Tuesday
28/3/23

• Chapter 3 :-

Biosynthesis and Degradation

* Polysaccharides :—

- Polysaccharides also called glycans differ from each other in the identity of their recurring monosaccharide units, in the lengths of their chains, in the type of bonds linking the monosaccharide units and in the degree of branching.

• Homo(Poly)saccharide → same polysaccharide molecules
• Heteropolysaccharide → diff. polysaccharide molecules.

* Polymerisation of starch :—

• Starch :—

- Starch is a storage Homopolysaccharde of D-Glucose residue i.e. found in the cytoplasm of plant cells.
- Starch is extensively hydrated because it has many exposed hydroxyl groups available to hydrogen bond with water.
- Starch consists of two types of polymers:
  (i) Amylose
  (ii) Amylopectin.

• Amylose is a linear polymer of D-glucose residue that all are connected via α-1,4-linkage.
- Molecular weight of amylose from few thounds to more than million.

• Amylopection → Branched polymer of D-glucose residue.
- Amylopectin chains contain a branch point linkage between D-glucose and that are

α-1,6 & sometimes at α-1,4 & branch point occurs about every 24-30 residue.

- Molecular weight upto 200 million dalton.

- A cluster of amylose & amylopetin molecule like that believe to be present in the starch granule in the plant cells. is as shocon in the figure.

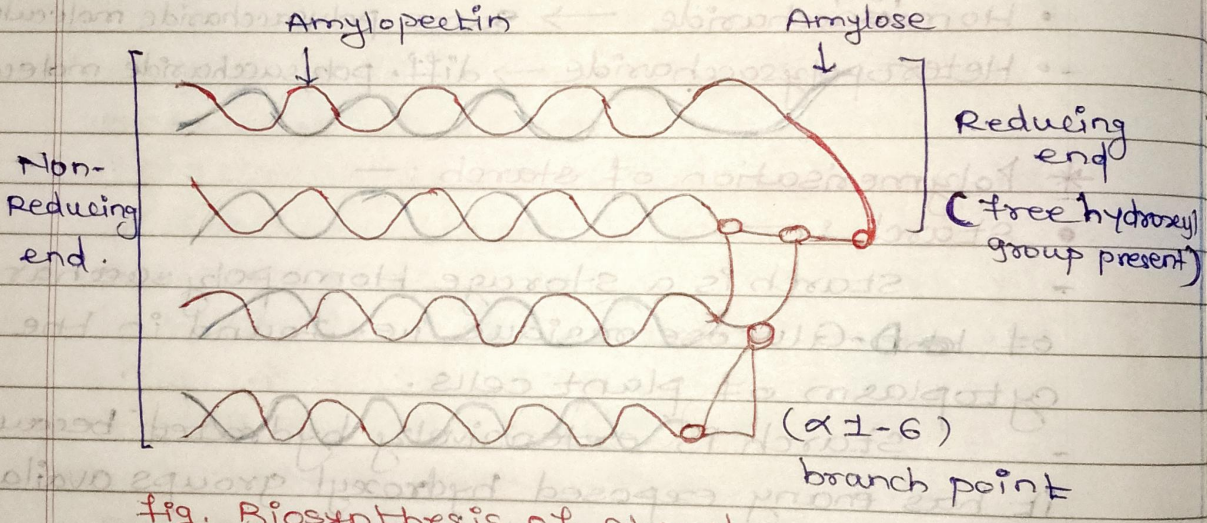


fig. Biosynthesis of starch

- About 10-30% amylose & 70-90% amylopectin is present in natural starches.
- Strands of amylopectin form a double helical structure with each other or with amylose strand.
- Glucose residues at the non-reducing end of the outer branches are remove enzymatically during the mobilization of starch for energy production.

• Peptidoglycan → murin
also called

• Amylose → The most stable three-dimensional structure for α-1,4 linkage of starch is a tightly coiled helix.
- The helix is stabilised by inter chain hydrogen bonds.
- The glucose residue in the chains are also able to form hydrogen bonds to surrounding solvent which keep the polymer in a solution.

