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Biochemistry 2

starchlipidproteinamine
ketone
isomers
carbohydrates
2

Doctor 2017 | Medicine | JU

◉ Sheet   ◯ Slides

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DOCTOR

Diala

Removing the α-amino group is essential for **producing energy** from any amino acid and is a necessary step in the catabolism of all amino acids.
Once removed, this nitrogen can be incorporated into other compounds or excreted as **urea**, with the carbon skeletons being metabolized. Only excess amino acids get metabolized to produce energy (AA are the last source of energy for the body)
We discussed transamination previously, now we will discuss oxidative deamination.

Oxidative Deamination of Amino Acids

-Cells **do not store amino acids**. All amino acids must be used by the cells.

-The amino groups of most amino acids are transferred to **glutamate** by **transamination** with α-ketoglutarate. **Glutamate** is the only amino acid that undergoes **rapid** oxidative deamination.
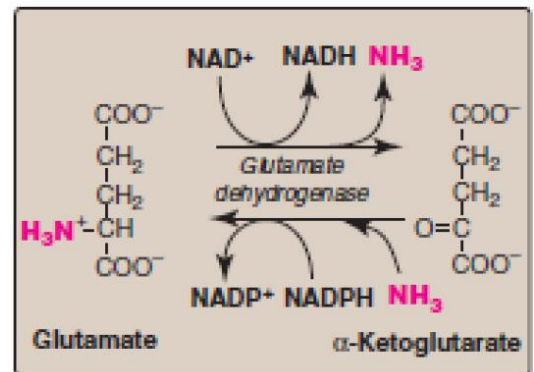
-Oxidative deamination reactions result in the release of the amino group as free ammonia. These reactions occur primarily in the **liver and kidney.** They provide **α- keto acids** that can enter the central pathways of **energy metabolism** and **ammonia**, which is a source of nitrogen in **hepatic urea synthesis.**

-Enzyme: **glutamate dehydrogenase** (a mitochondrial enzyme). It catalyzes the forward and backward reactions.

| Forward rxn (disposal of amino acids)	Backward rxn (synthesis of amino acids)
- Oxidative deamination (loss of NH3) - Carbon skeleton is oxidized - Coenzyme: **NAD+**	- Reductive amination (gain of NH3) - Carbon skeleton is reduced - Coenzyme: **NADPH**
The **direction of the reaction** depends on the relative concentrations of **glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes**. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia.	
Allosteric regulators: - GTP, which indicates a high energy level, is an allosteric **inhibitor** of glutamate dehydrogenase **-ADP** is an **activator**. Therefore, when energy levels are **low** in the cell (high ADP concentration), amino acid degradation by glutamate dehydrogenase is **high**, facilitating energy production from the carbon skeletons derived from amino acids.	

D-Amino Acid Oxidase

D-Amino acids are found in plants and in the cell walls of microorganisms but are **not used** in the synthesis of mammalian proteins. So other enzymes and coenzymes are used to metabolize them.
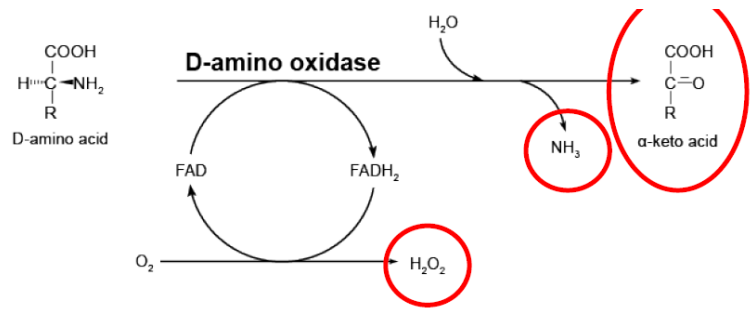
-D-Amino acids are efficiently metabolized by the **kidney and liver using a special enzyme.**

-**D-Amino acid oxidase (DAO)** is a **FAD** dependent peroxisomal enzyme that catalyzes the oxidative deamination of D-amino acids.

- the reaction produces **α-keto acids, ammonia, and hydrogen peroxide**.

-The α-keto acids can enter the general pathways of amino acid metabolism and be re-aminated to L-isomers or catabolized for energy.

