

Amino acid metabolism I

Catabolism of proteins

General catabolism of amino acids

Biochemistry I
Lecture 6

2009 (J.S.)

Digestion of dietary proteins in the GIT

Protein digestion begins in the stomach, where the acidic environment favours protein **denaturation**. Proteins undergo the primary proteolysis catalysed by gastric **pepsin** (the optimal pH 2).

Degradation continues in the lumen of the intestine owing to the activity of **pancreatic proteolytic enzymes** secreted as inactive proenzymes and converted into active enzymes within the intestinal lumen..

Gastric and pancreatic proteolytic enzymes

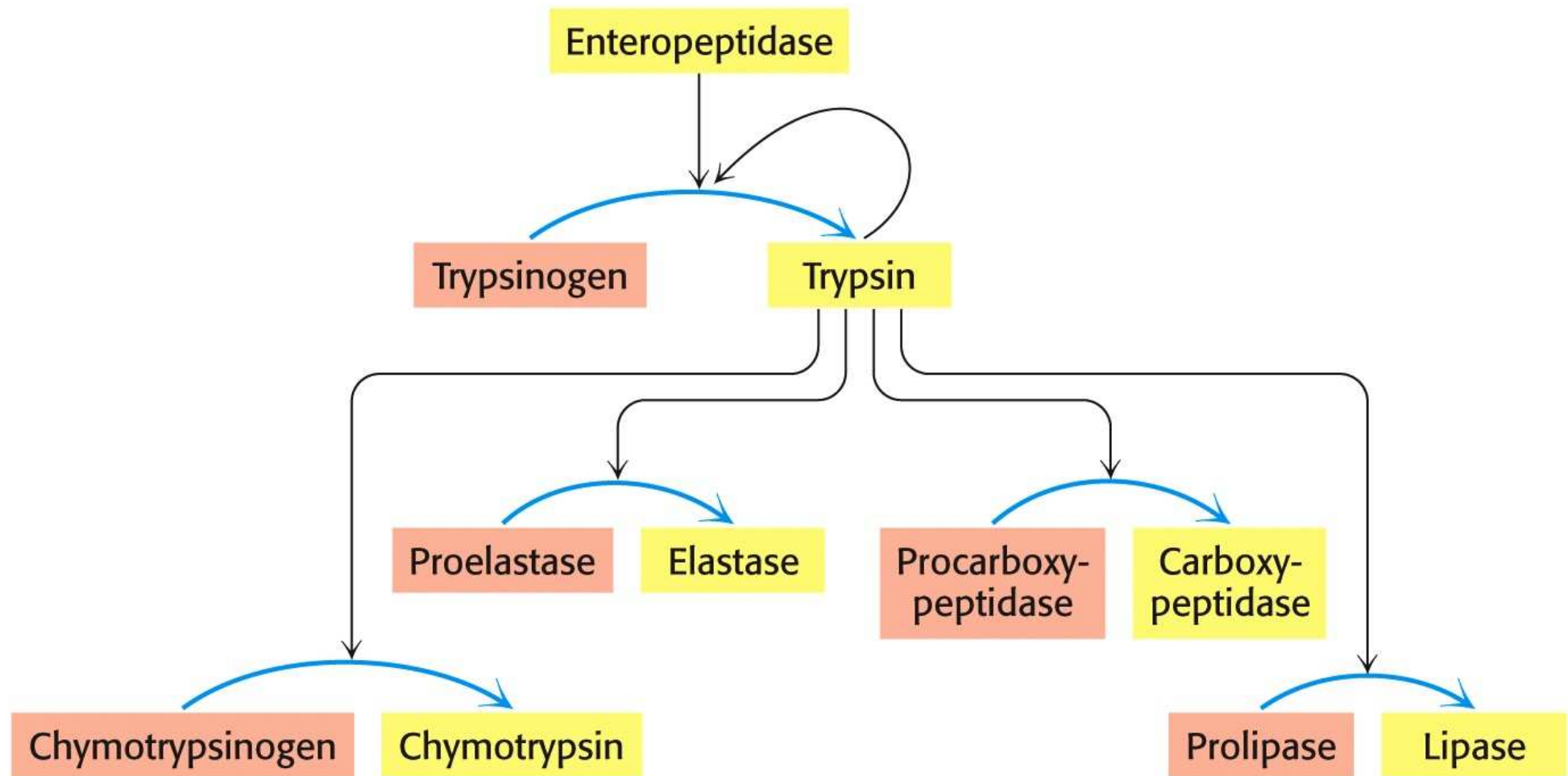
Site of synthesis	Proenzyme	Active enzyme
Stomach	Pepsinogen	Pepsin
Pancreas	Chymotrypsinogen	Chymotrypsin
	Trypsinogen	Trypsin
	Procarboxypeptidase	Carboxypeptidase
	Proelastase	Elastase

Digestion is then completed by **aminopeptidases** located in the plasma membrane (brush border) of the enterocytes.

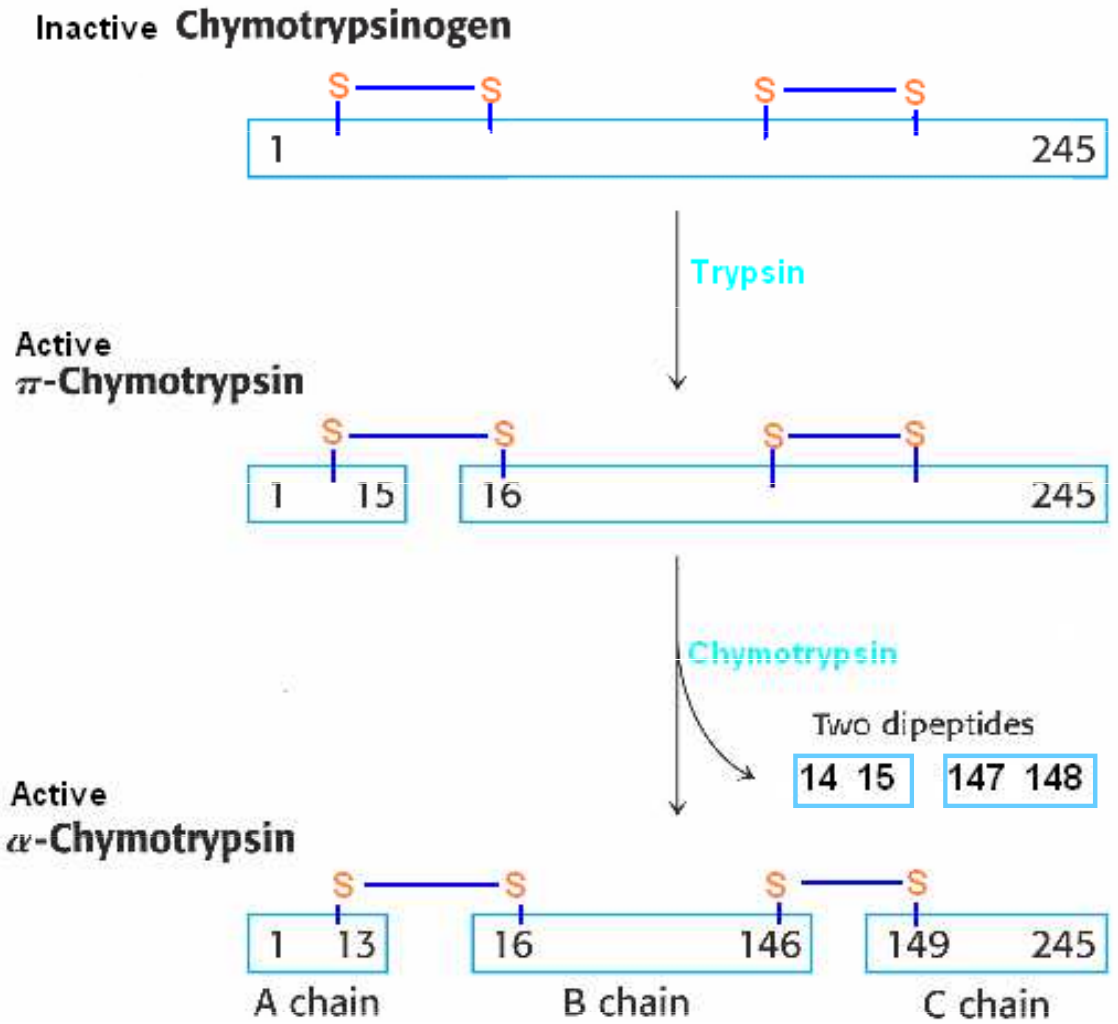
Single amino acids, as well as **di- and tripeptides**, are transported into the intestinal cells from the lumen and released into the blood.

The generation of trypsin leads to the activation of other proenzymes:

Enteropeptidase secreted by the mucosa of duodenum initiates the activation of the pancreatic proenzymes by activating trypsin, which then activates other proenzymes.



Proteolytic activation of chymotrypsinogen



The active site for binding a part of the substrate is not fully formed in the chymotrypsinogen. Proteolysis enables the formation of the substrate-binding site.