3/11/23 Friday

* Peptidoglycan Biosynthesis :—
- peptidoglycan is a heteropolymer due to the presence of sugar in it i.e. N-acetylmuramic acid (β-1,4-glycosidic linkage) and N-acetylglucosamine.
- peptide cross bridges are linked to N-acetylmuramic acid.
- Sugar & amino acids form a mesh like layer outside the plasma membrane of most of the bacteria forming cell wall.
- peptidoglycan confirm strength to the cell wall.
- peptidoglycan forms around 90% of the dry weight gram positive bacteria & only 10% of the gram negative bacteria.
and peptidoglycan layer is thick in gram positive & thin in gram negative bacteria.

• Structure → The peptidoglycan layer in bacterial wall is a crystal lattice structure & is made up of a glycan strand alternating residue of N-acetylglucosamine and ~~N-case~~ N-acetyl-muramic acid linked by 1-4 glycosidic bonds between C1 & C4 respectively.
- N-acetyl muramic acid is modified form of N-acetyl glucosamine in which a lactyl group has been

attach to the C_3 carbon & attach to each NAM is a tetra peptide.

- The tetrapeptide chains are cross linked by peptide bonds & the tetrapeptides are L-alenine, D-glutamine, L-Lysine, D-alenine.

- In the biosynthesis of peptidoglycan there is an involvement enzymatic reaction that takes place —

1) cytoplasm
2) inner side of cytoplasmic membrane
3) outer side of cytoplasmic membrane.

- Stages —

(I) formation of Disaccharide peptide monomer units.

(II) Polymerasation reaction accompanied by interactions of the newly made peptidoglycan material into cell wall.

- Biosynthesis :—

*Stage I → takes place in cytosol.

The two amino sugar that are the precursors of peptidoglycan i.e. NAM & NAG (NAM & NAG contains fructose-6-phosphate)

Step 1)

Glutamine donates amino group to sugar this in turns fructose-6-phosphate to Glucosamine-6-phosphate in the presence of enzyme called glucosamine-6-phosphate synthase.

Step 2) Isomerisation

Glucosamine-6-phosphate is converted

* UDP - N-acetylglucosamine → UDP - Glc - NAC,

to Glucosamine - 1 - phosphate in presence of phosphoglucosamine mutase enzyme.

Step 3)

An acetyl group is transfer from acety co-A to the amino group on the glucosamine-1-phosphate creating N-acetyl glucosamine-1-phosphate in the presence of enzyme Glucosamine-1-phosphate acyl transferase.

Step 4)

N-acetylglucosamine-1-phosphate which now a monophosphate attacks UTP (Uridine Tri-phosphate) which is a pyrimidine nucleotide it has ability to act as energy source.

In this particular reaction after the monophosphate has attack the UTP & and inorganic pyrophosphate is given of & is replaced by monophosphate creating UDP-N-Acetylglucosamine (UDP-GlcNAC)

- This Initial stage is used to create the precursor for the NAG in peptidoglycan.
- The enzyme is N-acetylglucosamine-1-phosphate Uridyl transferase.

Step 5)

- In this step UDP-Glc-NAC is converted into UDP-N-acetyl glucosamine enol pyruvate. (UDP-Glc-NAC-enol-pyruvate) by the addition of Lactyl group to the glucosamine.
- Also in this reaction the C3-hydroxyl group will remove a inorganic phosphate (Pi)

from the α- carbon of phospho-enol-pyruvate.
- In the presence of the enzyme MurA (It follows an addition-elimination mechanism)

Step 6)
- In this they undergo a reduction catalysed by MurB using one equivalent of NADPH and a solvent derived proton.
- This two electron reduction creates the Lactyl ether of UDP-N-acetylmuramic acid (UDP-MurNAc)

Step 7)
- In this stepwise assembly of the peptide stem of peptidoglycan is ensured by a series four essential enzyme known as Mur Ligases. (MurC, D, E, F) this provides addition of L-alanine - MurC, D-glutamic acid - MurD, L-Lysine - MurE, D-alanine - MurF.

