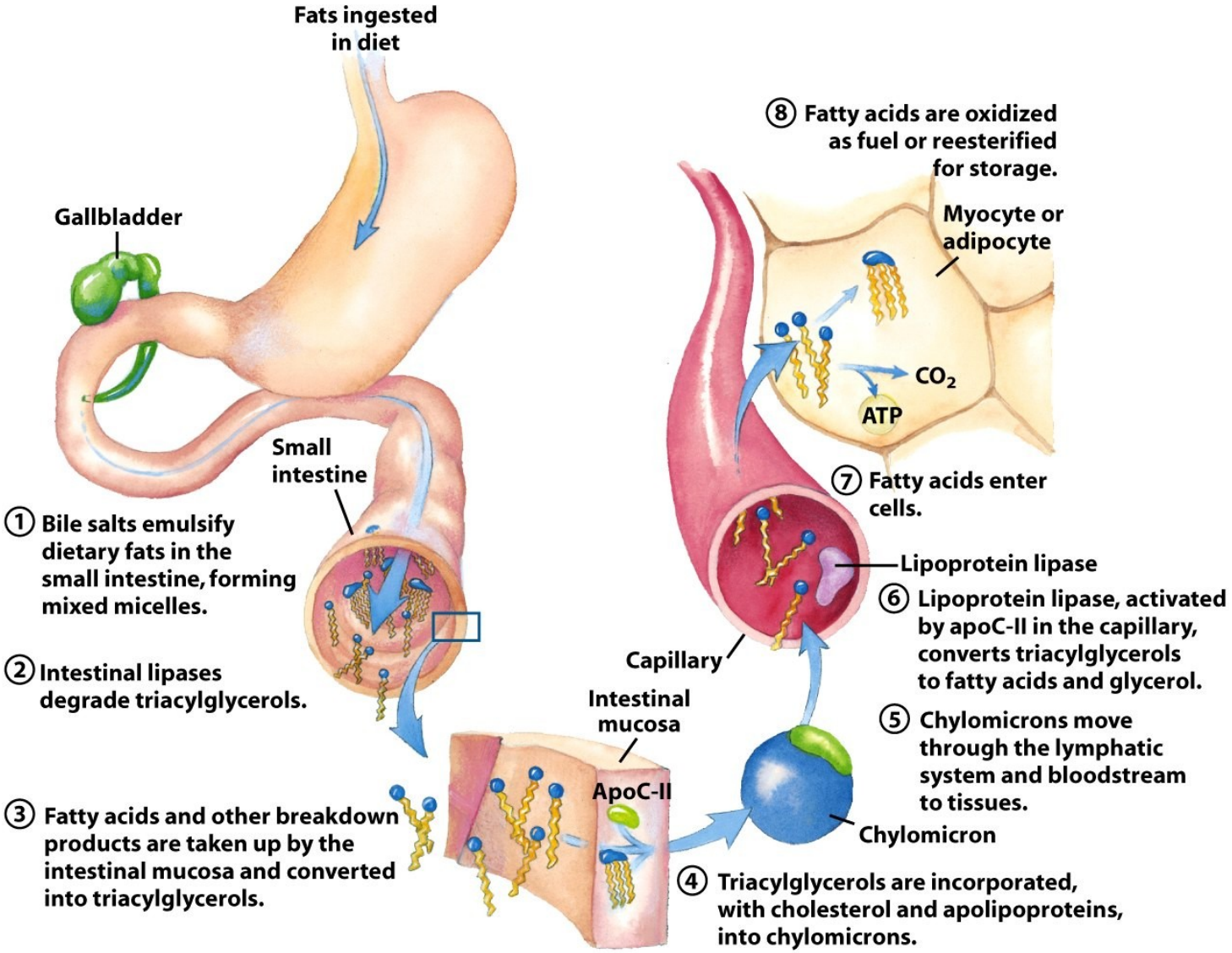
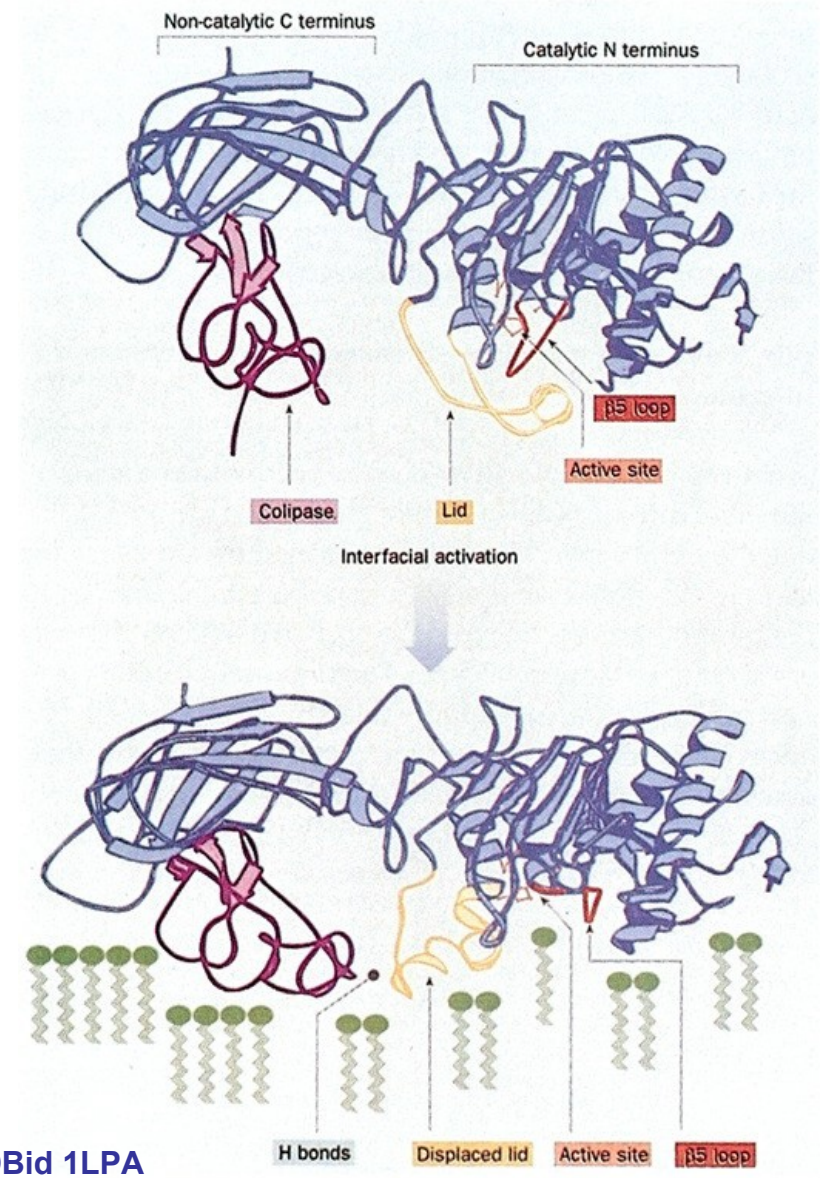
III. Metabolism

Fatty Acid Catabolism

Processing of Dietary Lipids in Vertebrates



Structure and Mechanism of Pancreatic Lipase



PDBid 1LPA

The enzymatic activity of pancreatic lipase is greatly increased by pancreatic colipase (1:1 complex)

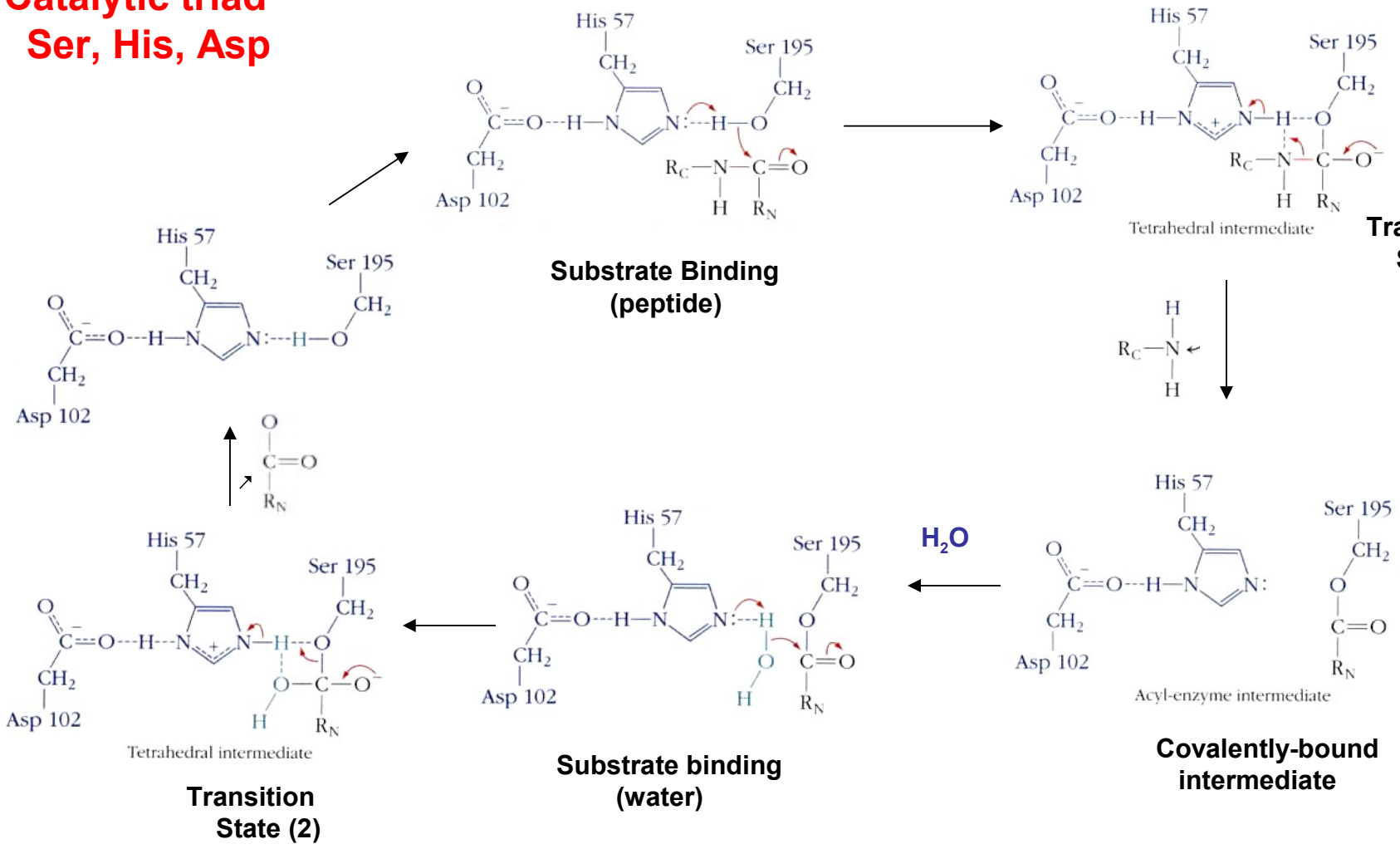
The catlytic center is covered by a 25 residue helical 'lid'.