Proteolytic enzymes of the GIT

attack peptide bonds of a number of amino acids, but they exhibit the preference for particular types of peptide bonds:

Proteinases (formerly called endopeptidases): Preferentially attacks **the bond after**:

Pepsin	aromatic (Phe, Tyr) and acidic amino acids (Glu, Asp)
Trypsin	basic amino acids (Arg, Lys)
Chymotrypsin	hydrophobic (Phe, Tyr, Trp, Leu) and acidic AA (Glu, Asp)
Elastase	an amino acid with a small side chain (Gly, Ala, Ser)

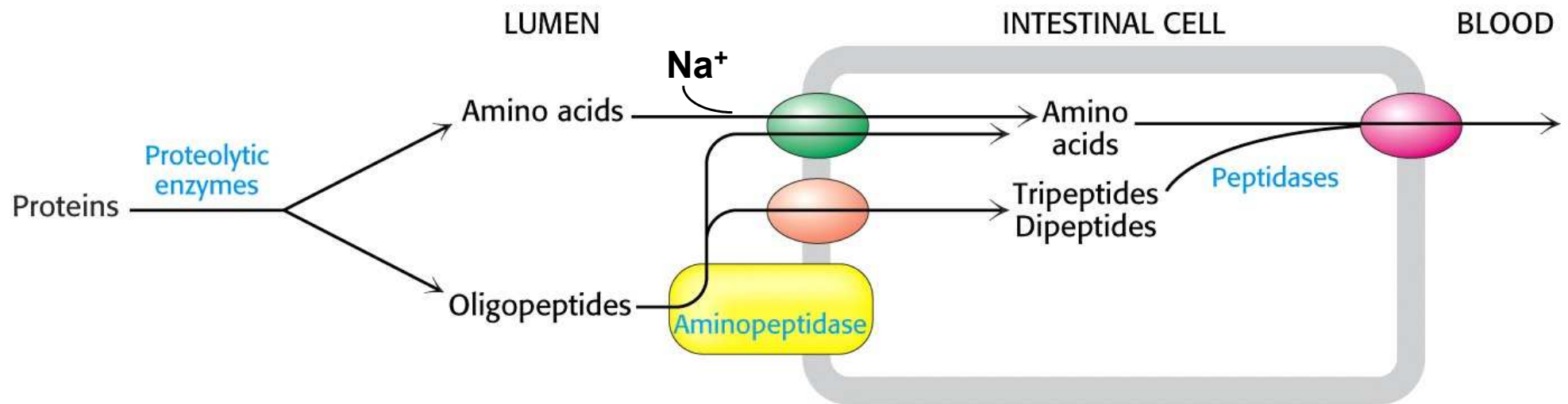
Peptidases (formerly exopeptidases):

<u>Carboxypeptidase A</u>	nearly all amino acids (<u>not</u> Arg and Lys)
<u>Carboxypeptidase B</u>	basic amino acids (Arg, Lys)
Leucine <u>aminopeptidase</u>	nearly all amino acids
Prolidase	proline
Dipeptidase	splits only dipeptides

The absorption of amino acids in the gut

The absorption of amino acids and peptides is an **active process**, **obligatorily coupled to the uptake of Na^+** . It is driven by the large difference in Na^+ concentration across the brush border membrane, that is maintained by the Na^+, K^+ -ATPase.

There are at least seven different Na^+ -linked amino acid transporters of different but overlapping specificities, and also separate transporters for di- and tripeptides.



Degradation of intracellular proteins

- eliminates abnormal proteins,
- permits the regulation of cellular metabolism by elimination of some enzymes and/or regulatory proteins that represent important metabolic control points.

Processes regulated by protein degradation

Gene transcription
Cell-cycle progression
Organ formation
Circadian rhythms
Inflammatory response
Tumor suppression
Cholesterol metabolism
Antigen processing

Normal intracellular proteins

are eliminated at rates that depend on their identity.

Long-lived proteins, *under physiological conditions*, are degraded at nearly constant rates, mostly nonselectively; *nutritional deprivation* increases rates of degradation and it is selective.

Short-lived proteins degradation is provided selectively by cytosolic ubiquitin system, or by other systems not yet known.

Systems which select proteins for degradation:

- The **N-end amino acyl residue** – in both prokaryotes and eukaryotes the half-life of a cytoplasmic protein varies with the identity of its N-terminal amino acyl:

Highly stabilizing residues ($t_{1/2} > 20$ hours)

Ala Cys Gly Met Pro Ser Thr Val

Intrinsically destabilizing residues

($t_{1/2} = 20$ to 30 minutes) Glu Ile Tyr

($t_{1/2} = 2$ to 3 minutes) Arg Asp Leu Lys Phe

- Some **sequences** of amino acyl residues in the primary structure.

Proteins rich in segments Pro-Glu-Ser-Thr (symbol PEST), so-called PEST proteins, are rapidly degraded in the cytosol, $t_{1/2}$ about 30 minutes.

During a prolonged fasting, proteins with sequences Lys-Phe-Glu-Arg-Gln (symbol KFERQ), KFERQ proteins, in the tissues that atrophy in response to starvation (e.g. liver, kidney) are specifically bound with the protein *prp73*, delivered to the lysosome, and degraded.

Cyclin destruction boxes – amino acid sequences that mark cell-cycle proteins for destruction.

- A highly destabilizing N-terminal residue such as Arg and Leu favours **rapid ubiquitination**, the ubiquitin-tagged proteins are degraded in the proteasomes.
- Other, more complex systems?

1 Protein degradation in lysosomes

Lysosomes contain a large number of proteolytic enzymes, **cathepsins**, which are active at pH 5 maintained by the vesicular H⁺ pump (and largely inactive at cytosolic pH values).

Extracellular proteins that enter the cell via endocytosis are hydrolysed in the phagolysosomes, which are formed by fusing the invaginated vesicles (simple endosomes or coated vesicles covered with clathrin in receptor-mediated endocytosis) with lysosomes.

Normal **long-lived intracellular proteins** are also degraded nonselectively in the lysosomes (**autophagy** – the digestion of cytoplasmic proteins by the cell's own lysosomes).

The protein degradation in lysosomes is **ATP-independent**. It is probably of less importance than the turnover of specific proteins in the ubiquitin pathway.

2 Cytosolic ubiquitin system (ATP-dependent)

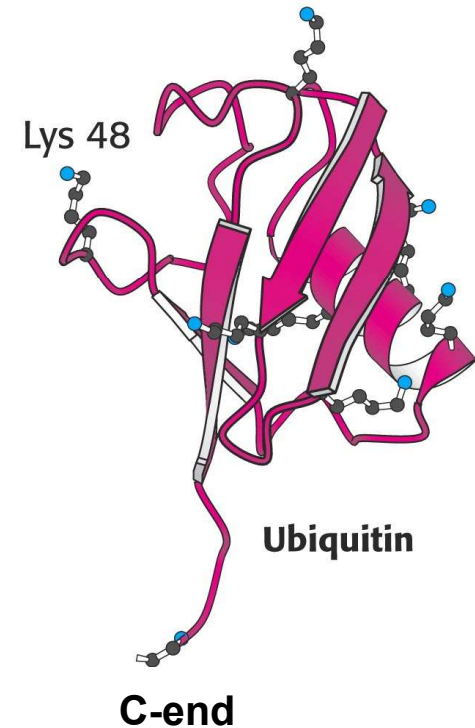
Ubiquitin, a small protein (*Mr* 8500) present in all eukaryotic cells, is the tag that marks proteins for destruction.

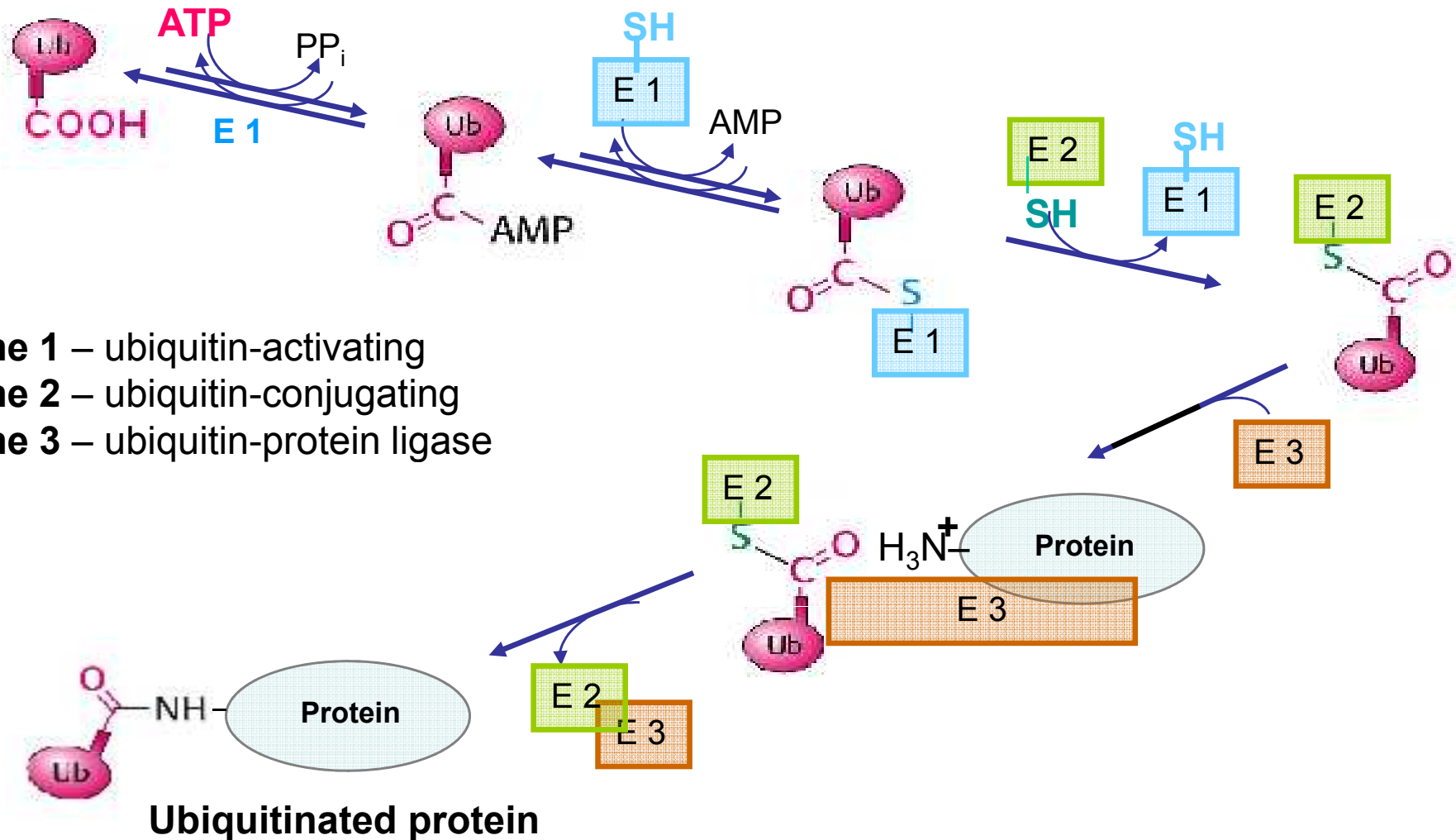
Ubiquitin is the most highly conserved protein known in eukaryotes. It is identical in humans, toad, trout, *Drosophila*. Yeast and human ubiquitin differs at only 3 of 76 amino acid residues.

