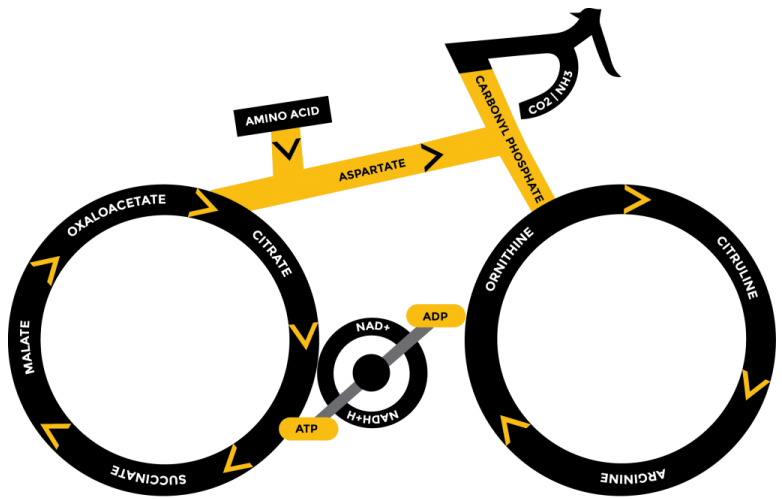
Amino acid degradation; urea cycle



Kristina Mlinac Jerković

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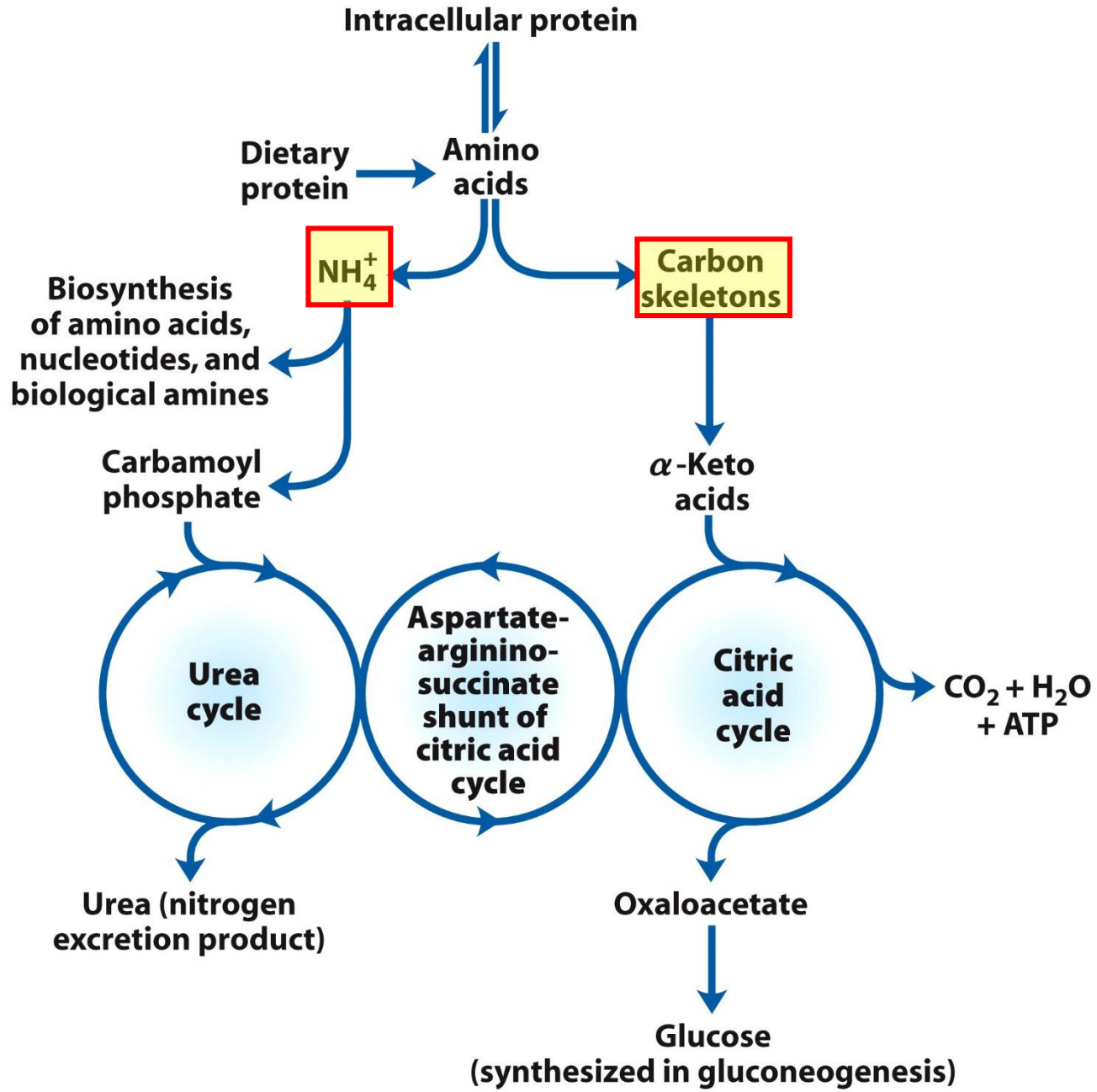
In animals, **amino acids undergo oxidative degradation in three different metabolic circumstances:**

1. During the normal synthesis and degradation of cellular proteins, some amino acids that are released from protein breakdown and are not needed for new protein synthesis undergo oxidative degradation.

2. When a diet is rich in protein and the ingested amino acids exceed the body's needs for protein synthesis, the surplus is catabolized; amino acids cannot be stored.

3. During starvation or in uncontrolled diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used as fuel.



Metabolic fates of amino groups

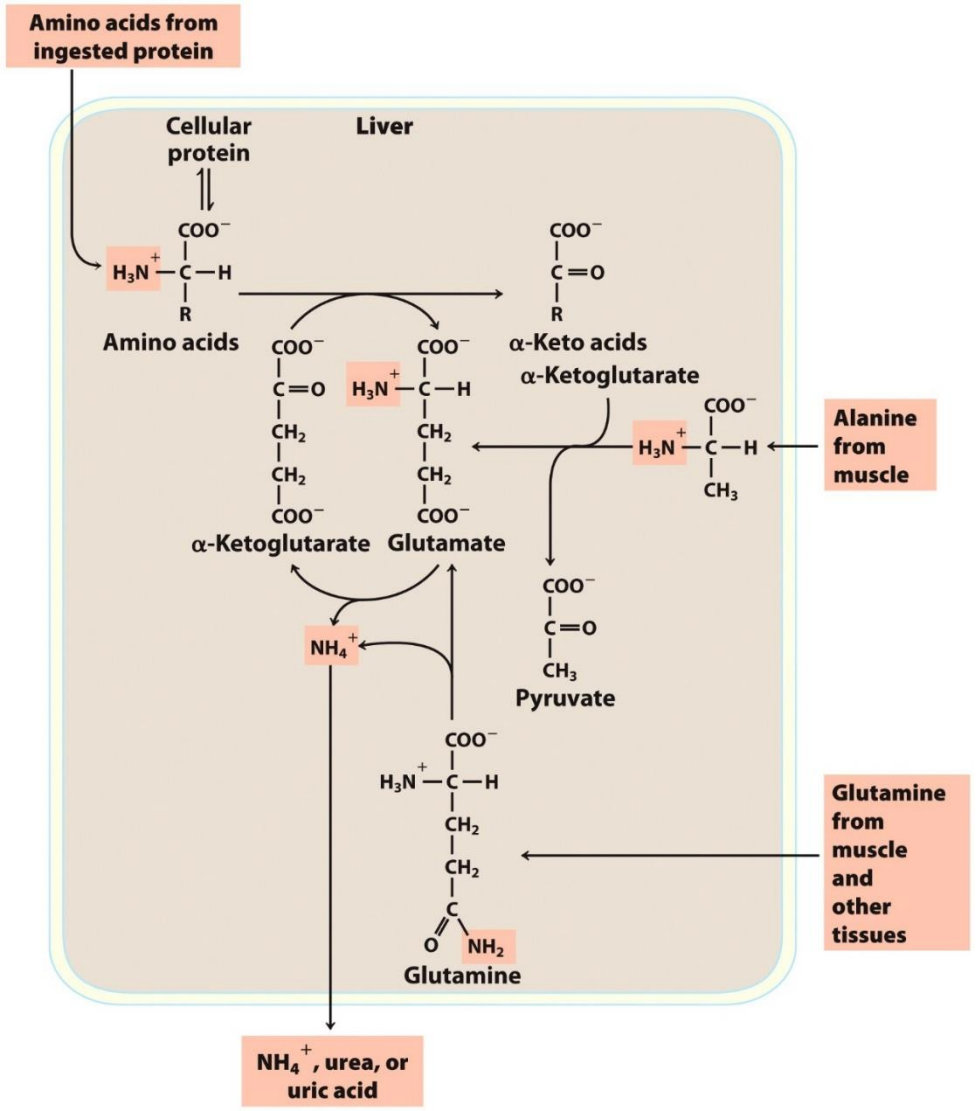
- The metabolism of nitrogen-containing molecules such as proteins and nucleic acids differs significantly from that of carbohydrates and lipids.

- Whereas the latter molecules can be stored and mobilized as needed for biosynthetic reactions or for energy generation, there is no nitrogen-storing molecule (one exception to this rule is storage protein in seeds).

