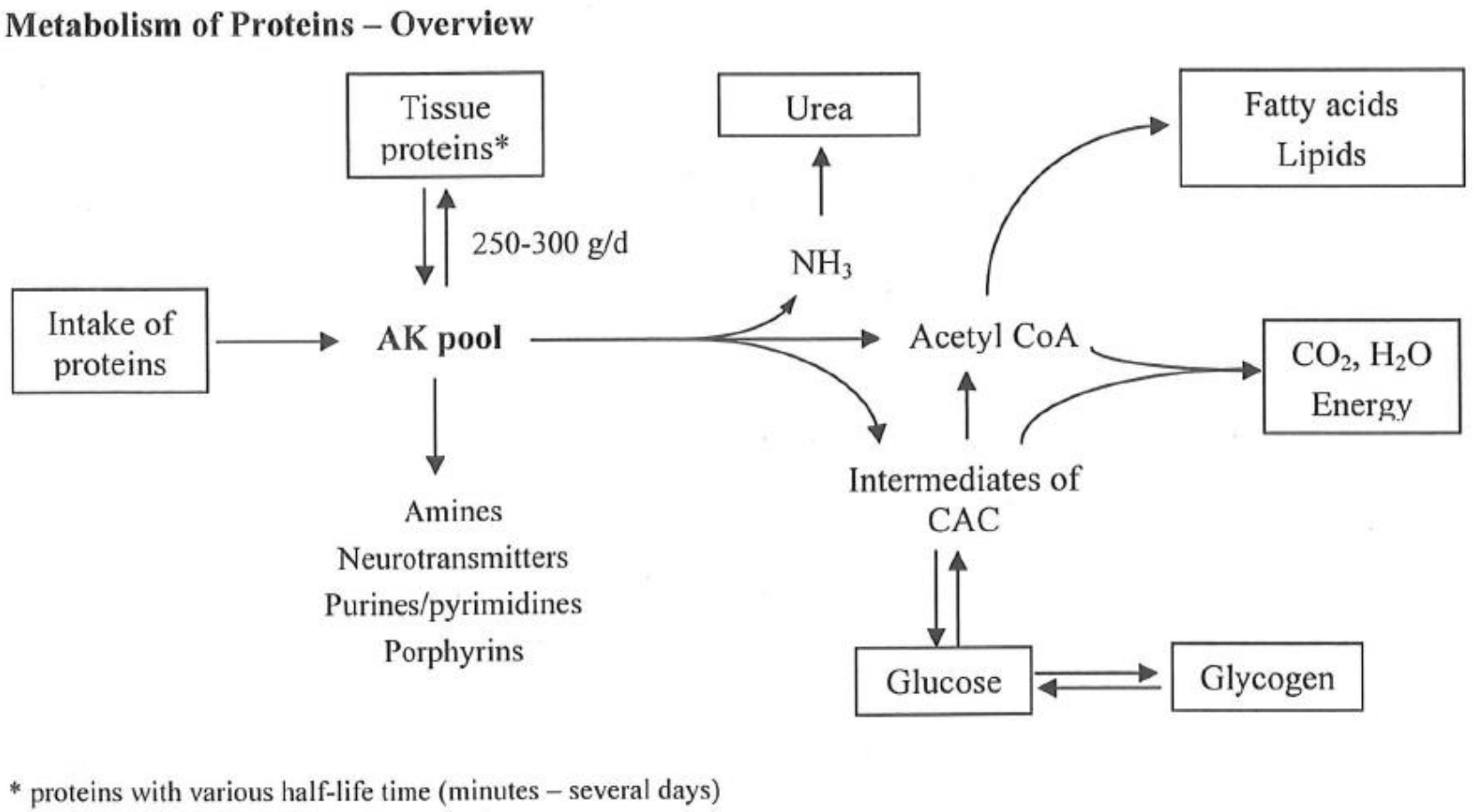
Metabolism of AA

Metabolism of proteins

Sources of AA:
a) **Exogenic proteins- food**
b) **Endogenic proteins**
c) AA biosynthesis of nonesential AA



AA pool

Sources:

1) Proteolysis of exogenic proteins from food
2) Proteolysis of tissue proteins
3) Synthesis of nonesential AA

Using of pool of AA:

1) Synthesis of plasmatic and tissue protiens
2) Synthesis of specific. N compounds
3) Deamination + utilisation of C scelet

Using of C scelet of AA:

1) Gluconeogenesis
2) Synthesis of FA and TAG
3) Metabolic fuel = oxidation in CAC to CO_2 = profit of energy

Degradation of proteins

Exogenic protiens – in lumen GIT, proenzyme
Stomach (pepsin, pepsinogen, activation by HCl
Intestine - **trypsin, chymotripsin, elastase, karboxypeptidase, aminopeptidase ect.**

Endogennic proteins in cells = intercelular degradation of proteins:
a) lysozome , b) ubiqitin-proteasome

Intracellular Degradation of Proteins

a) Lysosomal Protein Turnover

- Proteins degraded: extracellular (accepted by endocytosis),
  membrane bonded,
  intracellular under the stress (autophagy)

b) Ubiquitin – Proteasome Pathway (cytoplasm, nucleus)

- Proteins degraded: damaged or misfolded intracellular proteins
  proteins coded by viruses and other intracellular parasites
  transcription factors
  cyclins and other regulation proteins
  proteins with the short half-life


Proteasome

- Important for cell processes (growth, differentiation, signal transduction, apoptosis).

Degradation of proteins

Exogenic proteins

- Lumen GIT
- stomach – pepsin
- intestine – pankreatic proteasis (trypsin, chymotrypsin ect.)

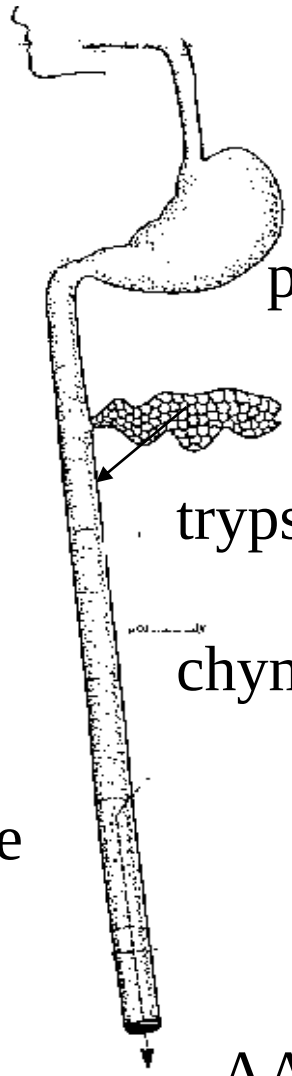
Endogenní proteiny

- Intracelular proteasis
- Two systems:

  1. Lyzosome
  2. Ubiqitin-proteasome

Digestion of exogenic proteins

Enzymes in GIT



pepsinogen (activation by HCl)

pH 1-2

pepsine

trypsine

chymotrypsin, elastasa

karboxypeptidase
aminopeptidase

AA – resorption to portal vein

Endogenic proteins with different biological time life

Protein	Time life
Ornithindekarboxylase	12 min
RNA polymerasa I	1,3 hor
Prealbumin	2 days
Laktátdehydrogenase	4 days
Albumin	19 days
IgG	23 days
Kolagen	years
Elastin	Whole life (?)

Lysozoms

- Degradation of proteins
- Independent on **ATP**
- **Nonspecific**
- **Extracellular**
- **endocellular**

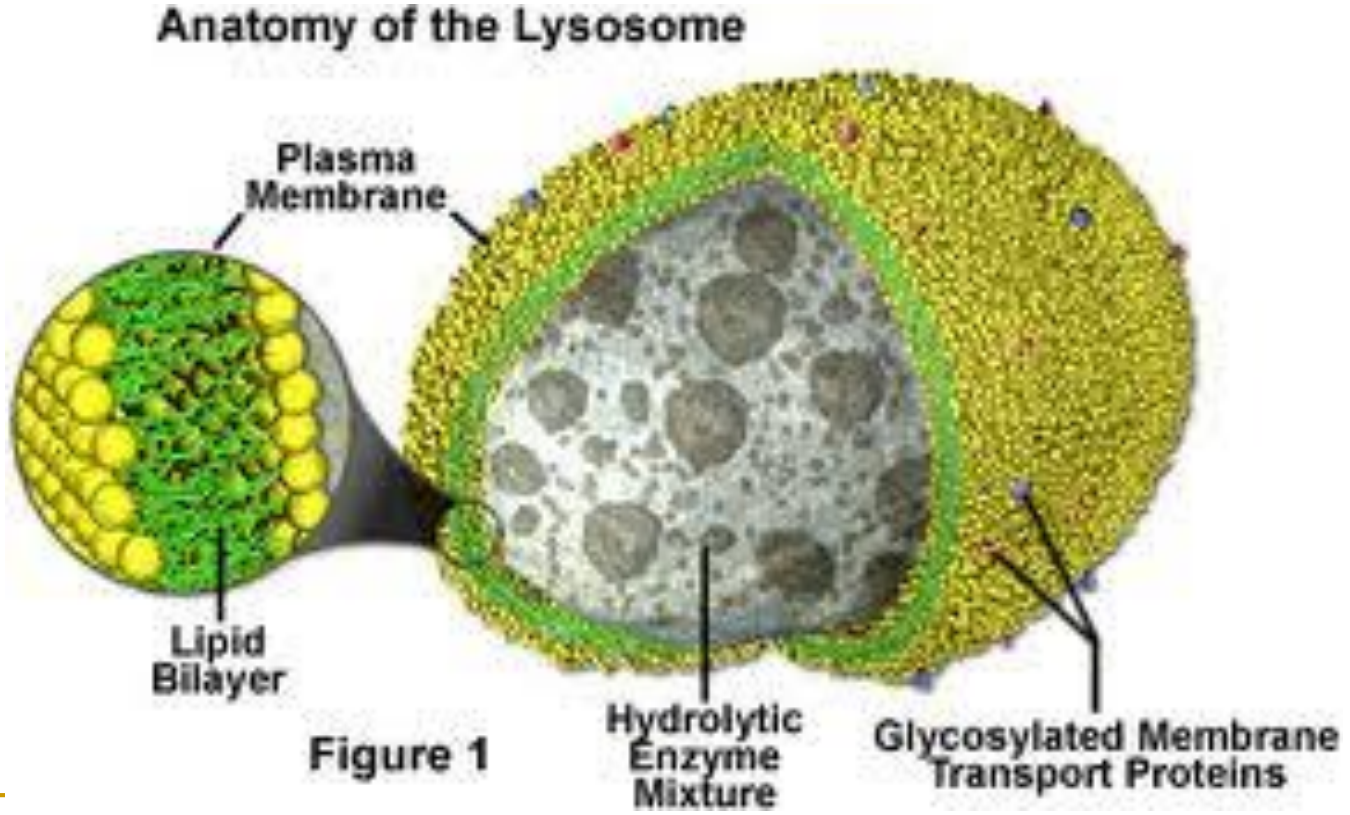


Figure 1

Degradation of endogenic proteins in lysosoms

- Independent on ATP, nonspecific
- Extracelular and membrane proteins
- Proteins with long half-life
- Extracelular glycoproteins (firt digestion of sialic acids)

Lyzosomal hydrolase cleave bond formed by condensation

Hydrolase	Type of bond
Glucosidase	glykosid
Galactosidase	glykosid
Hyaluronidase	glykosid
Arylsulfatase	sulphoester
Lysozym	glykosid
Kathepsin	**peptid**
Kolagenase	**peptid**
Elastase	**peptid**
Ribonuclease	phosphodiester
Lipase	ester
Fosfatase	phosphoester
Ceramidase	Amid

Ubiqitin (UB) –labelled proteins for degradation in proteasome

- Small proteins in al cells

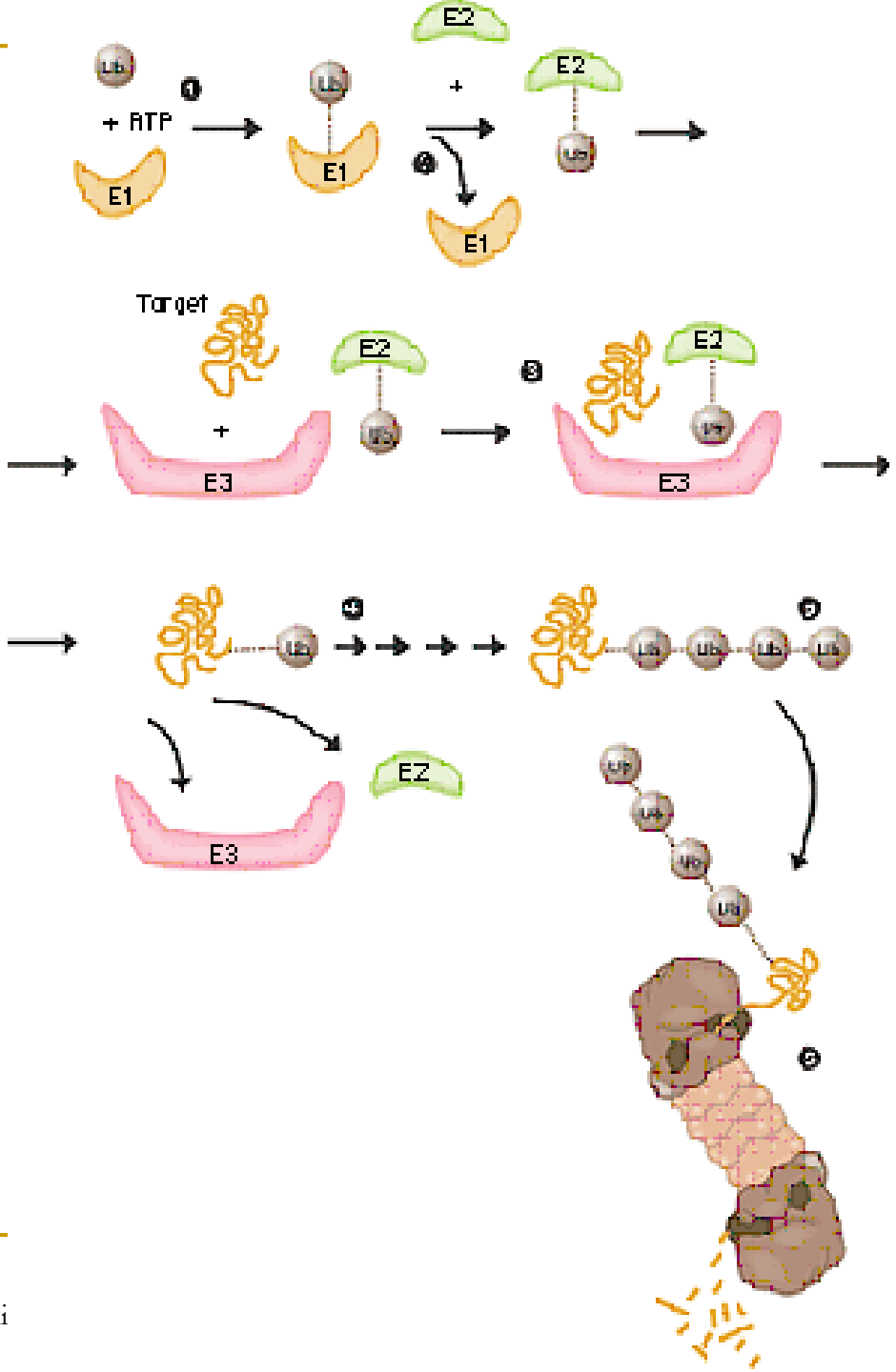
- C-terminus of UB bind to Lys of proteins - „kiss of death“
- Bnding of UB to proteins 3 phases -3 enzymes E_1, E_2 a E_3
- Binding of UB to E_1-SH require **ATP**
- UB - **polyubiquitination**
- Marked protiens is degradated in proteasome