The C-terminal glycine residue of ubiquitin (Ub) becomes covalently attached to the ϵ -amino groups of several lysine residues (isopeptide bonds) on a protein destined to be degraded.

The energy for the formation of these isopeptide bonds comes from ATP hydrolysis.

Three enzymes participate in the attachment of ubiquitin to each protein:





The ubiquitination reaction is processive – **chains of Ub are generated** by the linkage of the ϵ -amino group of lysine of one ubiquitin molecule to the terminal carboxylate of another. Chains of four or more ubiquitins are particularly effective in signaling degradation.

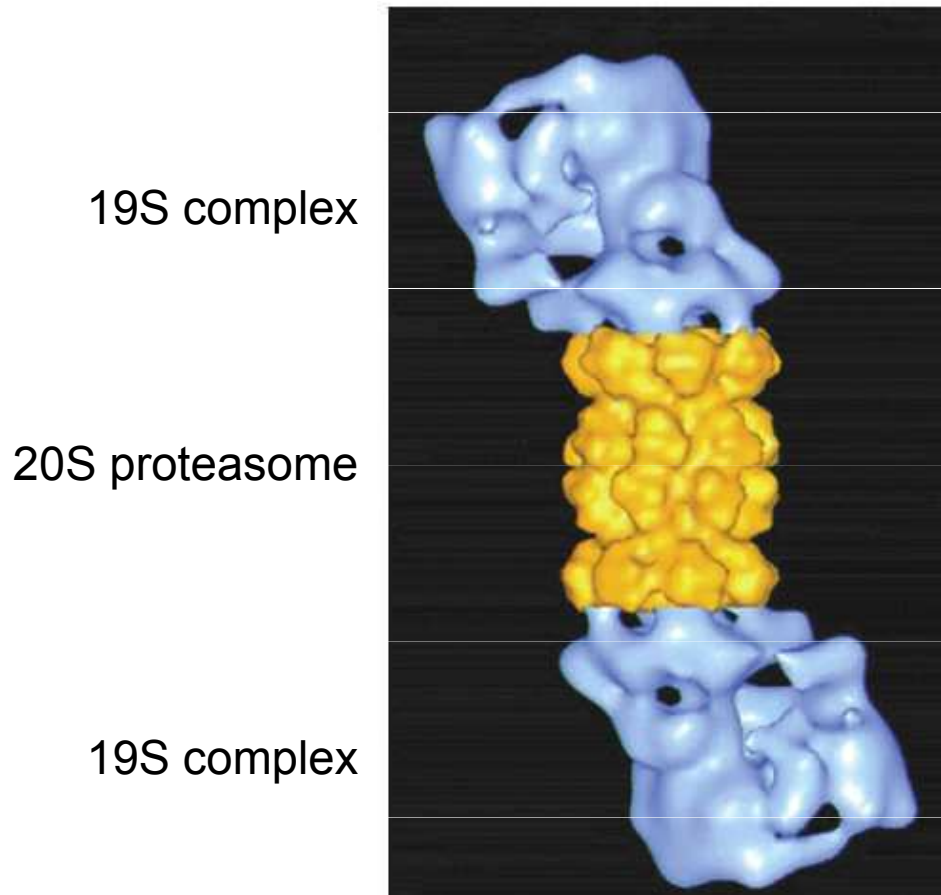
The ubiquitin-tagged protein heads towards proteasome.

The proteasome

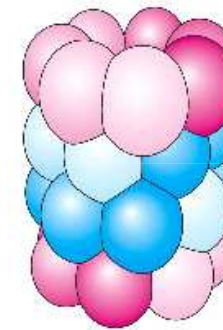
digests the ubiquitin-tagged proteins. This **ATP-driven** multisubunit process spares ubiquitin, which is then recycled.

The **26S proteasome** is a complex of a 20S proteasome (it exhibits at least 5 different proteolytic activities), and two 19S regulatory complexes.

26S proteasome



20S proteasome



The **20S proteasome** complex consists of 28 subunits arranged in four rings of subunits that stack to form a structure resembling a barrel. The active sites of the proteinases are on the interior of the barrel.

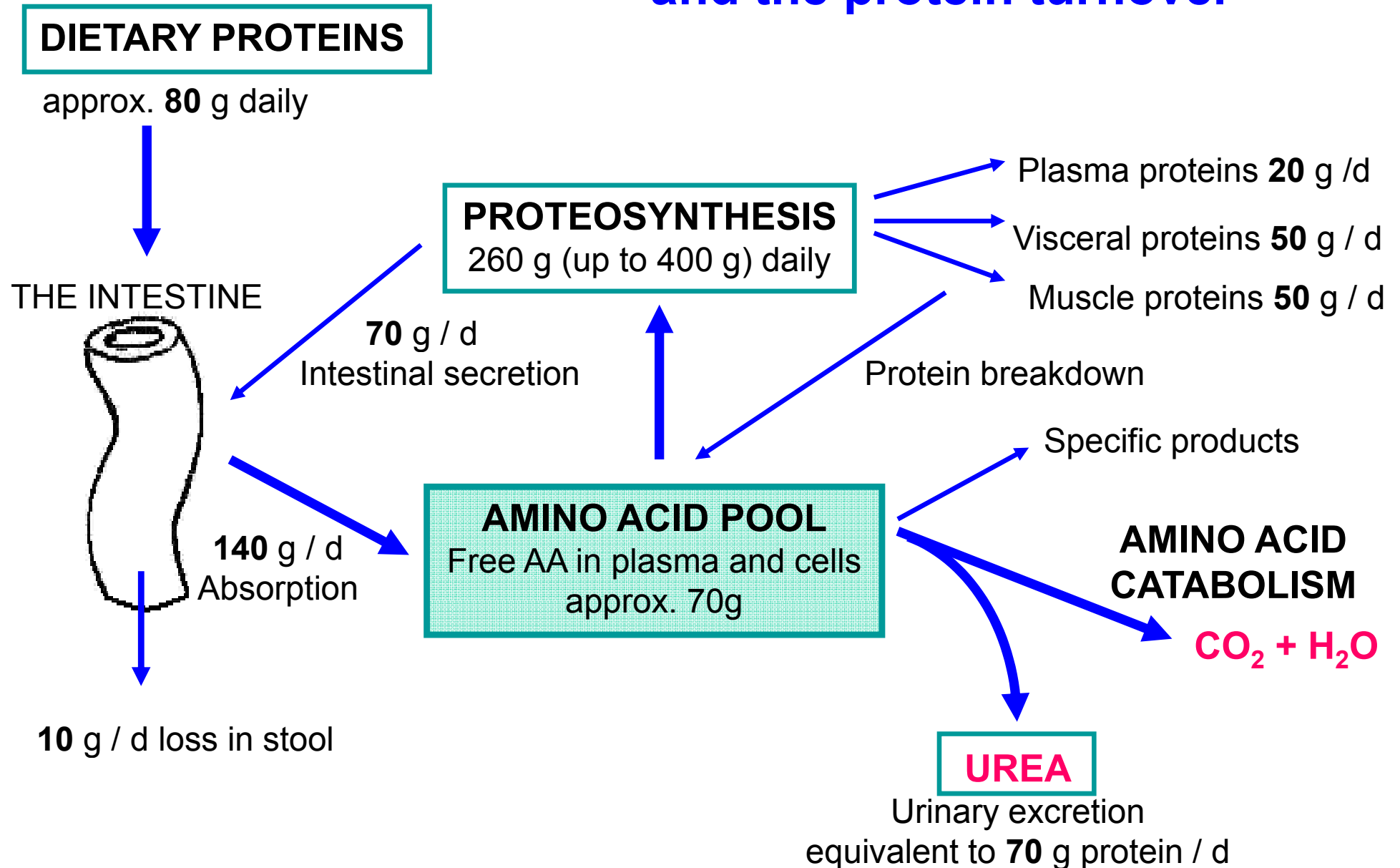
Substrates are degraded until they are reduced to **peptides** in lengths from seven to nine amino acyl residues.

