◉ Sheet ○ Slides

Subject :	Amino acids metabolism
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HOMOCYSTEINE AND VASCULAR DISEASE

Regarding to formation of cysteine and regeneration of methionine from homocysteine (in the last lecture), we will talk about a disorder in the metabolism which is homocysteinuria.

Homocysteinuria

Homocysteine in some genetic disorders can increase in the blood and this is due to the deficiency in cystathionine synthase (B6 requiring enzyme). This disorder has various forms such as: **B6 responsive** -which is rare- this means the abnormal enzyme has high KM so that when you increase the amount of B6 you will have higher activity, but most of them are **non-responsive**.

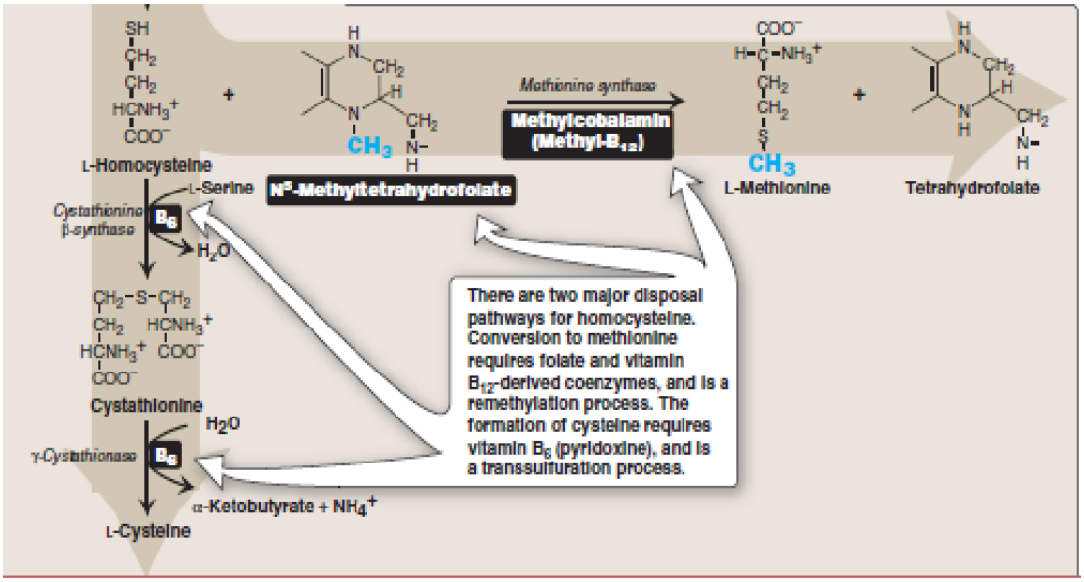
The second reason of homocysteinuria could be the deficiency in methionine synthase and in this disorder the patient will suffer from mental retardation and early myocardial infraction and pulmonary embolism (جلطه بالرئه).

They thought there is a relationship between the high amount of homocysteine and the cardiac disorders (because most of the people that have abnormal high level of homocysteine have myocardial infraction .. so many cardiologist used to request for the examination of homocysteine level in the blood but it seems the there is no direct relationship between increasing the amount of homocysteine and the cardiac disorders.

- The treatment here is:

  1- Taking folate (it is reduced to tetrahydrofolate which is important in the function of methionine synthase activation to convert homocysteine to methionine) and B12 (because it is important in the conversion of homocystiene to methionine and B12 needs tetrahydrofolate to be active) and B6 (important for conversion if homocysteine to cysteine).

  2-restrection of methionine diet.



SAM *S-Adenosyl methionine*

- Is required in several reactions (methylation reactions) such as:
  1-phosphotidylethanolamine convert to phosphotidylcholine .
  2-when norepinephrine converts to epinephrine.
  3-in nucleotides methylation as kind of protection.
  4-when aminoacetate converts to creatine (seen later).
  5-acetylserotonin to melatonin (hormone)
  All these reactions are done by methylation so they need SAM.