* Stage II :— takes place in cell membrane
- The N-acetyl muramic acid penta-peptide attach lipid carrier called bacto-prenol (LipidI) is a C-55 isoprenoid phosphate.
They are joined by pyrophosphate bond.
- UDP transfer NAG to bactoprenol PP-NAM penta-peptide. (Lipid III)
(penta-peptide → L-alanine sometimes added doubles)

• Bactoprenol → Lipid carrier.

- Interbridge formation occurs at the cytoplasmic side of membrane in the presence of enzyme MurG.
- The NAM-NAG penta peptide attach to the growing end of the nascent peptidoglycan chain.
i.e. Held at the membrane by bacto-prenol molecule.
- Then this bactoprenol donar moves back across the membrane it loses one phosphate becoming bactoprenol phosphate.
- It now ready to being a new cycle.

* - If a penta-glyciene inter bridge is required it is created using special glycl-Trna molecule (but not a ribosomes)

Thursday 6/4/23

* Fatty Acid Biosynthesis :—

• Fatty Acid —

- Fatty acids are class of compounds containing a long hydrophobic hydrocarbon chain and a terminal carboxylate group ($C\overset{O}{\overset{\|}{}}O^-$)
- They exist free in the body as well as fatty acyl ester molecule in more complex molecules such as triglycerides or phospholipids.
- Fatty acids can be oxidized in all tissues particularly liver & muscle to provide energy.
- They are also structural components of membrane lipids such as phospholipids & glycolipids.
- Esterified fatty acids in the form of triglycerides are stored in adipose cells.

• Sources of fatty acids —

1) Diet
2) Adipolysis
3) De-nove synthesis (from precursor)
[carbohydrate, proteins & other molecules obtain from diet in excess of the bodies need can be converted to fatty acids which are stored as Triglycerides]

*De-novo synthesis → Liver, kidney, Adipose tissue, brain, bone.

• Transportation of Acetyl Co-A —

- fatty acid synthesis requires considerable amount of acetyl co-A. nearly all acetyl co.A used in fatty acid synthesis is formed in mitochondria.
- Acetyl co.A has to move out from mitochondria to cytosol
- Acetate is a shuttled out of mitochondria as citrate. mitochondrial membrane is impermeable to acetyl co.A.

• Steps of fatty acid synthesis : →

• Step 1 —

- The input of fatty acid synthesis is acetyl Co-A, which is carboxylated to malonyl co-A the reaction is catalyzed by acetyl co-A carboxylase.

- All the remaining steps are catalyzed by fatty acid synthase complex.

• fatty acid synthase prosthetic groups —

(i) - The thiol (-SH) of the side chain of a cysteine residue of keto acyl synthase enzyme. (also called condensing enzyme)

(ii) - The thiol (-SH) of phosphopantetheine, equivalent in structure to part of coenzyme A.
- It is a component of acyl carrier protein (ACP)
- At the centre is the ACP (acyl carrier protein) with its phosphopantetheine arm ending in -SH.

- The long flexible arm of phosphopantetheine helps its thiol to move from one active site to another within the complex.
- It also tethering the growing fatty acyl chain to the surface of the synthase complex.
- carrying the reaction intermediates from one enzyme active site to the next.

• The 1st round of fatty acid biosynthesis —
- To initiate fatty acid biosynthesis, malonyl & acetyl groups are activated onto the enzyme fatty acid synthase.
- Initially, a priming molecule of acetyl Co-A combines with a cysteine -SH group of a KS enzyme catalysed by acetyl transacylase.
- Malonyl Co-A combines with the adjacent -SH on the 4- phosphopantetheine of ACP of the other monomers catalysed by

malonyl transacylase. (to form acetyl/acyl malonyl enzyme)

• Activation of acetyl Group —

- The acetyl group from acetyl Co-A is transferred to the cysteine-SH group of the β-ketoacyl ACP synthase.
- This reaction is catalyxed by acetyl Co-A transacetylase.

• The activation of the malonyl group —

- Transfer of the malonyl group to the -SH group of the ACP is catalysed by malonyl Co-A ACP-transferase.
- The charged acetyl & malonyl groups are now in close proximity to each other.

