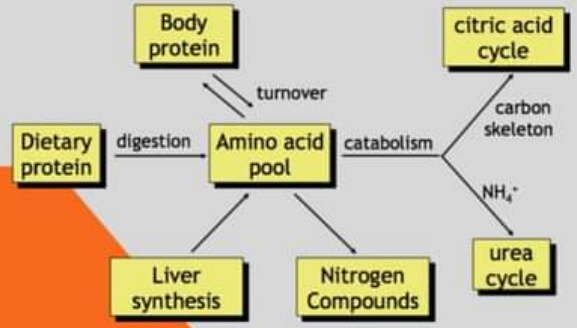
Metabolism of proteins & amino acids

by

Dr/ Heba M. Kareem



Essential amino acids :

Lysine, Leucine, Isoleucine, Valine, Methionine, Phenylalanine, Threonine, Tryptophan

Nonessential amino acids:

Alanine, glycine, aspartate , glutamate, serine, tyrosine, cysteine , proline , glutamine, aspargine

Histidine & arginine are **semi essential. They are** essential only for **infants** growth, **but** not for **old children** or **adults** where in adults **histidine** requirement is obtained by **intestinal flora**

Glucogenic	Ketogenic	Glucogenic&Ketogenic
Ala, Ser, Gly, Cys, Arg, His, Pro, Glu, Gln, Val, Met, Asp, Asn.	Leu , Lys	Phe,Tyr,Trp,Ile,Thr

METABOLISM OF AMMONIA

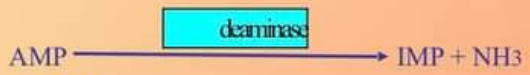
Ammonia is formed in body from:

a) ***From amino acids:*** 1.Transdeamination in liver

2. amino acid oxidases and amino acid deaminases in liver and kidney.

b) Deamination of physiological amines: by monoamine oxidase)histamine, adrenaline, dopamine and serotonine(

***C(Deamination of purine nucleotides*:** especially adenine nucleotides



a) ***Pyrimidine catabolism.***

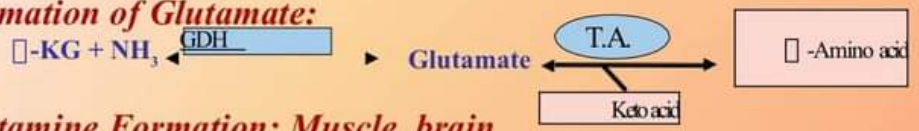
b) ***From bacterial action in the intestine*** **on dietary protein& on urea in the gut.**

NH_3 is also produced by glutaminase on glutamine .

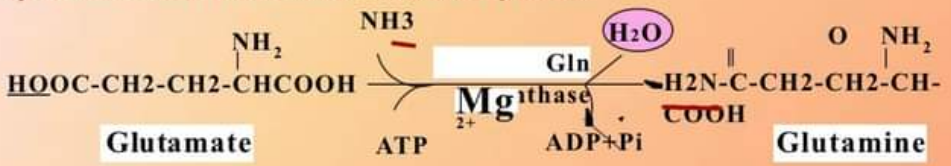
Metabolic Disposal of Ammonia

Ammonia is toxic to tissues it is fixed into nontoxic metabolite for reuse or excretion according to the body needs:

a) Formation of Glutamate:



b) Glutamine Formation: Muscle, brain



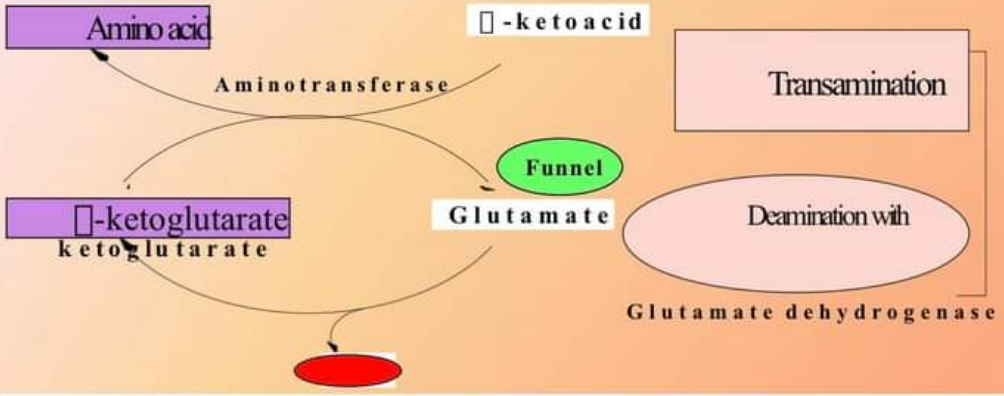
Glutamine is storehouse of ammonia & transporter form of ammonia.

In brain, glutamine is the major mechanism for removal of ammonia while **in liver** is urea formation.

..**Circulating glutamine** is removed by kidney, liver and intestine where it is deamidated by glutaminase .

c) Urea formation

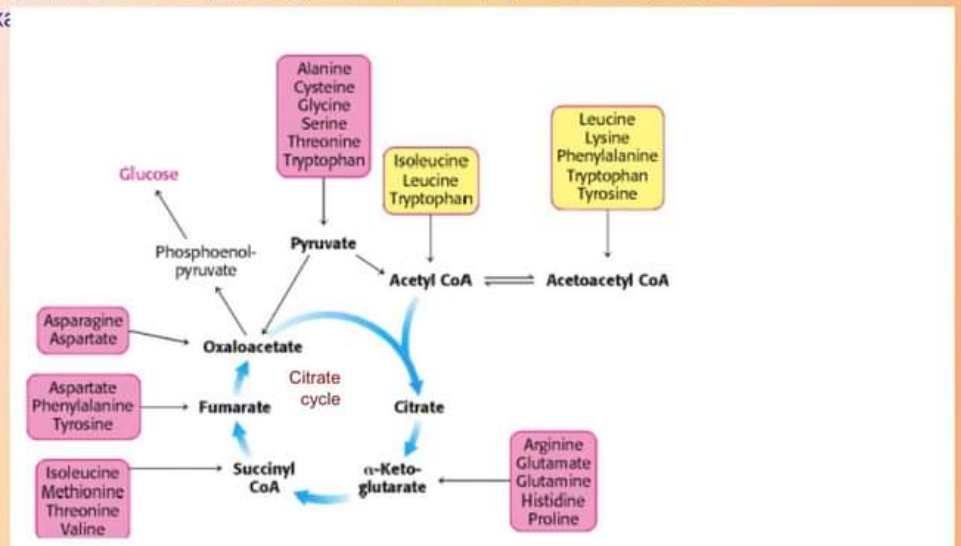
Transdeamination:



So… the most **important** and **rapid** way to deamination of amino acids **is first** transamination with -ketoglutarate **followed** by deamination of glutamate.