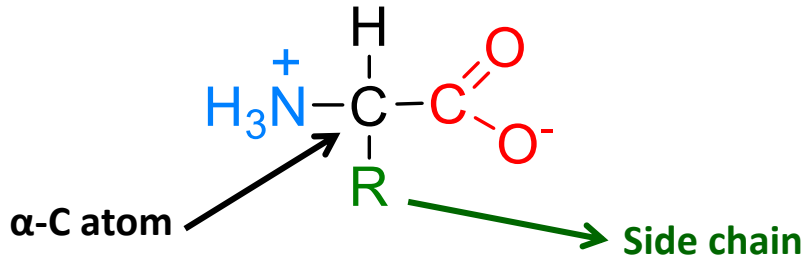
- Organisms must constantly replenish their supply of usable nitrogen to replace organic nitrogen that is lost in catabolism. For example, animals must have a steady supply of amino acids in their diets to replace the nitrogen excreted as urea, uric acid, and other nitrogenous waste products.

- Since excess dietary amino acids are neither stored for future use nor excreted, they are converted to common metabolic intermediates such as pyruvate, oxaloacetate, acetyl-CoA, and α-keto-glutarate.

- **Consequently, amino acids are also precursors of glucose, fatty acids, and ketone bodies and are therefore metabolic fuels.**

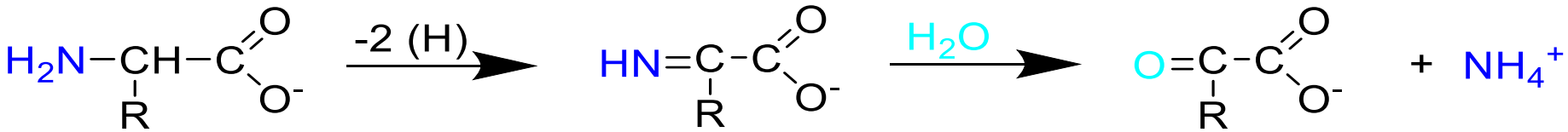


AMINO ACID – REMINDER



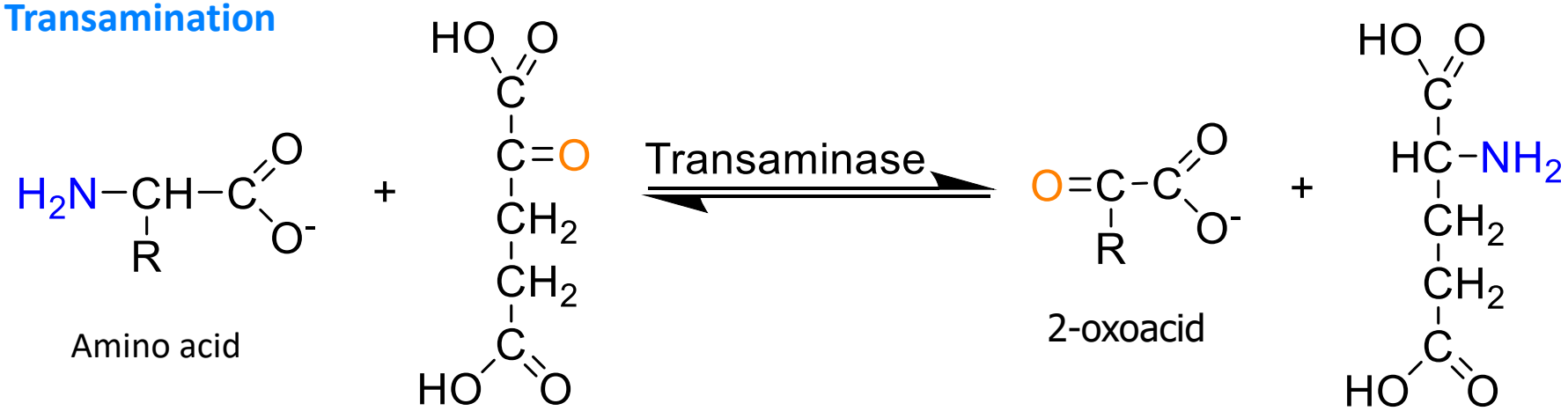
REACTIONS INVOLVING AMINO ACID NITROGEN

Deamination



Amino acid — Imino acid — Oxoacid

Transamination



Amino acid — 2-oxoglutaric acid (α-ketoglutaric acid) — 2-oxoacid — Glutamic acid

Deamination, release of -NH_2 in the form of **ammonia**

a. Oxidative deamination

- formation of $NADH+H^+$; reaction is catalyzed by **glutamate dehydrogenase (GDH)**

b. Hydrolytic deamination

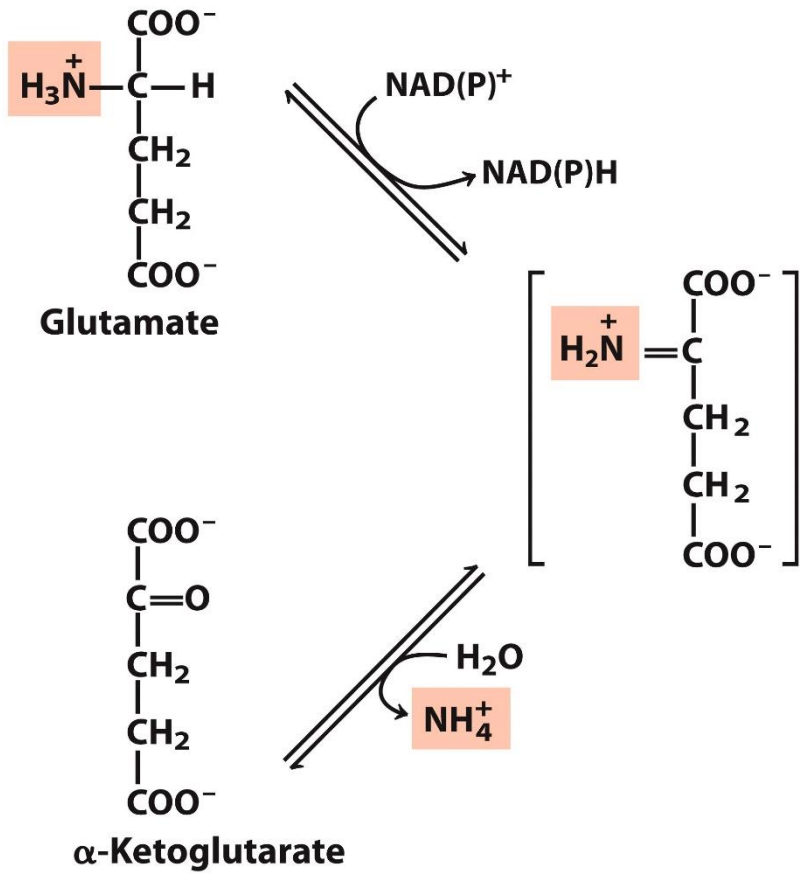
- amino groups are released by hydrolysis (asparagine and glutamine)

c. Eliminating deamination

- degradation of histidine and serine

Oxidative deamination of glutamate by glutamate dehydrogenase (GDH)

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



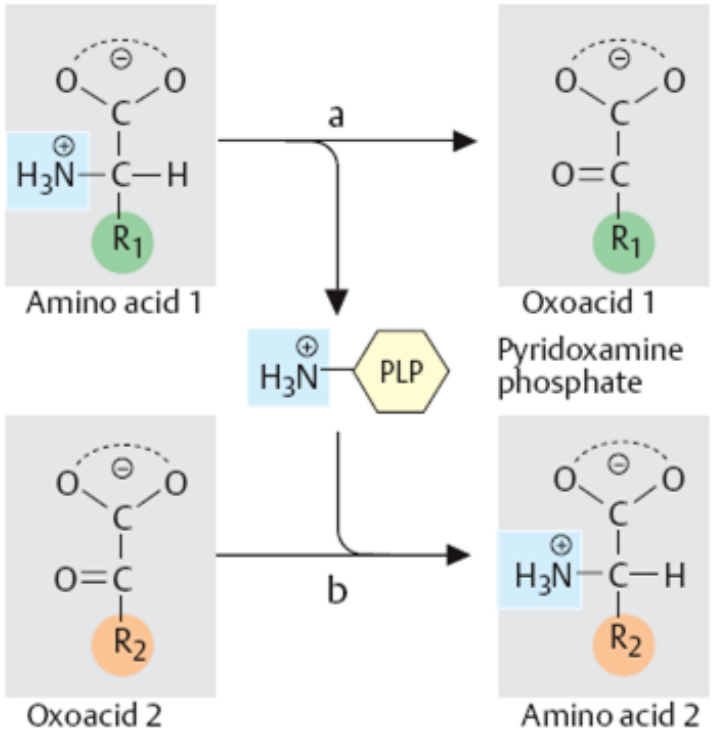
Transamination

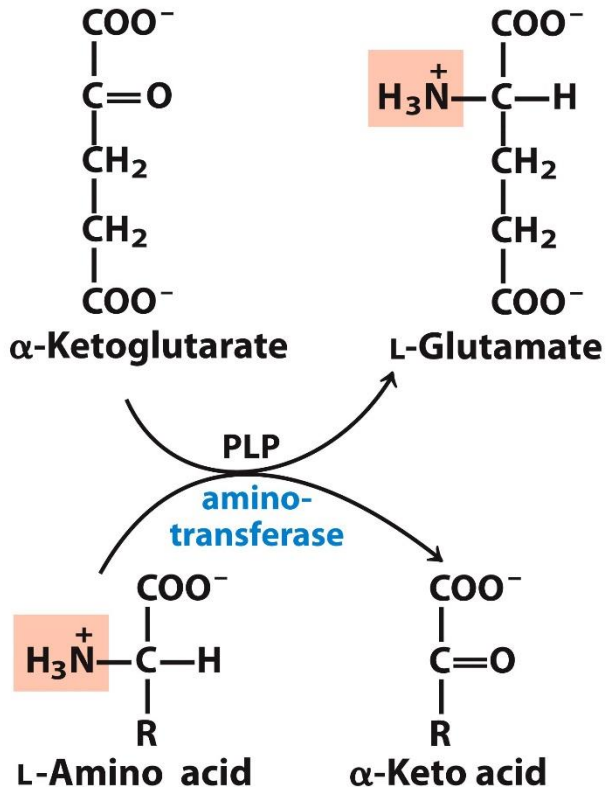
- No net deamination; amino groups from many a.a. are collected in the form of glutamate.
- Examples: branched-chain amino acids (Val, Leu, Ile); tyrosine, alanine and aspartate

- The predominant amino group acceptor is **α-ketoglutarate.**

- **Enzymes: amino-transferases, i.e. transaminases**

- Cells contain different types of aminotransferases which differ in their specificity for the L-amino acid (the enzymes are named for the amino group donor (alanine aminotransferase, aspartate aminotransferase..)