• Series of Reaction —

- After activation process, the reaction series are as follows :-

1) Condensation
2) Reduction
3) Dehydration
4) Reduction.

- These steps are repeated till a fatty acid with 16 carbon atom is synthesied.

1) Condensation —

- The acetyl group attacks the methylene group of the malonyl residue, catalyzed by S-keto acyl synthase & liberates CO_2 forming 3-ketoacyl enzyme (acetoacyl enzyme) freeing the cysteine -SH group.

- formation of acetoacyl ACP.
(due to condensation of acetyl & malonyl group)
- Reaction is catalysed by β-ketoacyl ACP synthase.

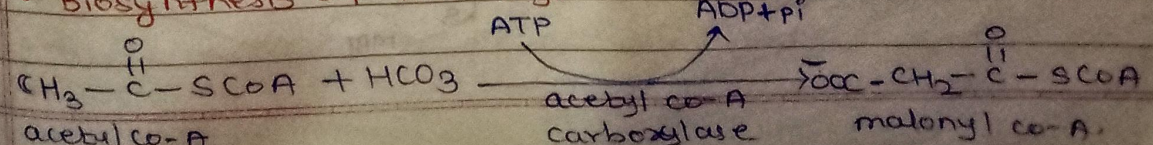
2) Reduction —
- Acetoacyl-ACP is reduced to β-hydroxy-butyryl ACP catalyzed by β-ketoacyl ACP reductase.
- $NADPH + H^+$ required for reduction.

3) Dehydration —
- It yeilds a double bond in the production of :- trans-Δ^2-butenoyl-ACP
- catalysed by enoyl-reductase.
- NADPH required.
- Reaction is catalysed by β-hydroxybutyryl ACP dehydrates.

4) Reduction —
- Reduction of double bond takes place to form butyryl-ACP.
- catalysed by enoyl reductase.
- NADPH required.

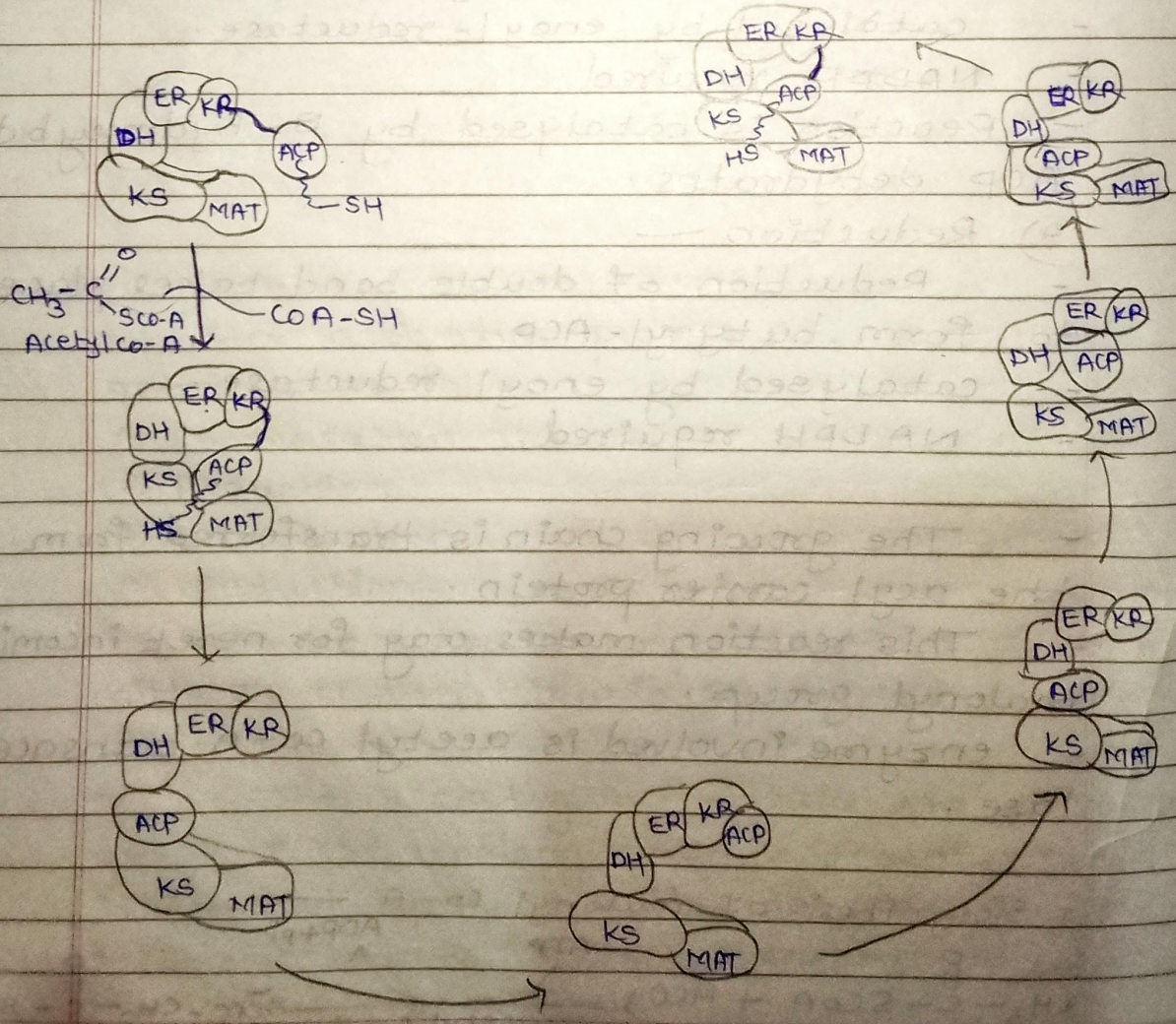
- The growing chain is transferred from the acyl carrier protein.
- This reaction makes way for next incoming malonyl group.
- enzyme involved is acetyl co-A transacetylase.