Binding to micelles causes structural changes:
(1) 'lid' uncovers active site
(2) β5 loop forms oxyanion hole

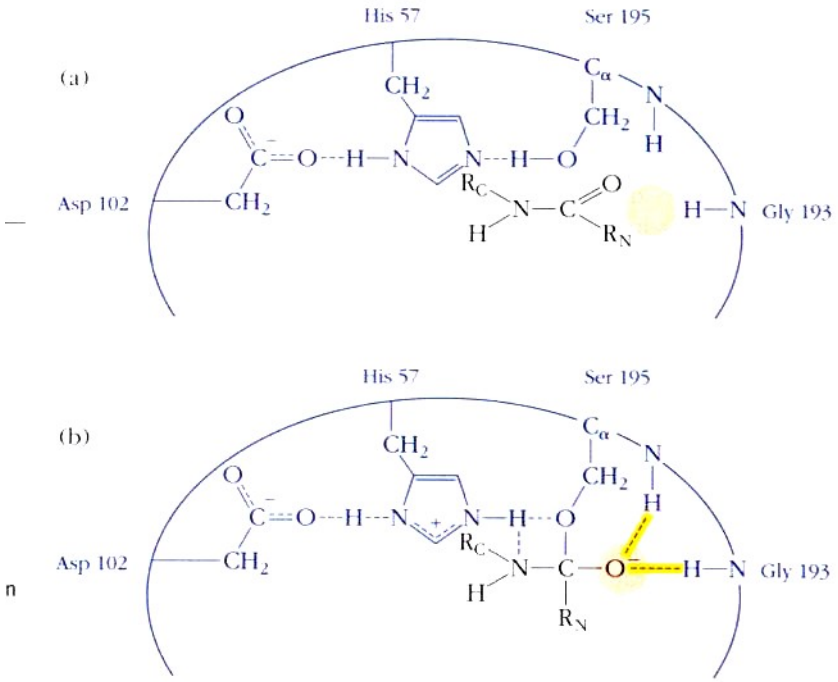
The active site contains a catalytic triad that closely resembles that in serine proteases.

Serine Proteases - Catalytic Mechanism

Catalytic triad
Ser, His, Asp



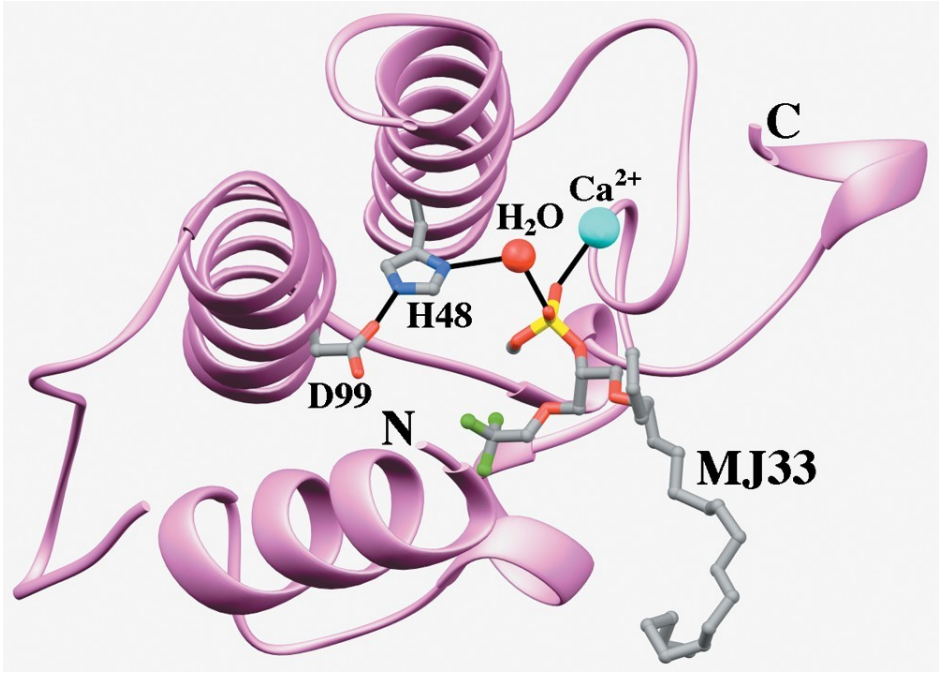
Serine Proteases - Catalytic Mechanism

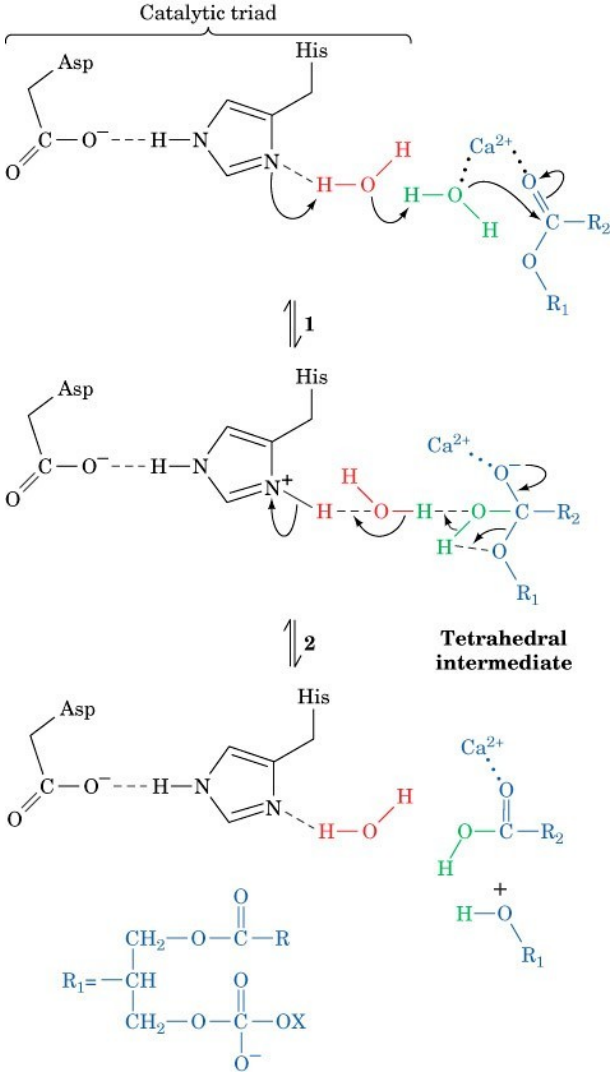


Transition state has negative charge that is stabilized by a pair of mainchain amines (the oxyanion hole)

