GLYOXYSOMES AND GLYOXYLIC ACID CYCLE

The discovery of Glyoxysomes: the work of Harry Beevers (1961)

Beevers and Breidanbach called these new organelles glyoxysomes.

- enzymes were found in an organelle fraction that was not mitochondria.

Introduction Of Glyoxysomes.

- Glyoxysomes Are Specialized Peroxisomes Found In Plants(particularly In Fat Storage Tissue Of Germinating Seed) And Also In Filamentous Fungi.
- Glyoxysomes PossessThe Key Enzyme Of Glyoxylate Cycle.
- Glyoxysomes Are Found In Contact With Lipid Bodies In Cotyledons Or Endosperm Where Fatty Acids Are Being Converted To Sugars During Germination.
- Thus Glyoxysomes Contain Enzymes That Initiate The Breakdown Of Fatty Acids And Additionally Posses The Enzyme To Produce Intermediate Products For Synthesis Of Sugars By Gluconeogenesis.
- The Seedling Uses These Sugars Synthesized From Fats Until It Is Mature Enough To Produce Them By Photosynthesis.
- Glyoxysomes Also Function In Photorespiration And Nitrogen Metabolism In Root Nodules.

Characteristics Of Glyoxysomes

- They have single membrane.
- Their matrix is finely granular.
- They have high equilibrium density in sucrose gradient centrifugation.

FUNCTIONS

- Functionally they contain enzyme (isocitrate lyase and malate synthase) of fatty acid metabolism involved in the conversion of lipids to carbohydrates during germination.
- This cycle allows seeds to use lipid as a source of energy to form root and shoot during germination.
- The lipid source of germinating seeds are used for the formation of the carbohydrates that fuel the growth and development of organisms
- It also functions in photorespiration and nitrogen fixation.
- It involves in Lipid Digestion.

There are three main and important functions of glyoxysomes that are:

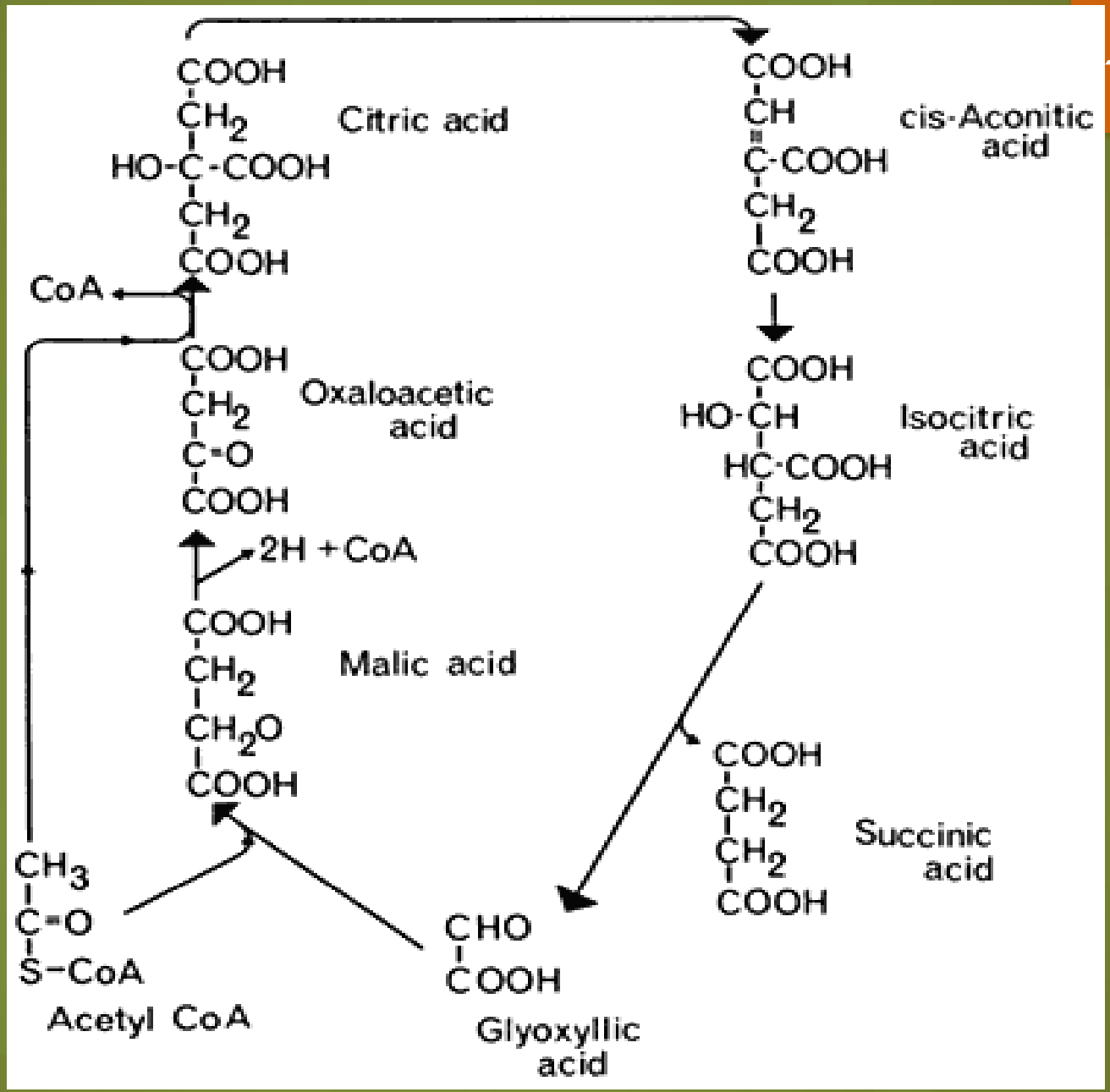
- **Beta-oxidation**
- **Glyoxylate cycle**
- **gluconeogenesis**