• Biosynthesis of malonyl-co-A —



- fatty acid synthesis —
- fatty acid synthase
- Acetyl-co-A serves as primer.
- addition of two carbon-units from malonyl Co-A.
- Each two-carbon unit added must be reduced by 2 NADPH + $2H^+$
- Reaction for the synthesis of palmitic acid (16 carbon acid)

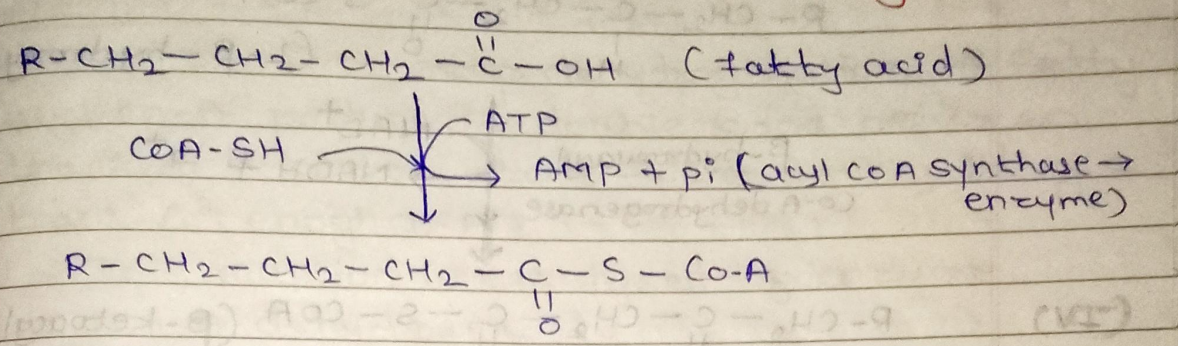
$$\text{Acetyl-CoA} + 7\,\text{Malonyl-CoA} + 14\,\text{NADPH} + 14H^+ \rightarrow \text{Palmitic acid} + 7CO_2 + 14\,\text{NADP}^+ + 8\,\text{CoA} + 6H_2O$$