Transition state stabilization within the oxyanion hole

Structure and Mechanism of Phospholipase A

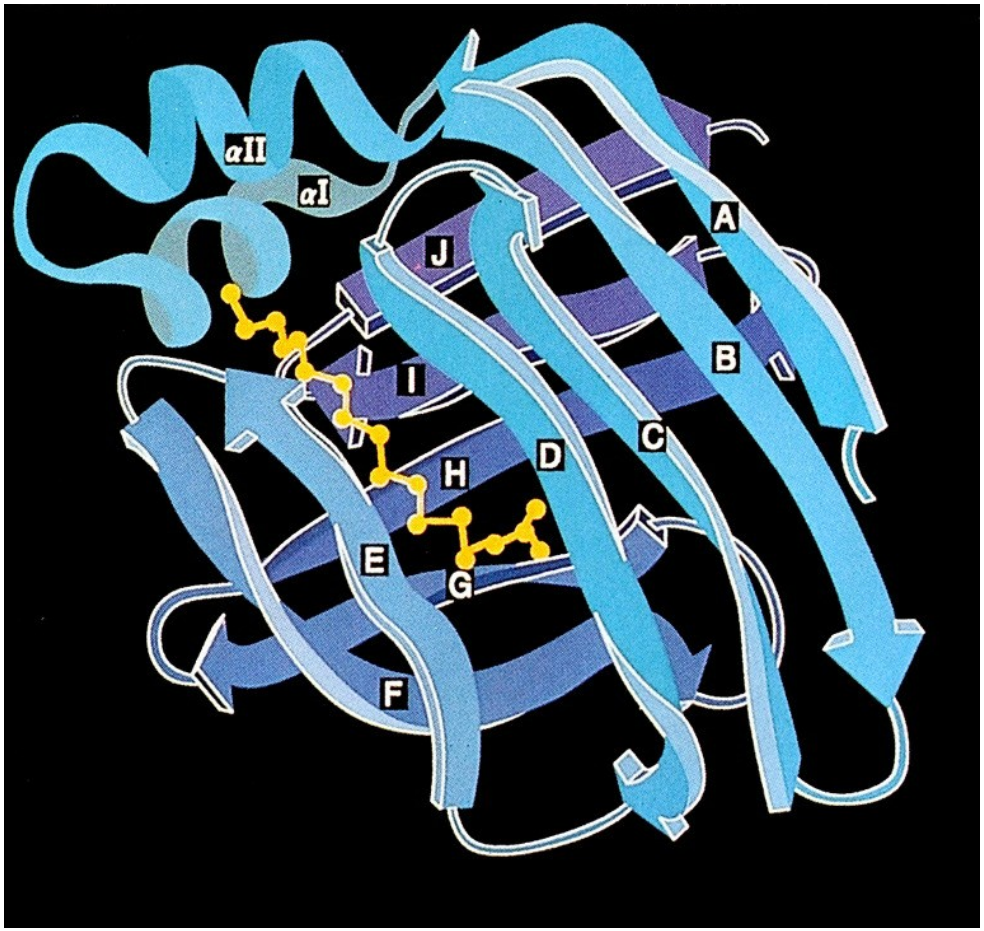




Intestinal Fatty Acid-binding Protein

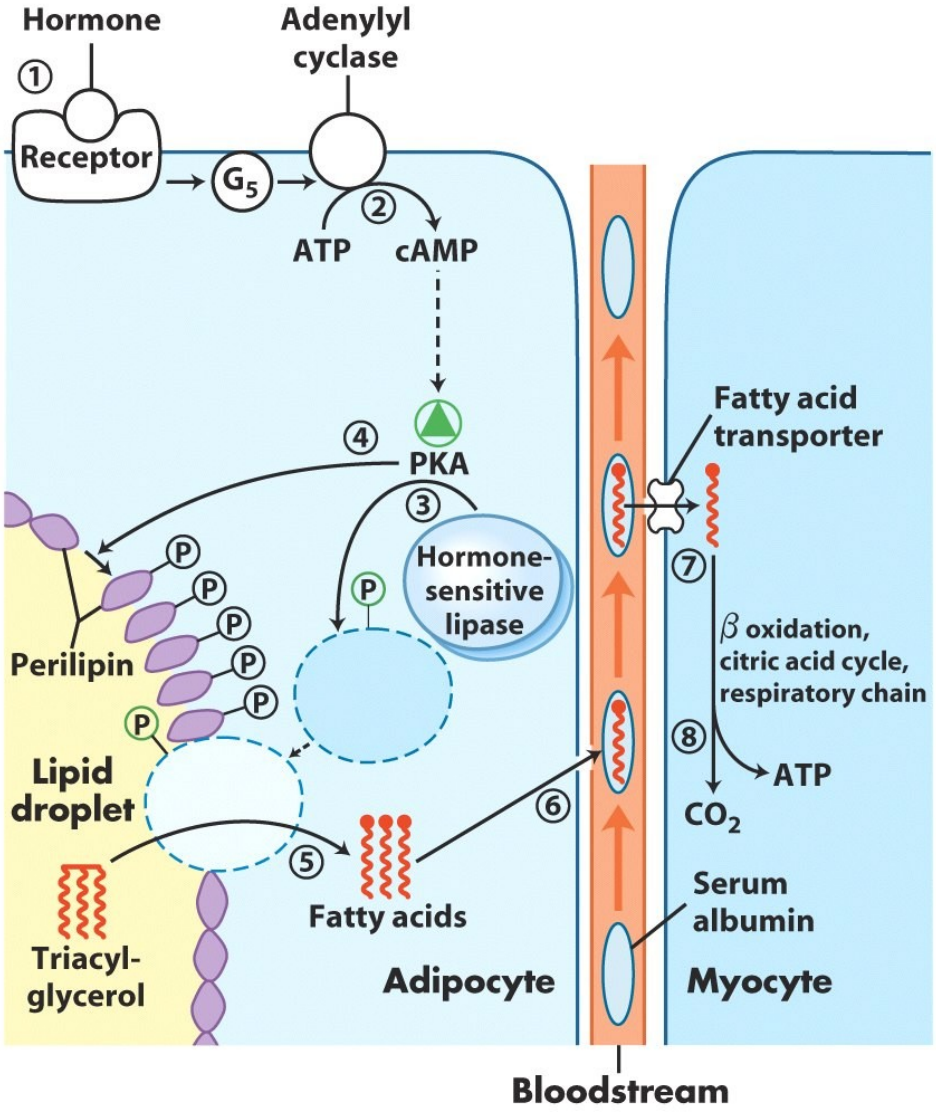
Inside the intestinal cells, fatty acids form complexes with intestinal fatty acid-binding protein (I-FABP).

Why?



To increase the effective solubility and to protect the cell from their detergent-like effects.

Mobilization of Stored Triacylglycerols

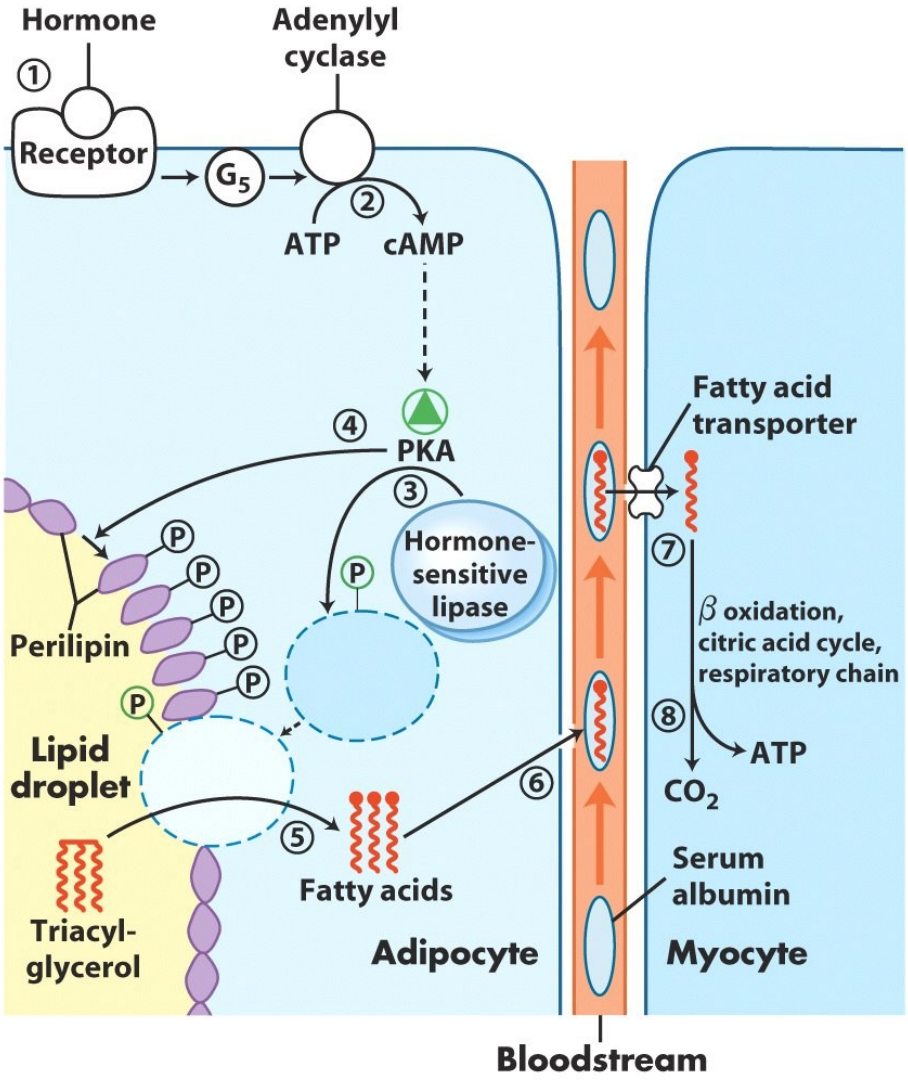


Neutral lipids are stored in adipocytes as lipid droplets
- core of sterol esters and triacylglycerols
- exterior is phospholipid monolayer

Lipid droplet surface is coated with perilipins
- restricts access to droplet

Step 1
Hormones (epinephrine, glucagon) secreted due to low blood glucose levels activate adenylyl cyclase.

Mobilization of Stored Triacylglycerols



Step 2

cAMP dependent protein kinase phosphorylates perilipin.

Step 3

Phosphorylated perilipin recruites Hormone-sensitive lipase (HSL) to lipid droplet surface.

Step 4

HSL hydrolyzes triacylglycerols to free fatty acids.

- **phosphorylation of HSL by protein kinase A increases activity (2-3 fold)**

Step 5

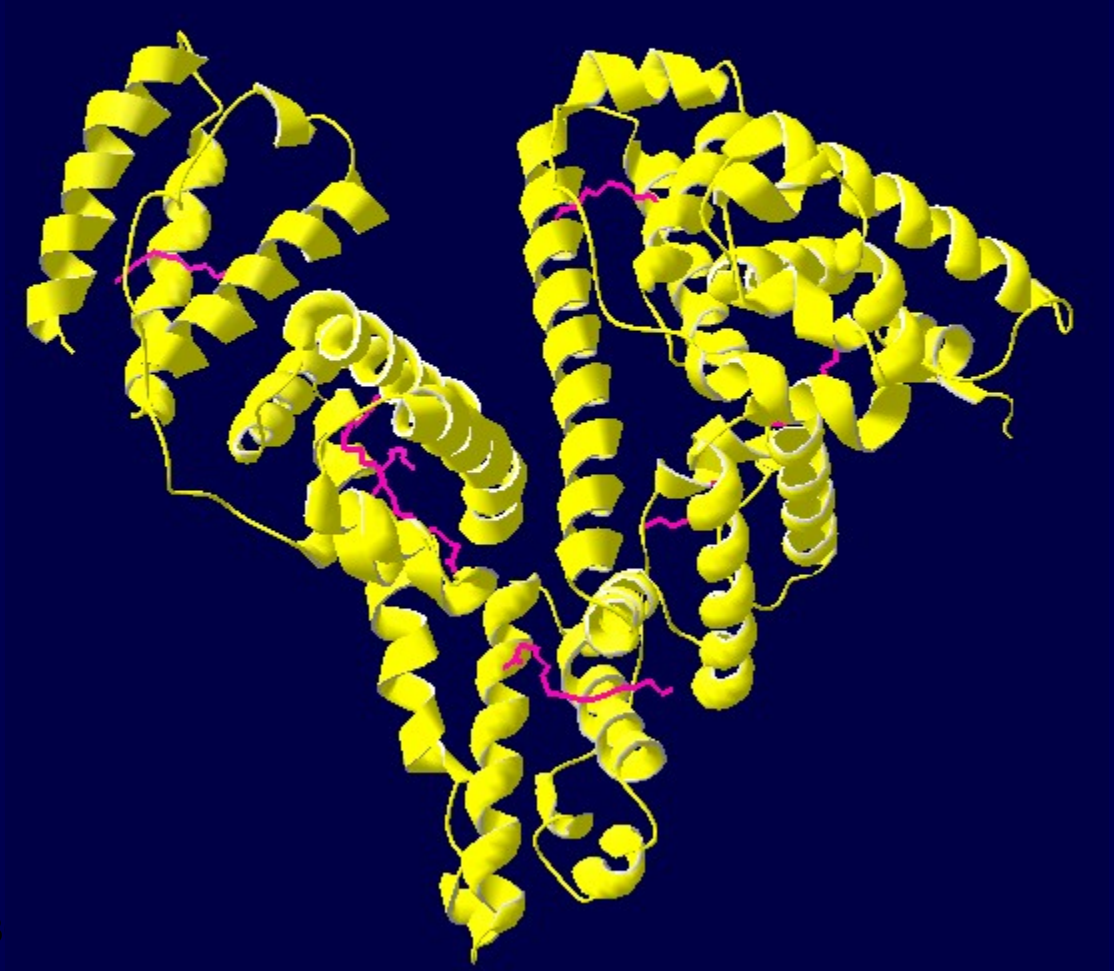
Free fatty acids are transported to bloodstream

- **non-covalently bind to serum albumin (up to 10 FAs/serum albumin) for transport throughout body**