Labelling of proteins by UB

E1 enzyme activated UB by ATP

E2 ubikvitin conjugation enzyme

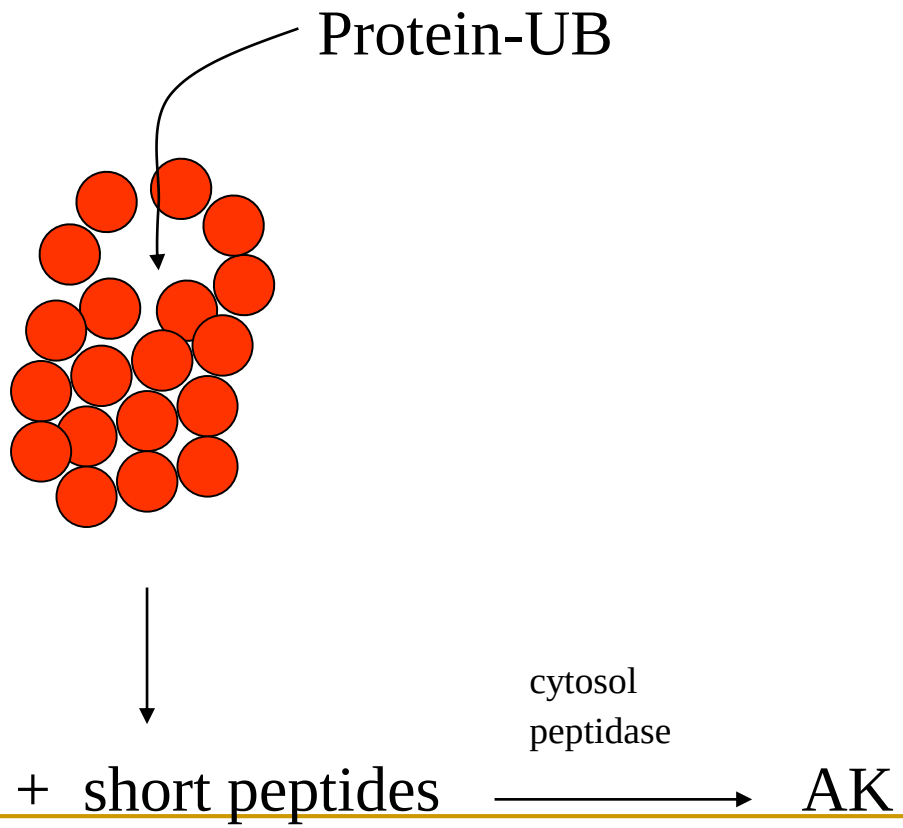
E3 ubiqitin-protein ligase



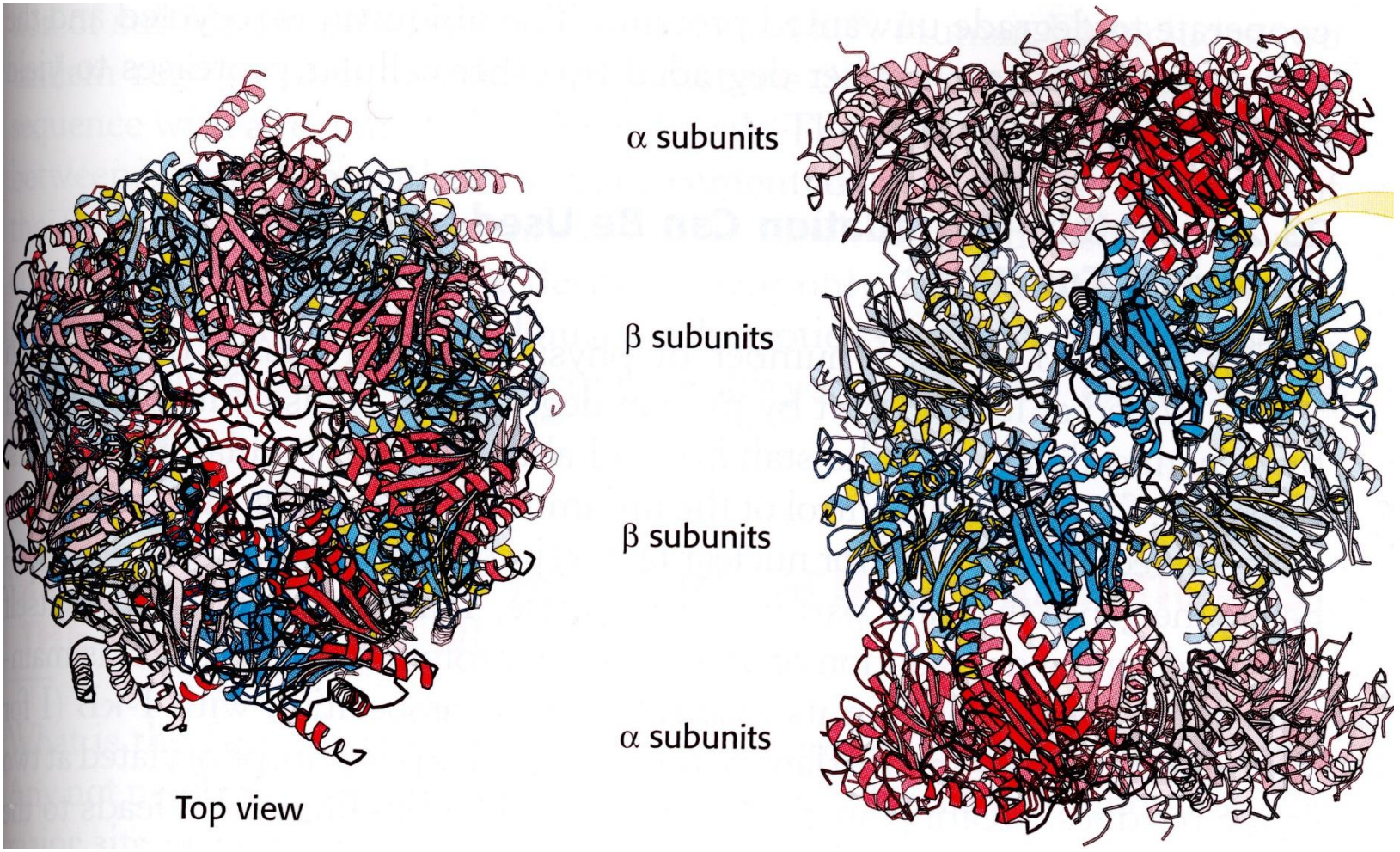
Proteasome

- proteasome most exclusively used in mammals is the cytosolic 26S proteasome, which is about 2000 kilodaltons (kDa) in molecular mass containing one 20S protein subunit and two 19S regulatory cap subunits.
- The core is hollow and provides an enclosed cavity in which proteins are degraded; openings at the two ends of the core allow the target protein to enter.
- Each end of the core particle associates with a 19S regulatory subunit that contains multiple ATPase active sites and ubiquitin binding sites; it is this structure that recognizes polyubiquitinated proteins and transfers them to the catalytic core.

Proteasome- degradation of proteins with short half-life, cyclins and other regulation proteins, transcription factors , damaged and misfolded proteins



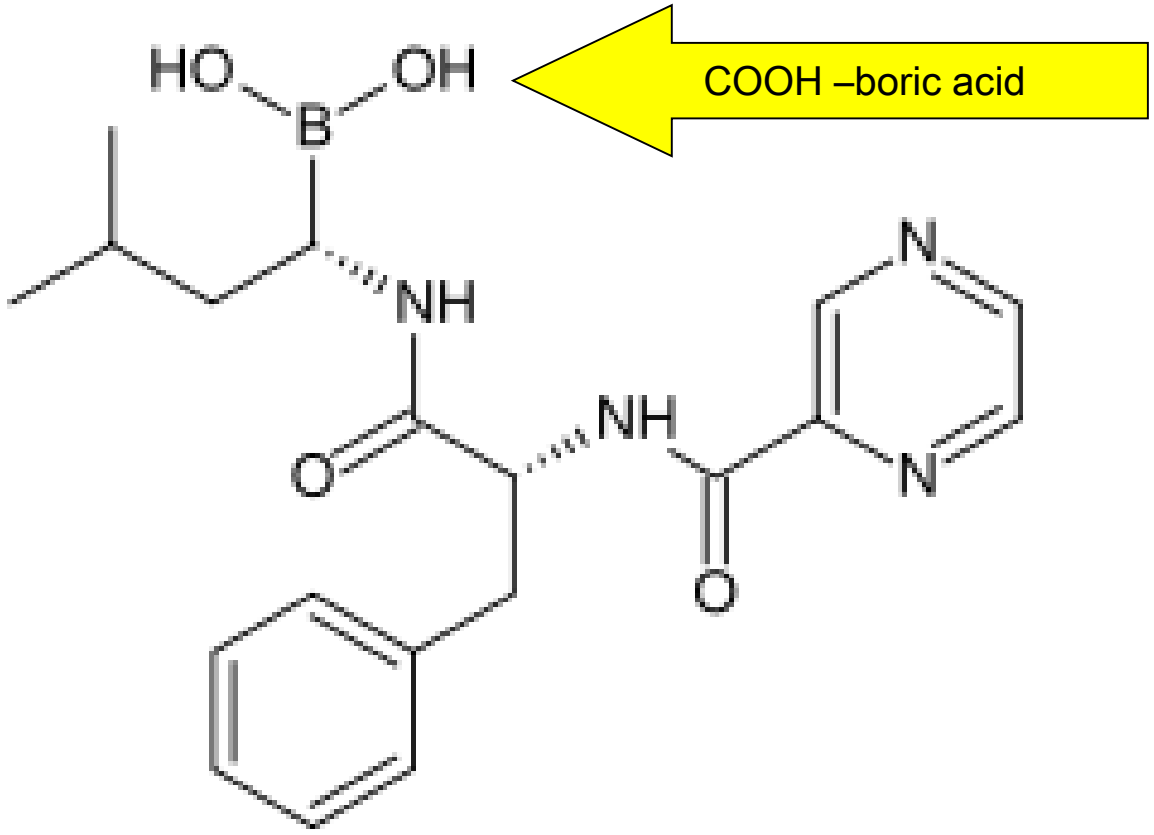
3D structure of proteasome



28 homolog subunits
Active sites - yellow

Bortezomib is inhibitor proteasome - atom bor –catalitic site (Thr)

(myeloma)



Syntetic tripeptid:

Caspase proteolytic cascade

- *c*ysteine-*asp*artic prote*ases* or **c**ysteine-dependent **asp**artate-directed prote**ases** are a family of cysteine proteases that play essential roles in apoptosis (programmed cell death), necrosis, and inflammation.[2]
- Caspases are essential in cells for apoptosis, or programmed cell death, in development and most other stages of adult life, and have been termed "executioner" proteins for their roles in the cell.
- Some caspases are also required in the immune system for the maturation of lymphocytes. Failure of apoptosis is one of the main contributions to tumour development and autoimmune diseases; this, coupled with the unwanted apoptosis that occurs with ischemia or Alzheimer's disease, has stimulated interest in caspases as potential therapeutic targets since they were discovered in the mid-1990s.

Biological value BV

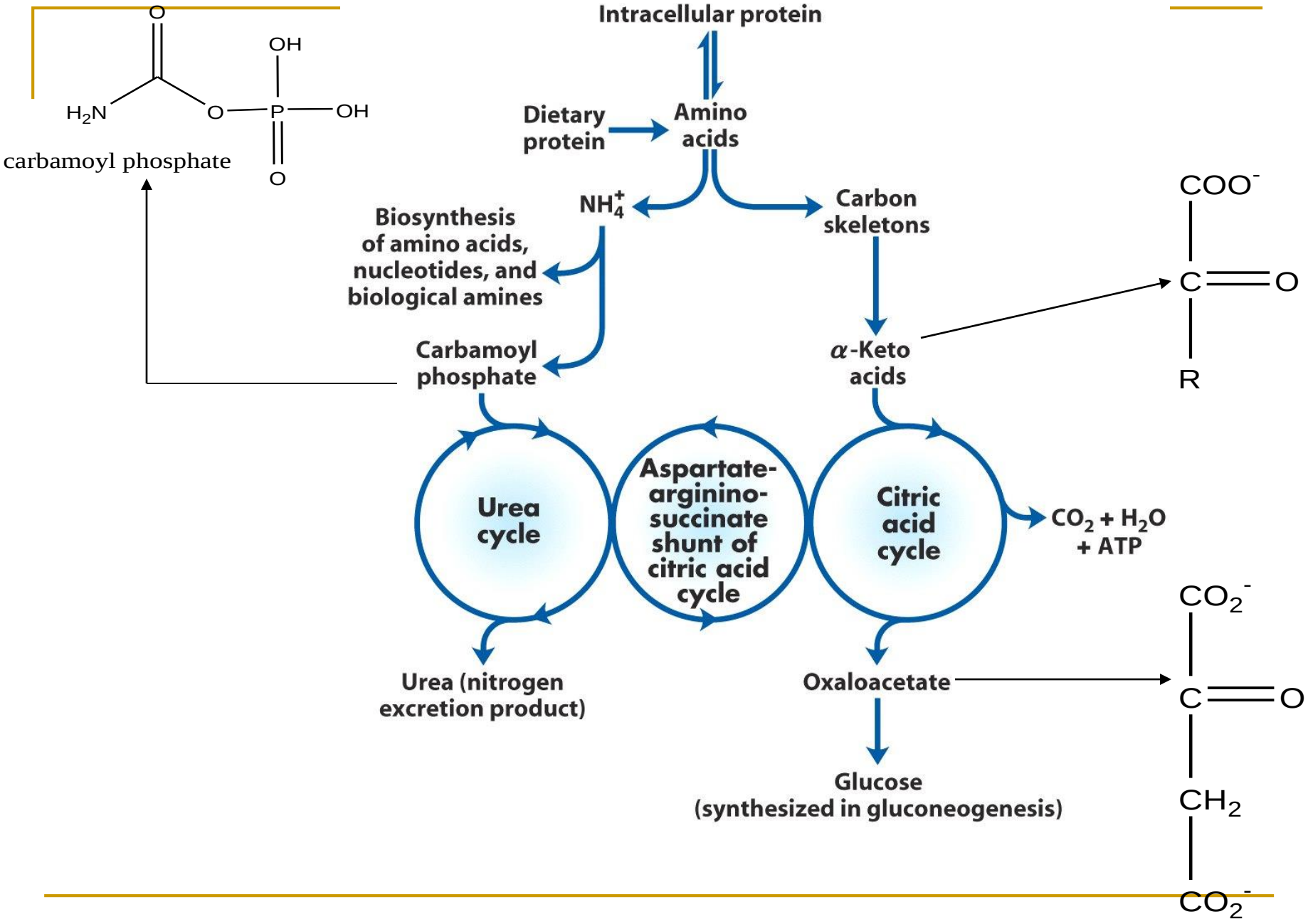
- is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism's body.
- It summarises how readily the broken down protein can be used in protein synthesis in the cells of the organism. Proteins are the major source of nitrogen in food, unlike carbohydrates and fats. This method assumes protein is the only source of nitrogen and measures the proportion of this nitrogen absorbed by the body which is then excreted. The remainder must have been incorporated into the proteins of the organisms body. A ratio of nitrogen incorporated into the body over nitrogen absorbed gives a measure of protein 'usability' - the BV.
- Unlike some measures of protein usability, biological value does not take into account how readily the protein can be digested and absorbed (largely by the small intestine). This is reflected in the experimental methods used to determine BV.
- **BV uses two similar scales:**
- The true percentage utilization (usually shown with a percent symbol).
- The percentage utilization relative to a readily utilizable protein source, often egg (usually shown as unitless).
- These two values will be similar but not identical.
- The BV of a food varies greatly, and depends on a wide variety of factors. In particular the BV value of a food varies depending on its preparation and the recent diet of the organism. This makes reliable determination of BV difficult and of limited use — fasting prior to testing is universally required in order to make the values reliable.
- BV is commonly used in nutrition science in many mammalian organisms, and is a relevant measure in humans.[1] It is a popular guideline in bodybuilding in protein choice.[2][3]

Esencial and semiesencial AA

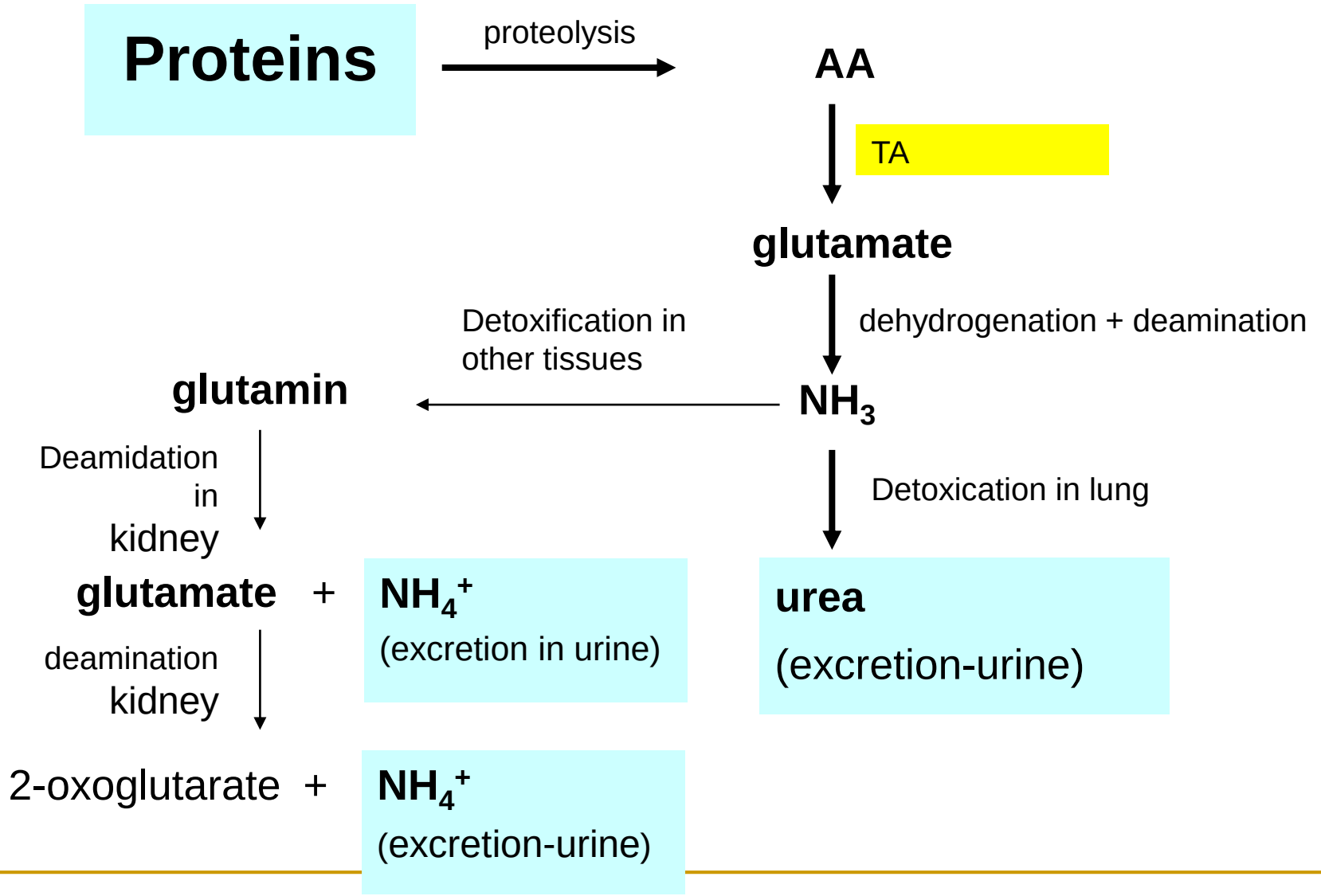
- valin
- leucin
- isoleucin
- threonin
- phenylalanin
- tryptofan
- lysin
- methionin

Semiesencial AA

- histidin, arginin -growth
- alanin, glutamin, taurin - stress
- cca 30 % met -cys
- cca 50 % phe - tyr

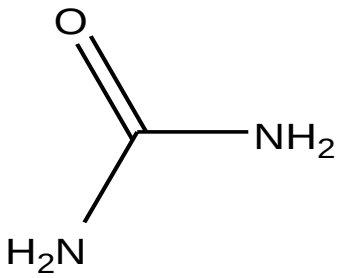

carbamoyl phosphate
Intracellular protein
Dietary protein
Amino acids
NH_4^+
Carbon skeletons
Biosynthesis of amino acids, nucleotides, and biological amines
Carbamoyl phosphate
α-Keto acids
Urea cycle
Aspartate-arginino-succinate shunt of citric acid cycle
Citric acid cycle
$CO_2 + H_2O$ + ATP
Urea (nitrogen excretion product)
Oxaloacetate
Glucose (synthesized in gluconeogenesis)

Catabolic pathway of AA/N



3 Stages of AA Breakdown

1. Deamination: Amino group is converted to ammonia or transferred to form Asp
2. Incorporation of N from Asp and NH_3^+ into urea
3. Conversion of AA carbons into metabolic intermediates



urea

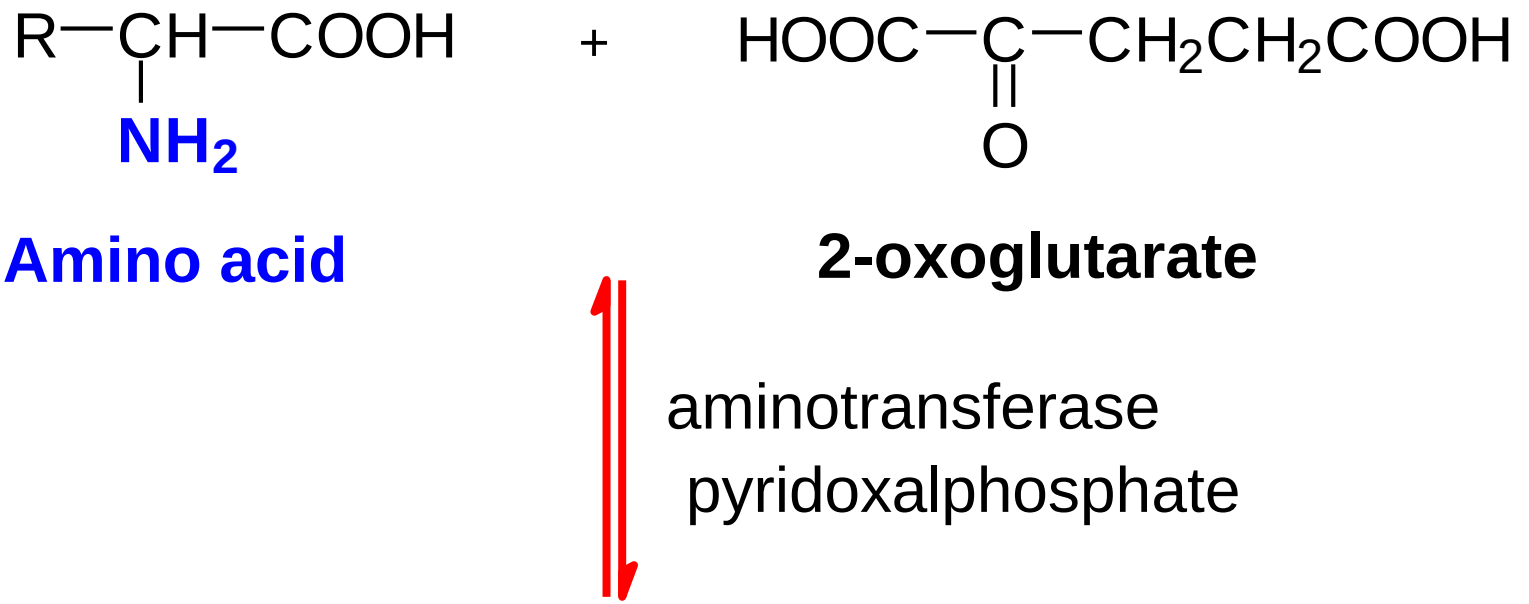
Transamination

transport -NH_2 group from 1 substrate to other

- Most of AA (no Lys, Thr, Pro, His, Trp, Arg, Met)
- Amino group- transport to keto group (2-oxo acid)