THE GLYOXYLATE CYCLE.

GLYOXYLATE CYCLE.

- The glyoxylate cycle was discovered in **1956** by **Kornberg** and **Krebs.**
- There was a problem that how acetyl co-A is converted into glucose. This problem was solved by the discovery of glyoxylate cycle. The acetyl co-A that is produced during the break down of fatty acid could be consumed by the TCA cycle but this would not result in the net formation of glucose precursor**.** Each molecule of acetyl co-A produce two molecules of carbon dioxide.
- The glyoxylate cycle was first revealed in the bacterium *Pseudomonas* that grows on two carbon compounds. After that it is shown in many micro-organisms and plants.
- The cycle is based on the presence of two key enzymes that are malate synthase and isocitrate lyase. Also it is based on the ^{14}C in organic acids, sugars and carbon dioxide.
- The glyoxylate cycle represents a modification of tricarboxylic acid cycle. During glyoxylate cycle two molecules of acetyl co-A are consumed and single molecule of succinic acid (a glucose precursor) is produced

- The glyoxylate cycle is an anaerobic pathway occurring in plant, fungi and protists.
- The glyoxylate cycles centers on the conversion of Acetyl-CoA to succinate for synthesis of carbohydrate.
- Glyoxylate cycle allows cell to neutralize simple carbon compounds as a carbon source when complex source such as glucose is not available.


COOH
CH2
HO-C-COOH
CH2
COOH
Citric acid
CoA
COOH
CH2
C=O
COOH
Oxaloacetic acid
2H + CoA
COOH
CH2
CH2O
COOH
Malic acid
CH3
C=O
S-CoA
Acetyl CoA
CHO
COOH
Glyoxyllic acid
COOH
CH
C-COOH
CH2
COOH
cis-Aconitic acid
COOH
HO-CH
HC-COOH
CH2
COOH
Isocitric acid
COOH
CH2
CH2
COOH
Succinic acid

ENZYMES IN GLYOXYLATE CYCLE:

There are five enzymes that are involved in this process:

Three of which are components of TCA cycle that are

1. Citrate synthetase
2. Aconitase
3. Malic dehydrogenase

The remaining two enzymes are the key enzymes of glyoxylate cycle that are

1. Isocitric lyase(isocitratase)
2. Malate synthetase

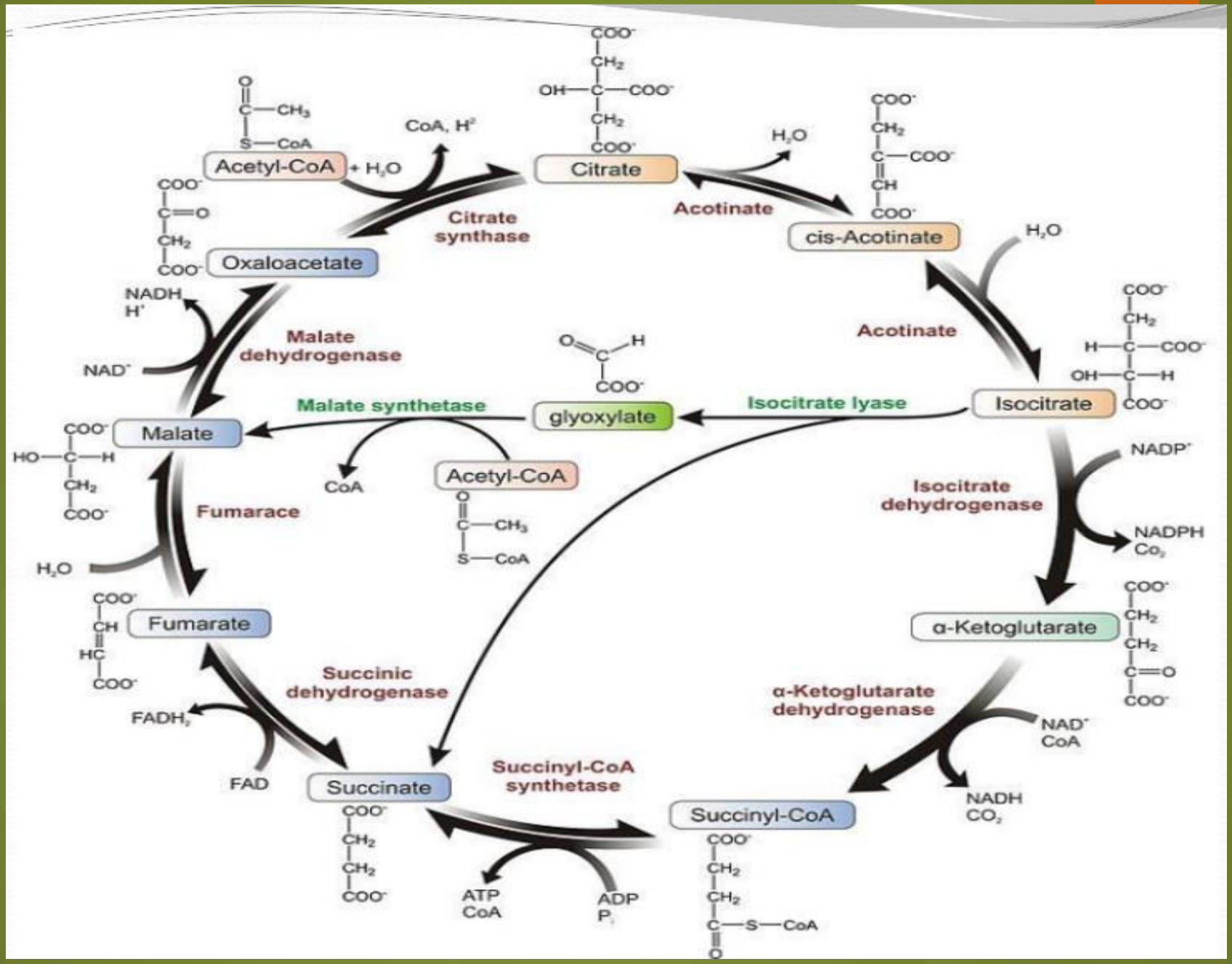
STEPS OF GLYOXYLATE CYCLE:

1. The first step is the condensation of oxaloacetate and acetyl co-A to produce citrate. This step is catalyzed by the enzyme **citrate synthetase.**
2. The second step is the conversion of citrate into isocitrate. The catalyzing enzyme is **Aconitase** in this step.
3. The third step of this cycle is the breakdown of isocitrate into succinate and glyoxylate. This step is catalyzed by the enzyme **isocitrate lyase**.
4. The fourth step is the production of malate. Glyoxylate which is the product of third step condensed with the second molecule of acetyl co-A to form malate. The enzyme for catalyzing this step is **malate synthetase**.
5. The fifth step in glyoxylate cycle is the oxidation of malate. This oxidation is carried out by the enzyme **malate dehydrogenase**. The malate is oxidized to oxaloacetate with the reduction of NAD+

The equation for all the cycle is:

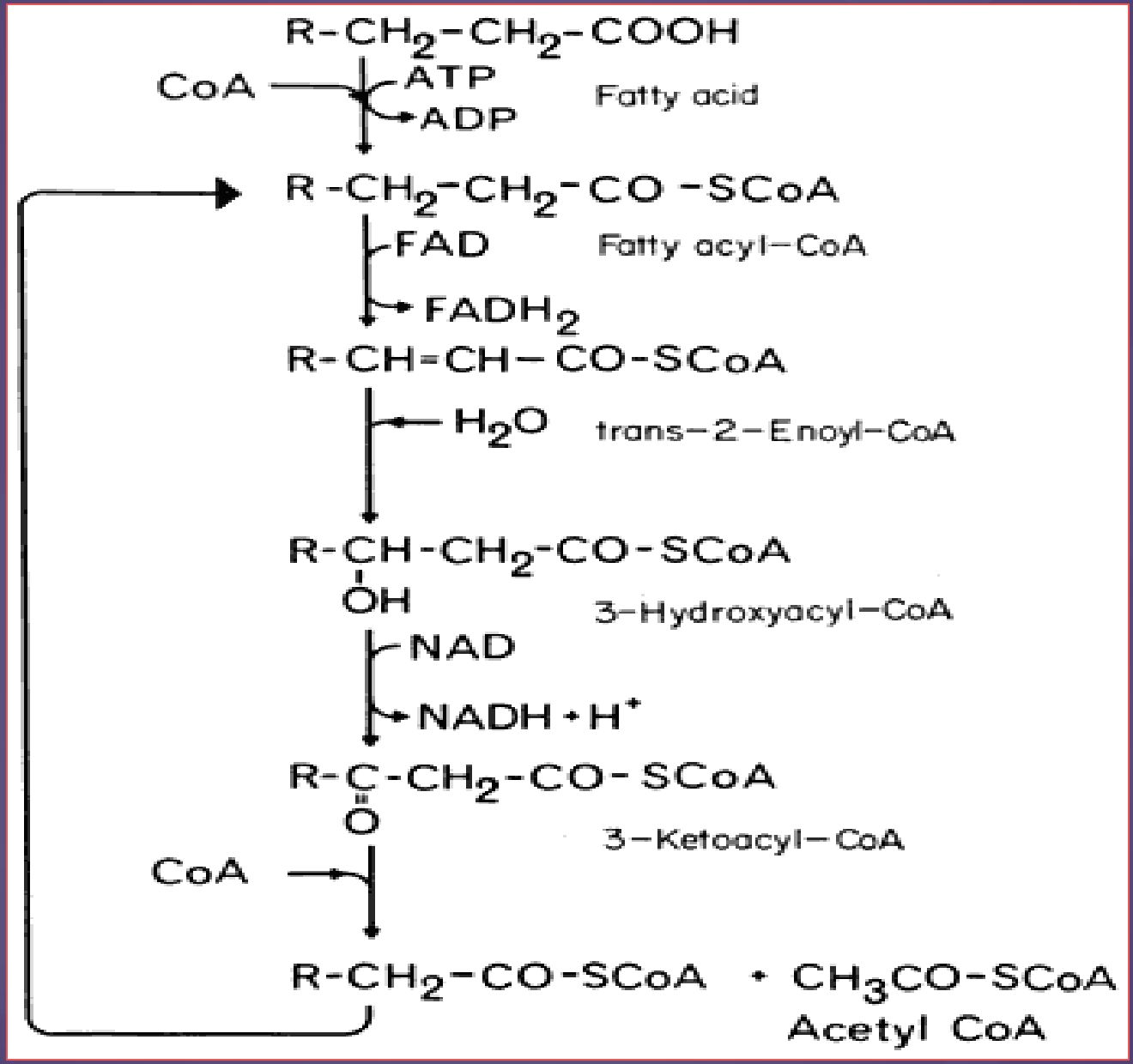
6. The oxaloacetate is than converted into phosphoenolpyruvate. The catalyzing enzyme is phosphoenolpyruvate carboxykinase

7. The phosphoenolpyruvate is than converted into glucose. In this way four molecules of acetyl co-A produce two molecules of succinate that converts to glucose along with the loss of two carbon dioxide molecules.