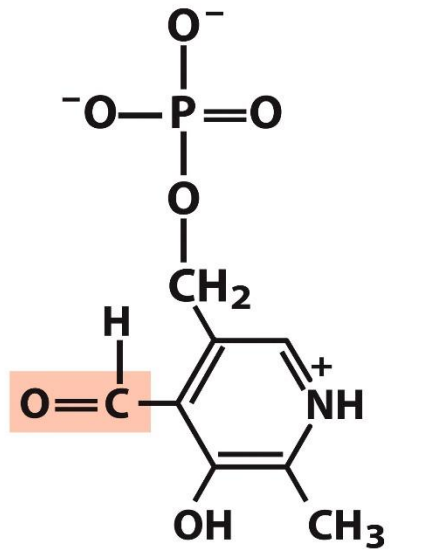


Cofactor of transaminases is pyridoxal phosphate (PLP)

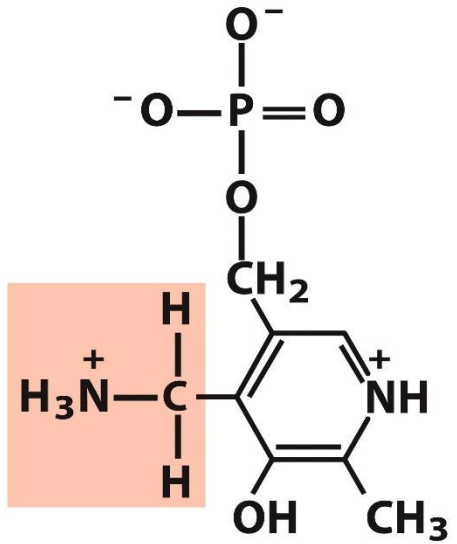
- The effect of transamination reactions is to **collect the amino groups from many different amino acids in the form of L-glutamate.**

- The glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products.

- To carry carry the amino group, aminotransferases require participation of an aldehyde-containing coenzyme, **pyridoxal-5'-phosphate (PLP)**, a derivative of **pyridoxine (vitamin B6).**



Pyridoxal phosphate (PLP)



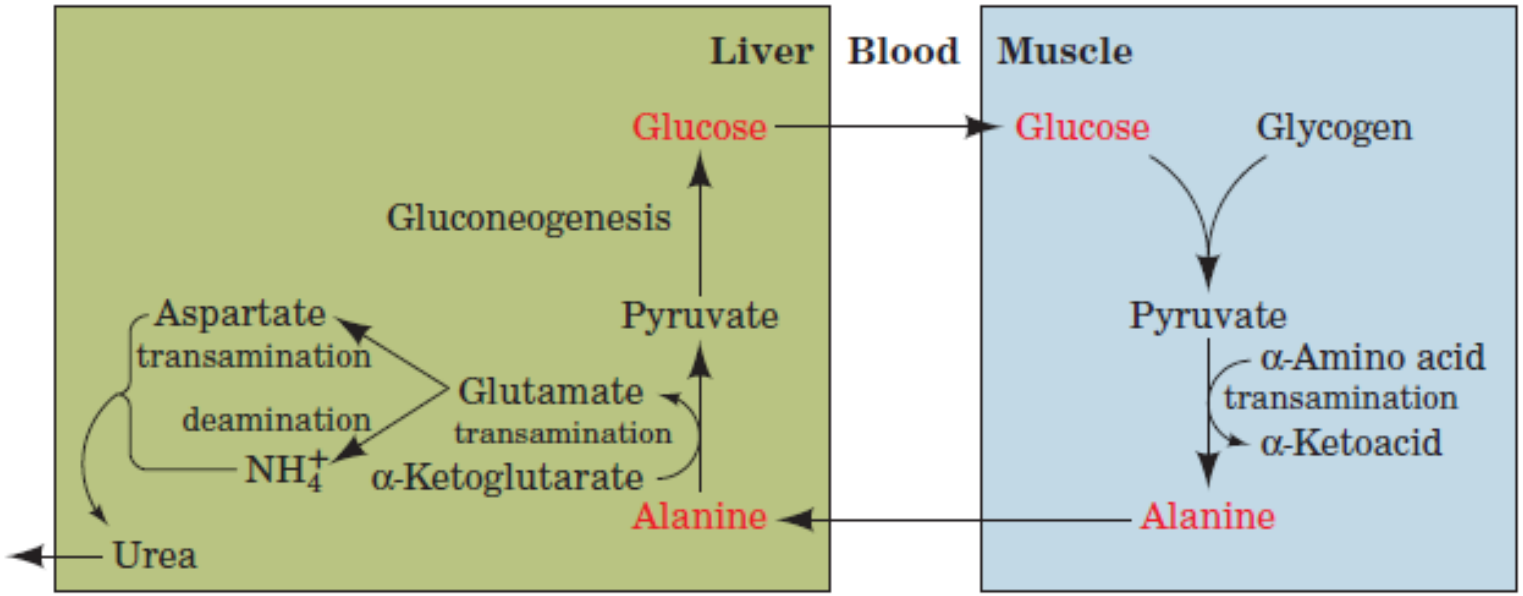
Pyridoxamine phosphate

- An exeption to using α-ketoglutarate as an amino group acceptor is a group of muscle aminotransferases that accept pyruvate as their keto acid substrate.

- The product amino acid, alanine, is released into the bloodstream and transported to the liver, where it undergoes transamination to yield pyruvate for use in gluconeogenesis - ***remember glucose-alanine cycle!***

- The amino group ends up in either ammonium ion or aspartate for urea biosynthesis.

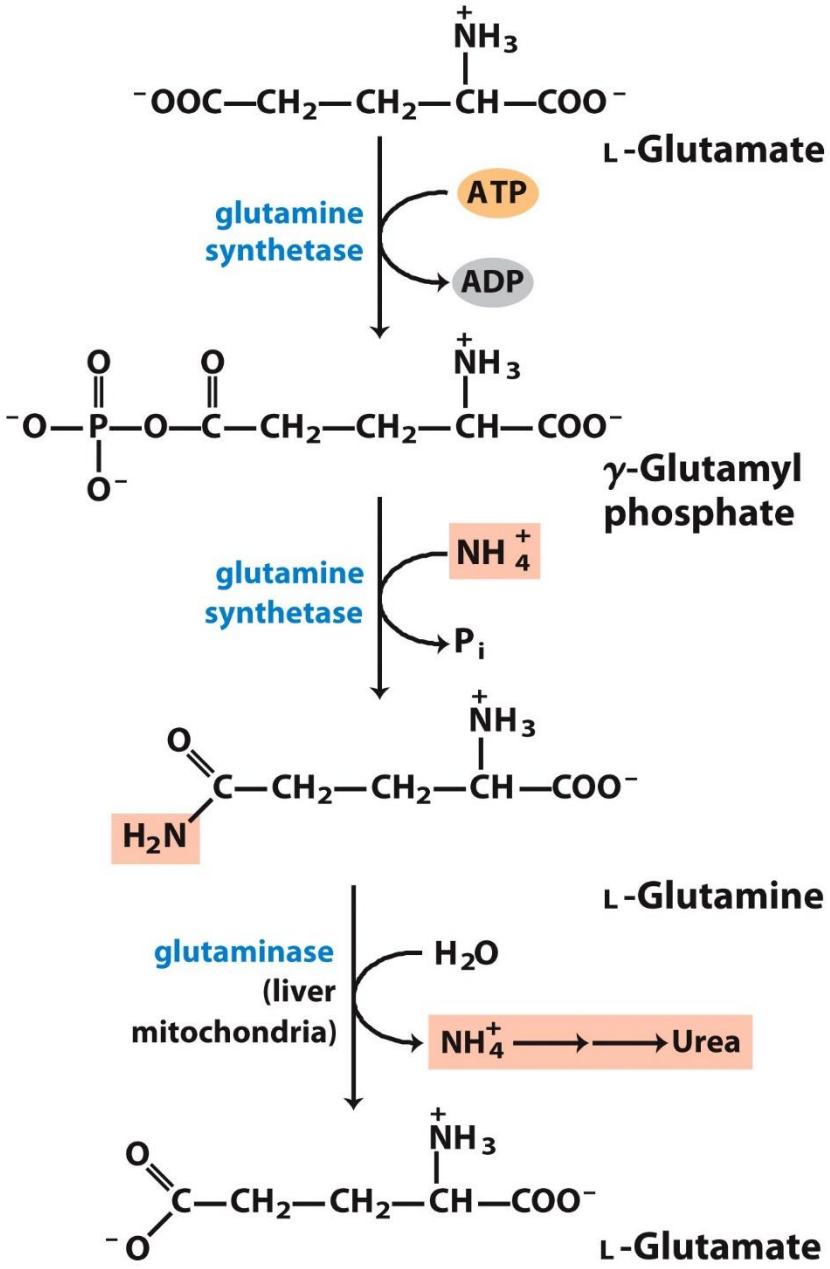
- Therefore, the glucose–alanine cycle functions to transport nitrogen from muscle to liver



- Nitrogen is also transported to the liver in the form of glutamine, synthesized from glutamate and ammonia in a reaction catalyzed by **glutamine synthetase.**

