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Protein and amino acid metabolism

SIBC511

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July 6th, 2007

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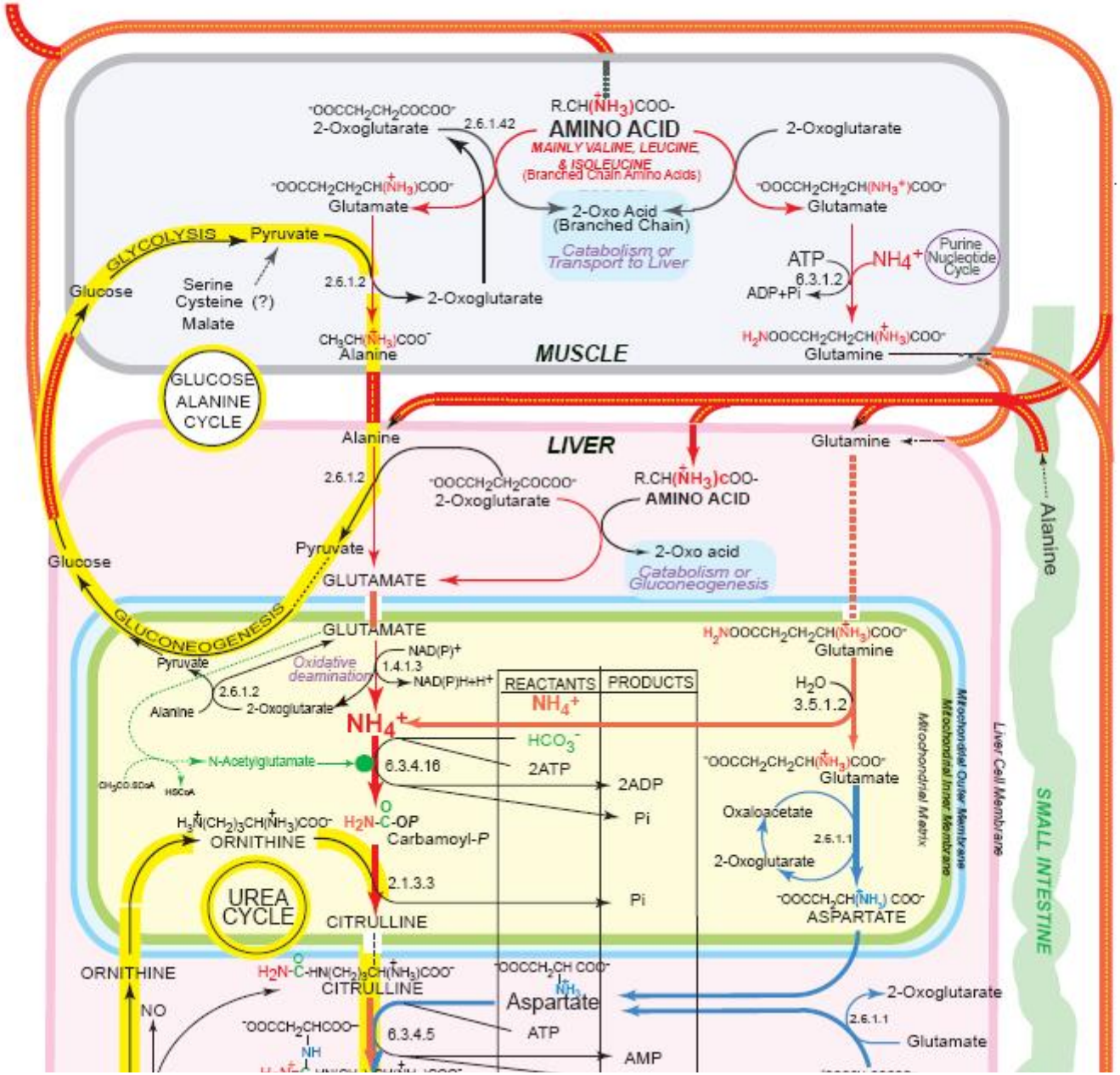
Topics

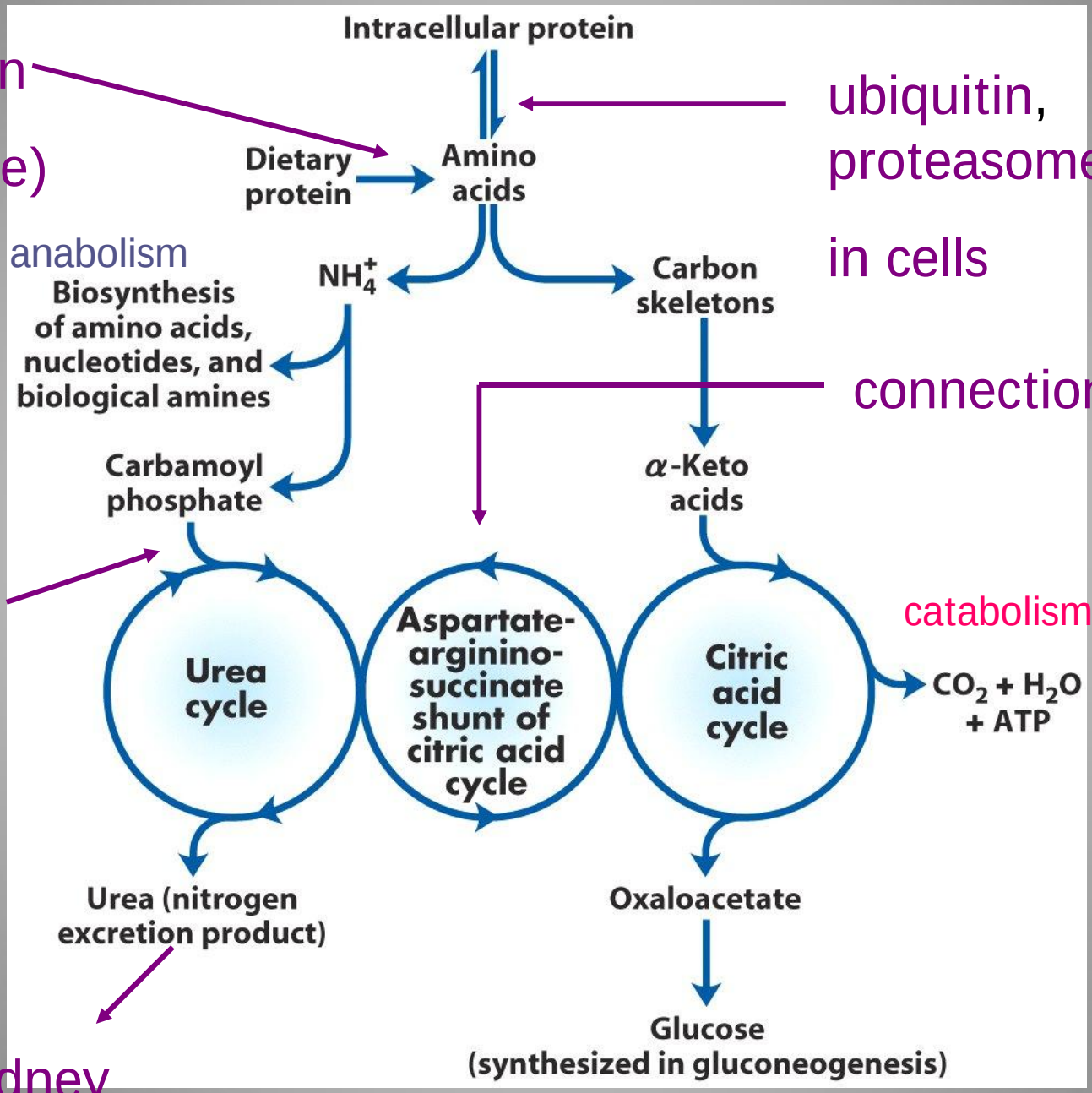
- Digestion and absorption of proteins
- Intracellular degradation of proteins
- Nitrogen metabolism
  - Nitrogen balance
- Amino acid degradation
- Urea cycle
- Metabolism of the carbon skeletons of amino acids
- Inherited diseases of amino acid metabolism
- Amino acid biosynthesis
- Conversions of aa to other biological amines

DEAMINATION OF AMINO ACIDS - THE UREA CYCLE



Digestion

(intestine)



ubiquitin, proteasome

in cells

connection

in liver

catabolism

via kidney

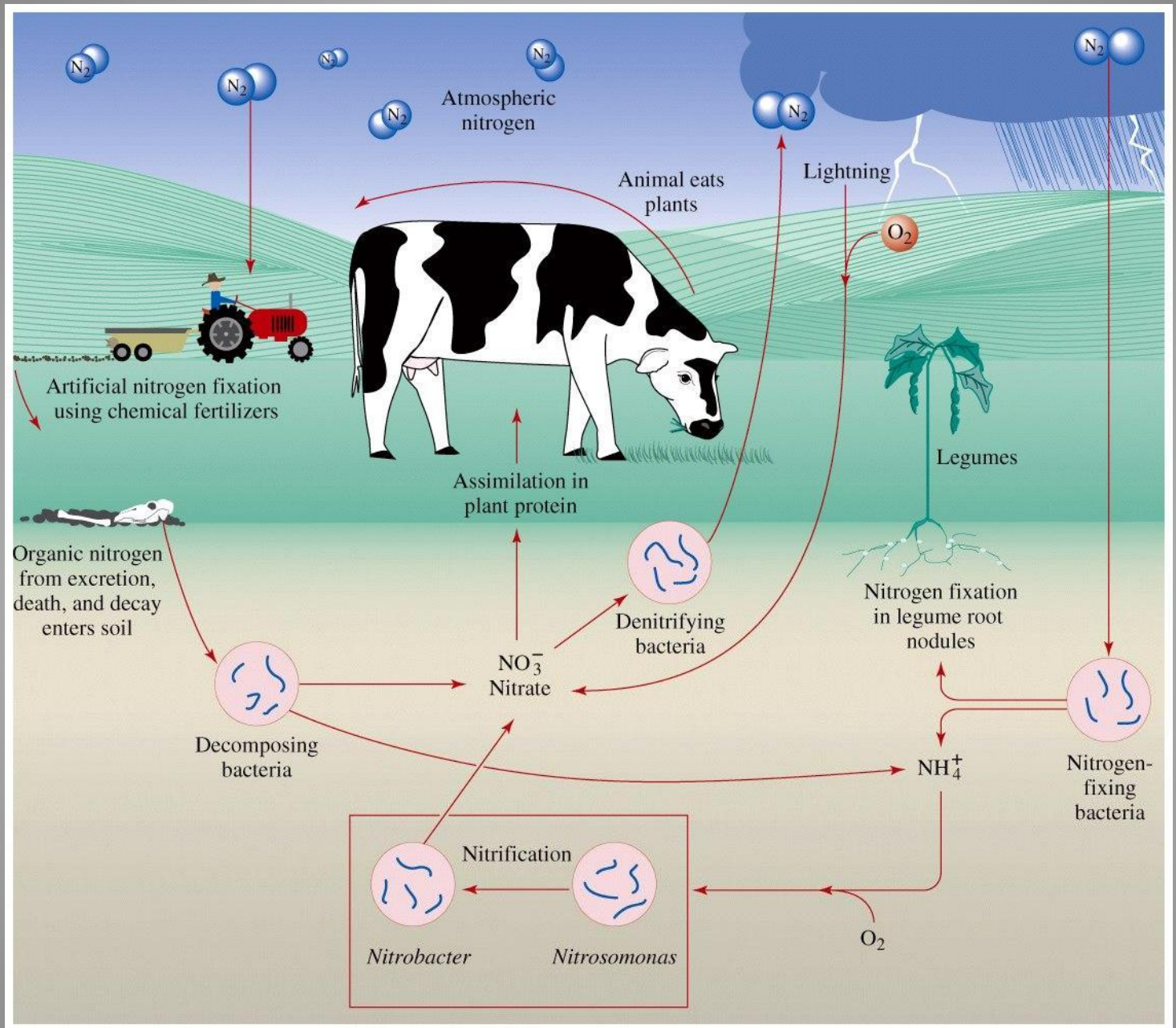
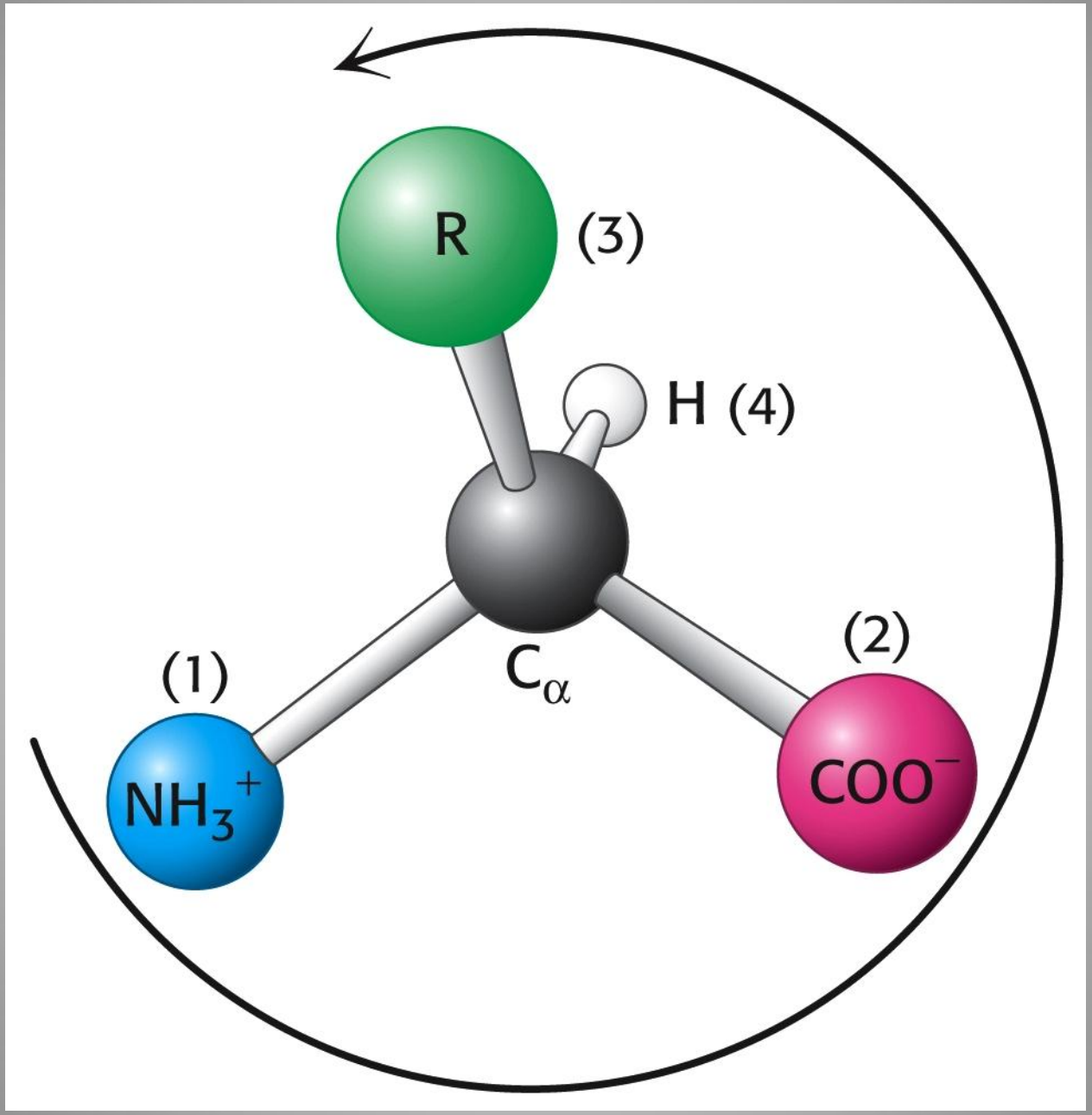
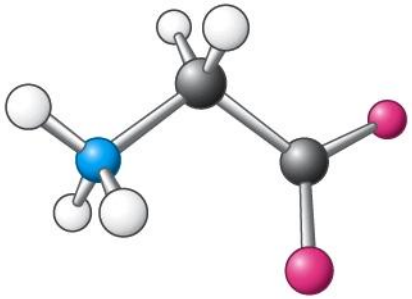
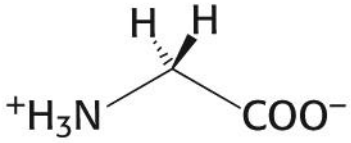


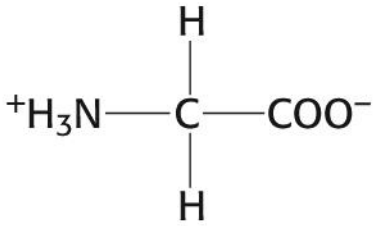
Figure 19-1 Concepts in Biochemistry, 3/e



Glycine (Gly, G)

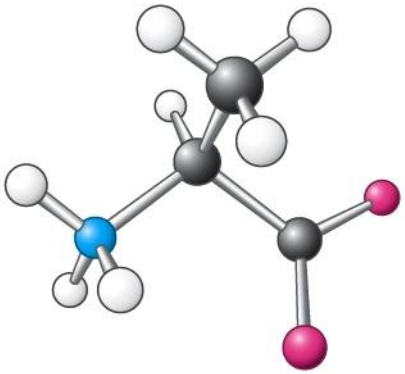


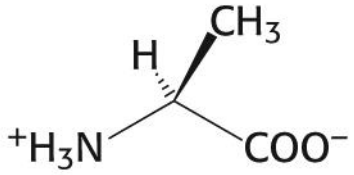


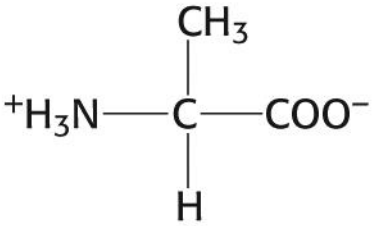


Glycine (Gly, G)

Alanine (Ala, A)

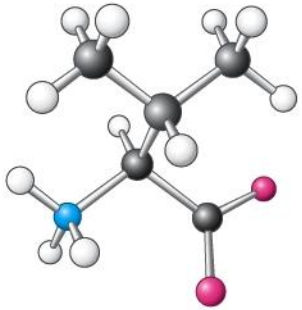


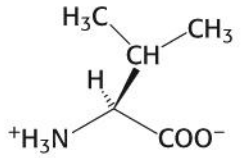


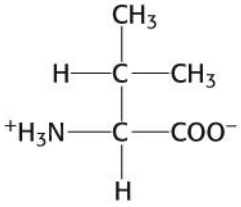


Alanine (Ala, A)

Valine (Val, V)

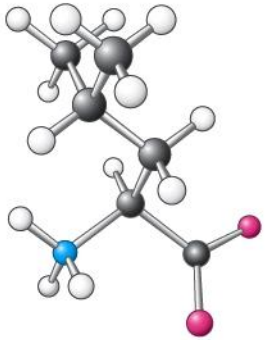


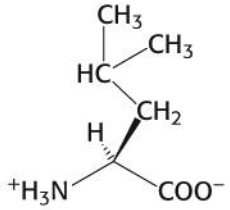


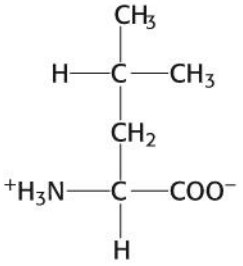


Valine (Val, V)

Leucine (Leu, L)

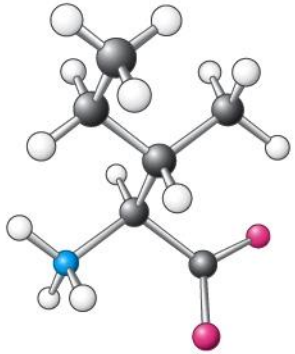


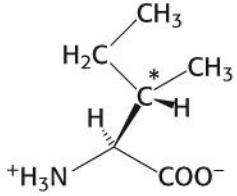


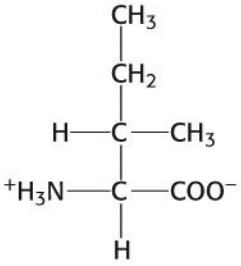


Leucine (Leu, L)

Isoleucine (Ile, I)

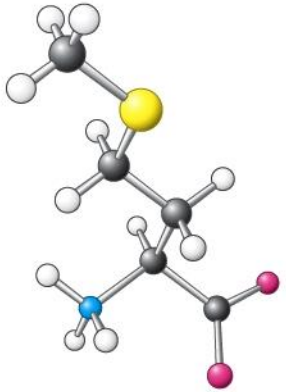


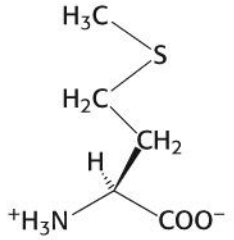


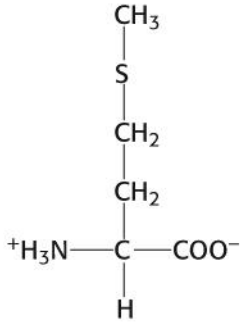


Isoleucine (Ile, I)

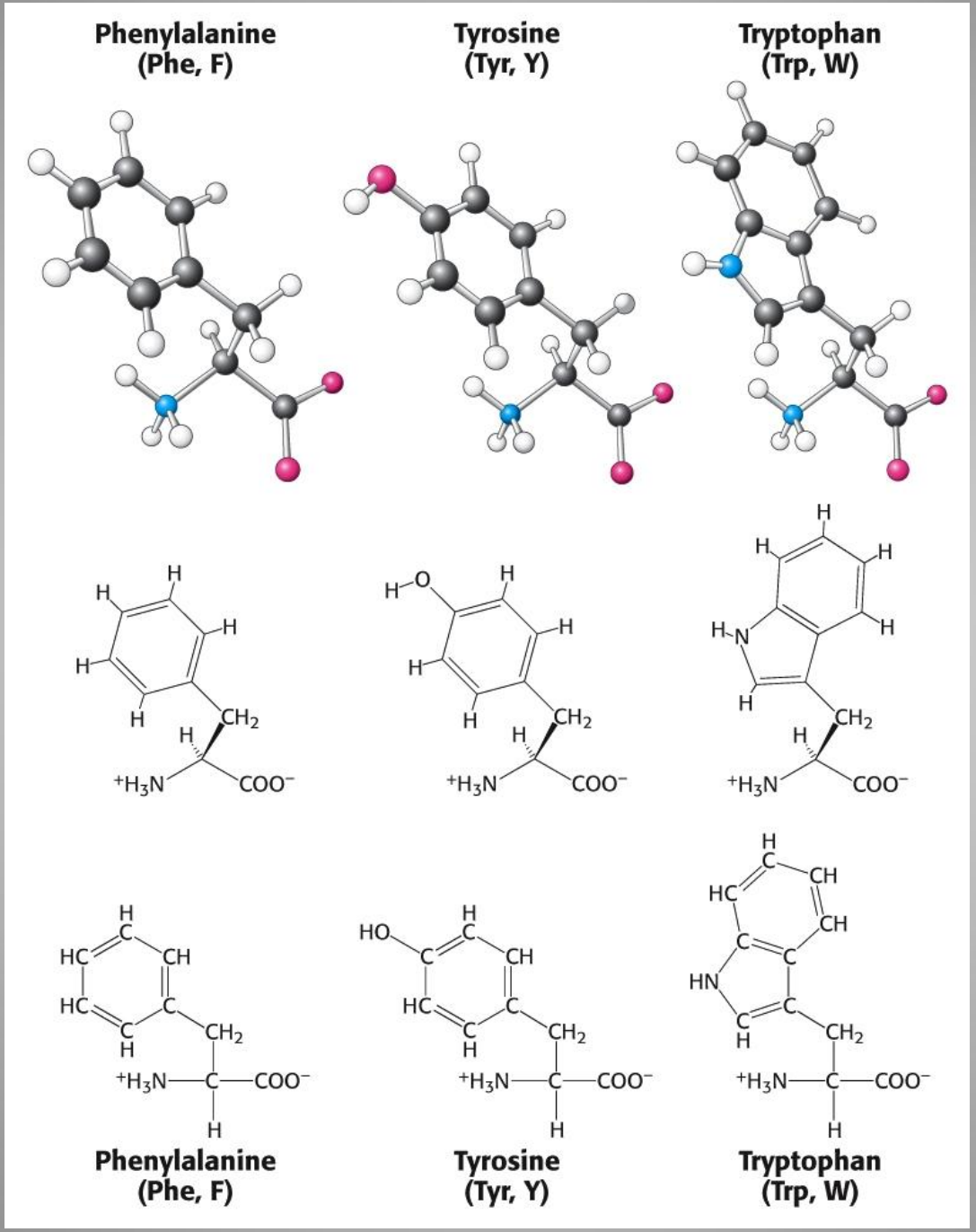
Methionine (Met, M)

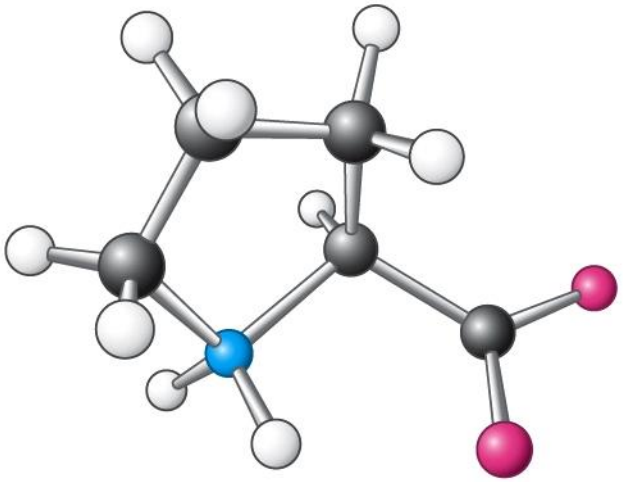


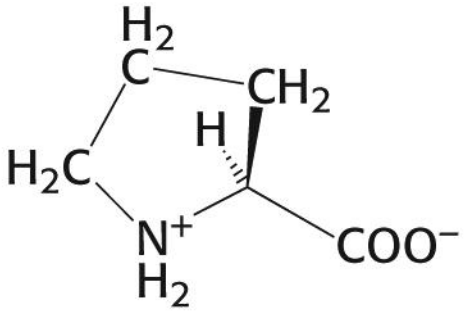




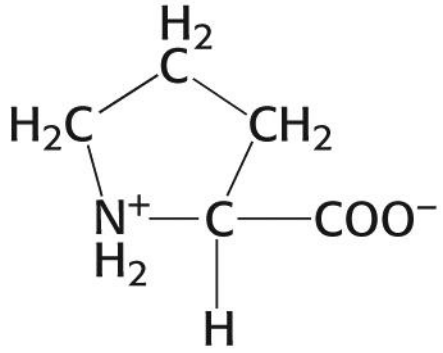
Methionine (Met, M)

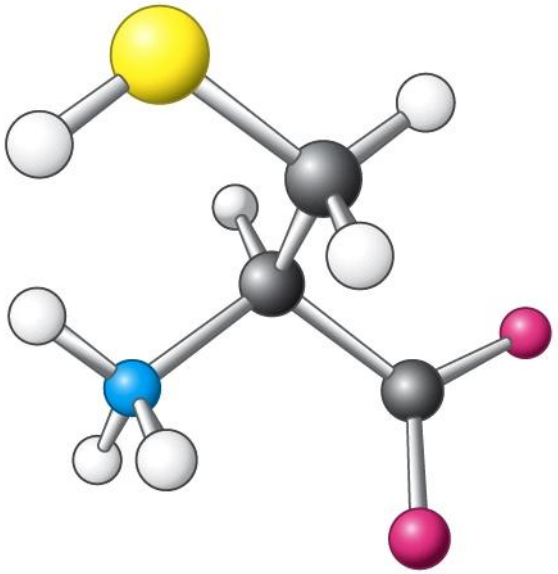


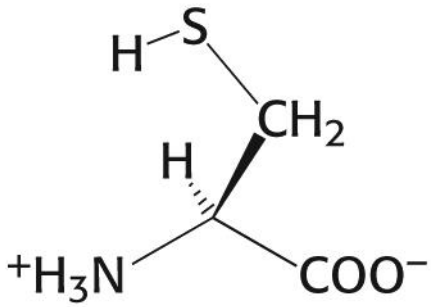




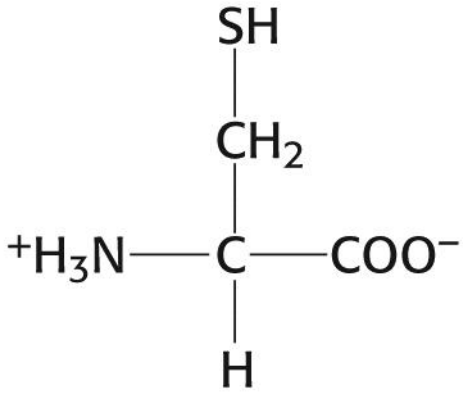
Proline
(Pro, P)



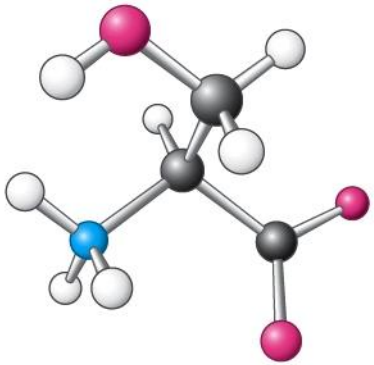


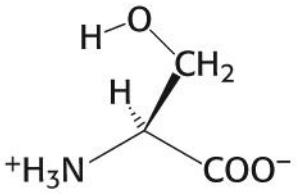


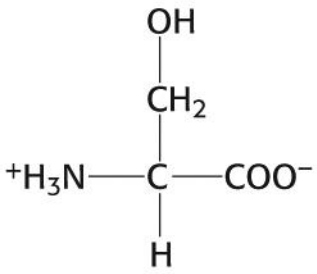
Cysteine
(Cys, C)



Serine (Ser, S)

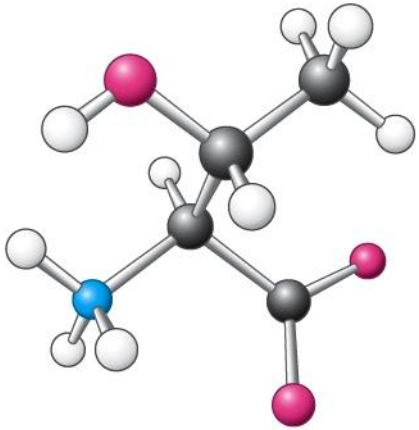


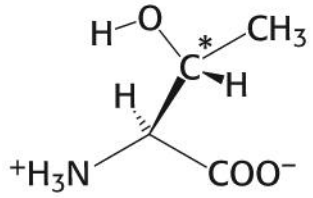


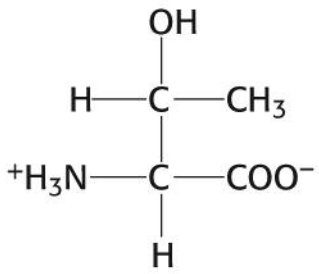


Serine (Ser, S)

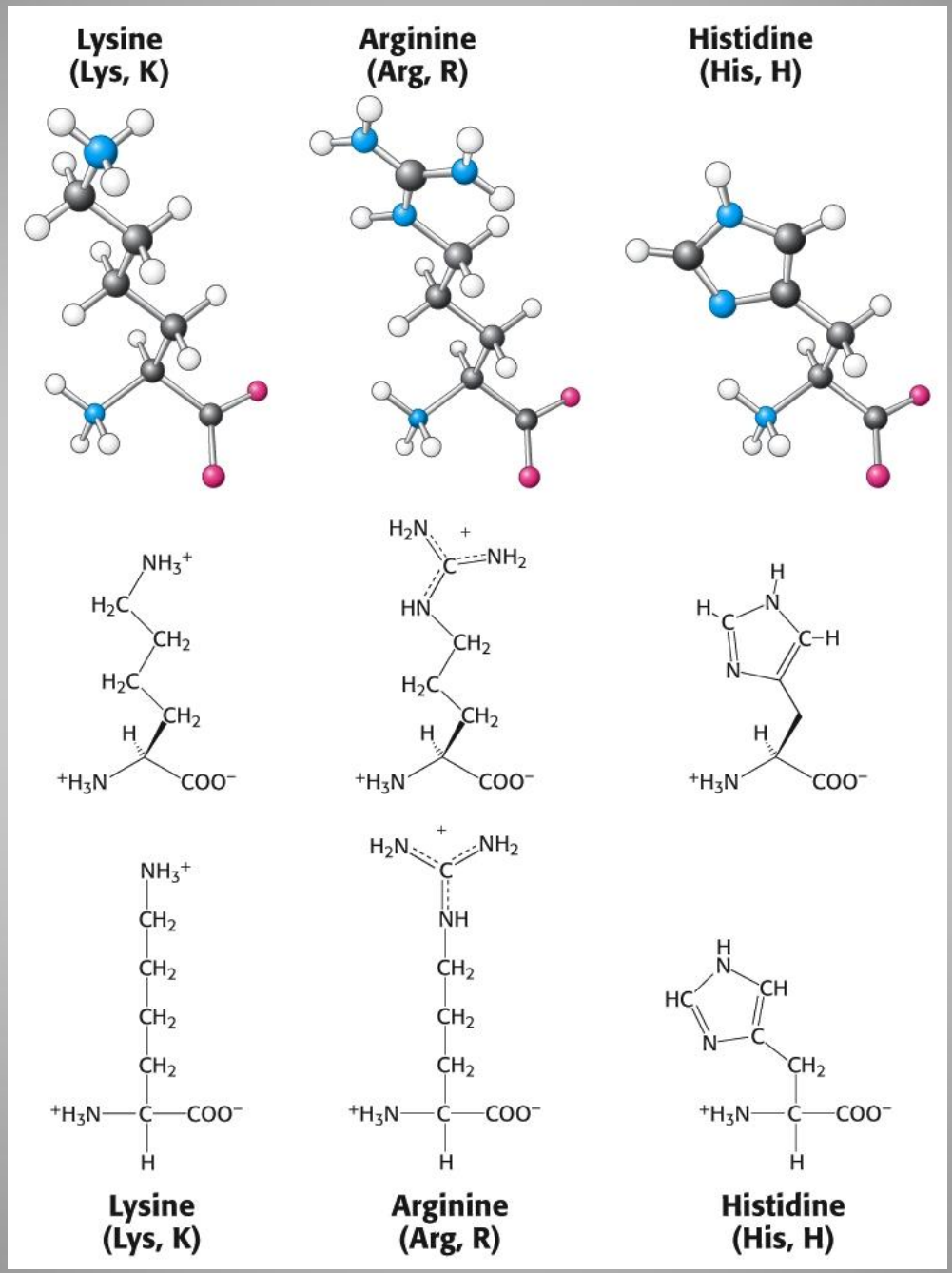
Threonine (Thr, T)

