

BIOCHEMISTRY

Subject

Final Exam - Chapter Twenty Three

للاستفسار والتسجيل

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The Metabolism of Nitrogen

✦ Introduction:

- Although nitrogen constitutes about 78% of atmospheric air, but the nitrogen found in the living organisms does not come directly from atmosphere.
- It comes through a bacteria living in the root nodules of leguminous plants, which convert the nitrogen molecules (N_2) to ammonia (NH_3) which incorporated in amino acid (glutamate).

✦ Amino acids play important roles in the metabolisms:

- Act as building blocks of proteins
- Contribute to the synthesis of nitrogenous bases of DNA, RNA, $NAD^+/NADP^+$
- Also they are important in the synthesis of oxygen binding pyrrole ring of hemoglobin, as well as hormones and neurotransmitters.
- The carbon skeletons of amino acids can be used to feed the citric acid cycle after deamination (removal of α -amino group, NH_3^+).
- May be used as precursors of other biomolecules including (glucose, fatty acids).

- Excess nitrogen in form of ammonia is toxic, so organisms must get rid of it:
 - ♦ **In mammals** → by **urea cycle**, where the nitrogen is concentrated in the form of **urea** (water soluble), which enters the bloodstream and filtered by kidney.
 - ♦ **In fish** → excrete excess nitrogen directly as **ammonia**, which rapidly diluted by water in the environment
 - ♦ **In birds** → concentrate it in the form of **uric acid**, which is water insoluble.

✚ What processes constitute nitrogen metabolism?

There are many compounds contain nitrogen in their structure (amino acids, porphyrins, and nucleotides), there are many important topics we should know about to understand the metabolism of nitrogen, these include:

- **Nitrogen fixation:** - (the conversion of molecular nitrogen to ammonia) here in this process the inorganic molecular nitrogen from the atmosphere (N_2) is incorporated first into ammonia and then into organic compounds in the organisms.
- **Nitrification:** - (nitrate (NO_3^-) reduction to ammonia), nitrate is another kind of inorganic nitrogen found in soil; "many fertilizer contain nitrate" and this process provide another way to obtain nitrogen.
- **Denitrification:** - the processes by which nitrate and nitrite ions are broken down to molecular nitrogen to the atmosphere.

Ammonia formed by nitrogen fixation or nitrification:

- After production of ammonia (NH_3), it is converted to organic nitrogen by plants, then this organic nitrogen passed to animals through food chains, then animal waste products (urea) are degraded to ammonia by microorganisms.
- Also the process of death and decay release ammonia in both plants and animals, denitrifying bacteria reverse the conversion of ammonia (NH_3) to nitrate (NO_3^-) and then recycle the nitrate (NO_3^-) as free nitrogen (N_2).

The topic of nitrogen metabolism includes:-

- ✓ Biosynthesis and breakdown of **amino acids**
- ✓ Biosynthesis and breakdown of **purines and pyrimidines**
- ✓ Metabolism of **porphyrines**

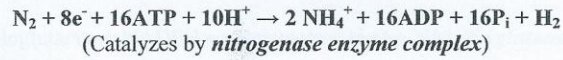
⚡ How is nitrogen incorporated into biologically useful compounds?

The process of nitrogen fixation (**reduction of N_2 to ammonia**) occurs in:

- ☞ **Nitrogen-fixing bacteria** found in the nodules on the root of leguminous plants (beans, alfalfa)
- ☞ **Many free living microbes.**
- ☞ **Some cyanobacteria.**

▪ Plants and animals cannot carry out nitrogen fixation

So the fixation process is a **reduction** process as shown in the following reaction:

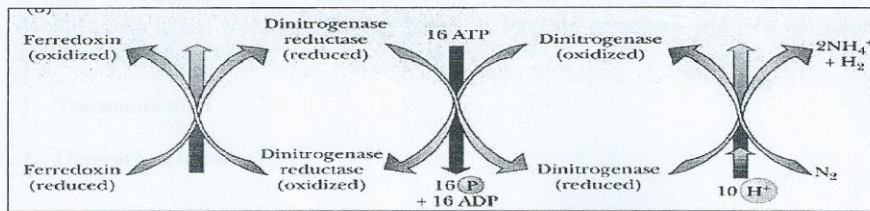


▪ In this reaction:

- NH_4^+ ammonium ion, (the conjugate acid of NH_3) is the form of nitrogen that is used in the synthesis of organic compounds.
 - Six electrons used to reduce molecular nitrogen to ammonia.
 - Two electrons used to reduce hydrogen ion to H_2 .
 - Nitrogenase enzyme complex found in nitrogen-fixing bacteria.
 - The reaction consumes a great deal of energy (about half of ATP produced from the photosynthesis in legumes is used to fix nitrogen).
- As this is a reduction reaction, it needs reducing agent to supply electrons, and different organisms vary in terms of reducing agent used to supply electrons for reduction of (N_2).
 - The **nitrogenase complex** includes several proteins:
 - **Ferredoxine**: - play an important role in electrons transport in photosynthesis
 - **Dinitrogenase reductase**: - an iron-sulfur protein (Fe-S)
 - **Dinitrogenase**: - iron-molybdenum protein (Fe-Mo)

The flow of electrons:

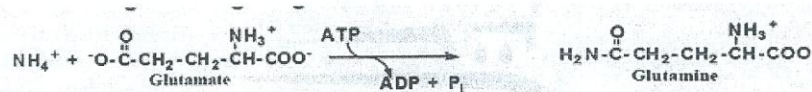
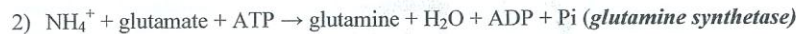
Ferredoxine → Dinitrogenase reductase → Dinitrogenase → Nitrogen



- The three dimensional structure of the nitrogenase is very complex, it is determined by X-ray crystallography for bacteria called *Azotobacter vinelandii*, where the Fe protein is a dimer (“the iron butterfly”) with the iron-sulfur cluster located at the butterfly’s head. The nitrogenase is even more complicated, with several types of subunits arranged into tetramers

⚡ How are amino acids synthesized?

- We said that after the production of ammonia, it is incorporated into organic compounds. The amino acids **glutamate** and **glutamine** play a central role in this process, how?
- Look to the following reactions:



- The first reaction as a **reductive amination** and the second reaction is **amidation**.
- In the first reaction the, glutamate is a major donor of amino groups, and α -ketoglutarate is a major acceptor of amino group.
- In the anabolism of other amino acids the α -amino group of glutamate and the side chain amino group of glutamine are shifted to other compound in **transamination** reactions. (This process also important in catabolism of amino acids).
- In amino acids synthesis we are not going to talk about all reactions that give rise to amino acids, we will group amino acids into families based on common precursors and then talk about two important type of reactions:

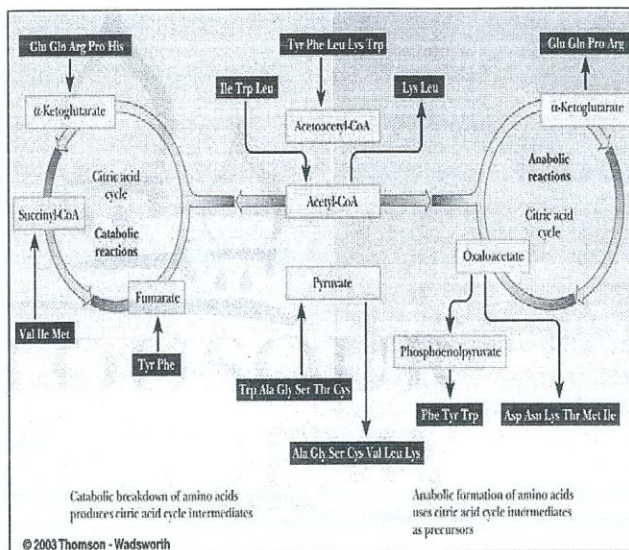
1. Transamination

2. One-carbon transfer

✚ The families of amino acids:

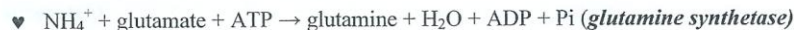
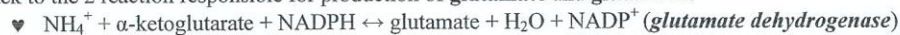
Amino acid family	Amino acids	Common precursor
Glutamate family	Glutamate, glutamine, proline, arginine	α -ketoglutarate
Aspartate family	Aspartate, asparagines, methionine, threonine, isoleucine, lysine	Oxaloacetate
Serine family	Serine, cysteine, glycine	3-phosphoglycerate
Pyruvate family	Pyruvate, valine, alanine, leucine	Pyruvate
Aromatic family	Phenylalanine, tyrosine, tryptophane	Phosphoenolpyruvate + erythrose-4-phosphate
Histidine family	Histidine	Ribose-5-phosphate

- We said that the citric acid cycle is **amphibolic**; it has a part in both catabolism and anabolism.
- The figure shows how **catabolic breakdown** of amino acids produces citric acid cycle intermediates and how **anabolic formation** of amino acids uses citric acid cycle intermediates as precursors.
- Also notice that the catabolism of amino acids give rise to metabolic intermediates such as **pyruvate, acetyl-CoA, α -ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate.**



✚ **Transamination reactions: the role of glutamate and pyridoxal phosphate:**

Back to the 2 reaction responsible for production of **glutamate** and **glutamine**:



- These reactions fix the inorganic nitrogen (NH_3), forming organic (carbon containing) nitrogen compounds, like amino acids, the enzymes responsible for these 2 reactions are:

- *glutamate dehydrogenase (GDH)*
- *glutamine synthetase (GS)*

- In fact, the combination of GDH and GS is responsible for most of assimilation of ammonia into organic compounds.
- K_M of (GS) is considerably lower than that of (GDH). This means that when the nitrogen is limiting, (as the case in plants); conversion of glutamate to glutamine is the preferred mode of nitrogen assimilation. This means that the supply of glutamate becomes depleted unless there is some way to replenish it. So, from where we get a continuous supply of glutamate?
- Look to the following reaction to get the answer:



- In this reaction, the reductive amination of α -ketoglutarate with the amide nitrogen of glutamine as a nitrogen source is the way to get glutamate.
- The enzyme catalyze this reaction (*glutamate synthase*) also known as *glutamate:oxoglutarate aminotransferase (GOGAT)*.
- The **reductant** here could be:
 - NADH, NADPH (in yeast and bacteria).
 - OR
 - Reduced ferredoxin (in plants).
- Transamination as a process means the transfer of amino groups from one molecule to another, and you should know that, the enzymes catalyze transamination reactions requires **pyridoxal phosphate** as a coenzyme.

How pyridoxal phosphate works in transamination reactions:

Step1: pyridoxal phosphate (PyrP) form a Schiff base with the amino group of substrate I (the amino group donor).

Step2: rearrangement followed by hydrolysis which remove product I (the α -keto acid corresponding to substrate I).

Step3: the coenzyme now carries the amino group (pyridoxamine).

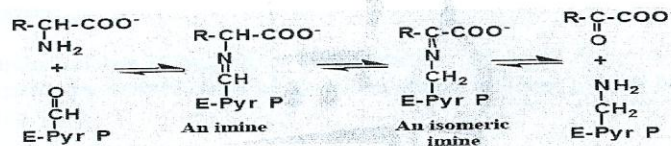
Step4: substrate II (another α -keto acid) then forms a Schiff base with pyridoxamine.

Step5: rearrangement followed by hydrolysis which give product II (an amino acid) and regenerate pyridoxal phosphate.

