P.G. Sem II CC 7 Unit 3 Submitted 3.6

β-oxidation of Fatty Acids Part I

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Introduction → In normal Condition, Bio-energy or ATP, required for normal physiological situation, comes from glucose oxidation and only during starvation or glucose deficiency, ATP is generated from Fatty acid oxidation. However, in normal physiological condition also, 40% of ATP generated comes from Fatty acid oxidation and during starvation, almost 100% of ATP comes from Fatty Acid oxidation. Fatty acid oxidation is also the chief source of energy for hibernating animals.

Fatty acids are oxidised to acetyl CoA and are also synthesized from acetyl Co-A, but the two pathways are entirely different. Enzymes of Fatty acid oxidation are present in mitochondria, whereas enzymes of fatty acid synthesis are present in smooth Endoplasmic Reticulum (SER) of cytosol. Secondly, when one pathway is active other pathway is suppressed. Most of the fatty acids are synthesized through β-oxidation.

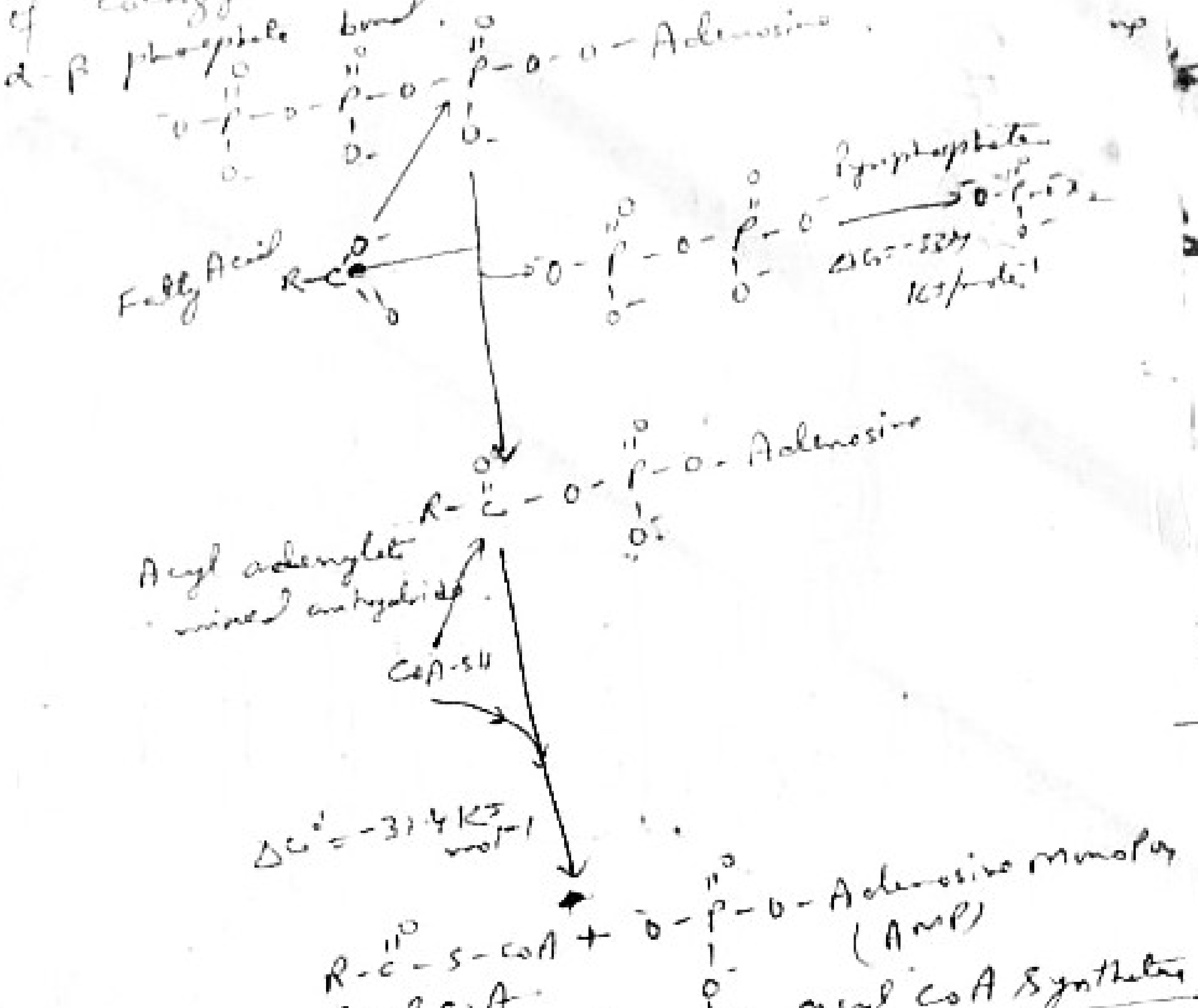
The most convincing experimental proof of β oxidation came from the works of Franz Knoop (1904), who for the first time, used metabolic technique.