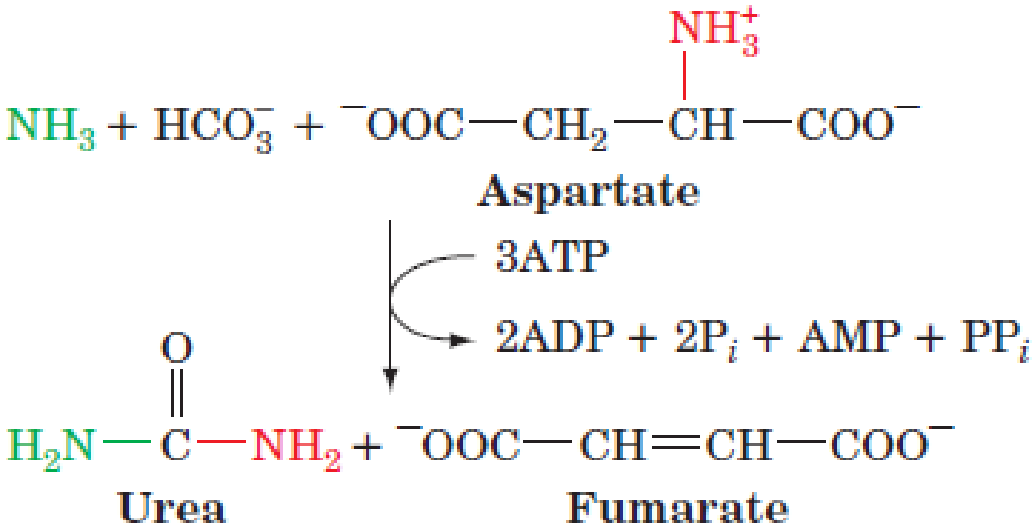
- The ammonia is released for urea synthesis in liver mitochondria or for excretion in the kidney through the action of **glutaminase.**

- **Amino acids glutamine and glutamate are the most abundant amino acids in tissues and circulation.**



Urea cycle

- Urea is synthesized **in the liver** by the enzymes of the **urea cycle.**
- It is then secreted into the bloodstream and sequestered by the kidneys for excretion in the urine.
- The overall urea cycle reaction is:

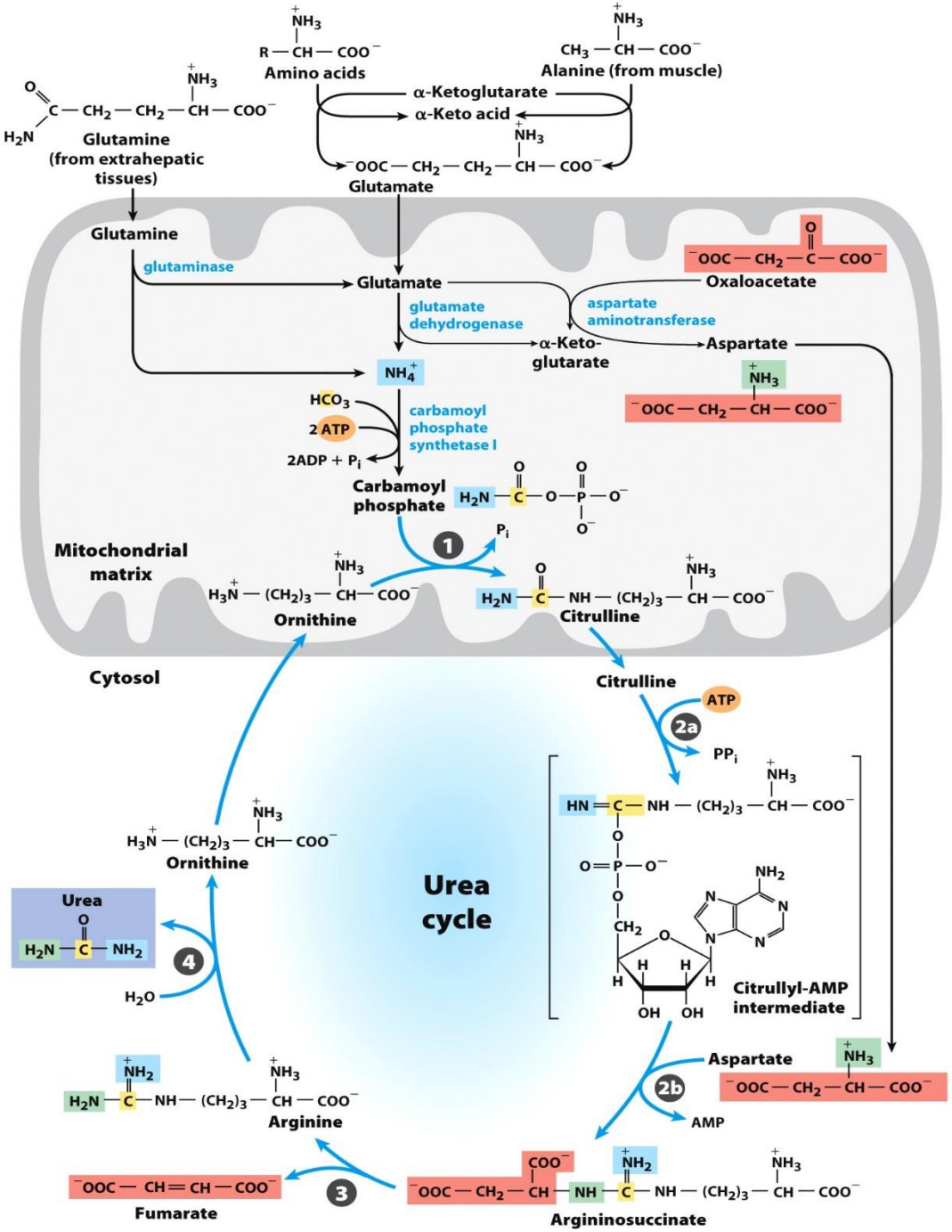


- The urea cycle was elucidated in outline in 1932 by Hans Krebs and Kurt Henseleit (**the first known metabolic cycle**; Krebs did not elucidate the citric acid cycle until 1937).

(1) Ornithine transcarbamoylase,
(2) argininosuccinate synthetase,
(3) argininosuccinase, and
(4) arginase.

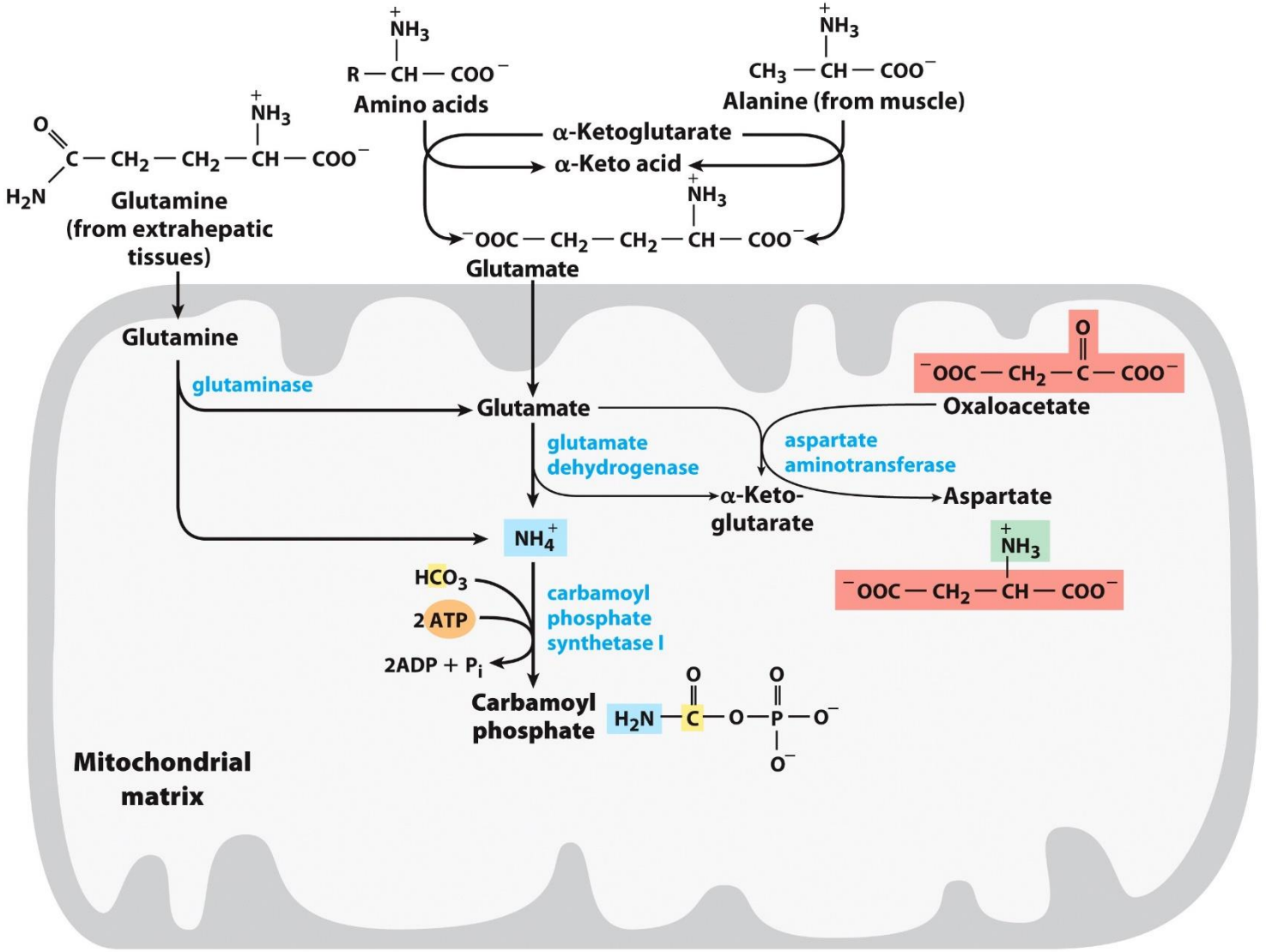
- Ornithine and citrulline are transported across the mitochondrial membrane by specific transport systems.