- The net reaction is that an amino acid (Substrate I) react with an α -keto acid (Substrate II) to form an α -keto acid (Product I) and an amino acid (Product II). The amino group has been transferred from substrate I to substrate II, forming the amino acid, product II.

NOTE: When not involved with one of the substrates, the pyridoxal group is bound in a Schiff-base linkage to an active site E-NH₂ group of lysine.

- Pyridoxal phosphate** is a versatile coenzyme that is also involved in other reaction, including decarboxylations, recemizations, and movement of hydroxymethyl groups.



✦ One-carbon transfer and the serine family:

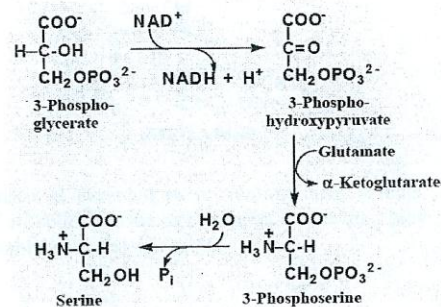
- One-carbon transfer reactions occur frequently in amino acid biosynthesis.

We will talk about the reactions that produce the amino acids of the serine family as an example of one-carbon transfer reactions.

- As you know serine family includes, **serine**, **cysteine**, and **glycine**, and the precursor for this family is **3-phosphoglycerate**.

✦ How serine is produced?

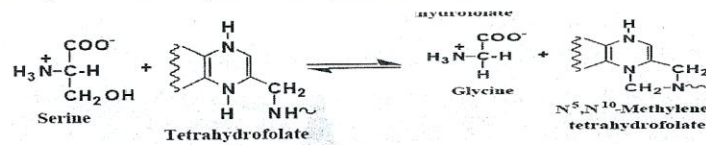
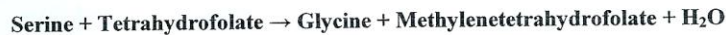
step1: the hydroxyl group on carbon 2 of **3-phosphoglycerate**, which produced from glycolytic pathway, is oxidized to a keto group, giving an α -keto acid.



step2: A transamination reaction in which glutamate is the nitrogen donor produces 3-phosphoserine and α -ketoglutarate.

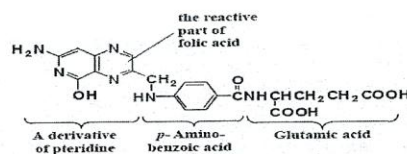
step3: hydrolysis of phosphate group gives serine.

- The conversion of serine to glycine involves the transfer of a one carbon unit from serine to an acceptor, the net reaction is:



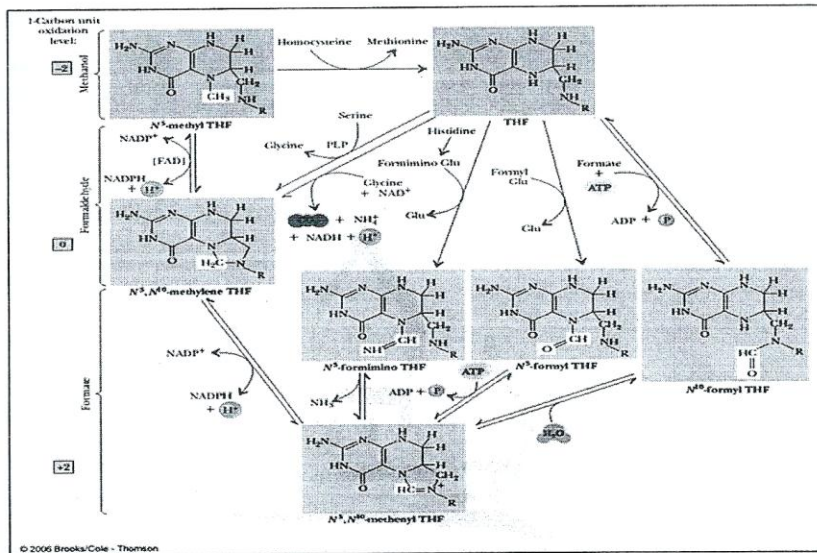
About this reaction:-

- This reaction is catalyzed by *serine hydroxymethylase* with pyridoxal phosphate as a coenzyme.
- Tetrahydrofolate** is the acceptor of this reaction, and it is a derivative of folic acid and frequently encountered carrier of one-carbon units in metabolic pathway (**do you remember biotin?**)
- Tetrahydrofolate** structure has three parts; a substituted **pteridine ring**, **p-aminobenzoic acid**, and **glutamic acid**.
- When one carbon unit bounded to tetrahydrofolate, **N⁵,N¹⁰-methylene-tetrahydrofolate** will be produced.
- In **N⁵,N¹⁰-methylenetetrahydrofolate**, methylene (one carbon) unit is bound to two of the nitrogen of the carrier.



- Note:** Folic acid is a vitamin that has been identified as essential in preventing birth defects, consequently, it is now recommended supplement of all child-bearing age women, and it may play a role in preventing heart diseases in both men and women over the age of 50.

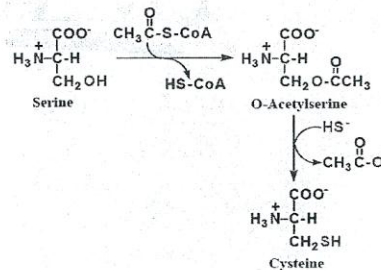
- The reactions that introduce one-carbon units into tetrahydrofolate (THF) link seven different folate intermediates that carry one-carbon units in three different oxidation states (-2, 0, and +2).



- The conversion of serine to **cysteine** (contain a **sulfur** atom) involves some interesting reactions. The source of sulfur in animals differs from that in plant and bacteria.

Plants and bacteria:

- In plant and bacteria, serine is acetylated to form O-acetylserine, by enzyme called **serine acetyltransferase**, with acetyl-CoA as the acetyl donor.
- Conversion of O-acetylserine to cysteine requires production of sulfide by a sulfur donor. The sulfur donor for plant and bacteria is **3'-phospho-5'-adenylyl sulfate**.

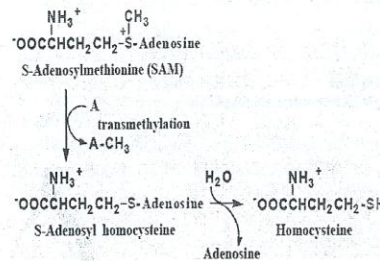


- The sulfate group (SO_4^{-2}) is reduced first to sulfite (SO_3^{-2}) and then to sulfide (S^{-2}), using NADPH as reducing agent.
- The sulfide, in the conjugate acid forms HS^- ; displace the acetyl group of the O-acetylserine to produce cysteine.

Note: animals do not have the enzymes that carry out the sulfate to sulfide conversion. So they form cysteine from serine by a different pathway.

In animals:

- Cysteine production in animals requires the amino acid **methionine** which is produced by reaction of the aspartate family in plants and bacteria, but cannot be produced by animals, and it must be obtained from dietary sources.
- So it is an **essential amino acid** because it cannot be synthesized by the body.



✚ **How cysteine is produced in animals:-**

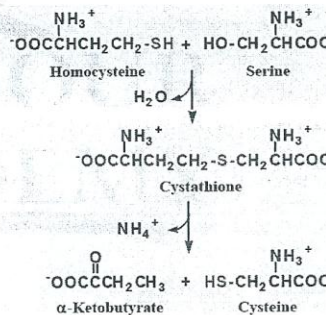
step1: the ingested methionine reacts with ATP to form **S-adenosylmethionine (SAM)**, which has a highly reactive methyl group.

step2: the methyl group from **S-adenosylmethionine** can be transferred to any one of a number of acceptors, producing **S-adenosylhomocysteine**.

step3: hydrolysis of **S-adenosylhomocysteine** will produce homocystein.

step4: serine and homocystein react to produce **cystathionine**.

step5: hydrolysis of **cystathionine** to produce cysteine, NH_4^+ , and α -ketobutarate.