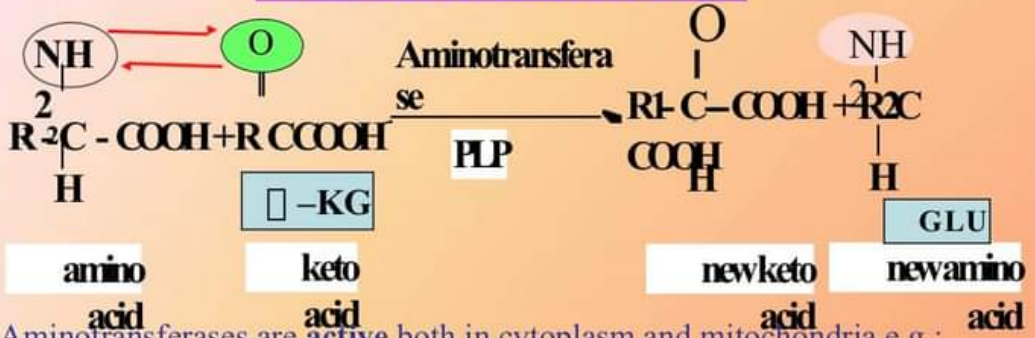
Therefore **glutamate through transdeamination serves to a funnel ammonia from all amino acids.**

The common metabolic intermediates that arised from the degradations of amino acids are: acetyl CoA, pyruvate, one of the cycle intermediates (-ketoglutarate, succinyl CoA, fumarate & oxa



re 23.21. Fates of the Carbon Skeletons of Amino Acids. Glucogenic amino acids are shaded red, and ket amino acids are shaded yellow. Most amino acids are both glucogenic and ketogenic.

Transamination:



Aminotransferases are **active** both in cytoplasm and mitochondria e.g.:

Aspartate aminotransferase (AST), Glutamate oxaloacetate transaminase **(GOT(**

Alanine aminotransferase (ALT), Glutamate pyruvate transaminase, **(GPT(**

In all transamination reactions, α-ketoglutarate (α –KG) acts as amino group acceptor.

Most, but not all amino acids undergo transamination reaction with few exceptions **(lysine, threonine and imino acids(**

Metabolic Significance of Transamination Reactions

- It is an **exchange of amino nitrogen** between the molecules without a net loss

This metabolically important because:

1) There is **no mechanism for storage** of a protein or amino acids.
2) In case of low energy)caloric shortage), the organism depends on **oxidation of the ketoacids** derived from transamination of amino acids.
3) It is important for formation of the **non-essential amino acids**

Metabolic Significant Aspects of Urea Cycle

A) ***Energy Cost:***. Three ATP molecules and four high-energy phosphate bonds are utilized in the reactions..

B) ***urea cycle is related to TCA cycle:***

1. CO_2
2. ***Aspartate arises via transamination of oxaloacetate with glutamate***. Thus, depletion of oxaloacetate will decrease urea formation
3. **Fumarate** enters TCA cycle

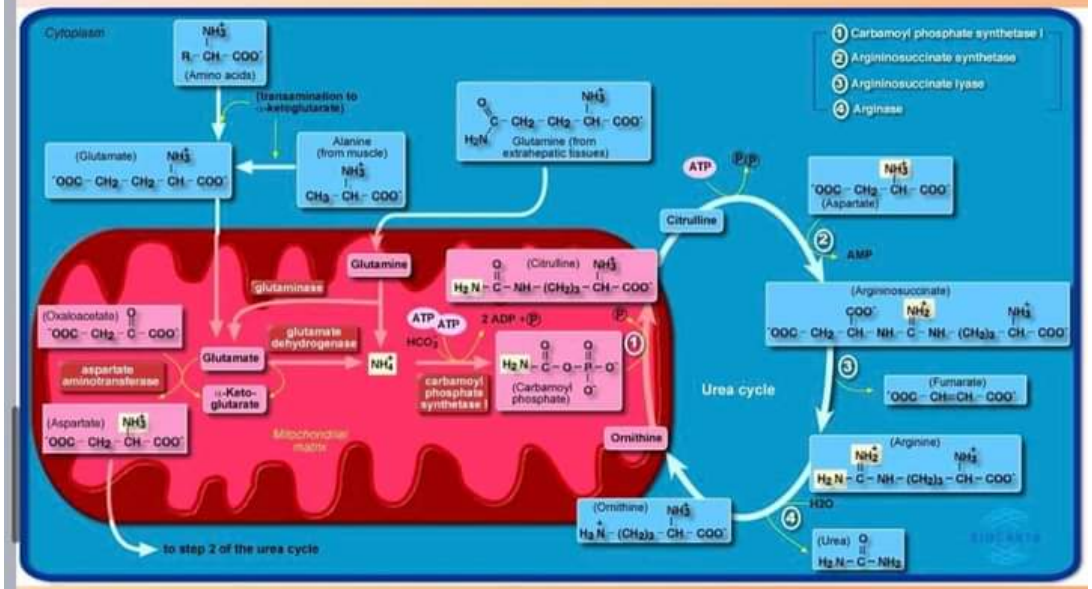
C) ***Sources of Nitrogen in*** urea :**free NH_3** and **aspartate**.

N.B. **glutamate** is the **immediate source** of both **NH_3** (via oxidative deamination by Glu. Dehyd.) and **aspartate** nitrogen (through transamination of oxaloacetate by AST).

Importance of Urea Cycle

1. **Formation of arginine (in organisms synthesizing arginine) & formation of urea (in ureotelic organisms, man) due to presence of arginase.**
2. **Liver** shows much **higher** activity of arginase than brain or kidney for formation of urea while **in brain or kidney** is the synthesis of arginine.
3. Synthesis of **non-protein amino acids** (ornithine and citrulline) in body.

The Urea Cycle



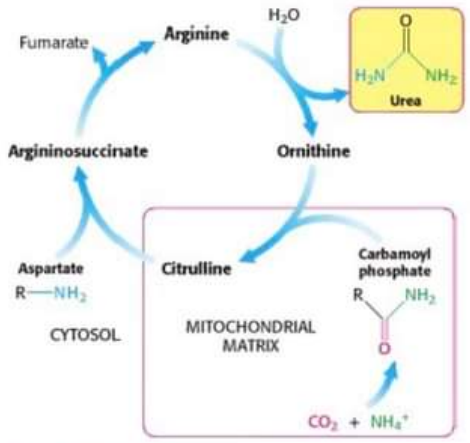
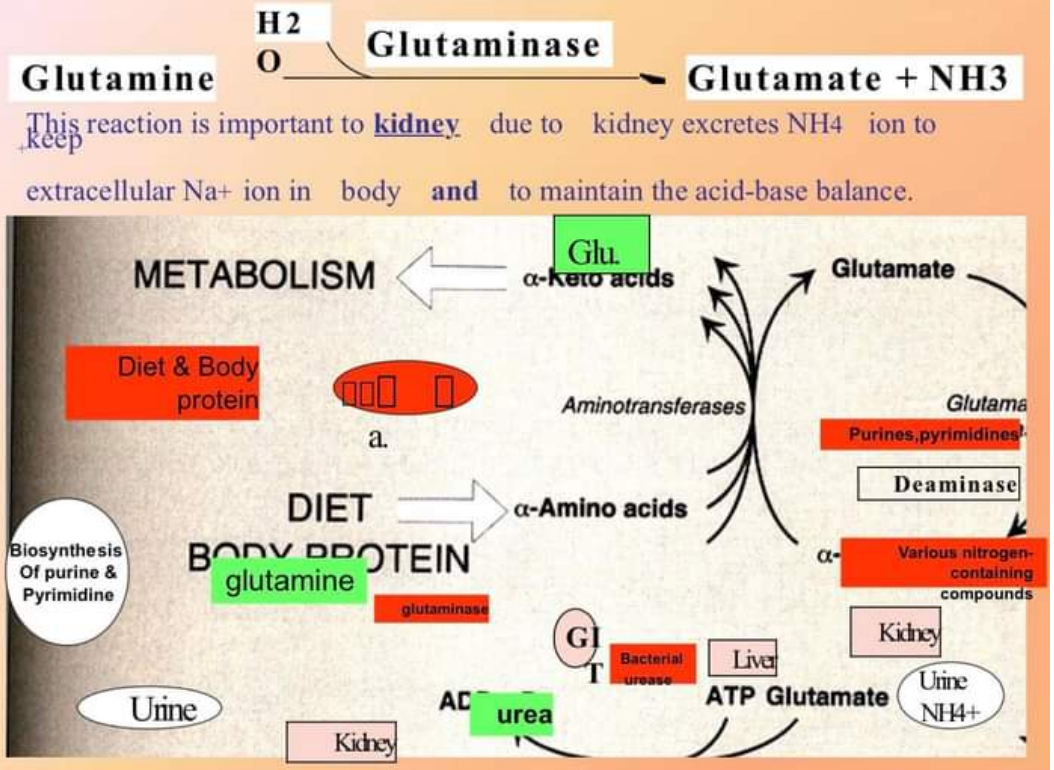


Figure 23.16. The Urea Cycle.



c) Urea Formation

Urea is the principal end-product of protein metabolism in humans.