- The fumarate product of the argininosuccinase reaction is converted to oxaloacetate for entry into gluconeogenesis via the same reactions that occur in the citric acid cycle but take place in the cytosol.



- Formation of carbamoyl phosphate in mitochondria is a key regulatory reaction and **carbamoyl phosphate synthetase I** is a regulatory enzyme of the urea cycle.

2 ATP molecules are consumed in this reaction:
1 ATP is used for activation of HCO_3^- and
1 ATP for phosphorylation of carbamate



„Krebs bicycle”

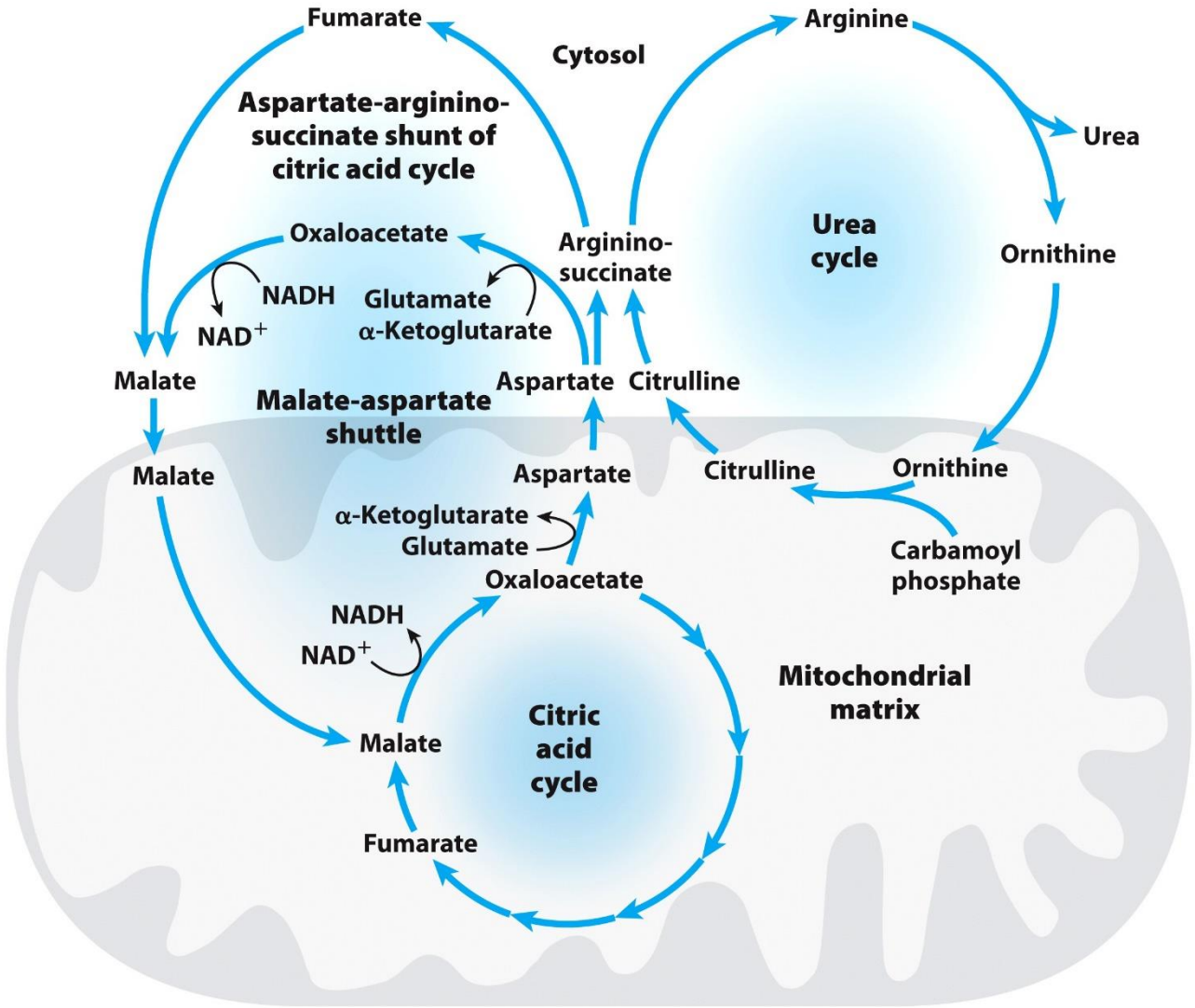
- Links between the urea cycle and citric acid cycle:

- CO_2 needed for urea synthesis is mostly produced by citric acid cycle.

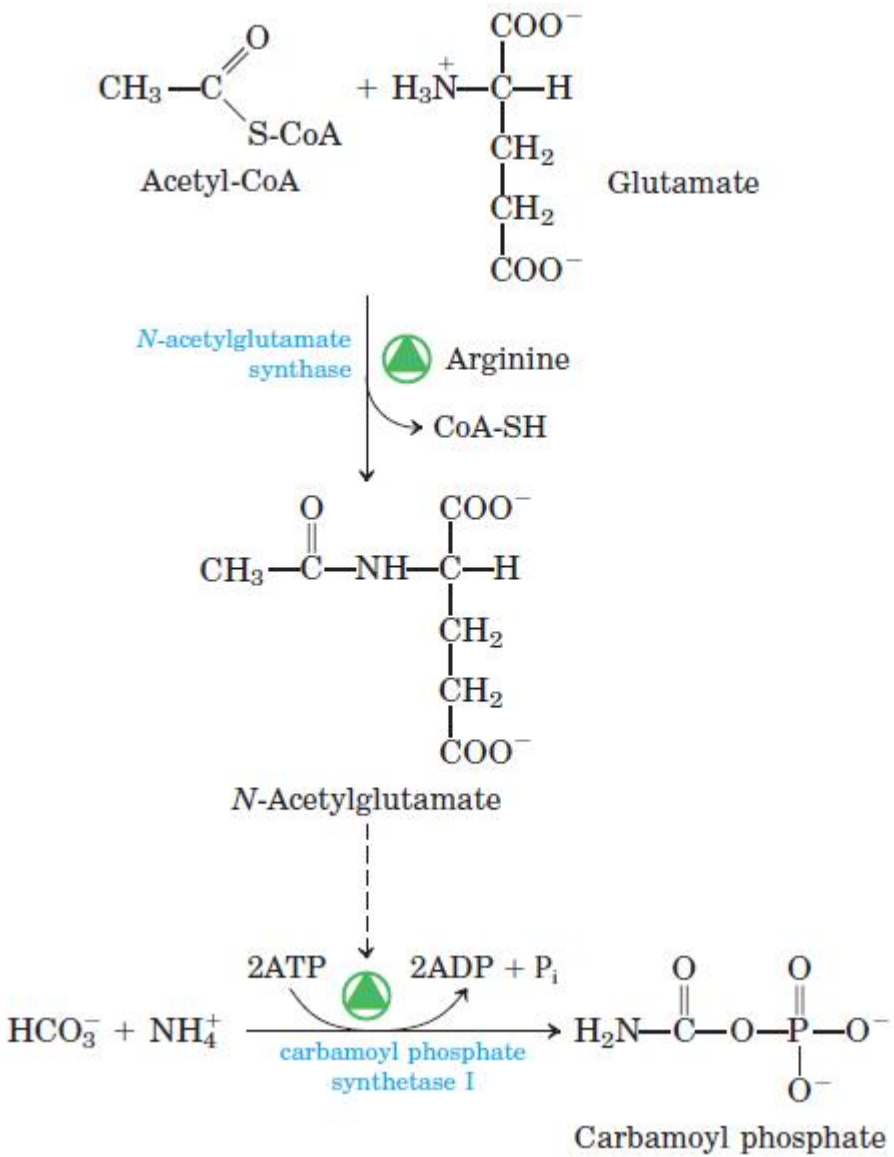
- Fumarate can be converted to oxaloacetate.

- Aspartate is formed from oxaloacetate by transamination with glutamate.

- ATP needed for urea cycle is derived from citric acid cycle.