- Increased DAO activity has been linked to increased susceptibility to schizophrenia.
 -L-amino acid oxidases exist and are found in some snake venoms.

Transport of Ammonia to Liver

In our bodies, the sources of ammonia (NH3) are:
- Bacterial action in the intestine: ammonia is formed from intestinal urea by bacterial urease. Ammonia is absorbed to the portal vein and transported to the liver to make urea.
- Amines: amines from diet, monoamines, neurotransmitters or hormones give rise to ammonia by amine oxidase.
- Purines and pyrimidines: in their catabolism, amino groups attached to the rings are released as ammonia.
- Oxidative deamination. It is the main source of ammonia.

-Ammonia is very toxic and if released as a free ammonia in the blood it will cause hyperammonemia (even at small concentrations). Hyperammonemia causes problems in the central nervous system.

-So ammonia produced by tissues need to be transported **safely** in the bloodstream to the **liver** to **form urea**,
-**Hepatocytes** are the **only** cells that can **produce urea** from ammonia.

There are **two** mechanisms to transport ammonia to liver:

1- Glutamine

-**Glutamine synthetase combines ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia.**

-This reaction **requires energy**.

-The glutamine is transported in the blood to the liver where it is cleaved by **glutaminase** to produce glutamate and free ammonia. The ammonia is converted to urea.

-This happens in all tissues

2- Transamination of pyruvate to form alanine

-Happens only in **muscles.**

Follow the steps on the diagram
a-When free ammonia is produced in muscle cells, it is combined with α-ketoglutarate too form glutamate by glutamate DH.

b- **Transamination of pyruvate happens and alanine is produced**. This is catalyzed by ALT.

c-Alanine moves to the bloodstream and reaches hepatocytes.

d-By the action of ALT, **pyruvate is produced by the deamination of alanine.**
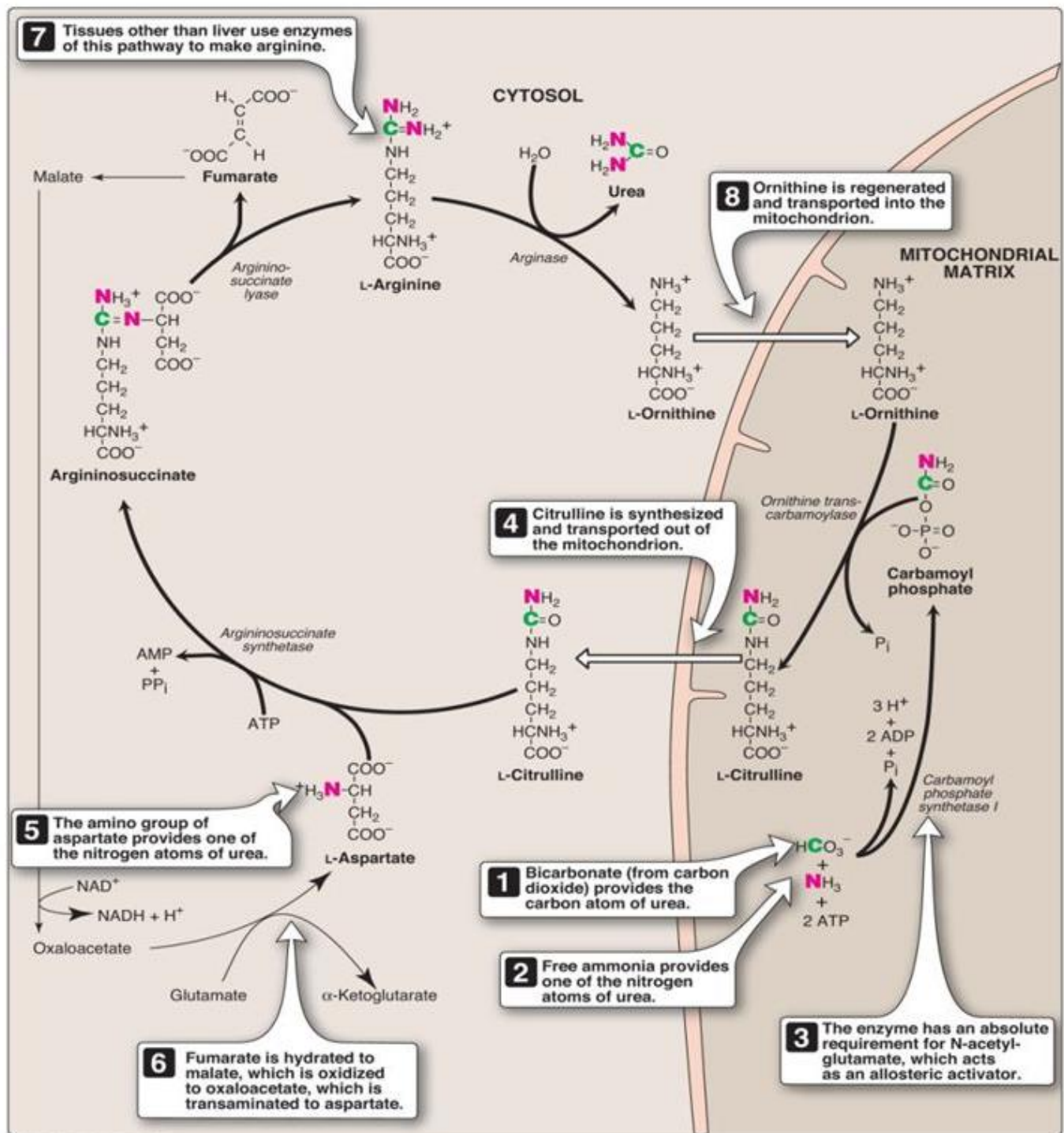
e-glutamate DH extracts ammonia from the glutamate produced.