Pathway of β-oxidation of Fatty Acids:

Lehninger, proved that fatty acids are oxidised in mitochondria but all fatty acids, particularly long chain fatty acids cannot pass through the inner mitochondrial membrane. Hence, for transfer of fatty acids, a special transport protein is present in the inner mitochondrial membrane. However, before this transfer, fatty acids are activated to form acyl CoA.

1. **Synthesis of Acyl CoA** — The process of β-oxidation of fatty acids begins with the activation of fatty acid; which is an energy consuming step in the presence of coenzyme A. All fatty acids are activated in SER but only small chain and medium chain fatty acids are activated in mitosol. The activation reaction is catalyzed by the enzyme acyl CoA synthetase found in SER and outer mitochondrial membrane. This enzyme uses coenzyme A and induces synthesis of thioester bond between carbonyl carbon

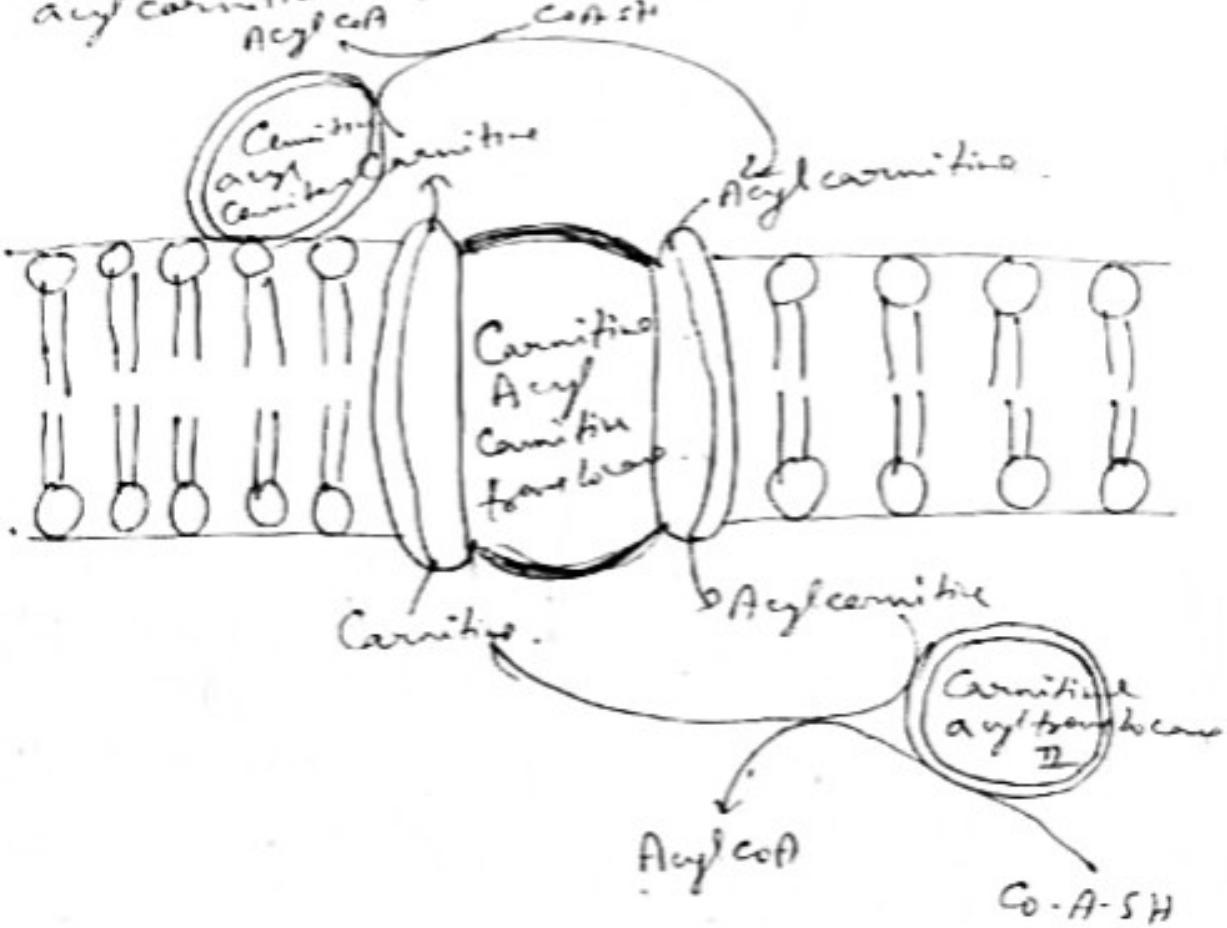
of fatty acid and suffers of the thiol group of coenzyme A. ATP provides energy from its α-β phosphate bond.



