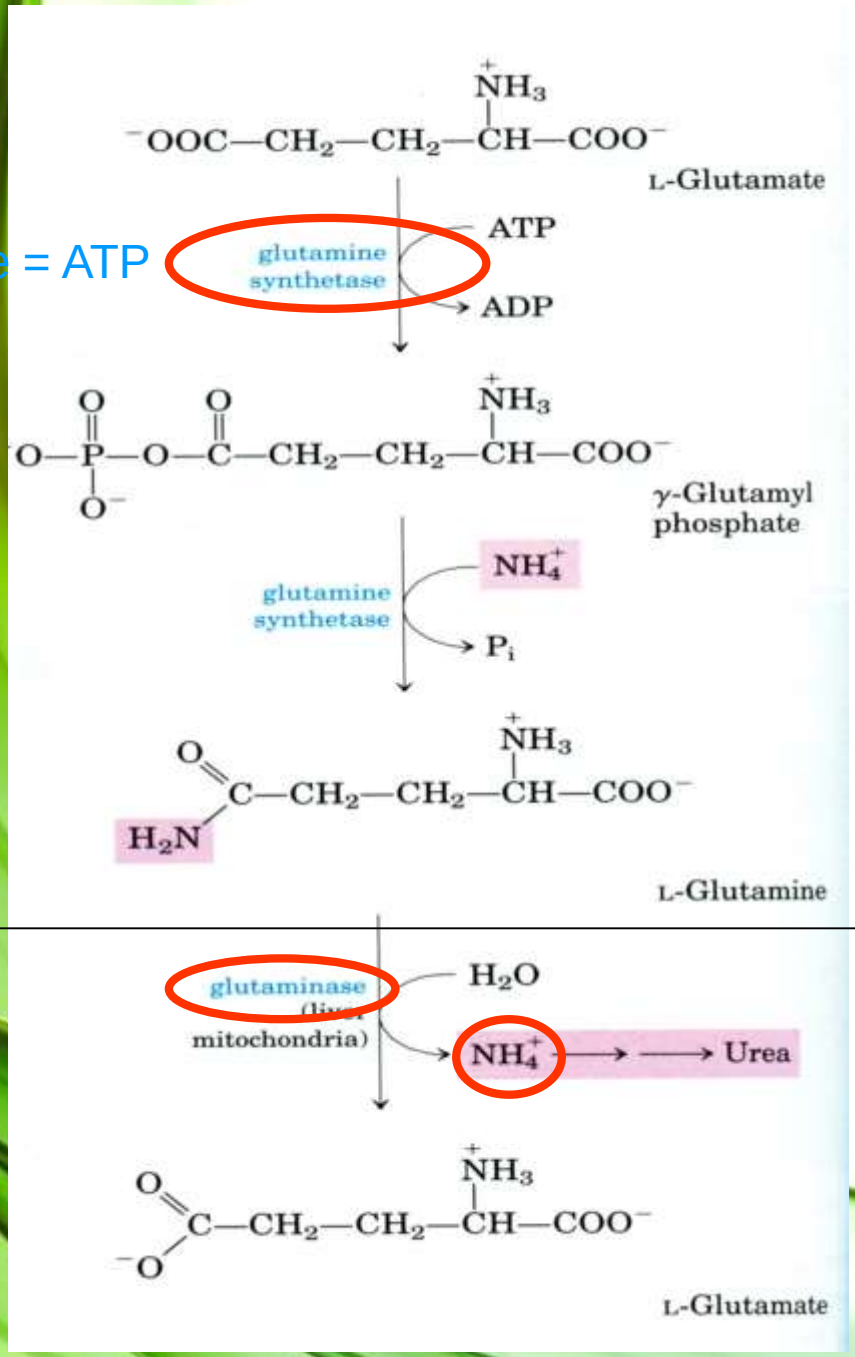
The amino groups from many of the α-amino acids are collected in the liver in the form of the amino group of L-glutamate molecules.

The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor

Nitrogen carriers

1. Glutamate
   - transferres one amino group WITHIN cells:
   - Aminotransferase → makes glutamate from α-ketogluta-rate
   - Glutamate dehydrogenase → opposite
2. Glutamine
   - transferres two amino group BETWEEN cells → releases its amino group in the liver
3. Alanine
   - transferres amino group from tissue (muscle) into the liver