f-The **pyruvate** produced enters **gluconeogenesis and not TCA cycle** to produce **glucose** which is transported to skeletal muscles and supplies them with energy.
This is called the **glucose alanine cycle.** **(this is beneficial to cells that are in a low energy state and in need of glucose).**
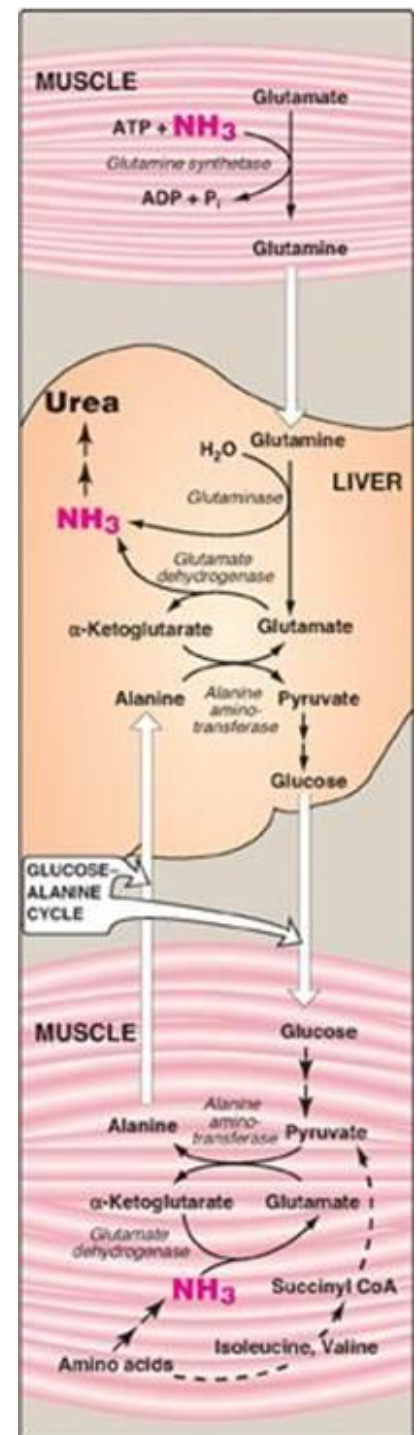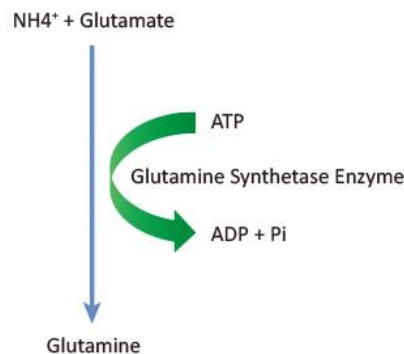
Urea cycle (in liver)

Please refer to this diagram to see the reactions and the intermediates.