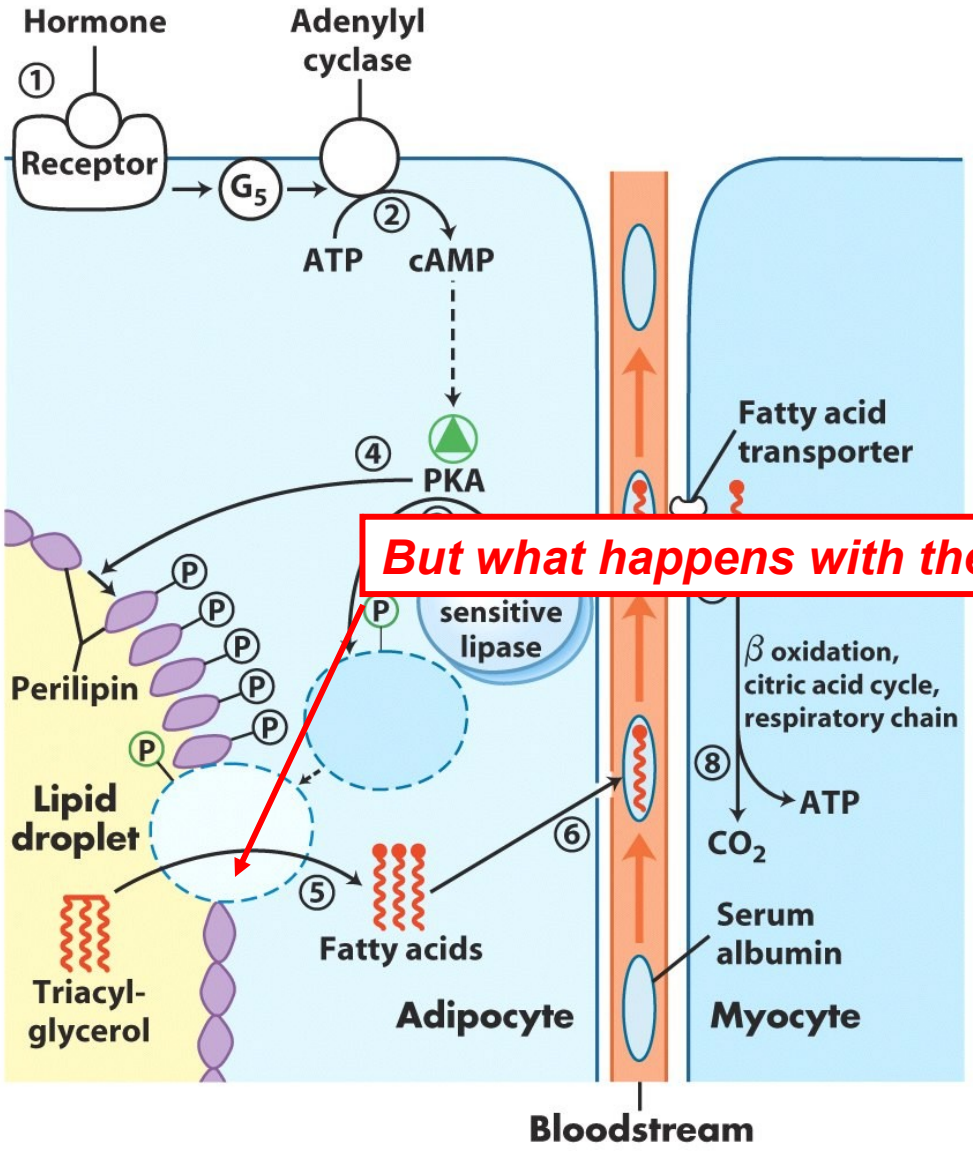
Released Fatty Acids Bind to Albumin

The free fatty acids are released into the blood stream, where they bind to albumin, a soluble 585-residue monomeric protein.

→ about half of the blood serum protein,



Mobilization of Stored Triacylglycerols



HSL hydrolyzes triacylglycerols to free fatty acids.

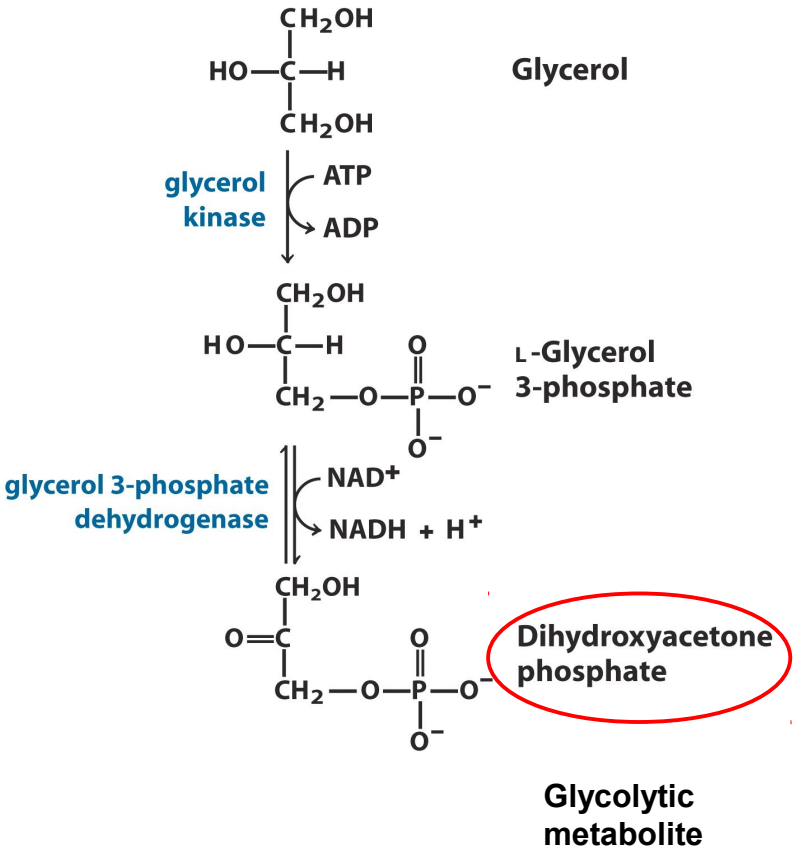
Phosphorylation of HSL by PKA can double or triple is activity.

acids pass into the bloodstream.

Released fatty acids bind to serum albumin (noncovalently).

The complex of up to 10 fatty acids per serum albumin is transported to the target tissues.

Entry of Glycerol Into the Glycolytic Pathway



~95% of the biologically available energy of triacylglycerols is due to the three fatty acid chains.
Only 5% is due to the glycerol moiety.

Two Steps:

1) Released glycerol is phosphorylated to glycerol-3-phosphate by **glycerol kinase.**

2) Glycerol 3-phosphate is oxidized to DHAP by **glycerol 3-phosphate dehydrogenase**

Fatty Acids Are Activated and Transported into Mitochondria

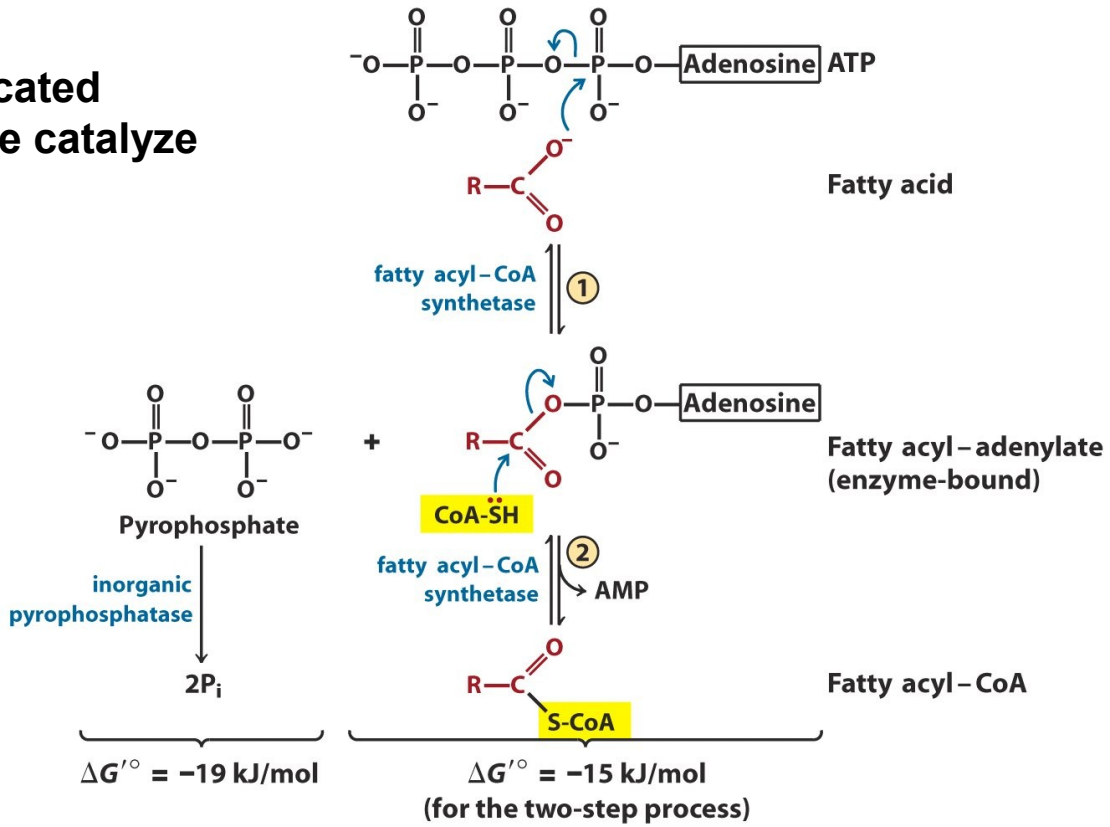
Fatty Acid Activation

Isozymes of acyl-CoA synthetase located in the outer mitochondrial membrane catalyze the formation of fatty acyl-CoA.

Requires Energy

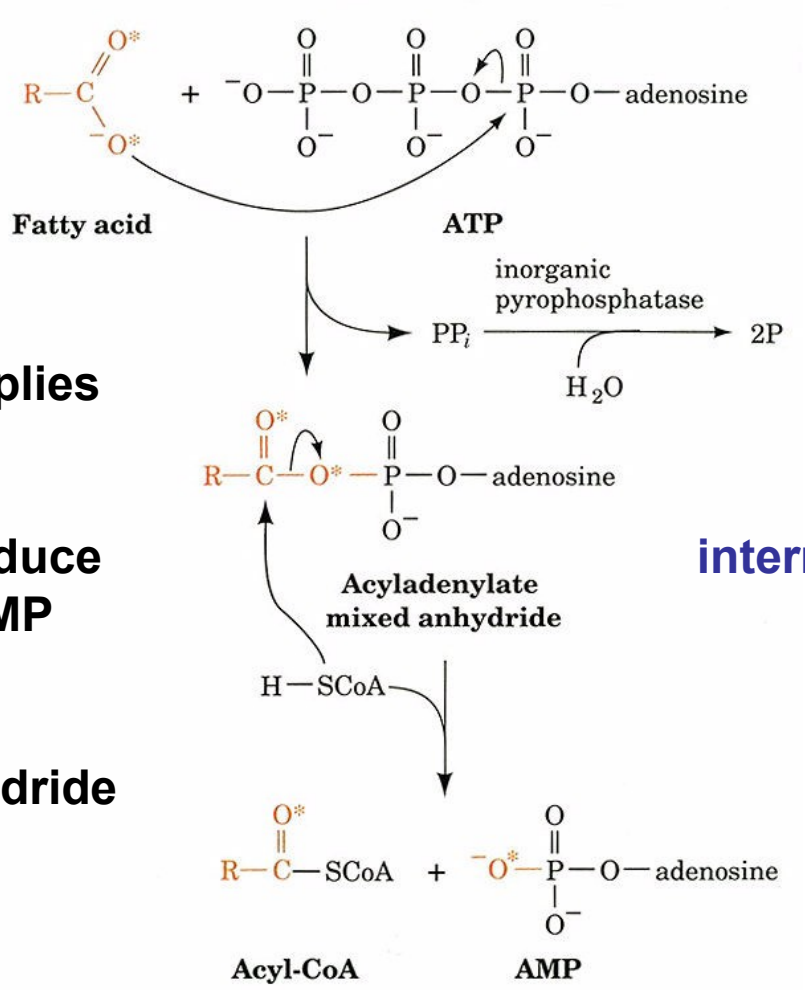
ATP is cleaved to AMP & PP_i; PP_i is then hydrolysed by inorganic pyrophosphatase to $2P_i$

Enzymes for fatty acid oxidation (animals cells) are located in the mitochondrial matrix.