There are three important carriers of one-carbon units:-
Biotin \rightarrow a carrier of CO_2
tetrahydrofolate (FH_4) \rightarrow a carrier of **methylene** and **formyl** group
S-adenosylmethionine \rightarrow a carrier of **methyl** group.



✦ What are the essential amino acids?

- Essential amino acids are amino acids that cannot be synthesized by the body and must be therefore obtained from the diet.
- If one of 20 amino acids is missing or in short supply, protein synthesis is inhibited.
- Although humans must obtain some amino acids from dietary sources, some organisms, such as *Escherichia coli* can synthesize all amino acids they need.
- In case of growing children, they need a dietary sources for essential amino acids at regular interval specially for **arginine**, and **histidine**, because a prolong deficiency in sources that contain essential amino acid lead to a disease known as **kwashiorkor** (not only starvation but breakdown of body's own proteins).

Essential for Humans	Nonessential for Humans
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

✦ How are amino acids catabolized?

We said that transamination is important in anabolism and catabolism of amino acids. So, it is important to remind ourselves that anabolic and catabolic pathways are not the exact reverse.

- In the first step of amino acids catabolism; the amino nitrogen of the original amino acid is transferred to α -ketoglutarate to produce glutamate (transamination process), leaving behind the carbon skeletons, the fates of carbon skeleton and the nitrogen can be considered separately.

➤ Disposition of the carbon skeleton:

We classify amino acids into two types according to the type of the end product that produced after the breakdown of their carbon skeleton:

1. **Glucogenic amino acid:** is the one that produce **pyruvate** or **oxaloacetate** on degradation (remember that oxaloacetate is the starting point for the production of glucose by gluconeogenesis), so can be converted to glucose.
2. **Ketogenic amino acids:** -is the one that breaks down to **acetyl-CoA**, or **acetoacetyl-CoA**, leading to formation of **keton bodies**.

Glucogenic	Ketogenic	Glucogenic and Ketogenic
Aspartate	Leucine	Isoleucine
Asparagine	Lysine	Phenylalanine
Alanine		Tryptophan
Glycine		Tyrosine
Serine		
Threonine		
Cysteine		
Glutamate		
Glutamine		
Arginine		
Proline		
Histidine		
Valine		
Methionine		

Notes:

- 1) oxaloacetate is a key intermediate in the breakdown of the carbon skeleton of amino acids because of its dual role in the citric acid cycle and in gluconeogenesis.
- 2) The amino acids degraded to acetyl-CoA and acetoacetyl-CoA are used in the citric acid cycle, but mammals cannot synthesize glucose from acetyl-CoA.
- 3) Depending on the previous fact we can distinguish between glucogenic and ketogenic amino acids. Glucogenic amino acids can be converted to glucose, with oxaloacetate as an intermediate, but ketogenic amino acids cannot be converted to glucose.
- 4) There are four amino acids listed as both glucogenic and ketogenic, because these amino acids have more than one pathway for catabolism.

➤ **Excretion of excess nitrogen:**

- The nitrogen portion of amino acids is involved in transamination reactions in both breakdown and biosynthesis.
- Excess nitrogen is excreted in one of three forms:
1) **Ammonia (as ammonium ion)** 2) **urea** 3) **uric acid**

✦ **The urea cycle:- (the pathway is confined to liver):**

- It is a central pathway in nitrogen metabolism. Nitrogens that enter this cycle come from several source.
- It is important to know that the source of nitrogen in urea cycle comes from:
 - Glutamate which release ammonia by **glutamate dehydrogenase**.
 - Free ammonia provided by mitochondrial **glutaminase** can also enter the cycle.