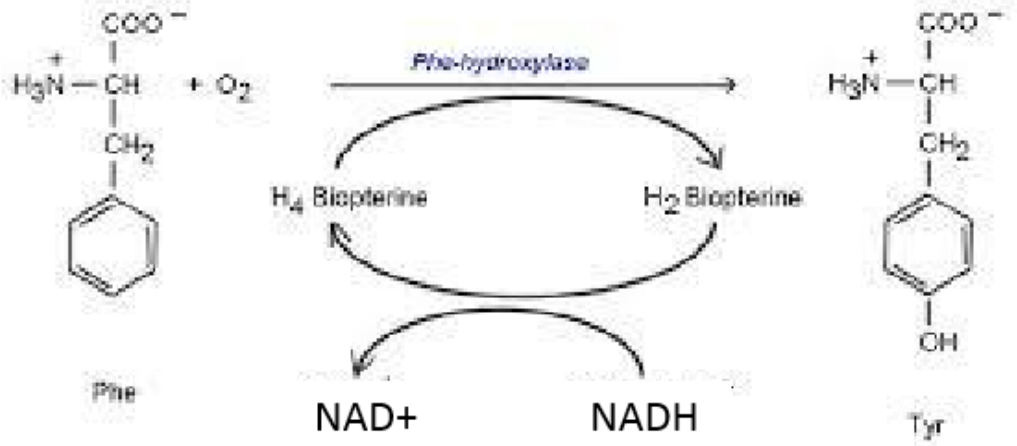
Note: it is important to remember the relationship between B12 and tetrahydrofolate and SAM (discussed previously).

This is the end of sulfer containing amino acids and now we will talk about:

TYROSINE (TYR) AND PHENYLALANINE (PHE)

It is an important group … phenylalanine is essential amino acid but tyrosine is not because we can make tyrosine from phynelalanine by **phenylalanine hydroxylase** (an important enzyme) that need the co-enzyme **tetrahydrobiopterin** (**BH4**) … so this enzyme help in conversion of phe to tyr → That means that a deficiency in the enzyme or the co-enzyme can lead to deficiency in synthesis of Tyr.

- Phenylalanine hydoxylase is a monooxygenase or mixed function oxygenase.
  (oxygenase mean that atom of molecular oxygen become hydroxyl group of TYR and the another one is reduced to water).
- This reaction illustrates how tyrosine comes from phenylalanine; but if phenylalanine amount is low then tyrosine become essential (this is similar to methionine and cysteine).



- Tyrosine undergoes transamination (loss of amino group) and produce **parahydroxyphenylpyruvate** by specific transaminase and then another hydroxylase (dioxygenase) (dioxygenase mean both oxygen atoms are incorporate in the substrate) then this will produce **homogentisate**.
  This compound will be metabolized by dioxygenase* to produce **fumarylacetoacetate** and this will be further metabolized to produce acetoacetate (wich is ketogenic) and fumarate (wich is glucogenic) .

  *when this dioxygenase is deficient (rare case) this will lead to **ALKAPTONURIA** … the high amount of homogentisate in the blood will go to the urine and this will cause formation of blackish layer on urine. If we exposed it to air the urine become black like ink.
- ALKAPTONURIA symptoms appear above age (34-40) (appear late); it appears in baby diapers so the diapers will be stained with black.

- Usually the patients will suffer from large joint arthritis and some of them may need replacement, buildup of dark pigment of cartilage and collagen tissue (it will appear black).

So this disease is asymptomatic until age of 40.

- Management: diet with low tyrosine and PHE.

Phenylketonuria

Deficiency in another enzyme which is **phenylalanine hydroxylase** is very important case to study and its incidence is 1 in 10000 therefore we should screen for this disorder in all newborns.