Fatty acyl-CoA esters are transported into the mitochondria or used to synthesize membrane lipids (cytosol).

Testing the Reaction Mechanism
(ie. Acyl CoA formation)



Activation of Fatty acids implies acyladenylate intermediate

^{18}O fatty acids (labeled) produce ^{18}O labeled acyl-CoA and AMP

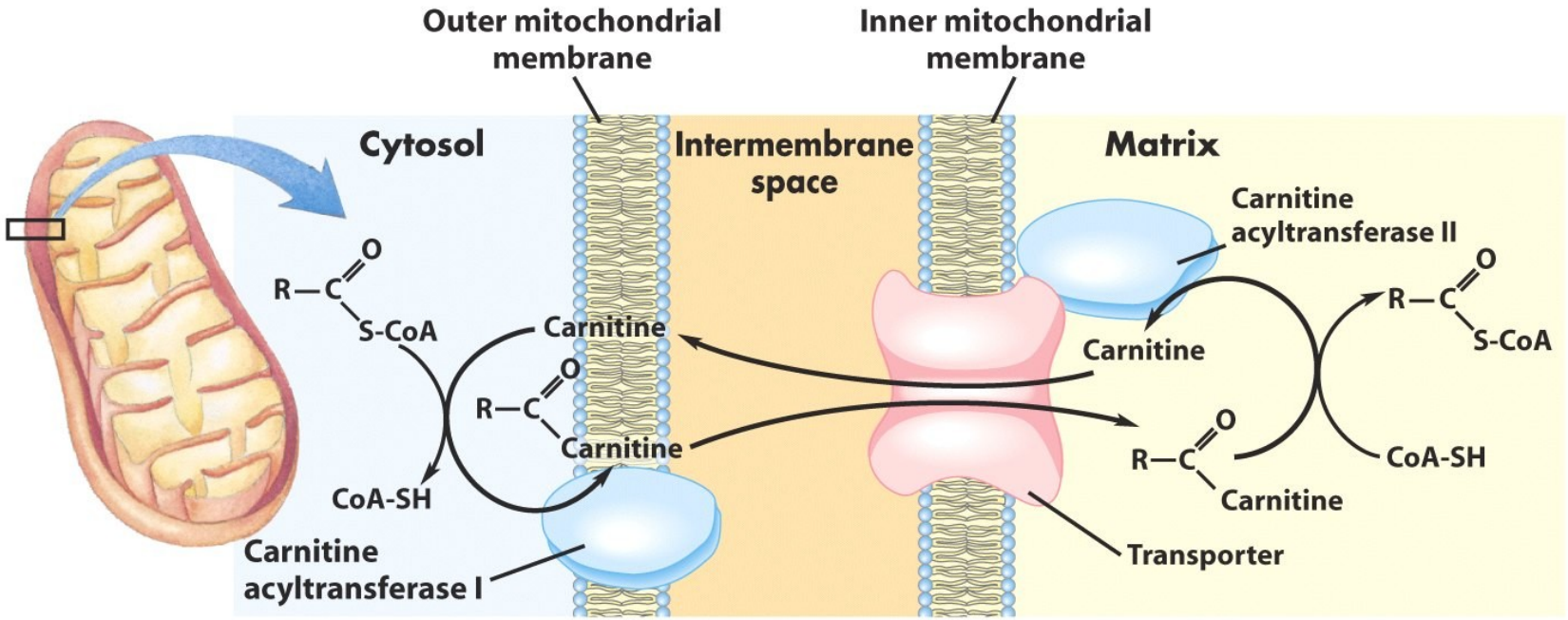
Labeling of both products consistent with mixed-anhydride reaction intermediate

intermediate

Fatty Acids Are Transported into the Mitochondrion

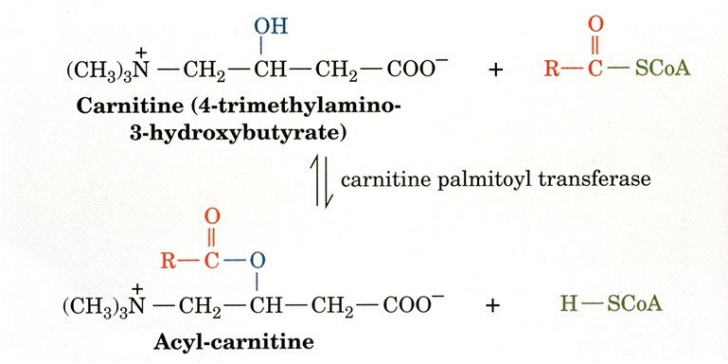
Fatty acid oxidation (animal cells) occurs in the mitochondrial matrix.

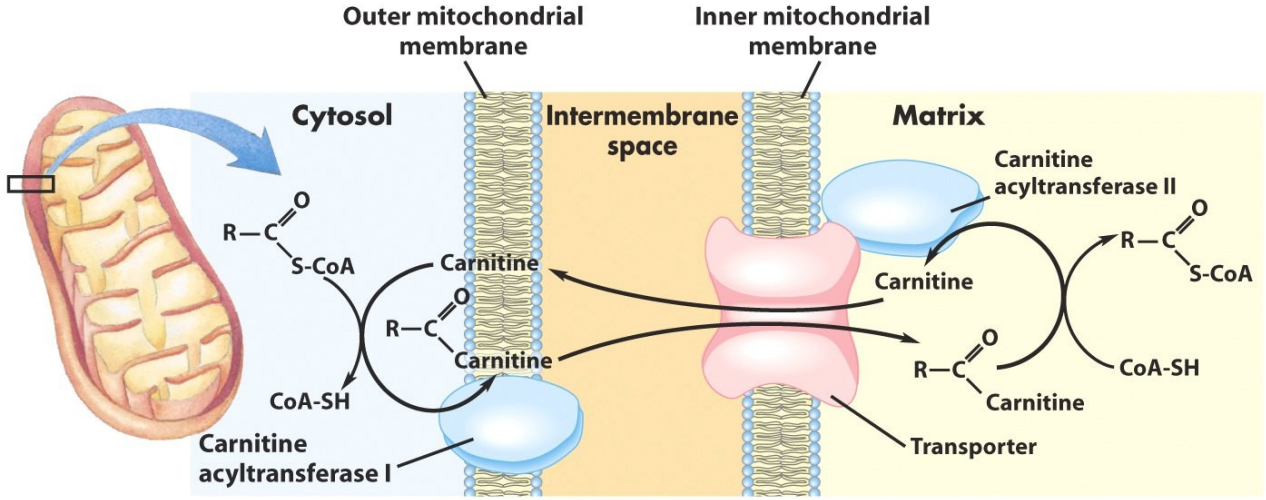
14C and longer fatty acids require Transporters to enter the mitochondria → the carnitine shuttle



Fatty Acids Are Transported into the Mitochondrion

Fatty acids are transiently attached to carnitine forming fatty acyl-carnitine.

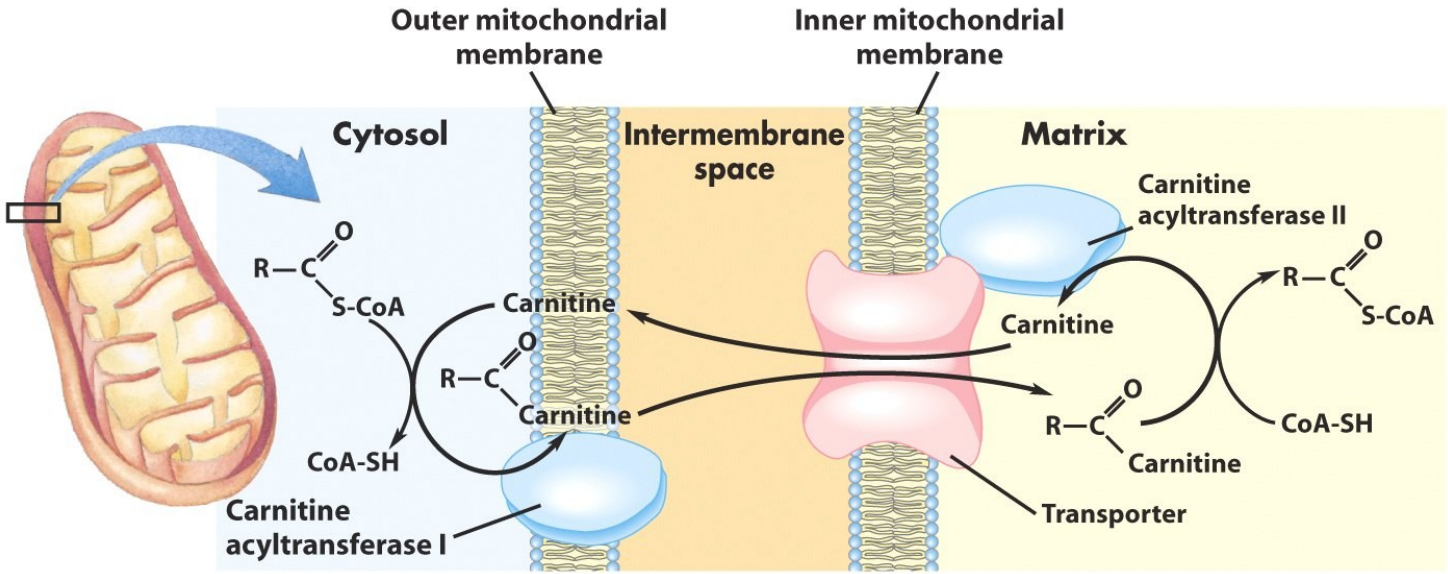




Fatty acyl-carnitine enters the matrix via the acyl-carnitine/carnitine antiporter.