The peptide products are further degraded by other cytoplasmic proteases to yield individual amino acids.

The 20S proteasome is a sealed barrel. Access to its interior is controlled by two **19S regulatory complexes** (each made up of 20 subunits), which bind specifically to polyubiquitin chains and exhibit **ATPase** activity. ATP hydrolysis may assist the 19S complex to unfold the substrate so that it can be passed into the center of the proteasome.

The 19S complex also cleaves off **intact ubiquitin molecules**.

The dynamics of amino acid metabolism and the protein turnover



The minimum protein requirement of adults

is above 0.4 g / kg daily, i.e. 28 g / d for a 70 kg man, supposing that the proteins given will be utilized with maximal efficiency.

The recommended daily protein supply for adults

is about **0.8 g / kg**, i.e. 56 g / d for a 70 kg man.

The protein **requirement of children is larger** than that of adults – 1.2-1.5 g / kg.

The effectiveness of different proteins in the diet

An empirical measure of the efficiency of a protein is the **biological value** – **the mass of body proteins that are synthesized from 100 g of a protein** (expressed as percentage).

It depends primarily on the sufficient content of the **essential amino acids** which the body cannot make itself, and on the digestibility of a protein.

Essential amino acids

Valine	Methionine
Leucine	Phenylalanine
Isoleucine	Tryptophan
Threonine	Histidine (for children)
Lysine	Arginine (for children)

Examples of the biological values of proteins in some foods(%) :

Eggs	97	Oat flakes	62
Milk	90	Wheat	53 (low in lysine)
Beef	77	Beans	46 (low in methionine)
Pork	71	Lentils	45 (low in methionine)
Casein	68	Gelatine	25 (very low in tryptophan)

Examples of protein content of foods (%) :

Cheese	25-30	Legumes	25-30
Curd	30	Yeast	11
Cottage cheese	20	Pasta products	8-10
Meat	20	Rice	7
Eggs	13	Potatoes	2
Cow's milk	3.3	Fruit, vegetables	0.5-2

The amino acid degradation

The first step in degradation of many standard amino acids is the removal of the α -amino group, i.e. **deamination** or **transamination**. The product is mostly the corresponding 2-oxoacid (α -ketoacid) and α -amino group is released as **ammonia** or **ammonium ion**.

Direct deamination of amino acids

Only **few amino acids** are deaminated directly:

Glutamate – **oxidative deamination** catalysed by [glutamate dehydrogenase](#)

Serin and **threonine** – deamination preceded by dehydration
catalysed by [serine dehydratase](#) or [threonine dehydratase](#).

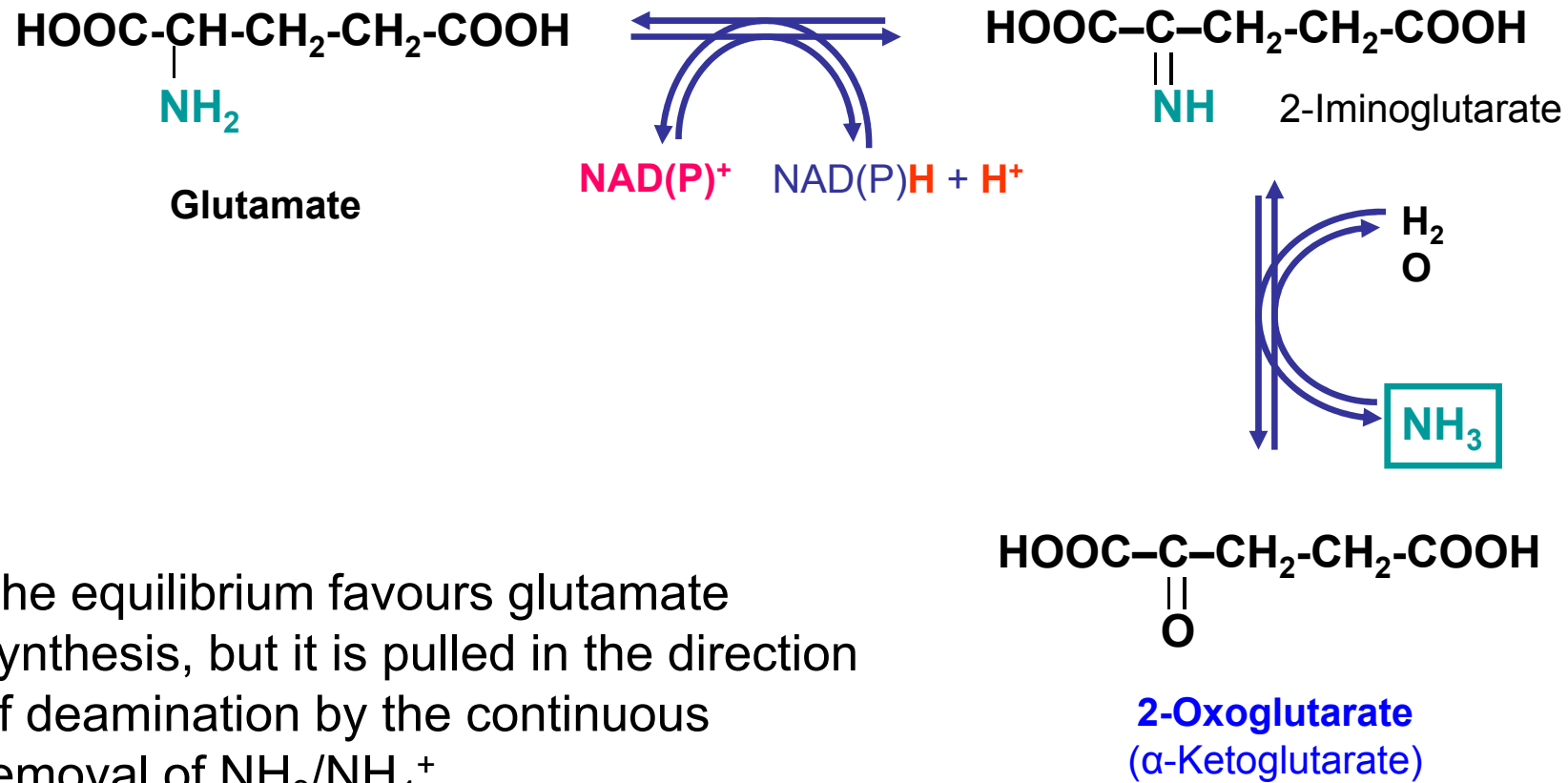
Histidine – deamination by elimination of NH_3 catalysed by [histidine ammonialyase](#).

In peroxisomes, aerobic deamination of various amino acids catalysed by [amino acid oxidase](#) is not very efficient.

Direct deamination is therefore unimportant for most L-amino acids; instead, their amino groups are removed **indirectly**, by **transamination**.

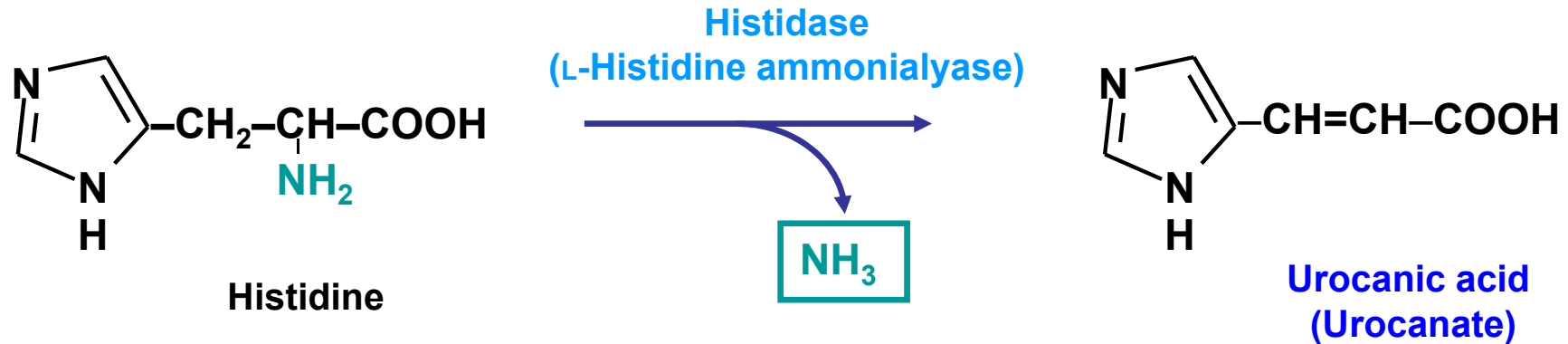
Direct oxidative deamination of glutamate by dehydrogenation

The reaction is catalysed by the mitochondrial enzyme **glutamate dehydrogenase (GLD)**. It requires either NAD^+ or NADP^+ as coenzyme, and its activity in mitochondria is high.