**Remember: phenylalanine hydroxylase convert PHE to TYR with help of O_2 and co-enzyme BH4 which will convert to dihydrobiopterin (BH2) and if I want to regenerate BH4 from BH2 then I need oxidation of NADH to NAD+ and reductase enzyme.

- Tetrahydrobiopterin is important in amino acid metabolism.

If there is deficiency in this enzyme then Phe (which is usually low in the body) will convert to phenylpyruvate (wich will cause PKU) and phenylpyruvate will convert to phenylacetate and Phenyllactate.

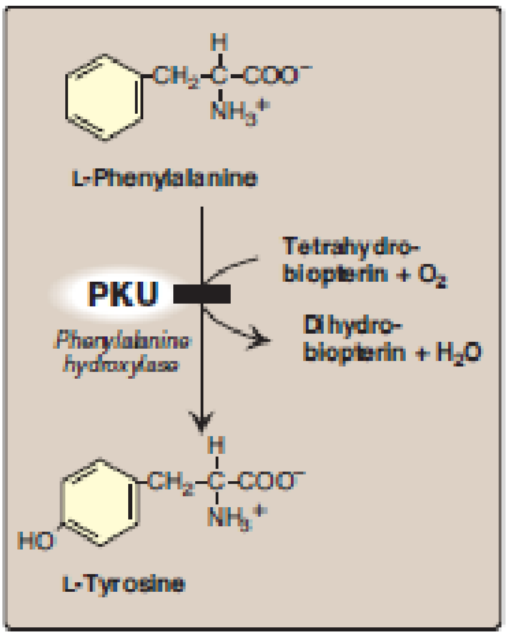
*Note: phenylpyruvate have ketone group so it will cause phenylketonuria (PKU).

And these compounds (phenylpyruvate and phenylacetate and phenyllactate) can cross the blood brain barrier and accumulate in the brain and cause mental retardation.

- Deficiency of this enzyme cause mental retardation this damage reaches maximum in 8 or 9 month.
- Early symptoms: vomiting, delay development, hypopigmentation, seizure, failure to talk or walk.
- The children should be monitored for the amount of Phe in their body.
- We do not do the test for Phe at birth? Because Phe will be cleared by placenta of the mother. So we wait after feeding the baby with milk (protein feeding) (after 48 hour) we can detect if there is

disorder in metabolism of PHE or not (by taking blood sample from the baby after 2 days of his/her life).

- If we do the test in the first day of newborn life it will give us false negative result.



- so we should restrict the amount of PHE for the baby that has PKU (we do not want to prevent the baby from taking PHE because it is essential for protein synthesis but we want to minimize it as possible as we can and we keep monitoring this amount in the blood).
- People with PKU should not take aspartame (*artificial sweetener*) because it contains PHE.
- Remember that newborns with PKU have normal level of Phe in the blood at birth.
- If there were no dietary restriction for newborn from the beginning >> the baby will be mental retard.
- But if the dietary was restricted then the baby will grow normally; and if at certain age he/she starts eating products with Phe >> they will not be mental retard ,instead there IQ (intelligence quotient indicate a person's mental ability) will start to reduce.

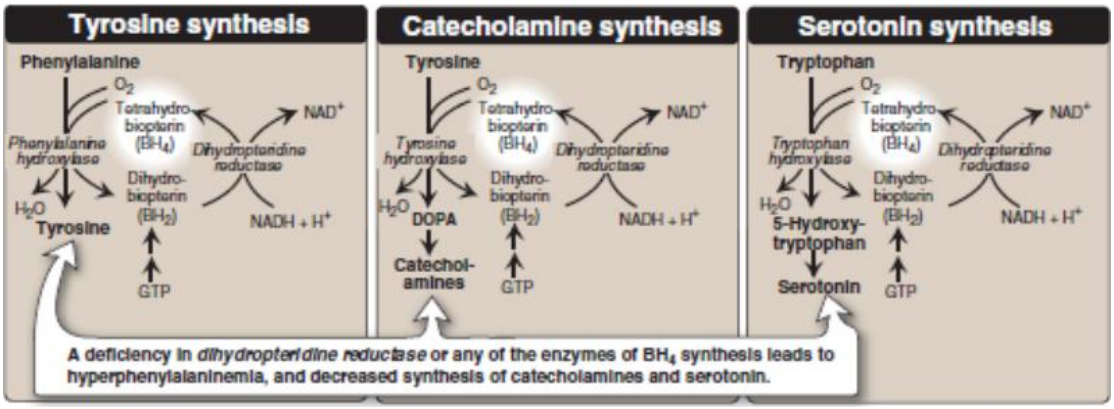
Mostly, PKU is caused by deficiency in enzyme **Phenylalanine Hydroxylase**; but it could be due to by other enzyme deficiencies.