SynthAtase = ATP

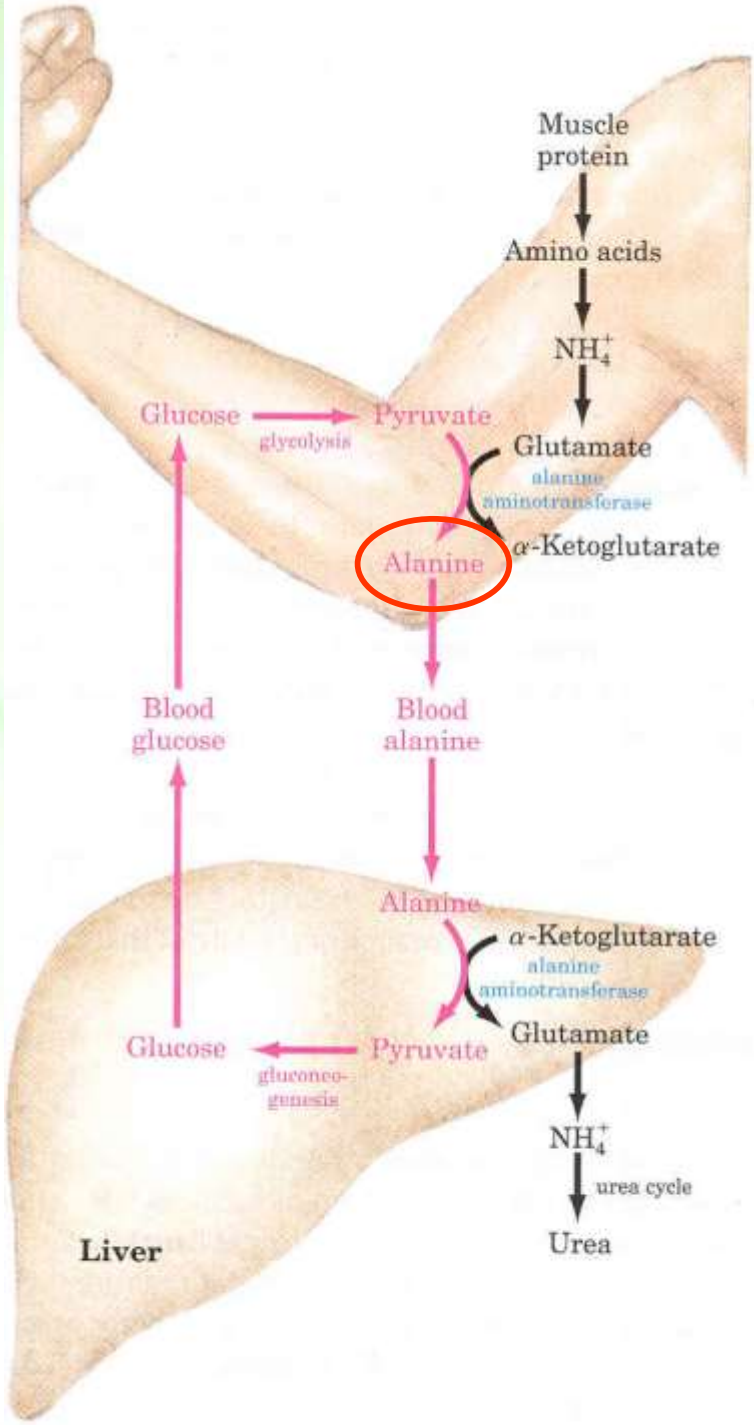


Move within cells

Move between cells

In liver

Glucose-alanine cycle



Alanine plays a special role in transporting amino groups to liver.

Ala is the carrier of ammonia and of the carbon skeleton of pyruvate from muscle to liver.
The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

Sources of ammonia for the urea cycle:

- Oxidative deamination of Glu, accumulated in the liver by the action of transaminases and glutaminase
- Glutaminase reaction releases NH_3 that enters the urea cycle in the liver (in the kidney, it is excreted into the urine)
- Catabolism of Ser, Thr, and His (nonoxidative deamination) also releases ammonia:

Serine - threonine dehydratase

Serine $\rightarrow\rightarrow$ pyruvate + NH_4^+

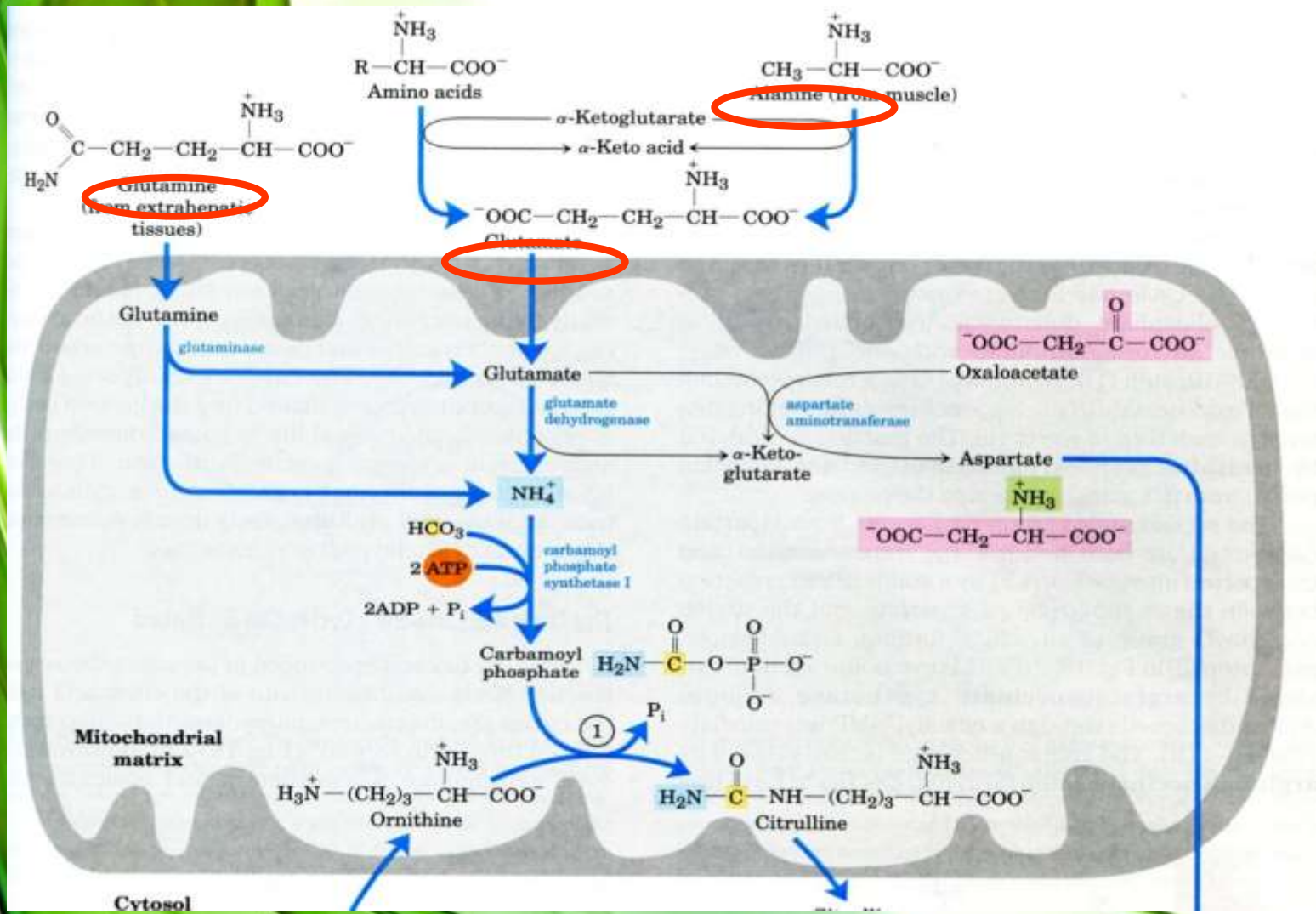
Threonine $\rightarrow\rightarrow$ α-ketobutyrate + NH_4^+

- Bacteria in the gut also produce ammonia.

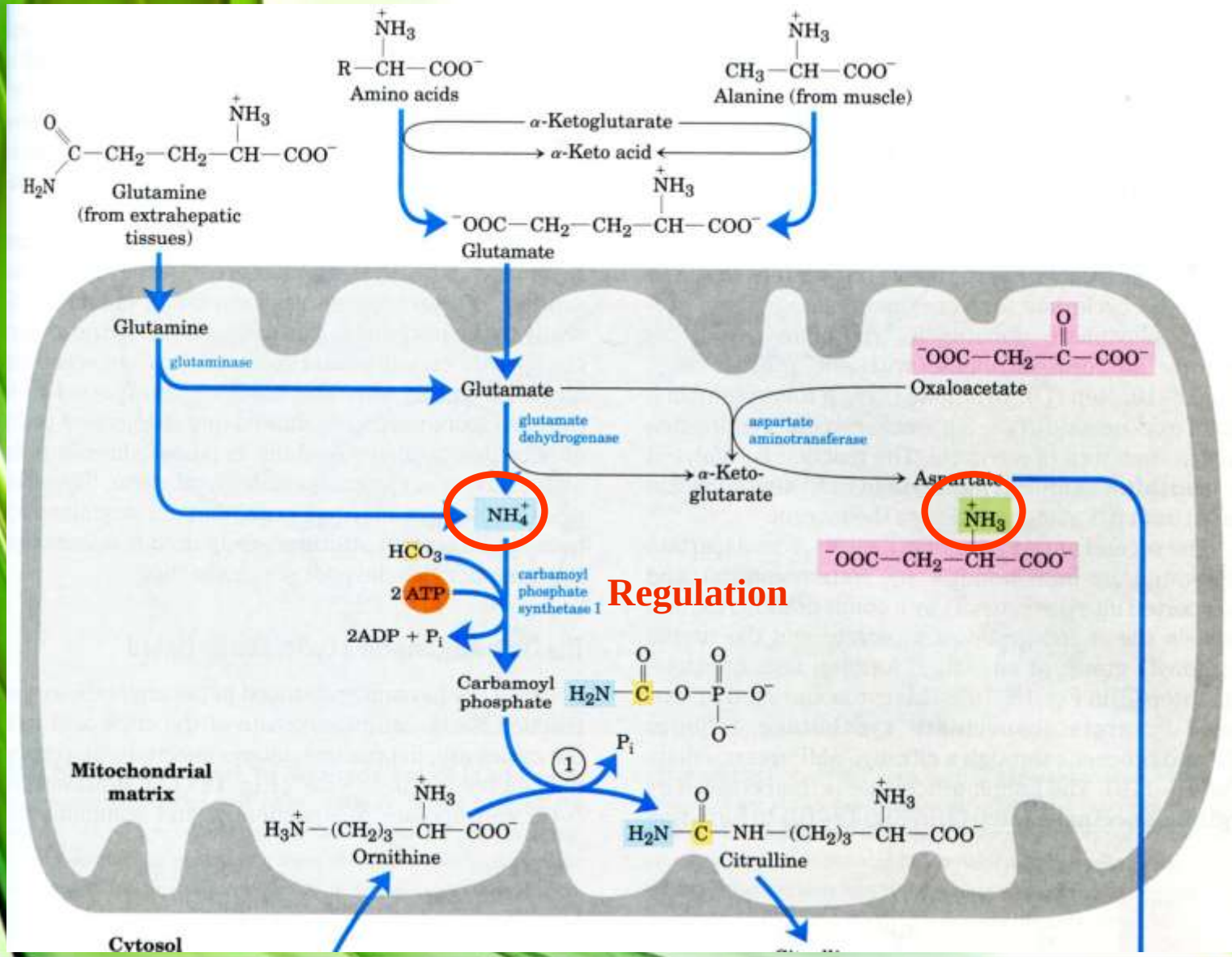
Review:

- Nitrogen carriers ⇨ glutamate, glutamine, alanine
- 2 enzymes outside liver, 2 enzymes inside liver:
  - Aminotransferase (PLP) → α-ketoglutarate → glutamate
  - Glutamate dehydrogenase (no PLP) → glutamate → α-ketoglutarate (**in liver**)
  - Glutamine synthase → glutamate → glutamine
  - Glutaminase → glutamine → glutamate (**in liver**)

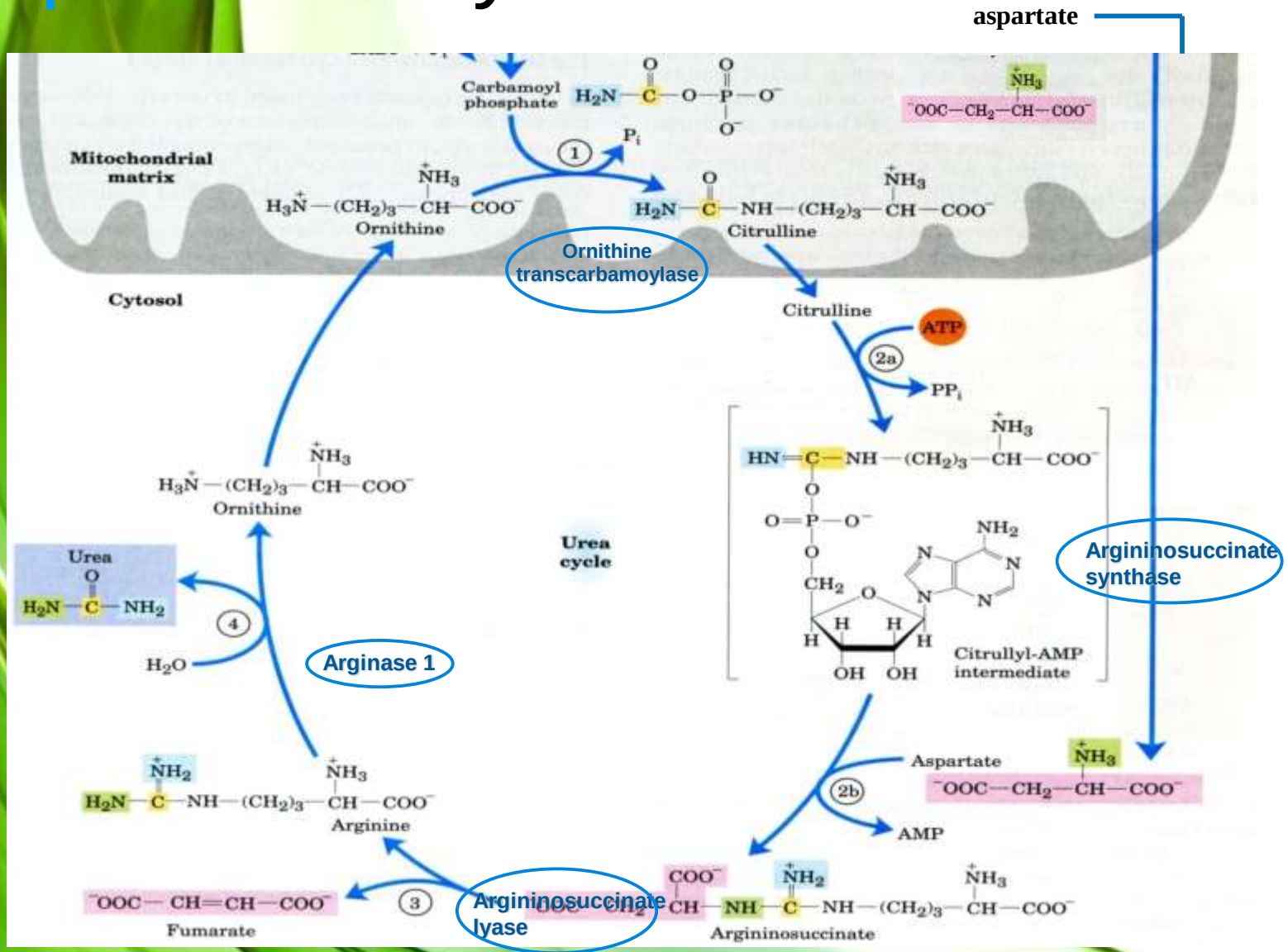
Step 3: entry of nitrogen to mitochondria