  (most 2-oxoglutarate)
- cofactor - **pyridoxalphosphate** – Schiff base
- **reversible reaction** $\Rightarrow$ synthesis of AA

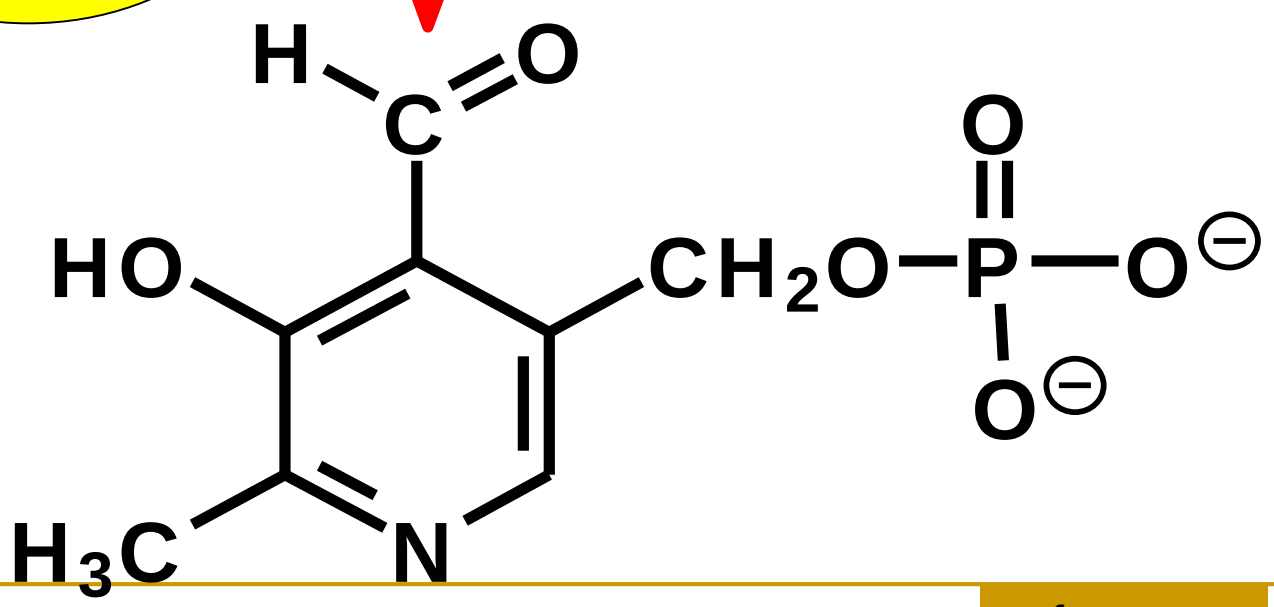
General equation of transamination reaction



Pyridoxalphosphate transfer -NH_2 from AA to 2-oxo glutarate

Enzyme-covalent bond, apoenzyme Lys

Reactive group

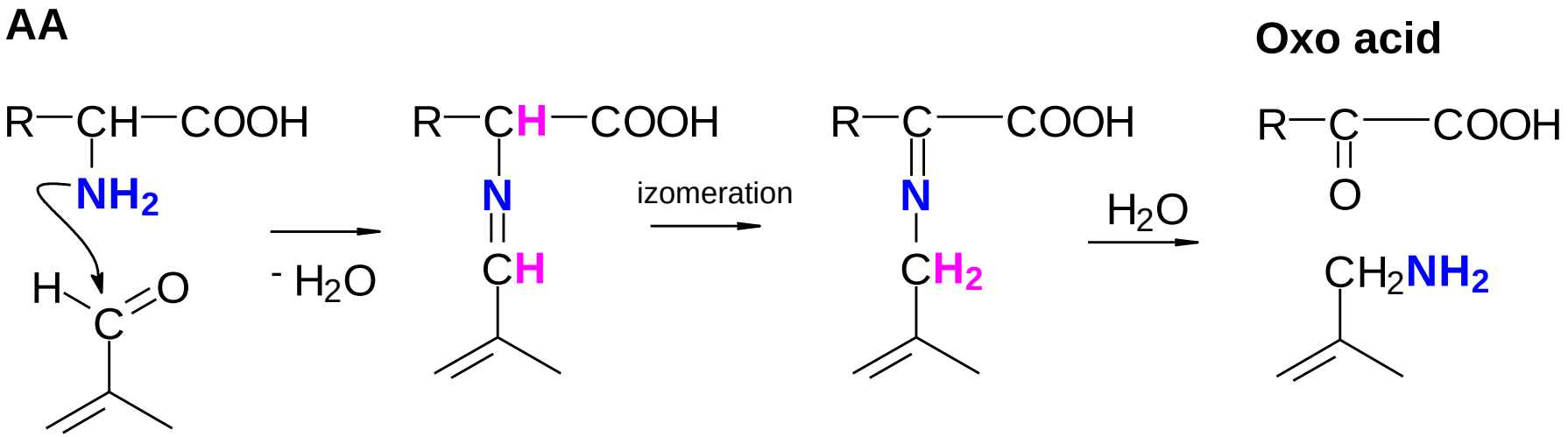


cofactor

1. phase transamination

AA → oxo acid

pyridoxal-P → pyridoxamin-P



pyridoxal-P

Schiff base

aldimin pyridoxal

Imino acid

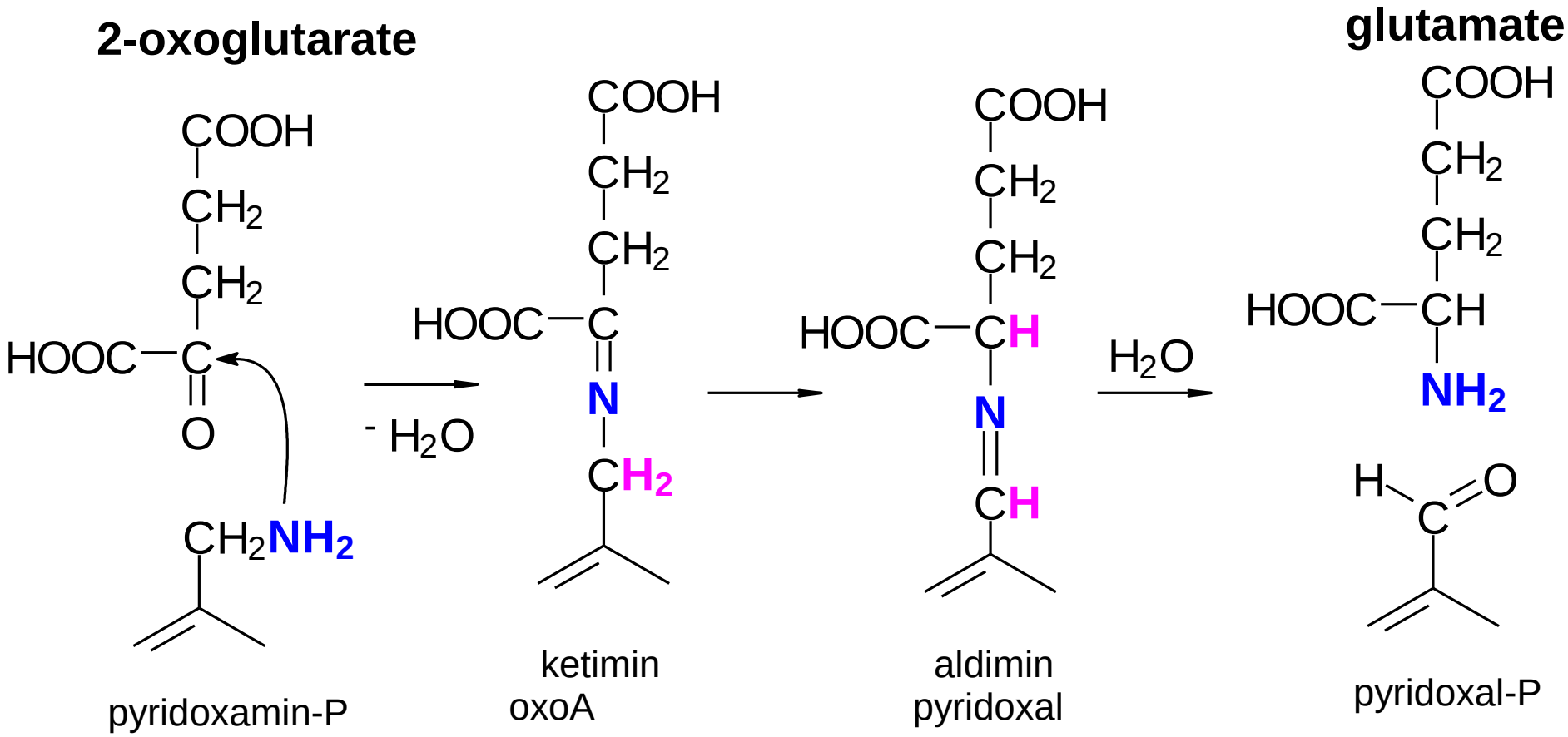
ketimin oxo A

pyridoxamin-P

2. phase transamination

2-oxoglutarate → glutamate

pyridoxamin-P → pyridoxal-P



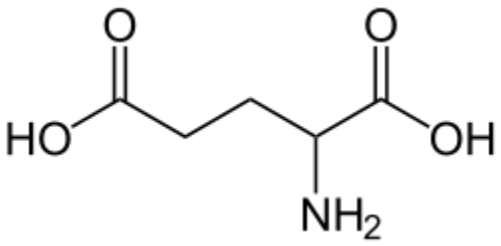
The transaminase enzymes

- are important in the production of various amino acids, and measuring the concentrations of various transaminases in the blood is important in the diagnosing and tracking many diseases. Transaminases require the coenzyme pyridoxal-phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid. Enzyme-bound pyridoxamine in turn reacts with pyruvate, oxaloacetate, or alpha-ketoglutarate, giving alanine, aspartic acid, or glutamic acid, respectively. Many transamination reactions occur in tissues, catalysed by transaminases specific for a particular amino/keto acid pair. The reactions are readily reversible, the direction being determined by which of the reactants are in excess. The specific enzymes are named from one of the reactant pairs, for example; the reaction between glutamic acid and pyruvic acid to make alpha ketoglutaric acid and alanine is called glutamic-pyruvic transaminase or GPT for short.
- Tissue transaminase activities can be investigated by incubating a homogenate with various amino/keto acid pairs. Transamination is demonstrated if the corresponding new amino acid and keto acid are formed, as revealed by paper chromatography. Reversibility is demonstrated by using the complementary keto/amino acid pair as starting reactants. After chromatogram has been taken out of the solvent the chromatogram is then treated with ninhydrin to locate the spots.
- Two important transaminase enzymes are AST (SGOT) and ALT (SGPT), the presence of elevated transaminases can be an indicator of liver damage. This discovery was made by Fernando De Ritis, Mario Coltorti and Giuseppe Giusti in 1955 at the University of Naples.[1][2][3]

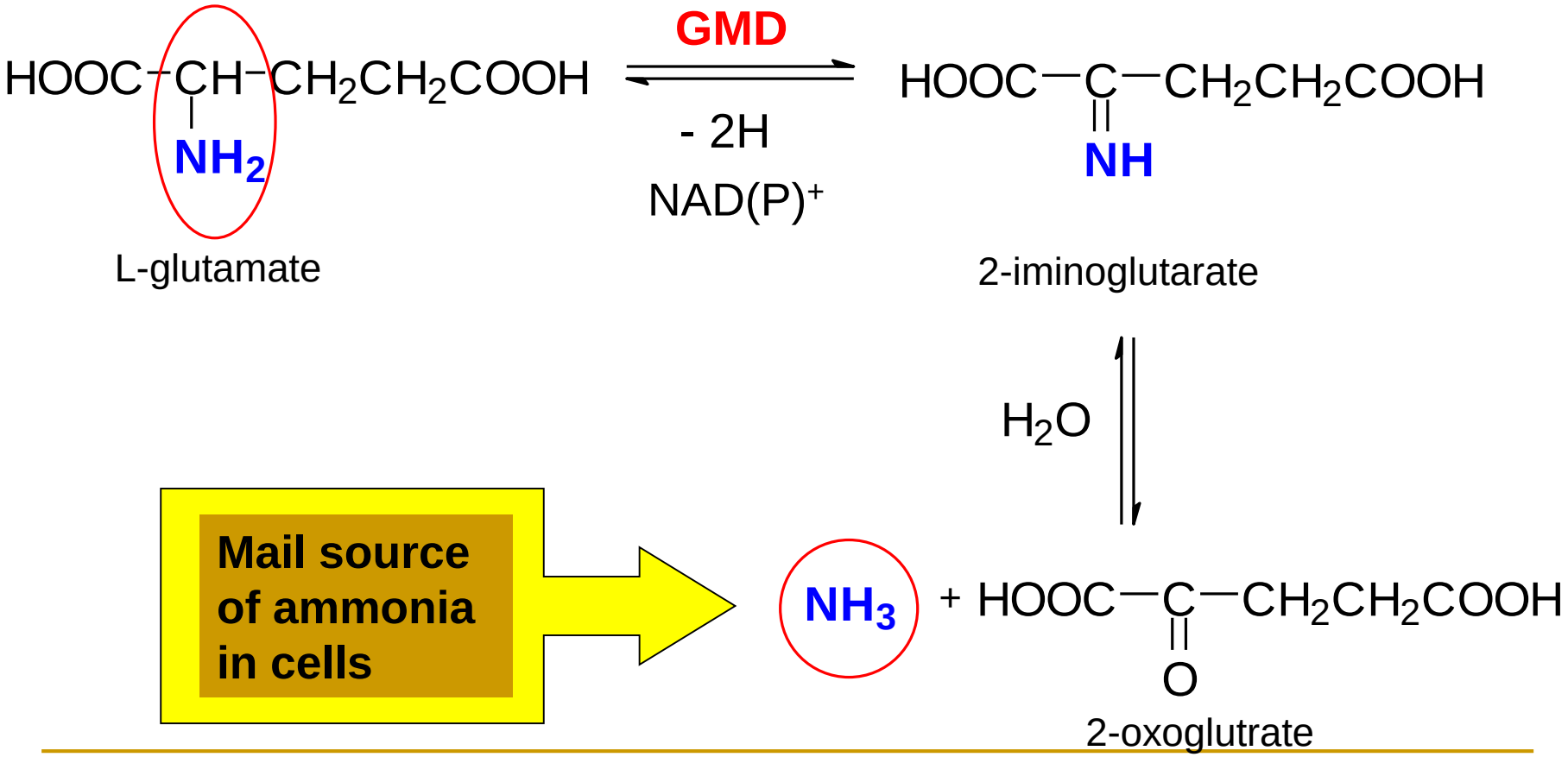
N of the most of AAs –transamination to glutamate

Deamination associated with dehydrogenation of Glutamate

Glutamic acid (abbreviated as **Glu** or **E**) is one of the 20-22 proteinogenic amino acids, and its codons are GAA and GAG. It is a non-essential amino acid. The carboxylate anions and salts of glutamic acid are known as **glutamates**. In neuroscience, glutamate is an important neurotransmitter that plays a key role in long-term potentiation and is important for learning and memory.[4]



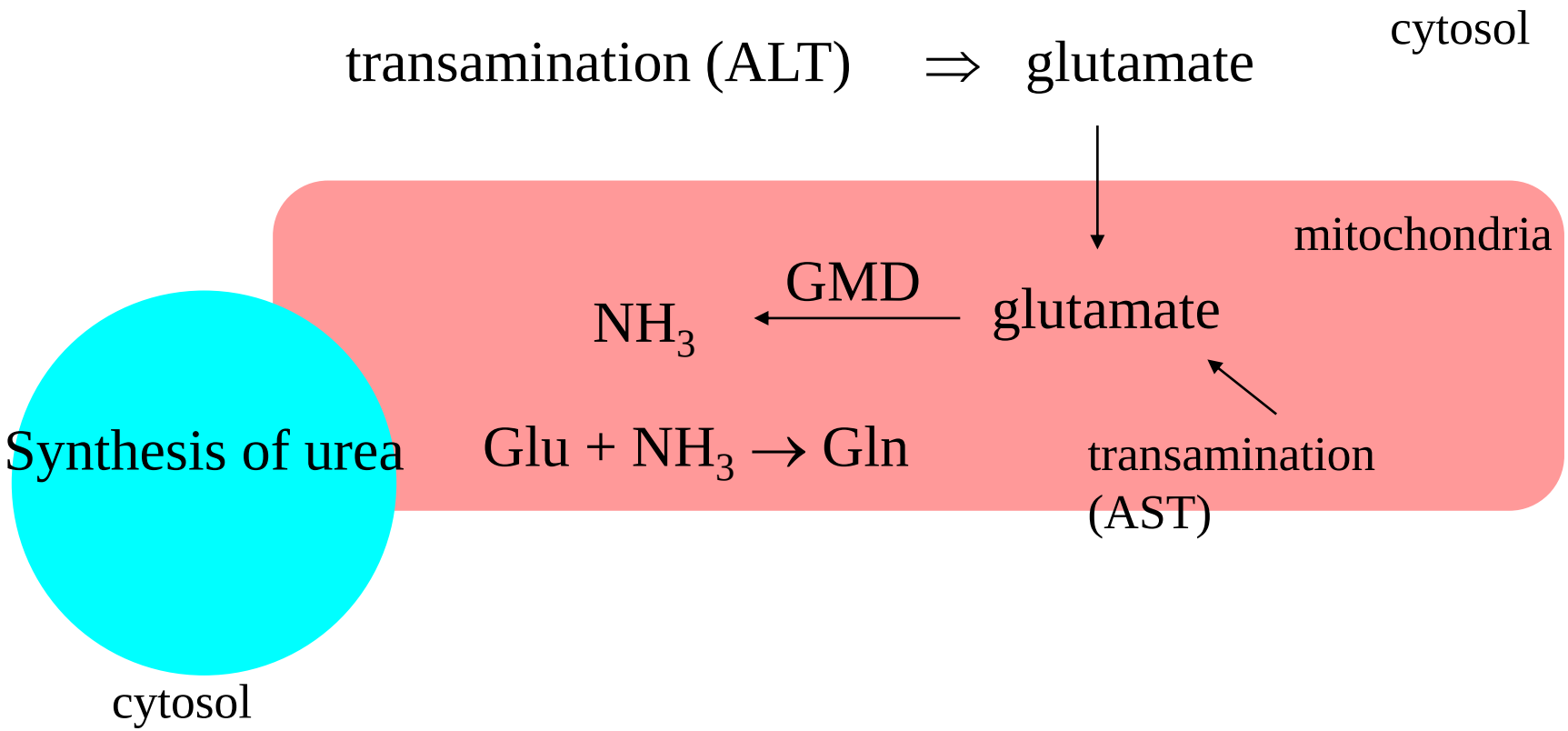
Deamination associated with dehydrogenation of Glutamate is reversible