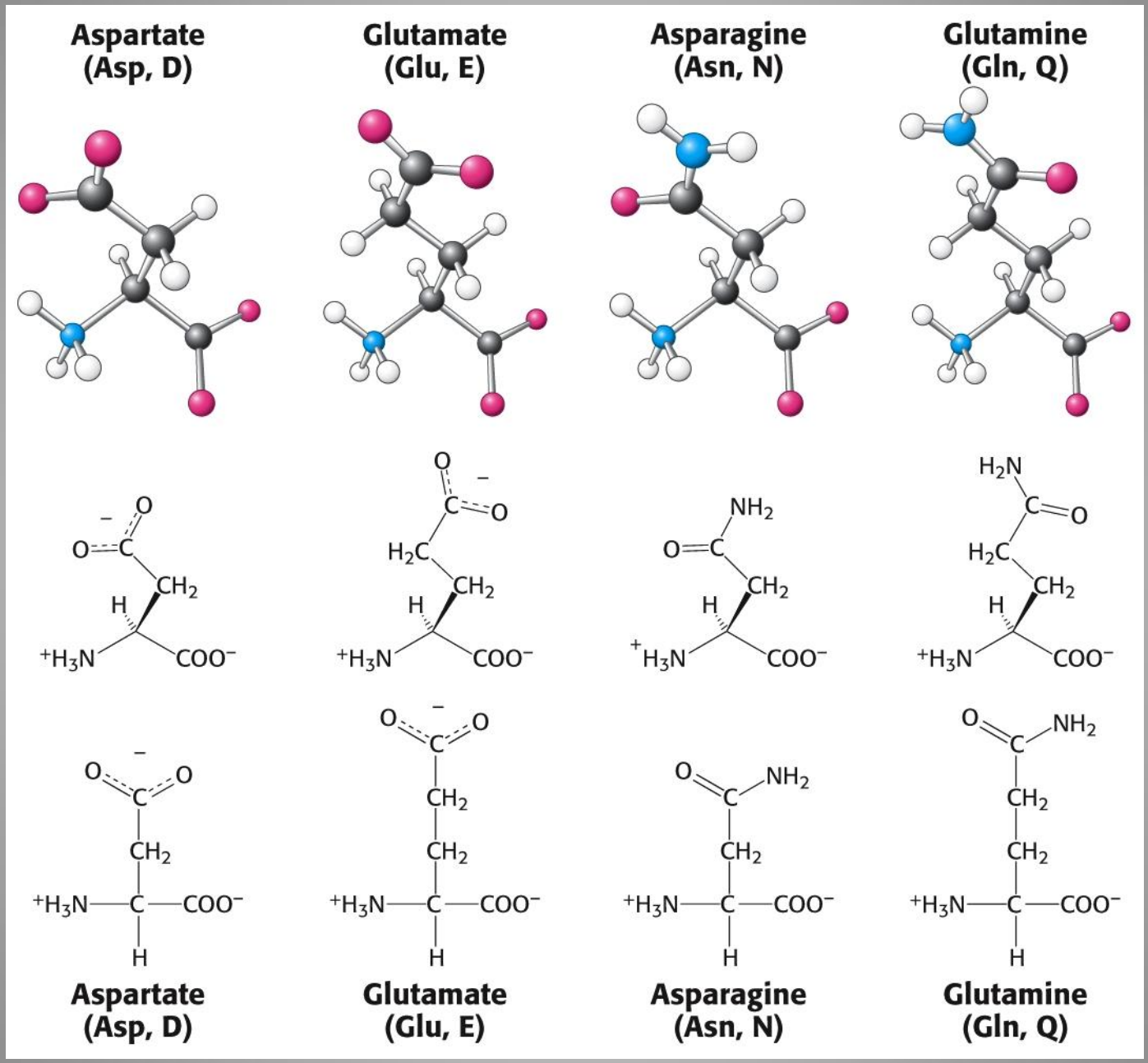






Threonine (Thr, T)





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Protein turnover (intracellular)

TABLE 23.2 Dependence of the half-lives of cytoplasmic yeast proteins on the identity of their amino-terminal residues

Highly stabilizing residues ($t_{1/2}$ > 20 hours)			
Ala	Cys	Gly	Met
Pro	Ser	Thr	Val
Intrinsically destabilizing residues ($t_{1/2}$ = 2 to 30 minutes)			
Arg	His	Ile	Leu
Lys	Phe	Trp	Tyr
Destabilizing residues after chemical modification ($t_{1/2}$ = 3 to 30 minutes)			
Asn	Asp	Gln	Glu

Source: J. W. Tobias, T. E. Schrader, G. Rocap, and A. Varshavsky. *Science*

"N-end rule"

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TABLE 23.3 Processes regulated by protein degradation

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

Table 23-3
Biochemistry, Sixth Edition

- Proteasome digests the ubiquitin-tagged proteins

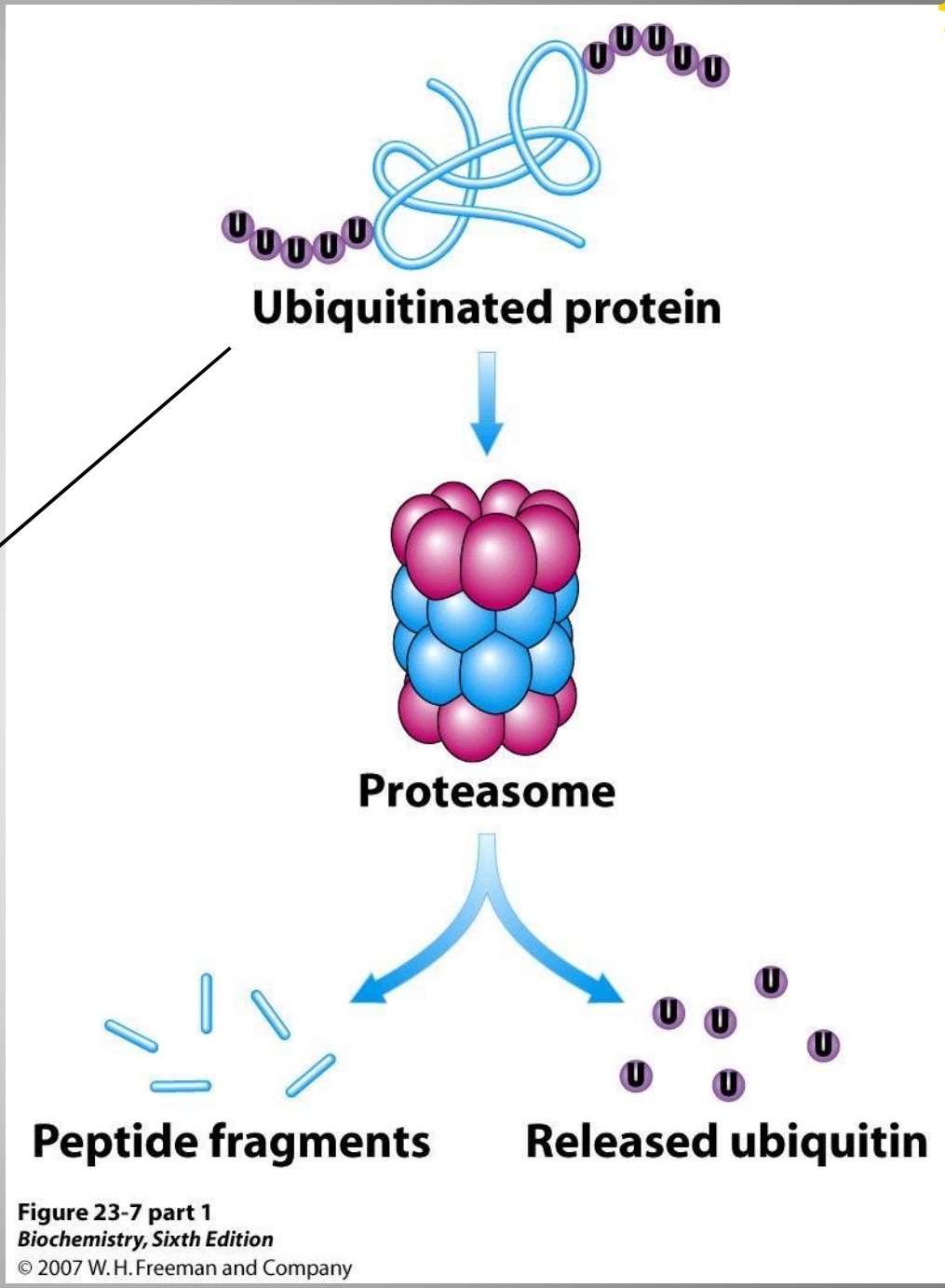
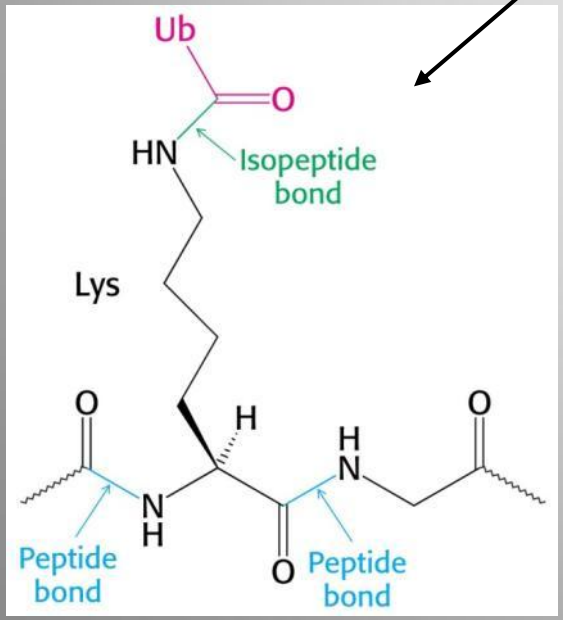


Figure 23-7 part 1
Biochemistry, Sixth Edition



- Ubiquitin (8.5 kD)
- Present in all eukaryotes
- Highly conserved
- Form isopeptide bond with target proteins

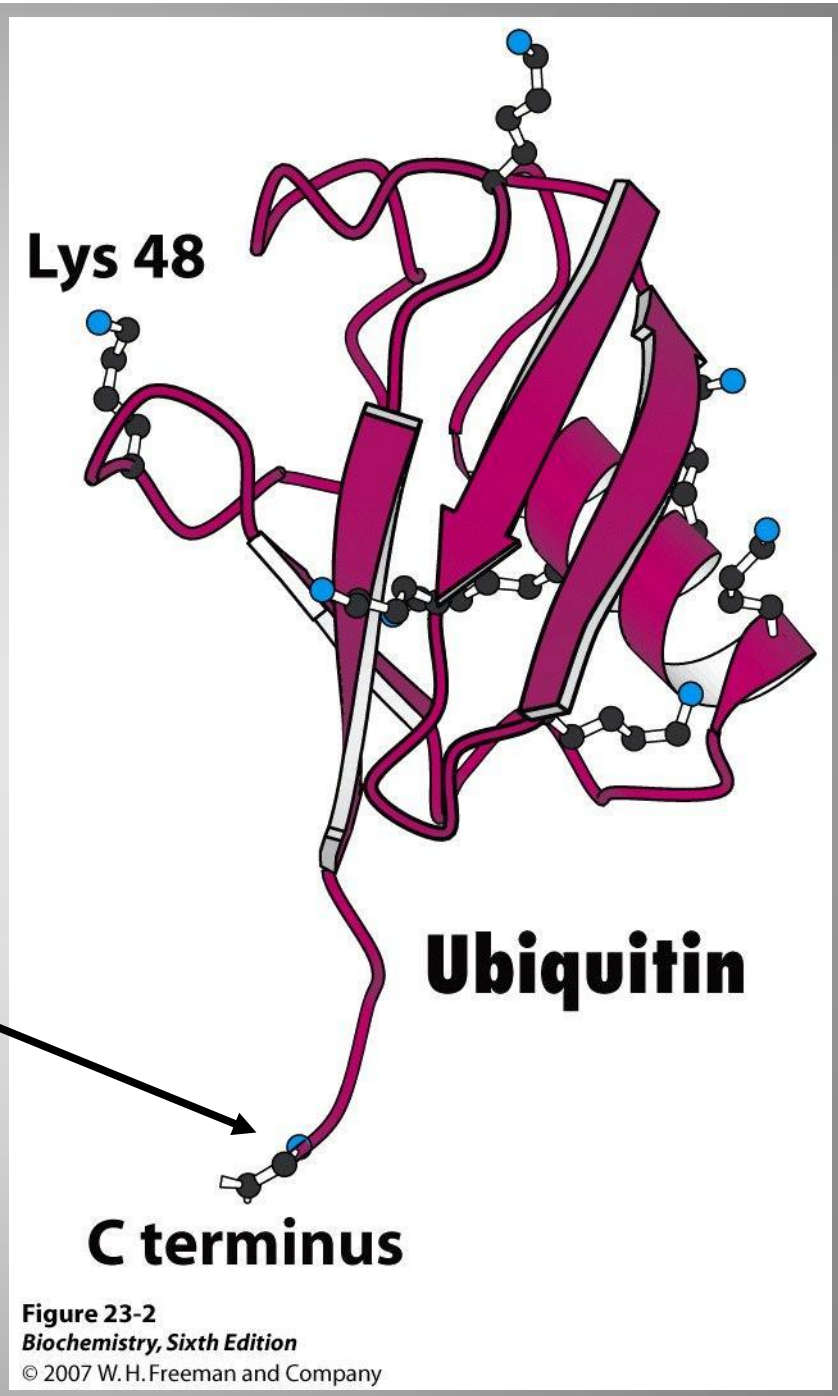


Figure 23-2
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Ubiquitin conjugation

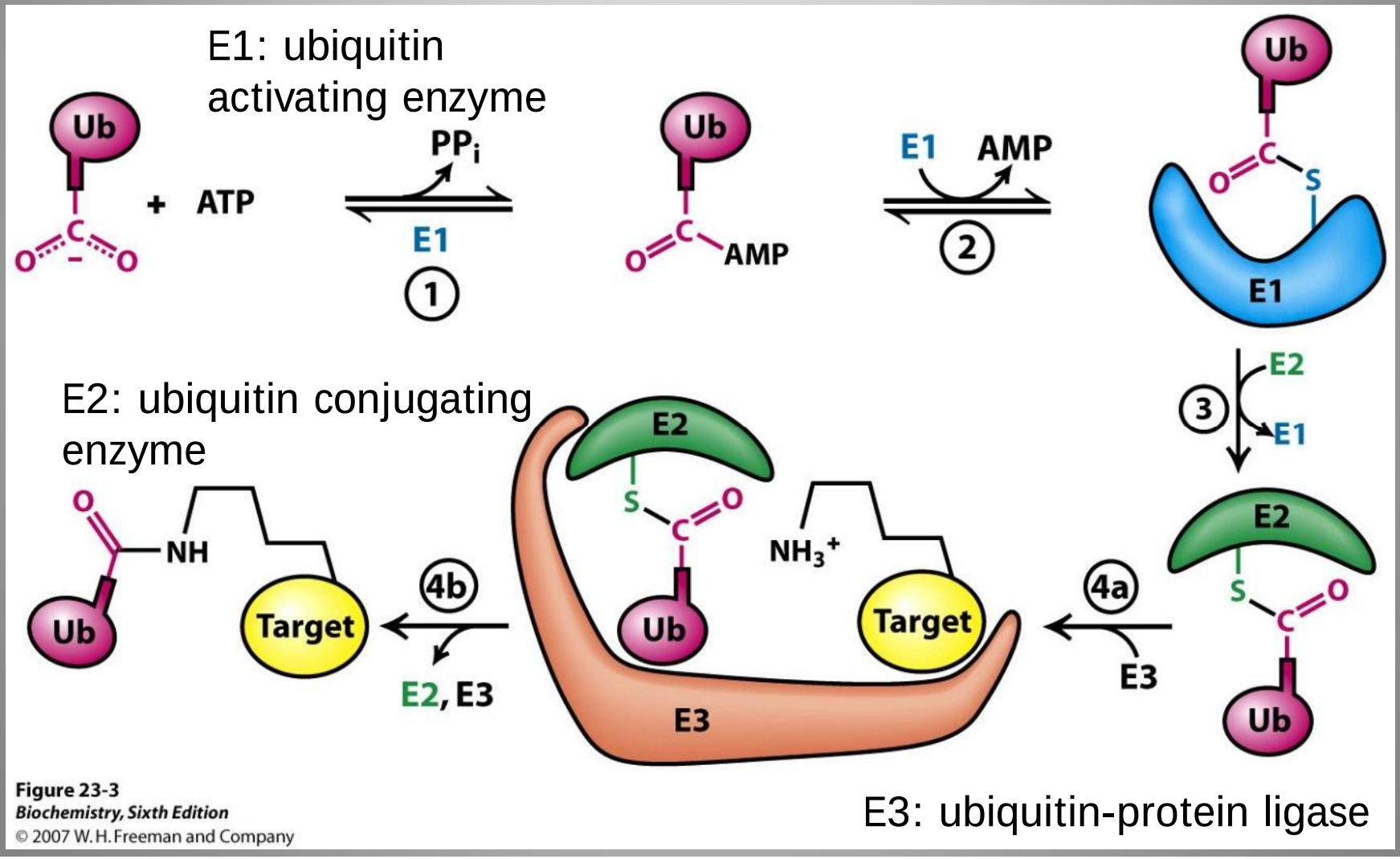

Figure 23-3
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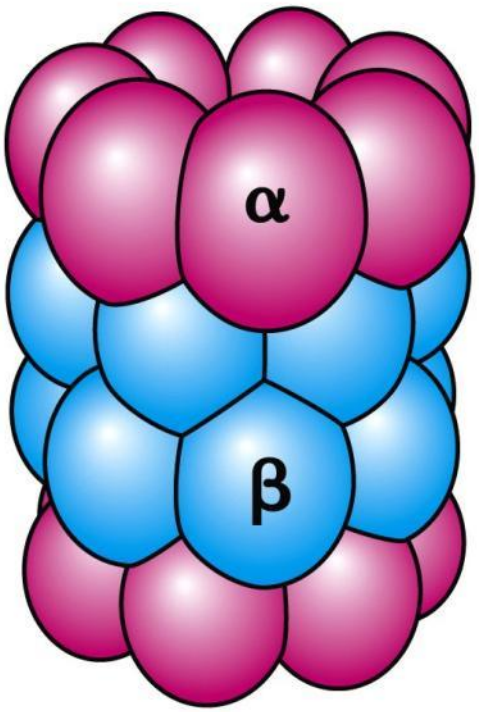
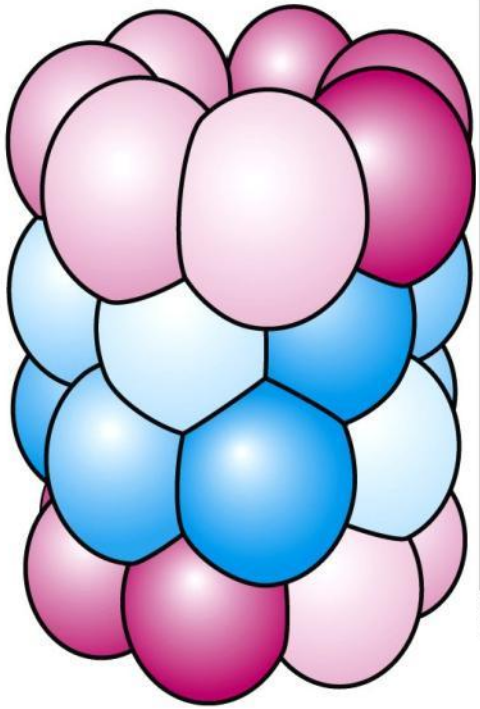
26S proteasome



Archaeal proteasome



Eukaryotic proteasome

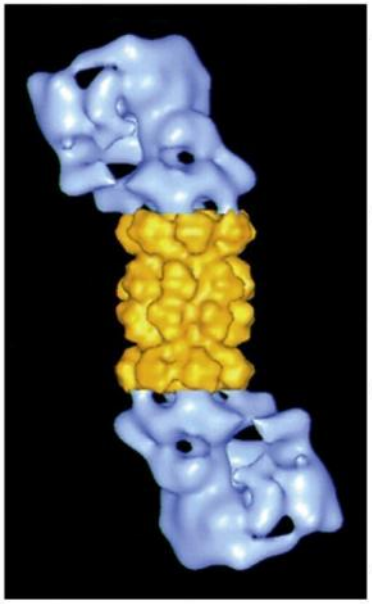


Figure 23-6
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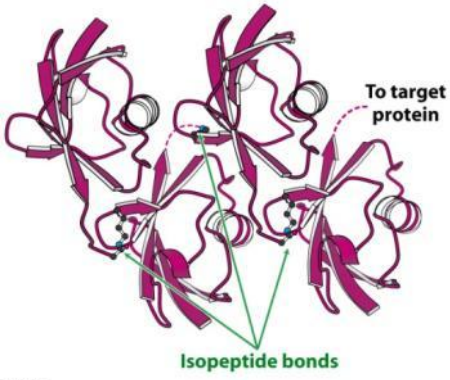

Figure 23-8
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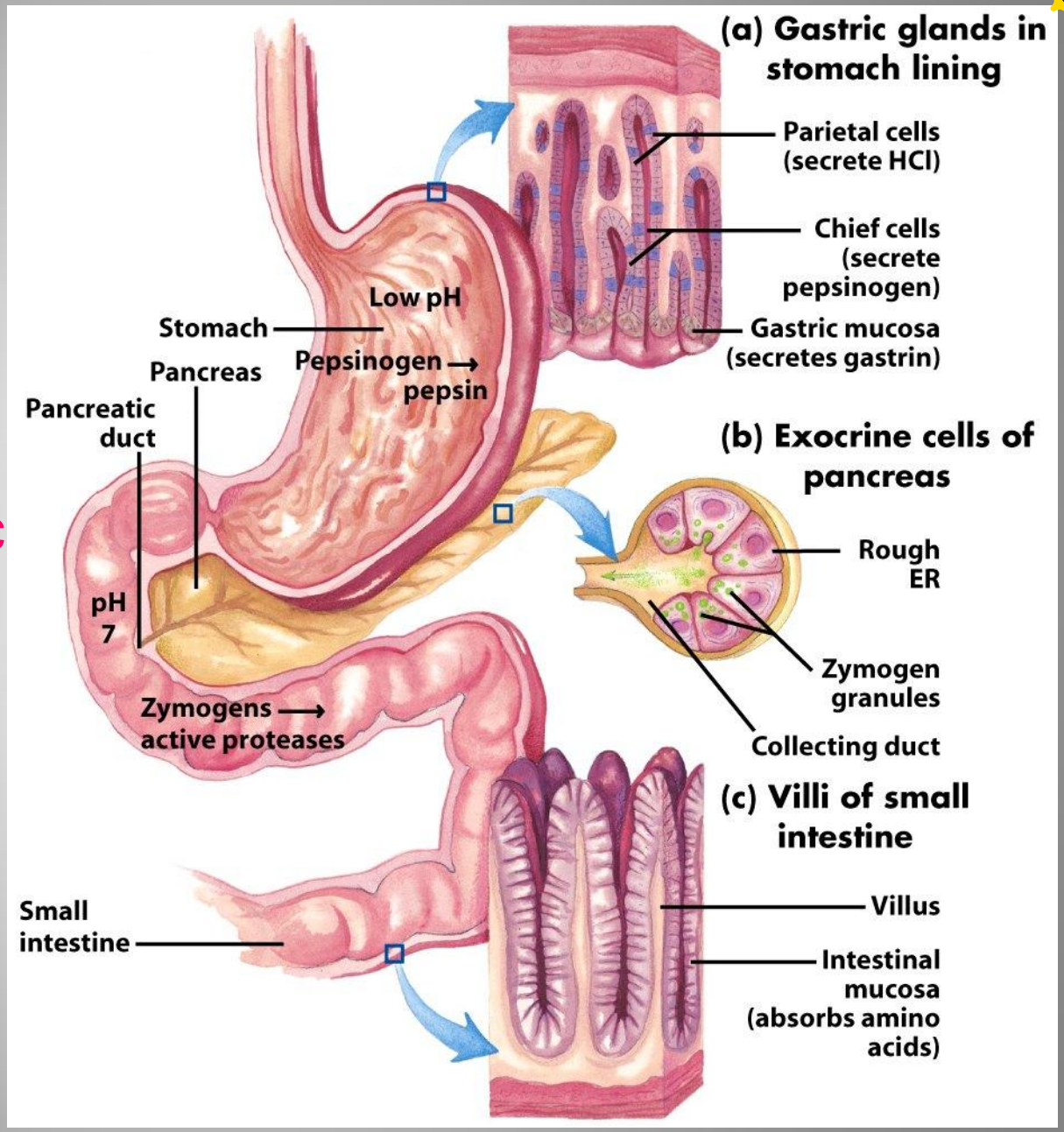
Applications

- E3, the reader of N-terminal residues
- Cyclin destruction in cell cycle
- Proteins rich in PEST sequence
- HPV encodes protein that activates E3
  - then E3 ubiquitinates p53 (tumor suppresor)→degraded → →cervical CA

Digestion

- acid denaturation
- pepsin,
  - primary proteolytic enzyme
- pancreatic enzymes
  - trypsin
  - chymotrypsin
  - elastase



Absorption

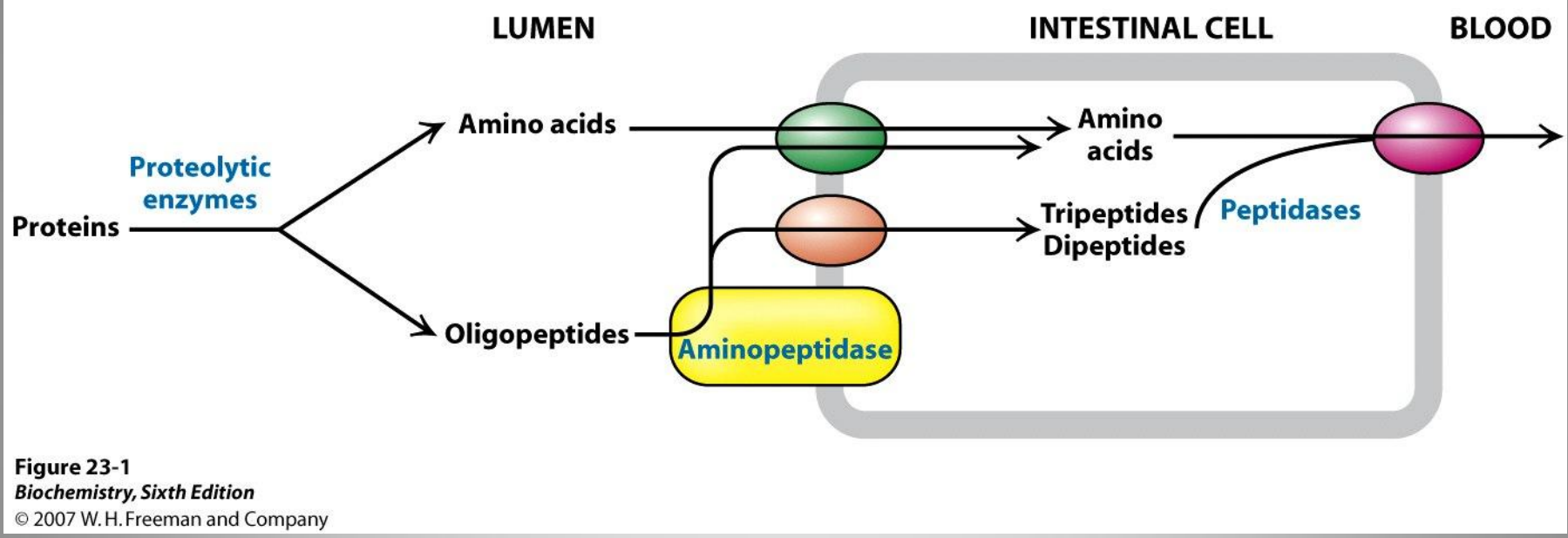
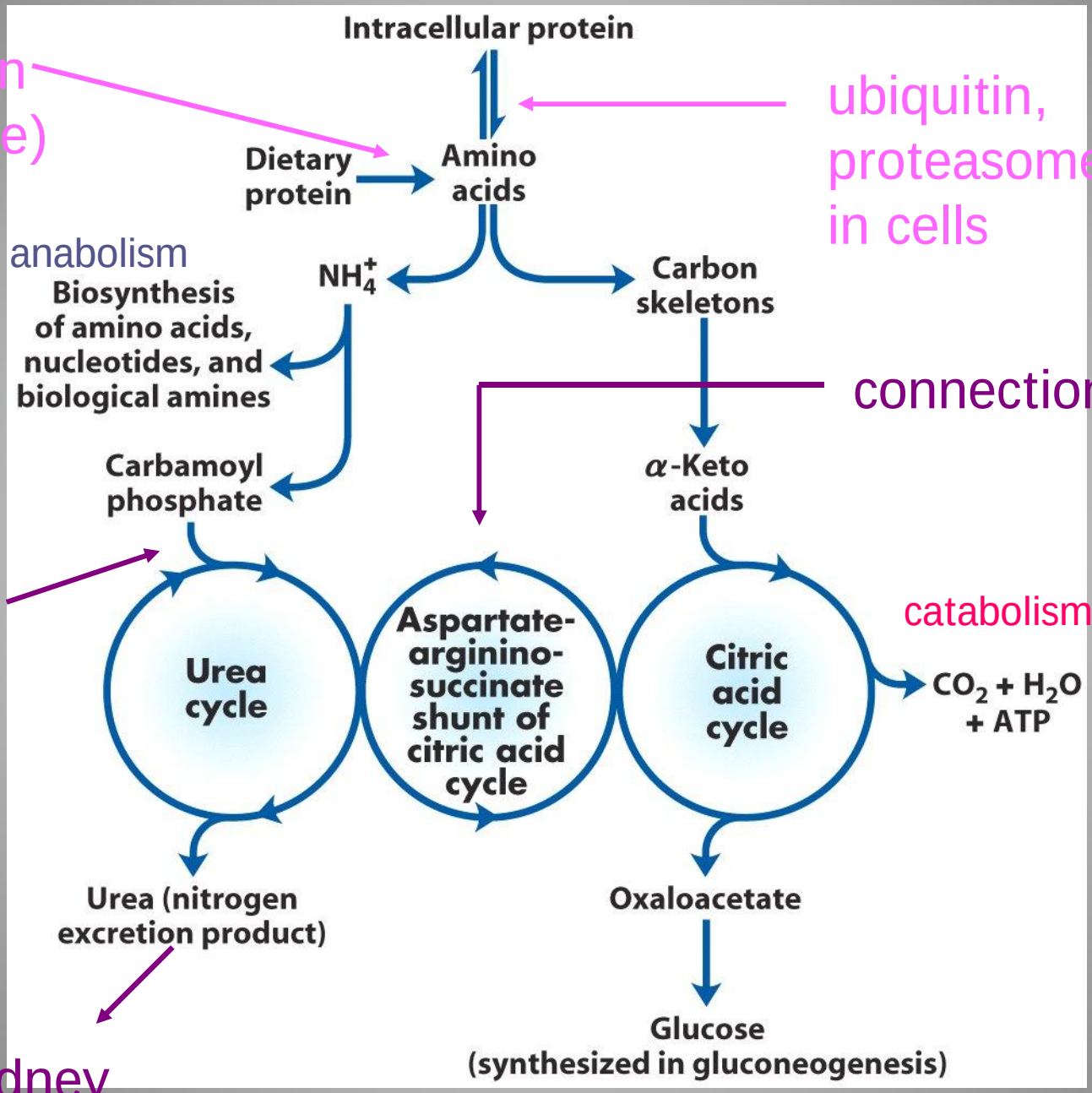