Fatty Acid Activation by acyl CoA Synthetase

2. **Transfer of acyl CoA across the inner mitochondrial membrane** — The acetyl moiety of the acetyl CoA is then transferred and ligated to a specific molecule, carnitine (β-hydroxy-γ-trimethyl amino butyrate) by the enzyme Carnitine acyltransferase I present on the outer surface of the inner mitochondrial membrane. Acyl Carnitine thus formed is transferred across the inner membrane by a carnitine carrier protein called Carnitine Acylcarnitine translocase. It also

transfers free carnitine from mitosol to cytosol. Acylcarnitine, once inside, is acted upon by the enzyme carnitine acyltransferase II by means of inner surface of inner membrane of mitochondria. This in the presence of coenzyme A brings about regeneration of acyl CoA and release of carnitine. Free carnitine is transported back to cytosol by carnitine acylcarnitine translocase.



Formation of acylcarnitine from acyl CoA and its transfer across inner membrane of mitochondria and regeneration of acyl CoA

STEPS OF B-OXIDATION IN MITOSOL

Acyl CoA transferred to mitosol or synthesized in mitosol is subjected to the action of four enzymes, collectively

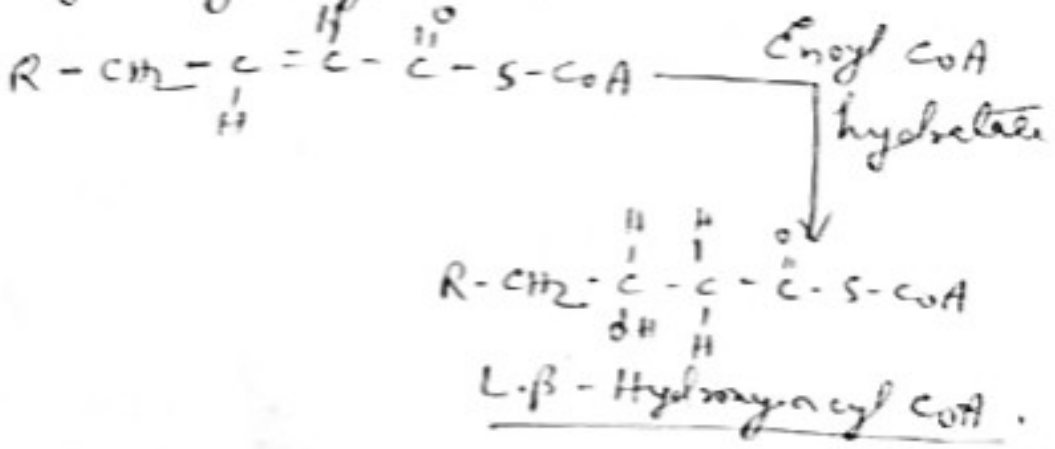
called "fatty Acid Oxidase". These four enzymes act in specific sequence and finally bring about removal of two carbon as acetyl CoA from acyl CoA. Repeated action of these enzymes bring about complete degradation of fatty acid to acetyl CoA. Obviously, palmitic acid is 16-carbon fatty acid and remains of palmitoyl CoA after complete oxidative degradation, will generate eight acetyl Co-A.