Glutamate dehydrogenase (GMD, GD, GDH)

- cofactor $NAD(P)^+$
- GMD is reversible, dehydrogenation NAD^+, hydrogenation $NADPH+H^+$
- 2 steps:
- **dehydrogenation** >CH-NH_2 imino group >C=NH
- **hydrolysis** iminogroup to oxo group and NH3

Cell localisation of AA transformation



2 sources of NH3 in organism

- **Dehydrogenation deamination of glutamate**

  in cells of many tissues

- **Bacterial degradation of proteins in large intestine**

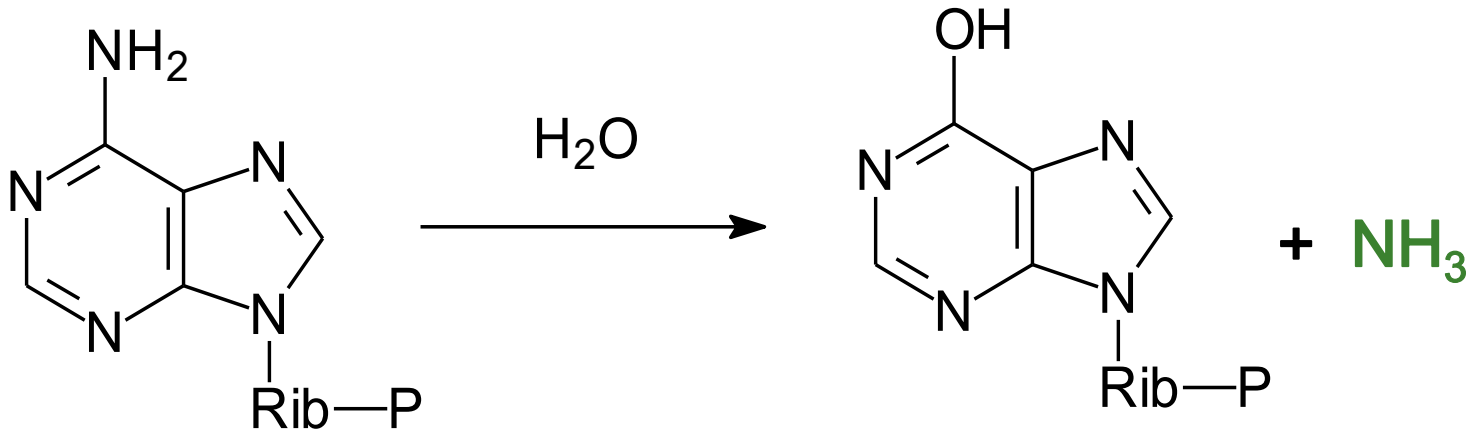
  NH3 difusion to vena portae blood $\Rightarrow$ high

  concetration of NH_3 $\Rightarrow$ remove by liver

Other sources of NH3 from other substrates

- Deamination of adenine
- Oxidation deamination of AA (H_2O_2)
- Desaturation of His
- Oxidation deamination of Lys
- Dehydratation deamination of Ser
- Oxidation deamination of biogenic amines

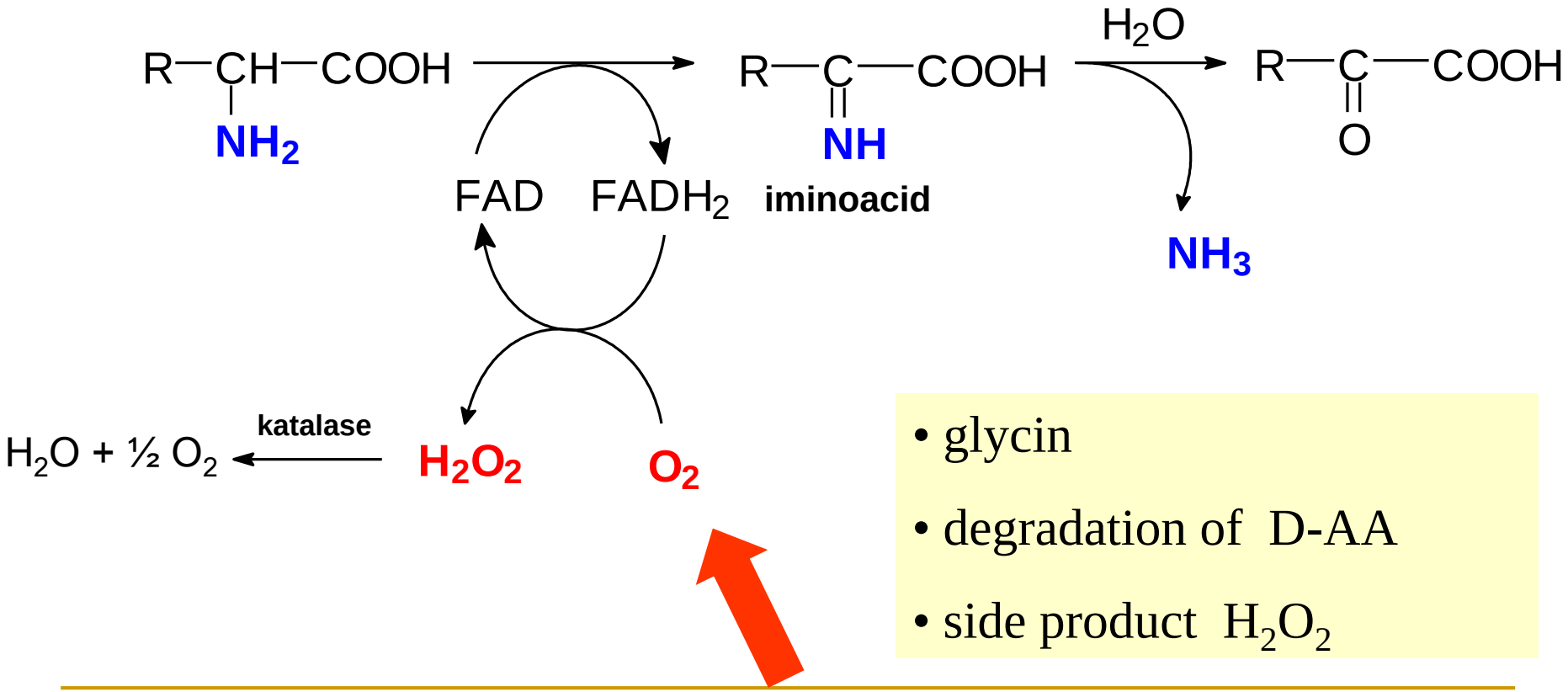
Deamination of adenine



adenosinemonoP

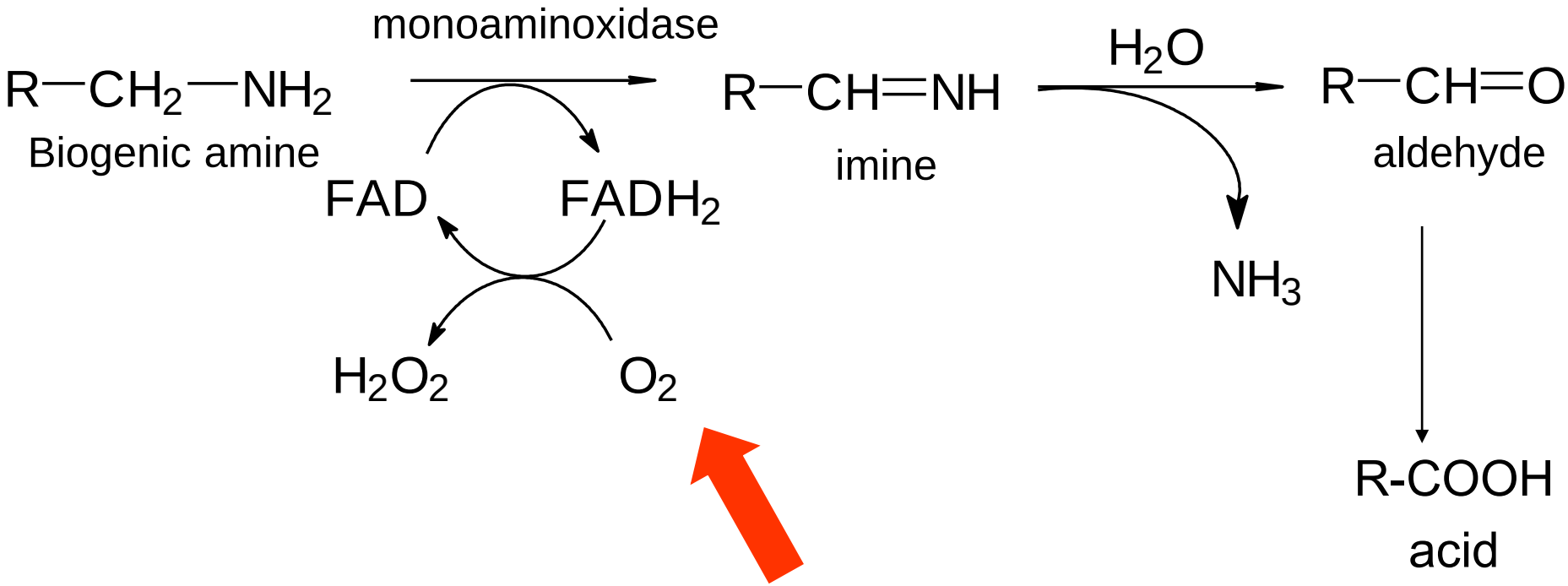
inosinemonoP

Oxidation deamination of some AA



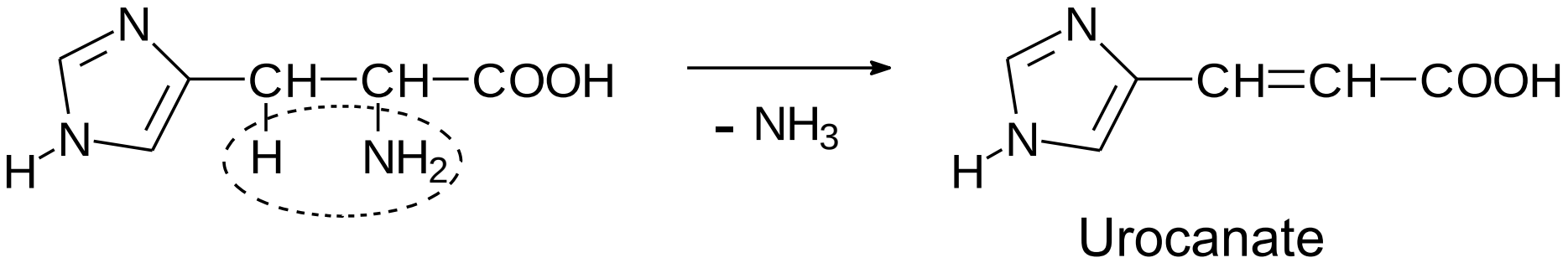
- glycin
- degradation of D-AA
- side product H_2O_2

Oxidation deamination of biogennic amines



Desaturation -deamination of His

- No transamination
- Histidine catabolism begins with release of the α-amino group catalyzed by histidase,
- introducing a double bond into the molecule.
- As a result, the deaminated product, urocanate, is not the usual α-keto acid associated with loss of α-amino nitrogens. The end product of histidine catabolism is glutamate, making histidine one of the glucogenic amino acids.



Urocanic acid

Other reaction with production of NH3

- **noenzymatic carbamylation of proteins (high concetration of urea in cells)** í.

  $Prot\text{-}NH_2 + NH_2\text{-}CO\text{-}NH_2 \rightarrow \mathbf{NH_3} + Prot\text{-}NH\text{-}CO\text{-}NH_2$

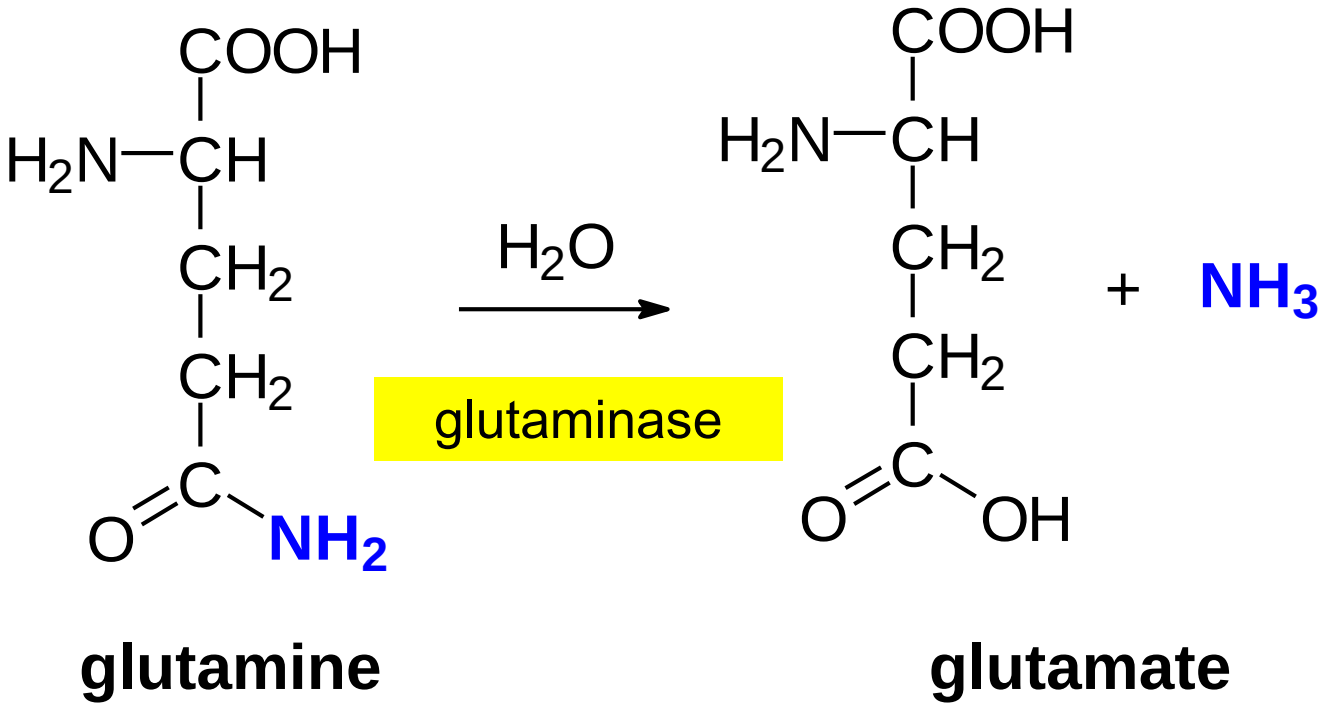
- **catabolismus pyrimidin base**

  cytosin/uracil $\rightarrow$ **NH_3** + CO_2 + β-alanin

  thymin $\rightarrow$ **NH_3** + CO_2 + β-aminoisobutyrate

- **Synthesis of hem** (4 porfobilinogen $\rightarrow$ **4 NH_3** + uroporfyrinogen)

Hydrolysis of amid group of Gln in kidney NH_4^+ - urine (deamidation)



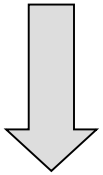
glutamine **glutamate**

Glutamine- non toxic form of NH3

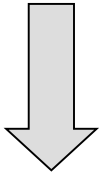
Patology- production of NH3

- **Bleeding to GIT** $\Rightarrow$ higher NH_3 in blood
- **uroinfection** – bacterial urease –catalysis of hydrolysis of urea

$$H_2N\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2\,NH_3 + CO_2$$



$$NH_3 + H_2O \leftrightarrows NH_4^+ + OH^-$$



Alcalic urine (pH 8) $\Rightarrow$ phosphate concrements

Acidobazic properties of NH_3

$pK_B\,(NH_3) = 4{,}75$ (weak base)

$NH_3 + H_2O \leftrightarrows NH_4^+ + OH^-$

$pK_A\,(NH_4^+) = 14 - 4{,}75 = 9{,}25$ (very weak acid)

At physiological pH ICT and ECT (7,40) :

98 % NH_4^+

2 % NH_3

NH_4^+ in body liquid

Body liquid	conc. NH_4^+ (mmol/l)	Metabolic origin of NH_4^+
urine	10 – 40	deamidation Gln + deamition Glu in kidney
Saliva	2 – 3	Hydrolysis of urine in mouth microbe
Portal blood	0,1 – 0,3	Decompose of proteins in large intestine, catabolismu Gln/Glu in enterocyte
Venous blood	< 0,03	Catabolism of AA in tissues

Jak omezit vznik amoniaku v lidském těle?

důležité při jaterním selhávání

1. **nízkoproteinová dieta**
2. **alterace střevní mikroflóry**

- **probiotika** – živé mikrorganismy, podporují kvasné procesy na úkor hnilobných (laktobacily, bifidobakterie) – kefír, acidofilní mléko ...
- **prebiotika** – nestravitelné složky potravy, které selektivně stimulují růst probiotik (oligofruktosa, inulin, vláknina)
- **střevní antibiotika** – lokálně působící (neomycin, metronidazol), krajní řešení, krátkodobé

- **Detoxification of NH3**

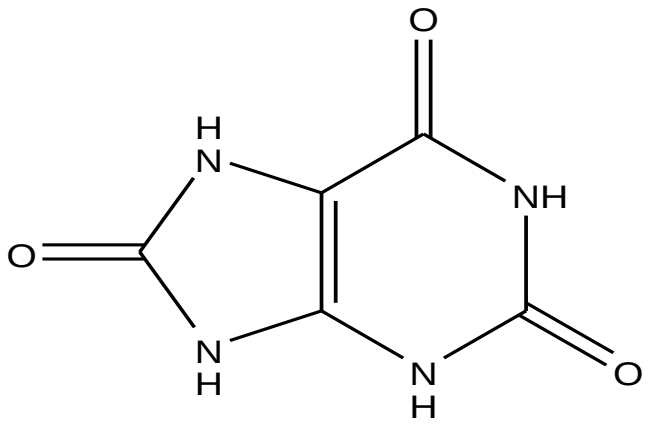
- 3 ways**:**
- 1) in **urea cycle**
- 2) formation of **glutamine**
- 3) formation of **glutamate**

3 products of detoxification of NH3

Characteristic	Urea	Glutamine	Glutamate
importance	★★★★★★	★★★★	★
Type of componds	diamide H_2CO_3	γ-amid Glu	α-amino acid
Reaction of synthesis	ureosynt. cycl	Glu + NH_3	red. amination 2-OG
Enzyme	5 enzymes	Gln-syntetase	GMD
energy need	3 ATP	1 ATP	1 $NADPH+H^+$
Cell localisation	mitoch. + cytosol	Mitoch.	Mitoch.
Organ	Only liver	liver, others	(CNS)

Production of Urea

- Organisms can excrete excess N as
  - Ammonia (ammonotelic; e.g., aquatic animals)
  - Urea (ureotelic; terrestrial animals)
    - Synthesized in liver by urea cycle (discovered by Hans Krebs, before he elucidated the TCA)
  - Uric acid (uricotelic: birds, reptiles, dinosaurs?)