It is important route for **detoxication** of NH3.

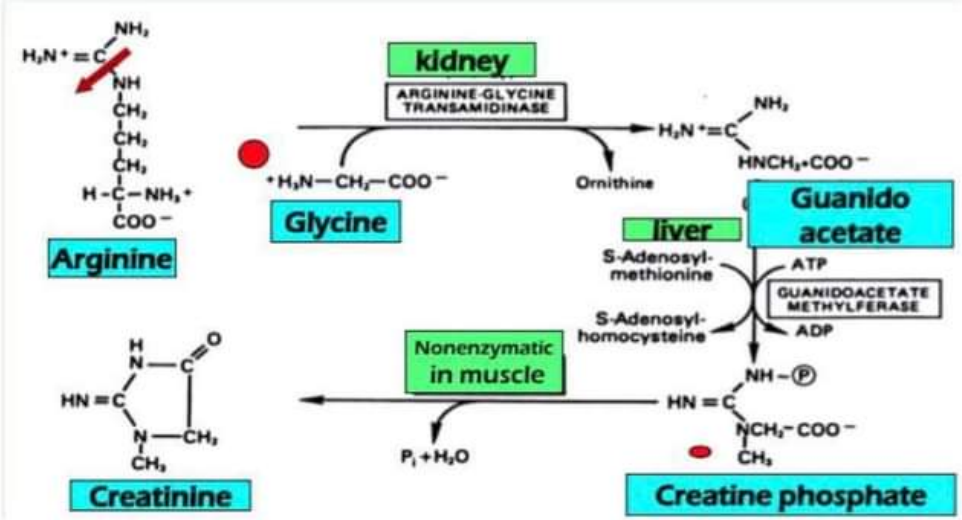
It is **operated** in liver, **released** into blood and **cleared** by kidney.

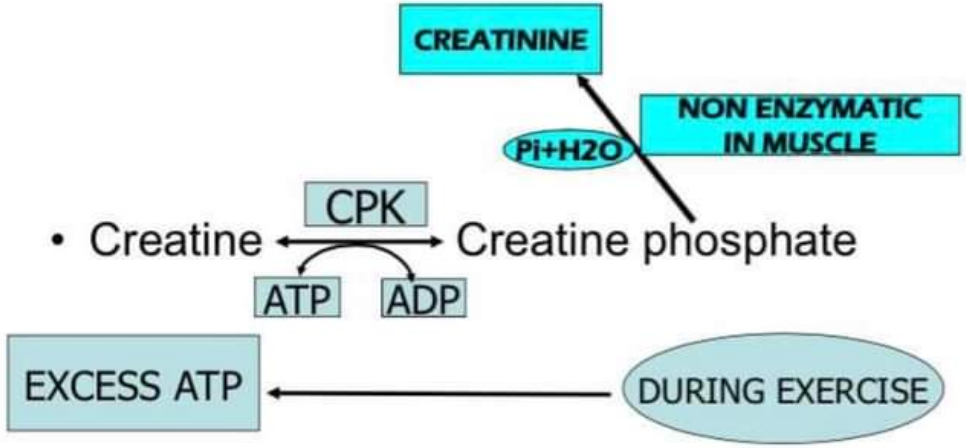
Urea is **highly soluble**, **nontoxic** and has a **high nitrogen content** (46%), **so** …it represents about **80-90% of the nitrogen** excreted in urine per day **in man**

Biosynthesis of urea in man is an energy- requiring process.

It takes place partially in mitochondria and partially in cytoplasm.

2. Formation of creatine (Methyl guanidoacetate)





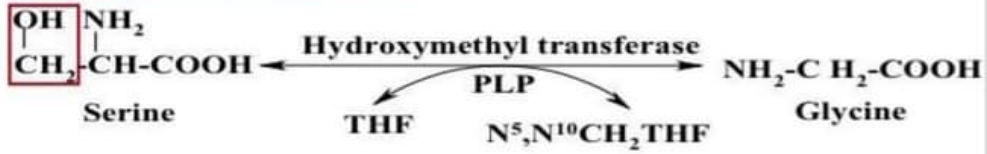
- Cr-P is the storage form of high energy phosphate in muscle
- Creatinine is excreted in urine & increases on kidney failure due to its filteration is decreased.
  Its level is constant per 24 hrs
  & is proportional to muscle mass in human.

METABOLISM OF INDIVIDUAL AMINO ACIDS

1. Metabolism of Glycine: nonessential, glucogenic.

Biosynthesis of glycine:

1



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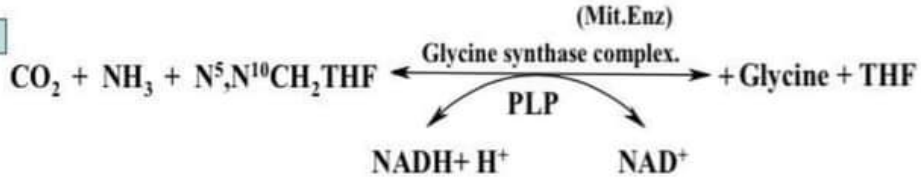


Special Functions of Glycine:

a-Protein, Hormones & enzymes.

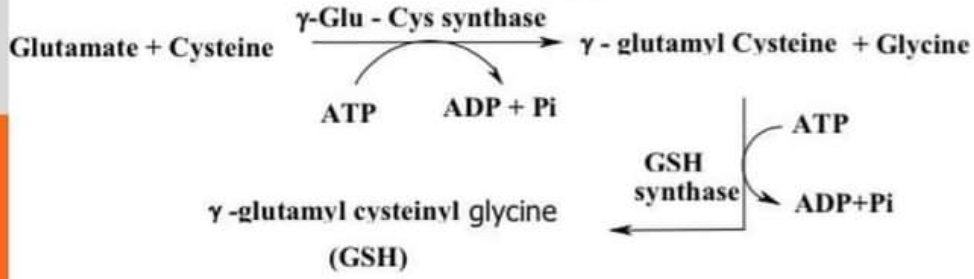
b- Heme c- Purines (C_4,C_5,N_7) d- Creatine

e- Glutathione

f- Conjugating reactions:

- Glycine + Cholic acid → glycocholate.
- Glycine + Benzoic acid → Hippuric acid

1.Formation of Glutathione (GSH) Dest.FR & Peroxides



Regulation of Urea Cycle

Activity of individual enzymes:

THE RATE LIMITING STEPS a) **carbamoyl phosphate synthase-1**

Ornithine transcarbamylase.

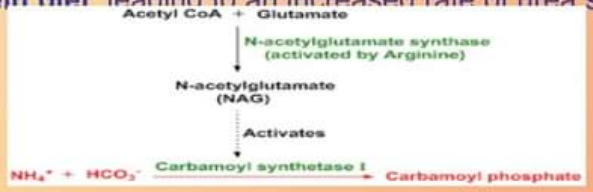
Arginase.

- ***N-acetylglutamate*** is activator for carbamoyl phosphate synthase-1

It enhances its **affinity** for ATP.