Figure 23-1
Biochemistry, Sixth Edition

- oligpeptides=di-, tripeptides--absorbable



Nitrogen balance

A well-nourished adult is said to be in nitrogen equilibrium or normal nitrogen balance if

Dietary intake of nitrogen = Nitrogen loss (through excretion and other processes, such as perspiration)

Positive nitrogen balance occurs when:

Dietary intake of nitrogen > Nitrogen loss

Negative nitrogen balance occurs when:

Dietary intake of nitrogen < Nitrogen loss

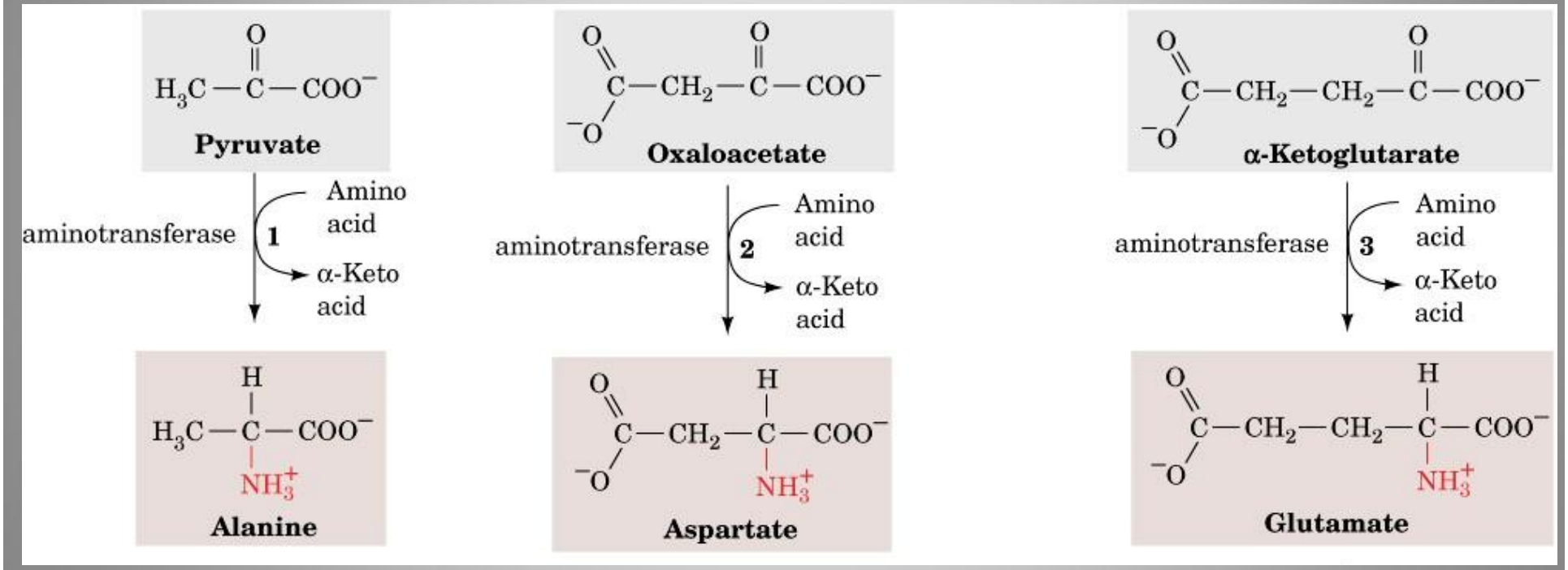
Nitrogen balance (application)

- Daily protein recommendation: 0.8 g/kg/day
- 1.4-1.7 g/kg/day for strength athletes to maintain muscle mass
- Balance between the rate of protein synthesis and protein breakdown
- Excess intake > 3 g/kg/day have negative effect

Amine group removal (deamination)

1. Transamination : transaminase
2. Oxidative deamination : glutamate dehydrogenase, L-amino acid oxidase
3. Dehydratase : removal of water first and then followed by deamination e.g serine dehydratase and threonine dehydratase

α-Amino acids and α-keto acids



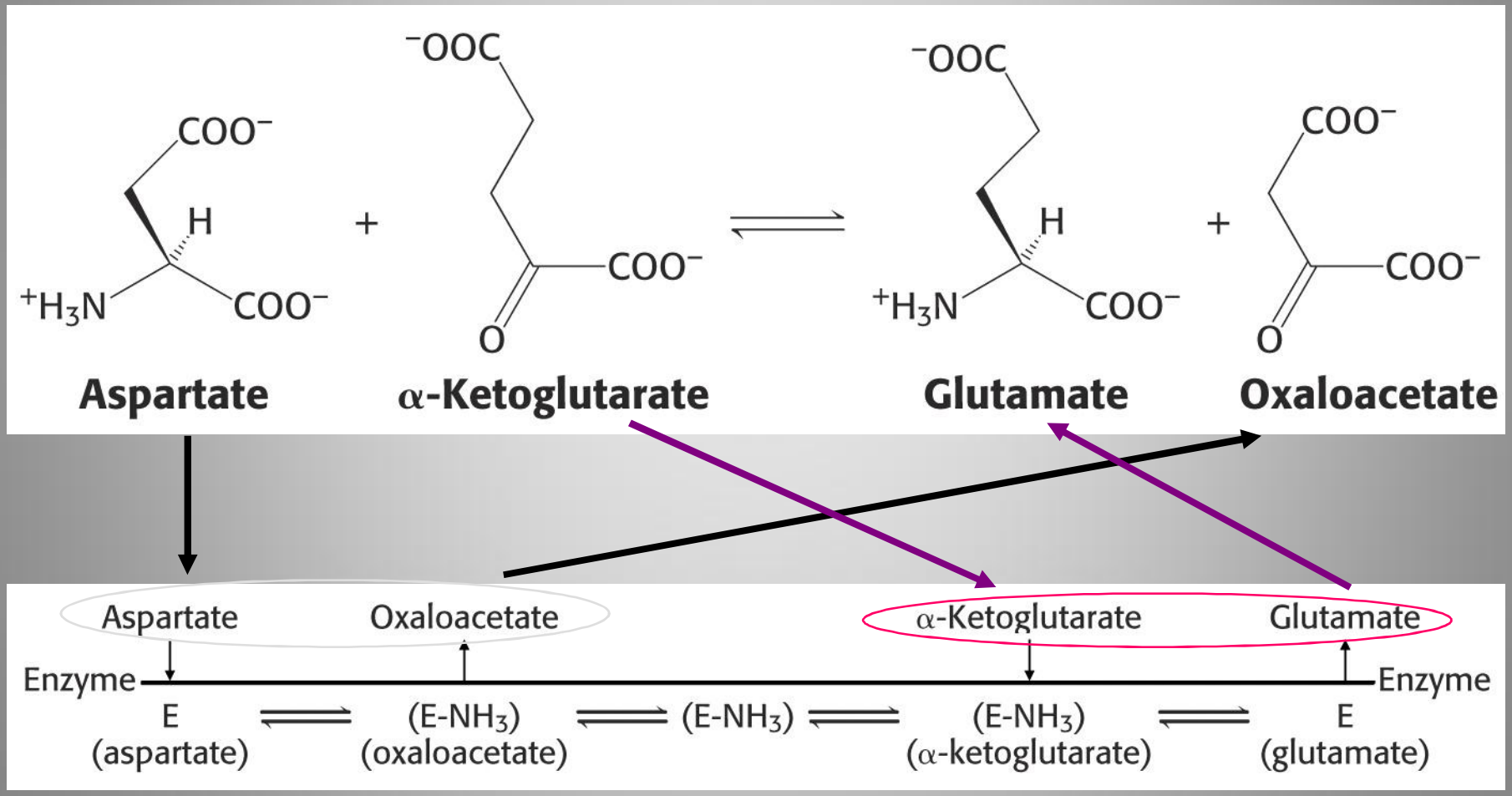
Transamination

- Enz: transaminase, aminotransferase
- Mec: transfer of α-amino group from α-amino acid to an α-ketoacid—usu. α-ketolgutarate
- Aspartate aminotransferase:

  aspartate + α-ketoglutarate ↔ oxaloacetate + glutamate
- Alanine amino transferase:

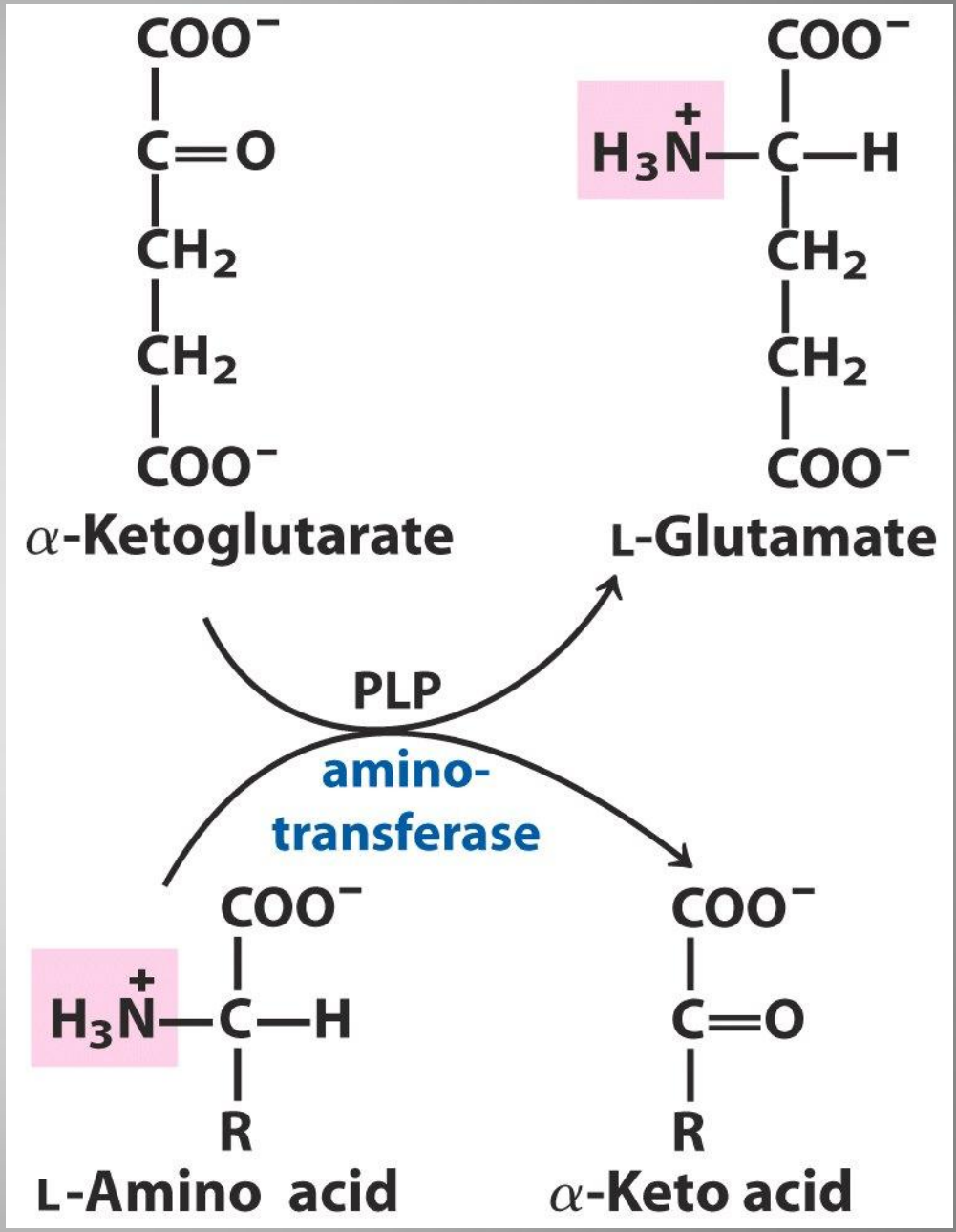
  alanine + α-ketoglutarate ↔ pyruvate + glutamate

Ping-pong reaction



Transamination

- α-ketoglutate often used by many transaminase
- all transaminases contain pyridoxal phosphate (prosthetic gr)
- Occur in most tissues



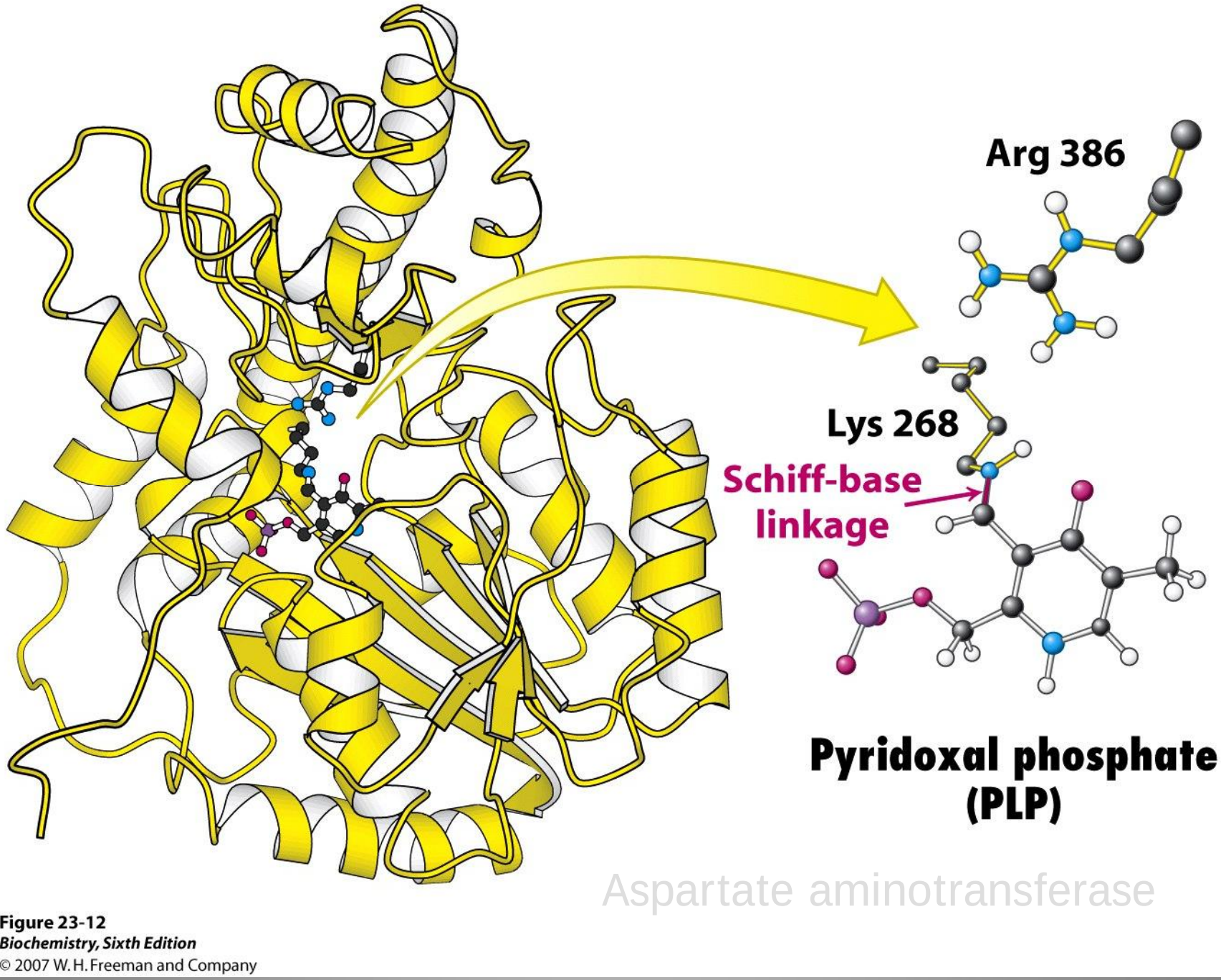


Figure 23-12
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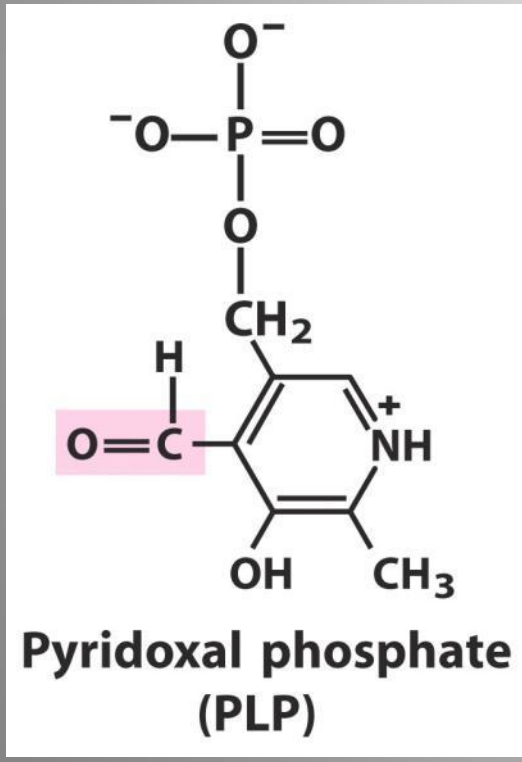
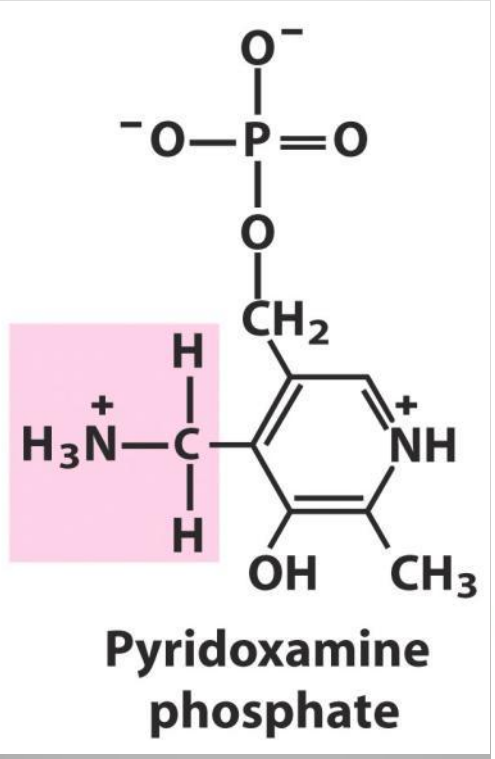
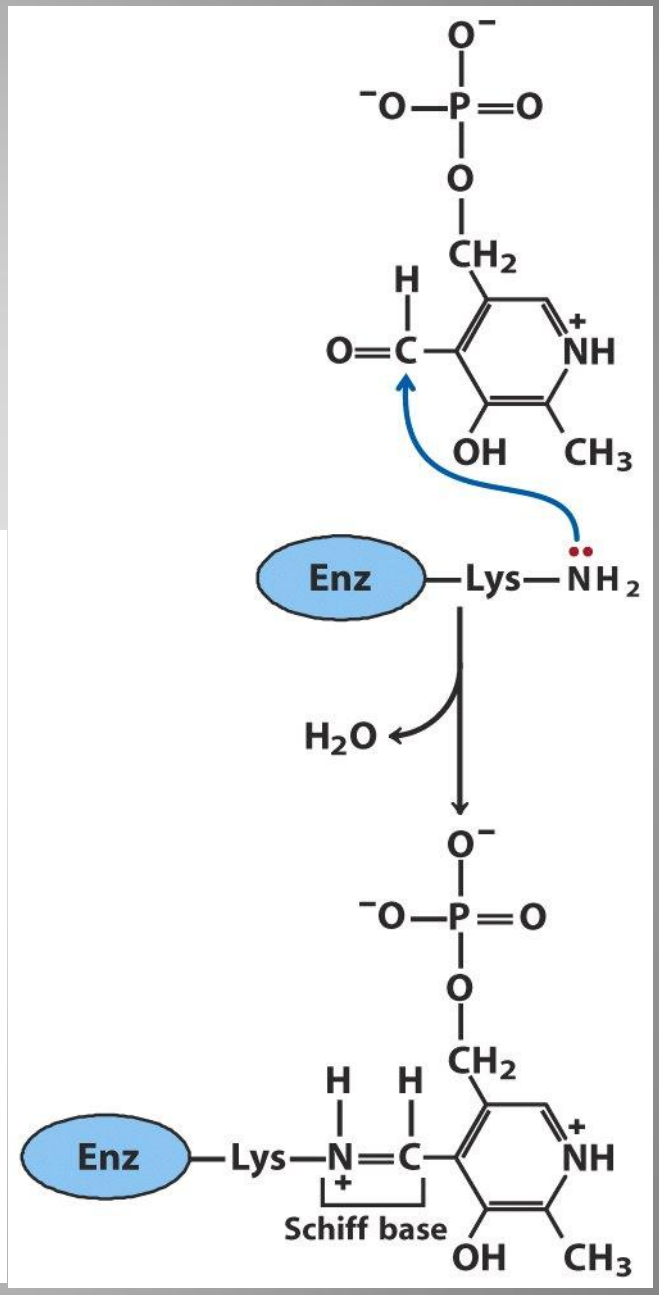
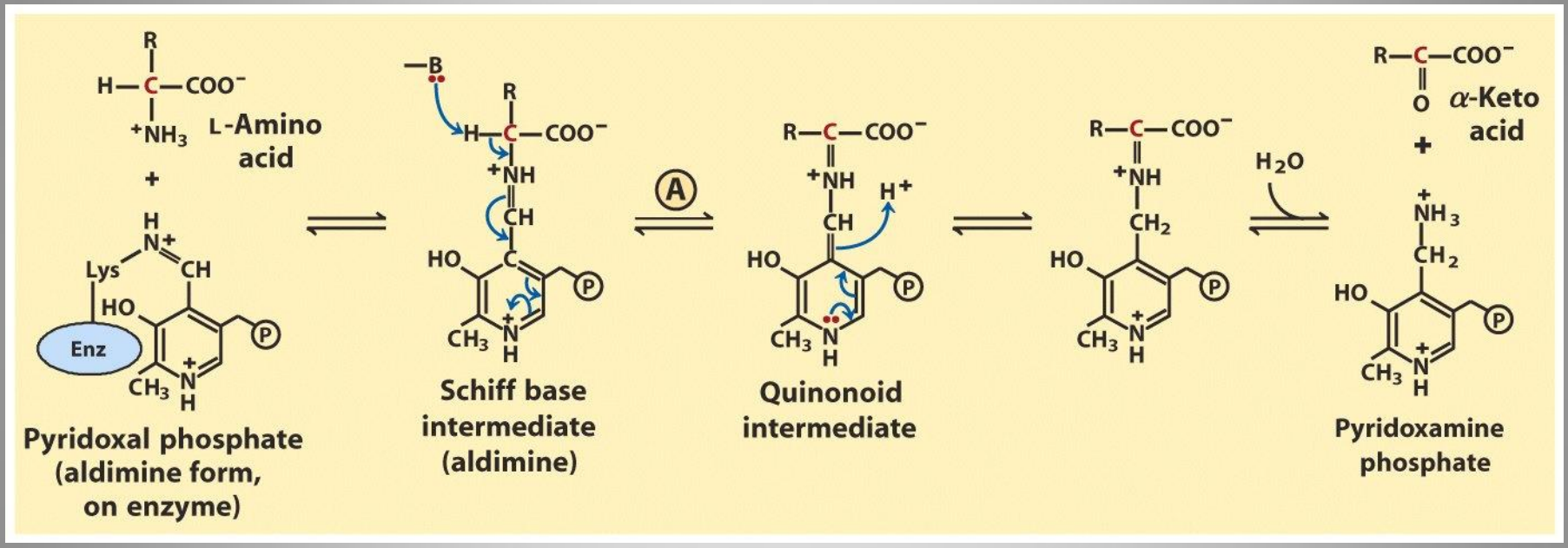
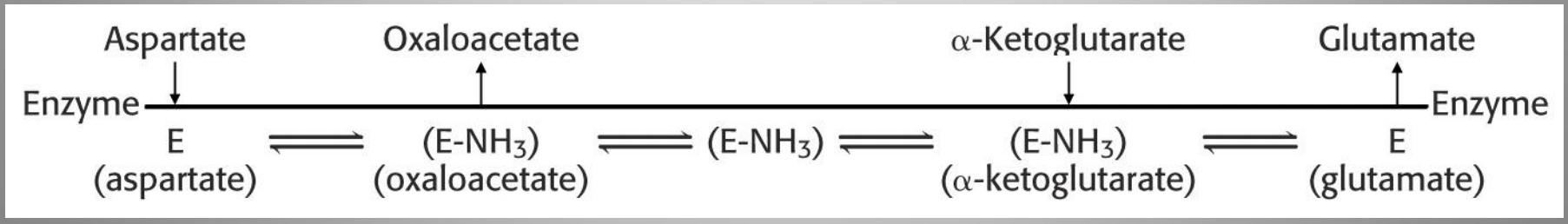
Vitamin B6



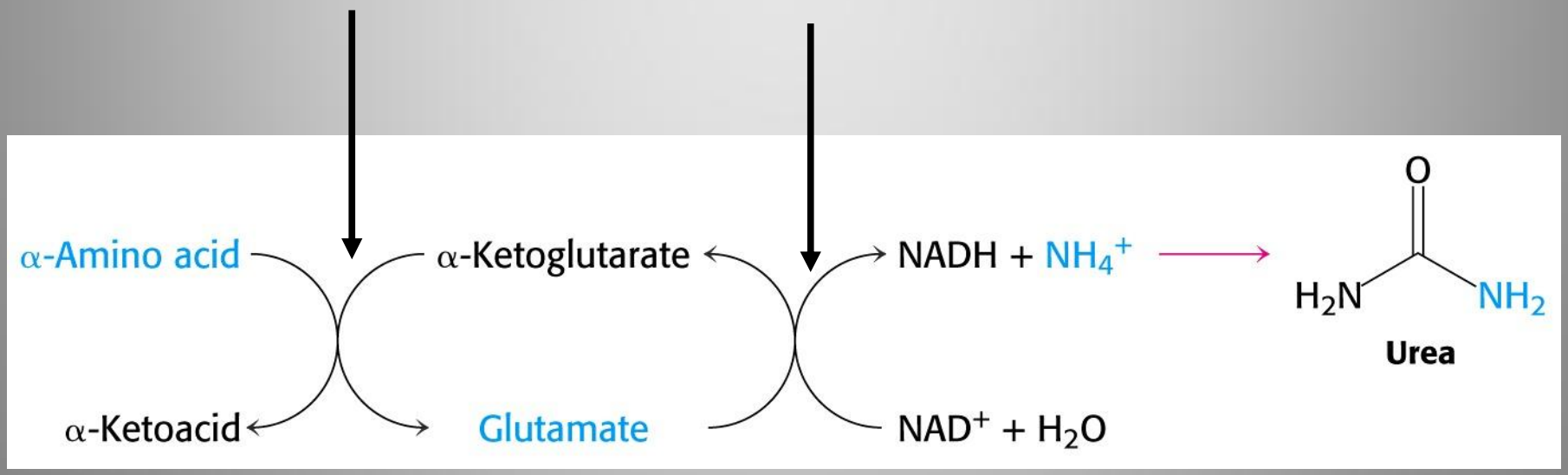





L-Amino acid
Pyridoxal phosphate (aldimine form, on enzyme)
Schiff base intermediate (aldimine)
Quinonoid intermediate
α-Keto acid
Pyridoxamine phosphate


Aspartate
Oxaloacetate
α-Ketoglutarate
Glutamate
Enzyme
Enzyme
E (aspartate)
(E-NH3) (oxaloacetate)
(E-NH3)
(E-NH3) (α-ketoglutarate)
E (glutamate)

Transamination followed by oxidative deamination



Glutamate DH

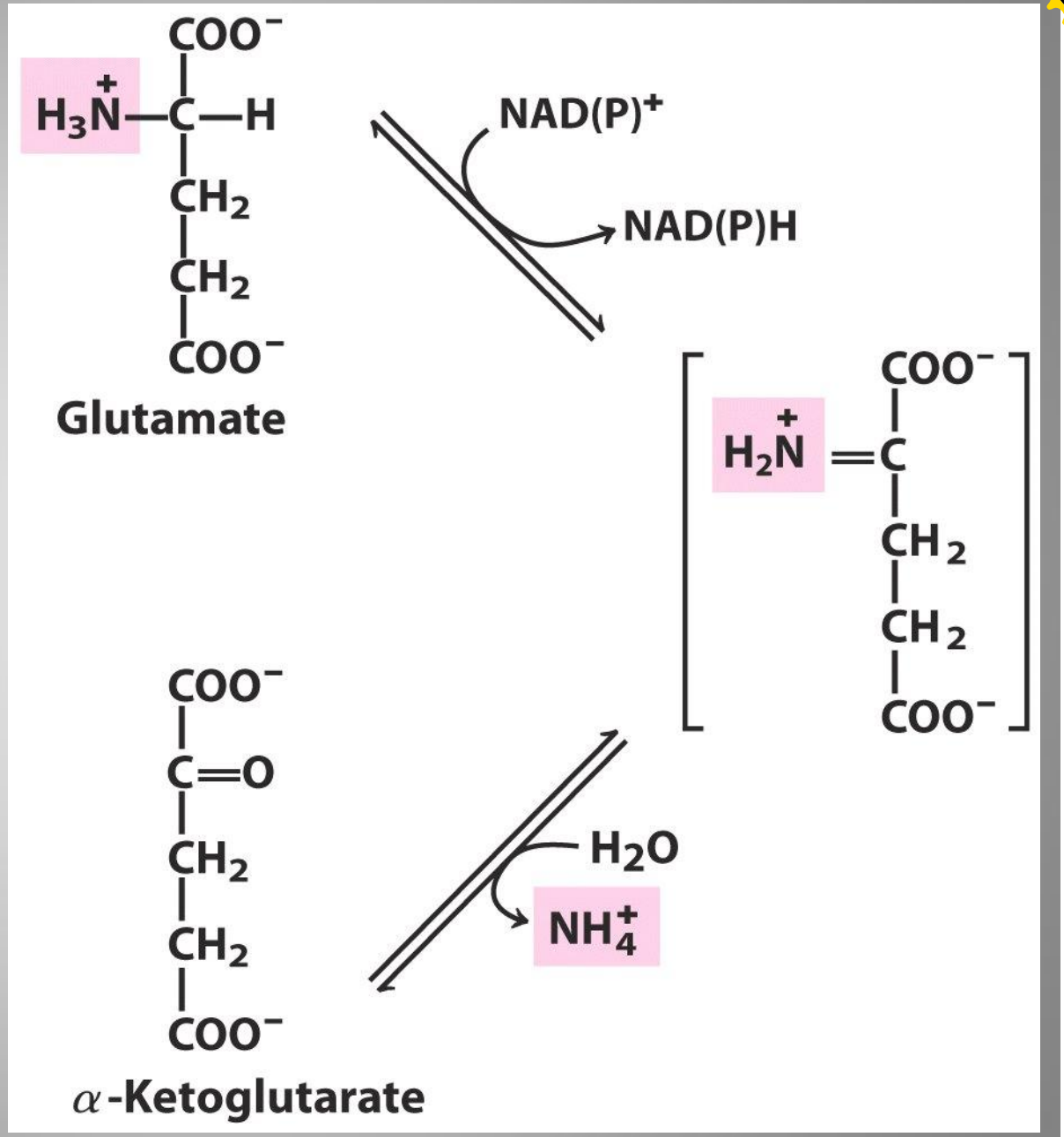
- Release free ammonium
- Occur in mitochondrion

Allosteric inhibitors

GTP, ATP

Allsteric activators

GDP, ADP



Active pathways:

1. Glycogen breakdown,
2. Glycolysis,
3. Citric acid cycle,
4. Oxidative phosphorylation,
5. Gluconeogenesis,
6. Urea cycle,