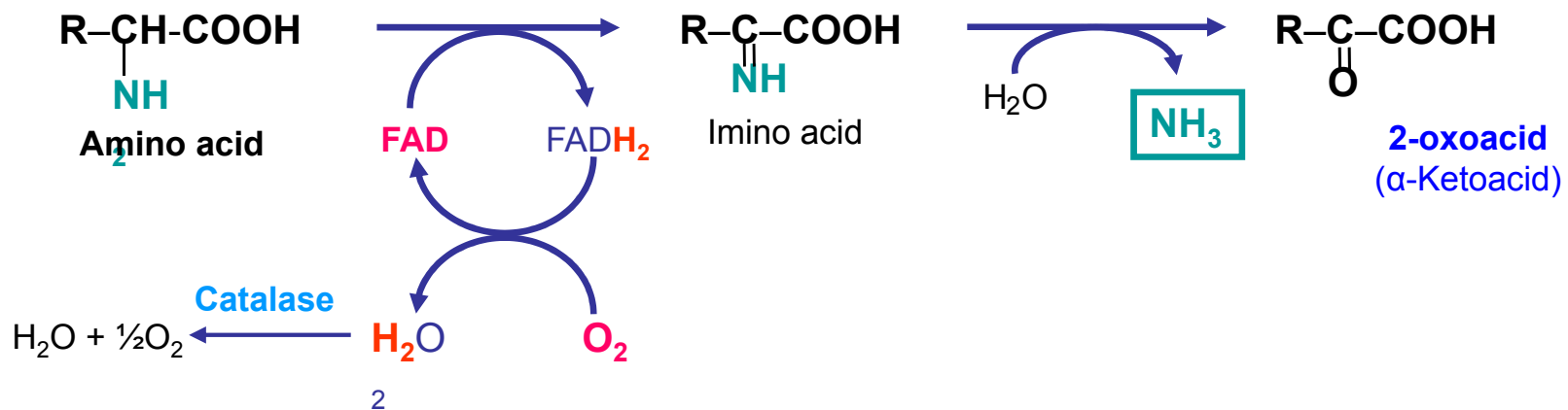
The equilibrium favours glutamate synthesis, but it is pulled in the direction of deamination by the continuous removal of $\text{NH}_3/\text{NH}_4^+$.

Direct deamination of histidine by elimination (desaturation)



Direct oxidative (aerobic) deamination of amino acids in peroxisomes

is catalysed by **L-amino acid oxidase**. In spite of this reaction seems to be similar to that catalysed by glutamate dehydrogenase, the amino acid oxidases are **flavoproteins** which require O_2 for reoxidation of FADH_2 , and they produce **hydrogen peroxide**.



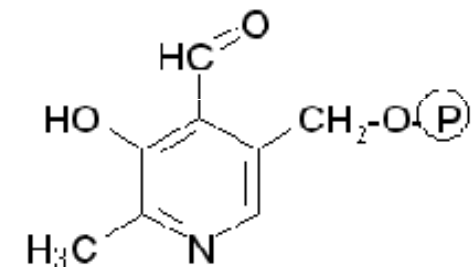
Transamination

The **α -amino group** of many amino acids that cannot be deaminated directly **is transferred to 2-oxoglutarate** (α -ketoglutarate) to form **glutamate**. The product glutamate is oxidatively deaminated by glutamate dehydrogenase to yield ammonium ion NH_4^+ .

These **transaminations are reversible** and can be used to synthesize amino acids from 2-oxoacids and glutamate.

Transaminations are catalysed by **aminotransferases**, the prosthetic group is **pyridoxal phosphate**.

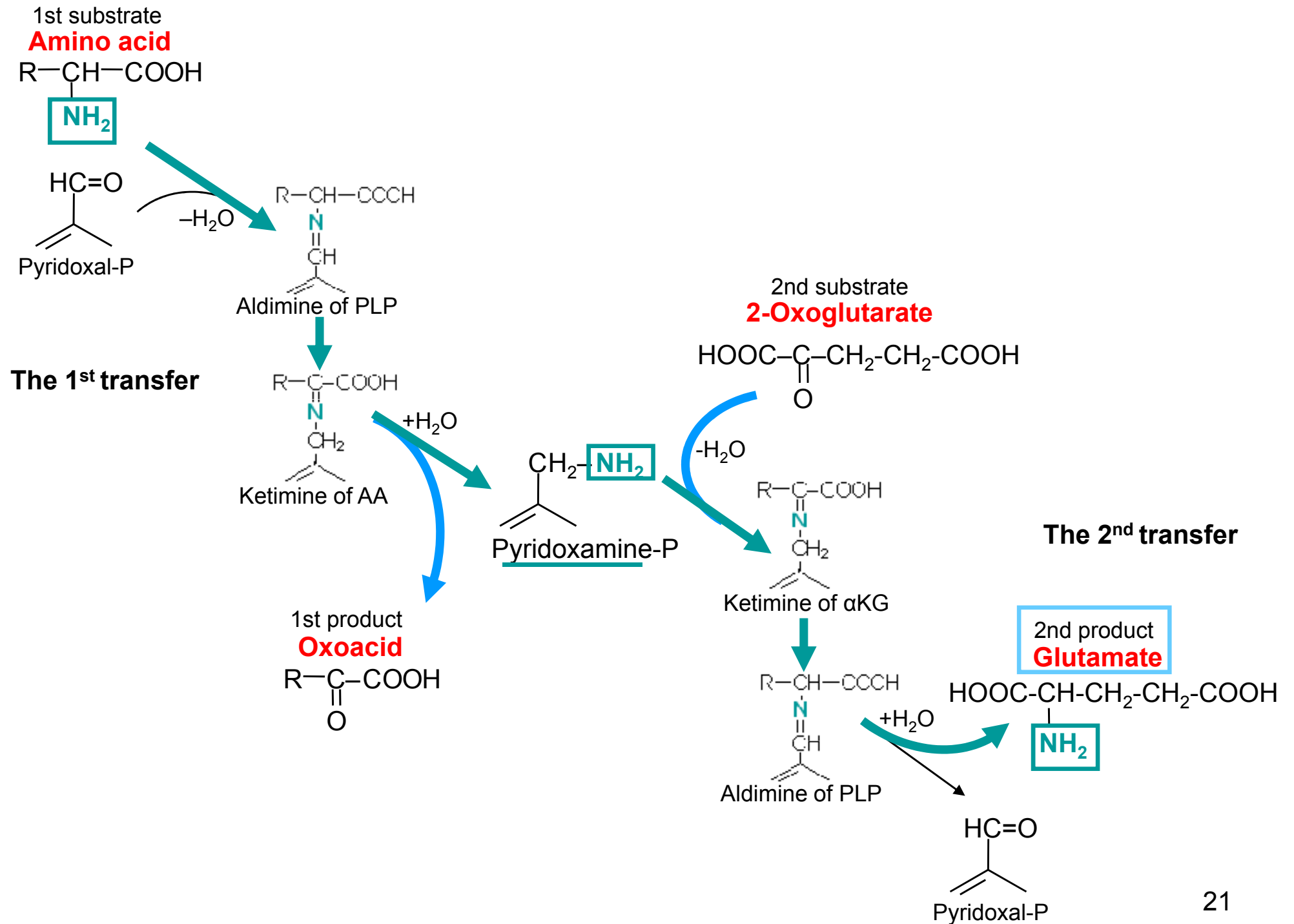
The aldehyde group enables binding to α -amino group of an amino acid so that **aldimine intermediates** (Schiff's bases) are formed.



Pyridoxal phosphate (PLP)

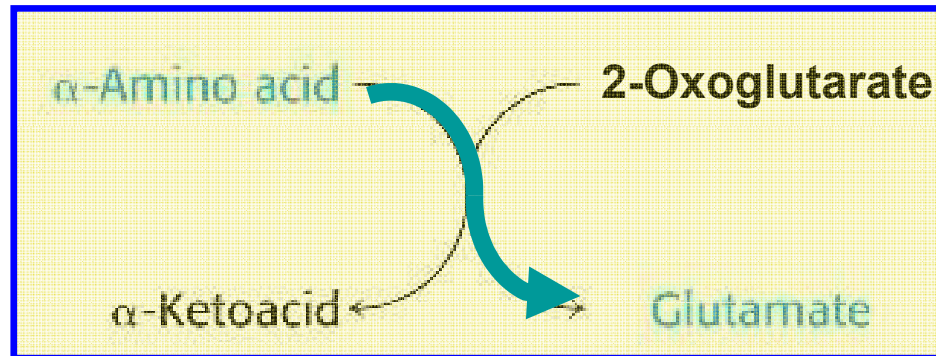
An aminotransferase reaction is shown on the next picture.

Transaminations are reactions with a **"ping-pong" mechanism**: the first product leaves the enzyme before the second substrate binds, in the meantime pyridoxal phosphate binds the transferred amino group (as pyridoxamine phosphate).