*Steps 1-3: building steps
Steps 4-5: cleaving steps

Step	Enzyme	Organelle	Reaction
1-formation of carbamoyl phosphate. The starting compound. **(Rate limiting step)**	**Carbamoyl phosphate synthetase I**	Mitochondria	**Carbamoyl phosphate** $CO_2 + NH_4^+ \xrightarrow{\text{2 ATP} \rightarrow \text{2 ADP + 1 P}}$ $H_2N-\overset{O}{\underset{}{C}}-O-\overset{O}{\underset{O^\ominus}{P}}-O^\ominus$ -Cleavage of two molecules of ATP. One for energy and the other as a source of phosphate. -Ammonia is provided by the **oxidative deamination of glutamate** by mitochondrial glutamate dehydrogenase. -The carbonyl group is from CO2 (in the form of HCO3-).
2-Formation of citrulline	**ornithine transcarbamoylase (OTC)**	Mitochondria	-The carbamoyl portion of carbamoyl phosphate is transferred **to ornithine** as the high-energy phosphate is **released as inorganic phosphate**. The reaction product, citrulline, is transported to the **cytosol.**
3-synthesis of argininosuccinate **(Largest molecule in the cycle)**	**Argininosuccinate synthetase**	Cytosol	-Argininosuccinate synthetase combines **citrulline with aspartate** to form **argininosuccinate**. -The α-amino group of aspartate provides the **second nitrogen** that is ultimately incorporated into urea **(the first came from ammonia).** -The formation of argininosuccinate is driven by the **cleavage of ATP** to AMP and pyrophosphate. -This is the **third and final** molecule of **ATP** consumed in the formation of urea. **-Note:** AST forms aspartate to supply the urea cycle
4-Cleavage of argininosuccinate	**argininosuccinate lyase**	Cytosol	-Argininosuccinate is cleaved **to arginine and fumarate.** -The arginine formed is the immediate precursor of urea. -Fumarate, which is the connecter between TCA and urea cycle, produced in the urea cycle is hydrated to malate. Malate then can either enter the mitochondrial through aspartate-malate shuttle and enter TCA cycle to form oxaloacetate that can be used to from glucose Or get converted to oxaloacetate in the cytosol that can be **converted to aspartate** via transamination and then **enter the urea cycle.**
5- Cleavage of arginine to ornithine and urea	**Arginase I**	Cytosol	-Arginase hydrolyzes arginine to **ornithine and urea.** -Ornithine is **the last intermediate** of the cycle. -A molecule of **water** is needed for this reaction.

Notes on urea cycle

-Urea cycle consumes energy unlike the Krebs cycle that produces energy.

- Urea is the major disposal form of amino groups derived from amino acids and accounts for about 90% of the nitrogen-containing components of urine.

-Urea diffuses from the liver, and is transported in the blood to the kidneys, where it is filtered and excreted in the urine.

-**Overall stoichiometry:**

 Aspartate + NH3 + HCO3– + 3 ATP + H2O →urea + fumarate + 2 ADP + AMP + 2 Pi + PPi

-Because four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea, the synthesis of urea is **irreversible**, with a **large negative ΔG.**

For one urea molecule:

- One nitrogen of the urea molecule is supplied by **free NH3** from the oxidative deamination of glutamate, and the other nitrogen by **aspartate.**

- The carbon and oxygen of urea are derived from CO2.

- 4 high energy P-bonds are consumed.

Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).

Regulation of urea cycle

-**N-Acetylglutamate** is an **activator** for carbamoyl phosphate synthetase (CPS I), the **rate-limiting step** in the urea cycle.

-**Arginine** is an activator for the **synthesis of N-acetylglutamate**, therefore it is also an activator of the urea cycle.

-High amounts of protein in the diet stimulate urea cycle, as the concentration of N-acytelglutamate increases in the liver after a protein rich meal (more arginine and glutamate are provided).