Glucose-alanine cycle

Cori cycle

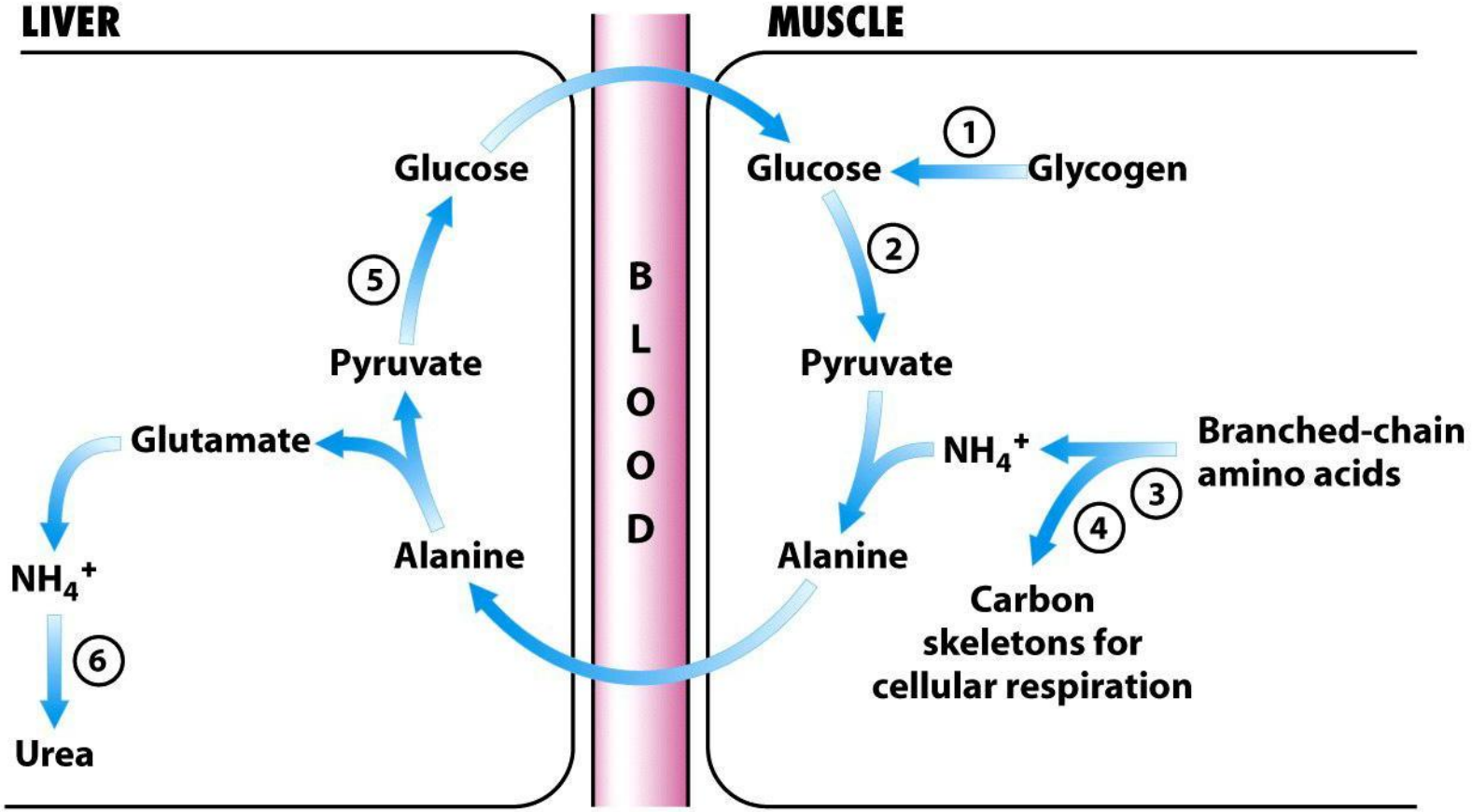


Figure 23-16
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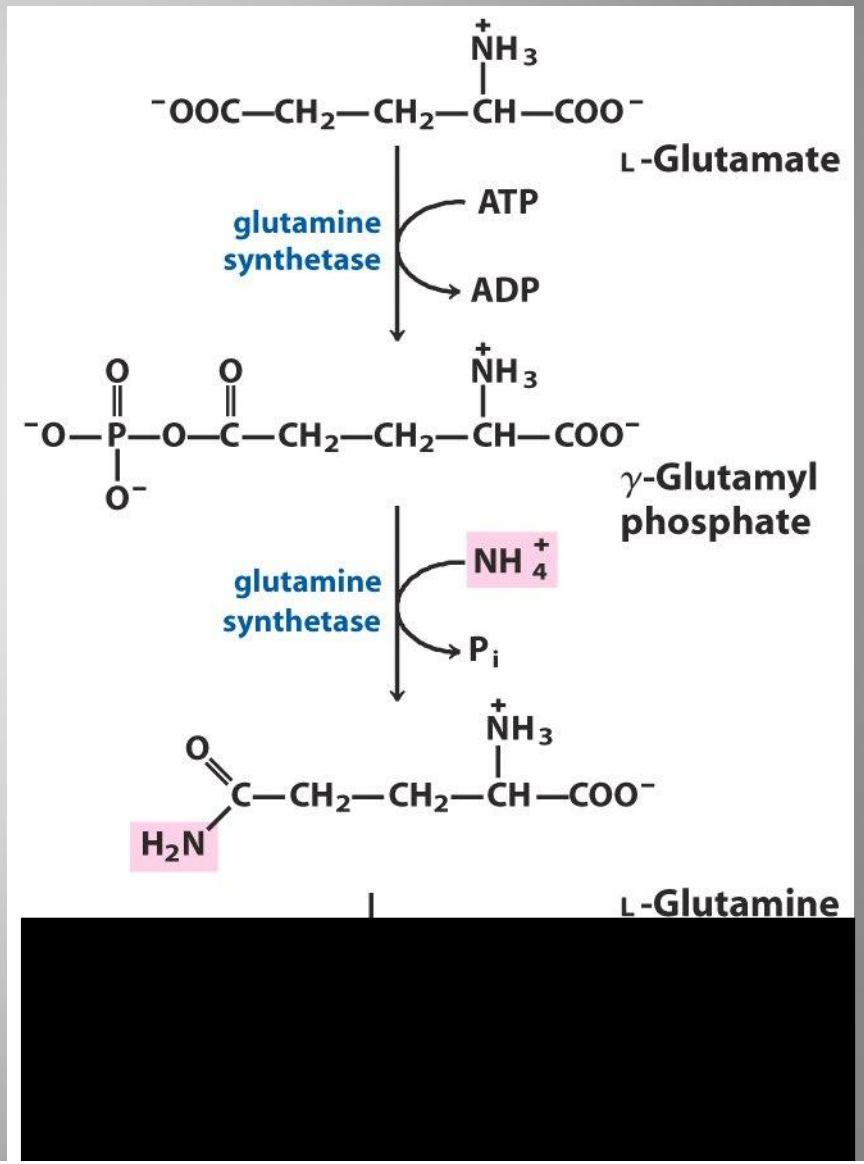
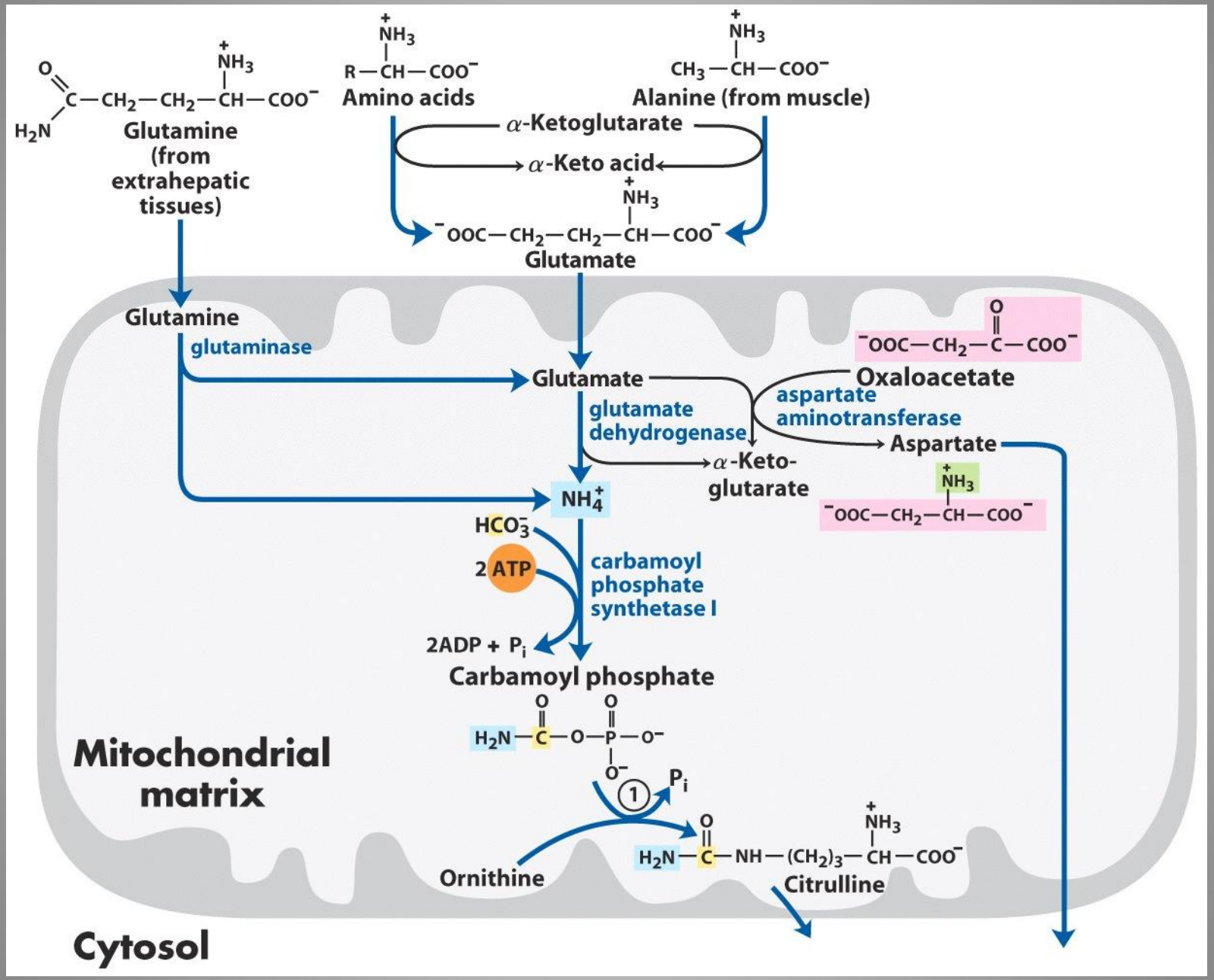
Nitrogen transport to liver

- Amino acid degradation occurs in tissues other than liver
- Muscle uses aa during prolong exercise and fasting
- Only liver contains all enzymes for urea cycle
- Muscle transports nitrogen in the form of alanine (via glucose –alanine cycle)
- Others transport nitrogen as glutamine via glutamine synthetase

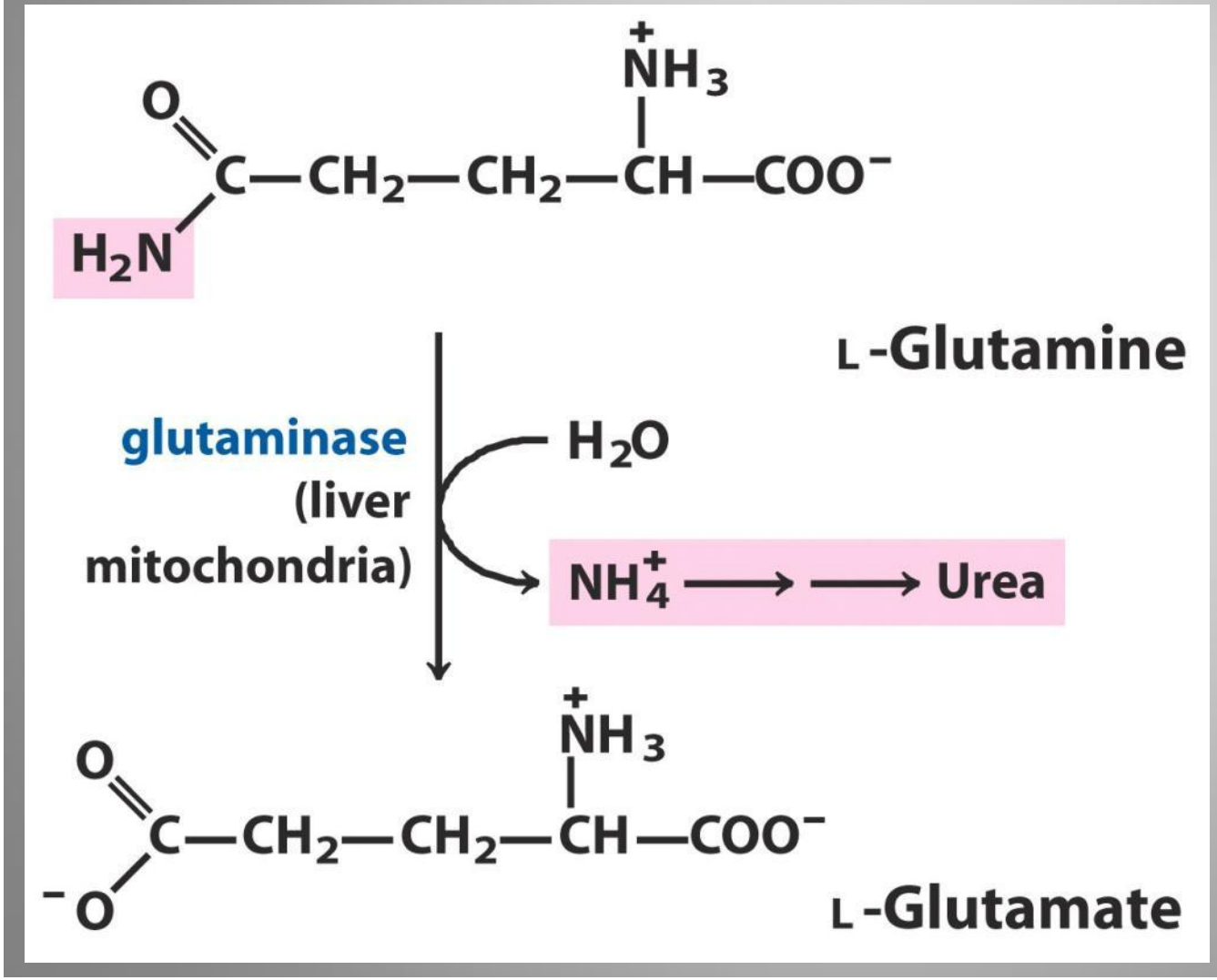
Glutamine

- Formation of glutamine from glutamate, ATP, ammonium
- Occurred in tissues other than liver
- Glutamine carries nitrogen to liver

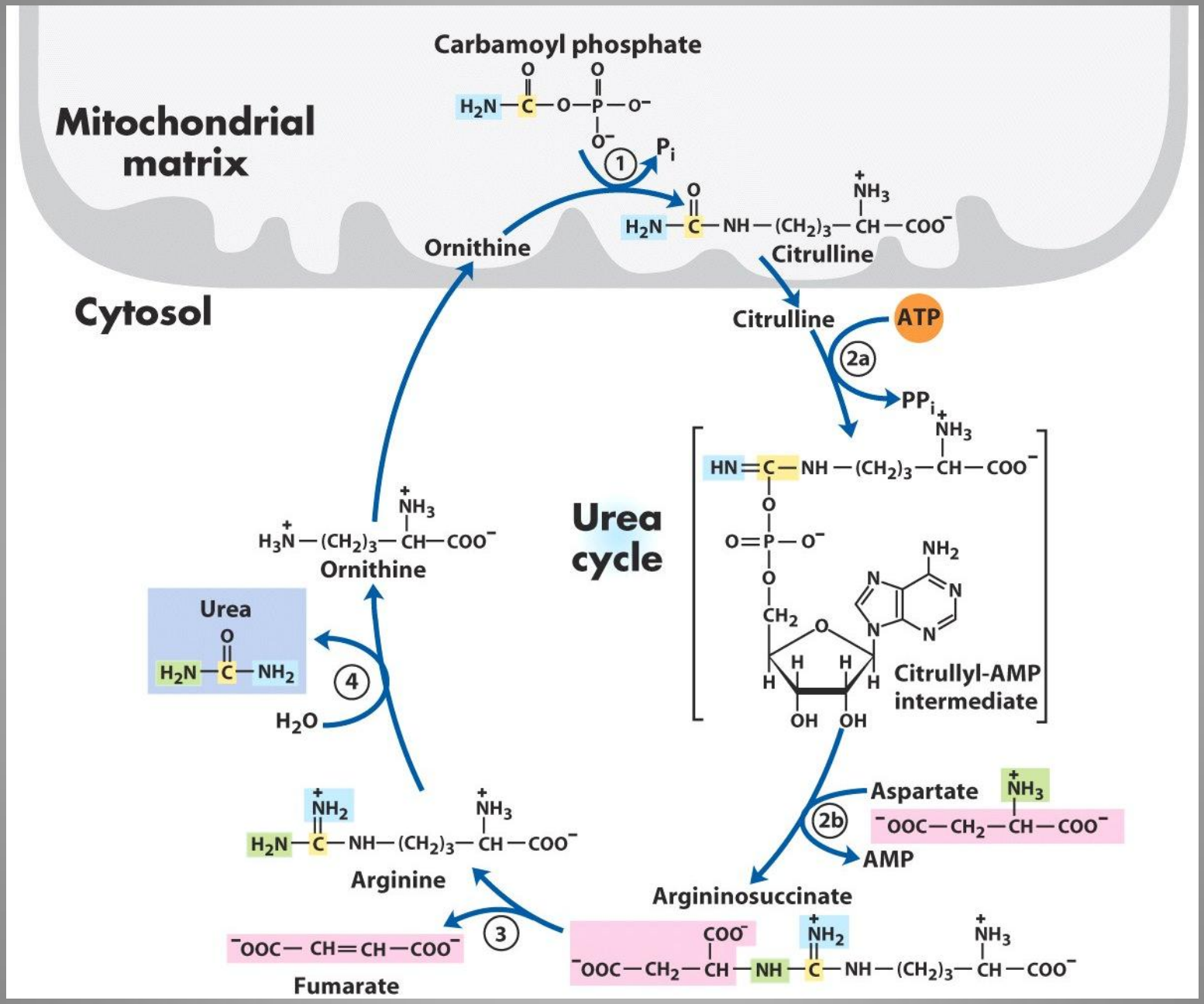


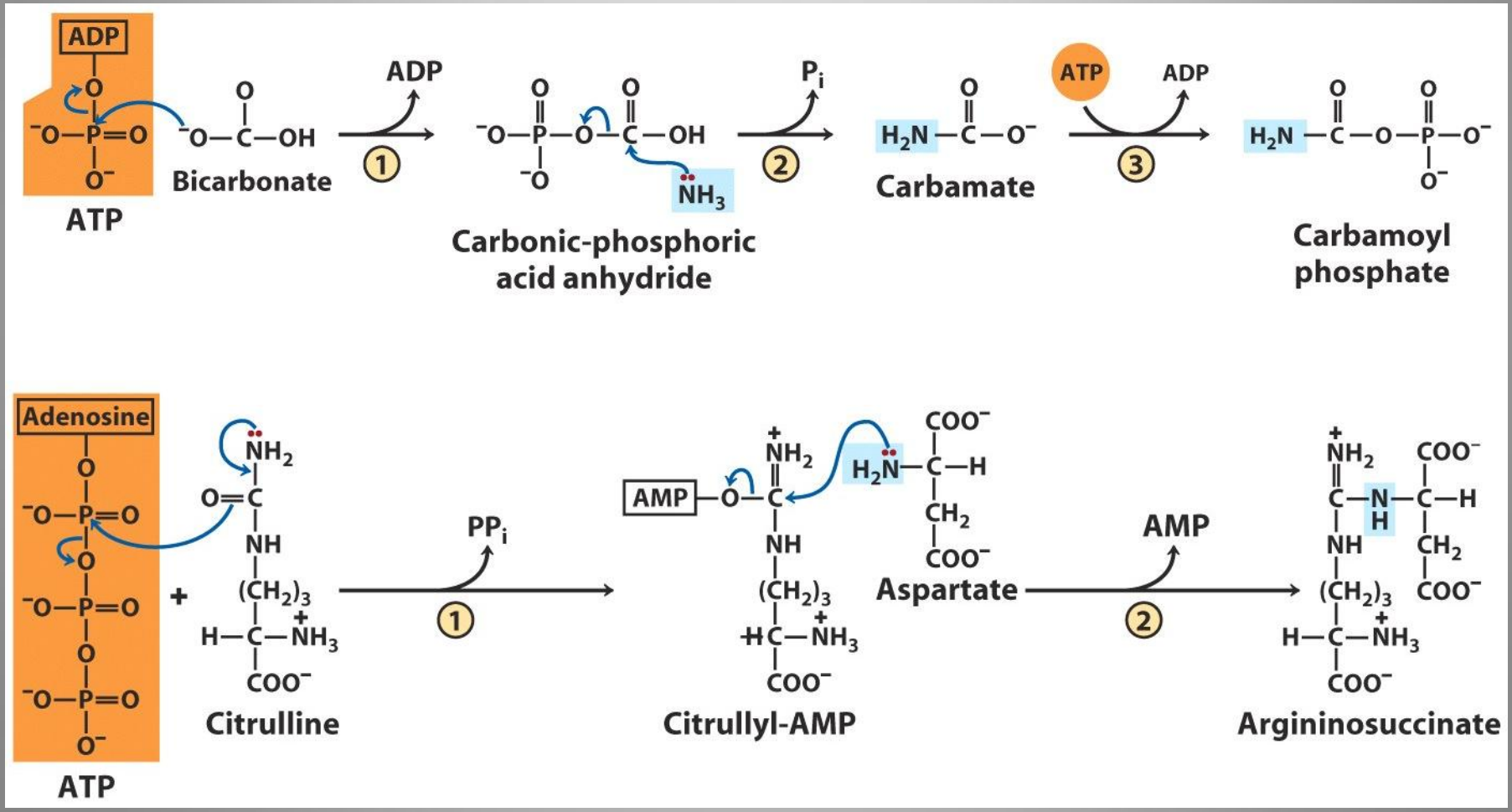

Glutamine
(from
extrahepatic
tissues)
Amino acids
Alanine (from muscle)
α-Ketoglutarate
α-Keto acid
Glutamate
Glutamine
glutaminase
Glutamate
Oxaloacetate
aspartate
aminotransferase
glutamate
dehydrogenase
Aspartate
α-Keto-
glutarate
NH4+
HCO3−
2 ATP
carbamoyl
phosphate
synthetase I
2ADP + Pi
Carbamoyl phosphate
Mitochondrial
matrix
Pi
Ornithine
Citrulline
Cytosol

Action of glutaminase



Glutaminase also present in kidney but for acid-base regulation


Carbamoyl phosphate
Mitochondrial matrix
Ornithine
Citrulline
Cytosol
Citrulline
ATP
PPi
Urea cycle
Citrullyl-AMP intermediate
Aspartate
AMP
Argininosuccinate
Arginine
Ornithine
Urea
H2O
Fumarate



Urea cycle

1=carbamoyl phosphate synthetase I:

2=ornithine transcarbamoylase: ornithine is carbamoylated citrulline

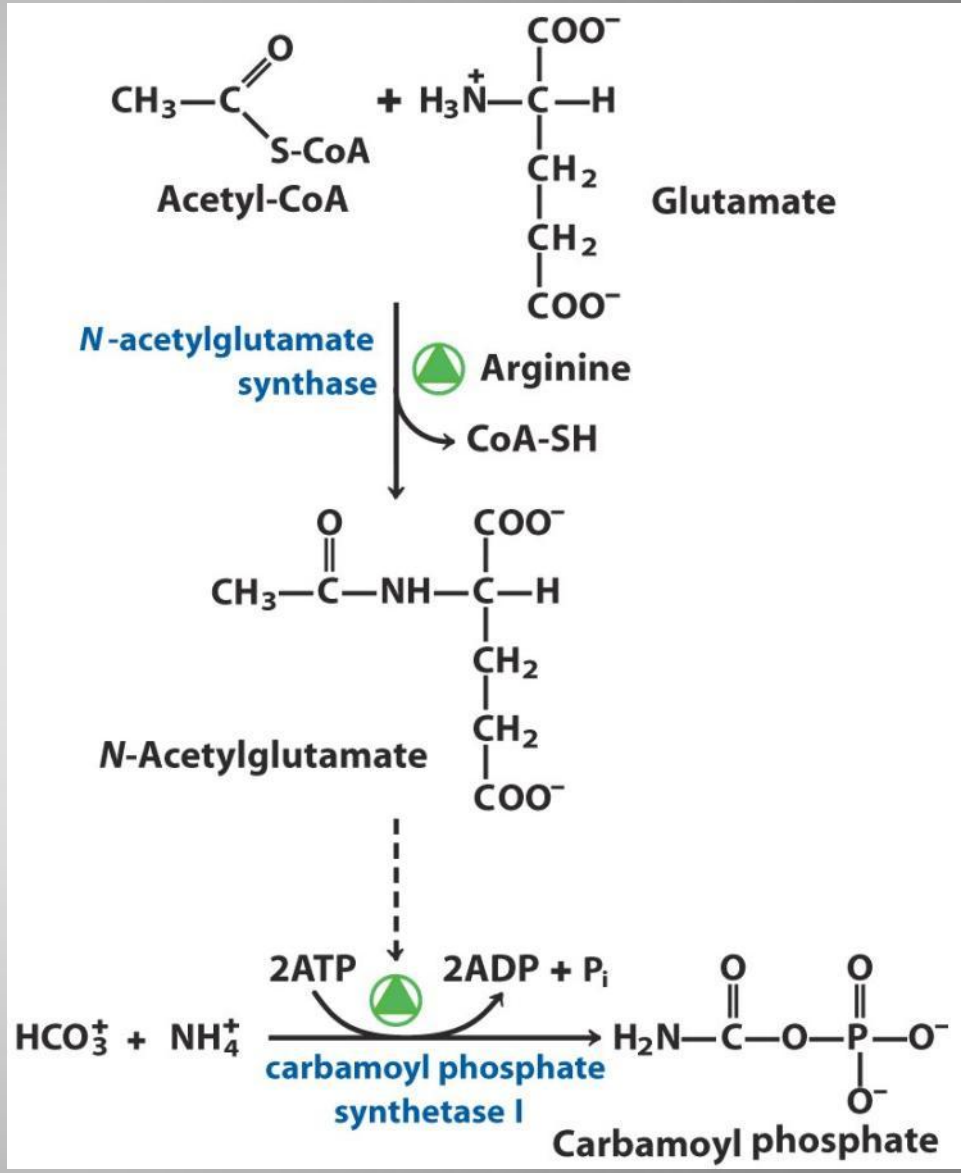
3=argininosuccinate synthetase: condensation of citrulline and aspartate

4=argininosuccinase: cleavage of argininosuccinate into arginine and fumarate

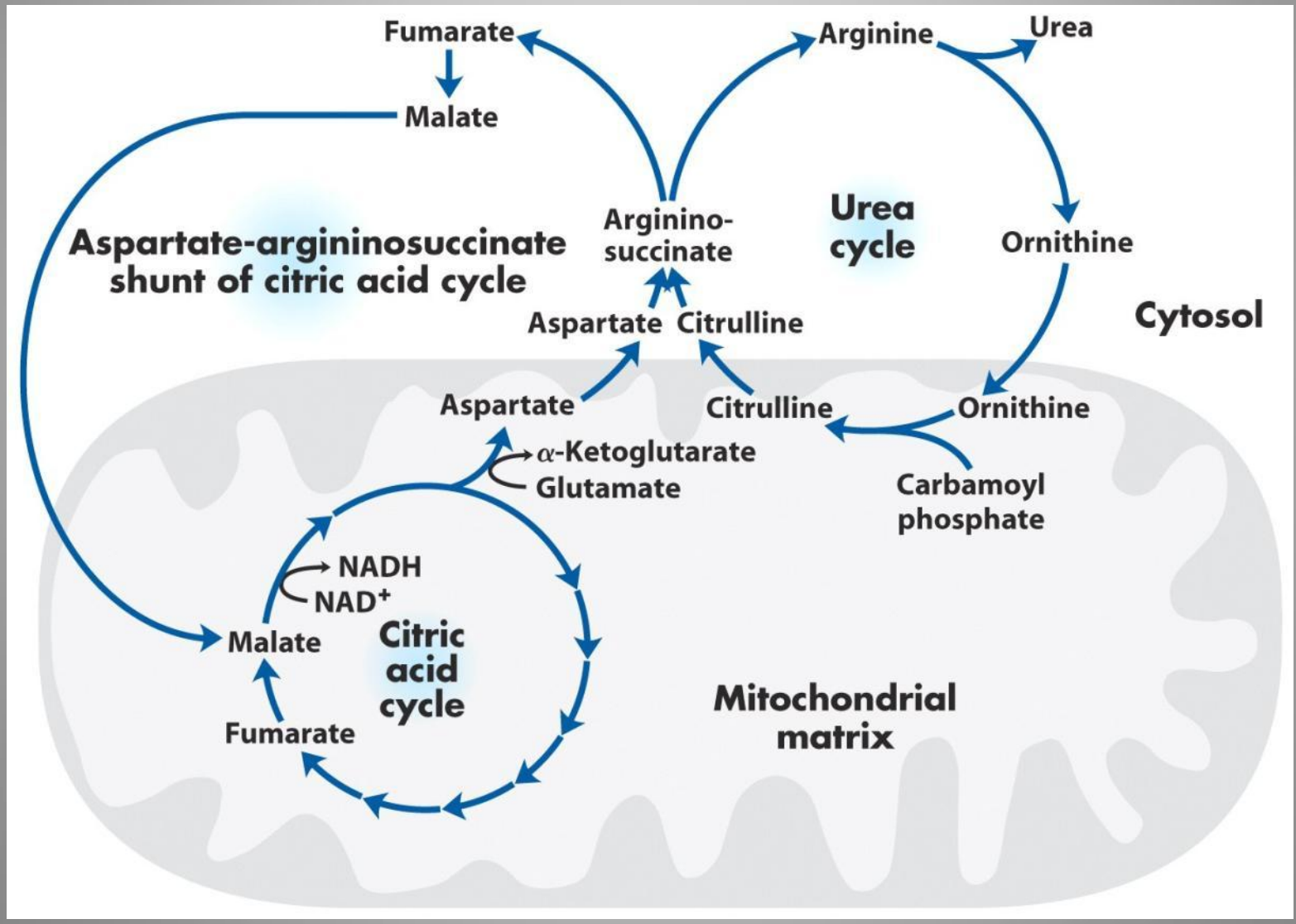
5=arginase: arginine is hydrolyzed to urea and ornithine

Regulation of urea cycle

CPSI needs N-acetyl glutamate as an allostric activator



Urea cycle and citric acid cycle



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Fates of oxaloacetate

- Fumarate $\rightarrow\rightarrow$ oxaloacetate = the link
  - Transamination to aspartate
  - Conversion into glucose via gluconeogenic pathway
  - Condensation with acetylCoA to form citrate
  - Conversion into pyruvate

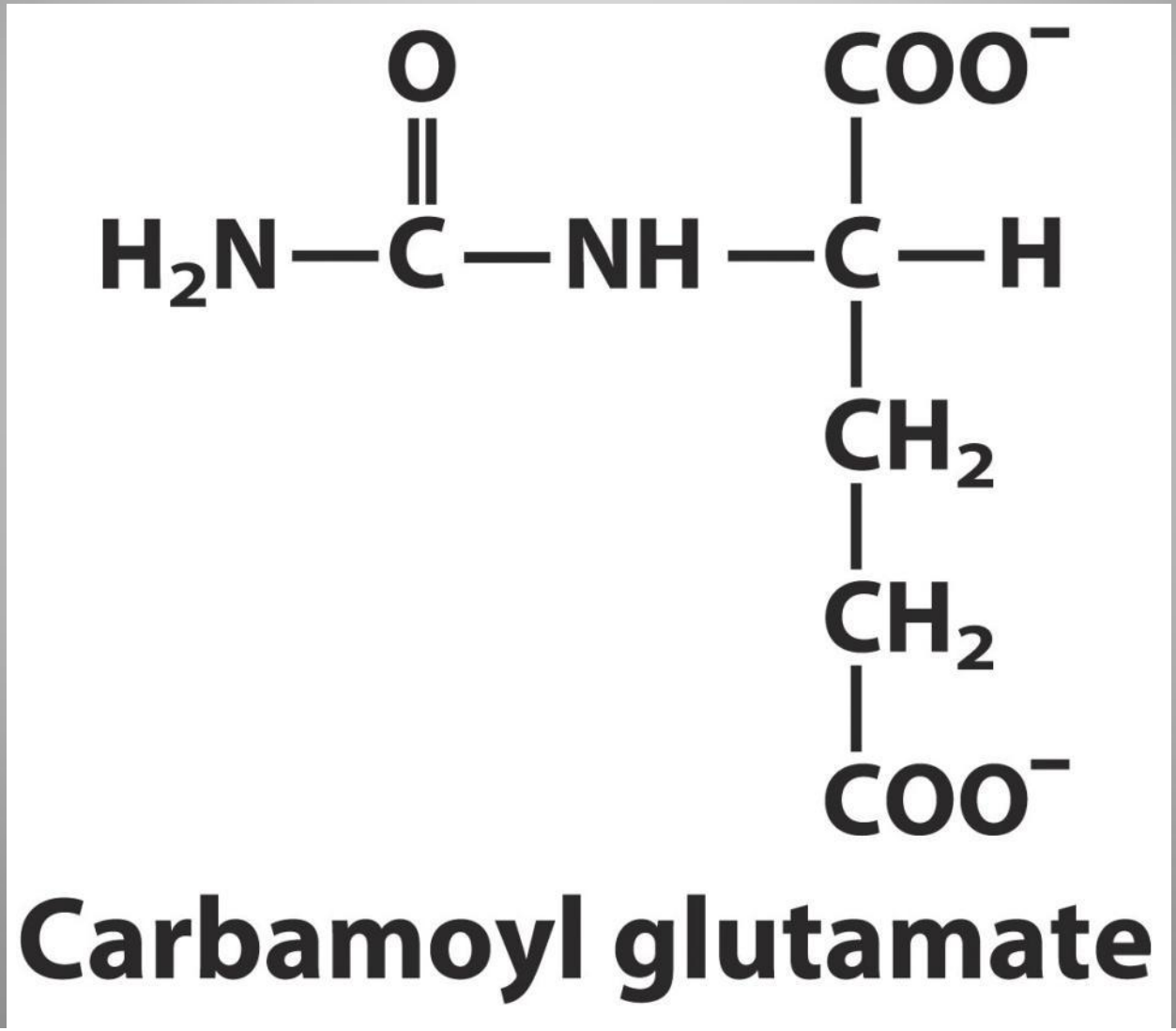
Ammonia

- Ammonia is toxic
  - Brain swelling (increase in the brain's water content)
  - Increase in intracranial pressure
  - Potential depletion of ATP
- ↑ammonia→