Acetyl-CoA
+ H2O
CoA, H+
Citrate
H2O
Acotinate
cis-Acotinate
Citrate synthase
Oxaloacetate
H2O
Acotinate
NADH
H+
NAD+
Malate dehydrogenase
Malate synthetase
glyoxylate
Isocitrate lyase
Isocitrate
Malate
CoA
Acetyl-CoA
NADP+
Isocitrate dehydrogenase
NADPH
CO2
Fumarace
H2O
Fumarate
α-Ketoglutarate
Succinic dehydrogenase
α-Ketoglutarate dehydrogenase
FADH2
NAD+
CoA
FAD
Succinate
Succinyl-CoA synthetase
Succinyl-CoA
NADH
CO2
ATP
CoA
ADP
Pi

β-OXIDATION:

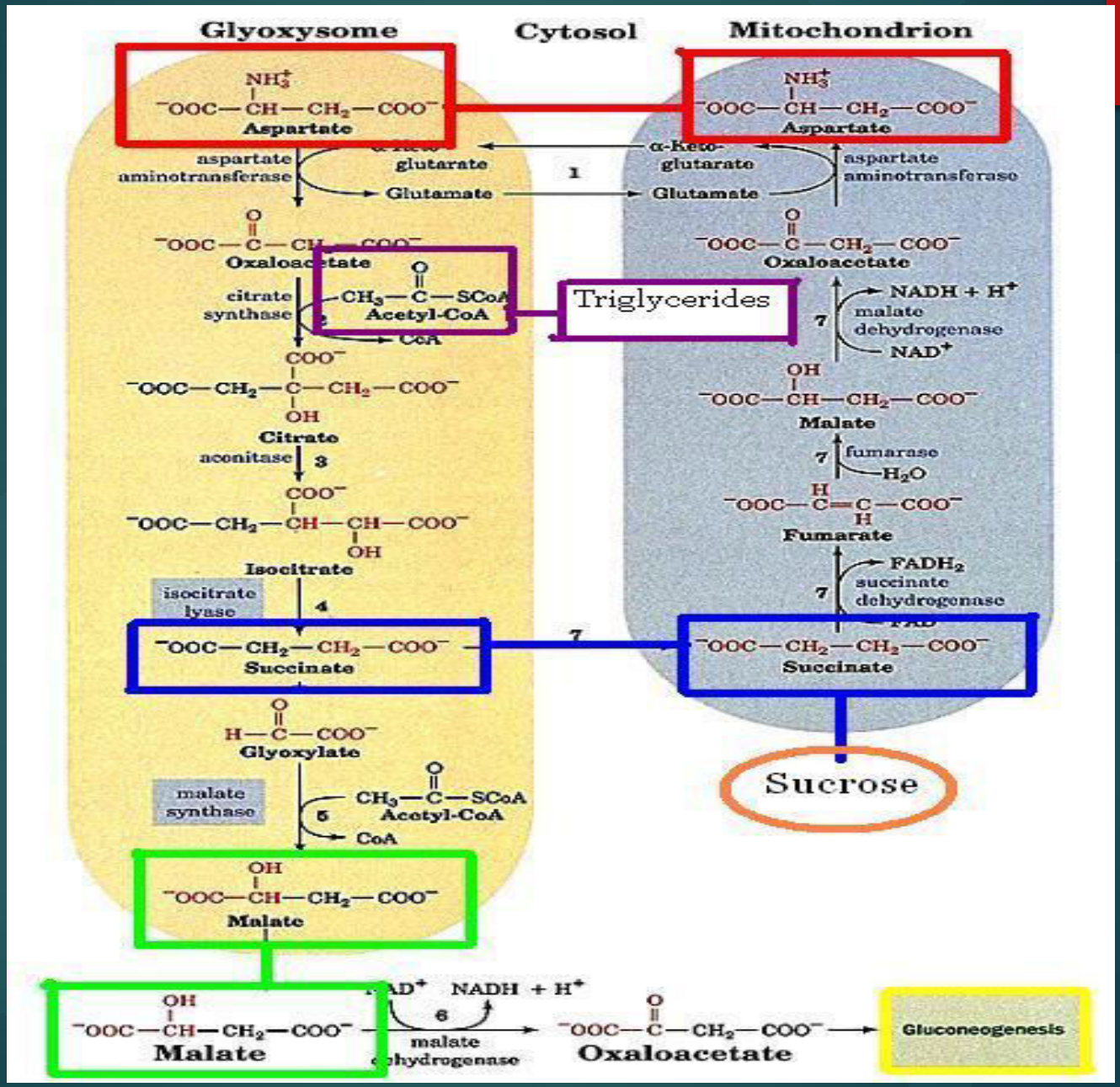
- The β-oxidation reaction involves the removal of 2-carbon fragments from long fatty acid chain and this process includes five steps.
- The β-oxidation pathway was demonstrated by **Stumpf** and **Barber**. They show that long chain aliphatic acids were oxidized to carbon dioxide.
- Thiokinase is the enzyme that in the presence of ATP activates the coenzyme-A that activates the substrate. Due to this activation the reaction sequence is initiated. Then the removal of carbon atoms continues with the formation of double bonds. The fatty acids which have even number of carbons, they produce only acetyl-coA. While the fatty acids which have odd number of carbons, they produce acetyl-coA as well as propiyonyl-coA.