In the figure below we notice that Dihydrobiopterin **BH2** needs to be reduced back to tetrahydrobiopterin **BH4** by the enzyme **Dihydropteridine Reductase**; Also, BH2 must be synthesized from **GTP** in several steps. So, any deficiency in an enzyme involved in the synthesis or the reduction will lead to Hyperphenylalaninemia as well.

Also, we notice that BH4 is required in other steps in the pathway of synthesizing Catecholamines and serotonin so they will decrease as a result of such deficiencies.

So the therapy requires not only dietary restriction but also supplementation of BH4, L-Dopa and 5-hydroxytryptophan (because L-Dopa and 5-hydroxytryptophan are products of the reactions catalyzed by enzymes that need BH4: **tyrosin hydroxylase** and **tryptophan hydroxylase**).

**therapy is not always so effective but it can be fine for some patients.

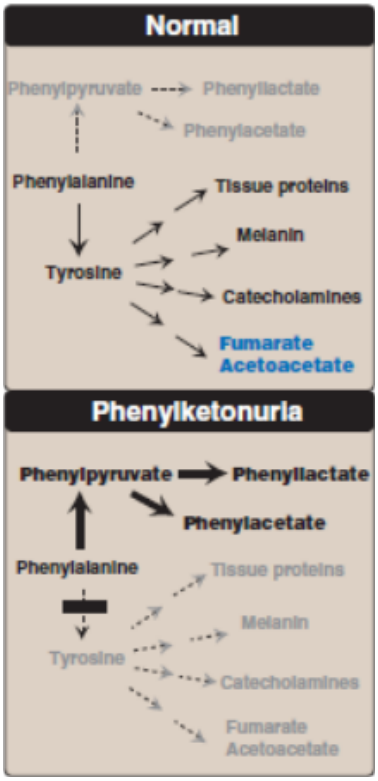


To sum up,

- A fraction of Phe is required for tissue protein but most of it to make Tyr.
- A fraction of Tyr is required for tissue protein but it is mainly a precursor to make many compounds such as Melanin pigment and Catecholamines or it will be metabolized to fumerate and acetoacetate.
- In PKU patients, Phe is not transformed into Tyr so most of it is transformed into Phenylapyruvate, Phenylacetate and Phenyllactate. Tyr in this case becomes essential amino acid.

Characteristics of classic PKU:

- ✓ Elevated phenylalanine, Phenylapyruvate, Phenylacetate and Phenyllactate in tissues, plasma, and urine.
- ✓ CNS symptoms: Mental retardation (IQ < 50), failure to walk or talk, seizures, hyperactivity, tremor, microcephaly, and failure to grow. As a result of accumulation of Phenyllactate,Phenylpyruvate and Phnelacetate which can cross the BBB.
- ✓ Hypopigmentation: fair hair, light skin color, and blue eyes because the hydroxylation of Tyr by tyrosinase (the first step in melanin formation) is competitively inhibited by the high levels of Phe.



• More than 40 mutations in the Enzyme **Phenylalanine Hydroxylase** were described. In most patients there are two different mutations (double heterozygous).