It is **synthesized** from acetyl CoA and glutamate.

its hepatic concentration increases after intake of a **protein diet** leading to an increased rate of urea synthesis.



- ***Activity of ornithine transcarbamylase*** is limited by the

-2 Regulation of the flux through the cycle:

1) **Flux of ammonia**:

1. by amino acids release from muscle (alanine, glutamine),
2. metabolism of glutamine in the intestine
3. amino acids degradation in the liver.

a) **Availability of ornithine.**

b) **Availability of aspartate:**

since aspartate is required in equimolar amounts with ammonia, this is satisfied by of transdeamination .

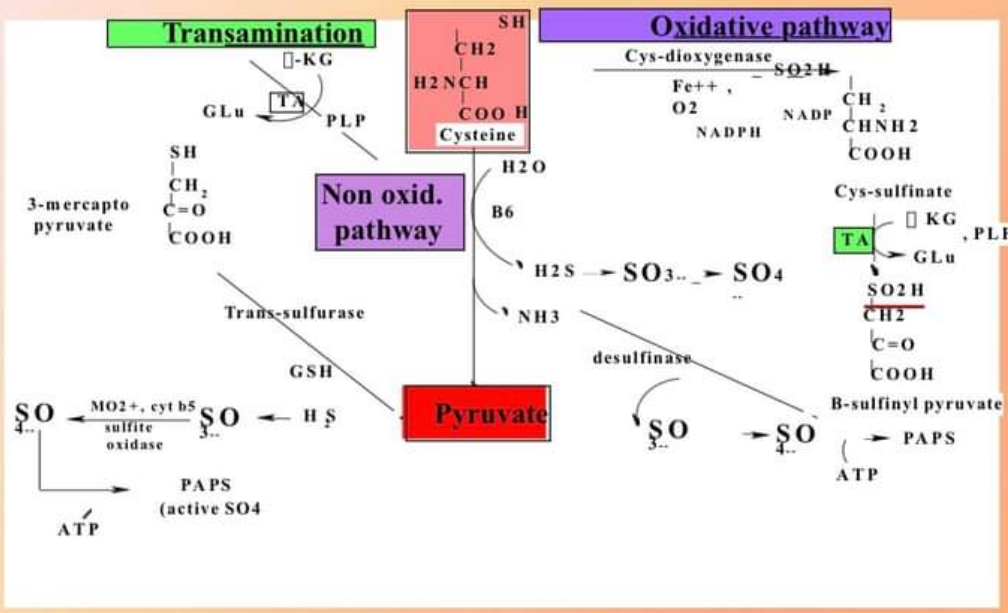
-3 Change in the level of Enzymes:

- **Arginase** & **other urea-forming enzymes** are adaptive enzymes thus a **protein-rich diet** will increase their **biosynthesis** rate
- & the opposite is true for **low protein diet.**

However, **in starvation**, where the body is forced to use its own tissue

- protein as fuel, there is an **increase in urea-forming enzymes.**

Degredative pathway of cysteine:



Biochemical functions of cysteine

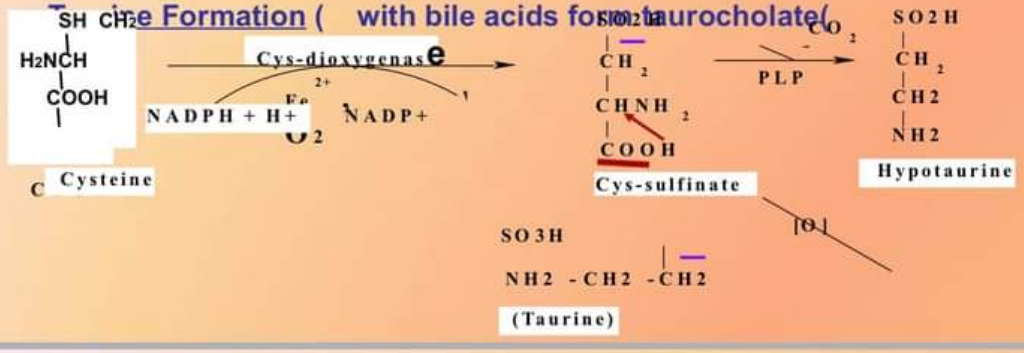
) **PAPS Formation: (3'-phosphoadenosine,5'-phosphosulphate)**active sulphate used in formation of sulfate esters of steroids, alcohol, phenol,some lipids, proteins and mucopolysaccharides

) **Sulfur of COASH, GSH, vasopressin, insulin**

3) **Detoxication reaction of bromo, chloro, iodobenzene, naphthalene and anthracene**

& of phenol, cresol, indol and skatol that is formed by the action of **intestinal bacteria** on **some amino a cids** in large intestine **with** formation of **ethereal sulfates which is water soluble and rapidly removed by the kidney**

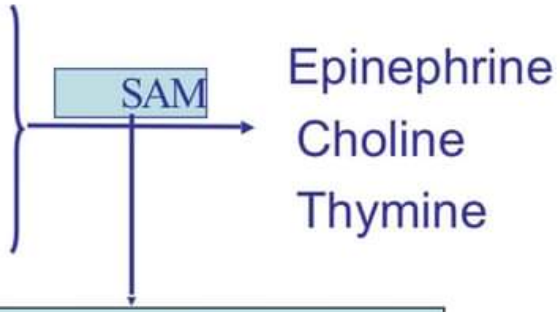
) **Taurine Formation (with bile acids forming taurocholate)**



In transmethylation there are:

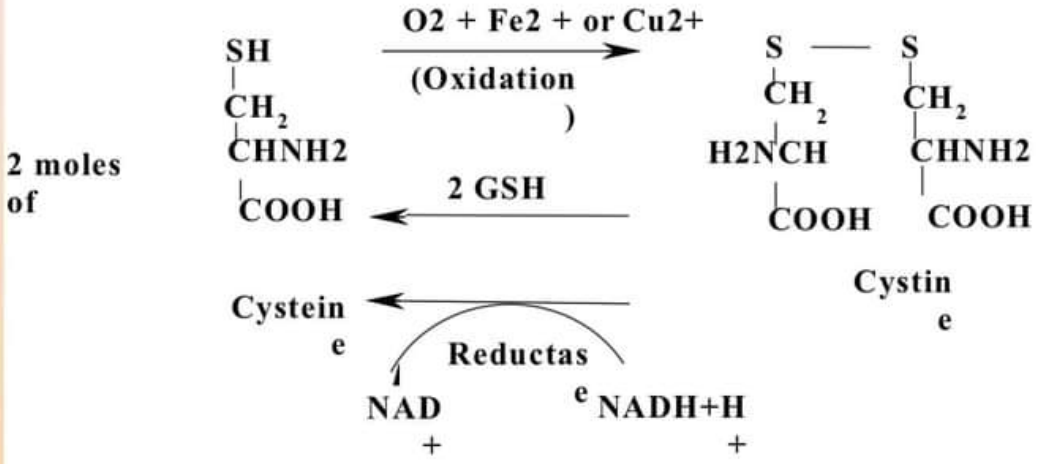
Methyl acceptors

(1) Guanidoacetic acid
(2) Norepinephrine
(3) Ethanolamine
(4) Uracil



Metabolism of Cysteine& Cystine:

- They are interconvertable &They are not essential
- can be synthesized from Met & Ser



3. Metabolism of Sulfur-Containing amino acids

(Methionine, cyteine & Cystine):

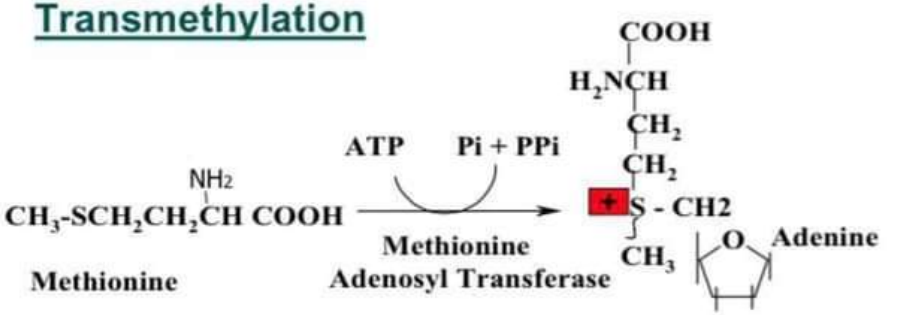
a) **Metabolism of methionine: (essential)**

Met. ⟶ Cysteine (diet.pr.)