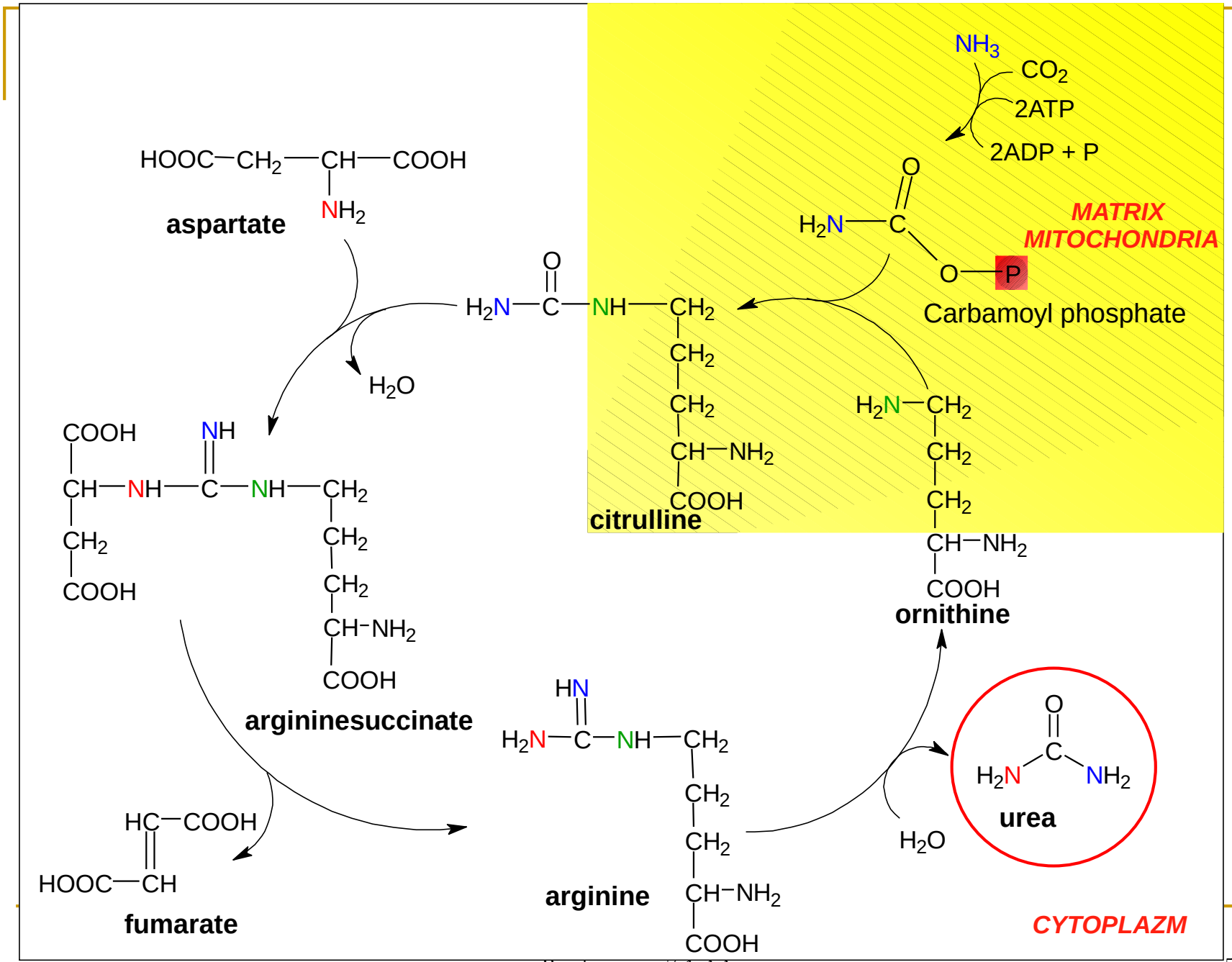


uric acid

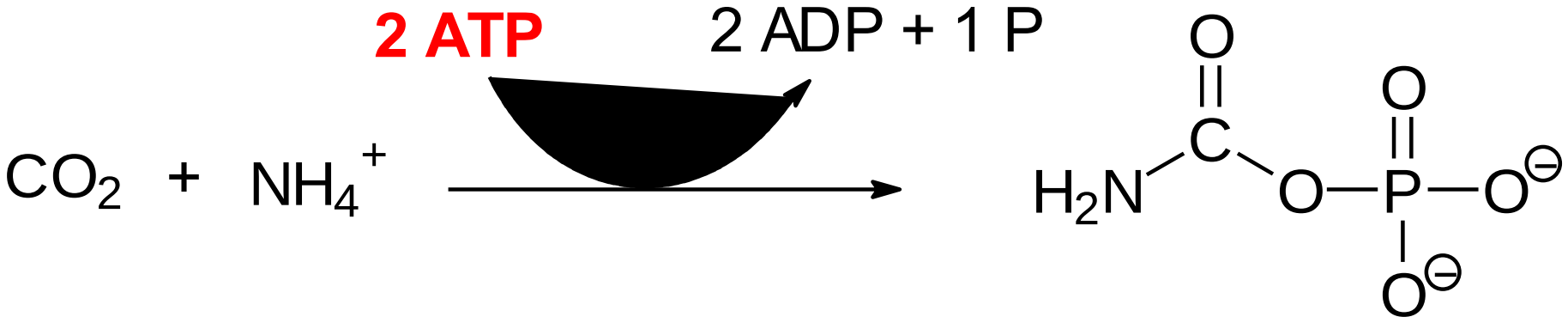
Synthesis of urea in liver

2 reaction in mitochondria

Other reactions in cytosol

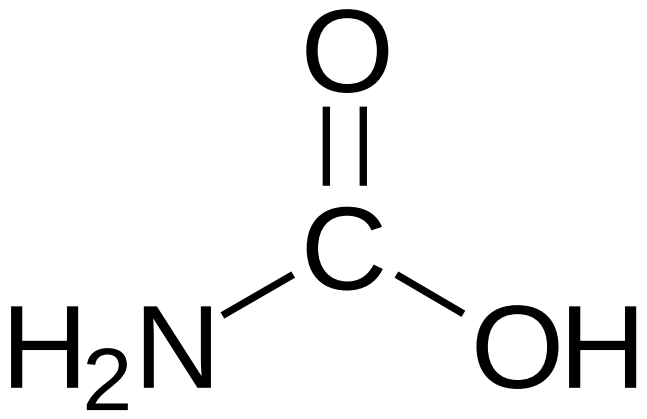

NH3
CO2
2ATP
2ADP + P
MATRIX
MITOCHONDRIA
Carbamoyl phosphate
aspartate
citrulline
ornithine
argininesuccinate
arginine
urea
fumarate
H2O
H2O
CYTOPLAZM

1. Carbamoylphosphate synthesis (matrix)



- carbamoylphsphatsynthetase, alloster. activator *N*-acetylglutamate
- matrix mitochondria
- **2 mols ATP**
- Amid bond + hybrid anhydrid
- **Macroergic compound**

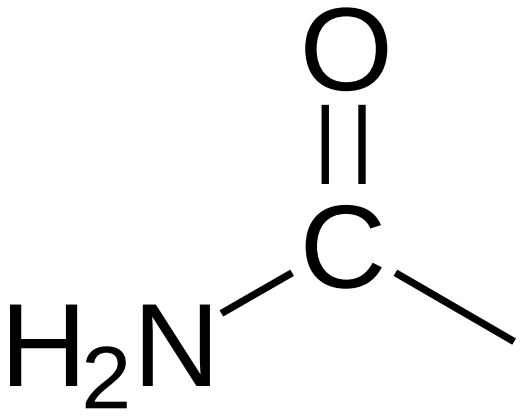
Carbamoyl is acyl carbamic acid



Acid carbamic

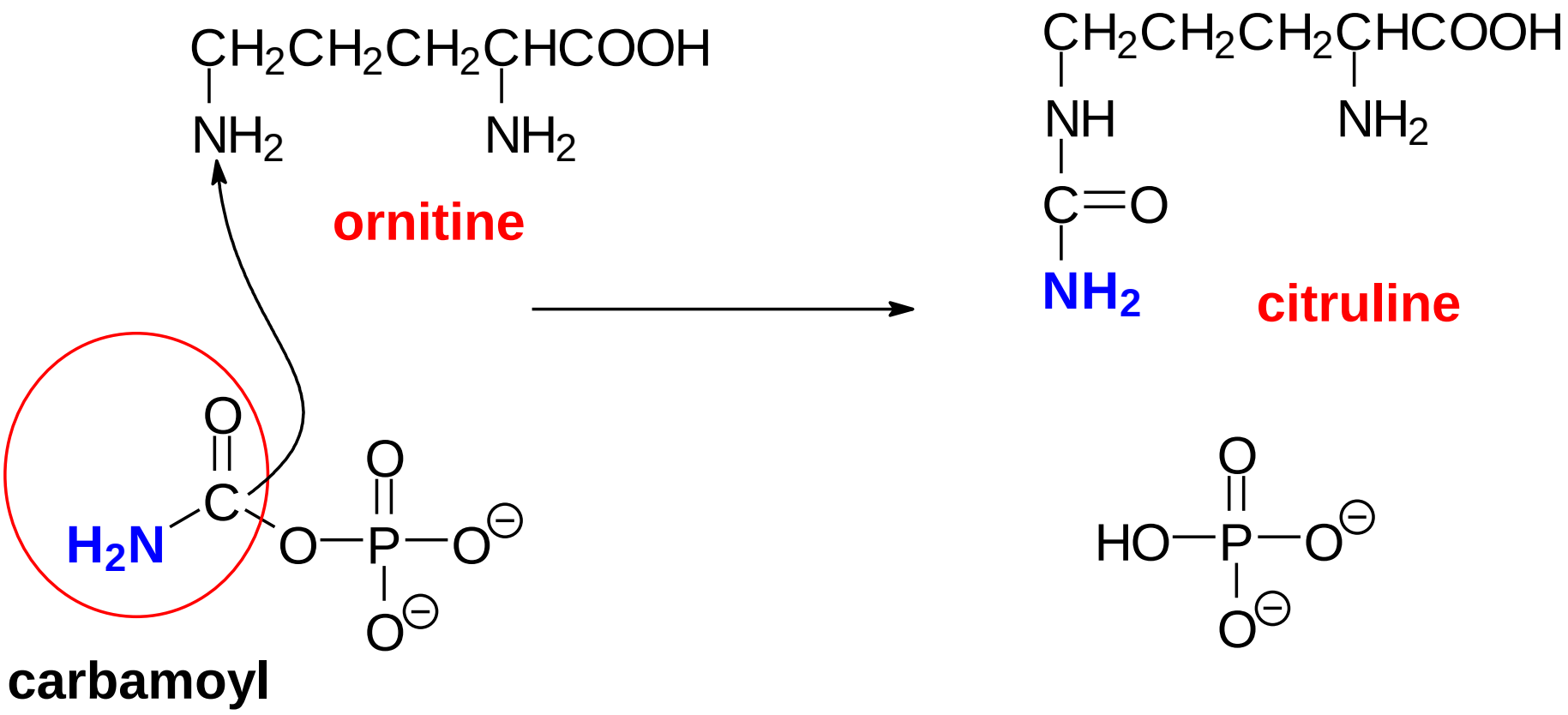
monoamid H2CO3

hypotetic

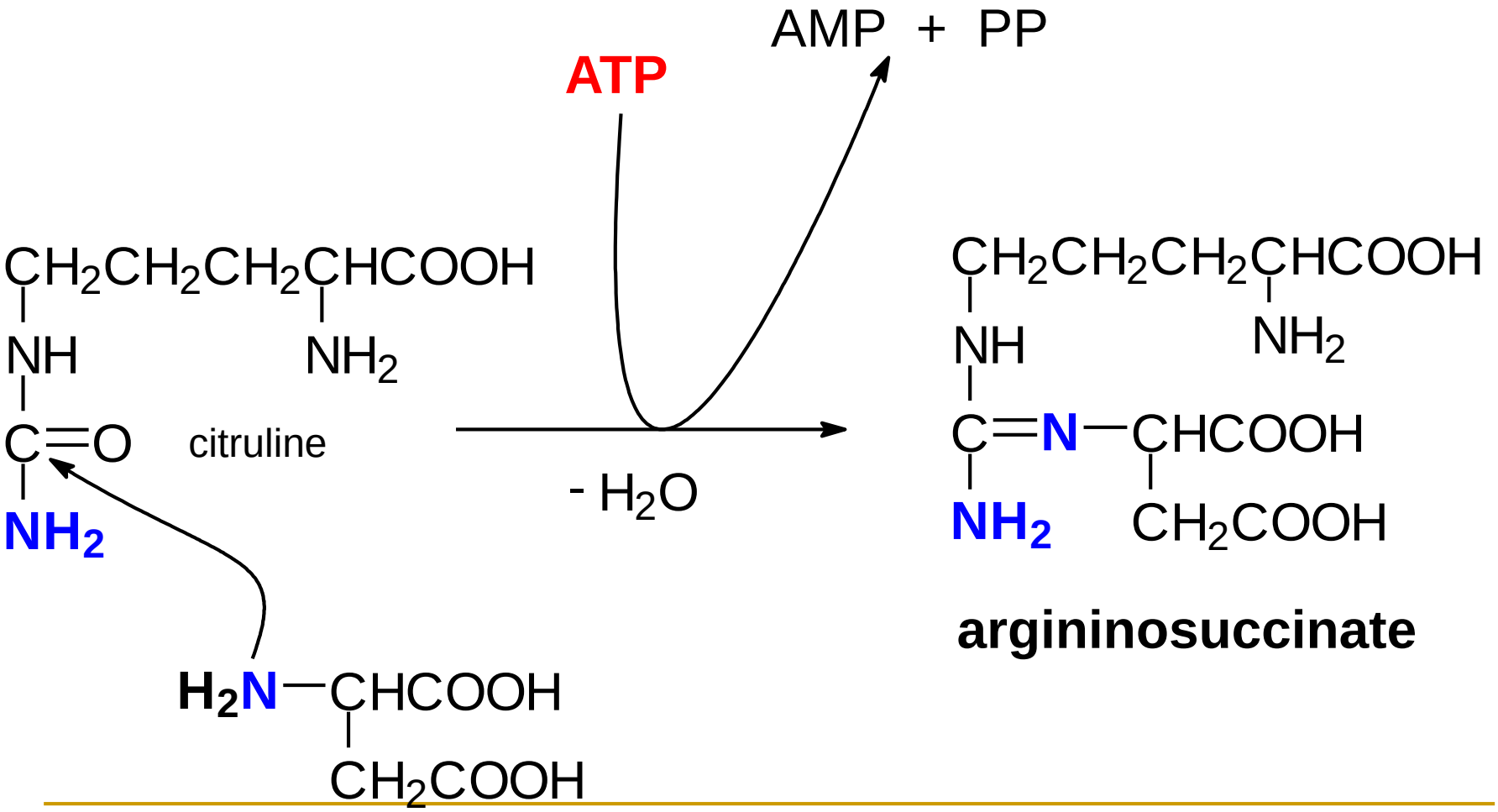


carbamoyl

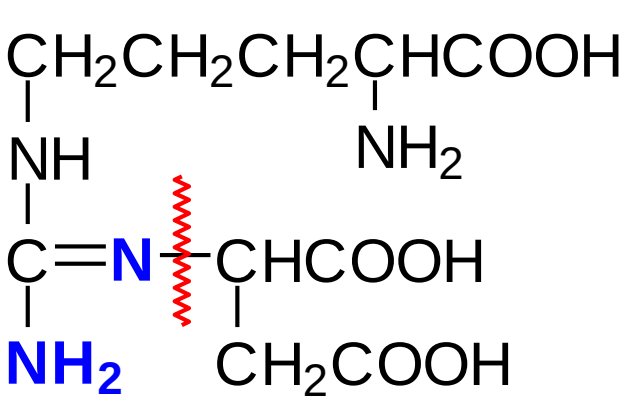
2. Citrulline formation (matrix)



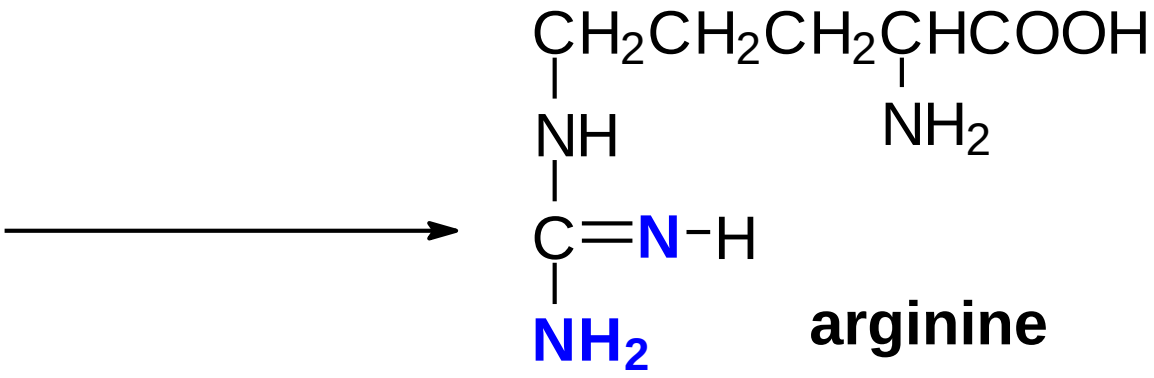
3. Argininosuccinate formation, NH2 in Asn (cytosol)