• Tyrosine becomes essential because we need Phenylalanine Hydroxylase to synthesize it from Phe.

Maternal PKU

Mothers that have PKU should be very careful because if the level of Phe in a pregnant woman was high it will affect the fetus causing mental retardation and congenital heart abnormalities because Phe is considered a teratogen (especially in the first semester).

Therefore, the lady -even before conception- has to monitor Phe level and keep it normal.

Biosynthesis of nonessential amino acids

Basically, it is the reverse of the metabolism process.

Essential: Phe, Val, Thr, Trp, Met, Leu, Ile, Lys & His.

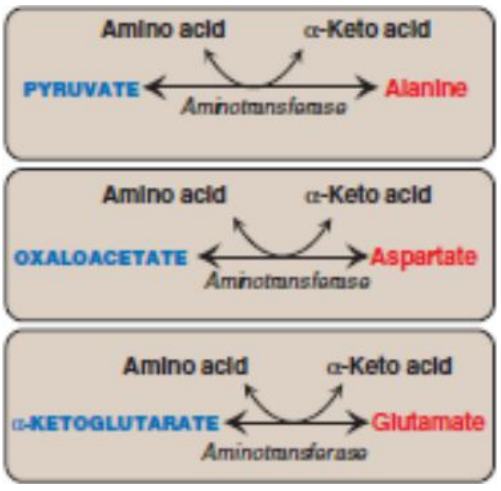
Nonessential: Ala, Arg, Asp, Asn, Cys, Glu, Gln, Gly, Pro, Ser & Tyr.
Any amino acid that starts with an A, G, C or S + Pro&Tyr.

Nonessential amino acids are synthesized from:
1.Metabolic intermediates
2.from the essential amino acids.

> Example: Tyr and Cys are synthesized from Phe and Met, respectively.
> If the level of of Phe and Met is low, Tyr and Cys will become essential.

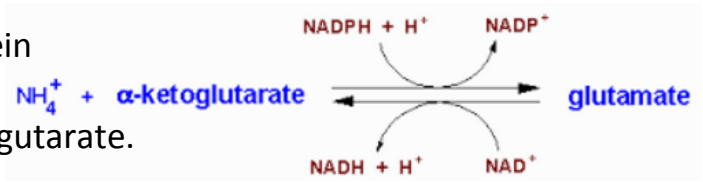
◘ Synthesis from the α-Ketocids

- Ala, Asp, and Glu are synthesized by transfer of an amino group to the α-keto acids pyruvate, oxaloacetate, and α-ketoglutarate, respectively.



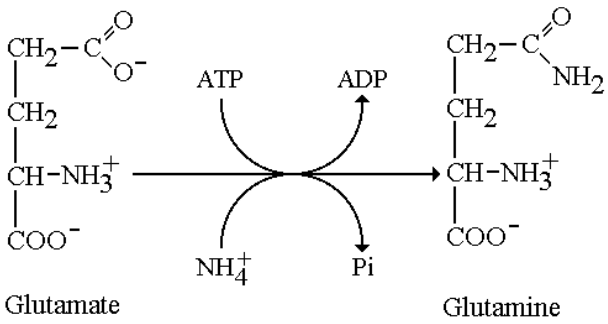
- Glu can also be synthesized by reductive amination (the reverse of oxidative deamination), catalyzed by glutamate dehydrogenase.

  * It is a reversible reaction.
  * When you ingest a high protein diet there will be higher conversion from Glu to α-ketogutarate.