- **2 principal metabolic pathways:**

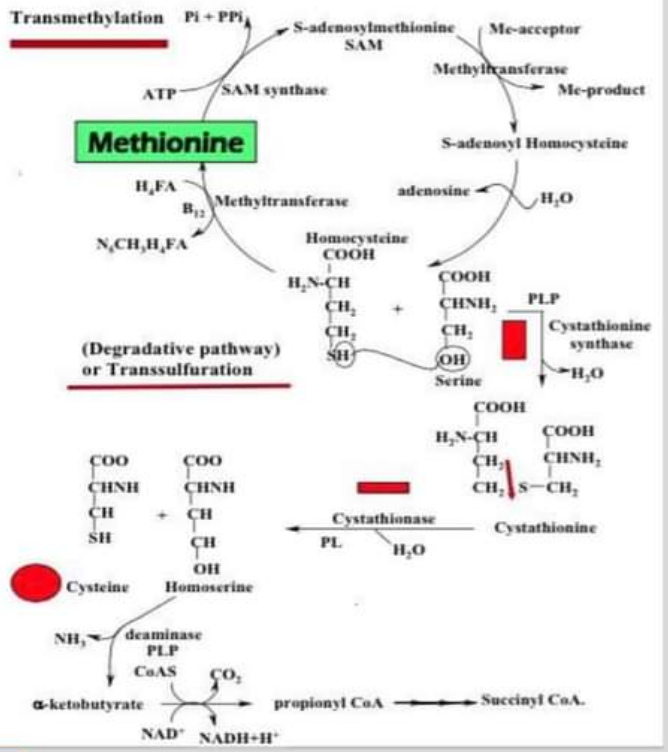
  Transmethylation and transsulfuration

- **Transmethylation**



Homocystinuria
Lack of
Cystathionine
synthase

C-skeleton of cysteine
From serine &
S from methionine



Fatty acids metabolism

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Biosynthesis of F.A.s

- F.A.s biosynthesis starts with **acetyl CoA**.

The enzyme systems are involved in this process.

1- Extra-mitochondrial (cytosolic) system. 2- Microsomal system.

Requirements; acetyl CoA, NADPH+H+ and group of enzymes called collectively fatty acid synthase complex.

Acetyl CoA formed from pyruvate within the mitochondria doesn't diffuse readily to the cytosol, the principal site of F.A. synthesis.

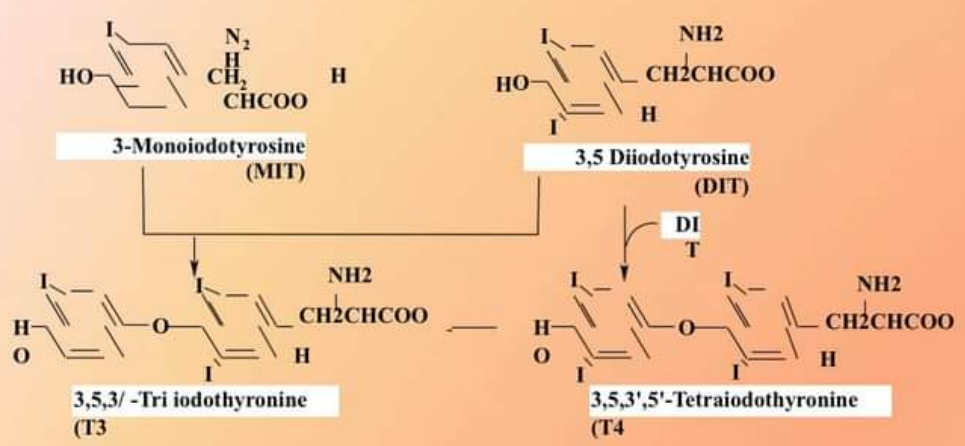
-Translocation to cytosol involves condensation with oxaloacetate to form citrate. (CITRATE SHUTTLE)

- When citrate is formed in excess of TCA cycle requirement, it diffuses into cytosol.
- By the aid of ATP citrate lyase, citrate is splitted down into acetyl CoA and oxaloacetate.

-**Acetyl CoA** may also pass through mitochondrial membrane into cytosol in the form of acetyl carnitine by carnitine acetyl transferase

-2Thyroid hormones:

Thyroxine Formation:



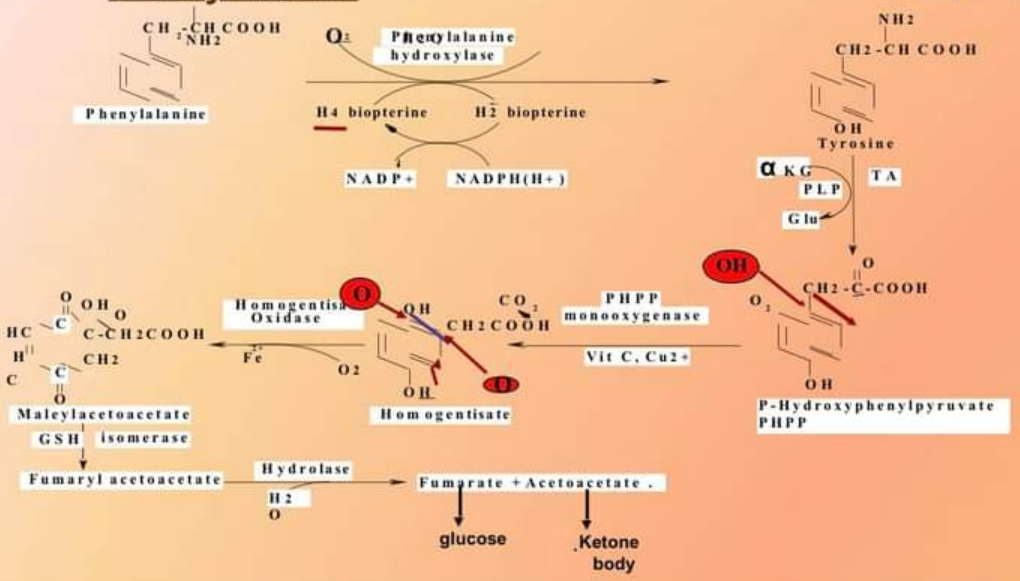
Amino acids as precursors of neurotransmitters

1. Serine Choline --- Acetyl choline.
2. Arginine ----------NO
3. Tryptophan----------Serotonin
4. Histidine----------Histamine
5. Phenyl alanine------dopa,dopamine, NE&E
6. Glutamic acid--------GABA