(α-ketoglutarate↓→glutamate↑)→glutamine↑

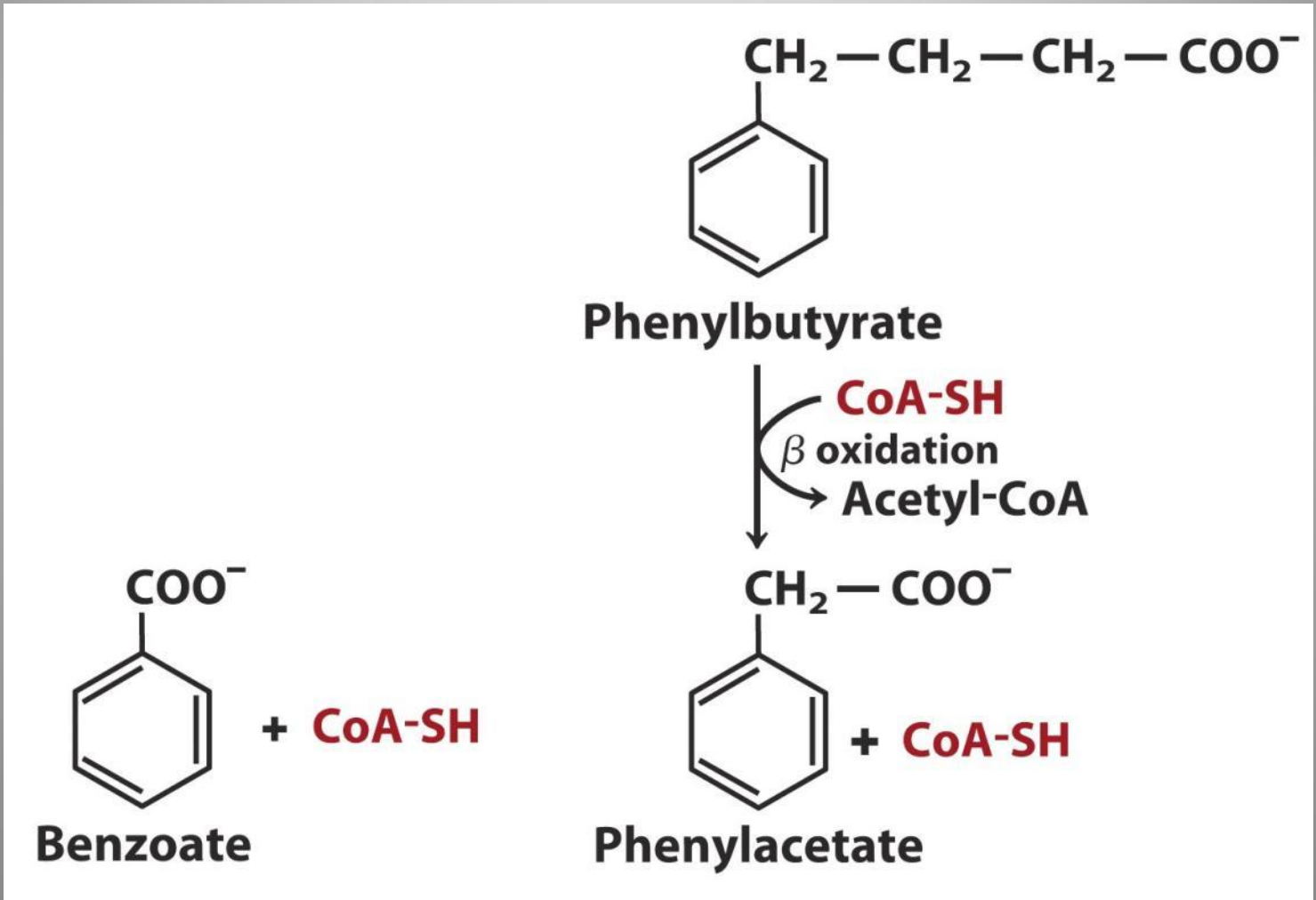
↑↑osmolyte in brain astrocyte

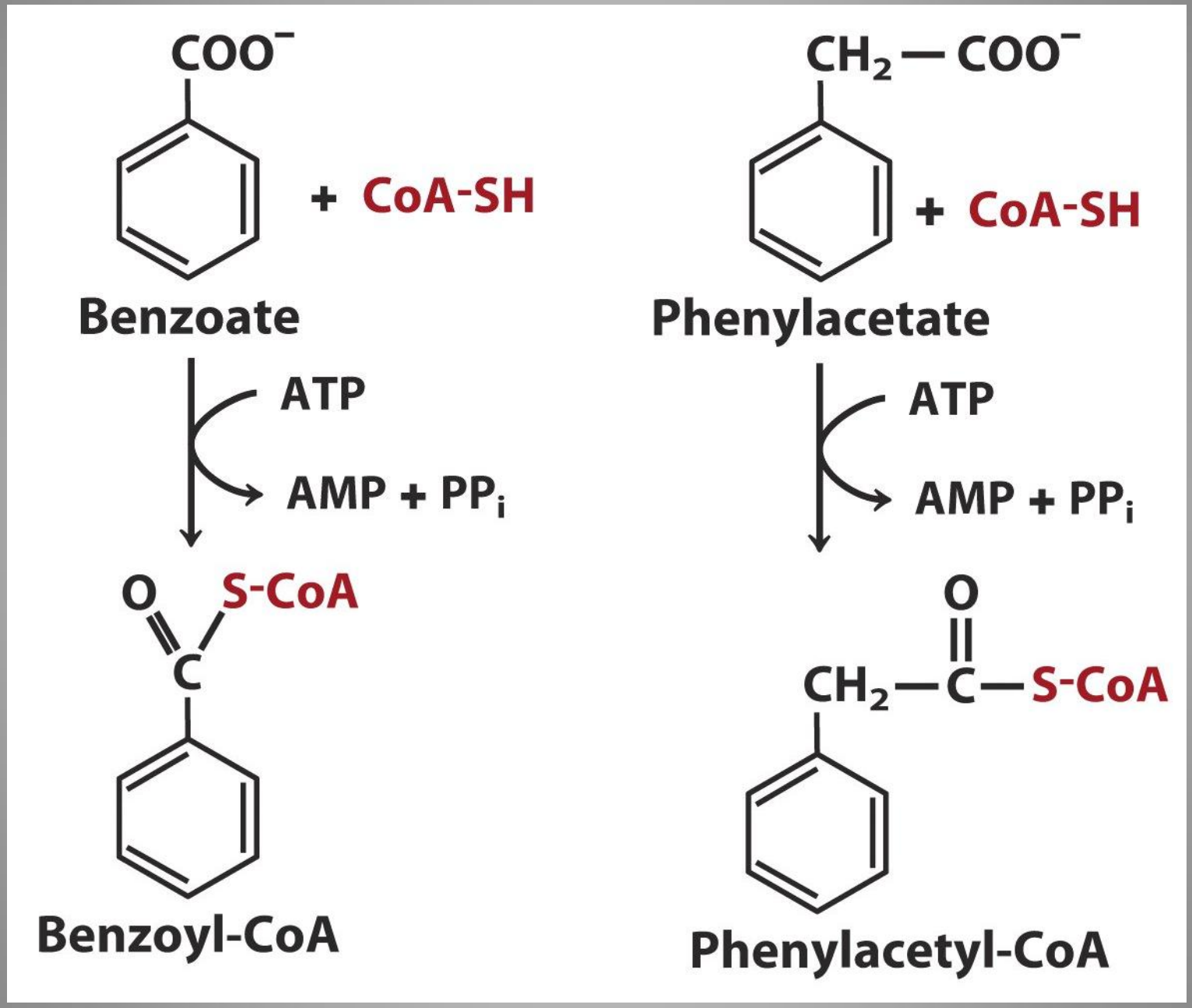
Treatment of urea cycle defect

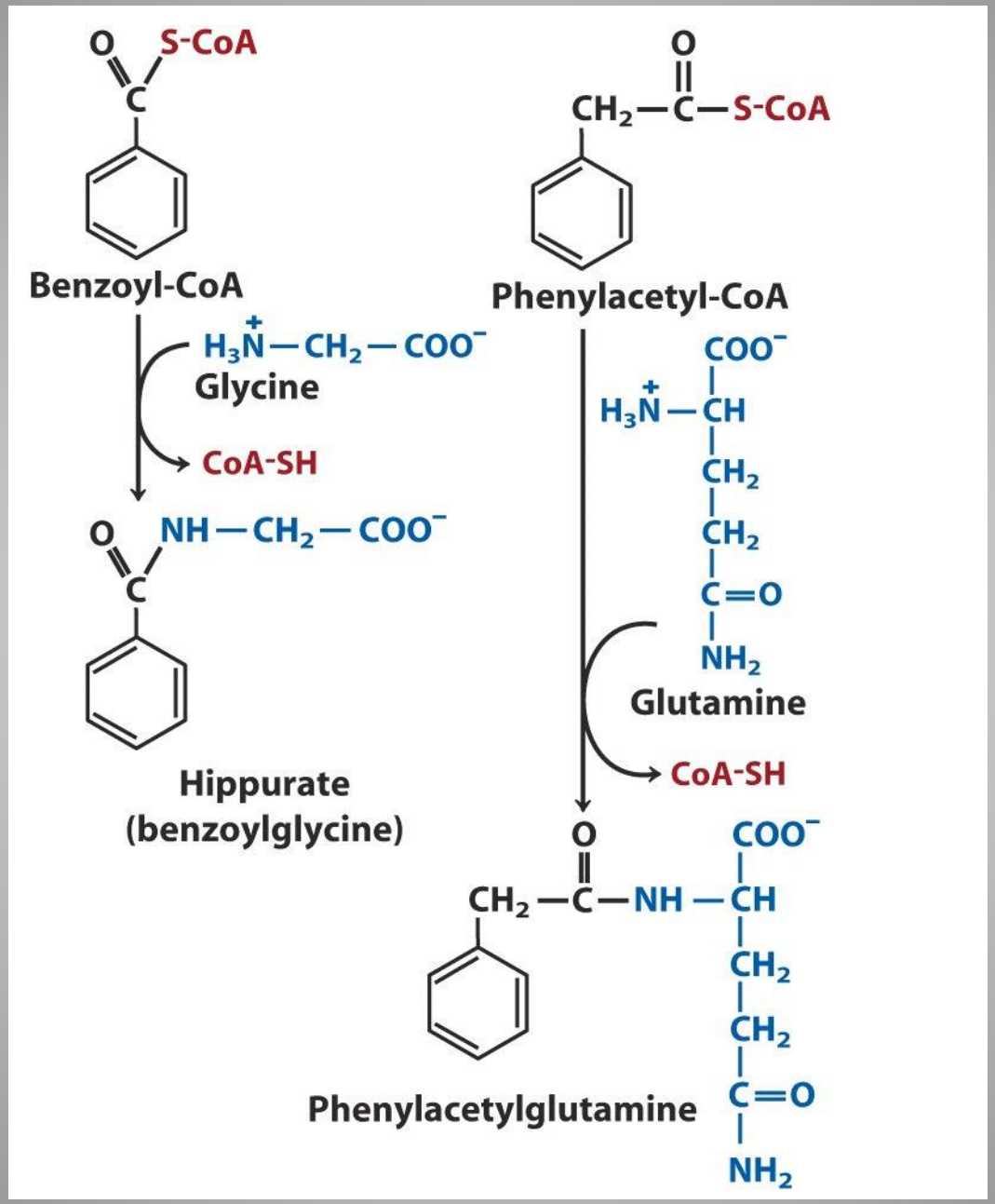


Carbamoyl glutamate

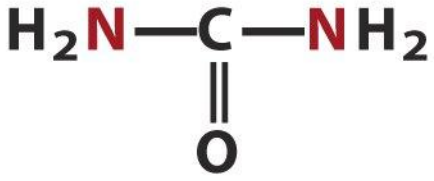
Treatment of urea cycle defect




COO⁻
+ CoA-SH
Benzoate
ATP
AMP + PPi
O
S-CoA
C
Benzoyl-CoA
CH2 — COO⁻
+ CoA-SH
Phenylacetate
ATP
AMP + PPi
O
CH2 — C — S-CoA
Phenylacetyl-CoA


S-CoA
Benzoyl-CoA
H3N+—CH2—COO−
Glycine
CoA-SH
NH—CH2—COO−
Hippurate
(benzoylglycine)
CH2—C—S-CoA
Phenylacetyl-CoA
COO−
H3N+—CH
CH2
CH2
C=O
NH2
Glutamine
CoA-SH
CH2—C—NH—CH
Phenylacetylglutamine

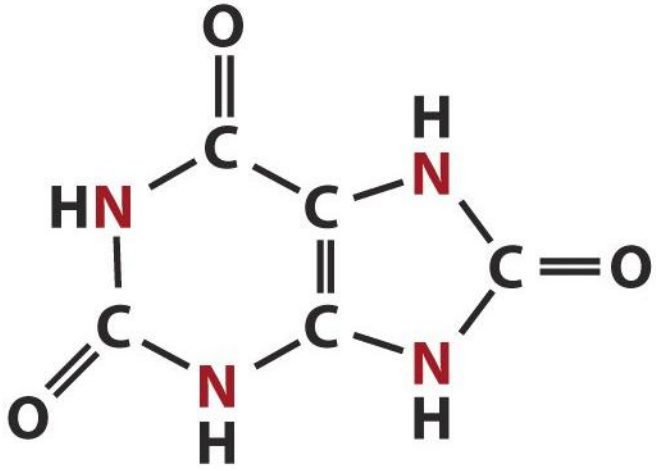
Ammonia (as ammonium ion)



Urea

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

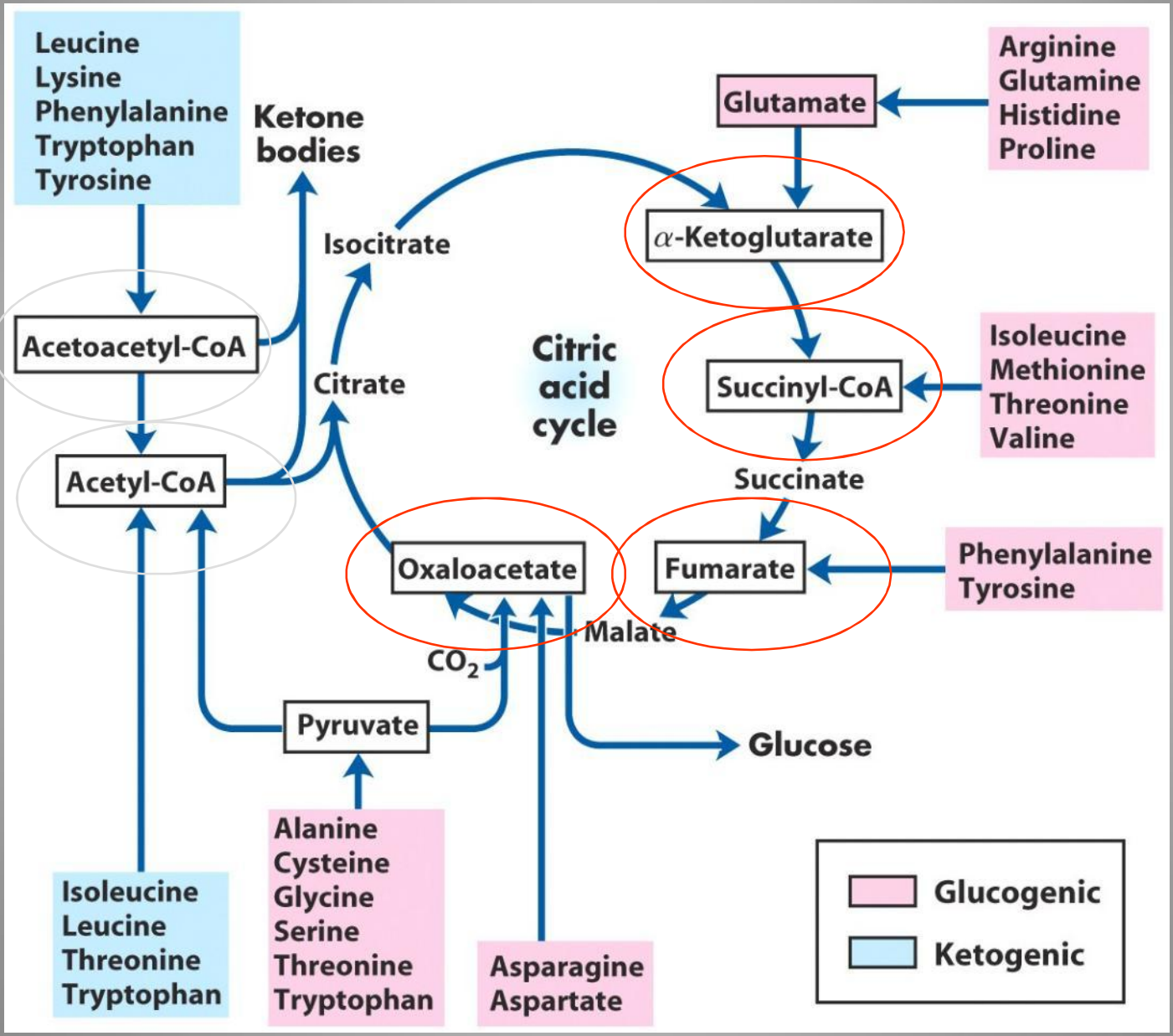
Ureotelic animals: many terrestrial vertebrates; also sharks



Uric acid

Uricotelic animals: birds, reptiles

Catabolism



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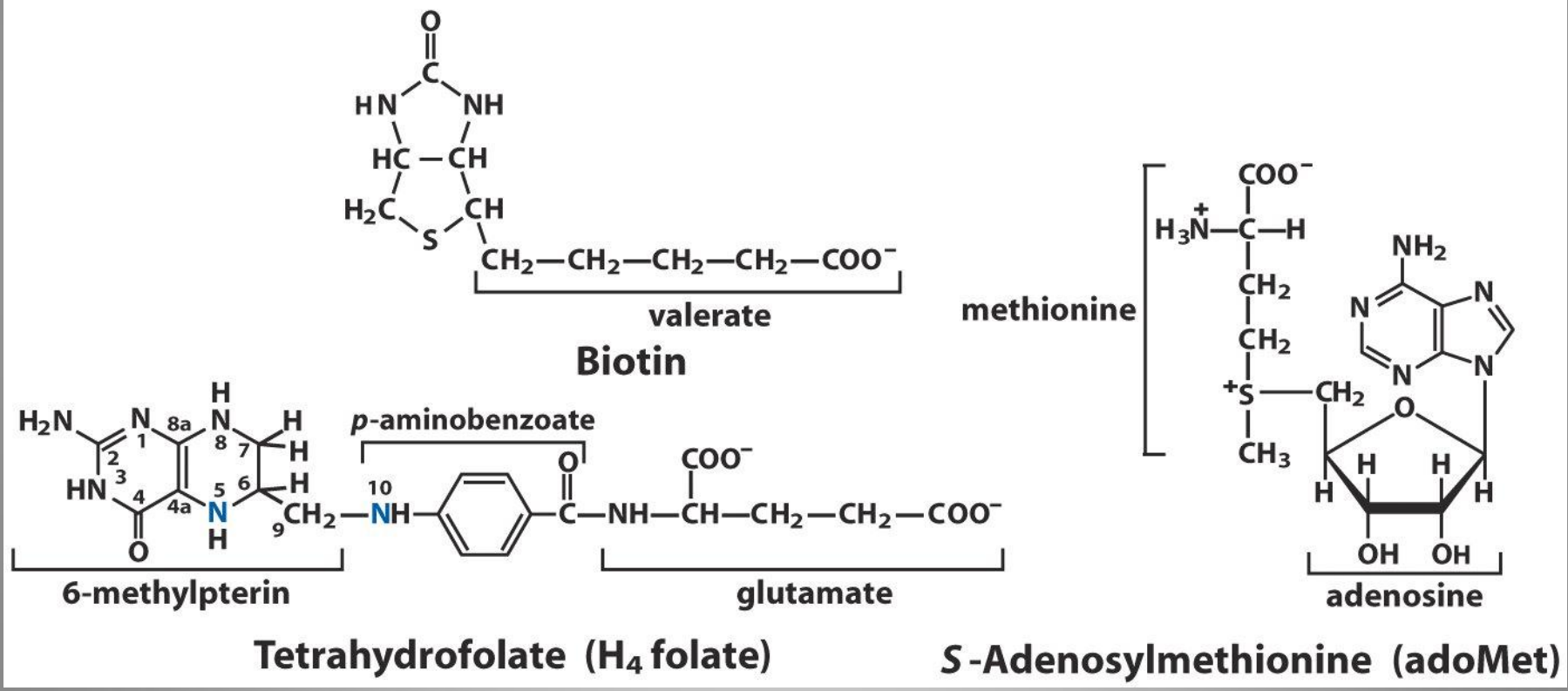
Catabolism

- Accounted for 10-15% of human energy production
- 20 pathways converge to 6 major products
  - 7 aa are broken down to acetyl-CoA
  - 5 aa to α-ketoglutarate
  - 4 to succinyl-CoA
  - 2 to fumarate
  - 2 to oxaloacetate

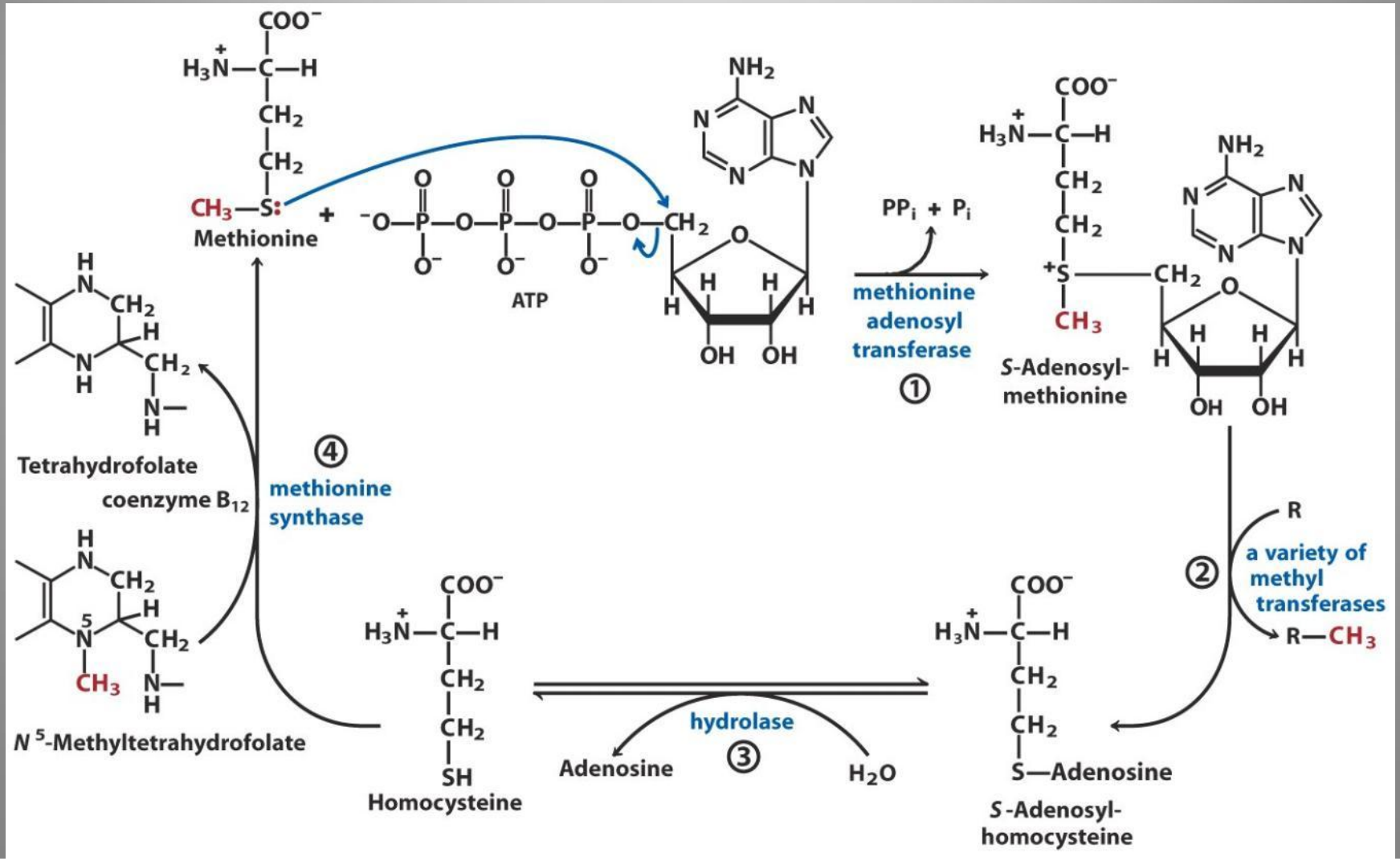
Catabolism

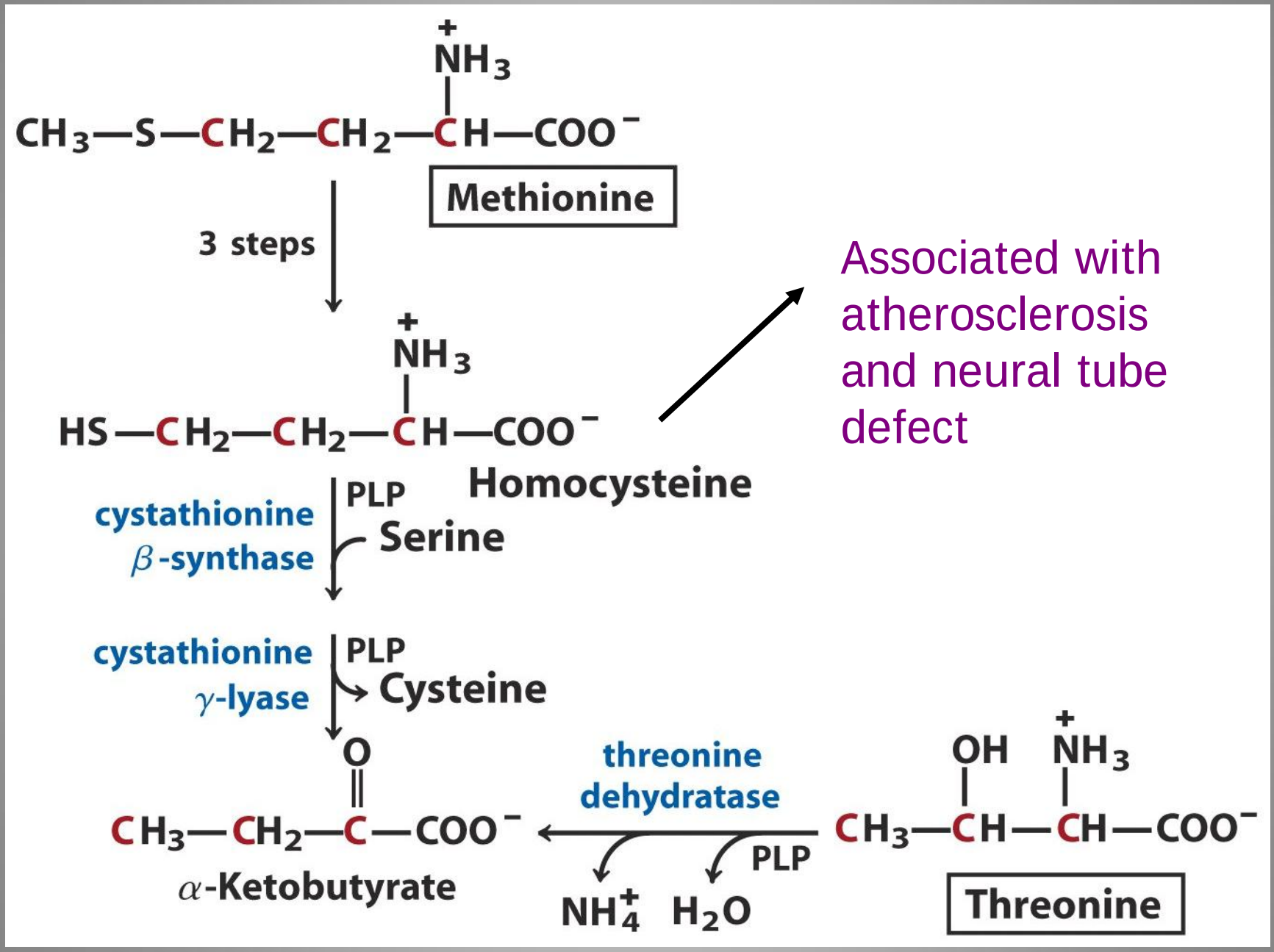
- Phe, Tyr, Ile, Leu, Trp, Thr, Lys→→acetoacetyl CoA/or acetyl CoA→→ketone bodies in liver (acetoacetate→acetone→β-hydroxybutyrate) = ketogenic
- Glucogenic aa = →→pyruvate, α-ketoglutarate, succinyl CoA, fumarate and/or oxaloacetate
- Trp, Phe, Tyr, Thr, Ile both ketogenic and glucogenic