Fatty Acids Are Transported into the Mitochondrion

Carnitine acyltransferase II transfers the fatty acyl group from carnitine to mitochondrial CoA
→ regenerates fatty acyl CoA within mitochondria.

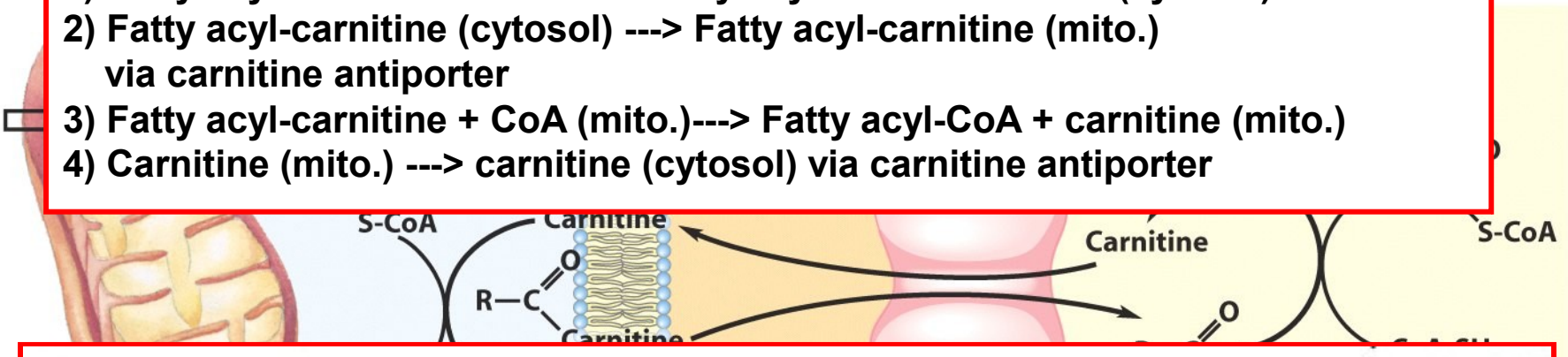


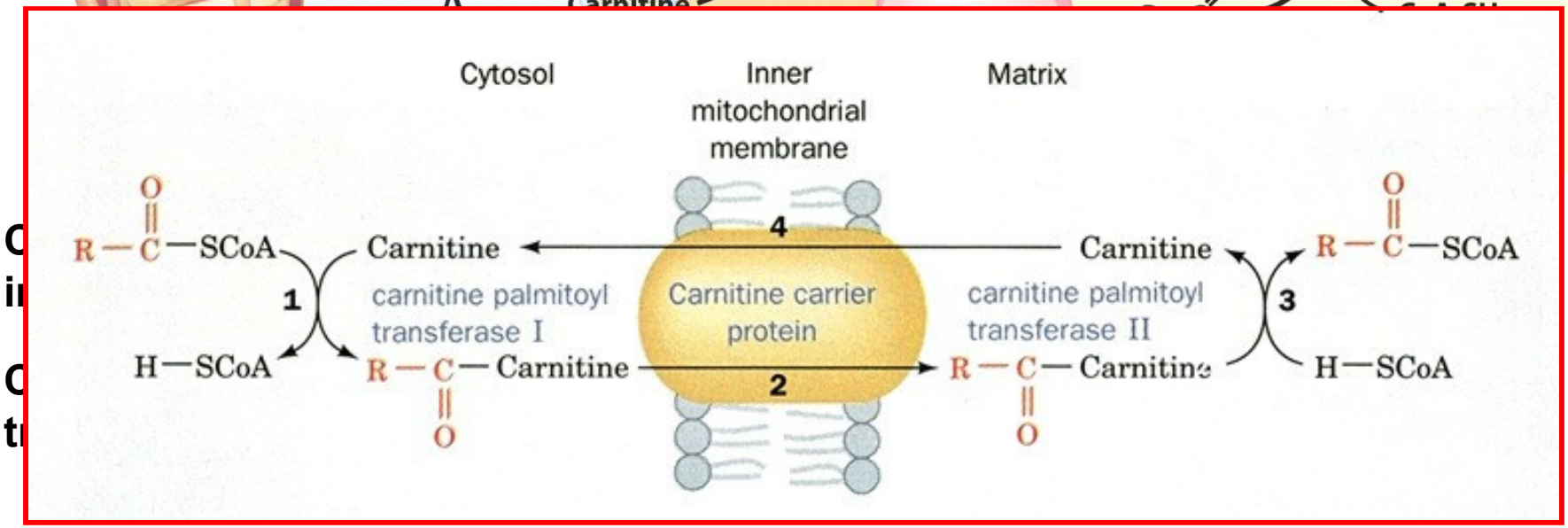
Carnitine exported from matrix via the acyl-carnitine/carnitine antiporter.

Fatty Acids Are Transported into the Mitochondrion

Acyl-CoA transport occurs via four steps:

1) Fatty acyl-CoA + carnitine ---> Fatty acyl-carnitine + CoA (cytosol)
2) Fatty acyl-carnitine (cytosol) ---> Fatty acyl-carnitine (mito.)
   via carnitine antiporter
3) Fatty acyl-carnitine + CoA (mito.)---> Fatty acyl-CoA + carnitine (mito.)
4) Carnitine (mito.) ---> carnitine (cytosol) via carnitine antiporter



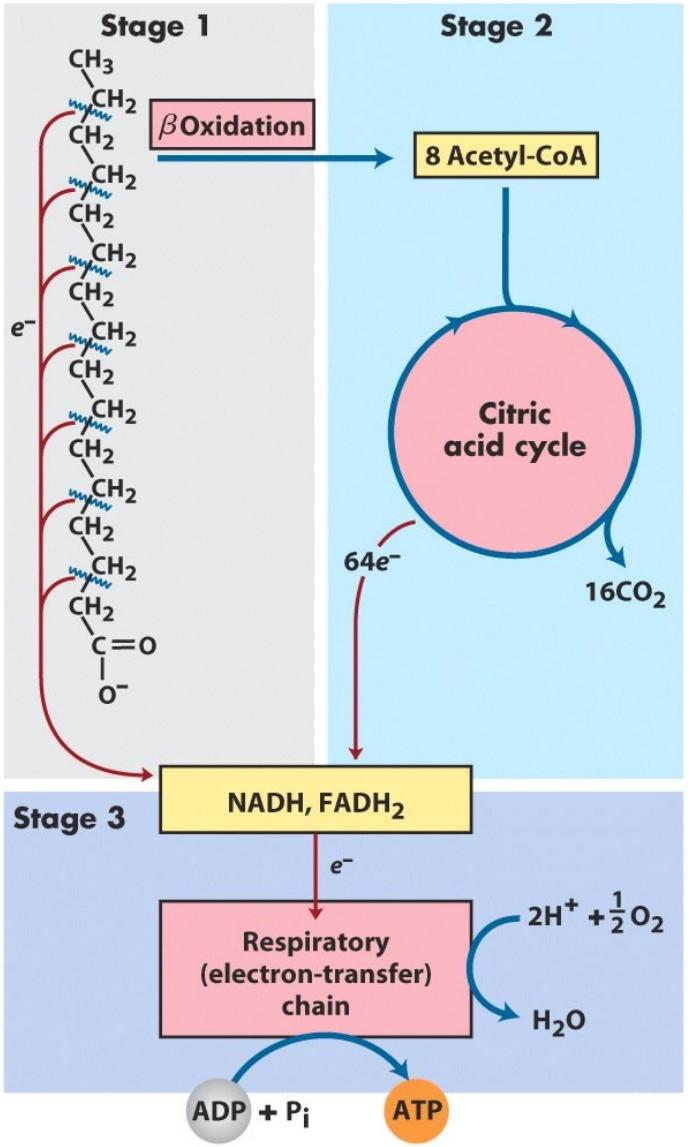


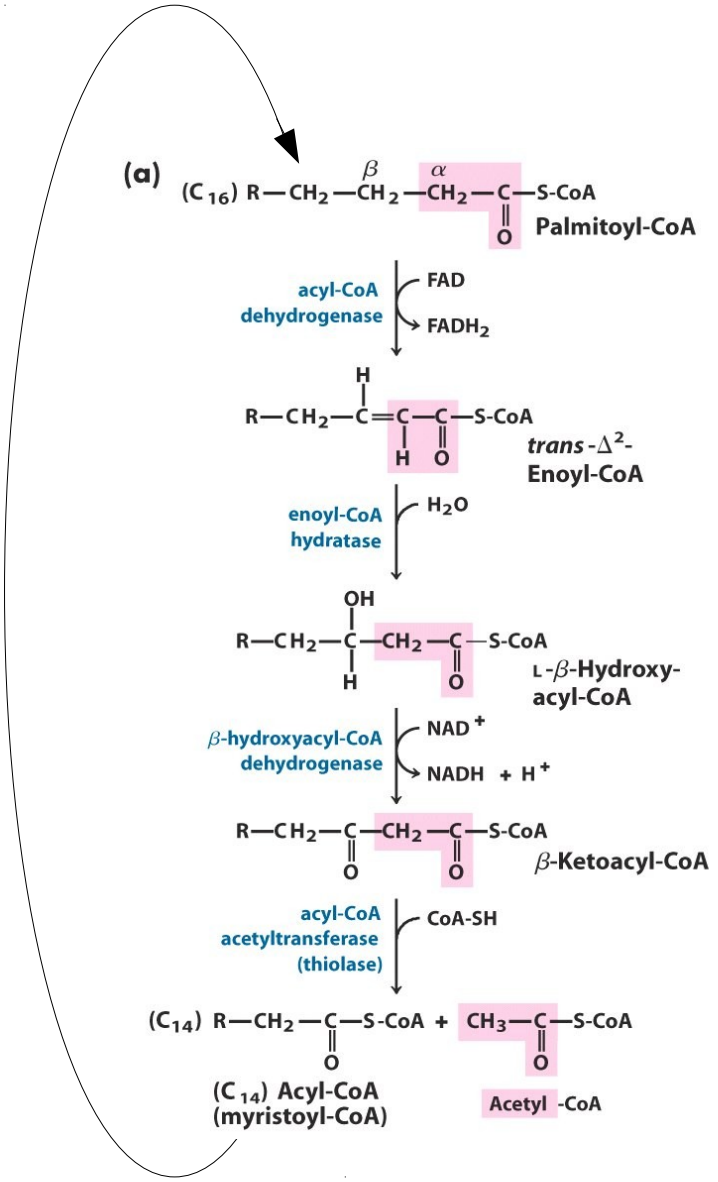
Oxidation of Fatty Acids

Stage 1:
Fatty acids are sequentially, converted to acetyl-CoA through the *β* oxidation of fatty acyl-CoA.

Stage 2:
acetyl groups are oxidized to CO_2 by the citric acid cycle.

Stage 3:
Electrons derived from oxidations in stages 1 and 2 enter the respiratory chain.