4. Aromatic amino acids

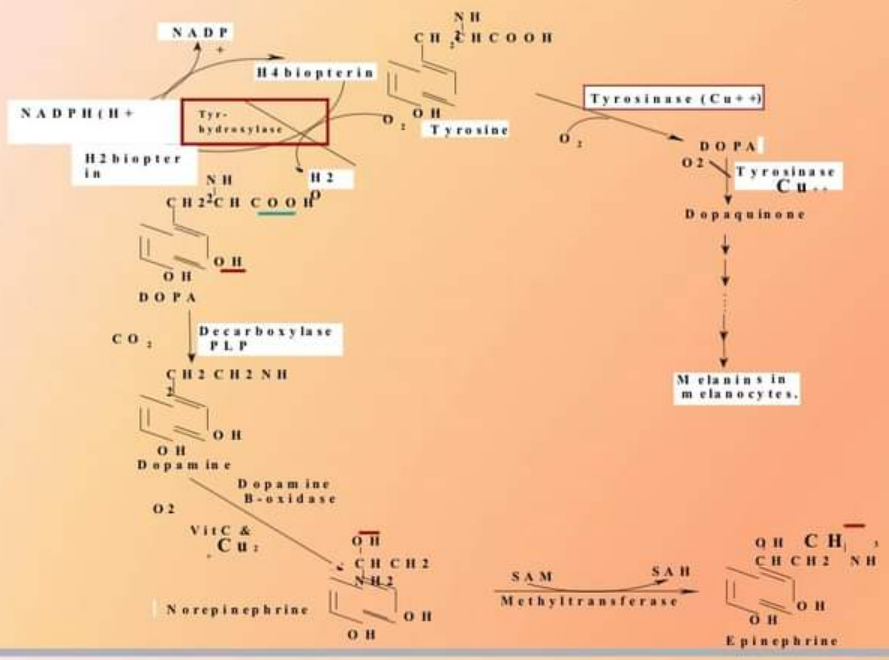
a) Metabolism of Phenylalanine

(glucogenic & ketogenic)



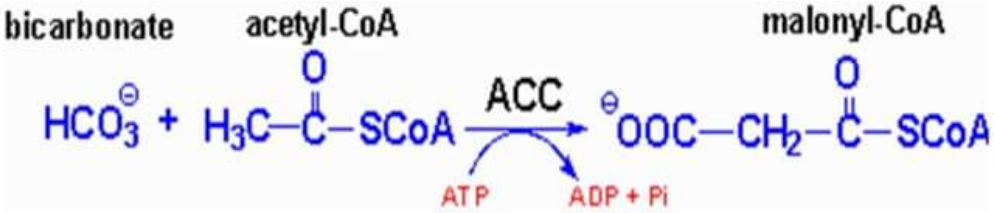
b) Tyrosine is a precursor of:

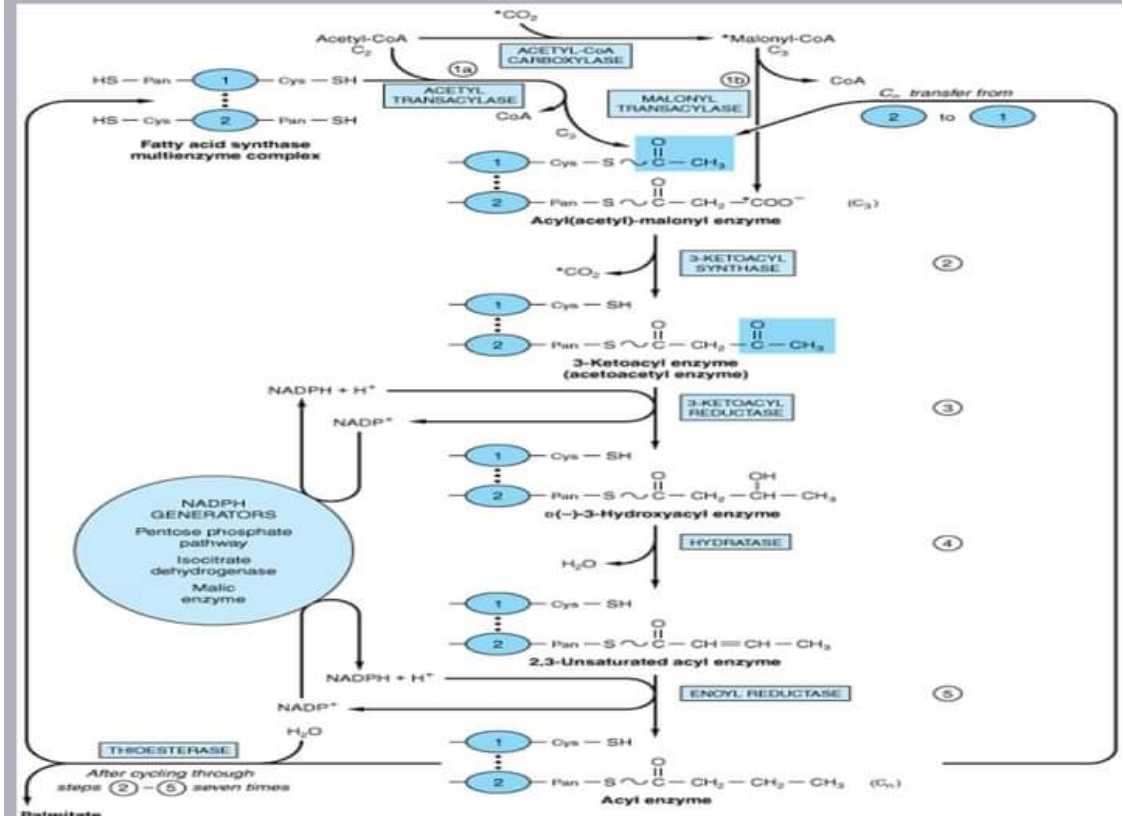
-1DOPA (3,4 dihydroxy phenylalanine(



-The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction, acetyl-CoA carboxylase (ACC), is the major site of regulation of fatty acid synthesis.

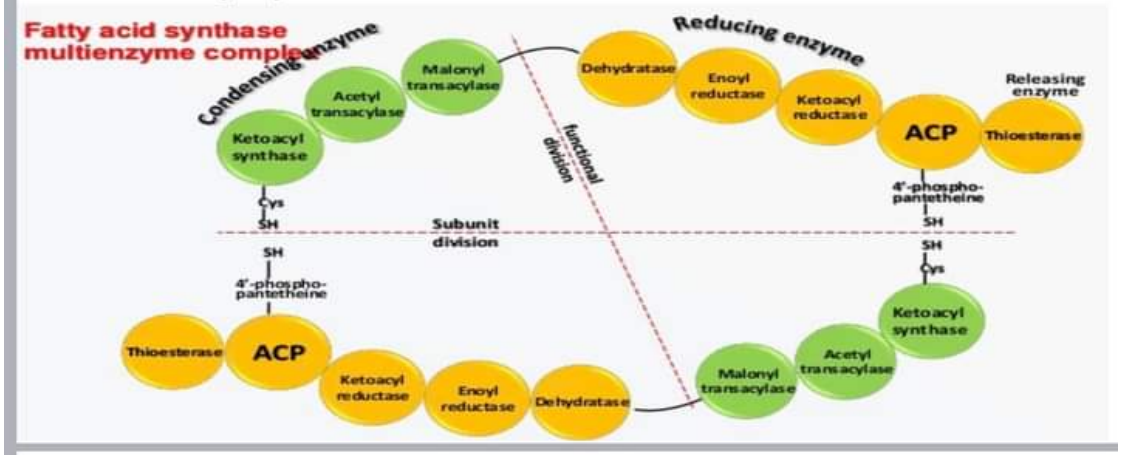
- Like other enzymes that transfer CO_2 to substrates, ACC requires a biotin co-factor.