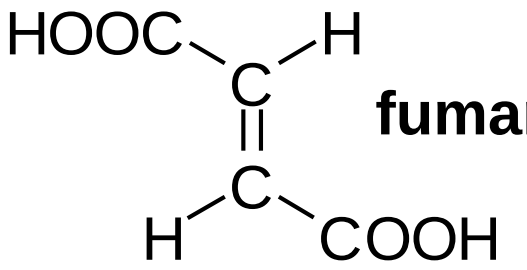
4. Cleavage of argininosuccinate



argininosuccinate

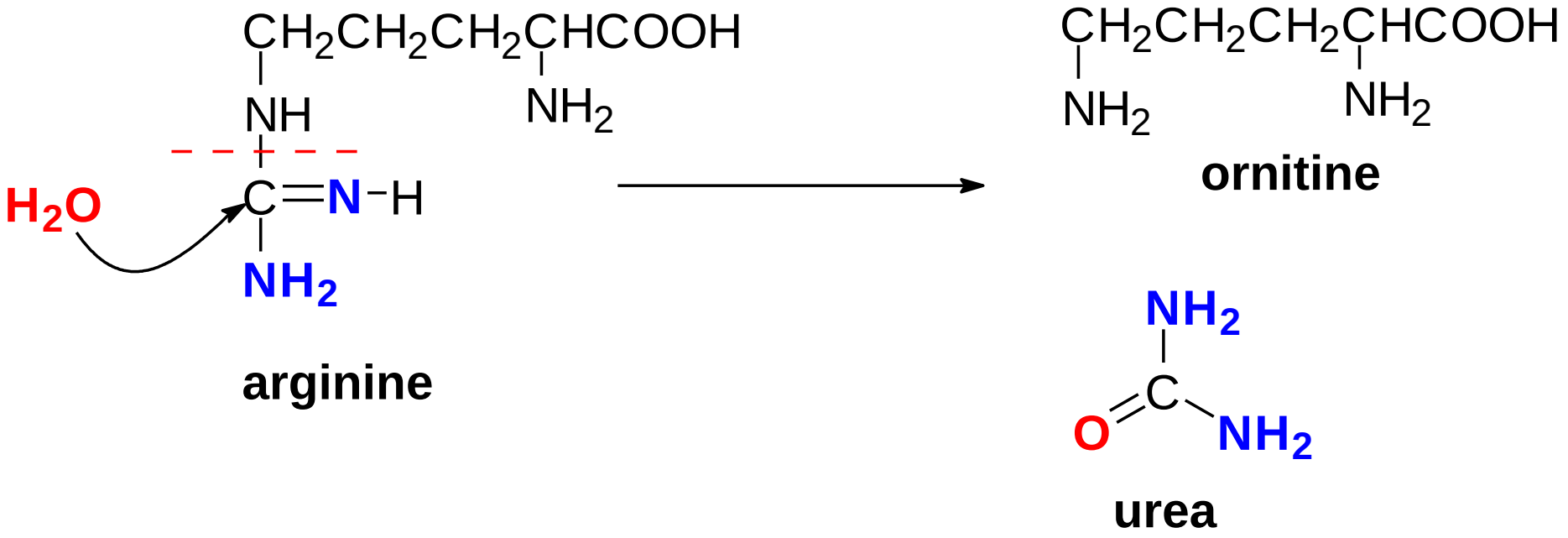


arginine

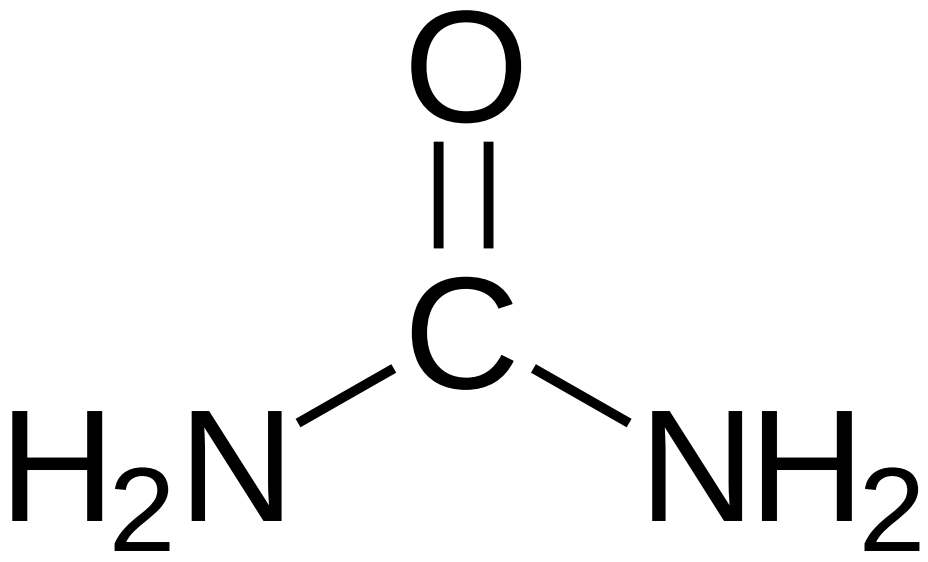


fumarate

5. Hydrolysis of arginine gives urea



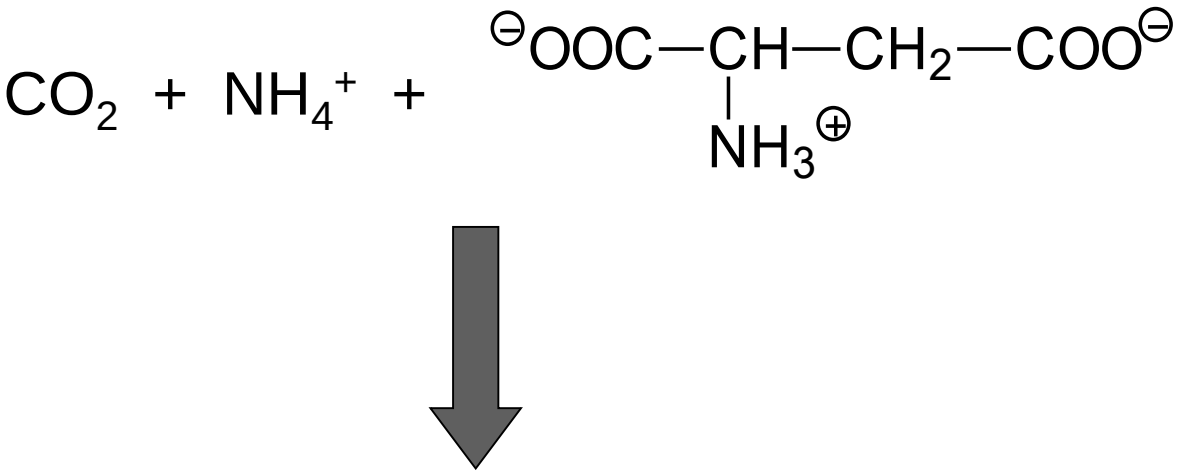
Metabolic origin of N in urea molecule



Free NH3 **aspartate**

Synthesis of urea in proton-productive action

$$CO_2 + NH_4^+ + \text{aspartate} \rightarrow \text{urea} + \text{fumarate} + H_2O + 2\,H^+$$



$$CO(NH_2)_2 + {}^-OOC\text{-}CH{=}CH\text{-}COO^- + H_2O + \mathbf{2\,H^+}$$

Urea is not electrolyt

- Polar substance $\Rightarrow$ very well soluble in water
- Very well transported –membrane (hydrophilic canal)
- Osmolarity of blood plasma:

  osmolarity $\approx$ 2 [Na^+] + [glukose] + [urea] mmol/kg H_2O
- In liver
- Excreted by urine – dependent on amount of proteins
- **330-600 mmol/d (20-35 g/d)**

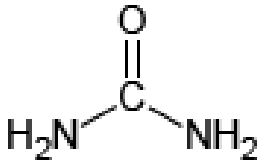
Urea in blood plasma(2-8 mmol/l)

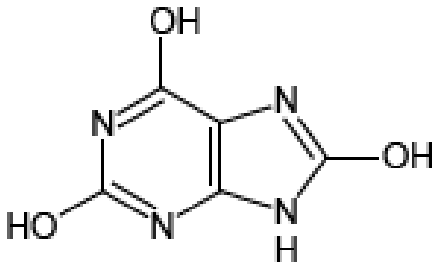
Higher concentration

- Defect of excretion (renal collapse)
- Extensive catabolism of proteins (sepsis, burn, polytrauma, tumors, fever …)

Lower concentration

- Lack of proteins in food
- Defects of production (liver collapse)



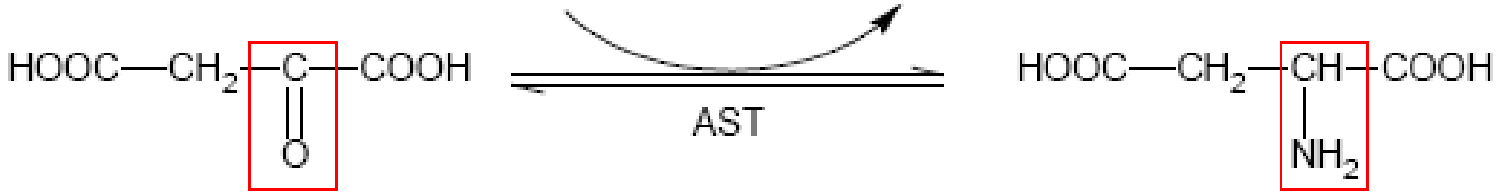


Character	Urea	Uric acid
Latine name	Urea	acidum uricum
Catabolit	AA	A,G
Behaviour n water	Noelectrolyte	Weak acid
pH	Neutra	Weak acidic
Solubility in water	Good	bed
Reduction protertyi	No	yes (antioxidante)
Body	Liver	Many tissues
In cells	mitoch. + cytosol	Cytosol
Concntration in plasma	2-8 mmol/l	150-400 µmol/l
Excretion of urine	20-35 g/d	0,5-1 g/d
% catabolic N	80-90	1-2

Regeneration of Aspartate

- PRODUCTION **transamination from oxalcetate**

(enzyme **AST – aspartateminotransferase**



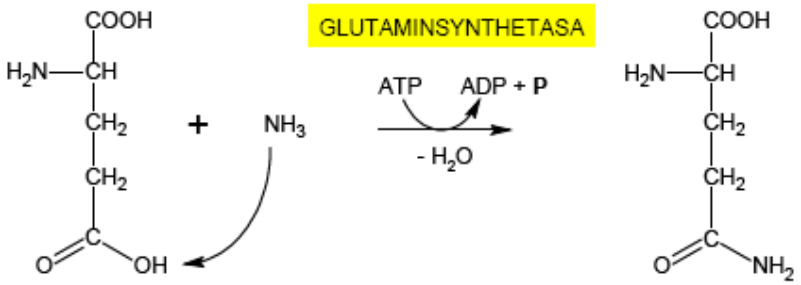
OA **Asp**

- OA- intermediate of CAC
- Substrate for TA
- Substrate for **2-oxoglutarate** (one reaction of CAC)

Synthesis glutamine

2. Way for detoxication of NH_3

- Synthesis of Gln
- needs 1 ATP.
- In many cells, in mitochrondia
- Transport form of NH3, Gln is transported to kidney—NH3

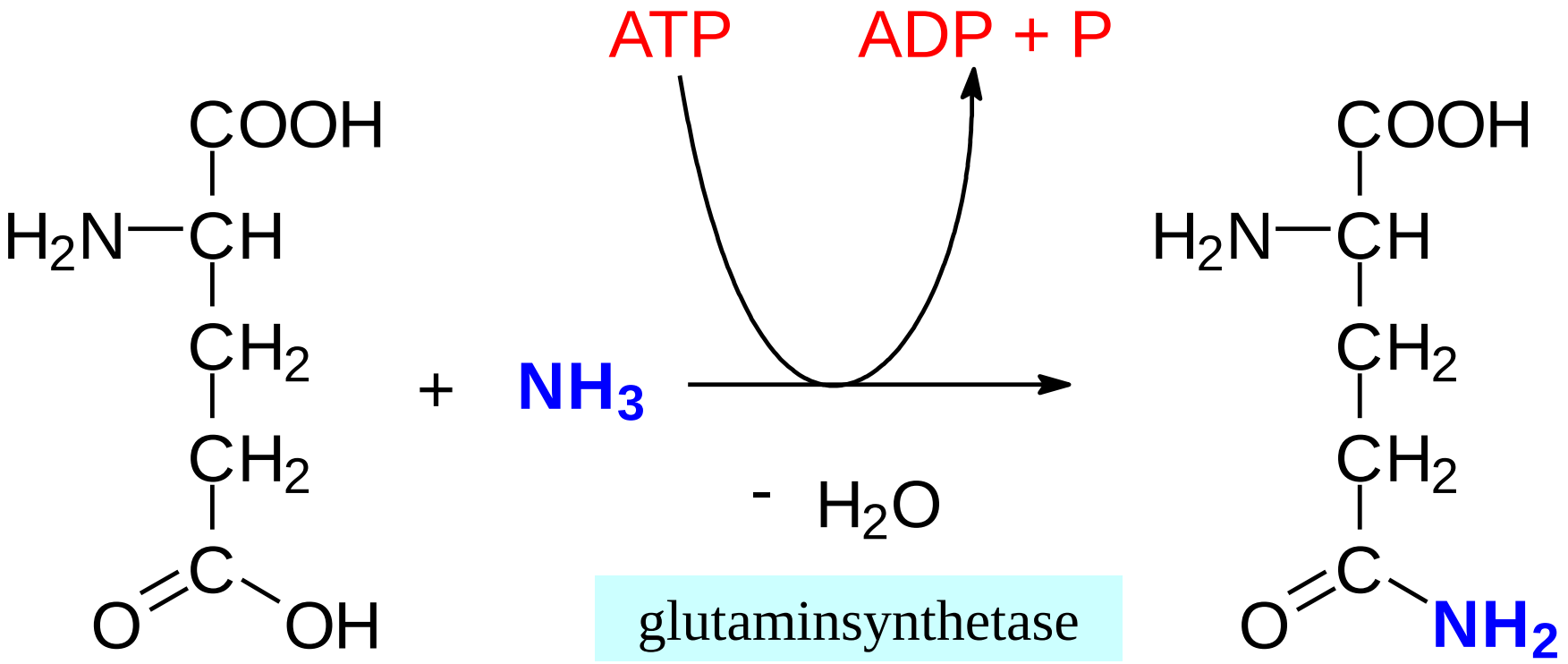


Glutamine, Ala – overrepresented AA in blood n postresorpted phasis

Function, Role in organism:
a) **Source of energy** for some cells (enterocyty, fibriblasty, lymfocyty, makrofágy)
b) **Source of N for synthesis** (purines, aminosugers…)
c) Source of **glutamate**

2. way detoxication NH_3

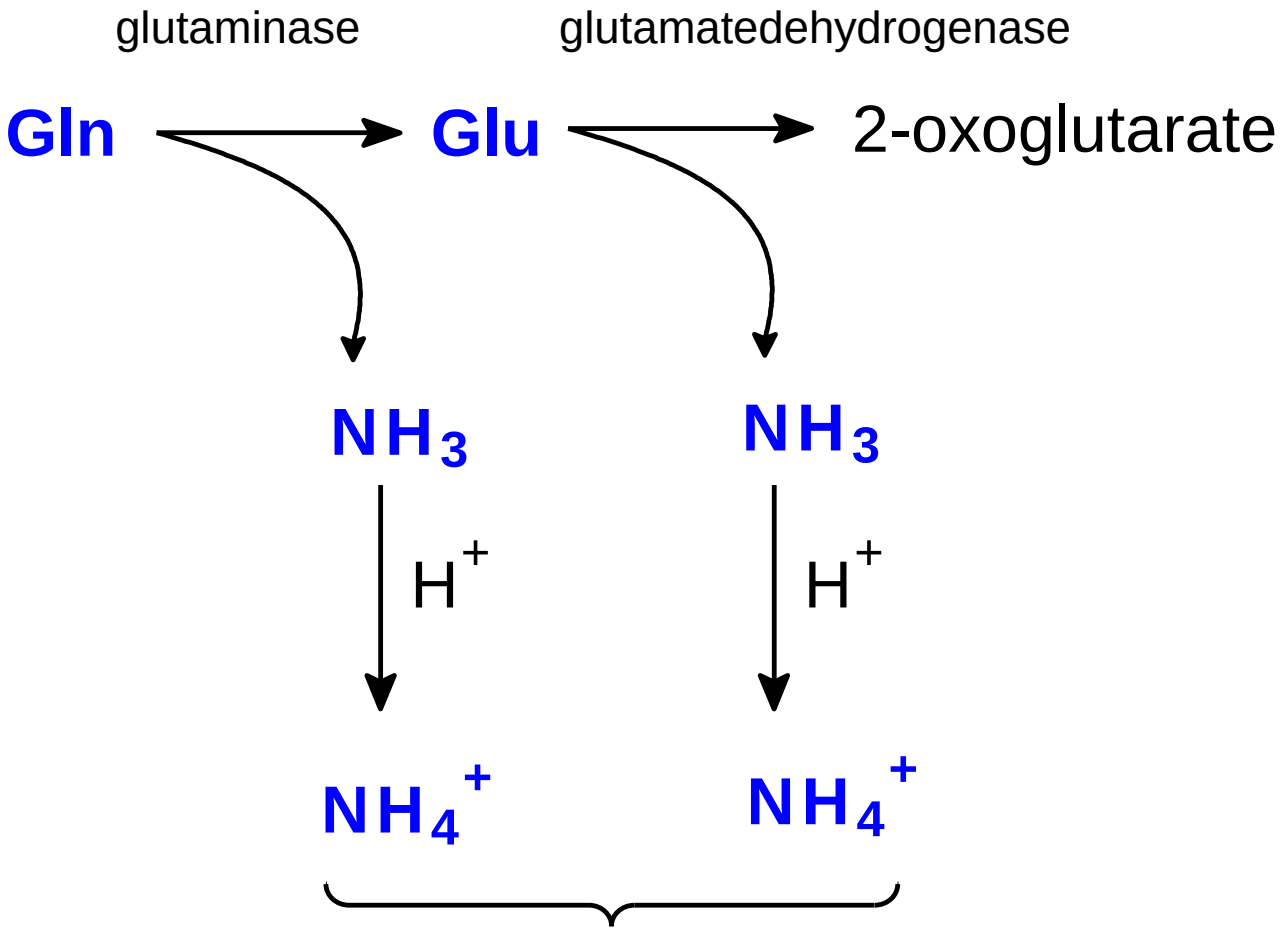
Synthesa glutamine



glutamate

glutamine

From glutamine in kidney release NH_4^+

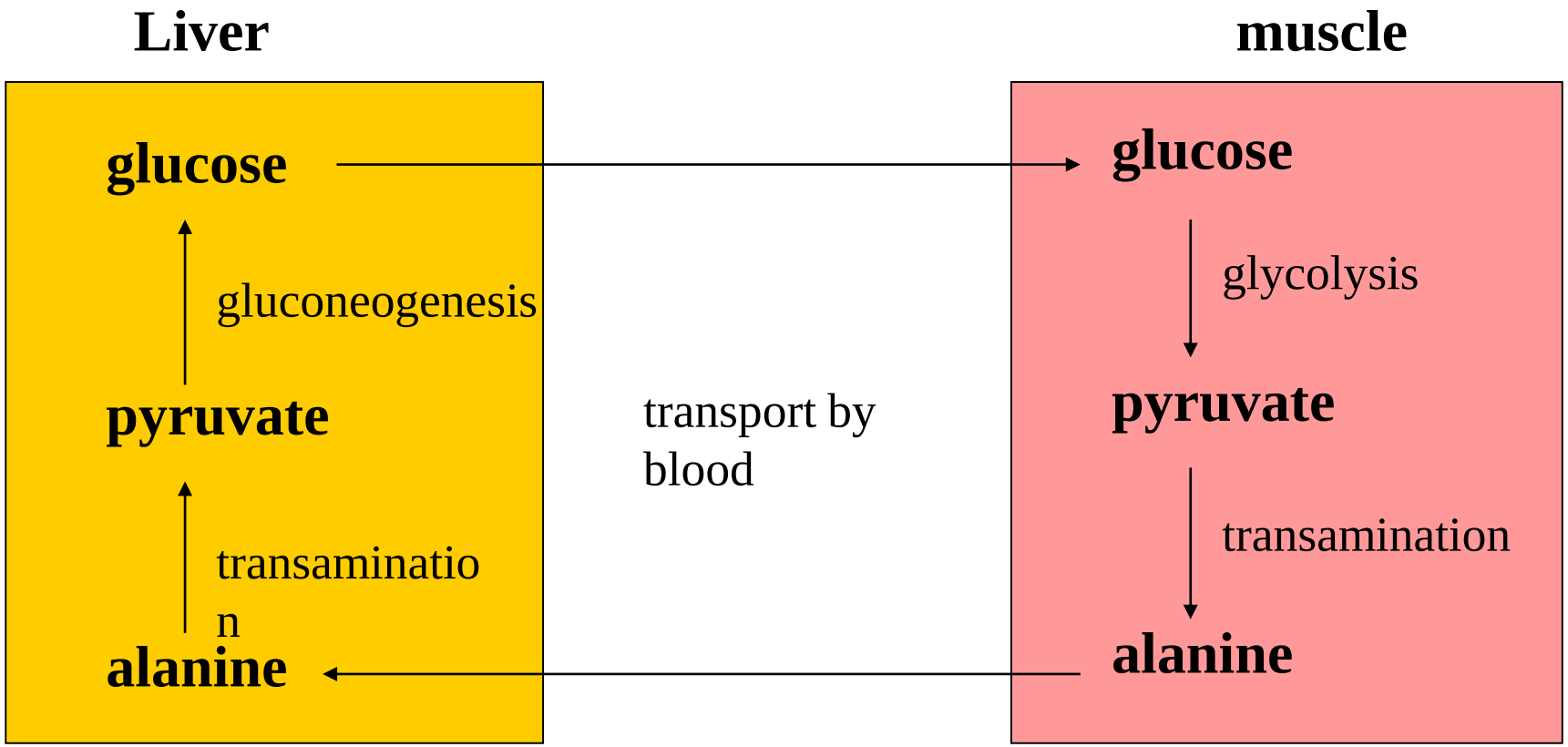


urea (pH ~ 5)