Enzyme cofactors in one-carbon transfer reaction

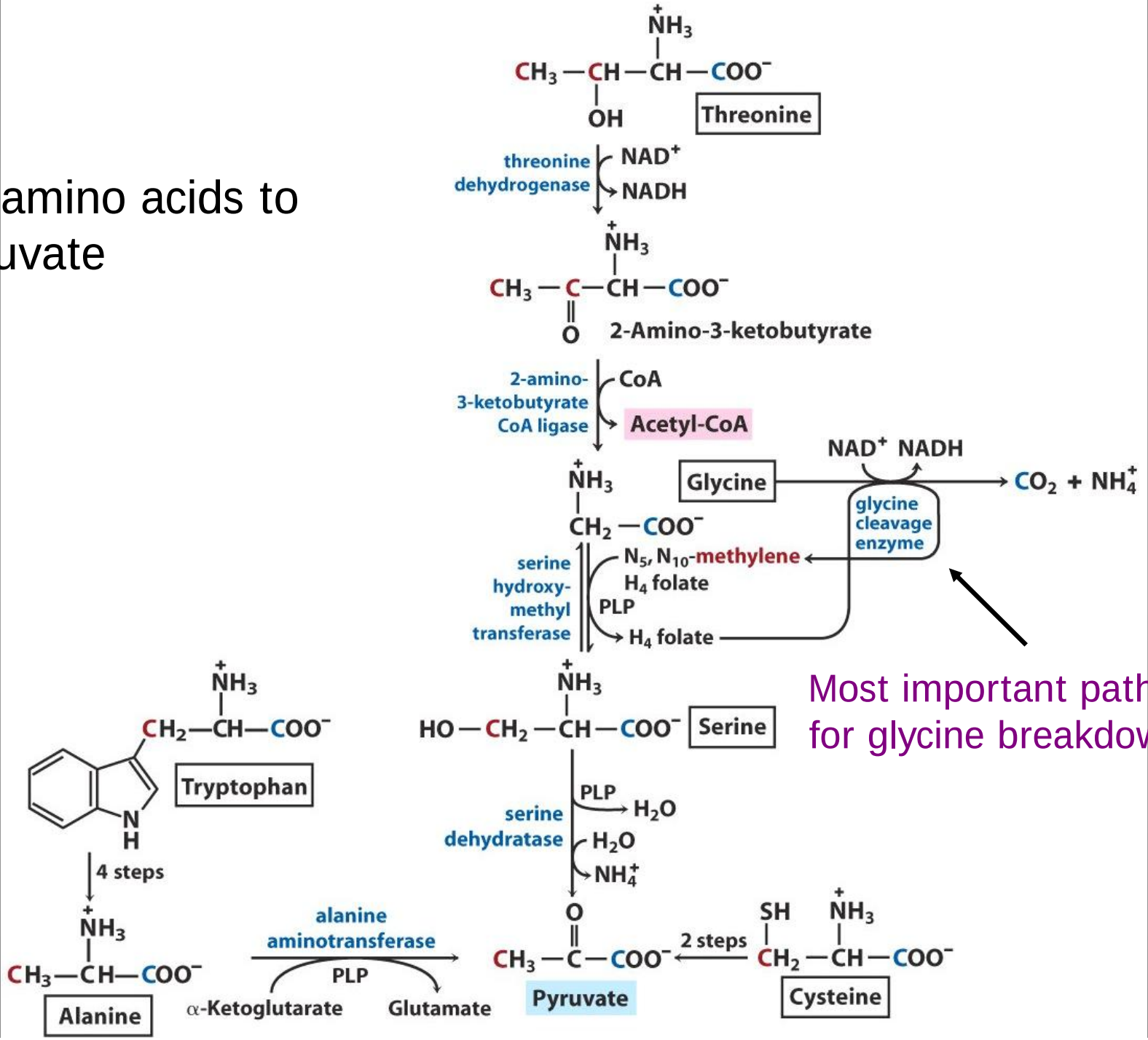


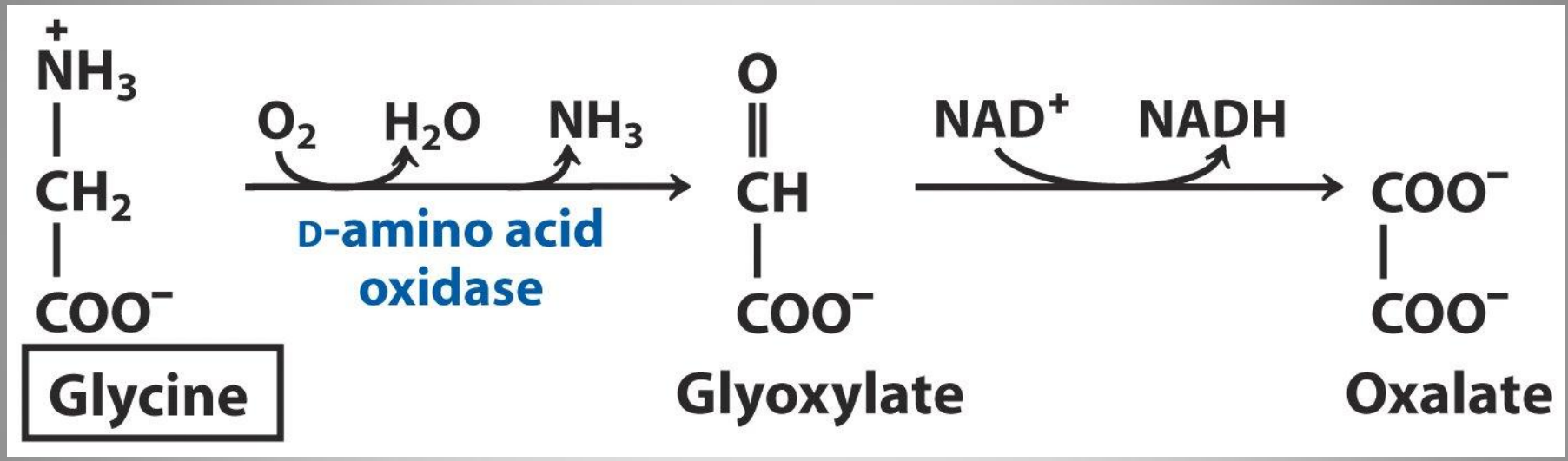
Metabolism of methionine




Methionine
3 steps
Homocysteine
Associated with atherosclerosis and neural tube defect
cystathionine β-synthase
PLP
Serine
cystathionine γ-lyase
PLP
Cysteine
α-Ketobutyrate
threonine dehydratase
PLP
Threonine

Six amino acids to pyruvate

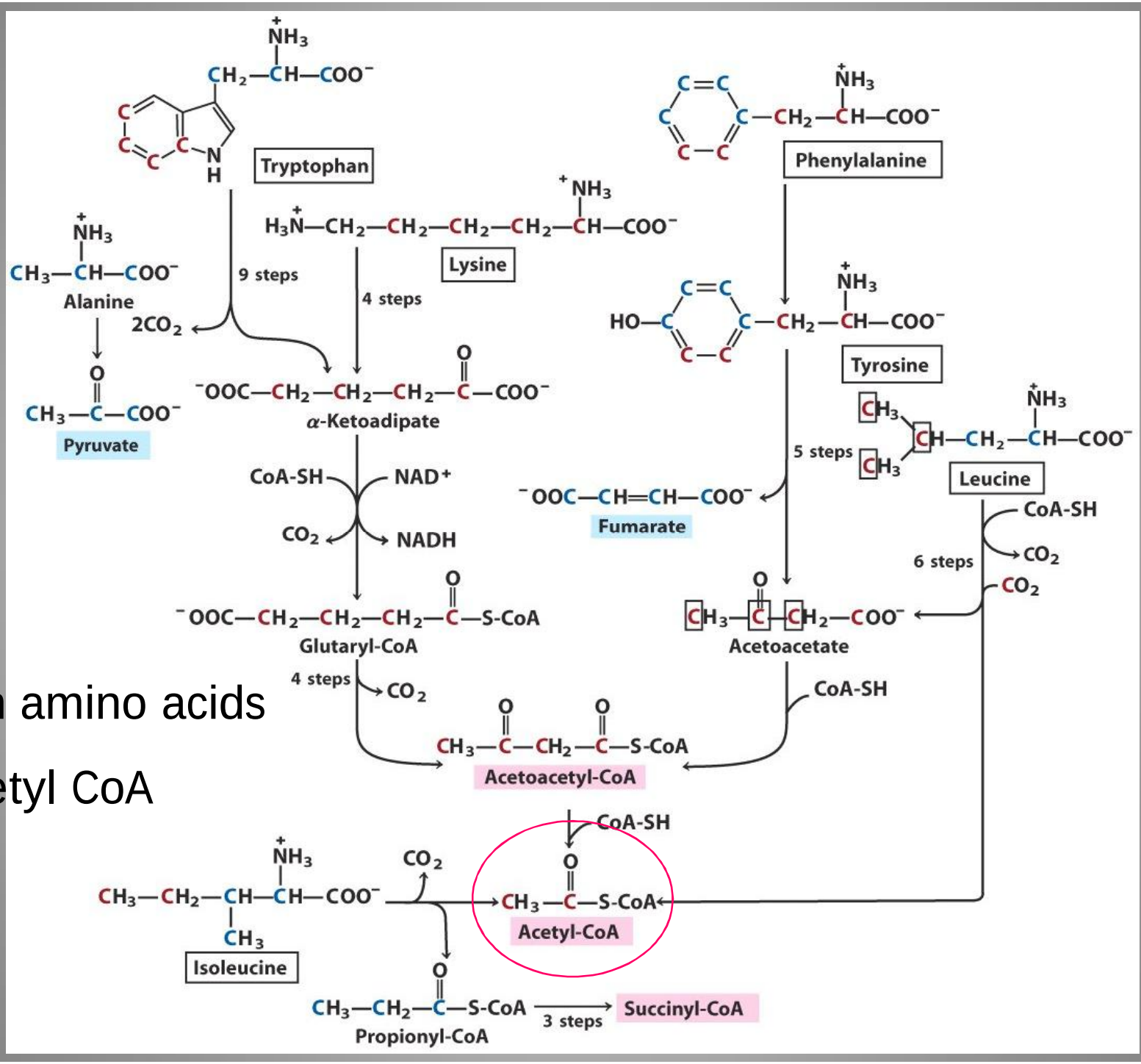




- High levels in kidney
- 75% kidney stones—calcium oxalate

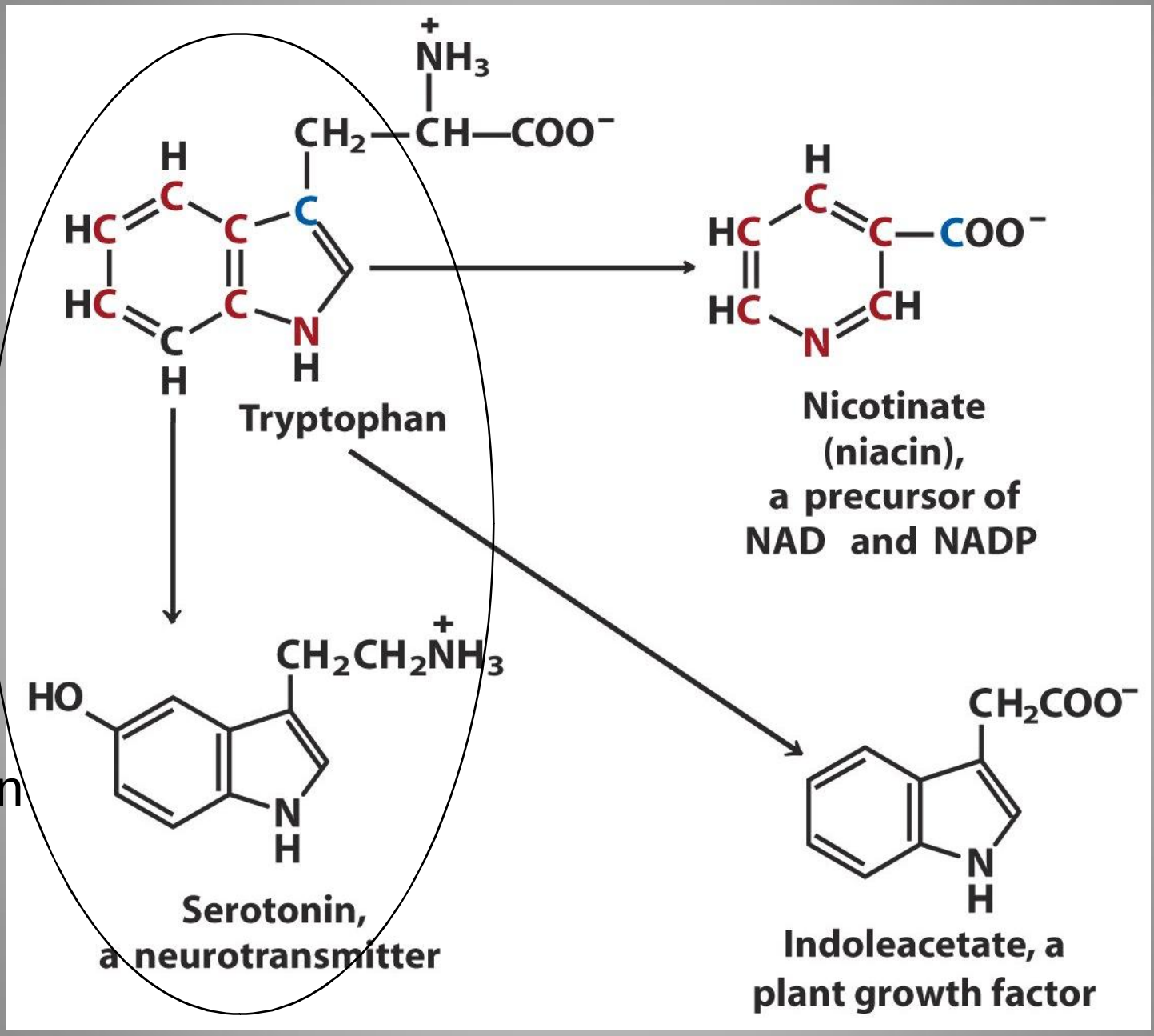
TABLE 18–2 Some Human Genetic Disorders Affecting Amino Acid Catabolism

Medical condition	*Approximate incidence (per 100,000 births)*	*Defective process*	*Defective enzyme*	*Symptoms and effects*
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation: white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase I deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathionine β-synthase	Faulty bone development; mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting; convulsions; mental retardation; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting; convulsions; mental retardation; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation



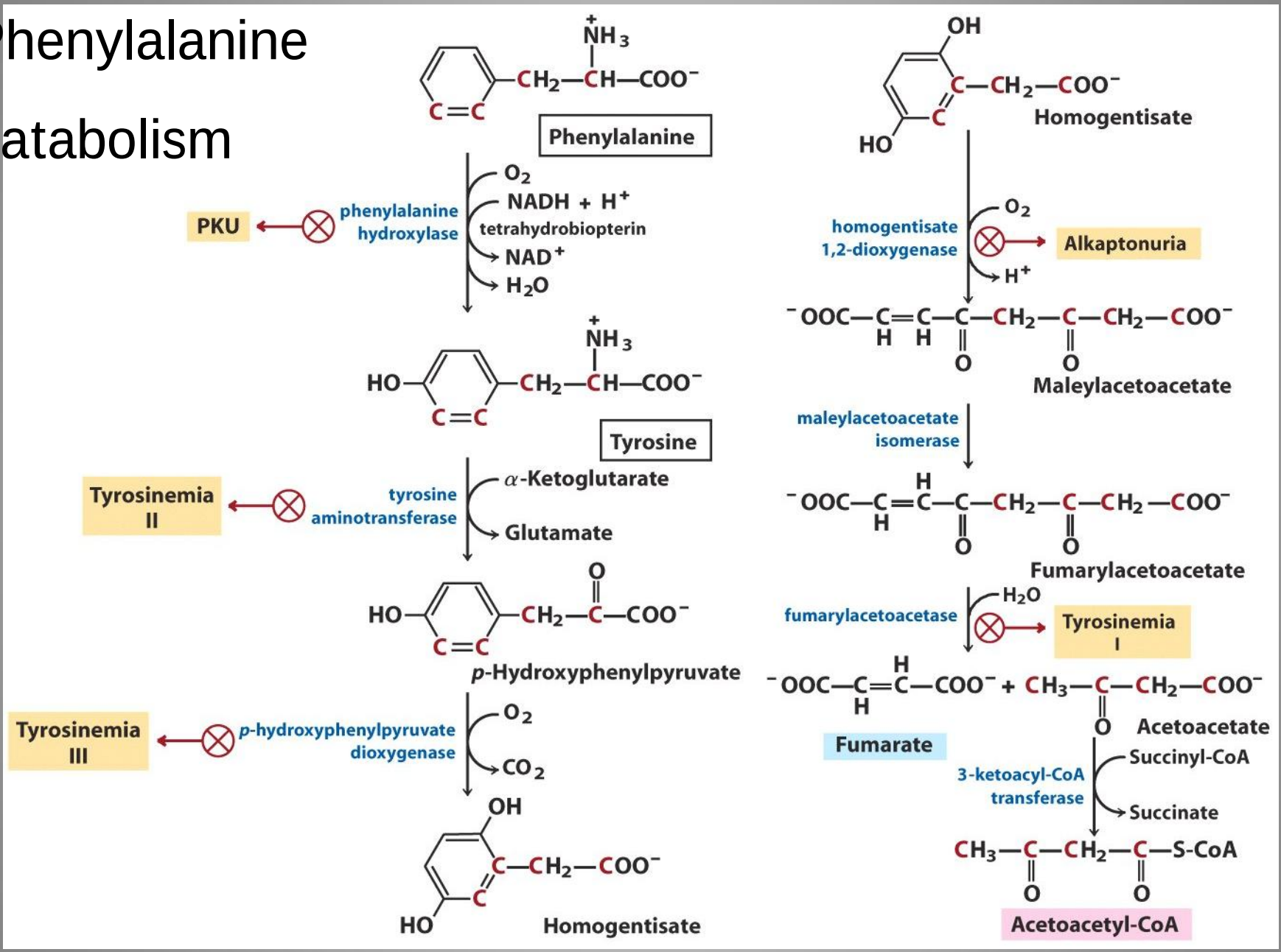
Seven amino acids

to acetyl CoA

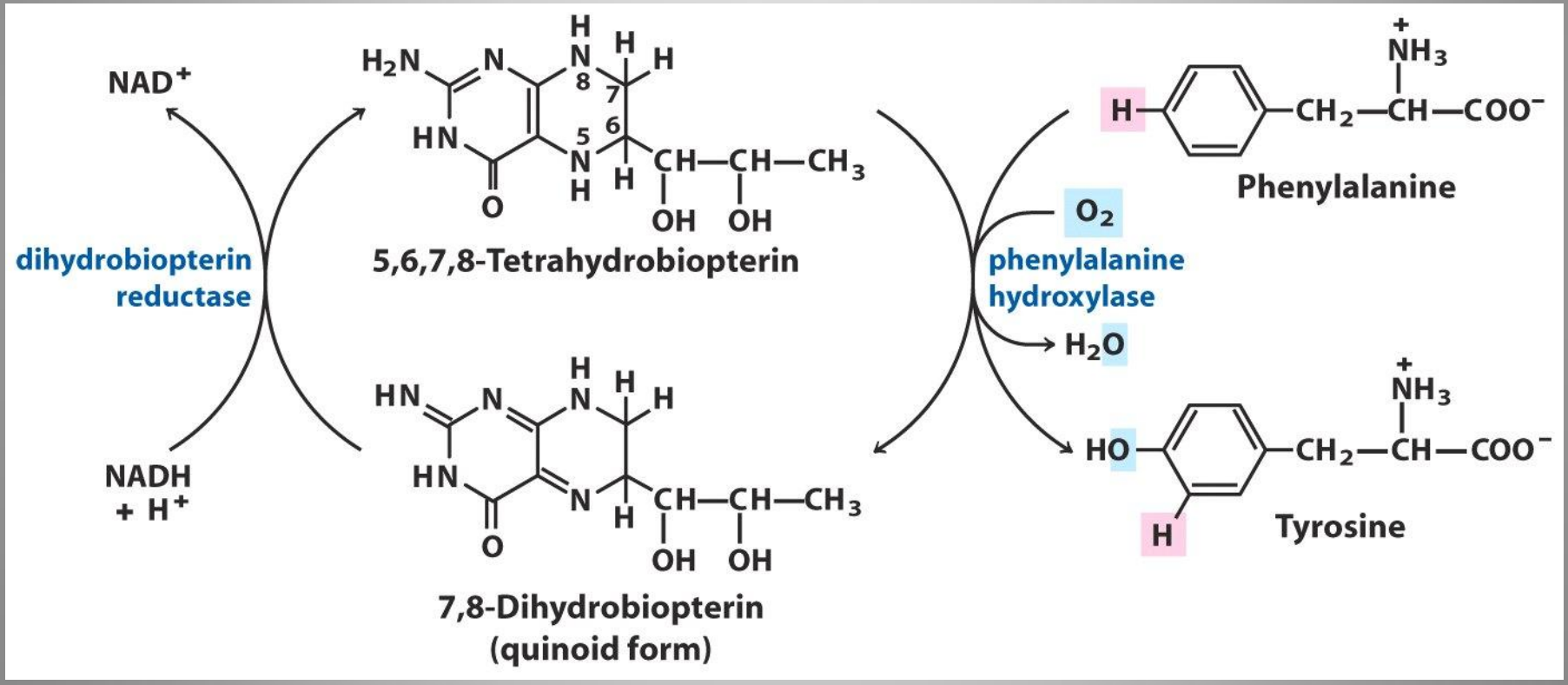


human

Phenylalanine catabolism

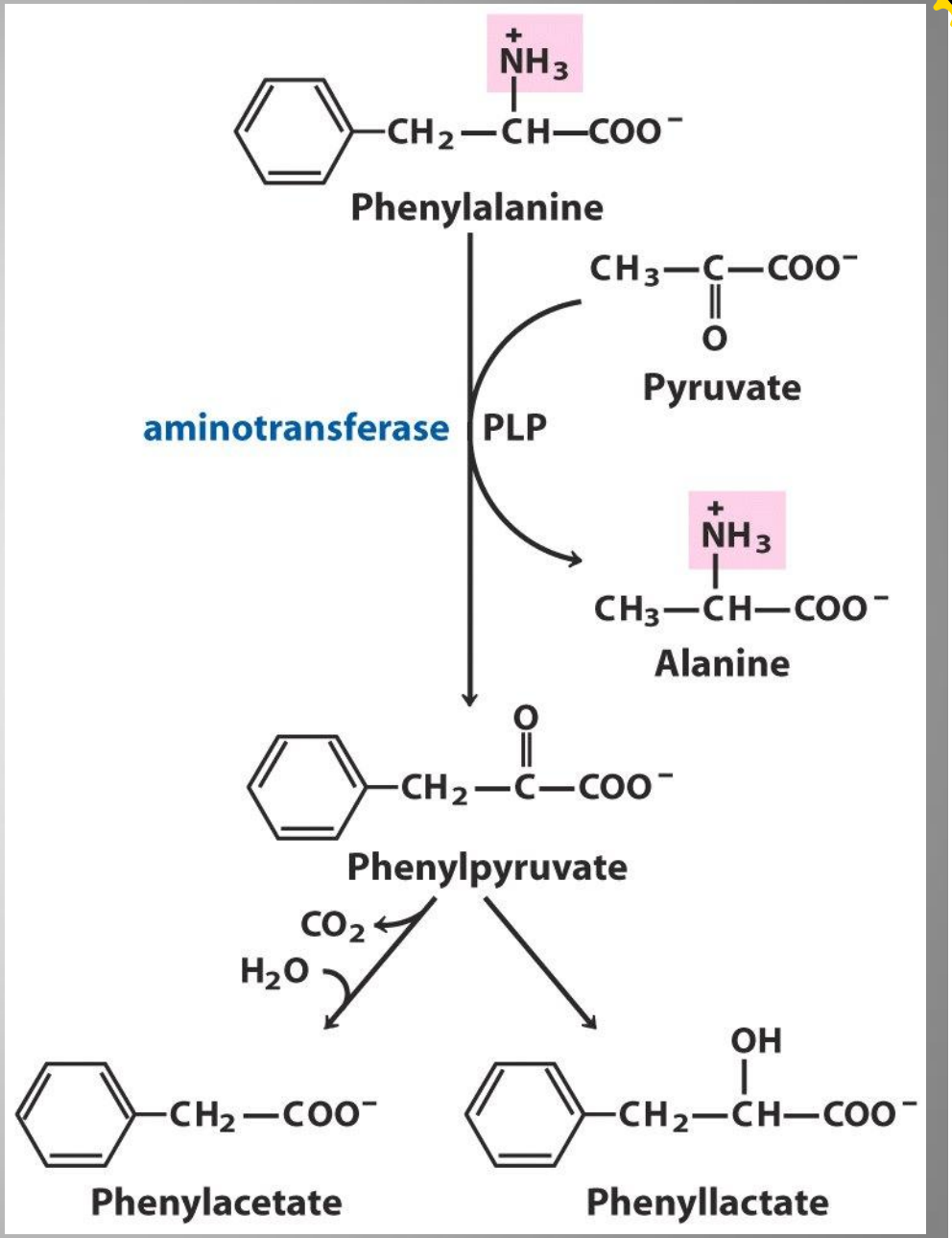


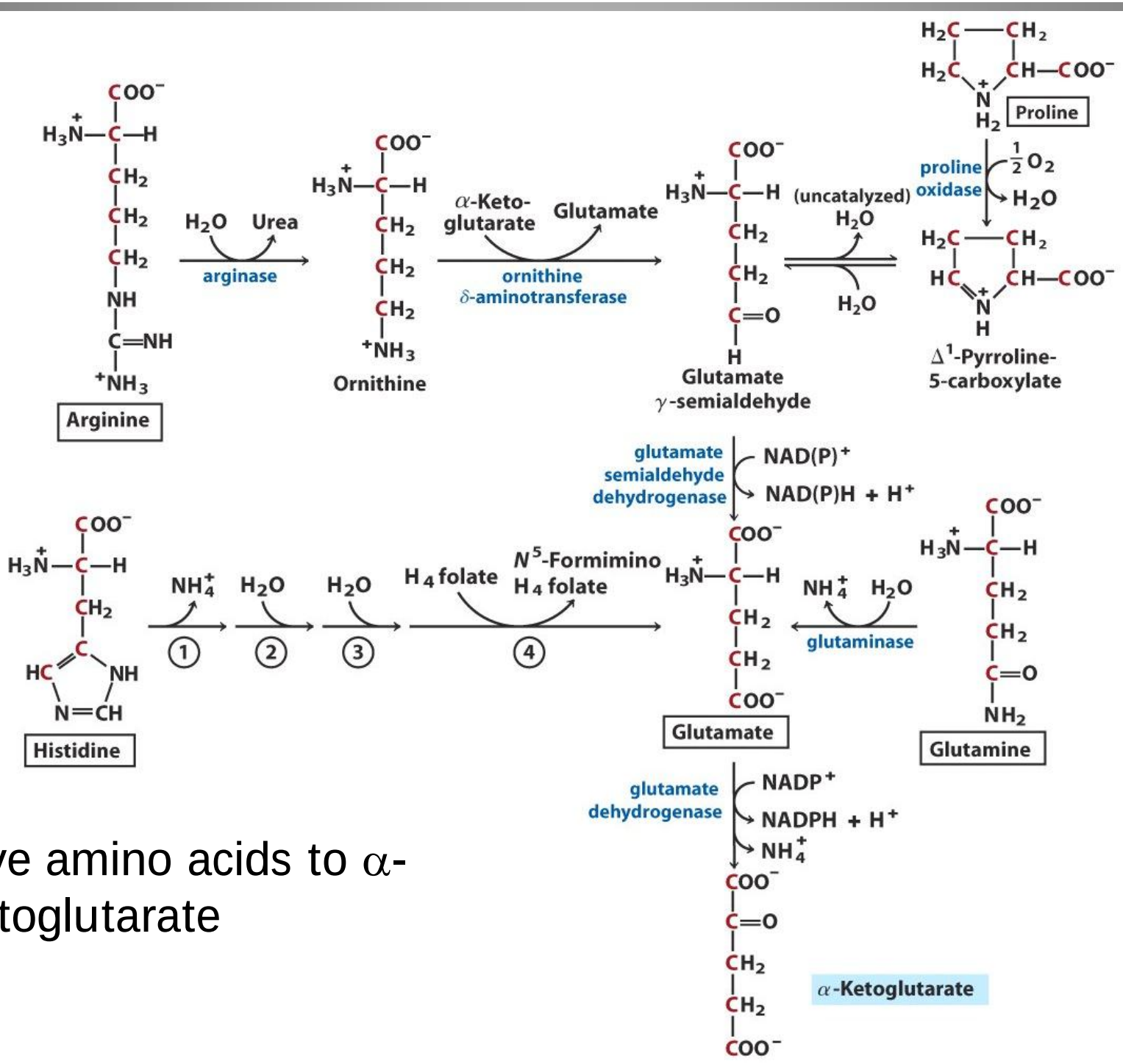
Phenylalanine hydroxylase



Minor pathway

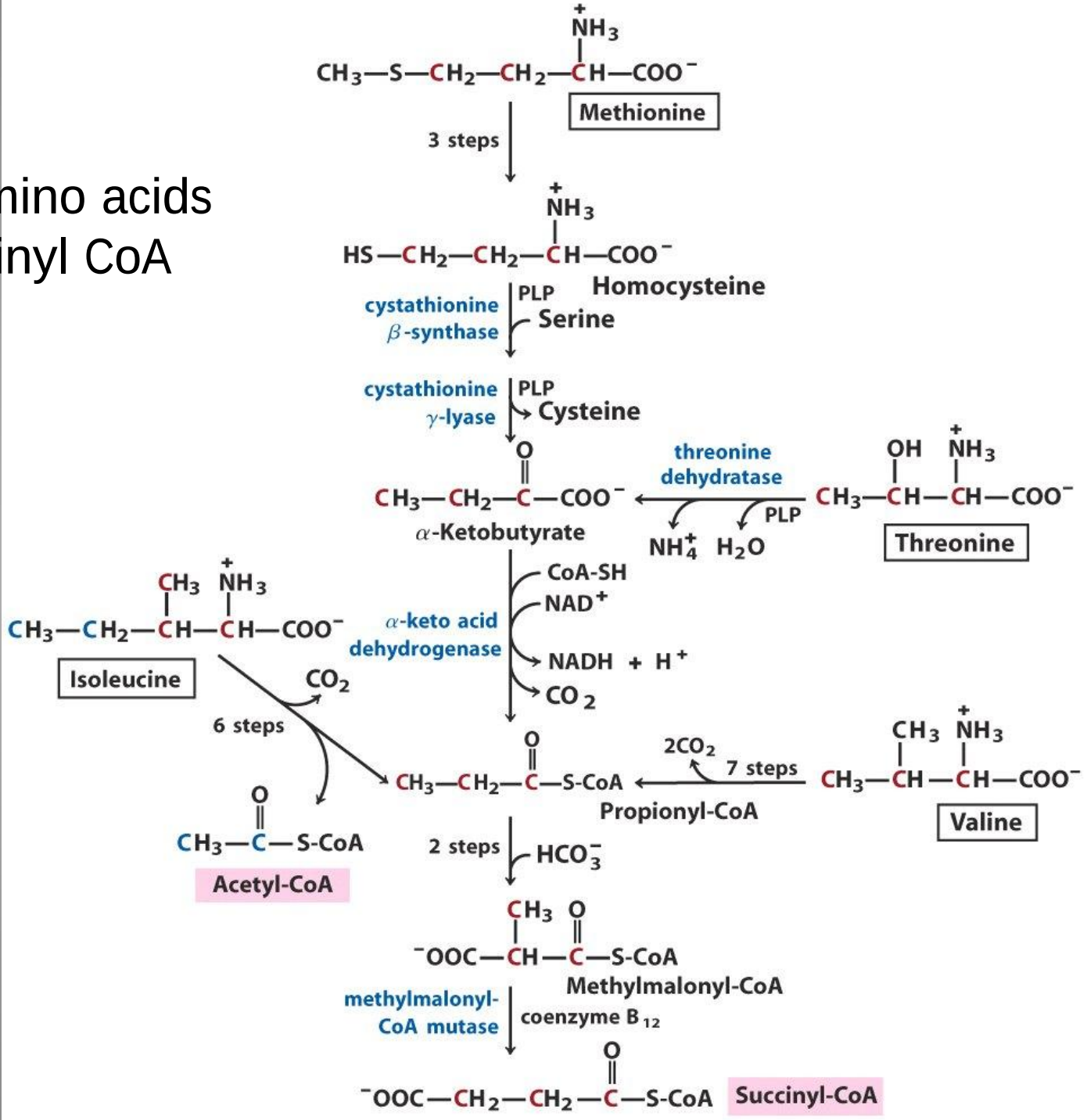
- In PKU phenylpyruvate accumulate in tissues, blood, urine
- Urine also contains phenylacetate and phenyllactate