- ACP is a protein contains the vitamin pantothenic acid in the form of phosphopantotheine. ACP is the part that carries the acyl group.
- Each monomer contains 2 –SH groups, one provided by phosphopantotheine and attached to ACP. The other is provided by cysteine attached to the enzyme 3- ketoacyl synthase.

The 2 monomers are arranged head to tail, so the -SH group of ACP of one monomer is very close to the -SH group provided by 3- ketoacyl synthase of the other monomer.



Extra-mitochondrial system

- It is the only system responsible for de novo synthesis of F.A.s from acetyl CoA, free palmitate is the main product.

Site:

Intracellular location: Cytosol.

8 molecules of acetyl CoA (C2) are used in the formation of palmitate (C16)

-7 acetyl CoA are converted into malonyl CoA by the enzyme acetyl CoA carboxylase.

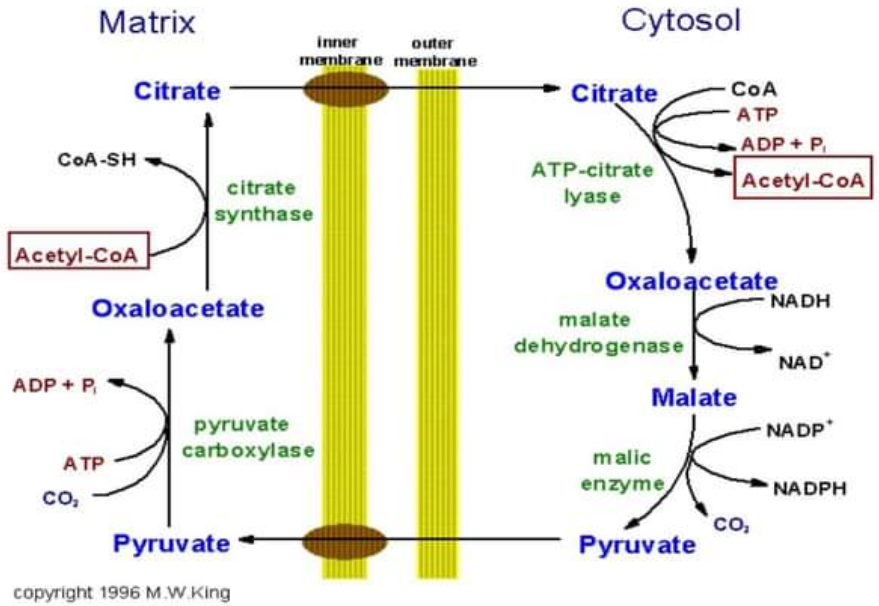
-The process occurs in 7 repeated cycles, each requires 2 mol of NADPH+H+ , and liberates 1 mol of CO2 and 1 mol of H2O.

Acetyl CoA + 7 Malonyl CoA + 14 NADPH+H+

⇓

Palmitic acid+ 8 COA+ 14 NADP+ 7 CO2+ 6 H2O ??

Translocation of acetyl CoA to cytosol



Note: Acetyl CoA used for fatty acid synthesis always derived from glucose and never from fatty acids. This is because insulin hormone secreted after meal stimulates both glucose oxidation (+acetyl CoA) and lipogenesis (=Fatty acid synthesis) and Inhibits lipolysis (+Fatty acid oxidation+Acetyl CoA).

NADPH+H+: It is provided by 3 sources:

a) Pentose phosphate pathway.

b) Action of cytoplasmic isocitrate dehydrogenase on isocitrate. It is similar to mitochondrial one but it uses NADP+ as hydrogen carrier.

c) Action of malic enzyme on malate to produce pyruvate:

Fatty acid synthase complex:

This enzyme is a dimer i.e. formed of 2 subunits.

Each unit, which is called monomer, contains 7 enzymes and a terminal protein called acyl carrier protein (ACP).

Long chain F.A.s transport

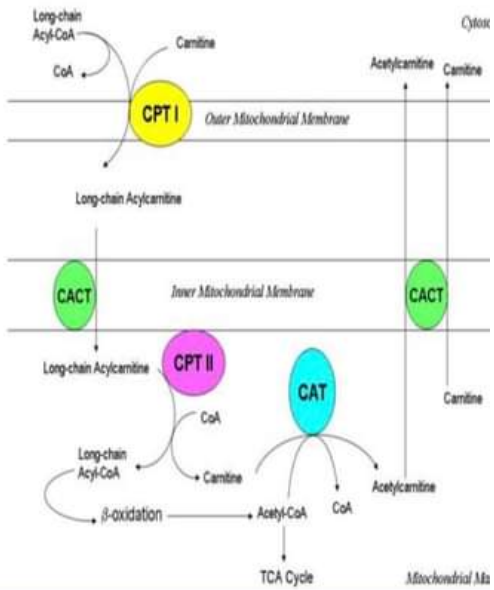
Carnitine:

It is β- hydroxy - γ- trimethylamino butyric acid

-The enzyme carnitine-palmitoyl transferase I transfers the acyl radical from the acyl-CoA to the hydroxyl group of carnitine, forming acylcarnitine

- It can cross the inner mitochondrial membrane in exchange with carnitine.
- Acyl carnitine transported to the inner mitochondrial membrane is accomplished by carnitine acyl-carnitine translocase.
- In mitochondria carnitine is regenerated by carnitine palmitoyl transferase II enzyme, and the active acyl CoA is now ready for oxidation and energy production.

Carnitine Shuttle



β –Oxidation

-In the mitochondrial matrix there is a group of enzymes called **F.A.s oxidase** that are responsible for F.A.s oxidation.

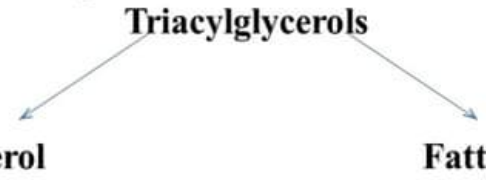
- The process is multi-cyclic, in each cycle 2 carbons as active acetate are removed.
- Also, two reduced coenzymes are produced ($FADH_2$ and $NADH+H^+$).
- Active acetate is oxidized in TCA cycle, producing also, reduced coenzymes.

-The net reduced coenzymes are oxidized via ETC for ATP production.

- β-oxidation occurs in many tissues except brain where F.A.s can not be uptaken by brain tissues and RBCs (because no mitochondria).
- It is termed β -oxidation since, it occurs through the sequential removal of 2-carbon units by oxidation at the β -carbon position of the fatty acyl-CoA molecule.

Catabolism of TAG (Lipolysis):

- Lipolysis is carried out by three enzymes present in adipose tissue :
  1. Hormone sensitive triacylglycerol lipase:
  2. Diacylglycerol lipase.
  3. Monoacylglycerol lipase.



- Glucose by gluconeogenesis.
- Pyruvate by glycolysis.
- Triacylglycerol by lipogenesis

- Oxidation to give energy.
- May remain in adipose tissue to be re-esterified into TAG

- **N.B.** Glycerol in adipose tissue cannot be used in re-esterification of fatty acids to form triacylglycerol due to deficiency of **glycerokinase enzyme.**

Fatty acid Oxidation

Oxidation of fatty acids occurs in the mitochondria.

-The CoA derivatives of long chain F.A. can not penetrate the inner mitochondrial membrane (short chain F.A.s & their acyl CoA can penetrate).