Glutamine and alanine has spetial importance

- The most representative AA in postresorption stage, muscular tissue
- Ala – significant substrate for gluconeogenesis
- Synthesis Glutamine – detoxification of NH3
- Glutamine break-up NH3 in tubular cells of kidney
- Glutamine –exlusive source f energy for some cells (enterocyt, fibroblast, lymfocyt, makrophag)
- Glutamine source of N for synthesis (purines, aminosugrs ...)
- Glutamine is source of glutamate (GSH, GABA, ornitine, prolin)

Glucoso-alanine cycle



GMD r is reversibe reaction

Main NH3 formation in tissue

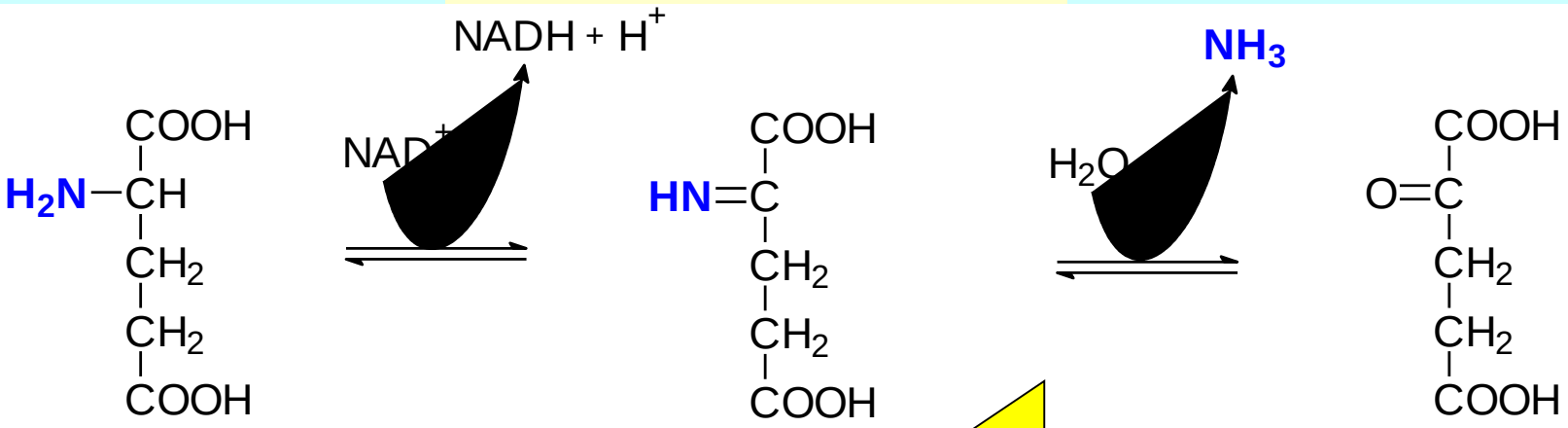
Dehydrogenation

glutamát

deamination

2-iminoglutarate

glutamate



3. Way of detoxication of NH3

Hydrogenation

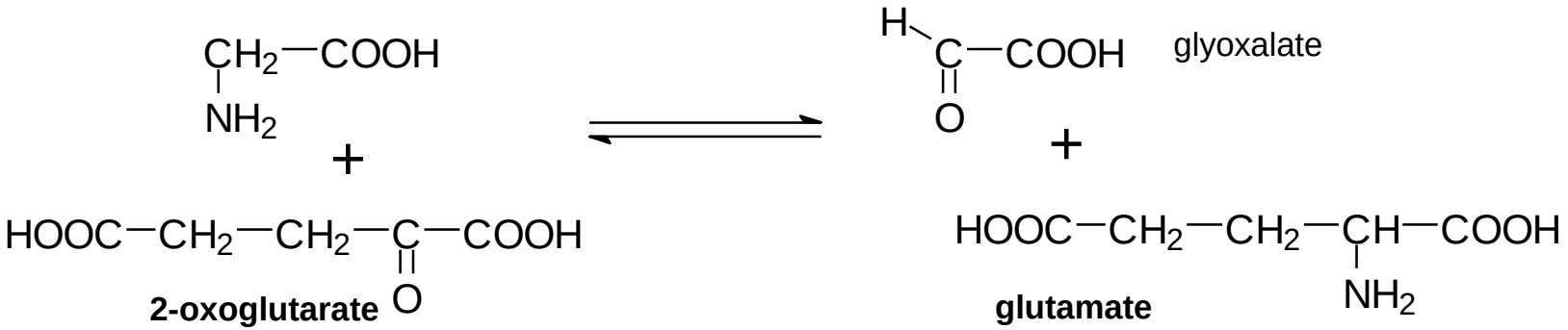
amination

2-oxoglutarate

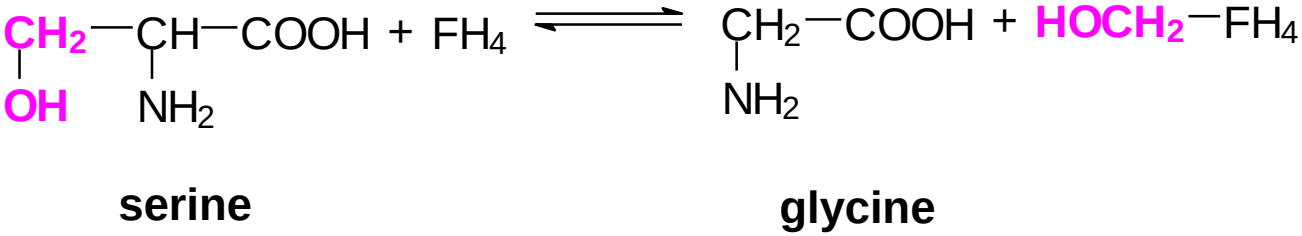
Synthesis of non essential AA

Synthese of glycine

1. Reverse of transamination reaction

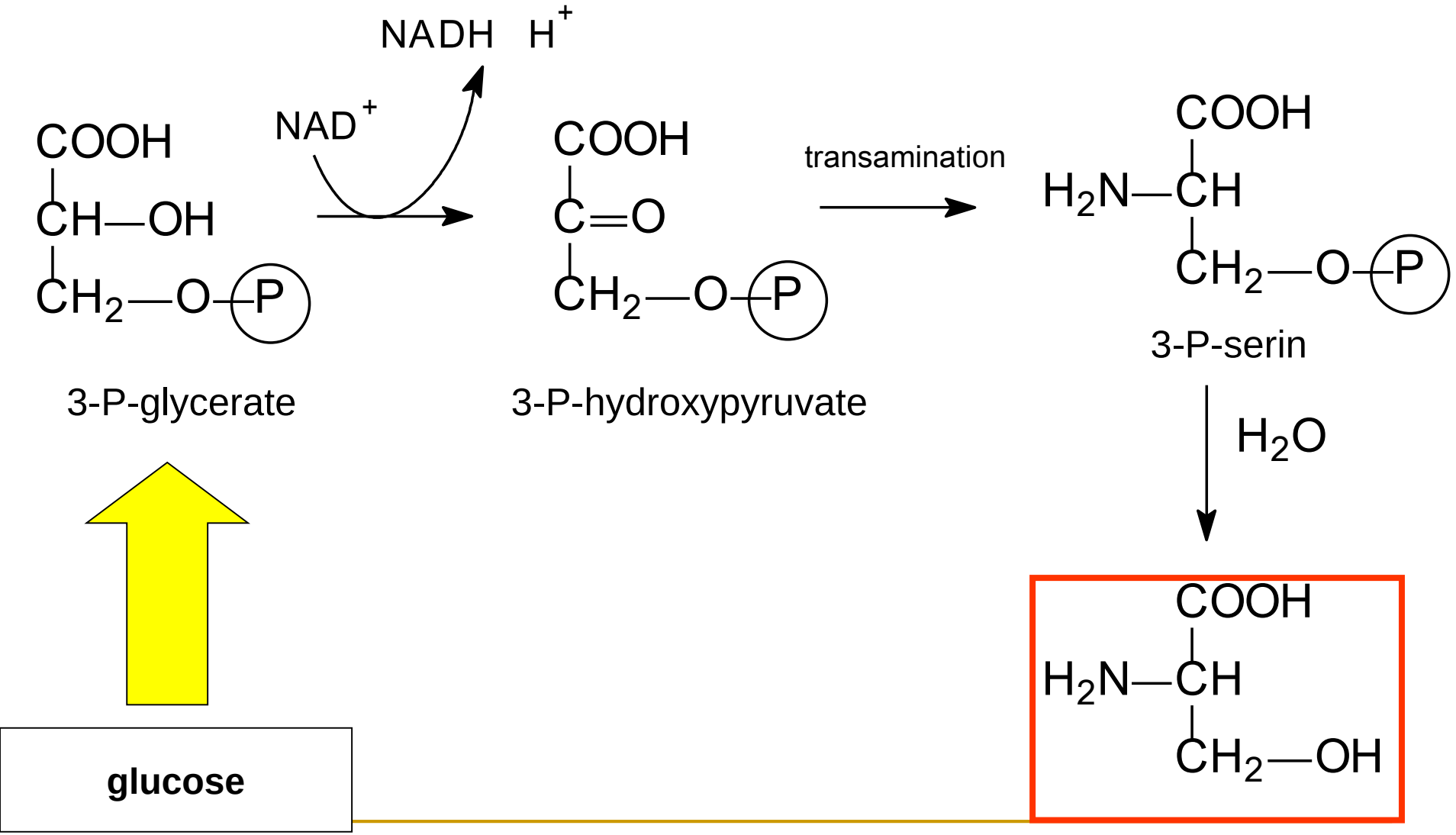


2. From Serine



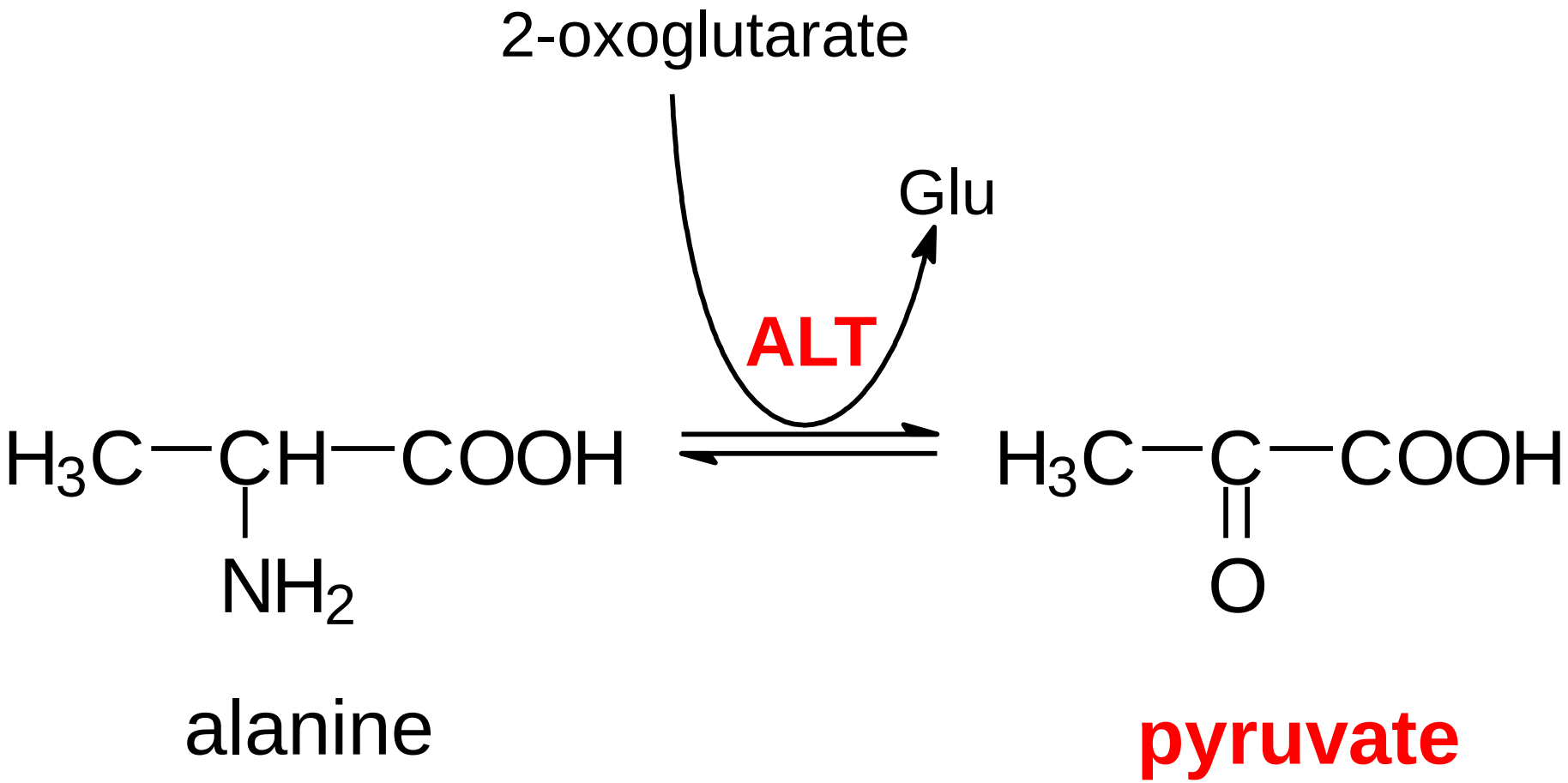
3. From Choline

Synthesis of Serine – from intermediate of glycolysis



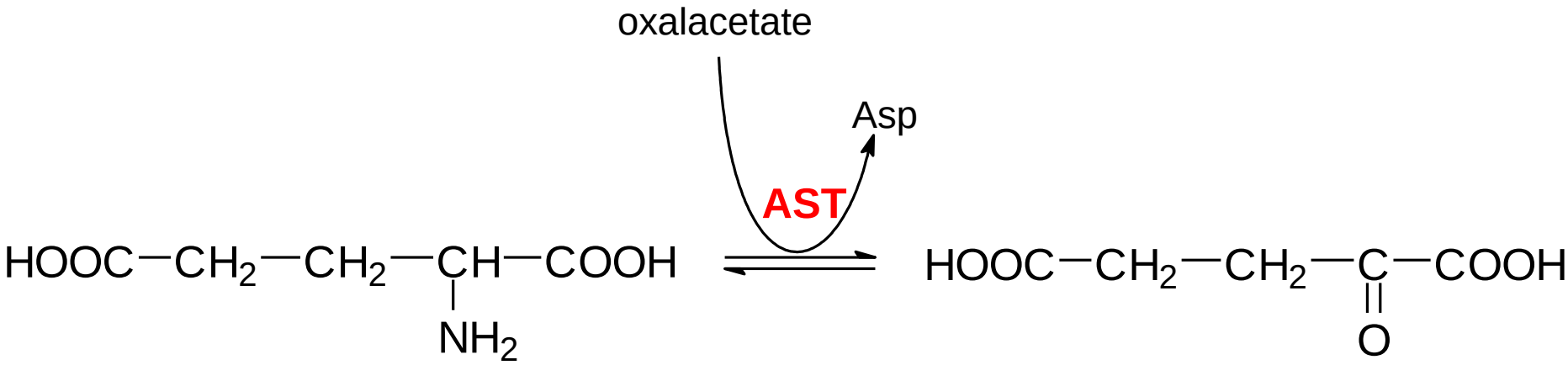
Synthesis of alanine from puruvate and glutamate

(ALT reversion reaction)



Synthesis of Aspartate from oxalacetate and glutamate

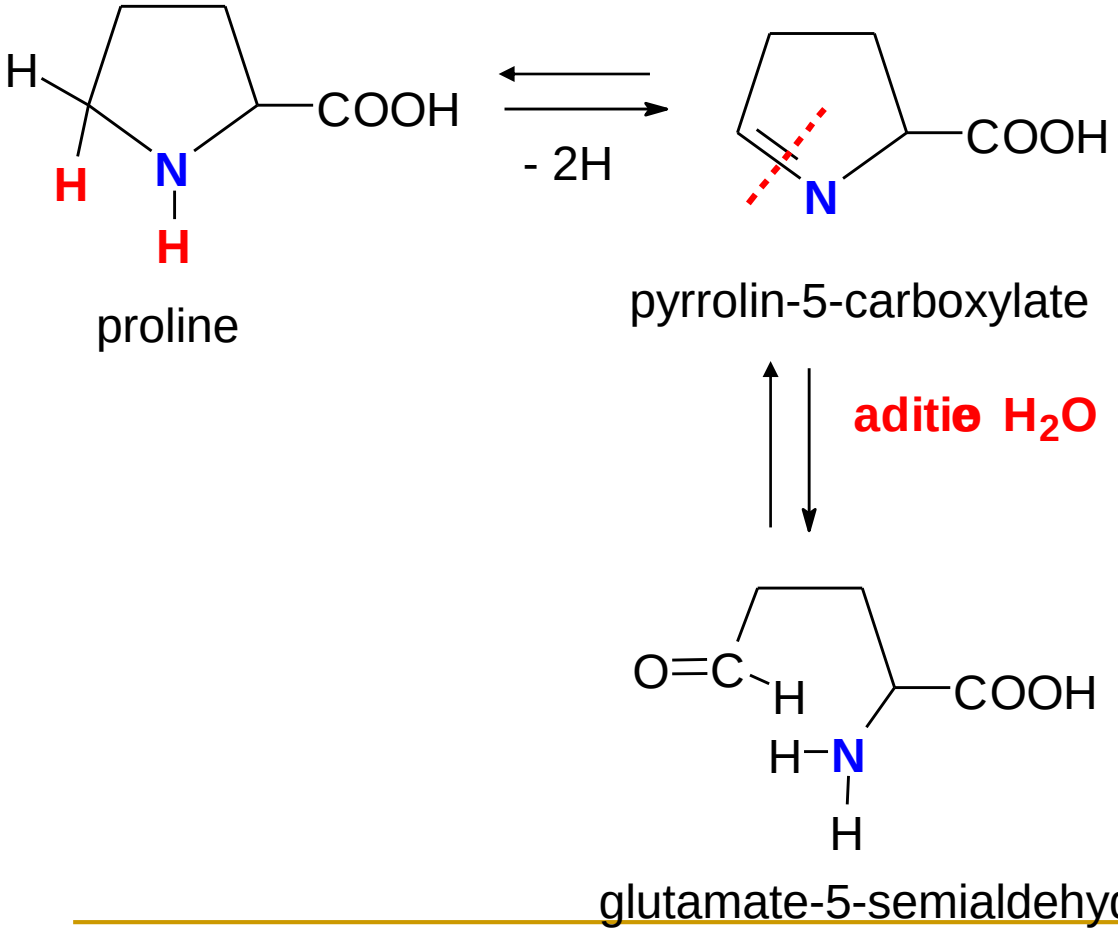
(AST reverse reaction)