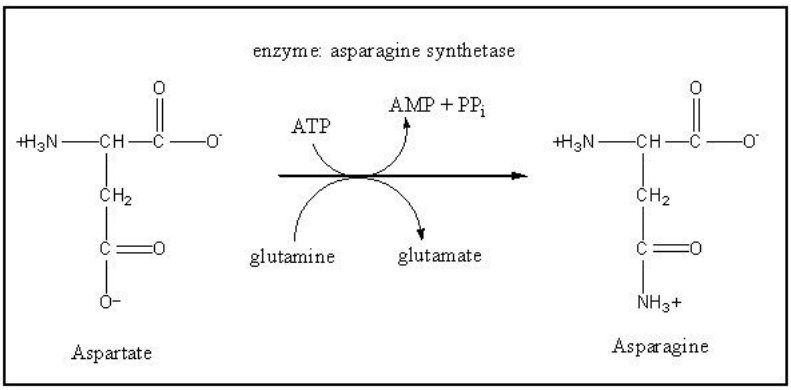


◘ Synthesis by amidation *(Formation of Amide linkage)*

- Gln is formed from Glu by glutamine synthetase.
  It requires ammonia and ATP.



- Asn is formed from Asp by asparagine synthetase, using glutamine as the amide donor.



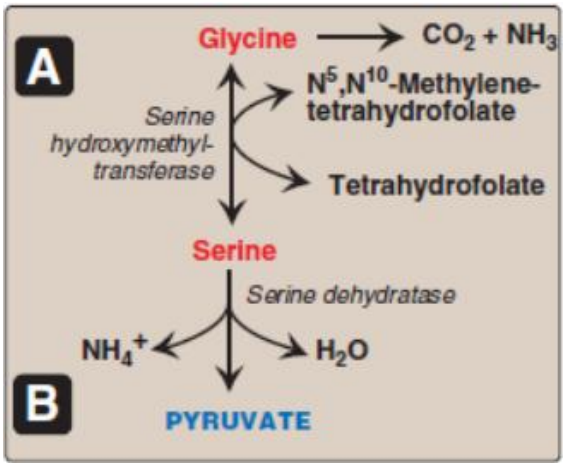
• In mammals, Aspartate amidation require Glutamine to donate an amino group, whereas in bacteria, it requires ammonia.

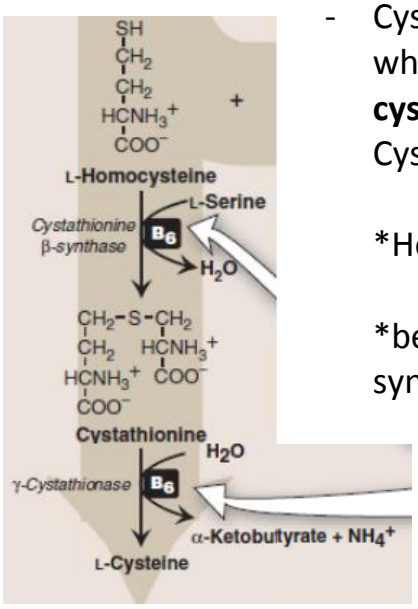
• The reaction can go back in the reverse direction to synthesize Gln from Glu, using Asn to donate the amino group.

◘ Serine, glycine, and cysteine

- Ser arises from **3-phosphoglycerate** (produced by glycolysis) that is oxidized by a dehydrogenase to **3-phosphopyruvate** → then it is transaminated to **O-phospho serine**. Serine is formed by hydrolysis of the phosphate ester by a phosphatase.
- If there is a plenty of Glycine, Ser can also be formed from glycine through transfer of a hydroxymethyl group by serine **hydroxymethyl transferase**.
  ***N5, N10-methylene- THF** is the one carbon donor.

- Gly is synthesized from serine by <u>removal of a hydroxymethyl</u> group, also by serine **hydroxymethyl transferase**.
  ***THF** is the one carbon acceptor.





- Cys is synthesized by two consecutive reactions in which <u>homocysteine combines with serine</u>, forming **cystathionine** that is <u>hydrolyzed</u> to α-ketobutyrate and Cys.