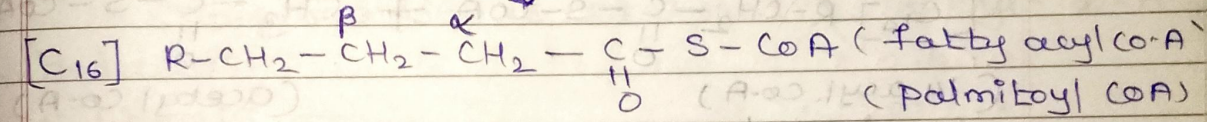


Saturday
6/5/23

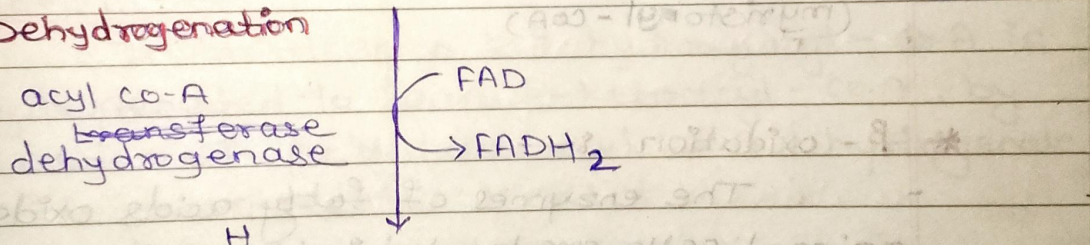
(~~fatty acid oxidation~~)