β Oxidation

Process occurs in four reactions:

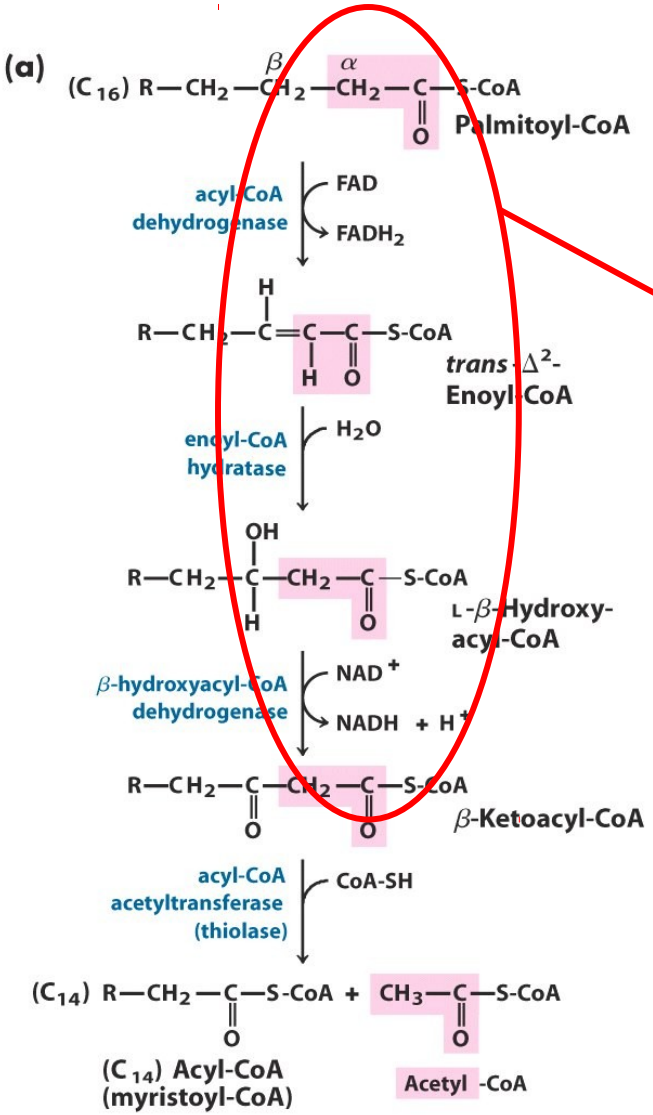
1. Formation of a trans-α,β double bond by acyl-CoA dehydrogenase
   - form $FADH_2$

2. Hydration of the double bond by enoyl-CoA hydratase

3. Formation of β-hydroxyacyl-CoA by 3-L-hydroxyacyl-CoA dehydrogenase.
   - produces β-keto group and NADH

4. Thiolysis reaction with CoA, catalyzed by β-ketoacyl-CoA thiolase
   - produces Acetyl CoA and a shortened (2C) fatty acyl-CoA

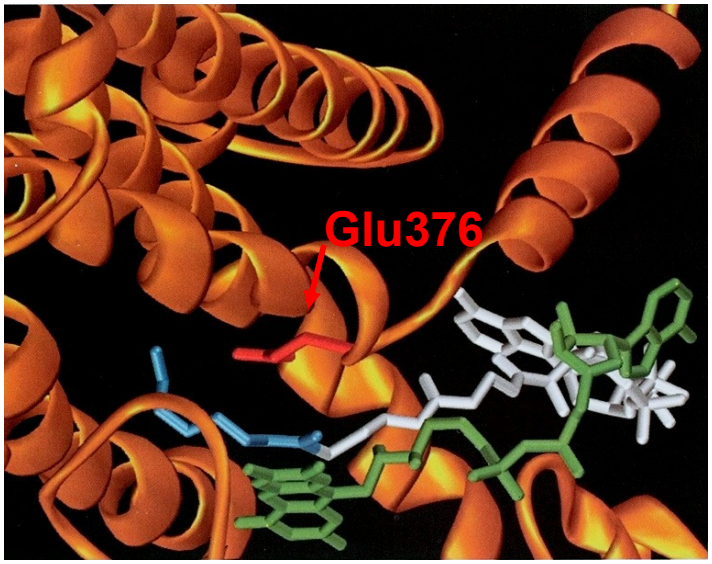
β Oxidation



Where have you seen something similar?

Steps 1-3 resemble (chemically) the citric acid cycle reactions that convert succinate to oxaloacetate.

β Oxidation (step 1)



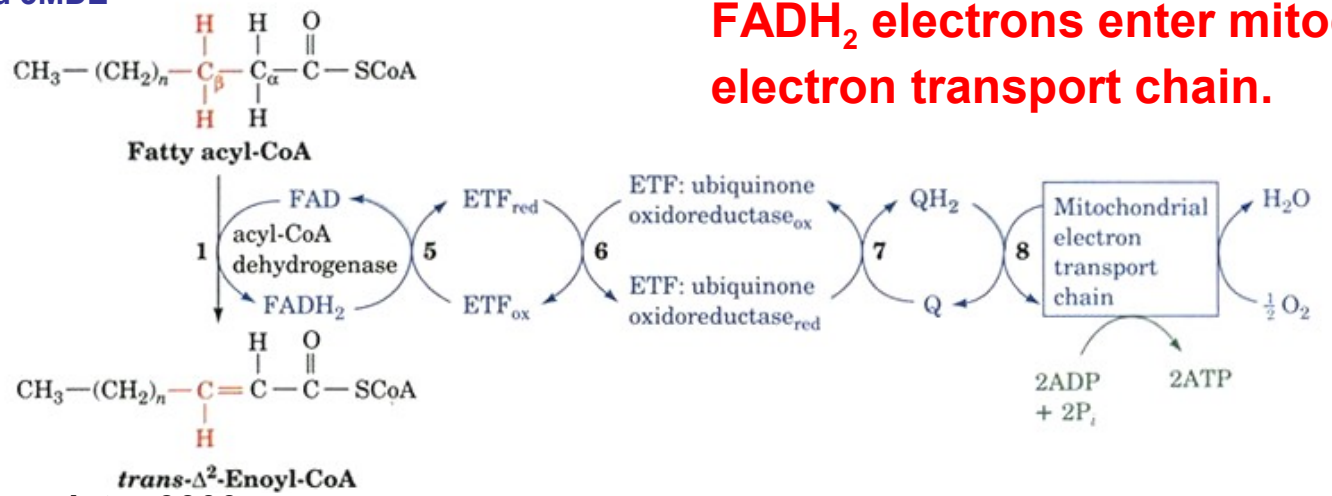
PDBid 3MDE

Mitochondria contain four acyl-CoA DH with different specificities:

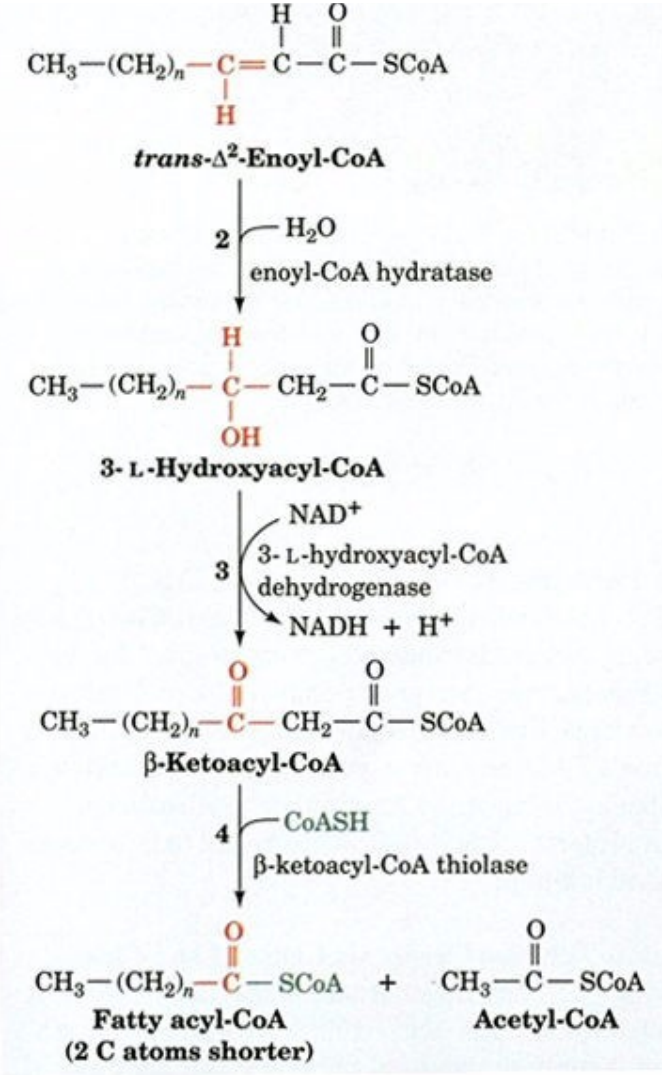
Fatty acyl-CoAs

short	C_4-C_6
medium	C_6-C_{10}
long	C_{10}-C_{12}
very long	C_{12}-C_{18}

$FADH_2$ electrons enter mitochondrial electron transport chain.



β Oxidation (steps 2-4)



Enoyl-CoA is processed by one of three systems (depending upon chain length):

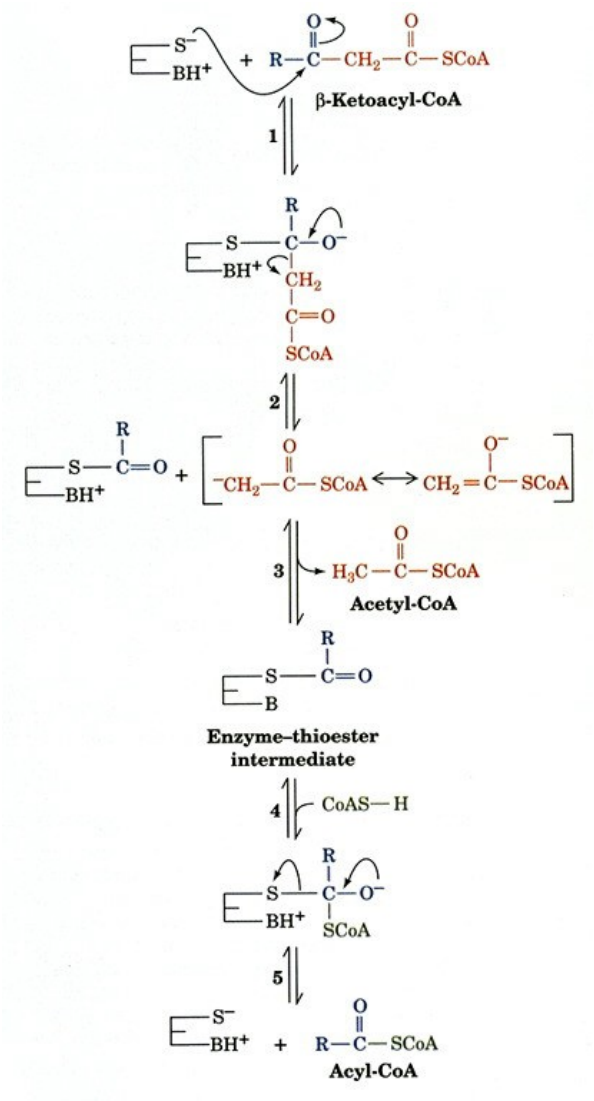
short-, medium-, or long-chain
2-enoyl-CoA Hydratases
hydroxyacyl-CoA dehydrogenases
β-ketoacyl-CoA thiolases

Short/Medium (C12 or less) fatty acyl chains are oxidized by a set of soluble proteins.