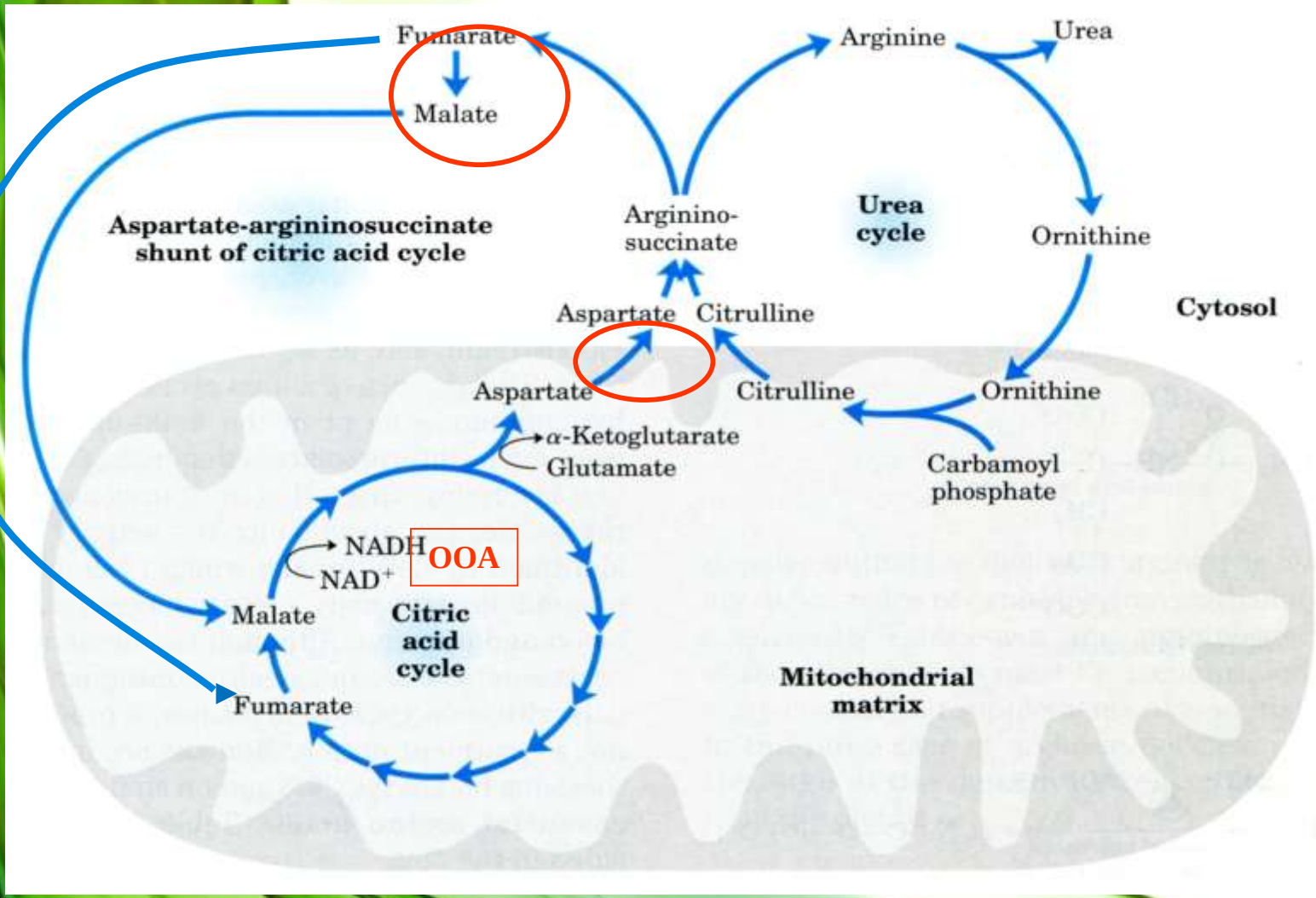
Step 4: prepare nitrogen to enter urea cycle



Step 5: Urea cycle



Oxaloacetate → aspartate



Urea cycle – review
(Sequence of reactions)

- ***Carbamoyl phosphate*** formation in mitochondria is a prerequisite for the urea cycle
  - (*Carbamoyl phosphate synthetase*)
- ***Citrulline*** formation from carbamoyl phosphate and ornithine
  - (*Ornithine transcarbamoylase*)
- Aspartate provides the additional nitrogen to form ***argininosuccinate*** in cytosol
  - (*Argininosuccinate synthase*)
- ***Arginine*** and ***fumarate*** formation
  - (*Argininosuccinate lyase*)
- Hydrolysis of arginine to ***urea*** and ornithine
  - (*Arginase*)