* β-oxidation (degradation of fatty acids) :-

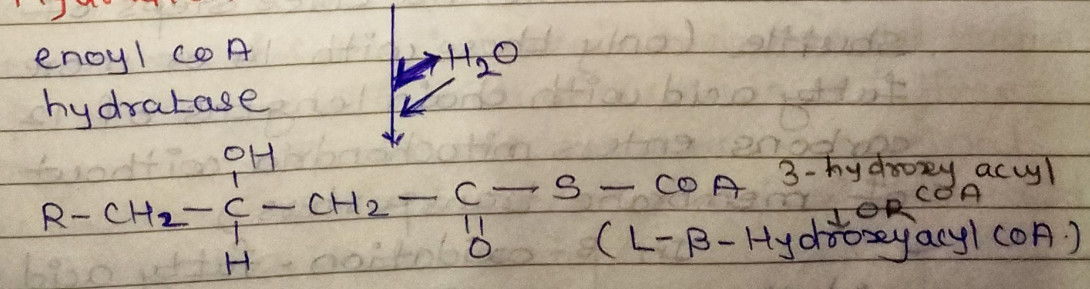




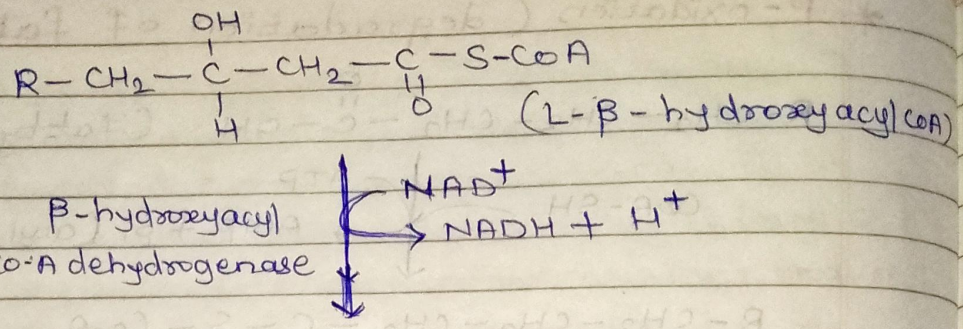
(I) Dehydrogenation



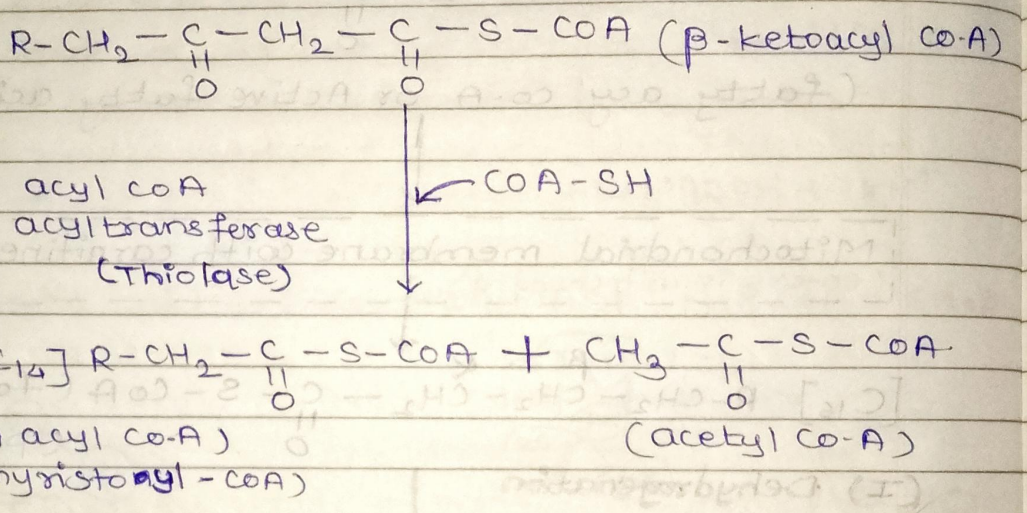
(II) Hydration



(III)



(IV)



* β-oxidation :—

- The enzymes of fatty acids oxidation in animal cells are located in the mitochondrial matrix hence fatty acid are activated in cytosol by conversion to fatty acyl Co-A enters in mitochondrial matrix via three steps of carnitine shuttle (only those with 14 or more carbon, fatty acid with chain lengths of 12 or fewer carbons enters mitochondria without the help of membrane transporters)
- In β-oxidation - fatty acid undergoes oxidative removal of acetyl co-A, starting from the carboxyl end of the fatty acyl chain.
- The β-oxidation of saturated fatty acid four basic steps :—

• **Step I** –

In first step dehydrogenation of fatty acyl CO-A produces a double bond between the α & β carbon atoms (C_2 & C_3) yeilding a trans-Δ^2 enoyl CO-A.

This reaction is catalysed by enzyme acyl CO-A dehydrogenase.

• **Step II** —

In the second step of β-oxidation cycle, water is added to the double bond of trans-Δ^2-enoyl-CO-A to form stereoisomer of β-hydroxyacyl CO-A (3-hydroxy acyl CO-A).

This reaction is catalysed by enoyl-CO-A hydratase.

• **Step III** —

In third step L-β-hydroxyacyl-CO-A is dehydrogenated to form β-ketoacyl-CO-A, by the action of β-hydroxyacyl CO-A dehydrogenase. NAD^+ is converted to $NADH + H^+$

NAD^+ is electron acceptor.

• **Step IV** —

The fourth & last step of the β-oxidation cycle is catalysed by acyl-CO-A acetyltransferase, more commonly called thiolase, which promotes reaction of β-ketoacyl-CO-A with a molecule of free coenzyme to split of the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl CO-A.

The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms.

- It undergo the similar set of reaction & in this way fatty acid containing even no. of carbon atom ultimately degraded by 2 carbon atom at a time to yeild acetyl Co-A
- So the seven cycles of β-oxidation requires to oxidize one molecule of palmitoyl co-A to eight molecules of acetyl co-A.

$$\text{palmitoyl-Co-A} + 7\,\text{Co-A} + 7\,\text{FAD} + 7\,\text{NAD}^+ + 7\,H_2O \longrightarrow 8\,\text{acetyl-Co-A} + 7\,FADH_2 + 7\,\text{NADH} + 7\,H^+$$

- Explain the role of carnitine in β-oxidation, add a note on β-oxidation and its energetics —

⇒ • Role of carnitine in β-oxidation :—

- The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix. The fatty acids with chain length of 12 or fewer carbons enter mitochondria without the help of membrane transporters. Those with 14 or more carbons, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes - They must first undergo the three enzymatic reactions of the carnitine shuttle.
- The first reaction in carnitine shuttle is catalysed by a family of isozymes present in the outer mitochondrial membrane, the acyl Co-A synthatase, which promote the general reaction.