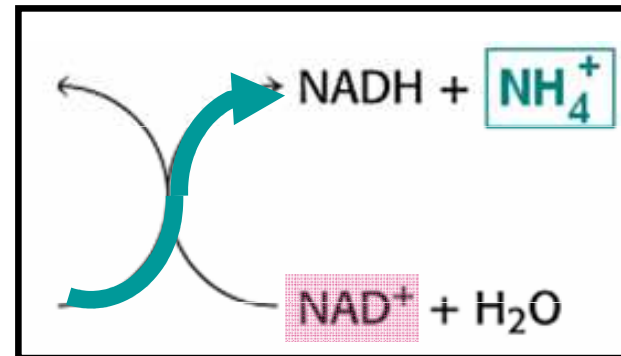


The product of transamination **glutamate is then deaminated directly** by the action of glutamate dehydrogenase (GLD):

Transamination:

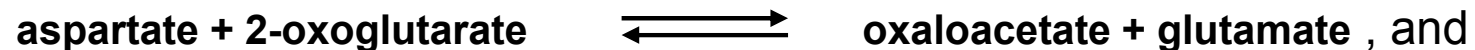


Oxidative deamination of glutamate

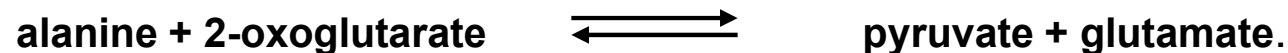


Among other aminotransferases, the two are important (e.g. assays in clinical biochemistry, see Practicals):

aspartate aminotransferase (AST), L-aspartate:2-oxoglutarate aminotransferase), which catalyses the reaction



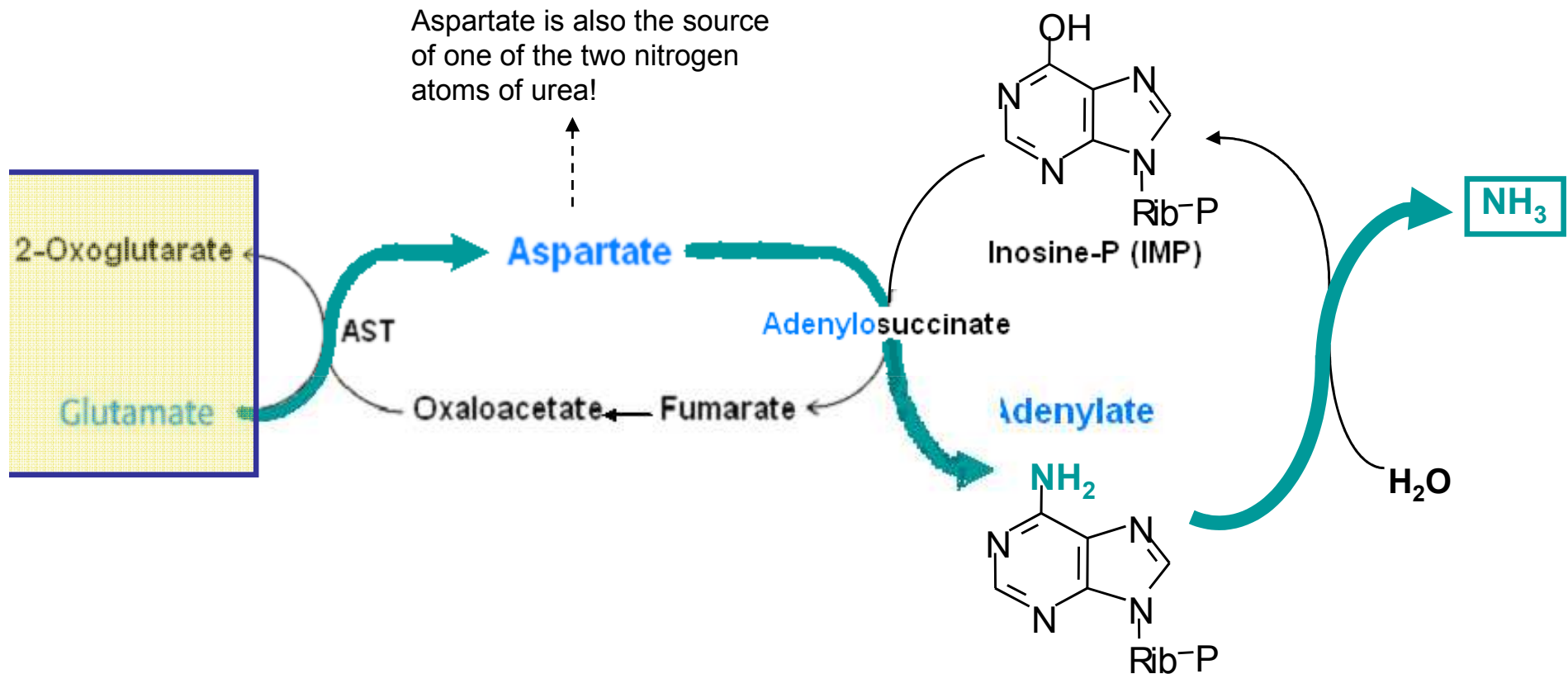
alanine aminotransferase (ALT), L-alanine:2-oxoglutarate aminotransferase), which in the liver catalyses the reaction



Indirect deamination of glutamate – so-called "purine nucleotide cycle"

In some tissues, in skeletal muscle particularly, glutamate is deaminated not directly but undergoes **transamination with oxaloacetate** to give aspartate and 2-oxoglutarate.

The **amino group of aspartate** is then transferred to hypoxanthine (the constituent of IMP, inosine-P), and the product (adenosine-P, AMP) **adenylate is deaminated** by **adenylate deaminase**.



In eight proteinogenic amino acids, transamination or oxidative deamination is not the usual first step in their degradation:

Serine and **threonine** are deaminated by the action of serine dehydratase, **histidine** undergoes deamination by desaturation – both reactions were already mentioned previously.

In five remaining amino acids, only some of their catabolites is deaminated.

Arginine – deamination occurs after transformation to ornithin,

lysine – after transformation to α -aminoadipate,

methionine – deamination of homoserine,

proline – deamination after conversion to glutamate,

tryptophan – after its transformation to kynurenine, alanine is released.

From the quantitative point of view, the most important reactions which produce ammonium ion NH_4^+ (and molecules NH_3) from the α -amino groups of amino acids **within the cells are:**

- 1 Direct deamination of glutamate (glutamate dehydrogenase reaction).
- 2 Deamination of aspartate within the "purine nucleotide cycle".
- 3 Aerobic glycine-cleavage system.
- 4 Direct deamination of histidine by desaturation.

Transport of "ammonia" in the blood

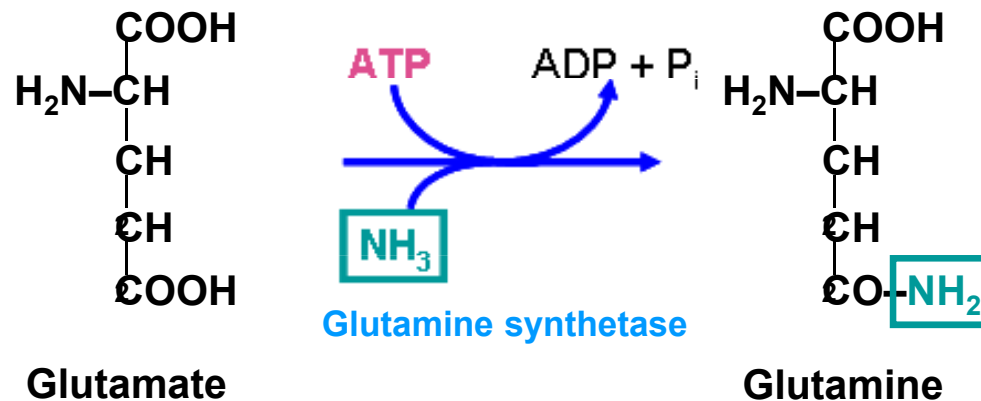
Ammonia is a weak base, molecules NH_3 are mostly protonated to ammonium ion NH_4^+ at physiological pH values. Therefore, we mean both NH_4^+ and NH_3 when speaking of ammonia.

Ammonia is toxic, particularly to the brain. Regardless of the intensive amino acid turnover in the body and high normal concentrations of ammonia (about $500 \mu\text{mol} / \text{l}$) in organs other than brain, the concentration of ammonia in the peripheral blood is quite low, up to $40 \mu\text{mol} / \text{l}$.

The major transport form of ammonia between tissues is non-toxic **glutamine** (concentration in blood plasma $400 - 700 \mu\text{mol} / \text{l}$).