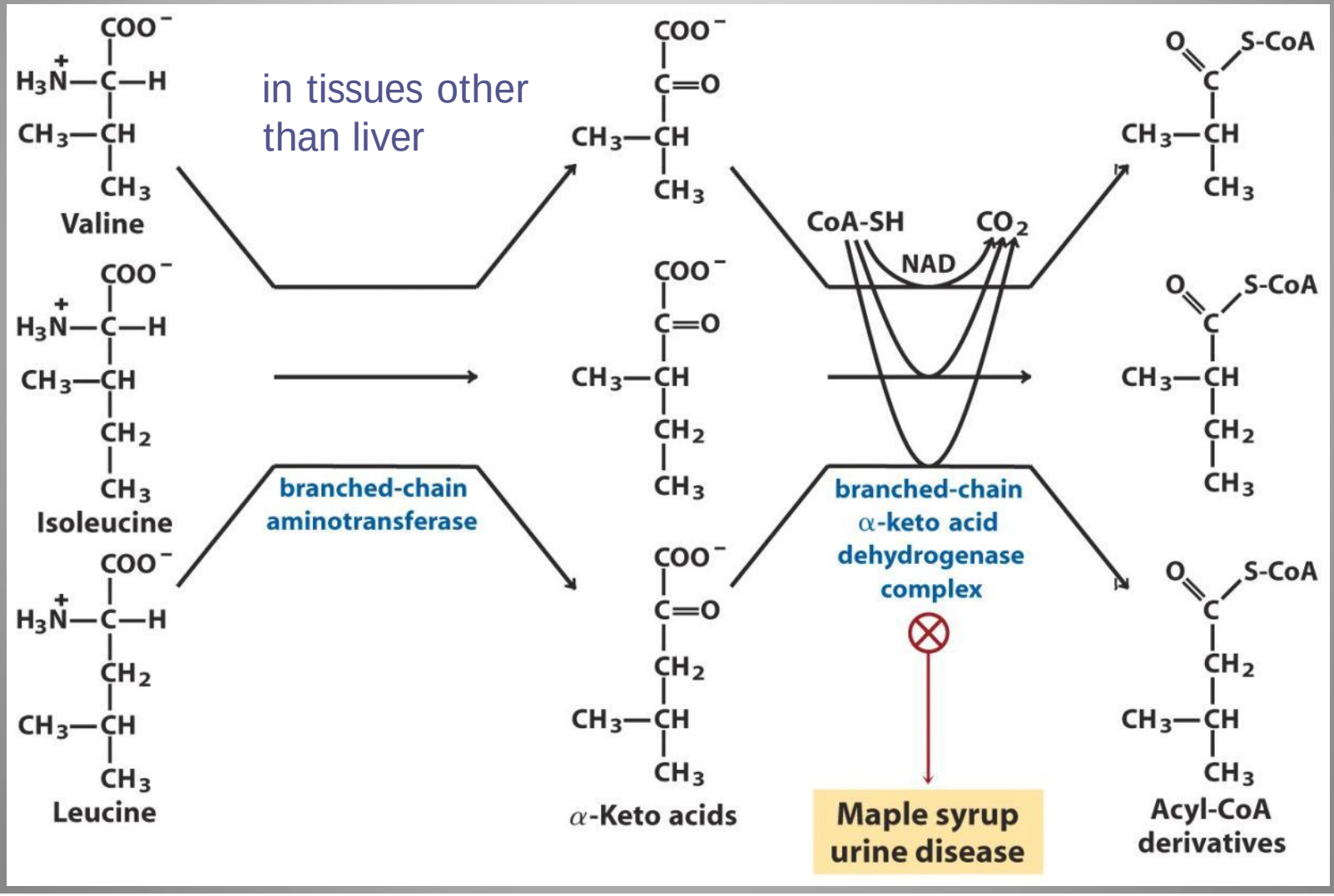


Five amino acids to α-ketoglutarate

Four amino acids to succinyl CoA



Branched-chain amino acids

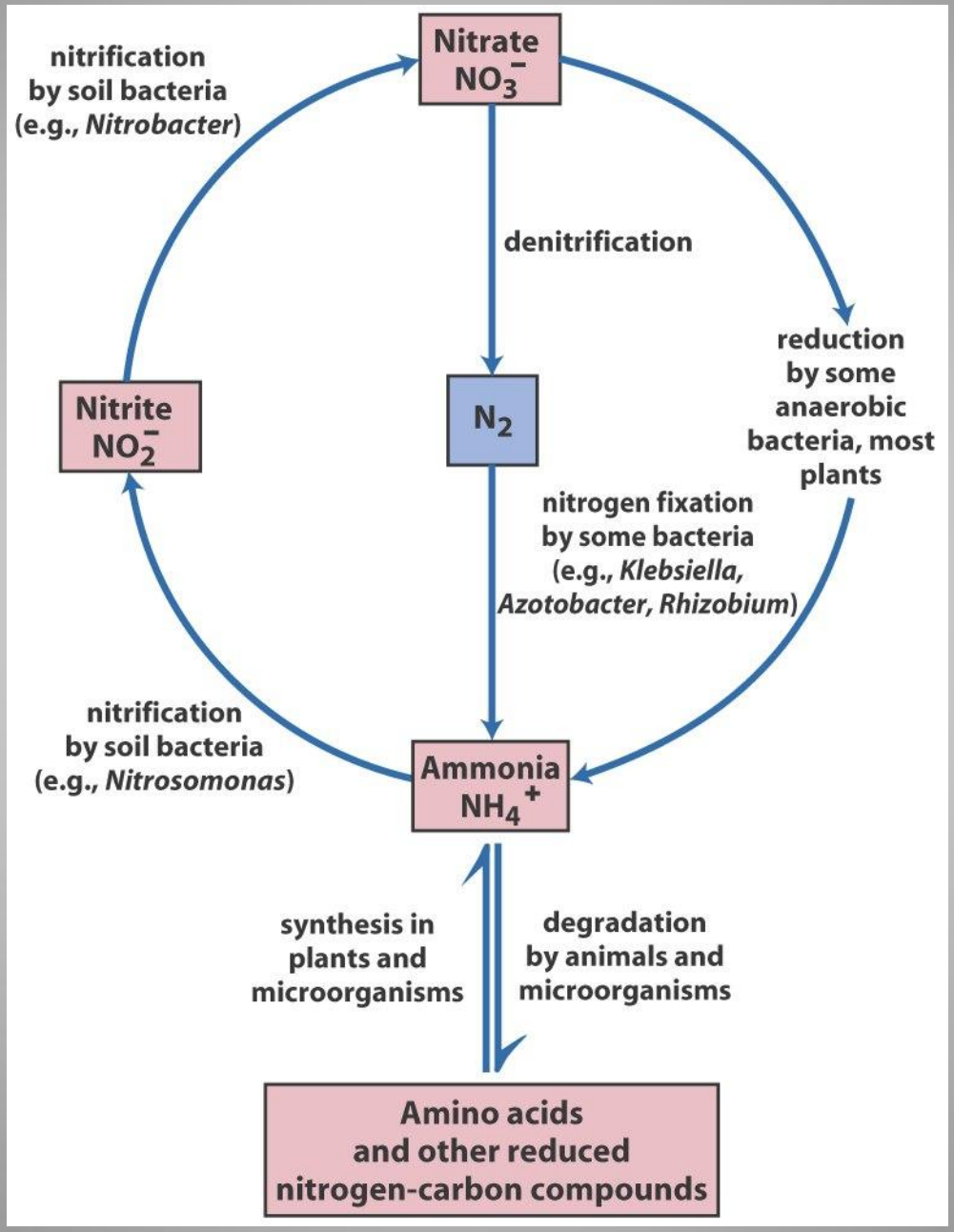


Pathways of amino acid degradation

- Removal of their amino groups
- Carbon skeletons undergo oxidation to common intermediates
- Some aa to glucose, some to ketone bodies, some to both
- Acetyl CoA, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate

Nitrogen cycle

- The majority source of N is the air (N_2)
- Nitrogen fixation: reduction of N_2 to NH_4^+
  - By nitrogen-fixing bacteria e.g. *Rhizobium*
- Nitrification: oxidation of NH_4^+ to nitrite and nitrate
  - By soil bacteria
- Denitrification: conversion of nitrate to N_2 under anaerobic condition



Nitrogenase complex

- Reduction of nitrogen to ammonia

$$N_2 + 3H_2 \longrightarrow 2NH_3$$

- $\Delta G'^{\circ}$=-33.5 kJ/mol
- Activation energy → $N \equiv N$
  - Bond energy = 930 kJ/mol
- Nitrogenase complex

$$N_2 + 10H^+ + 8e^- + 16ATP \longrightarrow 2NH_4^+ + 16ADP + 16P_i + H_2$$

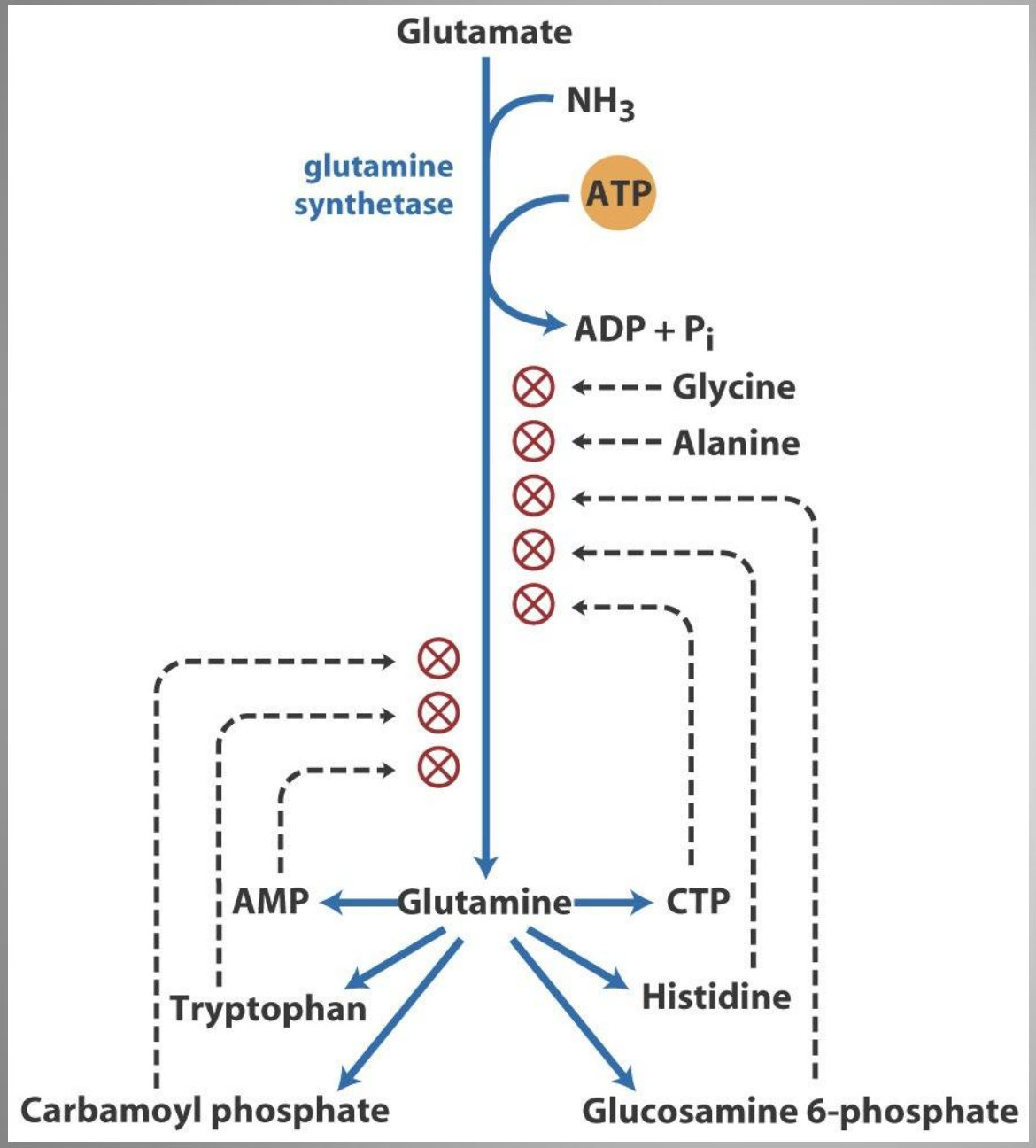
Ammonia→Glu, Gln

- Assimilation of NH_4^+ into glutamate requires two reactions
- Glutamine synthetase

$$\text{Glutamate} + NH_4^+ + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + P_i + H^+$$

- Glutamate synthetase

$$\alpha\text{-ketoglutarate} + \text{glutamine} + \text{NADPH} + H^+ \longrightarrow 2\,\text{glutamate} + \text{NADP}^+$$



Glutamine synthetase: primary regulatory in *E.coli*

Biosynthesis of amino acids

- Nonessential amino acids
  - Around half of 20 aa can be synthesized
- Essential amino acids

TABLE 22–1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate
Glutamate
Glutamine
Proline
Arginine

3-Phosphoglycerate
Serine
Glycine
Cysteine

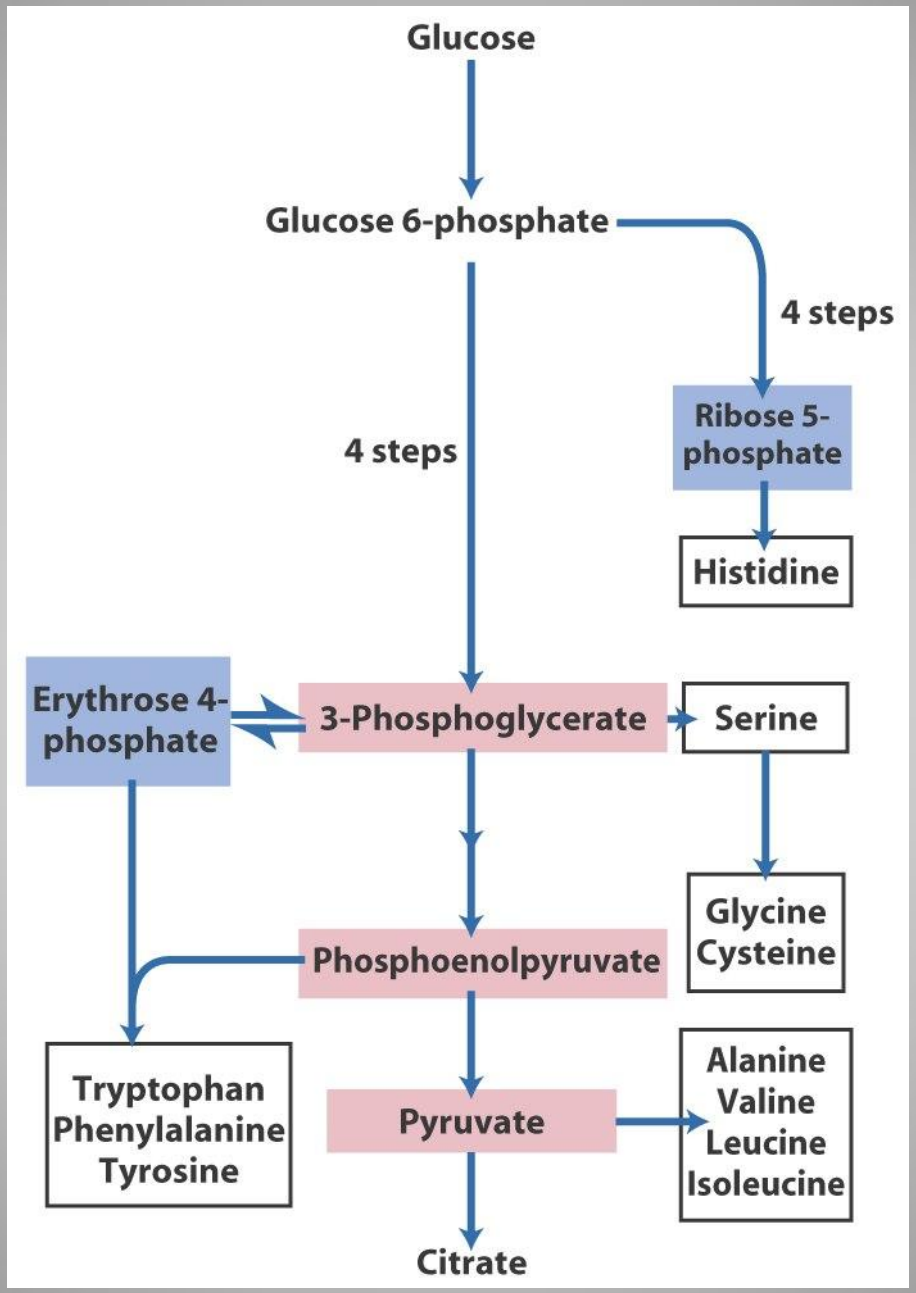
Oxaloacetate
Aspartate
Asparagine
Methionine*
Threonine*
Lysine*

Pyruvate
Alanine
Valine*
Leucine*
Isoleucine*

Phosphoenolpyruvate and erythrose 4-phosphate
Tryptophan*
Phenylalanine*
Tyrosine†

Ribose 5-phosphate
Histidine*

*Essential amino acids.
†Derived from phenylalanine in mammals.



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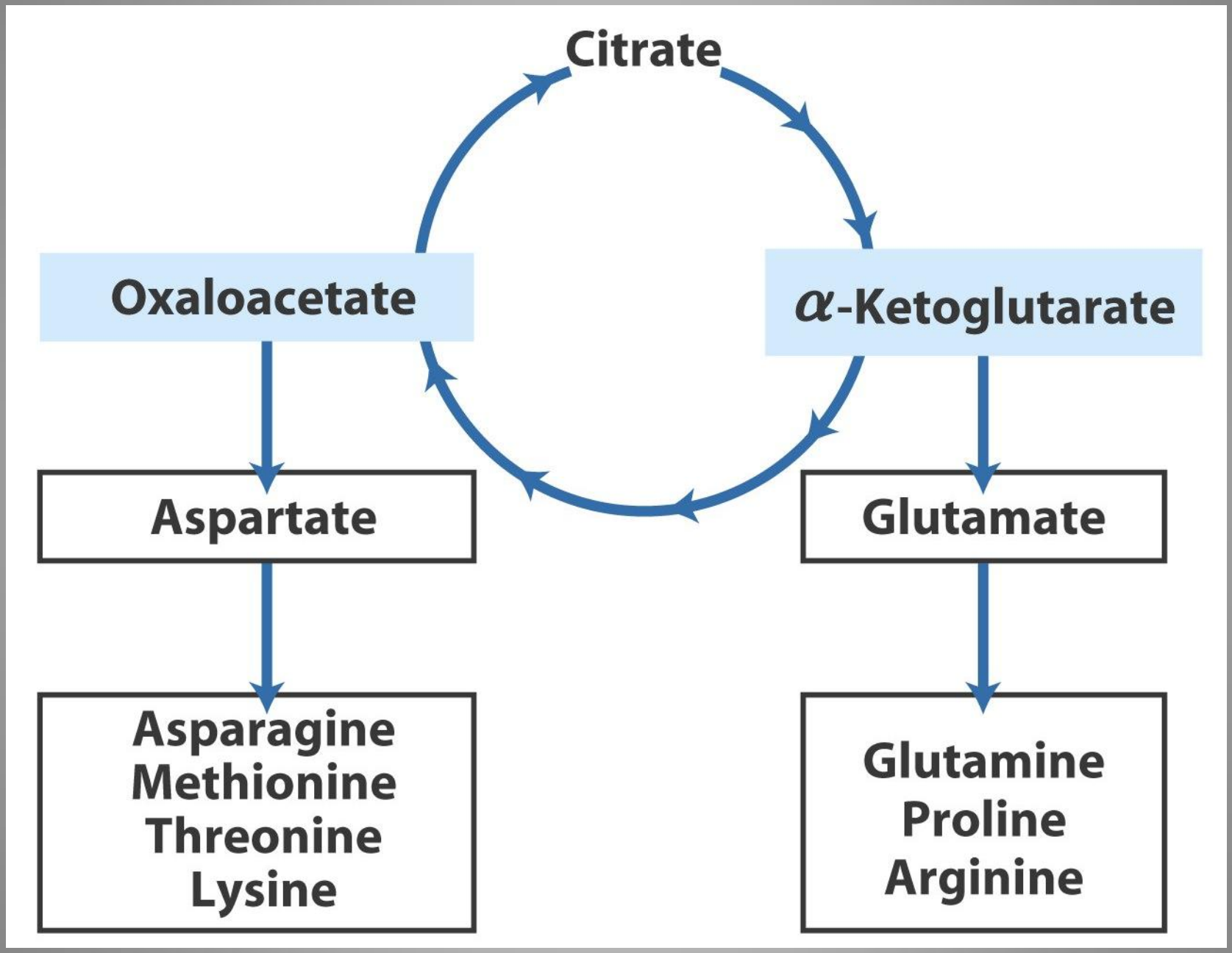

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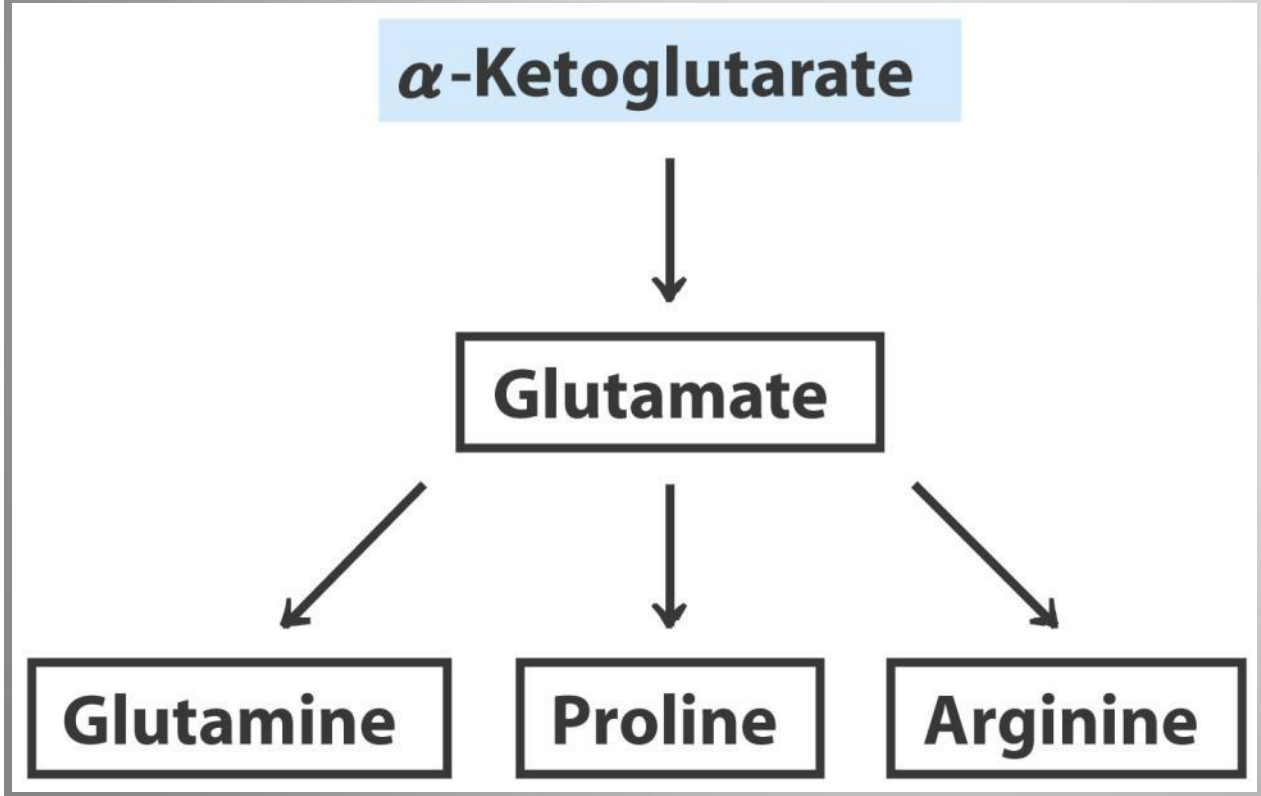
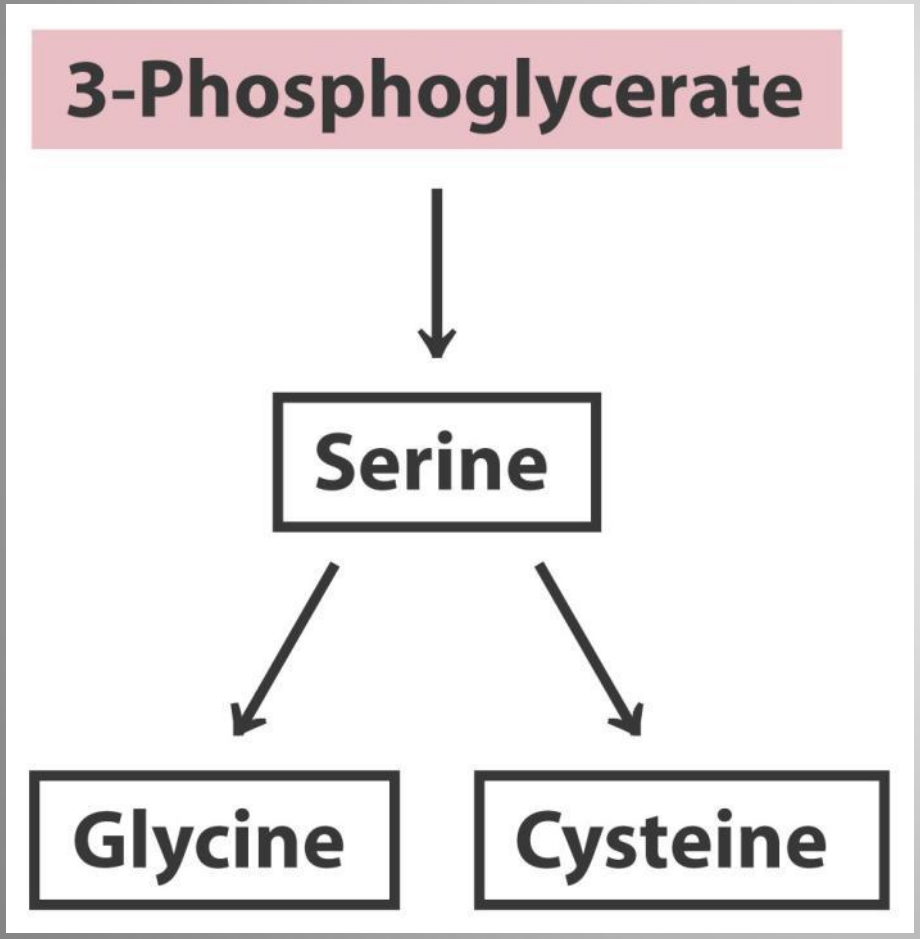
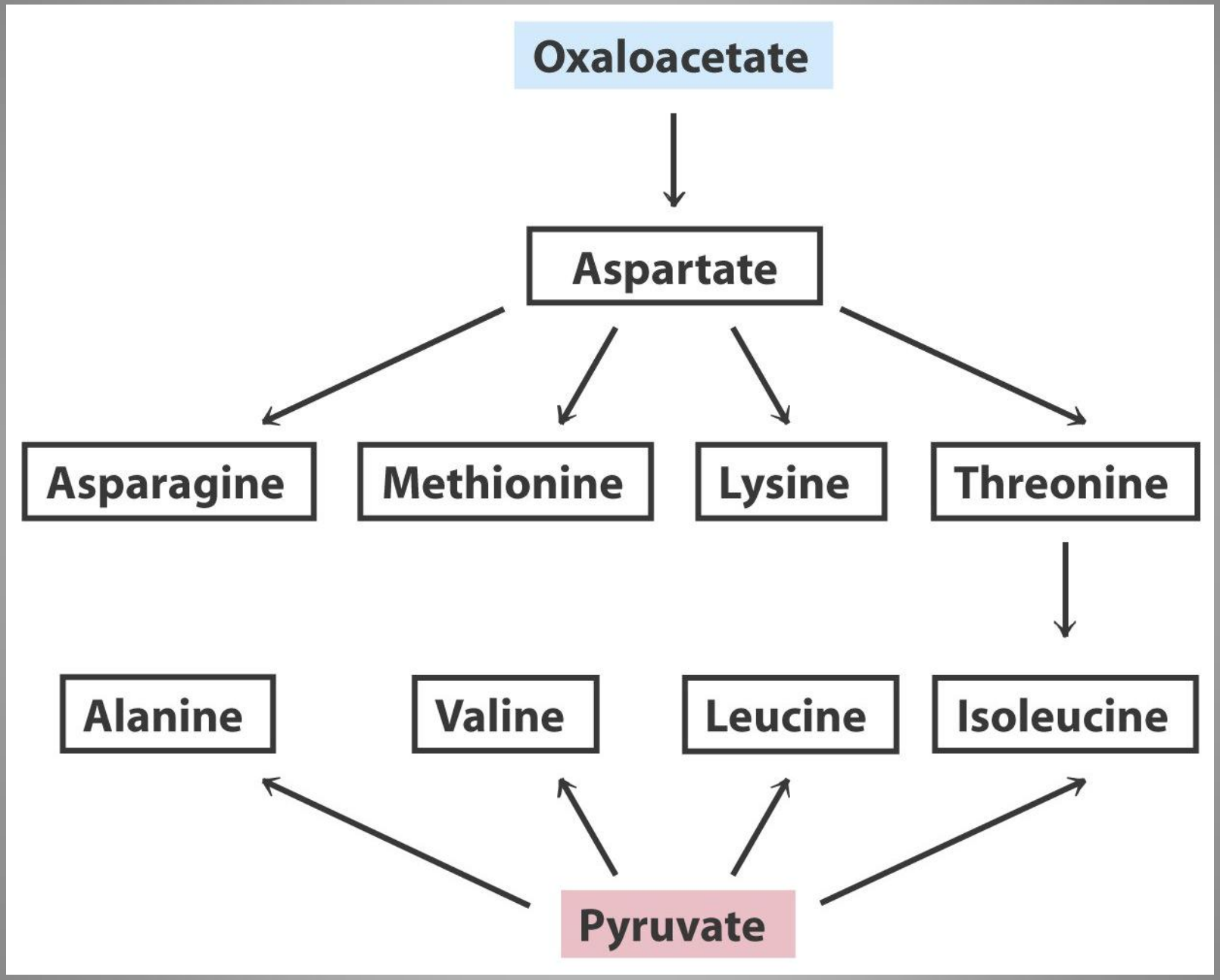


- Pro is a cyclized derivative of glutamate
- Arg is synthesized from glutamate via ornithine and the urea cycle
- Ornithine is interconverted to glutamate γ-semialdehyde by ornithine δ-aminotransferase



- Major pathway for synthesis of serine is the same in all organism.
- First by oxidation of hydroxyl group of 3-phosphoglycerate
- Transaminattion from glu yields 3-phosphoserine