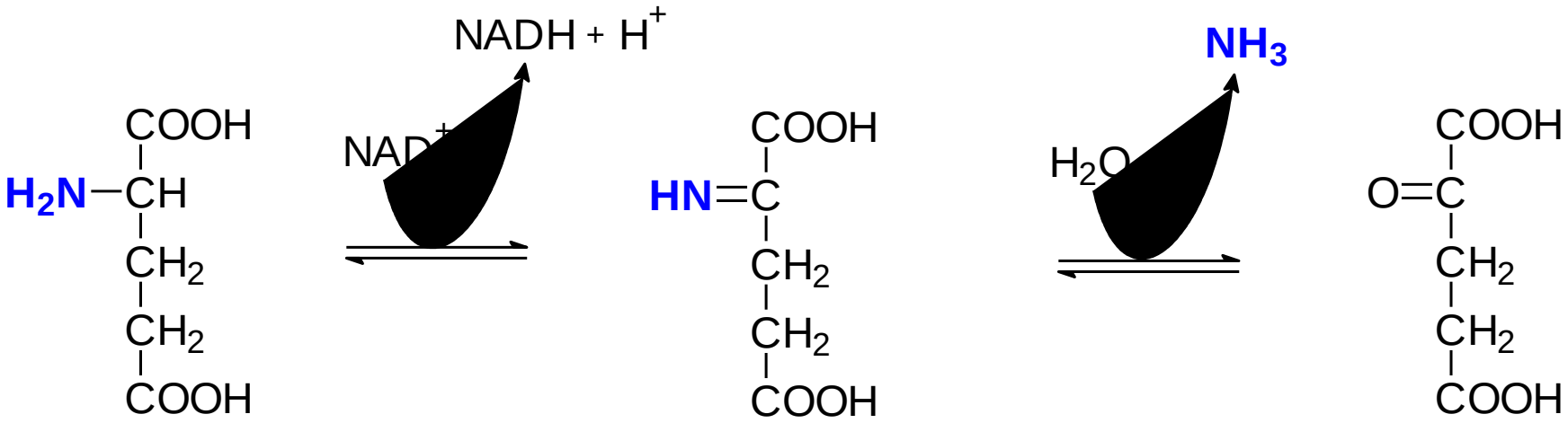
glutamate **2-oxoglutarate**

AST production of Asp for synthesis of urea

Synthesis of proline is contrary of catabolism

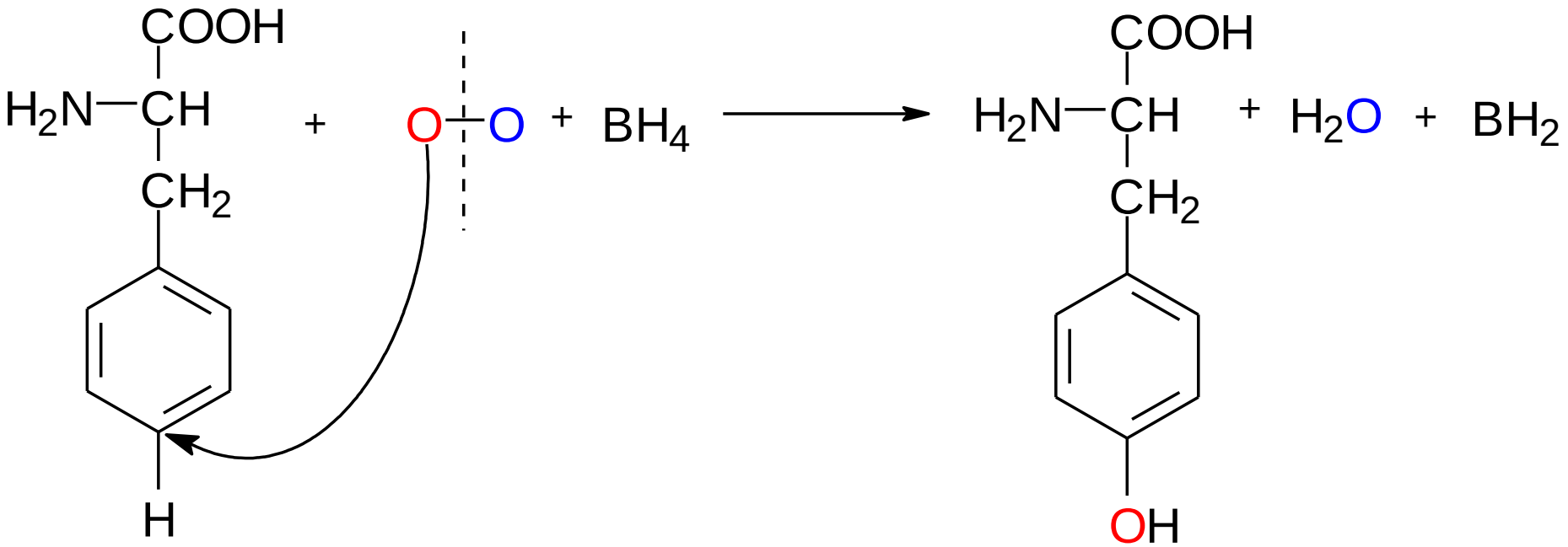


Glutamate reduction amination of 2-oxoglutarate (GMD opposite reaction)



L-glutamate 2-iminoglutarate 2-oxoglutarate

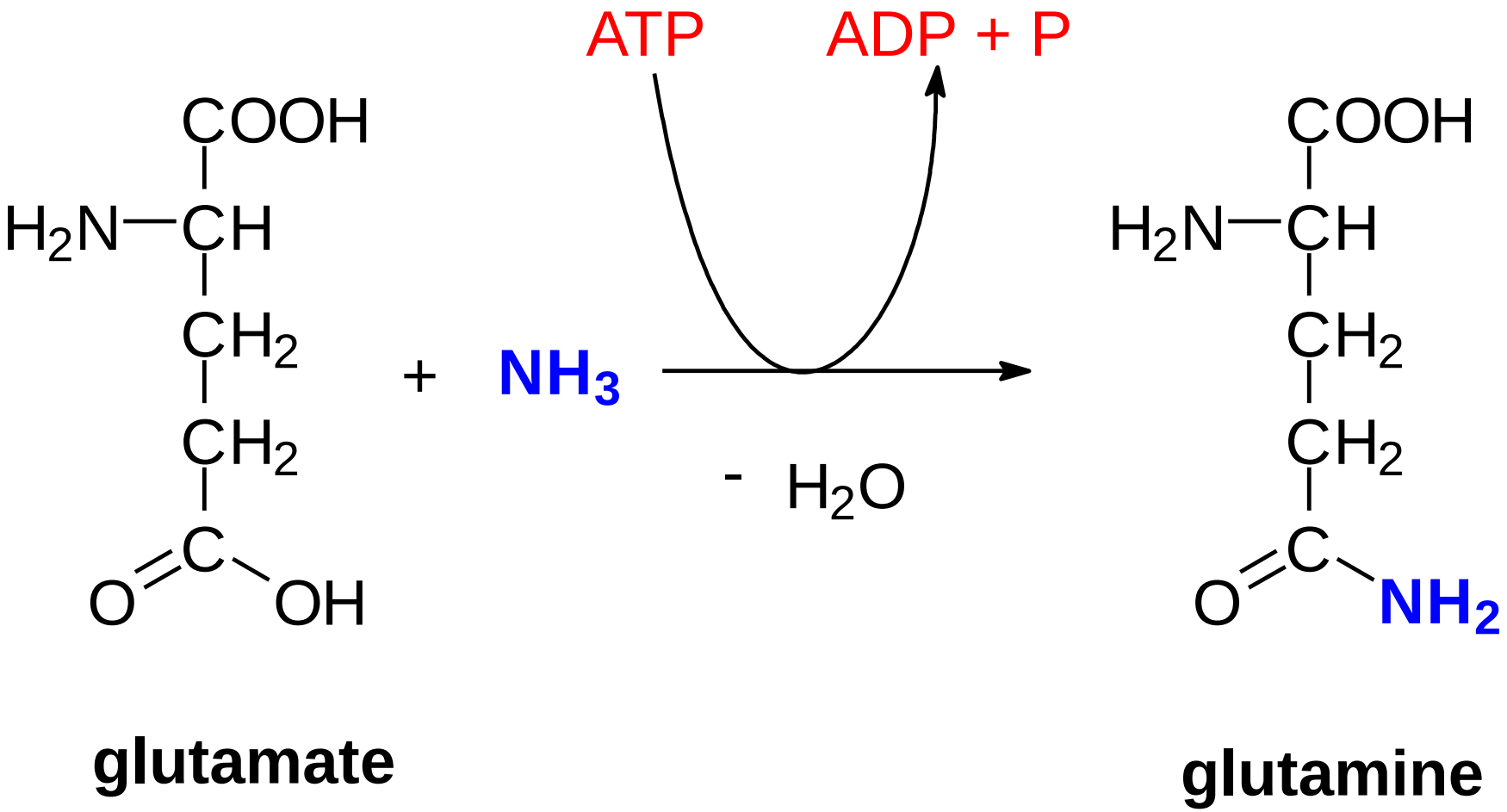
Hydroxylation of esential Phe – non esential Tyr



phenylalanine **tyrosine**

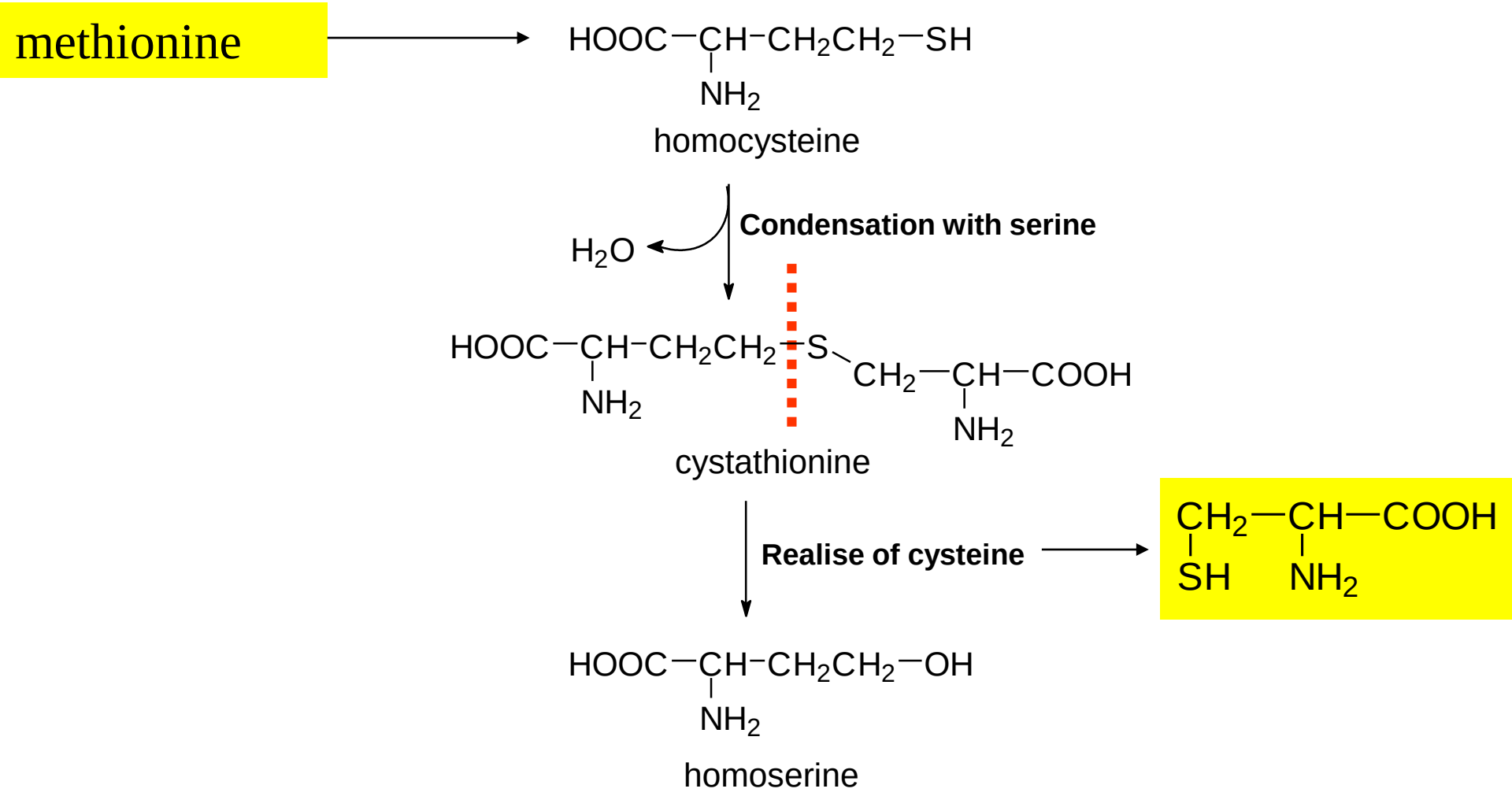
cofaktor tetrahydrobiopterine (BH_4) is donor of 2 H atoms, H2O formation

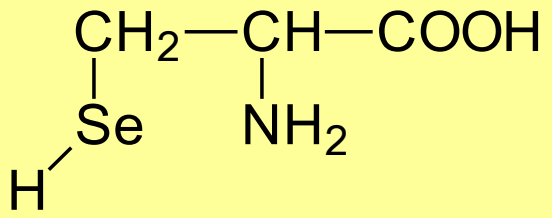
Glutamine formation from glutamate and NH3



Similar Asn (asparagine) from Asp

Cysteine formation from degradation of methionine



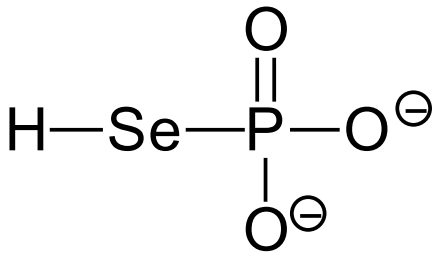


Selenocysteine formation by cotranslation of serine and selenoposphate

Serine-tRNA + selenophosphate → selenocysteine-tRNA + phosphate

SelenoP from selenid and ATP

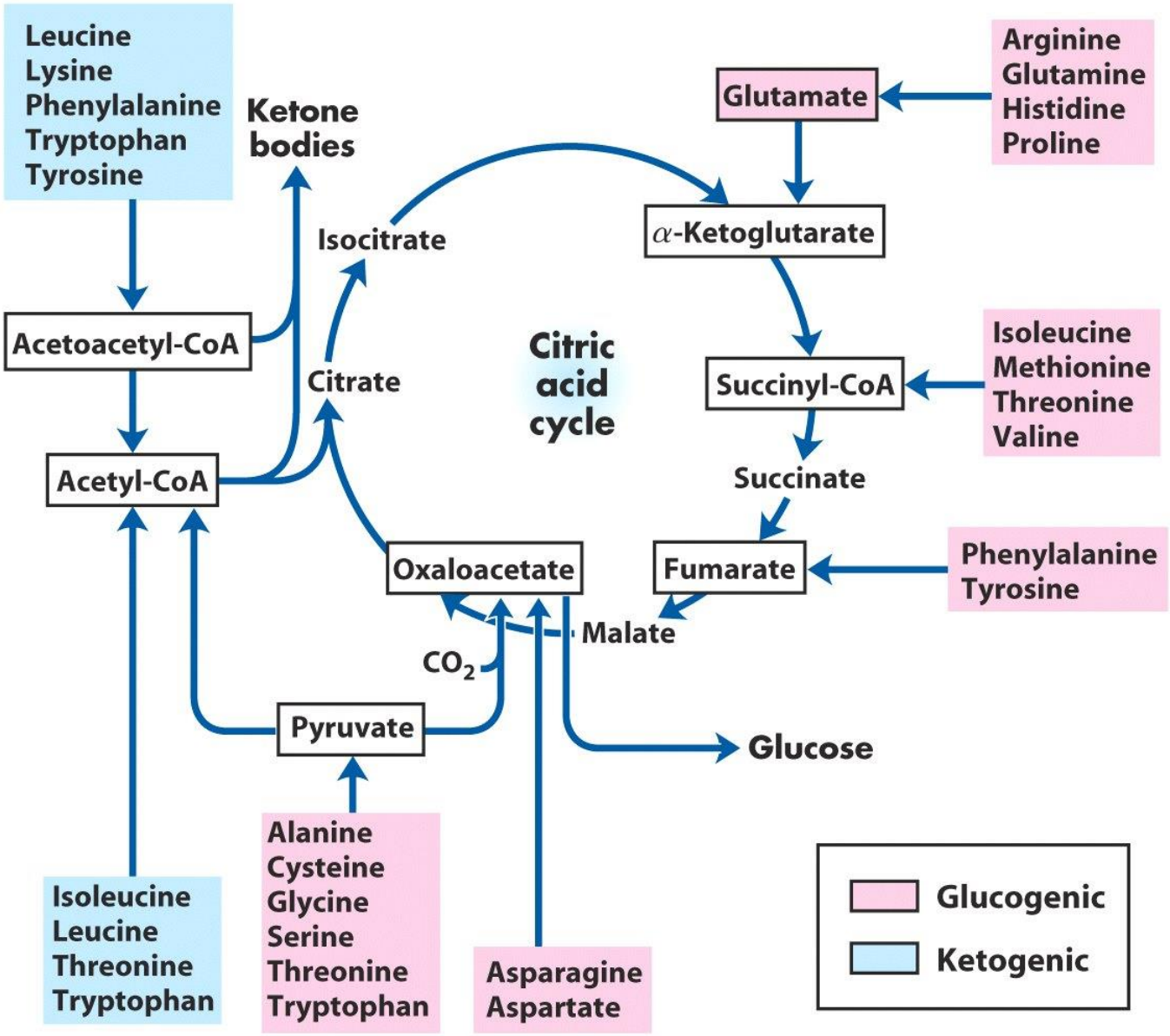
$$Se^{2-} + ATP + H_2O \rightarrow AMP + P_i +$$



Glutathionperoxidaea (2 GSH + H_2O_2 → 2 H_2O + G-S-S-G)

Dejodase of thyronine (thyroxin T4 → trijodothyronin T3)

Thioredoxin reduktase (ribose → deoxyribose)


Leucine
Lysine
Phenylalanine
Tryptophan
Tyrosine
Ketone bodies
Glutamate
Arginine
Glutamine
Histidine
Proline
Isocitrate
α-Ketoglutarate
Acetoacetyl-CoA
Citric acid cycle
Isoleucine
Methionine
Threonine
Valine
Citrate
Succinyl-CoA
Acetyl-CoA
Succinate
Oxaloacetate
Fumarate
Phenylalanine
Tyrosine
Malate
CO_2
Pyruvate
Glucose
Isoleucine
Leucine
Threonine
Tryptophan
Alanine
Cysteine
Glycine
Serine
Threonine
Tryptophan
Asparagine
Aspartate
Glucogenic
Ketogenic

	End Products	Amino Acid
Ketogenic	Acetyl CoA Acetoacetic Acid	Trp, Tyr, Thr, Ile, Leu, Lys, Phe
Glucogenic	Pyruvate	Ala, Cys, Gly, Ser, Thr, Trp
	α-ketoglutarate	Arg, Glu, Gln, His, Pro,
	Succinyl-CoA	Ile, Met, Val, Thr
	Fumarate	Asp, Phe, Tyr
	Oxaloacetate	Asp, Asn

Blue: Glucogenic and ketogenic
RED: ONLY Ketogenic