Hyperammonemia

-Hyperammonemia is a medical emergency, because ammonia has a direct **neurotoxic effect on the CNS**.
Elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death.

-There are **two** major types of hyperammonemia:

1. Acquired hyperammonemia: Liver disease is a common cause of hyperammonemia in adults and may be due to **viral hepatitis** or to **hepatotoxins** such as alcohol.
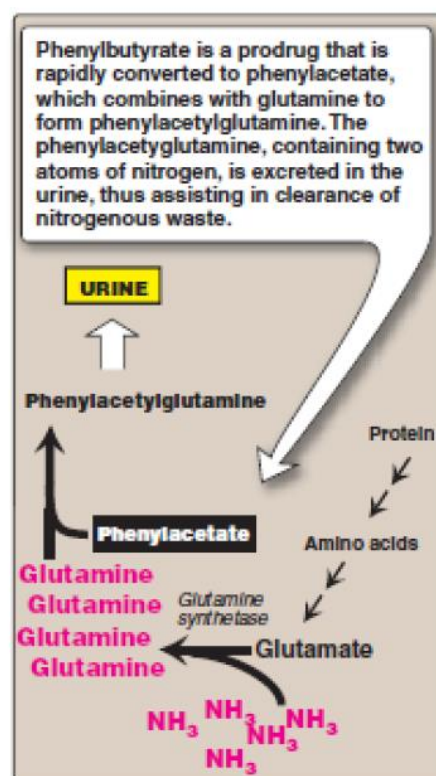
2. Congenital hyperammonemia: Genetic deficiencies of each of the five enzymes of the urea cycle have been found, with an overall incidence estimated to be 1:25,000 live births.

-**X-linked (not autosomal) ornithine transcarbamoylase (OTC)** deficiency is the **most common** of these disorders.
In each case, the failure to synthesize urea leads to hyperammonemia.

-**Treatment:**

- Restriction of dietary protein.

- Administration of compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine, has improved survival. For example, **phenylbutyrate** given orally is converted to **phenylacetate.** This **binds with glutamine** to form **phenylacetylglutamine**, which is excreted in urine.

Phenylbutyrate is a prodrug that is rapidly converted to phenylacetate, which combines with glutamine to form phenylacetylglutamine. The phenylacetylglutamine, containing two atoms of nitrogen, is excreted in the urine, thus assisting in clearance of nitrogenous waste.

Catabolism of the Carbon Skeletons of Amino Acids

-The catabolism of the amino acids involves the removal of α-amino groups, followed by the degradation of the resulting carbon skeletons.

-Amino acids are classified according to the intermediate their catabolism produces.

-The seven intermediate products are**: oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate.**

-These products directly enter the pathways of intermediary metabolism, resulting either in the **synthesis of glucose** or lipid or ketone bodies or in the **production of energy through their oxidation to CO2 by the tricarboxylic acid (TCA) cycle.**

1. Glucogenic amino acids

-Amino acids whose catabolism yields pyruvate or one of the intermediates of the **TCA Cycle**.
-These intermediates are substrates for **gluconeogenesis** and, therefore, can give rise to the net synthesis of glucose in the liver and kidney.

2. Ketogenic amino acids

Leucine and lysine (both start with L)
-Amino acids whose catabolism yields either **acetoacetate or one of its precursors (acetyl CoA or acetoacetyl CoA).**

-3-hydroxybutyrate and acetone are also ketone bodies.

	Glucogenic	Glucogenic and Ketogenic	Ketogenic
Nonessential	Alanine Arginine Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Essential	Histidine Methionine Threonine Valine	Isoleucine Phenyl-alanine Tryptophan	Leucine Lysine

This table is important

Note: some amino acids are both glucogenic and ketogenic.

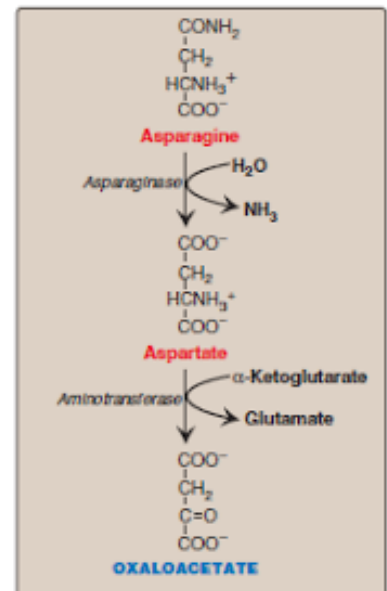## A-Amino Acids that form Oxaloacetate

-**Asparagine** is **hydrolyzed** by **asparaginase**, liberating ammonia and aspartate.