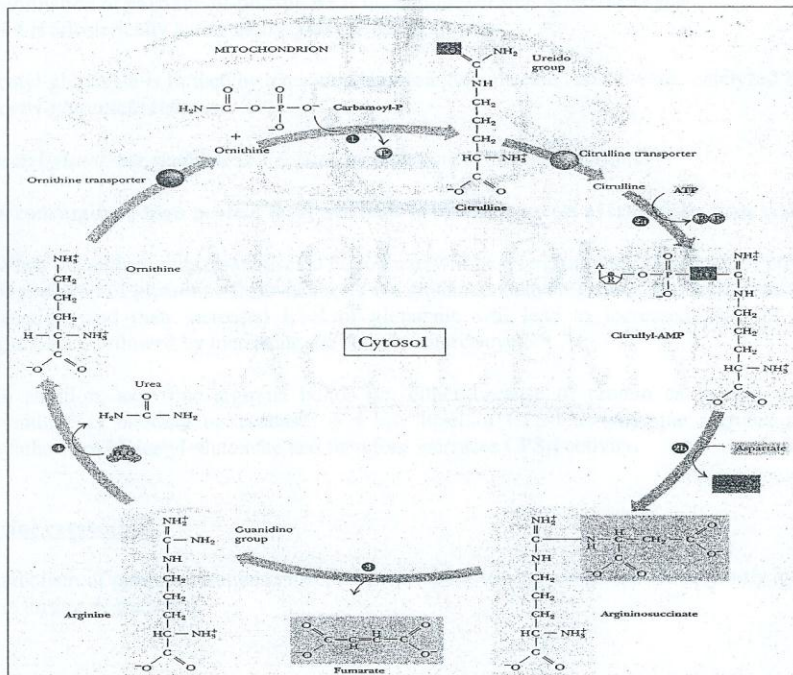
Let's start:

- Condensation reaction between the ammonium ion and the carbon dioxide to produce **carbamoyl phosphate**, this reaction occur in **mitochondria** and needs hydrolysis of 2 ATP.
- **Ornithine** enters the **mitochondria** by a transporter, and then reacts with **carbamoyl phosphate** to form **citrulline** (STEP 1).

- Citrulline then transported to the cytosol by a transporter.
- Citrulline react with **aspartate** to form **argininosuccinate**, this reaction also require ATP. (Note the amino group in the aspartate is asource of second nitrogen in urea cycle). (STEP 2)

NOTE: ATP that is consumed in this reaction result in formation of AMP + P*P*i.

- Argininosuccinate is split to produce **arginine** and **fumarate**. (STEP 3)
- Arginine hydrolysed to give **urea** and to regenerate **ornithine** which is transported back to the mitochondrion (STEP 4).
- We can look to urea cycle in another way; this can be done by considering arginine as the immediate precursor of urea and to see it as producing ornithine in the process. As a result, the rest of the cycle is the regenerating of arginine from ornithine.



The link between urea cycle and citric acid cycle:

- The synthesis of fumarate is a link between the urea cycle and the citric acid cycle.
- Fumarate is an intermediate of citric acid cycle and can be converted to oxaloacetate.
- Transamination reaction can convert oxaloacetate to Aspartate, providing another link between the two cycles.
- Four high-energy phosphate bonds are required because of the production of pyrophosphate in the conversion of Aspartate to argininosuccinate.

Note: the discoverer of the both pathways is Hanks Krebs.

Control of urea cycle:

- The major control point is mitochondrial enzyme *carbamyol-phosphate synthetase I (CPS-I)*.
- The formation of **carbamyol-phosphate** is the committed step in the urea cycle.
- **CPS-I** is allosterically activated by **N-acetyl-glutamate**.
- N-acetyl-glutamate is formed by a reaction between glutamate and acetyl-CoA, catalyzed by *N-acetyl-glutamate synthase*.
- *N-acetyl-glutamate synthase* is activated by high concentration of arginine.

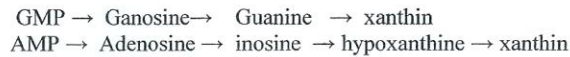
So how consuming a high protein meal will lead to nitrogen excess excretion by urea synthesis?