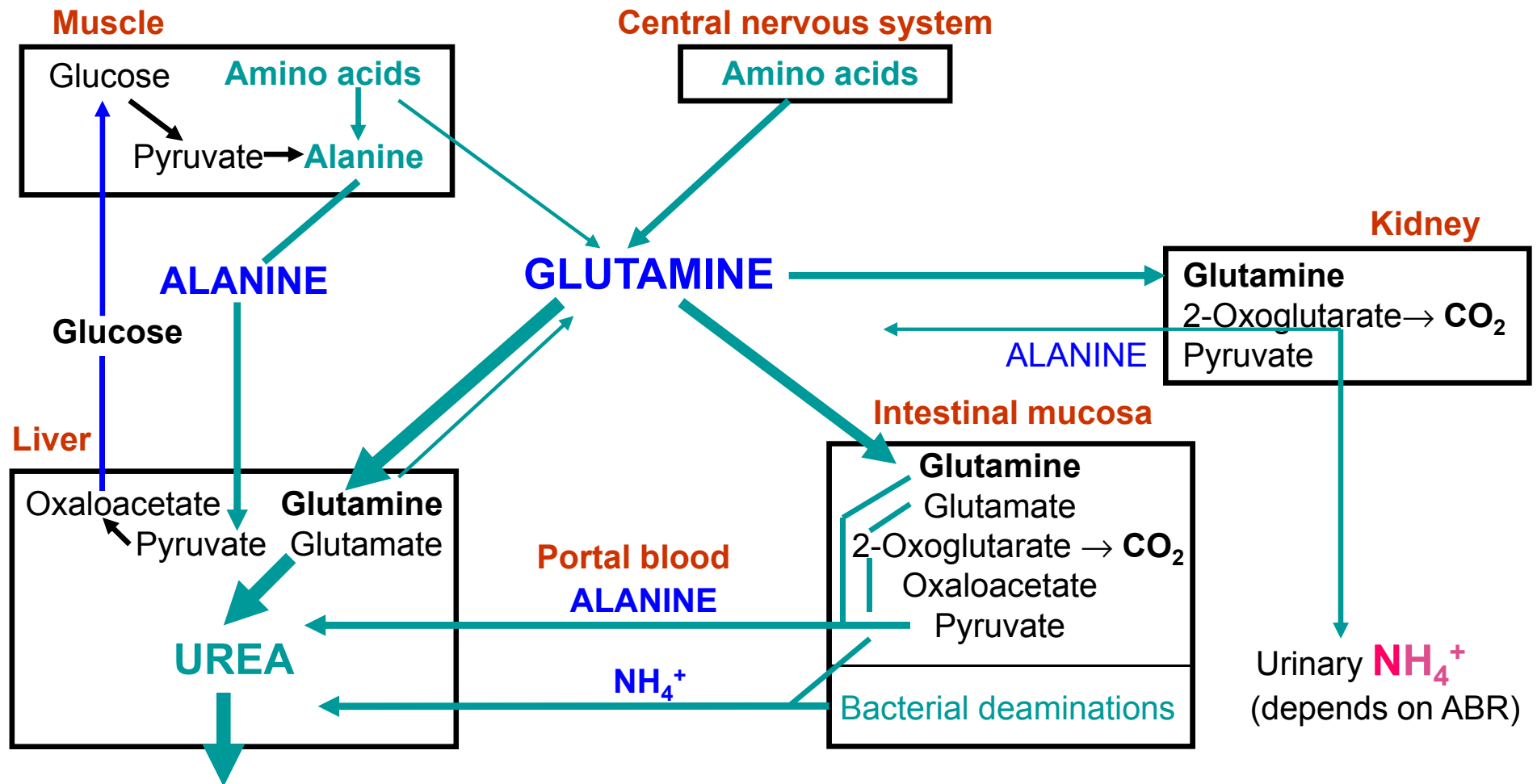
Synthesis of glutamine

The hydrolysis of glutamine to glutamate is catalysed by **glutaminase**.



An additional form of amino groups transfer from peripheral tissues, above all skeletal muscles, to the liver is **alanine** (the glucose-alanine cycle).

Alanine concentration in blood plasma is $300 - 400 \mu\text{mol} / \text{l}$.



The transfer of $-NH_2$ from **muscle** to liver is provided mainly by the **glucose-alanine cycle** which is analogous to the glucose-lactate (Cori) cycle..

Glutamine is a major energy nutrient for **small intestine mucosa**. The nitrogen atoms of $-NH_2$ are transferred to liver as NH_4^+ (its concentration in portal blood is very high) and alanine.

The fate of ammonium ion NH_4^+ in vertebrates

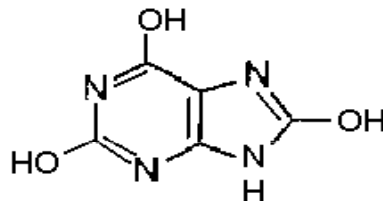
NH_4^+ formed in the breakdown of amino acids

Most aquatic vertebrates
(bony fishes)
The larvae of amphibia
Ammonotelic animals



Birds
Snakes
Lizards
Uricotelic animals

Uric acid

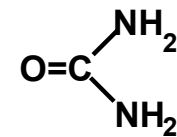


Most terrestrial vertebrates

Sharks
Adult frogs

Ureotelic animals

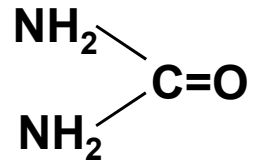
Urea



The ureosynthetic cycle

In most terrestrial vertebrates, the **liver cells detoxify NH_4^+ ions** originating

in the catabolism of amino acids **by the synthesis of urea.**



Urea

Urea (carbamide, carbonic acid diamide) is a **non-electrolyte, highly soluble** in water. It diffuses easily across biological membrane, and therefore it is evenly distributed in all biological fluids and excreted into urine..

The ureosynthesis is one of the unique functions of the liver cells. These cells take up glutamine and ammonium ions from the blood, they can also deaminate glutamate or other amino acids.

The carbon atom of urea is derived from hydrogencarbonate anion, one of the nitrogens comes from ammonium ion, the other is obtained from aspartate.

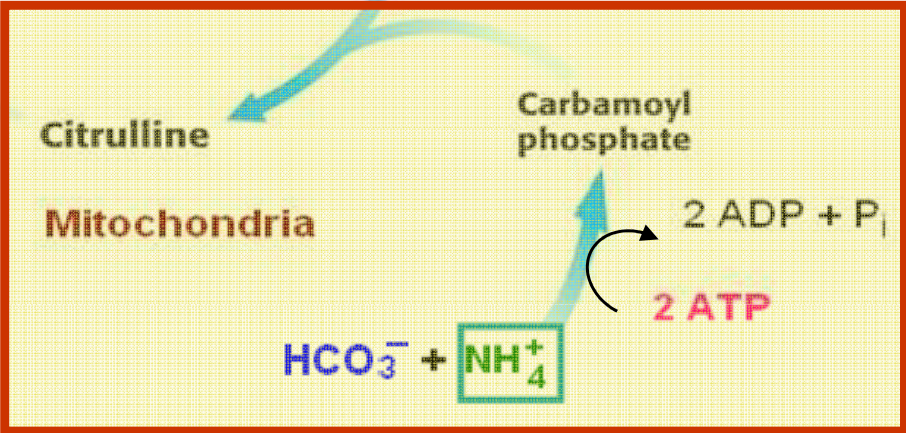
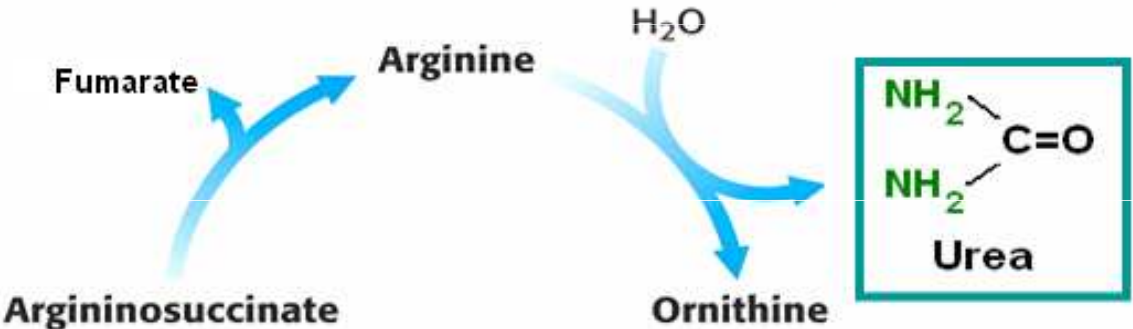
Ureosynthesis is an **endergonic process** – 3 ATPs are hydrolysed to 2 ADPs, 1 AMP and 4 P_i in the synthesis of one molecule of urea.

It is recommendable to remember, from the physiological standpoint, that the ureosynthesis is a proton-producing process which consumes hydrogencarbonate, a buffer base.

The simplified pathway of ureosynthesis

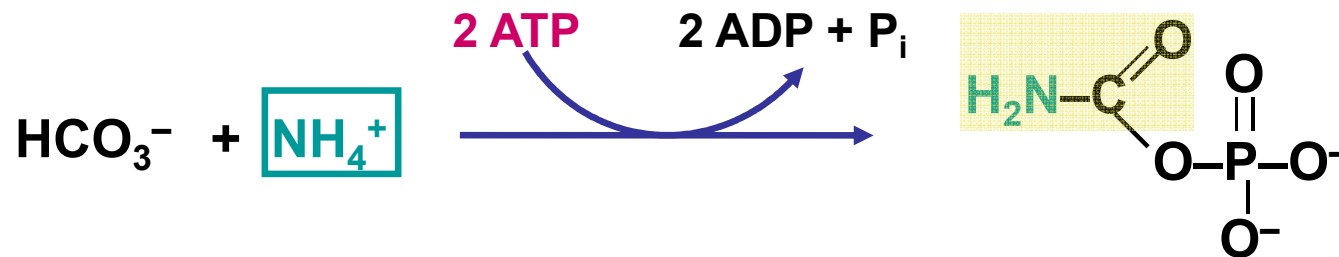
(the ornithine or Krebs-Henseleit cycle)

LIVER CELLS
CYTOSOL



Urea is synthesized in five steps:

1 Synthesis of carbamoyl phosphate in the mitochondrial matrix

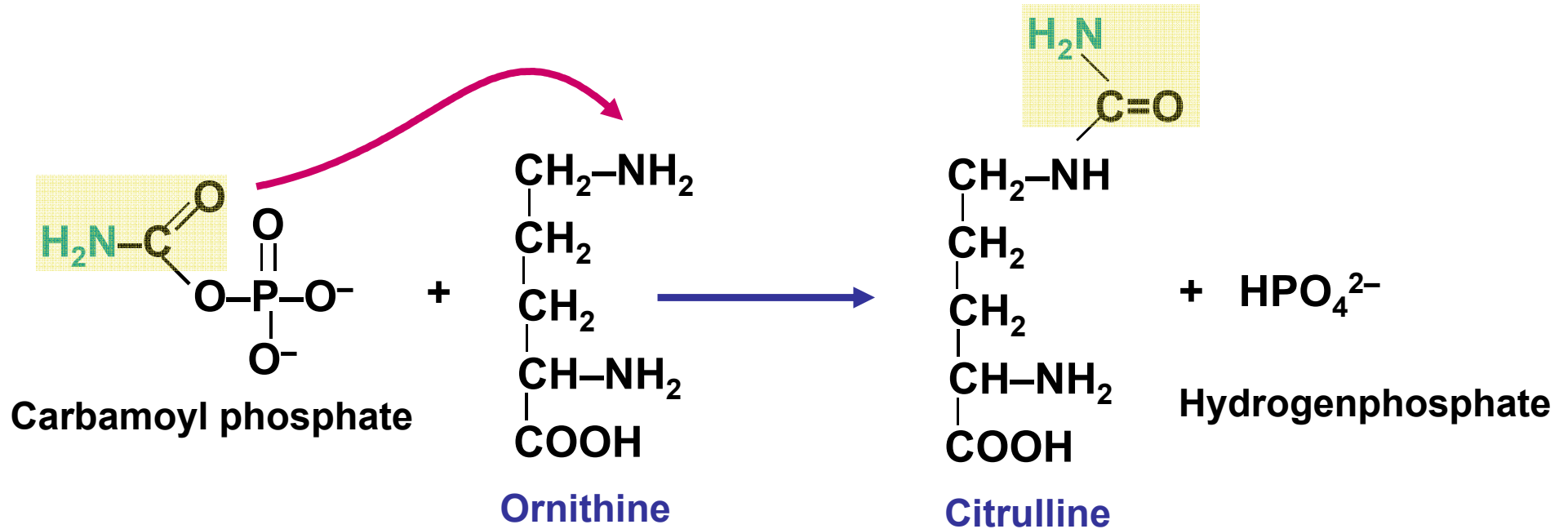


Carbamoyl phosphate
(a mixed anhydride,
a high-energy compound)

The reaction is catalysed by **carbamoyl phosphate synthetase I** which requires essentially the allosteric activator – **N-acetylglutamate**.

The cytosolic form of the enzyme – carbamoyl phosphate synthetase II – utilizes glutamine instead of ammonia and catalyses the first step of the pyrimidine base synthesis.

2 The transfer of carbamoyl to ornithine

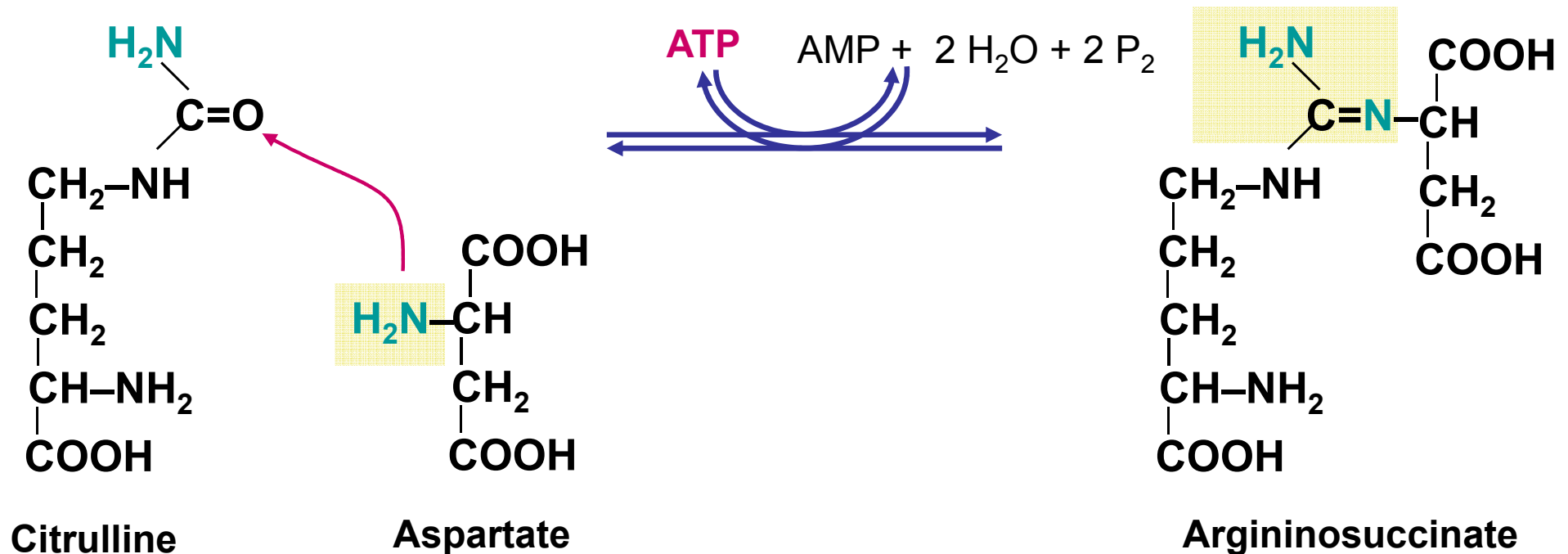


The reaction is catalysed by **ornithine transcarbamoylase** to form citrulline.

Both ornithine and citrulline are amino acids, but they are not used as building blocks of proteins.

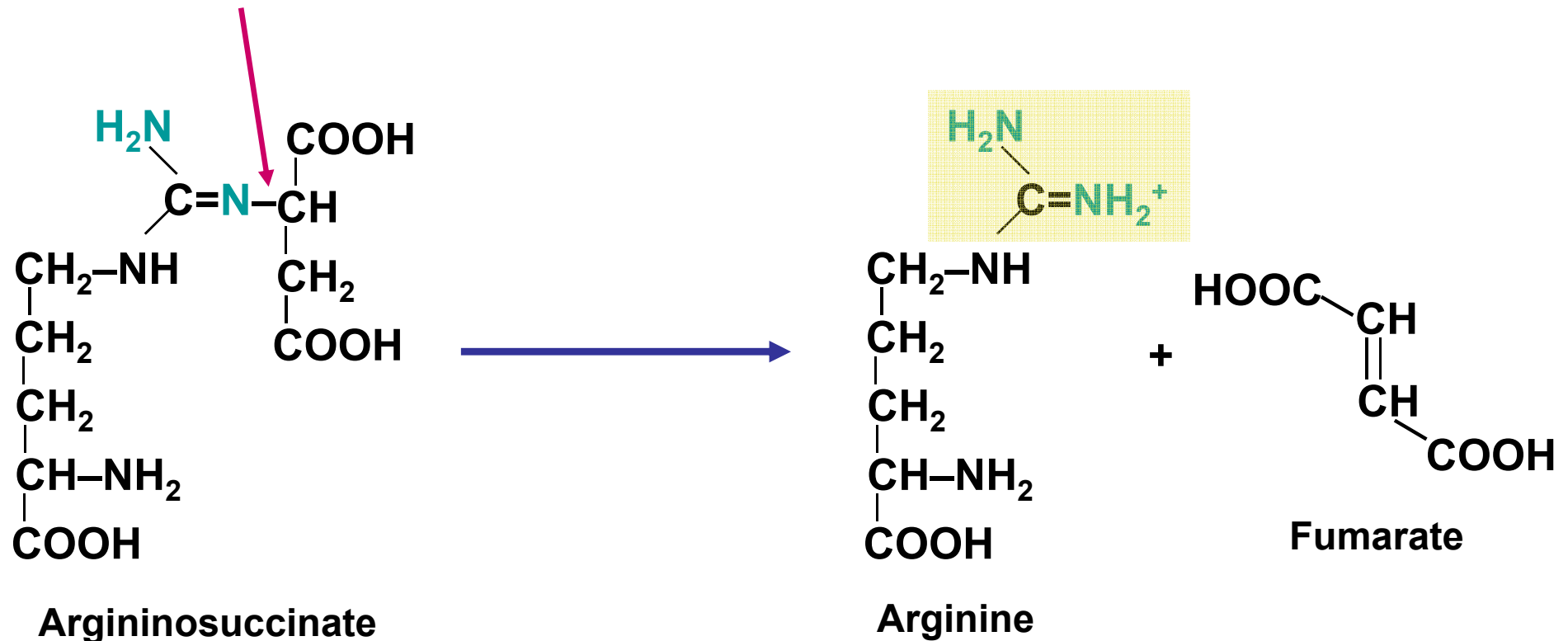
The next three reaction of the cycle take place in the cytoplasm.

3 The transport of citrulline to the cytoplasm and condensation with aspartate



The reversible reaction is catalysed by **argininosuccinate synthetase** and is driven by the cleavage of ATP into AMP and diphosphate and by the subsequent hydrolysis of diphosphate.

4 The cleavage of argininosuccinate into arginine and fumarate



Argininosuccinase cleaves argininosuccinate into arginine and fumarate. The carbon skeleton of aspartate is preserved in the form of fumarate.

The synthesis of fumarate by the urea cycle is important because it **links the urea cycle and the citric acid cycle**: Fumarate after hydration to malate may enter mitochondrion and be oxidized to oxaloacetate, which can undergo transamination to aspartate. .

5 The hydrolysis of arginine generates urea and ornithine