∴ fatty acid + CoA + ATP ⇌ fatty acyl Co-A + AMP + pi

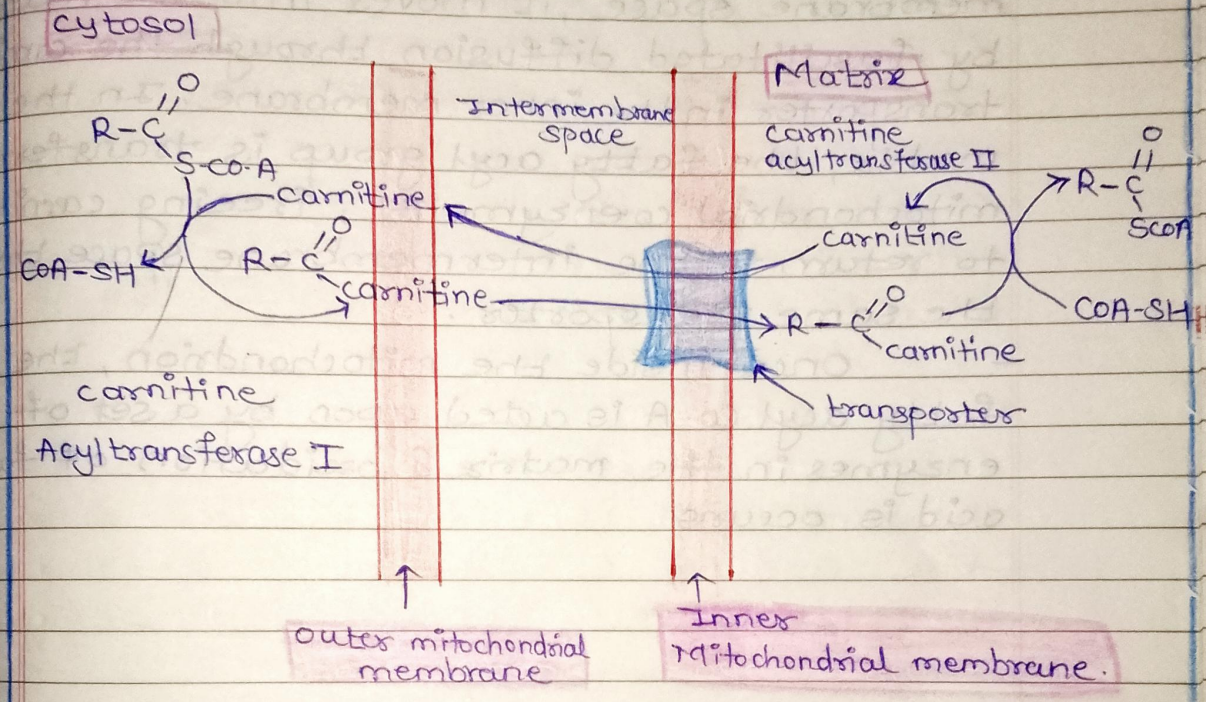


fig. fatty acid entry into mitochondria via the acyl carnitine / carnitine transporter.

Thus, acyl co-A synthatase catalyse the formation of a thioester linkage between the fatty acid carboxyl group & the thiol group of coenzyme A to yeild a fatty acyl-Co-A, coupled to the cleavage of ATP to AMP and pi.

Fatty acyl co-A esters formed for mitochondrial oxidation are transiently ~~added~~ attached to the hydroxyl group of carnitine to form fatty acyl-carnitine.

The second reaction of the shuttle. This transesterification is catalysed by carnitine ~~acyltransfe~~ acyltransferase I in the outer membrane.

- After fatty acyl-carnitine is formed at the outer membrane or in the inter-membrane space, it moves into the matrix by facilitated diffusion through the carnitine transporter in the inner membrane. In the matrix, the fatty acyl group is transferred to mitochondrial coenzyme A, freeing carnitine to return to the intermembrane space through the same transporter.
- Once inside the mitochondrion, the fatty acyl Co-A is acted upon by a set of enzymes in the matrix & oxidation of fatty acid is occurs.