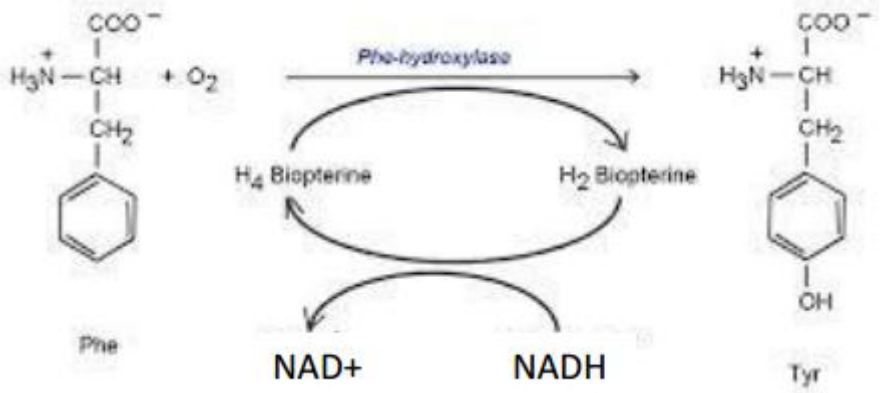
  *Homocysteine is derived from Met

  *because Met is an essential amino acid, Cys can be synthesized if the Met dietary intake is adequate.

◘ <u>Tyrosine</u>

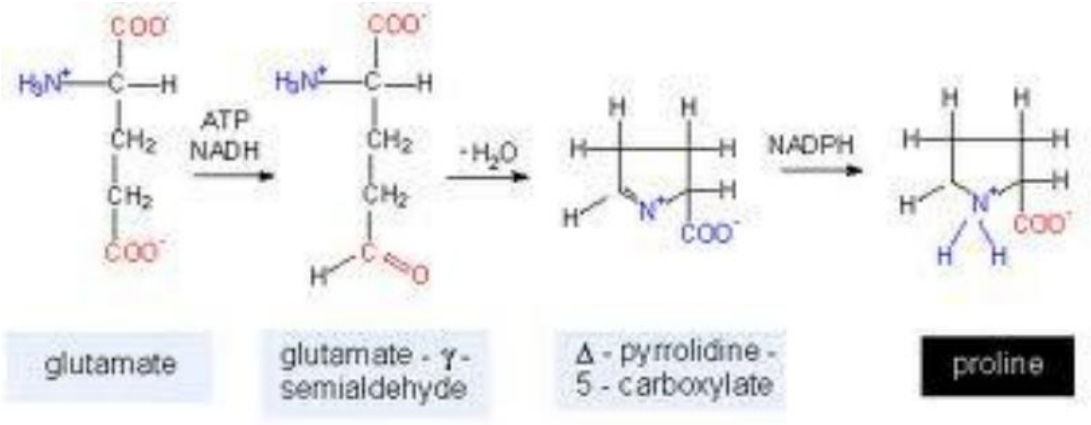
- Tyr (non-essential AA) is formed from Phe (essential AA) by phenylalanine hydroxylase.
- The reaction requires molecular oxygen and the coenzyme tetra hydrobiopterin (BH4)
- BH4 can be synthesized from GTP.

- One atom of molecular oxygen becomes the hydroxyl group of Tyr, and the other atom is reduced to water.
- BH4 is oxidized to dihydrobiopterin (BH2).
- BH4 is regenerated from BH2 by NADH-requiring dihydro pteridine reductase.



◘ Proline

- Glutamate is converted to proline by cyclization and reduction
  1. The carboxylic group is reduced to aldehyde producing **glutamate-γ-semialdehyde**. *(γ because it is on γ carbon).*
  2. We remove H_2O by a dehydration reaction to reduce the number of bonds allowing the molecule to form a cycle by a reaction between the amino group and the side chain.
  3. A reduction process on the nitrogen occurs by adding hydrogens taken from NADPH.



SUCCESS IN LIFE COMES WHEN YOU SIMPLY REFUSE TO GIVE UP, WITH GOALS SO STRONG THAT OBSTACLES, FAILURE AND LOSS ONLY ACT AS MOTIVATION