The four enzyme of fatty acid oxidation act in sequence and induce four different reactions as follows —

1. Dehydrogenation of acyl Co-A - Dehydrogenation is the first reaction catalyzed by acyl Co-A dehydrogenase. This enzyme is of different types with molecular weight ranging from 170-180 KD, specific for different lengths of acyl CoA. However, they have been categorised into three types — specific for short, medium and long chain fatty acids. These enzymes bind tightly but noncovalently to Prosthetic group FAD. Thus, this is completely a dehydrogenation reaction.

Oxidation of α and β carbons of acyl CoA by acyl CoA dehydrogenase

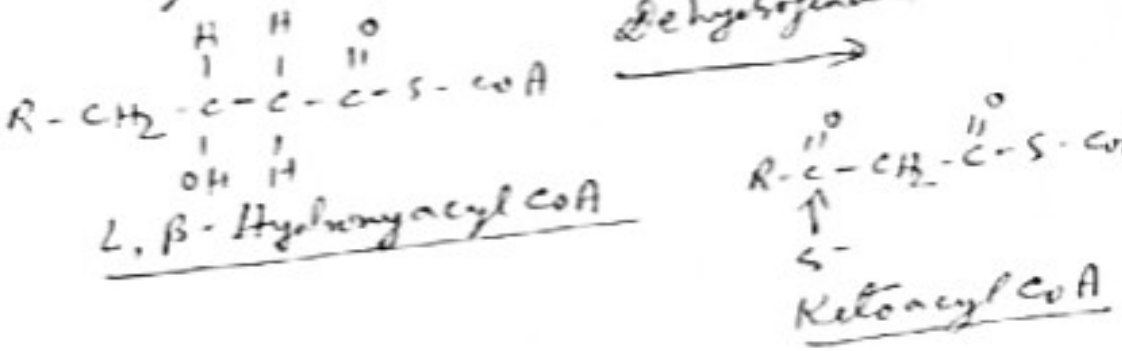
2. Hydration of Enoyl CoA → The Δ^2 trans enoyl CoA formed above undergoes trans hydration of α and β-carbons by the enzyme Enoyl CoA hydratase also called crotonase. Hydration of α and β-carbons is stereospecific and converts Δ^2 trans form only to L, β-hydroxyacetyl CoA.



L-β-Hydroxyacyl CoA.