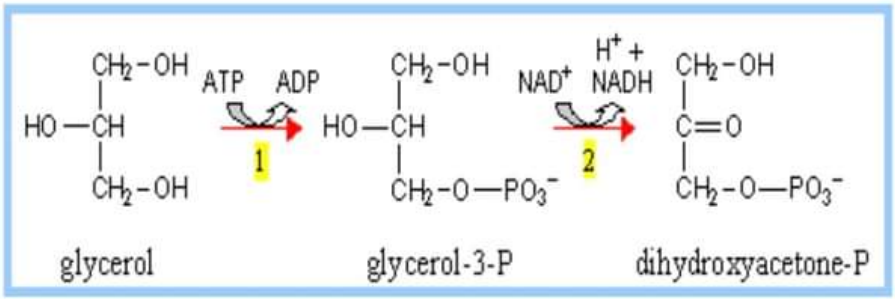
- The transport of fatty acyl-CoA into the mitochondria is accomplished via an acyl – carnitine intermediate

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \xrightarrow{\text{thiokinase}} \text{Acyl-CoA} + PP_i + \text{AMP}$$

- Fatty acids results from TAG hydrolysis in adipose tissue are taken up by most tissues and must be activated in the cytoplasm before being oxidized in the mitochondria.
- Activation is catalyzed by fatty acyl-CoA synthetase or (thiokinase).

Synthesis of TAG (Lipogenesis):

- Fatty acids are stored for future use as triacylglycerols in all cells, but primarily in adipocytes of adipose tissue.
-Triacylglycerols constitute molecules of glycerol to which three fatty acids have been esterified.
-The major building block for the synthesis of triacylglycerols, in tissues other than adipose tissues is **glycerol-3-phosphate** which is formed from glycerol by glycerokinase or from glycolysis.
- Adipocytes and muscles lack **glycerol kinase,** therefore, dihydroxyacetone phosphate (**DHAP**), produced during glycolysis, is the precursor for triacylglycerol synthesis in adipose tissues.



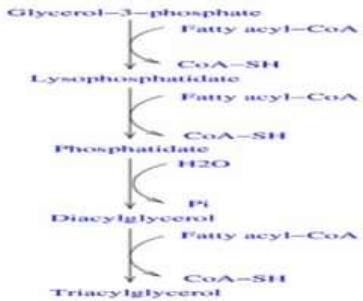
The fatty acids incorporated into triacylglycerols are activated to *acyl-CoA* through the action of acyl-CoA synthetases.

-Two molecules of acyl-CoA are esterified to glycerol-3-phosphate to yield 1,2-diacylglycerol phosphate (commonly identified as phosphatidic acid).

-The phosphate is then removed, by phosphatidic acid phosphatase, to yield 1,2-diacylglycerol, the substrate for addition of the third fatty acid.

-**N.B.** After meal, insulin is secreted which stimulate glycolysis which supplies **DHAP** that converted to glycerol phosphate in adipose tissue so **lipogenesis is stimulated.**

Triacylglycerol Synthesis



Fatty acids are synthesized and degraded by different pathways

Degradation (β-Oxidation)	Synthesis
1- In the mitochondria matrix	- In the cytosol
2- Intermediates are linked to CoA	- Intermediates are linked to an acyl carrier protein (ACP) complex
3- No linkage of the enzymes involved	- Enzymes are joined in one polypeptide chain -> FA synthase
4- The oxidants are NAD^+ and FAD	- The reductant is NADPH
5- Degradation by C_2 units -> Acetyl-CoA	- Elongation by addition of malonyl ACP + release of CO_2
	- Synthesis stops at palmitate (C16), additional enzymes necessary for further elongation

4- During hemodialysis which removes carnitine from blood.

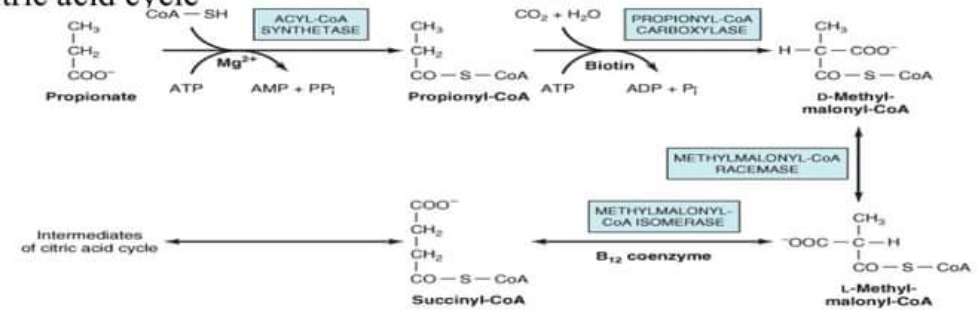
-Symptoms include muscle cramps during exercise, severe weakness

- Muscle weakness related to importance of fatty acids as long term energy source
- Hypoglycemia and hypo ketosis are common findings
- Diet containing medium chain fatty acids is recommended since they do not require carnitine shuttle to enter mitochondria.

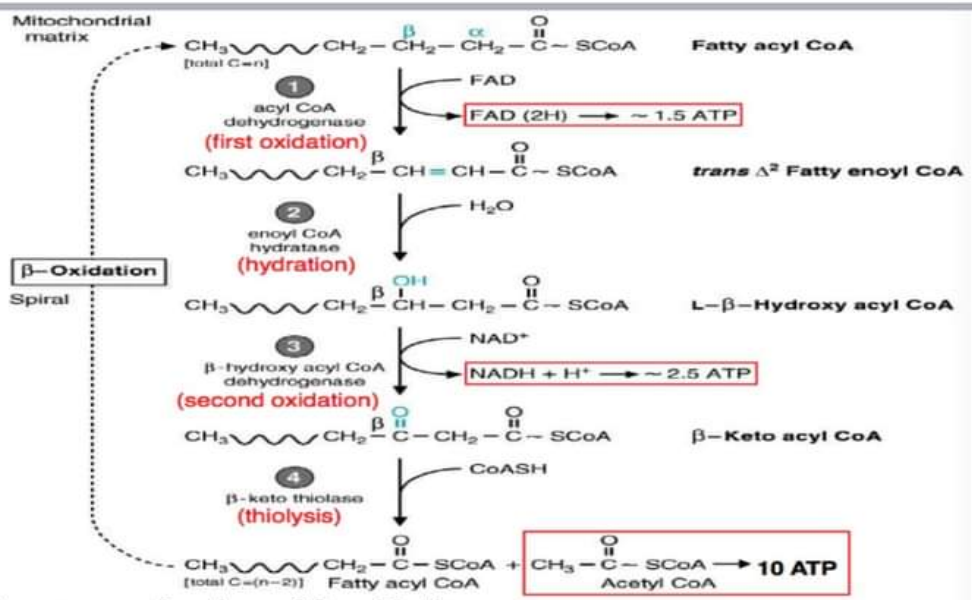
β- oxidation of odd chain fatty acids

-Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation, producing acetyl-CoA, until a three-carbon (propionyl - CoA) residue remains.

-This compound is converted to Succinyl-CoA, a constituent of the citric acid cycle



-The propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.



Energy production of β-oxidation

-Calculation formula of energy production for fatty acid oxidation :

= {(N/2 – 1) X 4 ATP} + (N/2 X 10 ATP) – 2 ATP

where N represents the number of carbon atoms of fatty acid.

Regulation of fatty acid oxidation:

Through energy production: ↑ATP → inhibit ETC → inhibit β-oxidation.

Importance of β-oxidation:

1- Energy production.

2- Production of acetyl CoA which enters in many pathways

3- Ketone body formation : Acetoacetyl CoA is the last 4 carbon atoms in the course of β-oxidation, it may be converted into acetoacetate; one of ketone bodies.

Disorders associated with impaired β-oxidation

1- Carnitine deficiency: It leads to accumulation of toxic amounts of free fatty acids and branched-chain acyl groups

- It occurs in patients with:

1- liver disease.

2- malnutrition.

3- In those with increased requirement of carnitine as sever infection and burns.

4- During hemodialysis which removes carnitine from blood.