Regulation of the urea cycle



1. Short-term regulation at **carbamoyl phosphate synthetase I**, by allosteric activator *N*-acetylglutamate;

2. Long-term regulation at the gene level (enzyme synthesis) is associated with dietary protein amount - ↑ proteins, ↑ urea cycle enzyme activity; or during starvation and breakdown of muscle proteins (↑ urea cycle enzyme activity);

3. Long-term hormonal regulation - glucagon and glucocorticoids activate the synthesis of urea cycle enzymes.

Summary of urea cycle:

1. Carbamoyl phosphate contains high-energy bond (upon hydrolysis, energy released is: – 51.4 kJ mol^{-1})

2. In one turn of urea cycle:

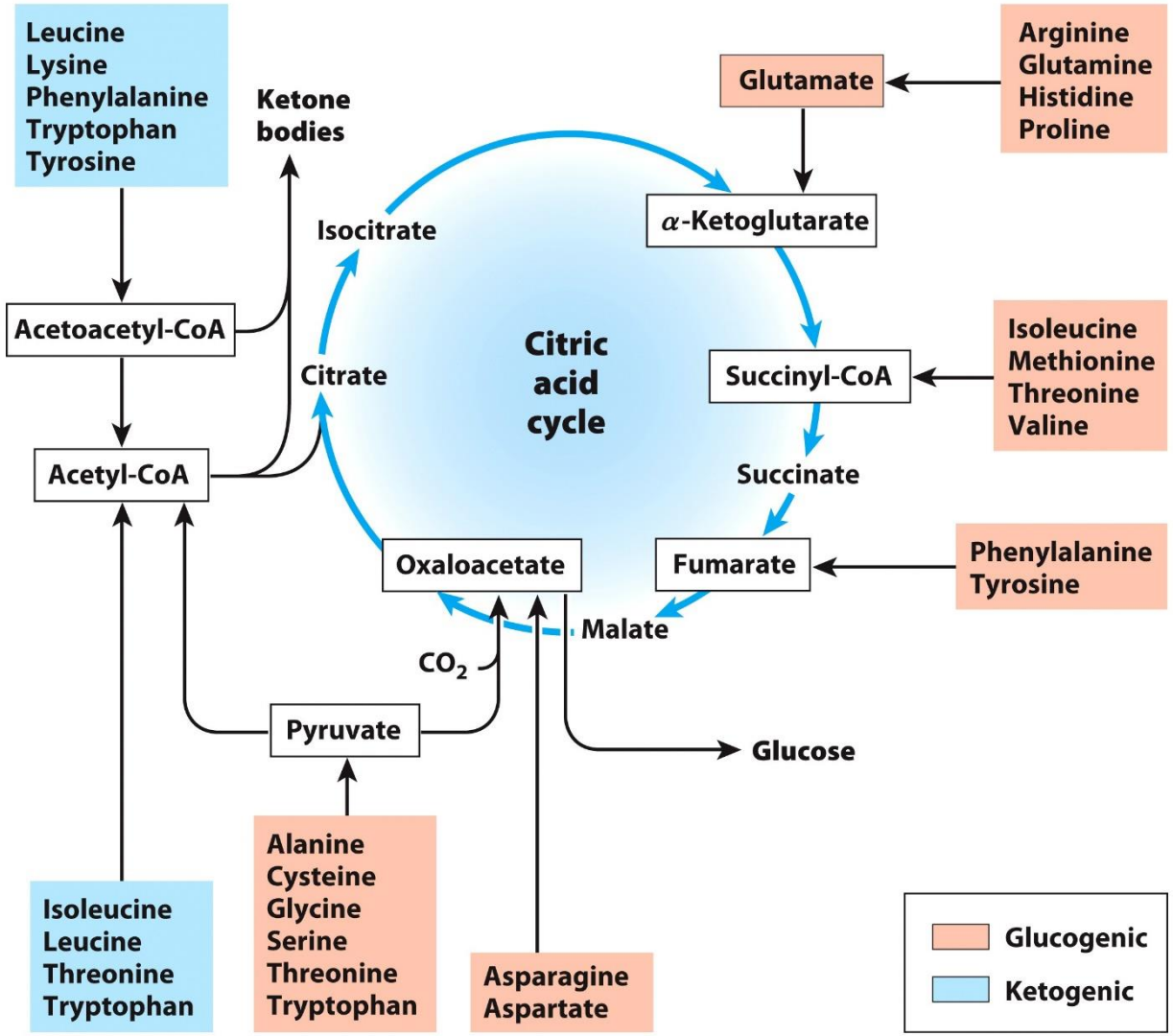
- 2 molecules of ammonia are consumed;
- 1 molecule of CO_2 is consumed;
- 1 molecule of urea is formed;
- 1 molecule of ornithine is regenerated for another turn of the cycle;
- 3 ATP and 4 high-energy bonds are consumed;

$$2NH_4^+ + HCO_3^- + 3ATP^{4-} + H_2O \longrightarrow \text{urea} + 2ADP^{3-} + 4P_i^{2-} + AMP^{2-} + 2H^+$$

Differences between carbamoyl phosphate synthetase I and II (CPS I and CPS II)

	CPS I	CPS II
Intracellular localisation	Mitochondria	Cytosol
Tissue localisation	Liver cells	Cells of most tissues
Function	**Urea biosynthesis**	**Pyrimidine biosynthesis**
Activation	*N*-acetylglutamate	Phosphoribosyl-pyrophosphate (PRPP)
Source of nitrogen	Ammonia	Amide nitrogen of glutamine

DEGRADATION OF CARBON SKELETON OF AMINO ACIDS



7 metabolites are produced by degradation of amino acid carbon skeletons:

Glucogenic metabolites
- pyruvate
- oxaloacetate
- fumarate
- succinyl-CoA
- α–ketoglutarate

Ketogenic metabolites
- acetyl-CoA
- acetoacetyl-CoA

- Some of amino acids are **glucogenic**, **two of them are exclusively ketogenic (leucine and lysine)**, several of amino acids are both glucogenic and ketogenic

Degradation of amino acids to pyruvate

- Alanine, cysteine, glycine, serine, threonine, tryptophan.

- Then pyruvate can then be converted to either acetyl-CoA (a ketone body precursor) or oxaloacetate (a precursor for gluconeogenesis); thus **amino acids catabolized to pyruvate are both ketogenic and glucogenic.**

Degradation of amino acids to acetyl-CoA / acetoacetyl-CoA

- Leucine, isoleucine, lysine, phenylalanine, tryptophan, tyrosine, threonine.

- The degradative pathways of two of these seven amino acids deserve special mention: Trp and Phe.

- **Tryptophan breakdown is the most complex of all the pathways of amino acid catabolism** in animal tissues; portions of tryptophan (four of its carbons) yield acetyl-CoA via acetoacetyl-CoA; some of the intermediates in tryptophan catabolism are precursors for the synthesis of other biomolecules (including nicotinate, a precursor of NAD and NADP in animals and serotonin, a neurotransmitter).

- The **breakdown of phenylalanine is noteworthy because genetic defects in the enzymes of this pathway lead to several inheritable human diseases.** Phenylalanine, after its hydroxylation to tyrosine, is also the precursor of dopamine, a neurotransmitter, and of norepinephrine and epinephrine, hormones secreted by the adrenal medulla; melanin, the black pigment of skin and hair, is also derived from tyrosine.

Degradation of amino acids to α-ketoglutarate

- Arginine, hystidine, proline, glutamate, glutamine.

Degradation of amino acids to succinyl-CoA

- Methionine, isoleucine, threonine, valine.

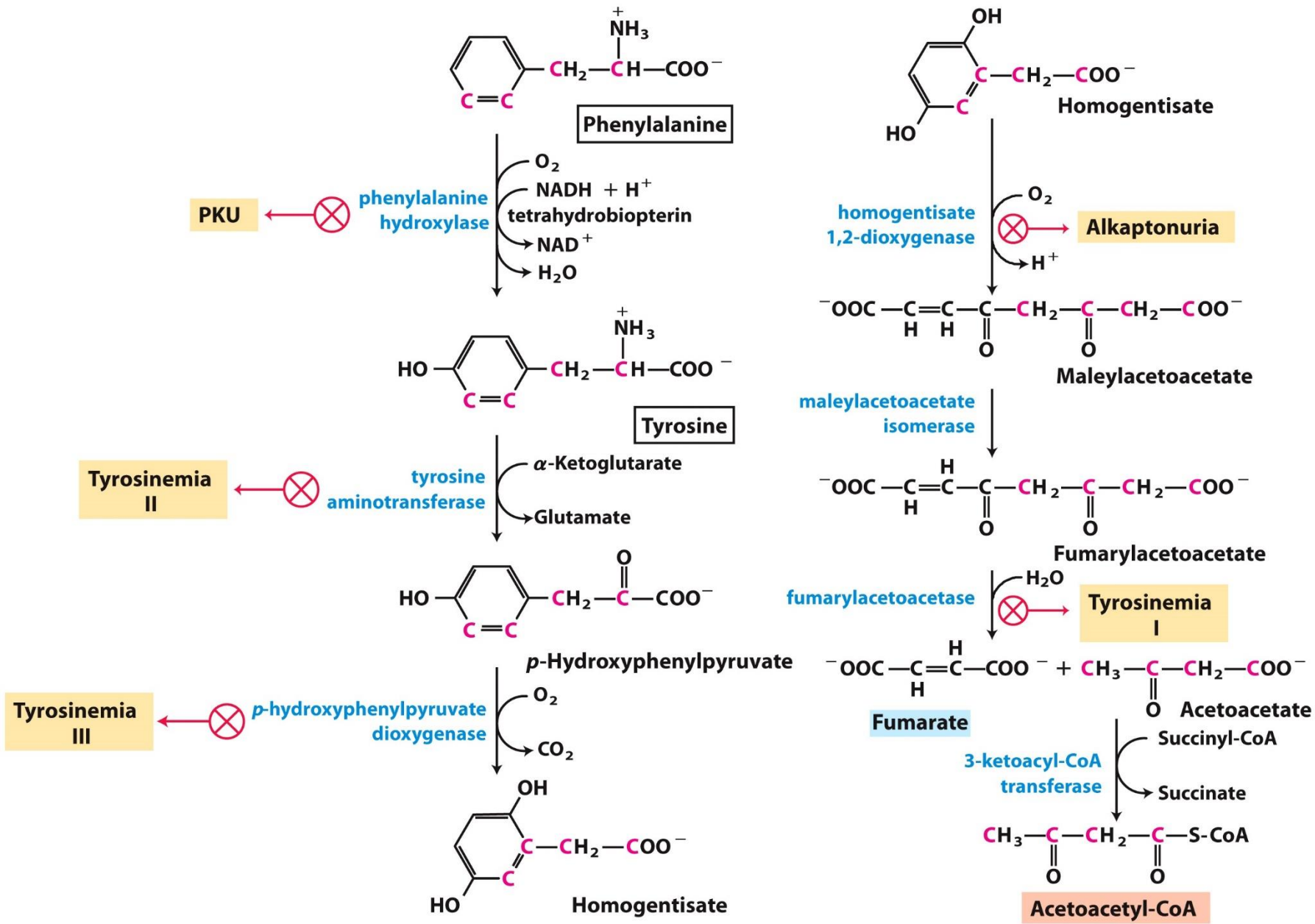
Degradation of amino acids to oxaloacetate

- Asparagine, aspartate.