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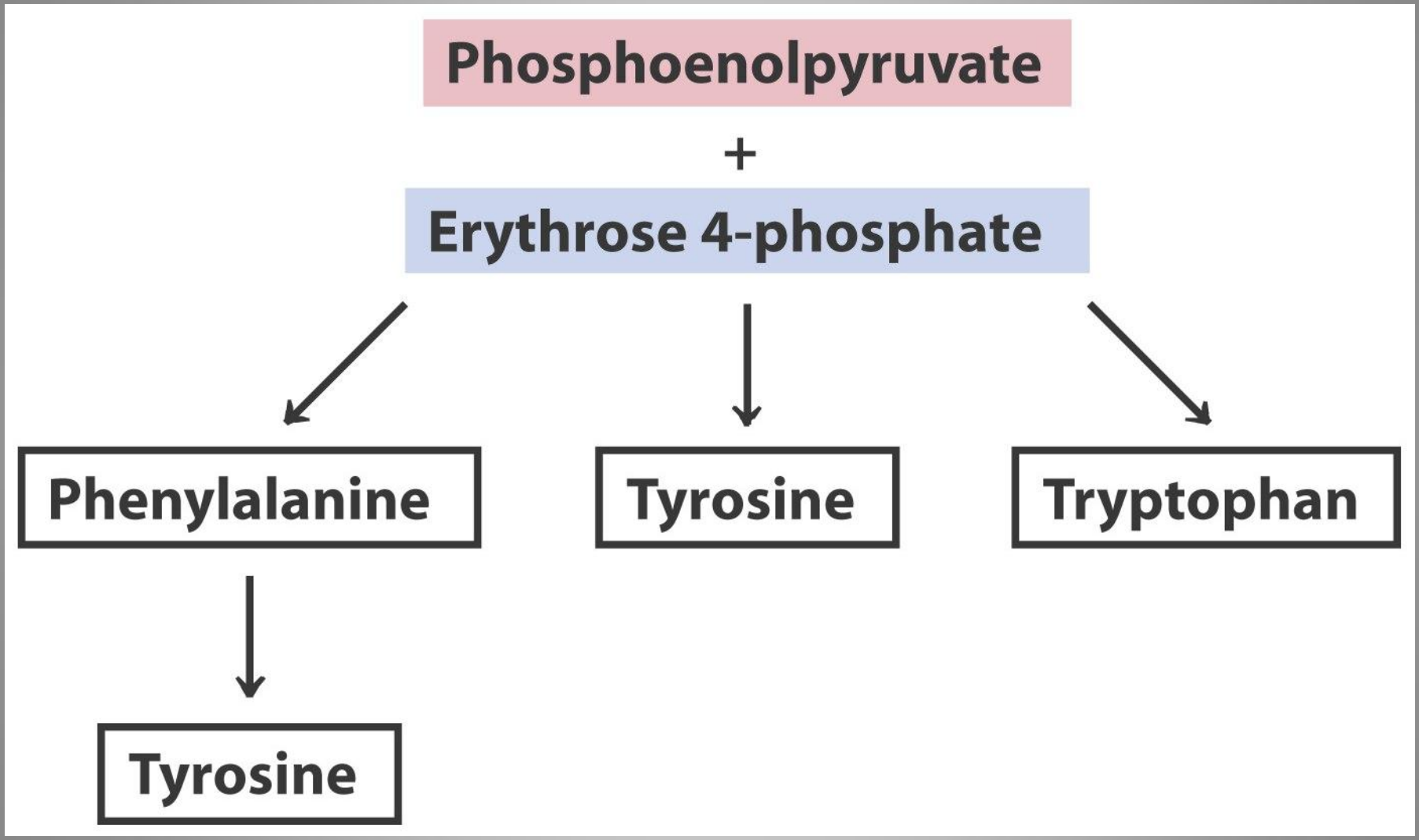
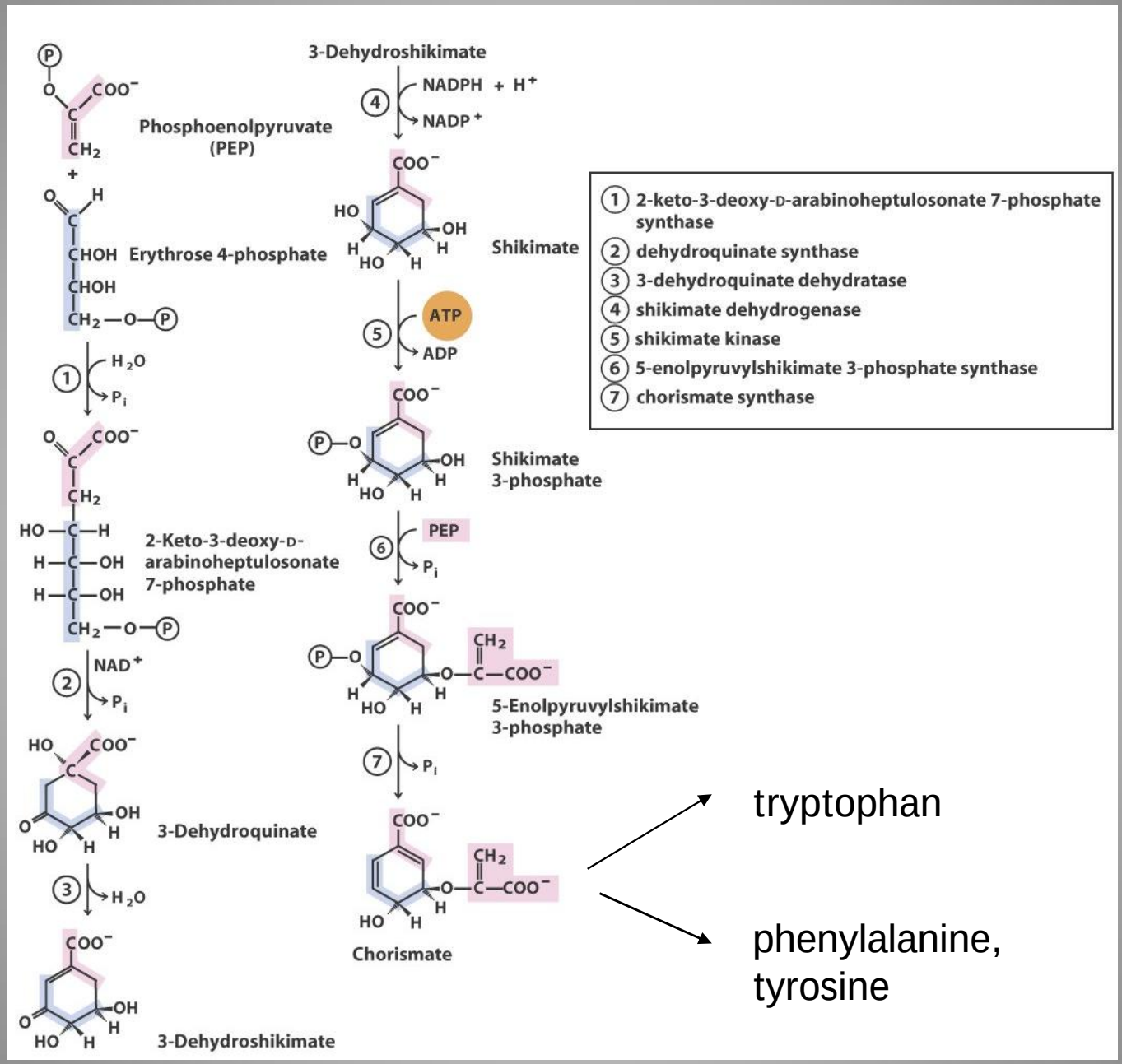
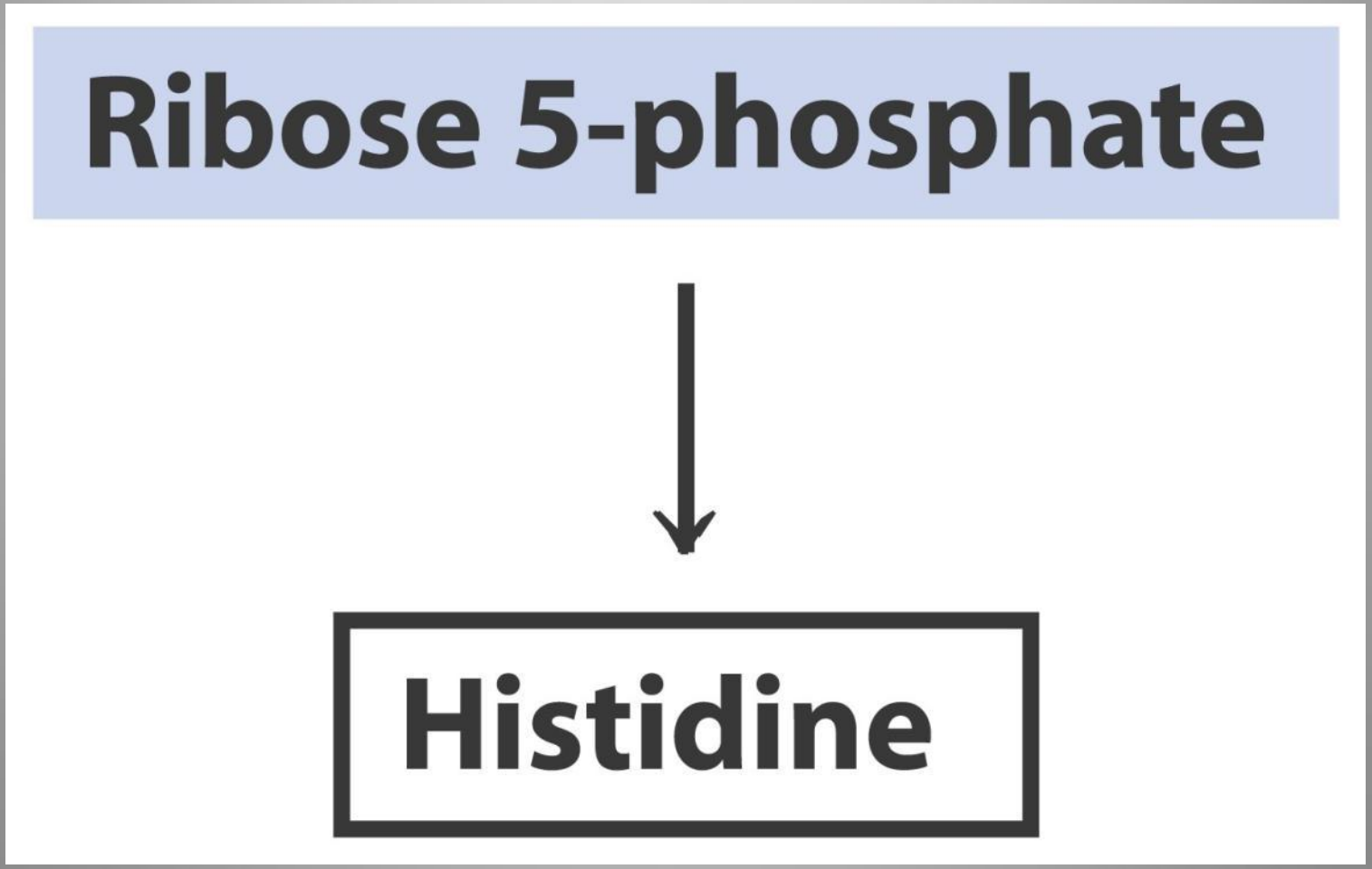
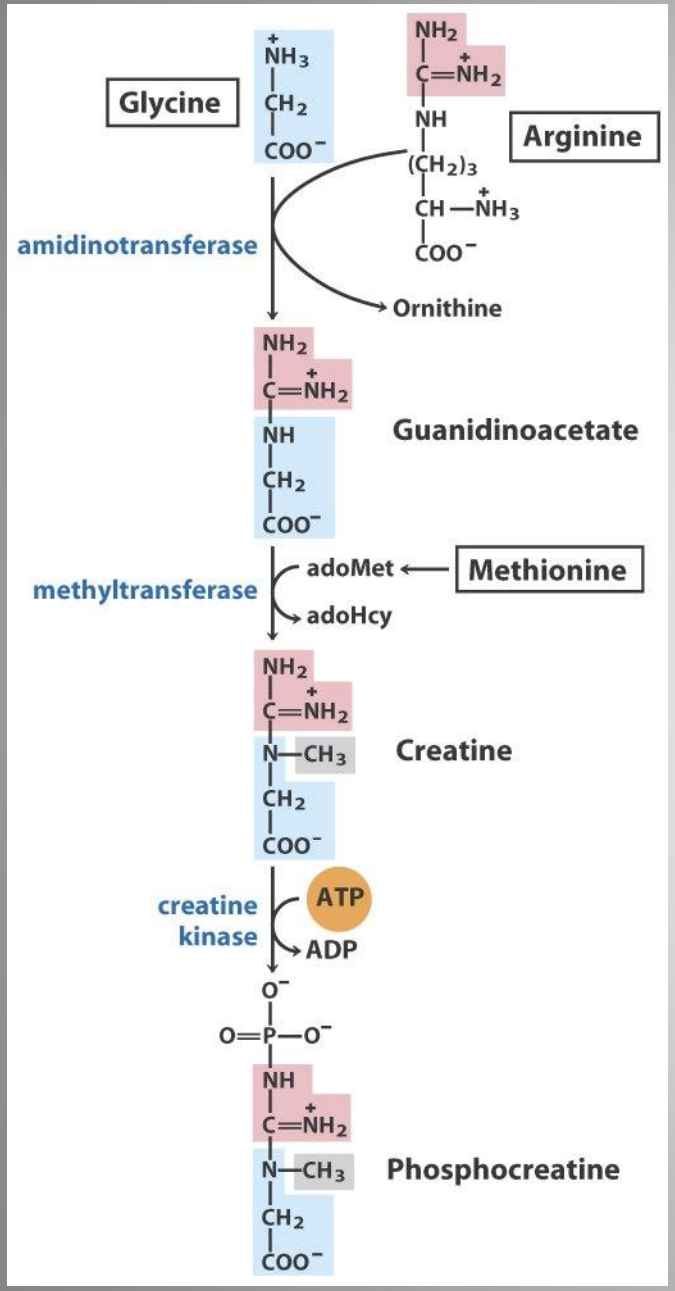



Phosphoenolpyruvate (PEP)
Erythrose 4-phosphate
2-Keto-3-deoxy-D-arabinoheptulosonate 7-phosphate
3-Dehydroquinate
3-Dehydroshikimate
3-Dehydroshikimate
NADPH + H+
NADP+
Shikimate
ATP
ADP
Shikimate 3-phosphate
PEP
5-Enolpyruvylshikimate 3-phosphate
Chorismate
1 2-keto-3-deoxy-D-arabinoheptulosonate 7-phosphate synthase
2 dehydroquinate synthase
3 3-dehydroquinate dehydratase
4 shikimate dehydrogenase
5 shikimate kinase
6 5-enolpyruvylshikimate 3-phosphate synthase
7 chorismate synthase
tryptophan
phenylalanine, tyrosine

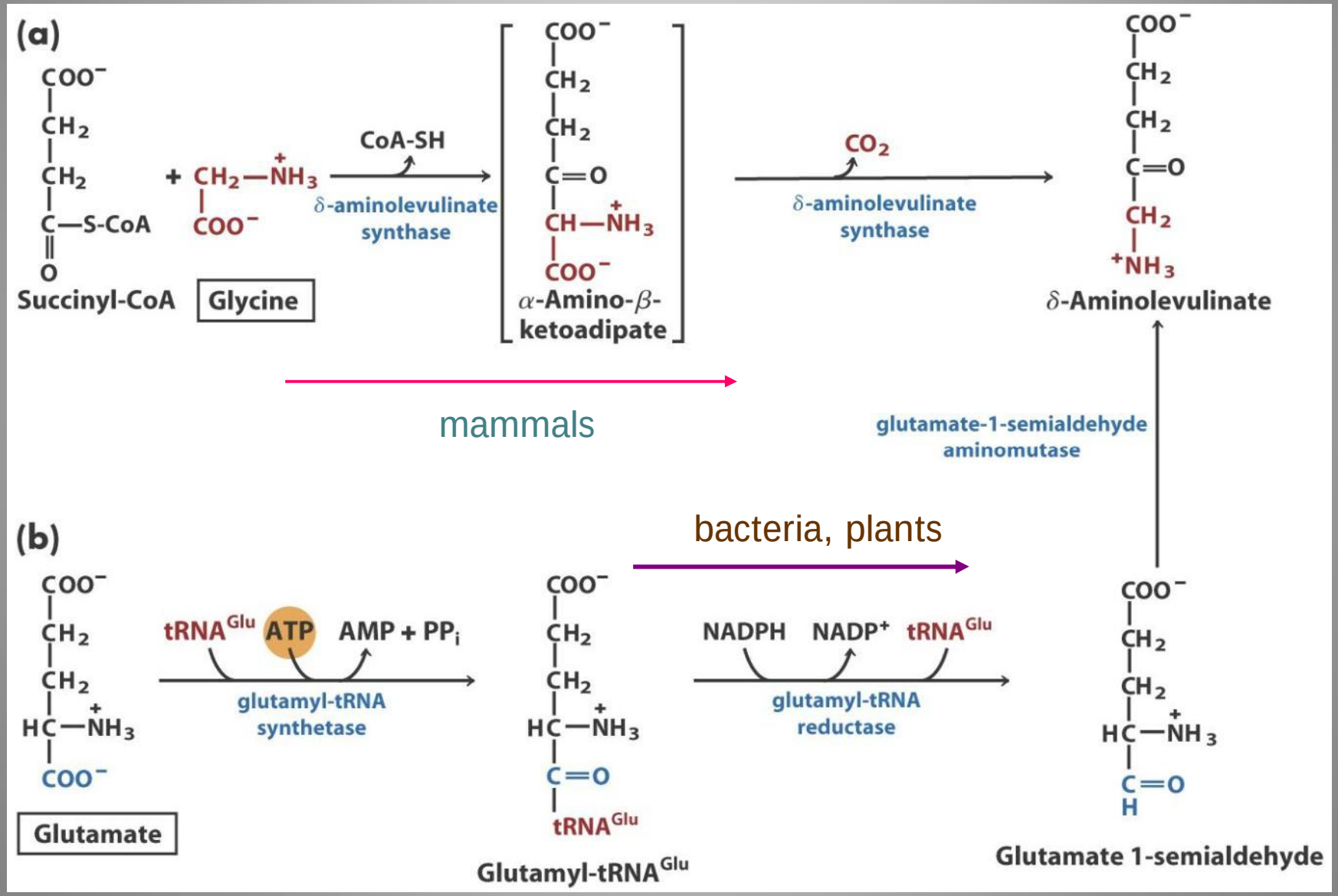
PRPP contributes five carbons, the purine ring of ATP contributes a nitrogen and a carbon, and glutamine supplies the second nitrogen atom.



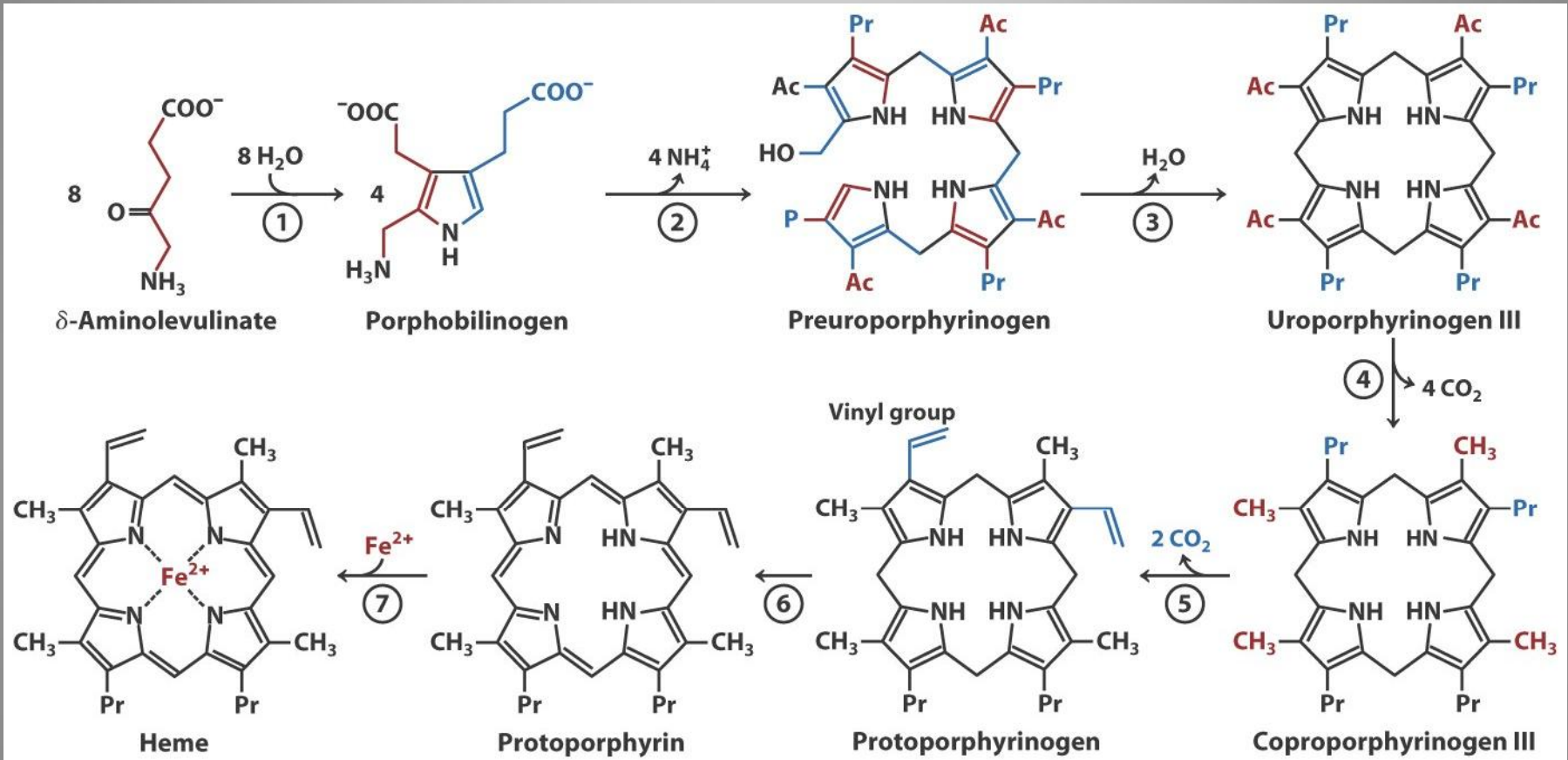


- Synthesis of high energy compounds: Creatine phosphate

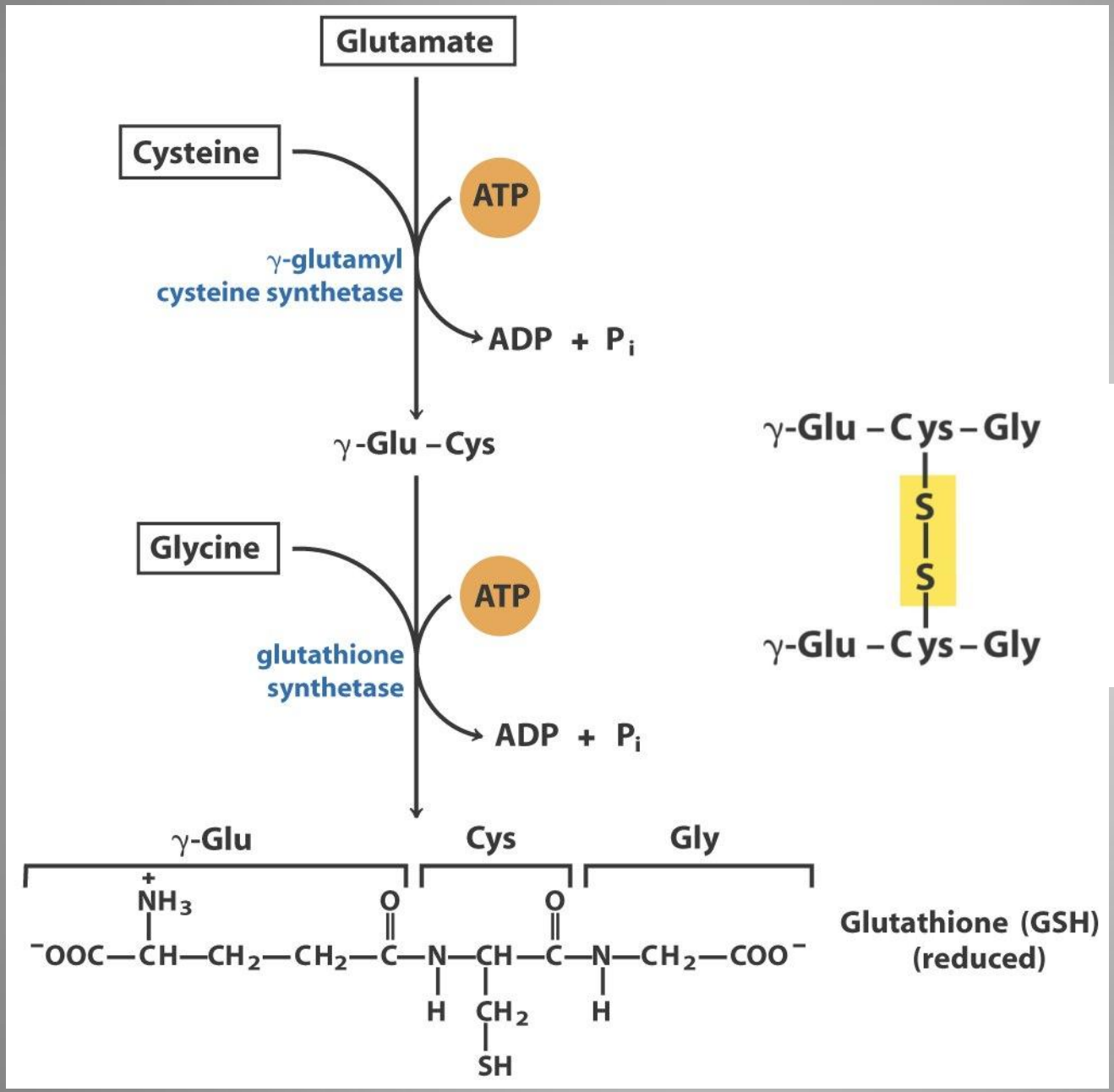
Glycine as a precursor of porphyrins

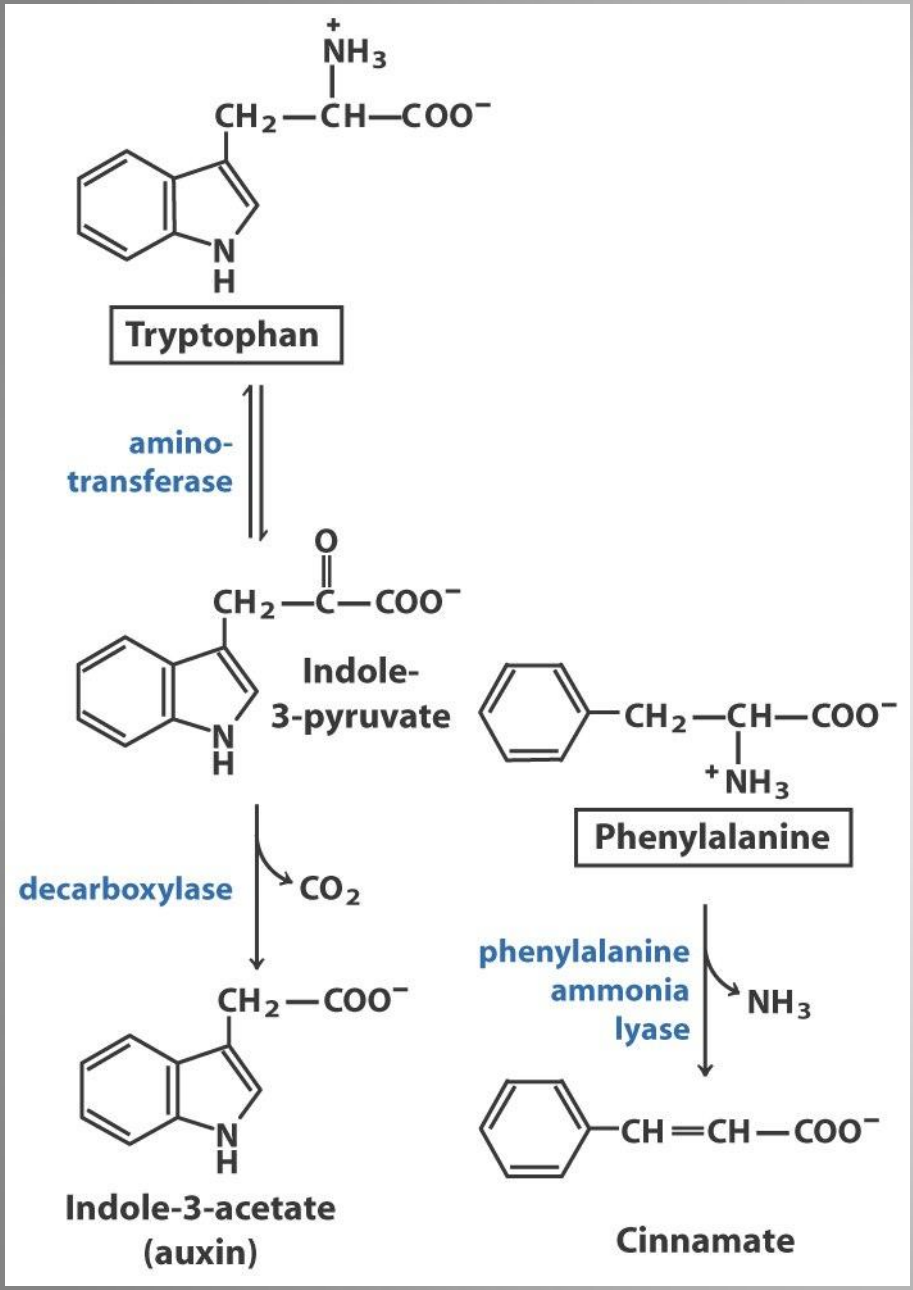


Biosynthesis of heme

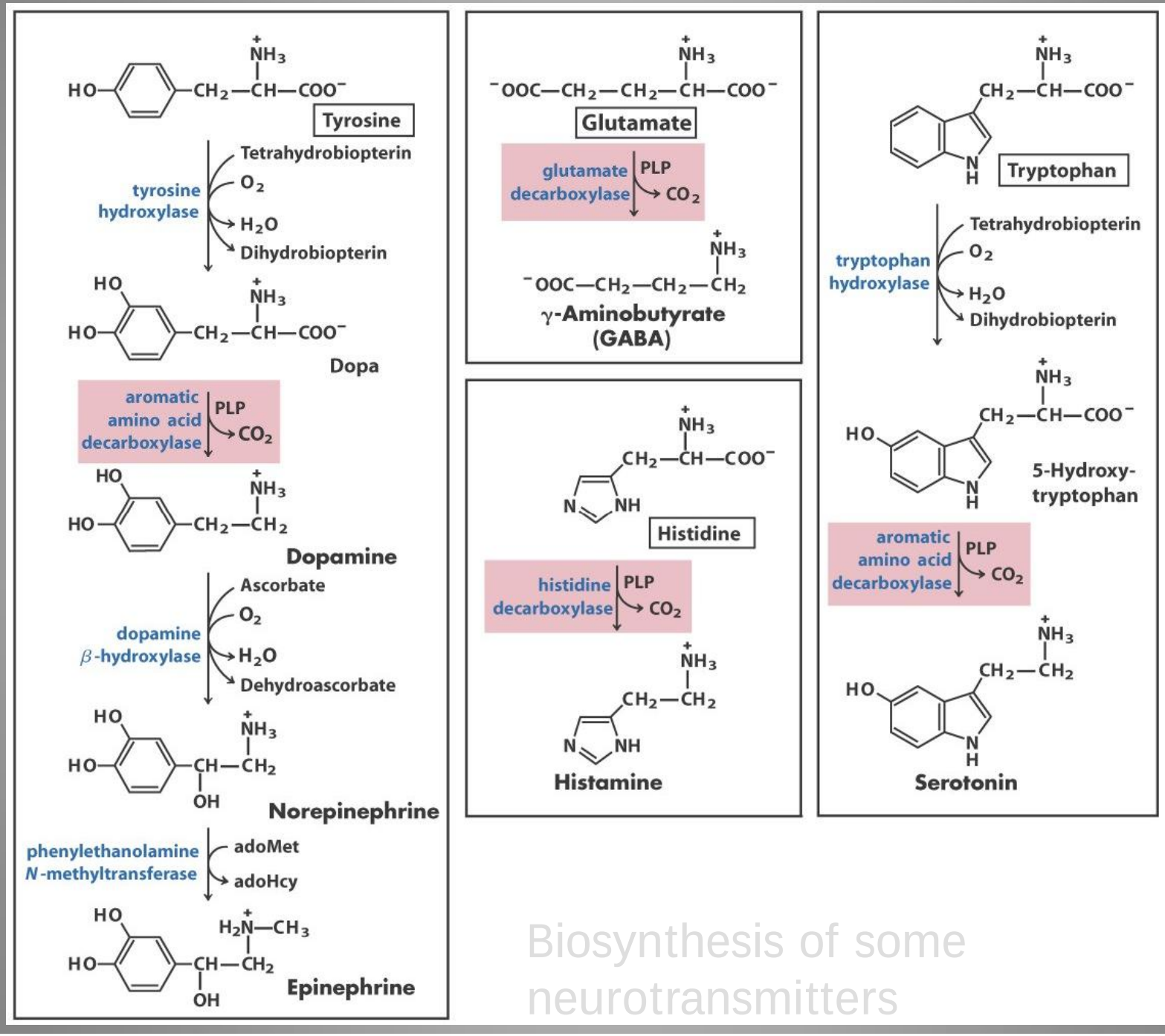


1	porphobilinogen synthase
2	uroporphyrinogen synthase
3	uroporphyrinogen III cosynthase
4	uroporphyrinogen decarboxylase
5	coproporphyrinogen oxidase
6	protoporphyrinogen oxidase
7	ferrochelatase

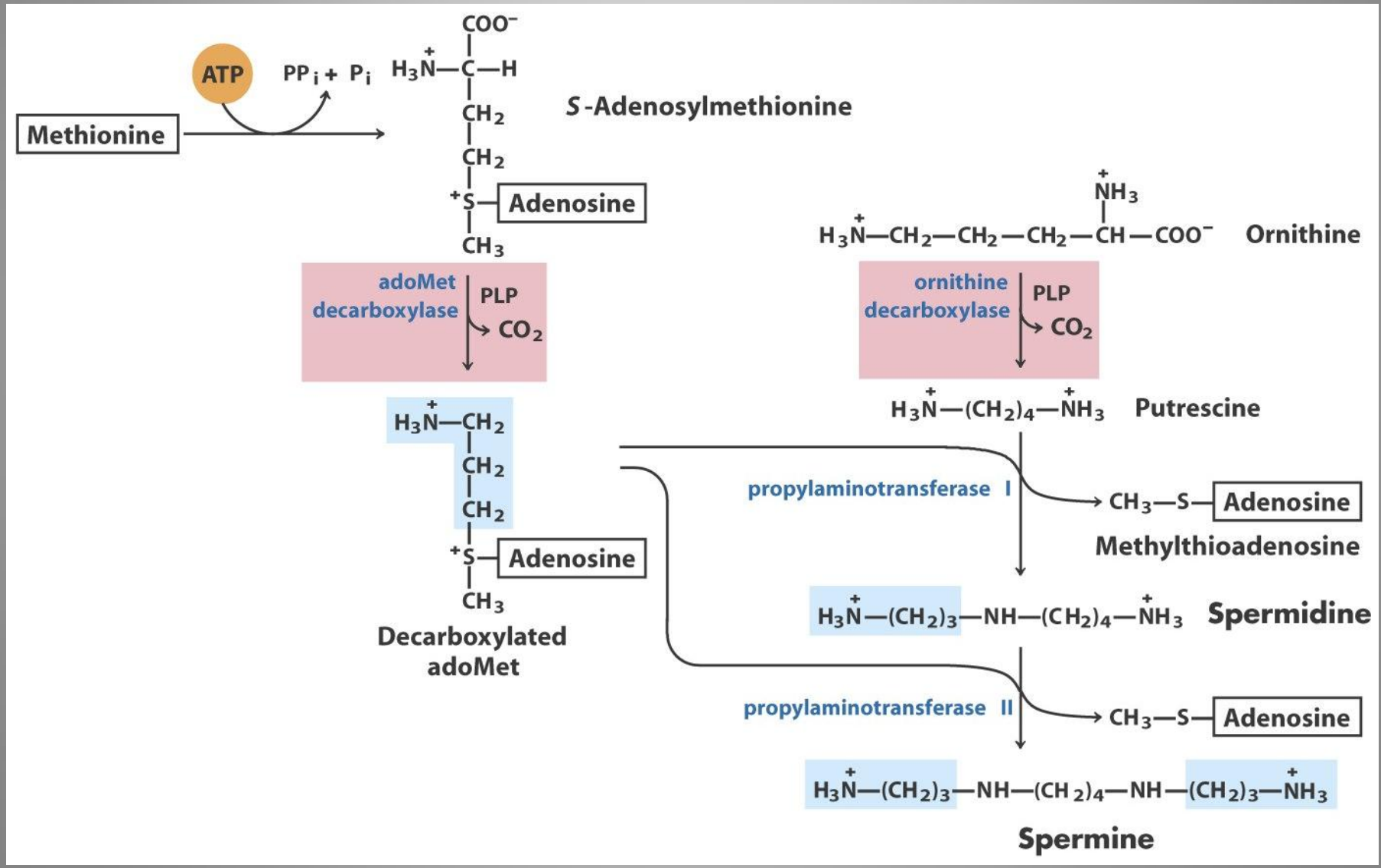




Biological amines from aa decarboxylation



Biosynthesis of some neurotransmitters


ATP
PPi + Pi
Methionine
Adenosine
S-Adenosylmethionine
adoMet decarboxylase
PLP
CO2
Decarboxylated adoMet
Ornithine
ornithine decarboxylase
Putrescine
propylaminotransferase I
Methylthioadenosine
Spermidine
propylaminotransferase II
Spermine

Biosynthesis of NO