-**Aspartate** loses its amino group by **transamination** to form oxaloacetate.

-Asparagine can be synthesized from aspartate by adding an amine group.

B-Amino acids that form α-ketoglutarate via glutamate

1. Glutamine: This amino acid is hydrolyzed to glutamate and ammonium by the enzyme glutaminase. Glutamate is converted to α-ketoglutarate by transamination or through oxidative deamination by glutamate dehydrogenase.

2. Proline: is oxidized to glutamate. Glutamate is transaminated or oxidatively deaminated to form α-ketoglutarate. Proline's ring structure need to be opened first.

3. Arginine: This amino acid is hydrolyzed by arginase to produce **ornithine** (and urea). This reaction occurs as part of the **urea cycle.**
Ornithine is subsequently converted to **α-ketoglutarate.**

4. Histidine: This amino acid is **oxidatively deaminated** by **histidase** to **urocanic acid**.

-Which then forms **N-formiminoglutamate** (named after the double bond with nitrogen).

-FIGlu donates its **formimino group** to **tetrahydrofolate** (active form of folic acid) and becomes 5-formiminotertahydrofolate. **Glutamate is produced.**

-Individuals deficient in folic acid excrete increased amounts of FIGlu in the urine. The FIGlu excretion test has been used in diagnosing a deficiency of folic acid.
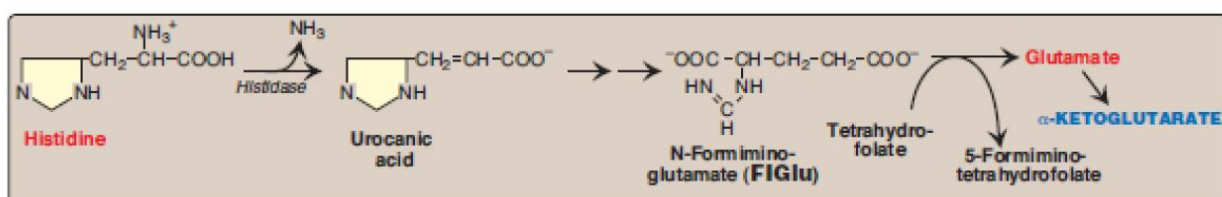
C-Amino acids that form pyruvate

1. **Alanine:** This amino acid loses its amino group by transamination to form pyruvate by the action of ALT.
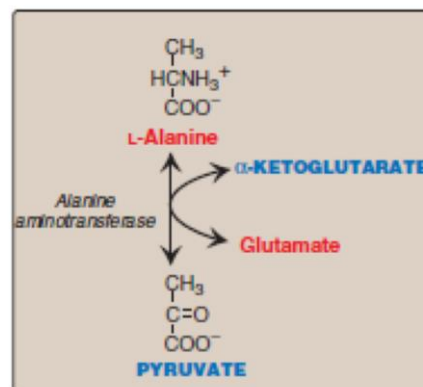
2. **Serine:** Serine can be converted to pyruvate by **serine dehydratase that removes water and ammonia.**
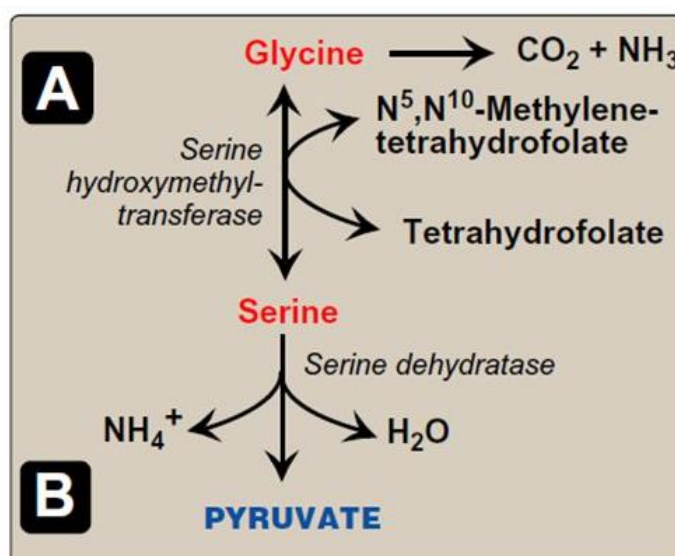
3. **Glycine:**
It is the simplest AA. This amino acid can be:

 a-converted to serine by the reversible addition of a **methyl and a hydroxyl** group from N5,N10-methylenetetrahydrofolate. The enzyme is named according to the groups it transfers (methyl and hydroxyl)
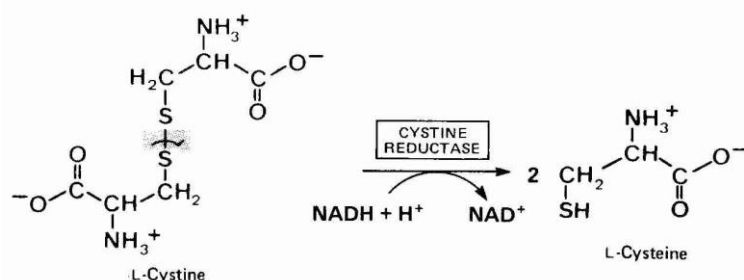 Or
 b- oxidized to CO2 and NH3.

4. **Cysteine:**
-Cystine (oxidized form) is **reduced** to **2 cysteine** molecules. NADH acts as a reductant.

- Cysteine undergoes **desulfuration** (removal of SH group) to yield pyruvate.

5. **Threonine:** is converted to pyruvate or to α-ketobutyrate, which forms succinyl CoA due to its larger size.

D-Amino acids that form Fumarate

Phenylalanine and tyrosine: these two have very similar structures. Phenylalanine has a benzene ring. Tyrosine has phenol.

-**Hydroxylation (addition of OH)** of phenylalanine produces **tyrosine**.

-This reaction is catalyzed by **phenylalanine hydroxylase**.
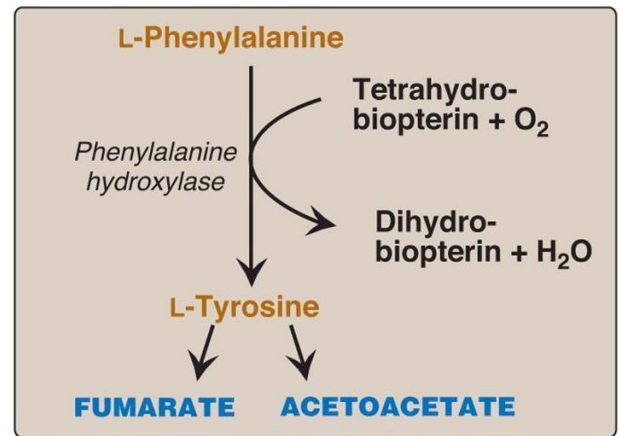
-The metabolism of phenylalanine and tyrosine merge, leading ultimately to the formation of **fumarate and acetoacetate**.

-**Therefore, phenylalanine and tyrosine are both glucogenic and ketogenic.**

-**Inherited deficiencies** in the enzymes of phenylalanine and tyrosine metabolism lead to the diseases phenylketonuria, tyrosimenia, and alkaptonuria as well as the condition of albinism.

E-Amino acids that form succinyl coenzyme A (a TCA cycle intermediate and a glucogenic compound)

Valine and isoleucine: These amino acids are **branched-chain** non-polar amino acids that generate propionyl CoA, which is converted to succinyl CoA by biotin- and vitamin B12–requiring reactions.

Threonine: is dehydrated to α-ketobutyrate, which is converted to propionyl CoA and then to **succinyl CoA**.
Threonine can also be converted to **pyruvate.**

Methionine: it is converted to S-adenosylmethionine (SAM), the major methyl-group donor in one-carbon metabolism. (will be discussed in the next lecture).

Good Luck