Degradation of amino acids to fumarate

- Phenylalanine, tyrosine.

Catabolic pathways for phenylalanine and tyrosine

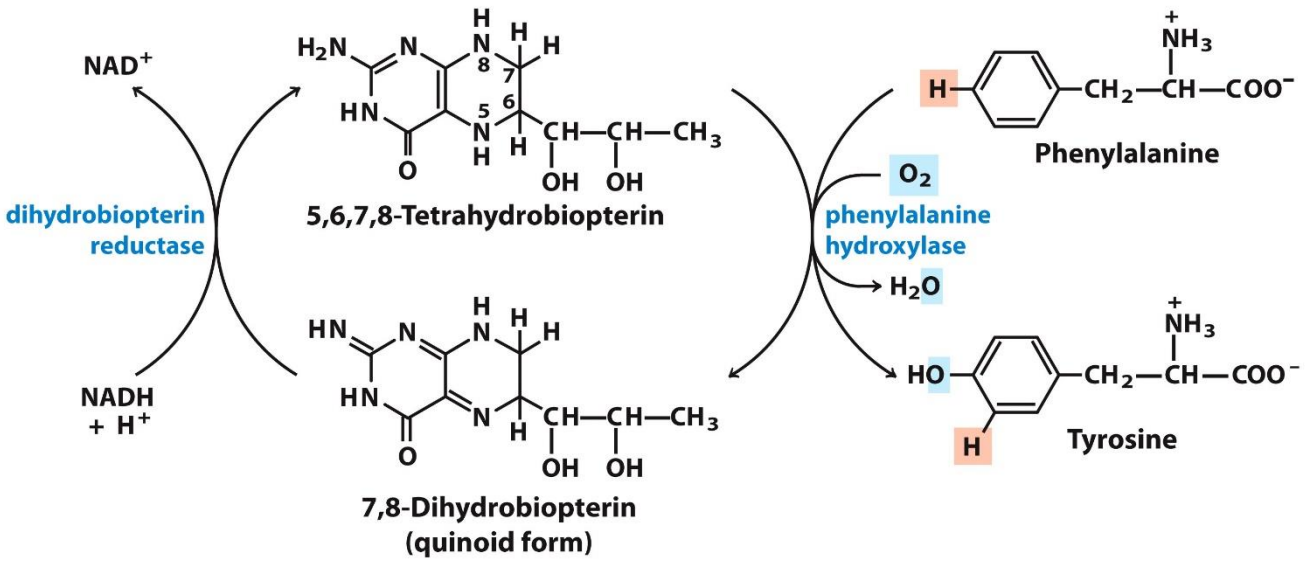


PHENYLKETONURIA (PKU)

- Phenylketonuria is a consequence of an enzyme deficiency: mono-oxigenase phenylalanine hydroxylase.

- The enzyme needs coenzyme tetrahidrobiopterin (transfers e- from NADH to O2 and is converted to its oxidized state).

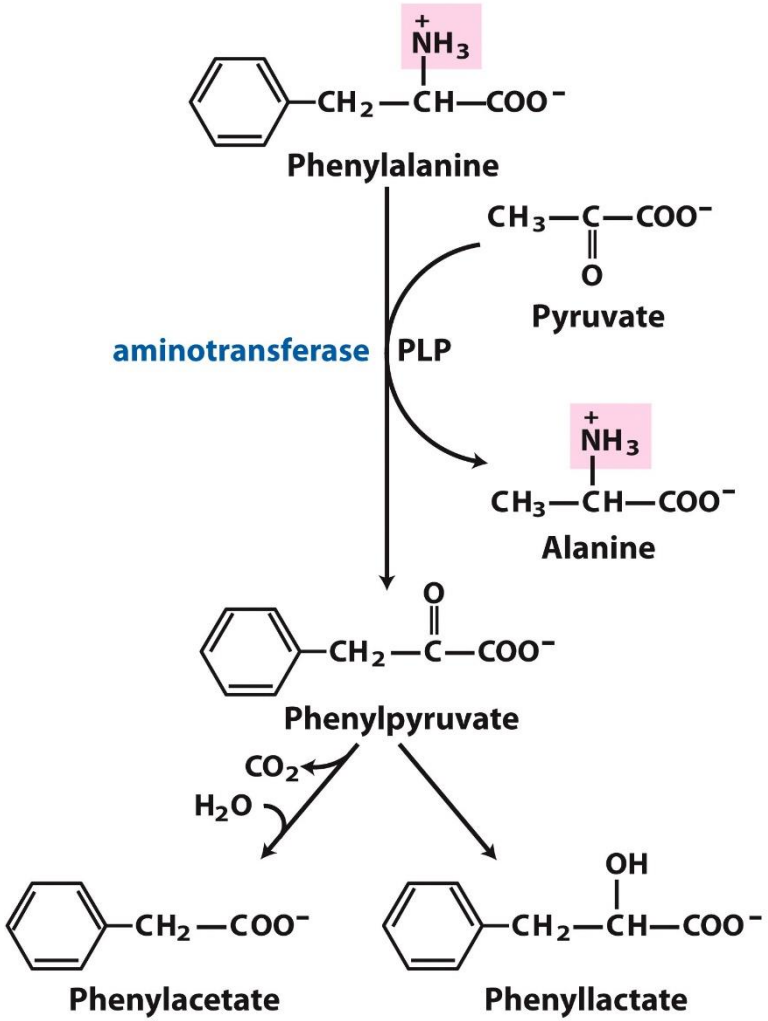
- Less common forms of PKU: deficiency of dihydrobiopterin-reductase.



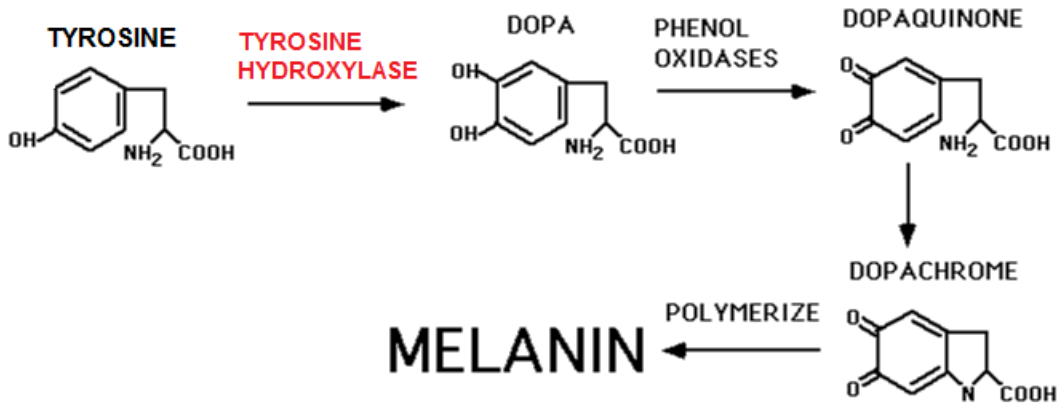
PHENYLKETONURIA (PKU)

- Concentration of phenylalanine in blood and tissues is increased.
- Phenylalanine is further metabolized to phenylpyruvate, phenyllactate, phenylacetate (increased concentration of all these metabolites may be found in the blood in PKU patients).
- Pathophysiological changes include disorders in brain development resulting with intellectual deficits.
- Obligatory newborns screening!

- Therapeutical approaches: restriction of dietary sources containing phenylalanine.