The overall chemical balance of the biosynthesis of urea

$NH_3 + CO_2 + 2ATP \rightarrow$ carbamoyl phosphate + 2ADP + Pi

Carbamoyl phosphate + ornithine → citrulline + Pi

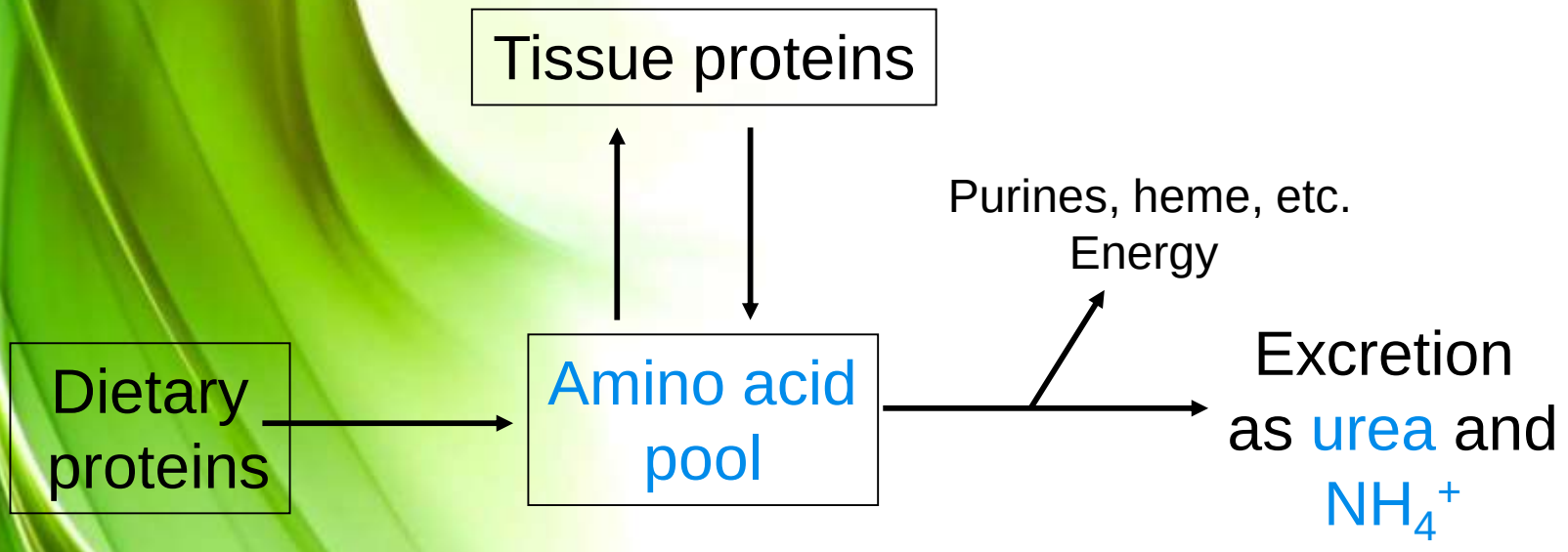
Citrulline + ATP + aspartate → argininosuccinate + AMP + PPi

Argininosuccinate → arginine + fumarate

Arginine → urea + ornithine

Sum: $2NH_3 + CO_2 + 3ATP \rightarrow$ urea + 2ADP + AMP + PPi + 2Pi

Nitrogen balance



The amount of nitrogen ingested is balanced by the excretion of an equivalent amount of nitrogen. About 80% of excreted nitrogen is in the form 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.