- When the amino acids catabolism is high, there will be a large amount of glutamate produced by degradation of glutamine from synthesis via glutamate dehydrogenase, and from transamination reaction, and then increased level of glutamate will lead to increased level of N-acetyl-glutamate, followed by increasing the activity of urea cycle.
- In addition, any time arginine builds up, either because of protein catabolism or because ornithine is building up because of a low level of CPS-I activity, the arginine stimulates synthesis of N-acetyl-glutamate and therefore increases CPS-I activity.

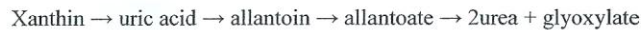
❖ Purine catabolism :

The catabolism of purin nucleotides proceed by hydrolysis to nucleoside and subsequently to free base which is further degradation.

- Purin nucleotide is converted to free base and then to xanthine as in the following reaction:



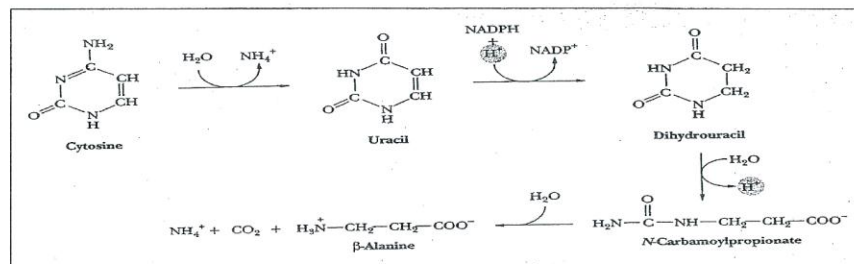
Catabolism of xanthin:



- Xanthin is oxidized to uric acid, which represent the end product in bird, reptiles, insects, primates, and human and can be excreted by kidney
- In terrestrial animal uric acid converted to allantoin then excreted.
- In fish allantoin further converted to allantoat.
- In other microorganism and some amphibians allantoate converted to two urea and one glyoxylate molecule.
- In human, the case of over production of uric acid and its deposits that accumulate in the joints of the feet and hands causes **Gout disease**.
- The best treatment to gout disease is **allopurinol drug**, which inhibits the degradation of hypoxanthine to xanthin and conversion of xanthin to uric acid and consequently prevents the buildup of uric acid deposits.

❖ Pyrimidine catabolism:

- Pyrimidine nucleotides are broken down first to nucleoside and then to the base, as purin nucleotides.





Questions

1- Which of the following is true:

- Amidation is preferred than amidation when NH_3 is limiting
- Glutamine synthetase has lower K_m than glutamate dehydrogenase
- Glutamate:oxoglutarate aminotransferase converts glutamine to glutamate
- Transamination reactions require pyridoxal phosphate as a coenzyme
- All of the above

2-Which of the following amino acid is not from pyruvate family:

- Valine
- Isoleucine
- Leucine
- Alanine
- B and C

3-Which of the following amino acid is produced from ribose-5-phosphate:

- Histidine
- Asparagine
- Serine
- Tyrosine
- Lysine

4-The catabolism of amino acids give rise to which metabolic intermediates:

- Pyruvate
- Oxaloacetate
- Fumarate
- Succinyl-CoA
- All of the above

5-Tetrahydrofolate is involved in which reaction:

- Serine \rightarrow Glycine
- Glycine \rightarrow Cysteine
- Serine \rightarrow Cystine
- Glutamate \rightarrow Glutamine
- Glutamine \rightarrow Glutamate

6-What is the reductant that is used in converting Glutamine to Glutamate in plants:

- NADPH
- FADH
- Reduced ferredoxin
- NADH
- Reduced glutathione

Answer sheet

Question #	Answer
1-	e. All of the above
2-	b. Isoleucine
3-	a. Histidine
4-	e. All of the above
5-	a. Serine --> Glycine
6-	c. Reduced ferredoxin

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10
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اسماء

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