3. Oxidation of (β-carbon of) L,β-hydroxyacyl CoA

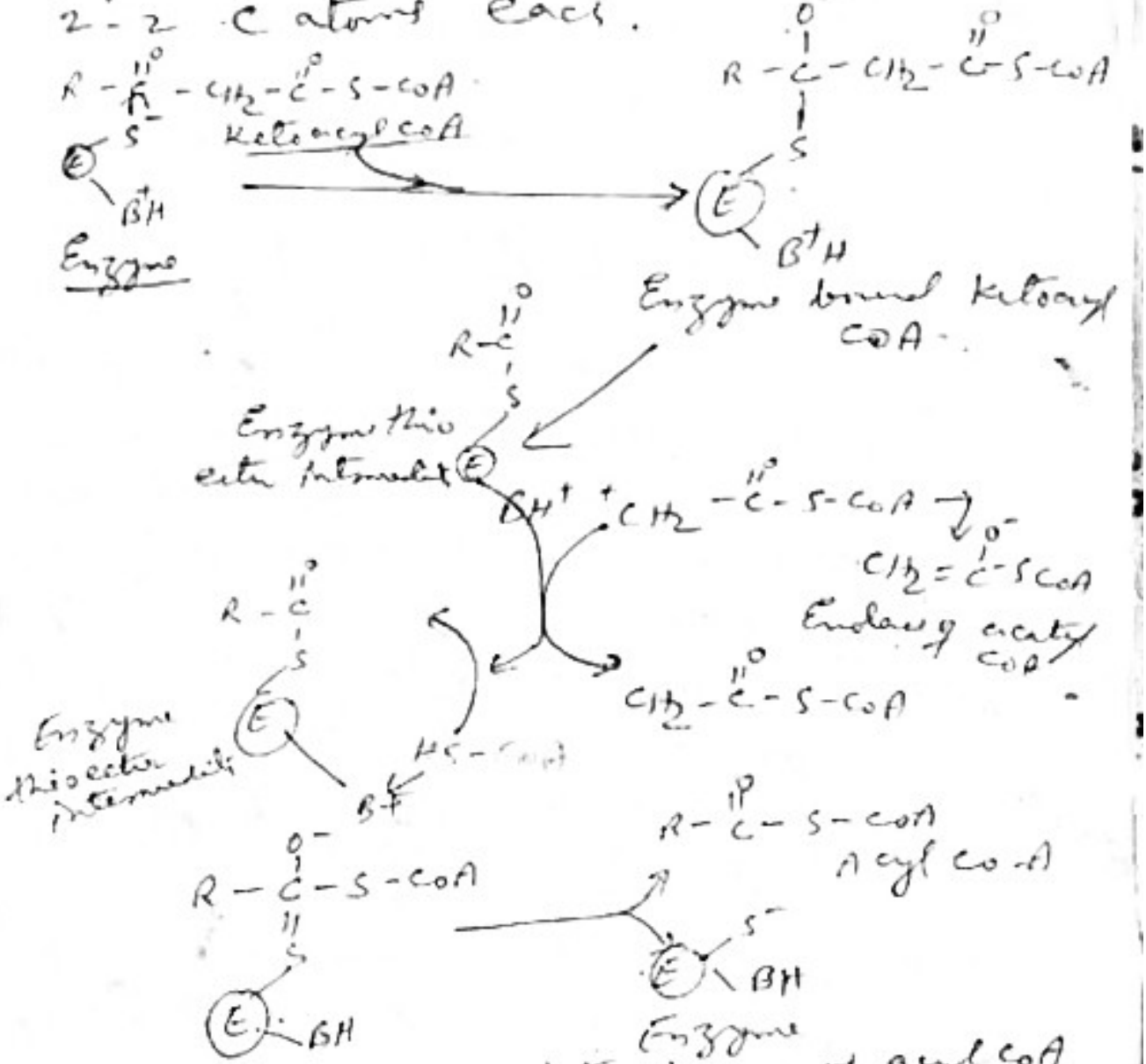
The enzyme L,β-hydroxyacyl CoA dehydrogenase acts specifically on the L,β-hydroxyacyl CoA and brings about dehydrogenation of the hydroxyl group of β-carbon. The enzyme requires (NAD^+) to receive the reducing equivalents. Reduced NAD, thus formed, transfers its electrons to the respiratory chain for ATP synthesis. Oxidation of L,β-hydroxyacyl CoA leads to the formation of β-Ketoacyl CoA.



4. Thiolytic cleavage of β-Ketoacyl CoA → This is the last step of β-oxidation of fatty acids. The enzyme acyl CoA acetyl transferase or thiolase (also called β-ketothiolase) splits the C-C bond of β-Ketoacyl CoA by the thiolytic cleavage, thereby, releasing acetyl CoA and another acyl CoA which is shorter in length by two carbons than that of the initial acyl CoA. The reaction begins with the attack of β-carbonyl carbon of the substrate by the thiolytic group of the enzyme, thus forming

thiocster bond between the enzyme and the substrate. This enzyme-substrate complex dissociate into enolate form of acetyl CoA and an enzyme thiocster intermediate. The base of the enzyme loses its proton to the enolate of acetyl CoA forming acyl CoA and thiol group of a free coenzyme.

Fatty acid is thus degraded to a fatty acid shorter by two carbons each time repeating it finally to two molecules of 2-2 C atoms each.



Mechanism of thiolytic cleavage of acyl CoA by thiolase