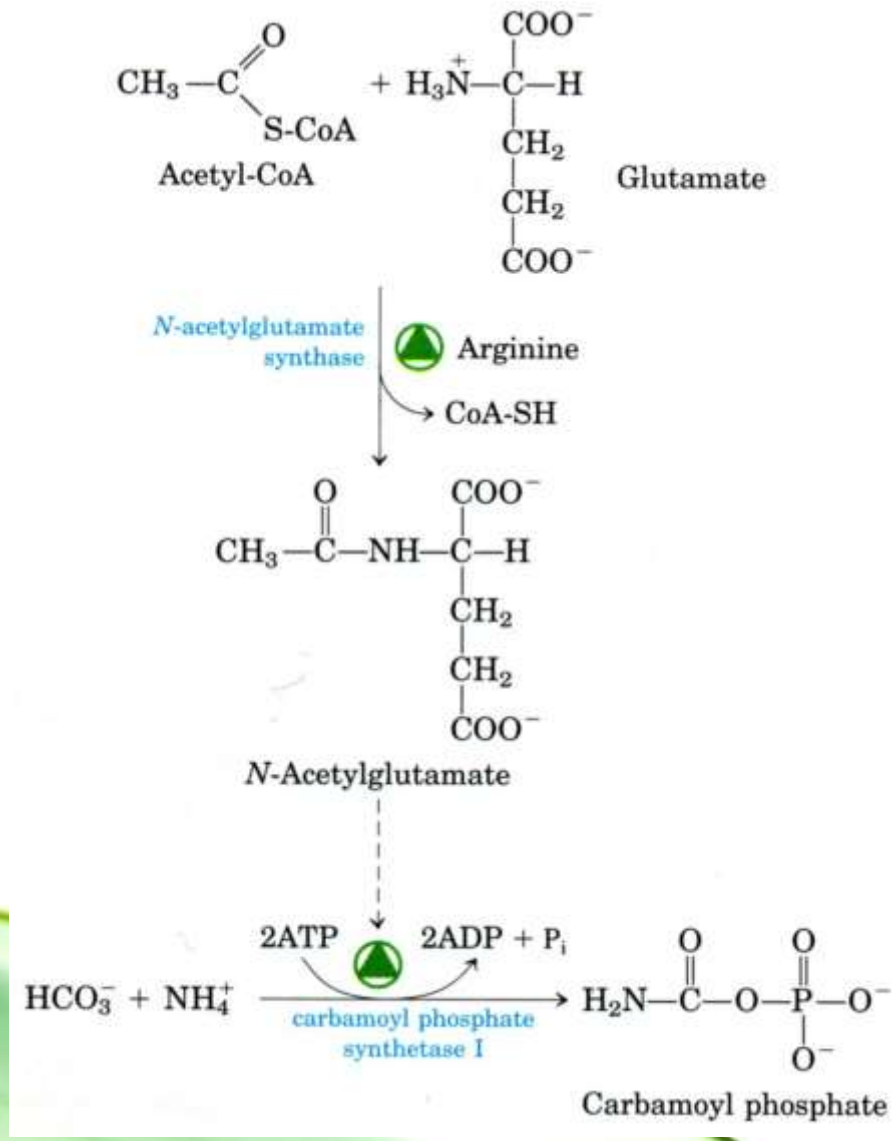
Regulation of urea cycle

- 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

Regulation of urea cycle

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

Ammonia toxicity

Ammonia encephalopathy

- Increased concentration of ammonia in the blood and other biological fluids → ammonia difuses into cells, across blood/brain barrier → increased synthesis of glutamate from α-ketoglutarate, increased synthesis of glutamine
- α-ketoglutarate is depleted from CNS → inhibition of TCA cycle and production of ATP
- Neurotransmitters – glutamate (excitatory neurotr.) and GABA (inhibitory neurotr.), may contribute to the CNS effects – bizarre behaviour

Deficiencies of urea cycle enzymes

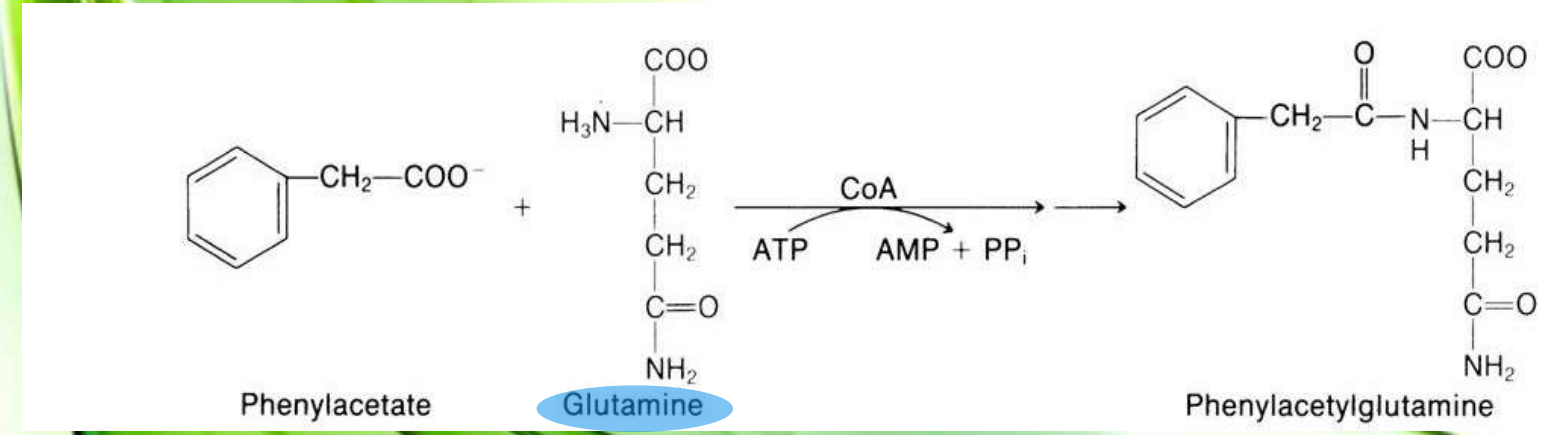
- Infant born with total deficiency of one or more enzymes survive at least several days.
- Many enzymes deficiencies are partial → enzymes have altered K_m values.
- Case are known of deficiencies of each enzymes.
- Interruption of the cycle at each point affected nitrogen metabolism differently - some 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.**

N-acetylglutamate synthase deficiency:

- Deficiency or genetic mutation of enzyme (autosomal recessive) → urea cycle failure.
- A severe neonatal disorder with fatal consequences, if not detected immediately upon birth.
- Hyperammonemia and general hyperaminoacidemia in a newborn (liver contain no detectable ability to synthesize N-acetylglutamate).
- Early symptoms include lethargy, vomiting, and deep coma.
- ***Treatment*** with structural analog N-carbamoyl-L-glutamate – activates CPS-I, mitigates the intensity of the disorder,

Carbamoyl phosphate synthetase (CPS I) deficiency:

- autosomal recessive metabolic disorder, associated with mental retardation and developmental delay.
- Hyperammonemia has been observed in 0 – 50% of normal level of CPS-I synthesis in the liver.
- Treatment with *benzoate* and *phenylacetate* ⟶ hippurate and Phe-Ac-Gln are excreted in the urine:



Ornithine transcarbamoylase (OTC) deficiency

- The most common urea cycle disorder, resulting in a mutated and ineffective form of the enzyme.
- X-linked recessive disorder caused by a number of different mutations in the OTC gene – males are generally more seriously affected than females (males are asymptomatic as heterozygotes).
- Complications with OTC may include mental retardation and developmental delay.