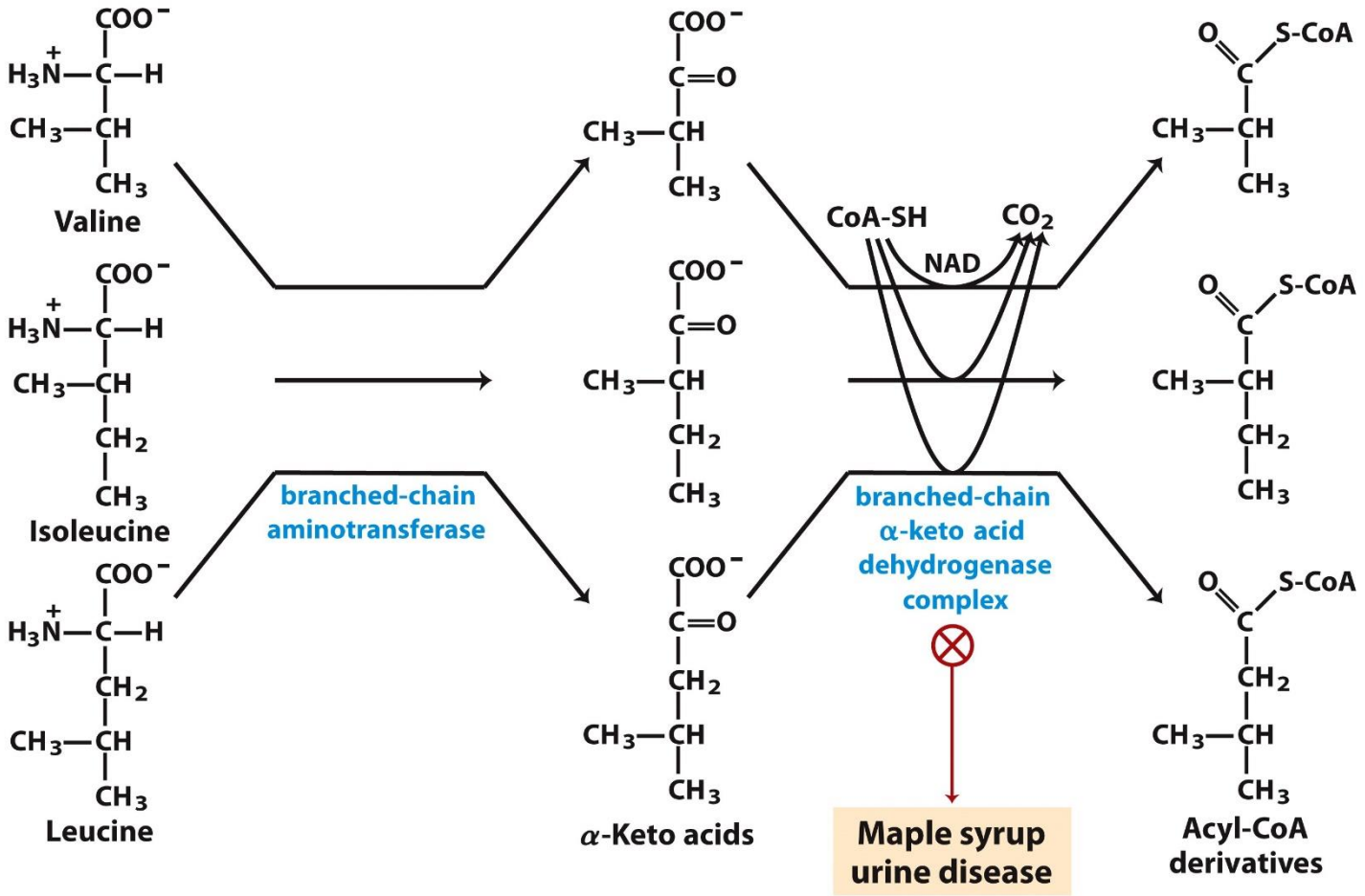


ALBINISM – tyrosine degradation disorder leading to inability to synthesize pigment melanin



Branched-Chain Amino Acids (BCAAs) Are Not Degraded in the Liver

- Transaminases catalyzing the first step of degradation of valine, isoleucine and leucine are not expressed in the liver.

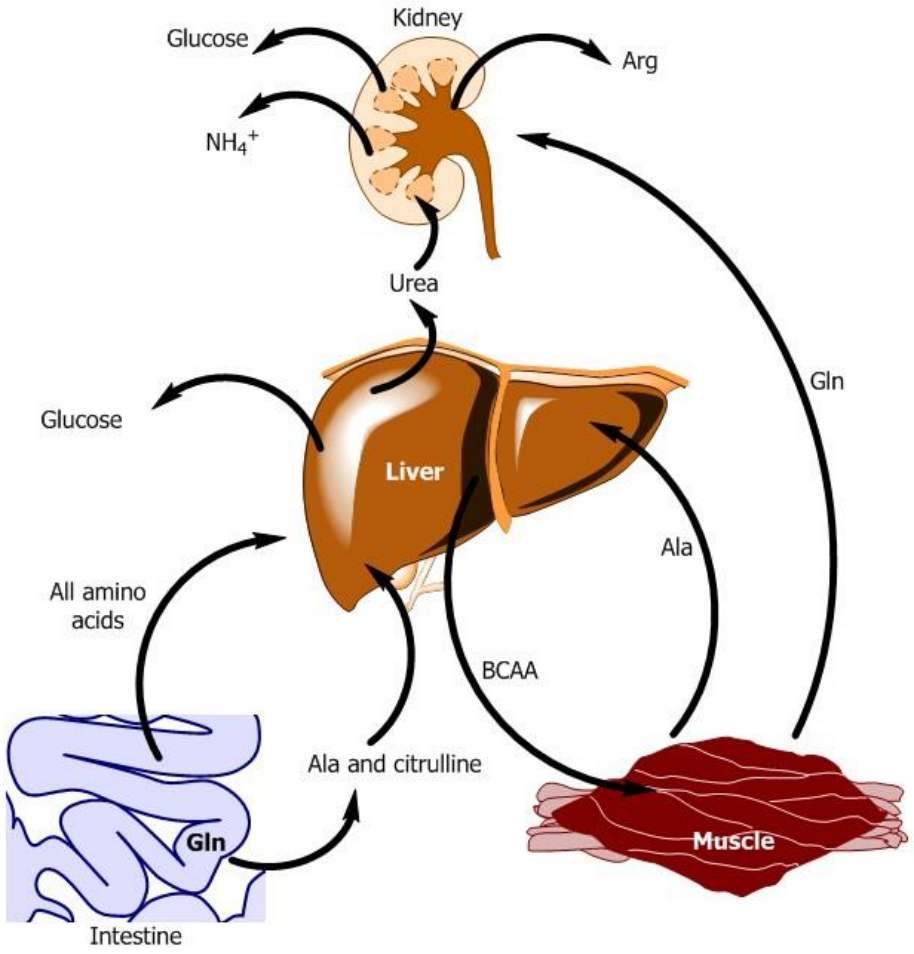


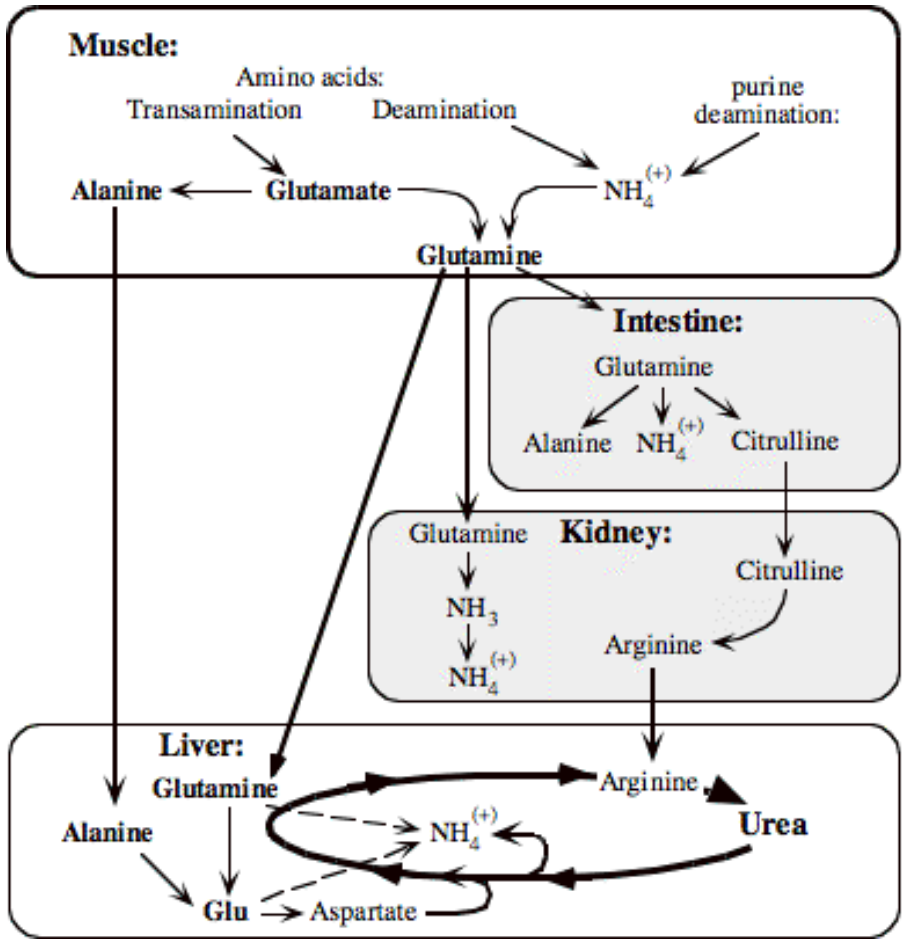
For illustration

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

Overview of Amino Acid Catabolism:
Interorgan Relationships





Literature used to prepare the presentation

1. D.L. Nelson and M.M. Cox: **Lehninger Principles of Biochemistry**, 6 th edition, W.H. Freeman and Company, USA, 2013.

2. D. Voet i J.G. Voet: **Biochemistry**, 4th edition, John Wiley & Sons Inc., USA, 2010.

3. J. Koolman & K.H. Roehm: **Color atlas of Biochemistry**, 2nd Edition, Thieme, 2005.

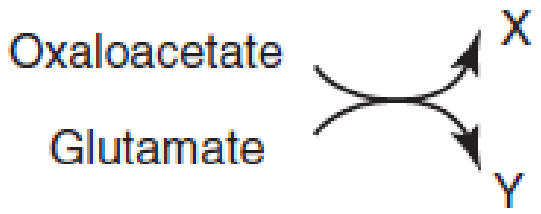
ANIMATIONS AND ONLINE SOURCES:

1. http://www.deconstructingthetour.group.shef.ac.uk/krebs-bicycle/
2. https://seqcore.brcf.med.umich.edu/mcb500/aasyl/aametab.html
3. http://www.wiley.com/college/fob/quiz/quiz21/21-6.html
4. http://www.wiley.com/college/fob/quiz/quiz20/20-8.html
5. http://www.wiley.com/college/fob/quiz/quiz20/20-7.html

Review questions/questions you should know the answers to:

1. What is the regulatory reaction for urea cycle? Where does urea cycle take place - which organ or organs?
2. Name 5 α-amino acids that are required in the urea cycle.
3. How many ATPs are needed to make one molecule of urea?
4. Describe the Krebs bicycle. What compound links the citric acid and urea cycles?
5. In the transamination reaction shown below, which of the following are the products X and Y?

   a) Alanine, α-ketoglutarate.
   b) Glutamate, α-ketoglutarate.
   c) Aspartate, α-ketoglutarate.
   d) Pyruvate, aspartate.
   e) Pyruvate, alanine.



6. Which metabolites are produced by degradation of amino acid carbon skeletons?
7. What are the two exclusively ketogenic amino acids?
8. Name some examples of human disorders of amino acid metabolism.