R-CH2-CH2-COOH
CoA
ATP
ADP
Fatty acid
R-CH2-CH2-CO-SCoA
FAD
Fatty acyl-CoA
FADH2
R-CH=CH-CO-SCoA
H2O
trans-2-Enoyl-CoA
R-CH-CH2-CO-SCoA
OH
3-Hydroxyacyl-CoA
NAD
NADH + H+
R-C-CH2-CO-SCoA
O
3-Ketoacyl-CoA
CoA
R-CH2-CO-SCoA + CH3CO-SCoA
Acetyl CoA

GLUCONEOGENESIS

- The principal pathways of gluconeogenesis are therefore catalogued in a single organelle, the glyoxysome. The complete pathway of gluconeogenesis however involves at least three organelles, spherosome, glyoxysome and mitochondrion.
- The reactions are initiated by the hydrolysis of lipids in the spherosome, the catalyzing enzyme is lipase. The glycerol and fatty acids produced diffuse out of the organelle.
- Glycerol is utilized directly by the EMP pathway in the cytosol and takes part in the sucrose synthesis, while the fatty acids diffuse into glyoxysomes which are located near the spherosome.

- In the glyoxysomes the fatty acids are oxidized in the *b* -oxidation pathway and the acetyl-Co which was formed is converted to succinate by the action of the glyoxylate cycle enzymes located in the organelle. While the *b* -oxidation pathway is reversible, the equilibrium is apparently maintained in a catabolic direction by the removal of peroxide, produced in the oxidation step of the pathway, by the catalase present in the glyoxizome. Reduced NAD^+ formed in these reactions is probably oxidized in the mitochondria.
- Succinate formed in the glyoxisome is further broken-down to oxaloacetate and finally to phosphoenolpyruvate in the mitochondria subsequently succinic dehydrogenase and fumarate are not component enzymes of the glyoxylate cycle and are absent from glyoxysomes.


Glyoxysome
Cytosol
Mitochondrion
Aspartate
aspartate aminotransferase
α-Keto-glutarate
Glutamate
1
Oxaloacetate
citrate synthase
Acetyl-CoA
CoA
Triglycerides
NADH + H+
malate dehydrogenase
NAD+
7
Citrate
aconitase
3
Malate
fumarase
H2O
Fumarate
FADH2
succinate dehydrogenase
FAD
Isocitrate
isocitrate lyase
4
Succinate
Glyoxylate
malate synthase
5
Sucrose
6
Gluconeogenesis

Difference between Peroxisomes and Glyoxysomes

	PEROXISOMES	GLYOXYSOMES
DEFINITION	A small organelle that is present in the cytoplasm of many cells and that contains the reducing enzyme catalase and usually some oxidases.	Glyoxysomes are specialized peroxisomes found in plants (particularly in the fat storage tissues of germinating seeds) and also in filamentous fungi.
ORIGIN	They can be derived from the endoplasmic reticulum and replicate by fission	by the fission or budding of preexisting glyoxysomes
STRUCTURE	This organelle is surrounded by a lipid bilayer membrane which encloses the crystalloid core.	They are similar in appearance to peroxisomes but differ markedly in their enzyme composition

	PEROXISOMES	GLYOXYSOMES
FUNCTION	• Break down of very long chain fatty acids through beta-oxidation. • Metabolism of hydrogen peroxide	• In glyoxysomes the fatty acids are hydrolyzed to acetyl-CoA by peroxisomal β-oxidation enzymes. • Glyoxysomes possess additionally the key enzymes of glyoxylate cycle.
FOUND IN	found in liver & kidney cell which metabolize alcohol	They are found in plants, protists and fungi.