Argininosuccinate synthase deficiency – citrullinemia (citrullinuria)

- autosomal recessive metabolic disorder, inability to condense citrulline with aspartate.
- Accumulation of citrulline in blood and excretion in the urine.
- Type I citrullinemia - usually becomes evident in the first few days of life.
- Type II citrullinemia - the signs and symptoms usually appear during adulthood and mainly affect the nervous system.
- Therapy – specific supplementation with arginine for protein synthesis and for formation of creatin and ornithin.

Argininosuccinate lyase deficiency (argininosuccinate aciduria)

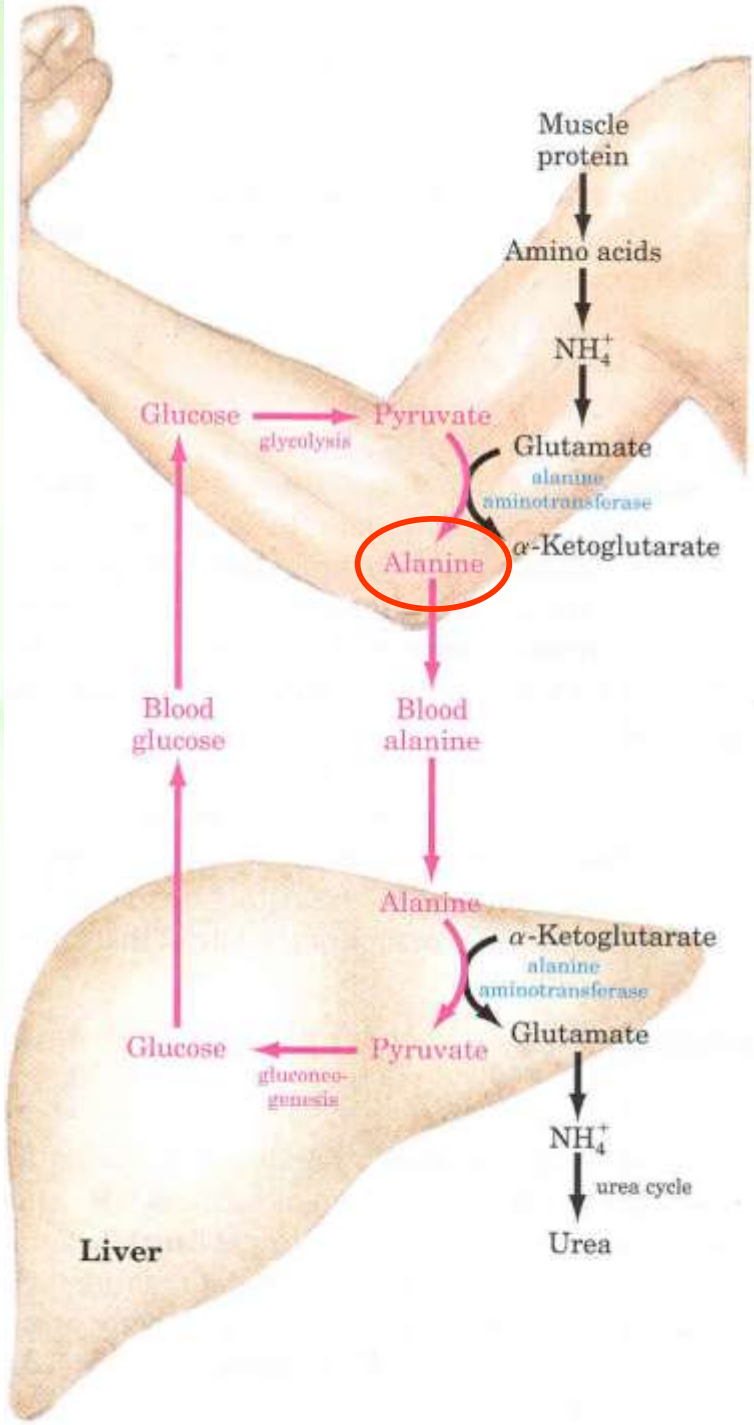
- Rare autosomal recessive disorder, argininosuccinate is excreted in large amount in urine.
- The severity of symptoms varies greatly, it is hard to evaluate the effect of therapy – useful is dietary restriction of nitrogen.

Arginase deficiency (argininemia)

- Rare autosomal recessive disorder that cause many abnormalities in development and function of CNS.
- Accumulation and excretion of arginine in urine and arginine precursors and products of arginine metabolism.
- Therapy – low nitrogen compounds diet (including essential amino acids

Which of amino acid carries the amino group from muscles to the liver?

Glucose-alanine cycle



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References

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- Lippon Cott illustrated reviews by D.R. Ferrier(2018)
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