Long-chain version of these enzymes form a multienzyme complex ($\alpha_2\beta_2$) associated with the mitochondrial inner membrane.

- **α-subunits contain the enoyl-CoA hydratase and *β*-hydroxyacyl-CoA activity.**
- ***β*-subunits contain the thiolase activity.**

Mechanism of β-Ketoacyl-CoA thiolase (step 4)

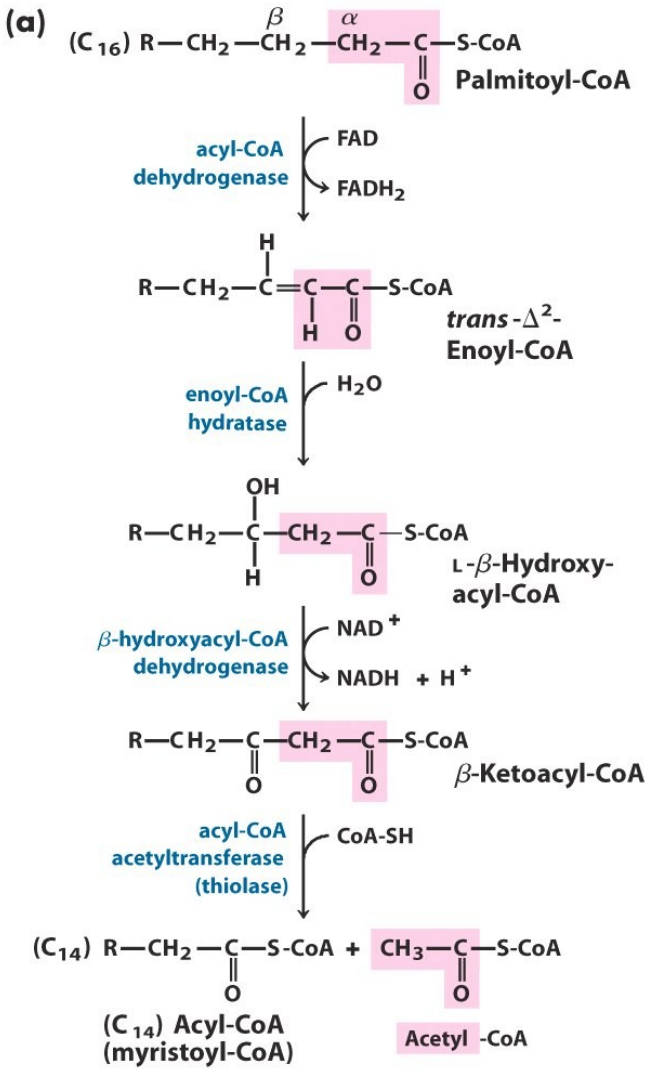


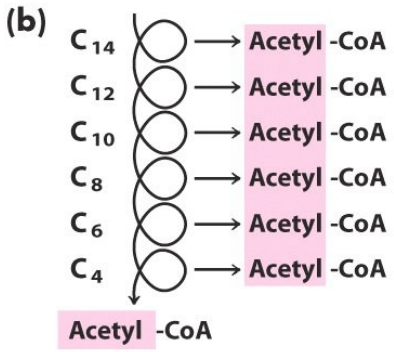
Final stage of the fatty acid β-oxidation is the thiolase reaction.

Formation of acetyl-CoA and an acyl-CoA shortened by 2 carbons.

1. Thiol attacks acyl-CoA β-keto group
2. C-C bond cleavage forming acetyl-CoA carbanion
3. Protonation of acetyl-CoA
4 & 5. CoA displaces enzyme thiol Releasing acyl-CoA

β Oxidation





The four β-Oxidation Steps are repeated to yield multiple acetyl-CoA.

Palmitoyl-CoA + 7CoA + 7FAD + 7NAD⁺ + 7H$_2$0
→ 8 acetyl-CoA + 7FADH$_2$ + 7 NADH + 7H⁺

Transfer of electrons from FADH$_2$ and NADH to O$_2$ result in ~28 ATPs and 7 H$_2$O

Note: still have 8 acetyl-CoA

Acetyl-CoA is Further Oxidized in the Citric Acid Cycle

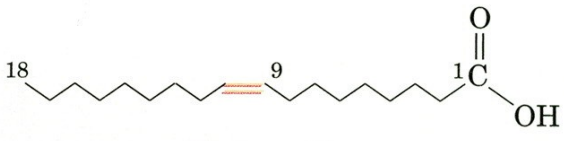
TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	*Number of NADH or $FADH_2$ formed*	*Number of ATP ultimately formed**
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

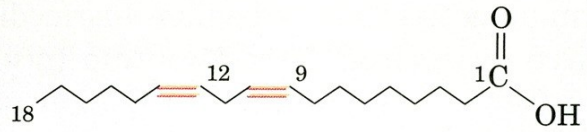
Assuming that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ and 2.5 ATP per NADH.

Oxidation of Unsaturated Fatty Acids

Oxidation of unsaturated fatty acids requires two additional reaction

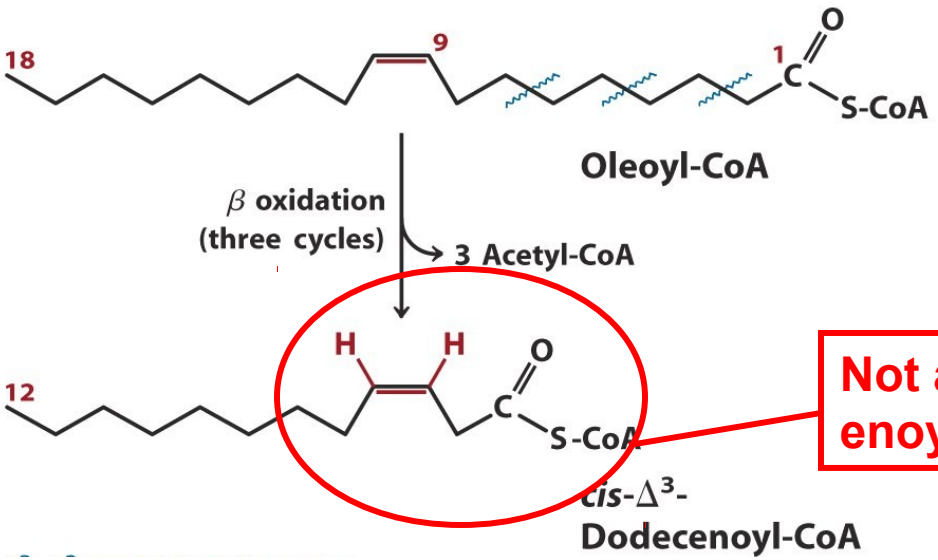


Oleic acid
(9-*cis*-Octadecenoic acid)



Linoleic acid
(9,12-*cis*-Octadecadienoic acid)

Problems in the Oxidation of Unsaturated fatty acids.



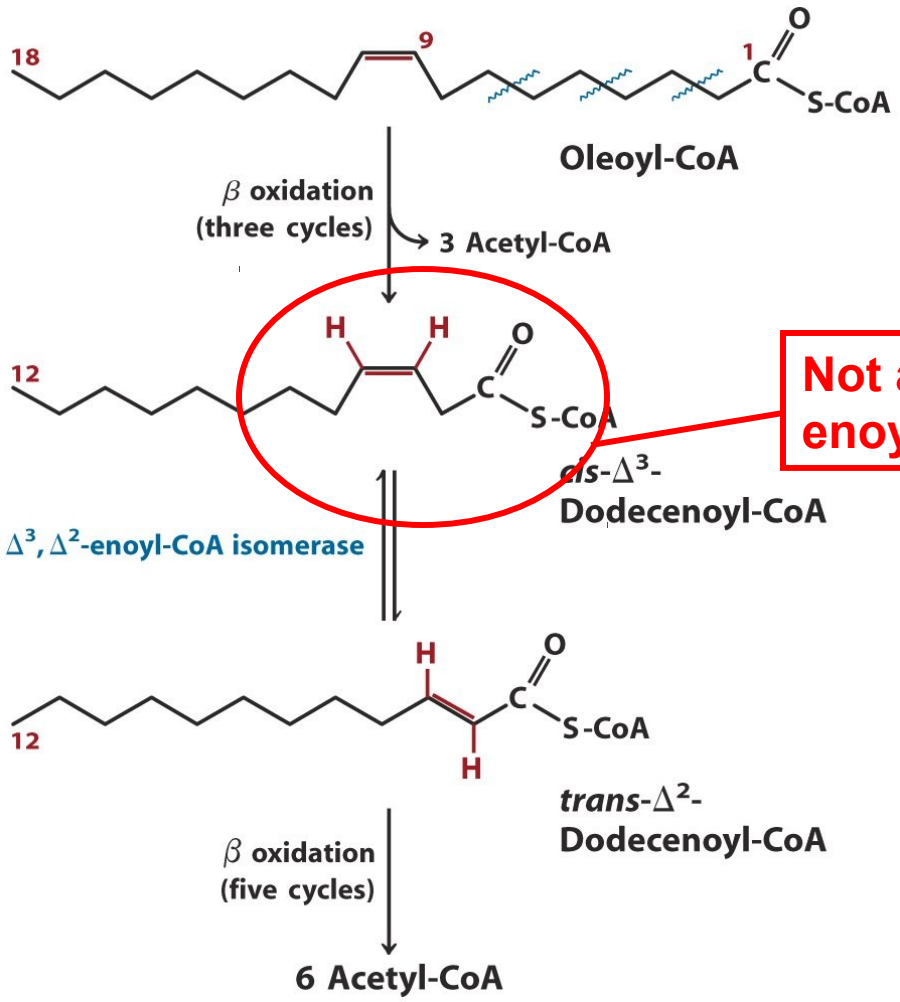
Problem 1

cis double bonds!

Not a substrate for enoyl-CoA hydratase

cis-Δ^3 fatty acids are not an enoyl-CoA hydratase substrate

Problems in the Oxidation of Unsaturated fatty acids.



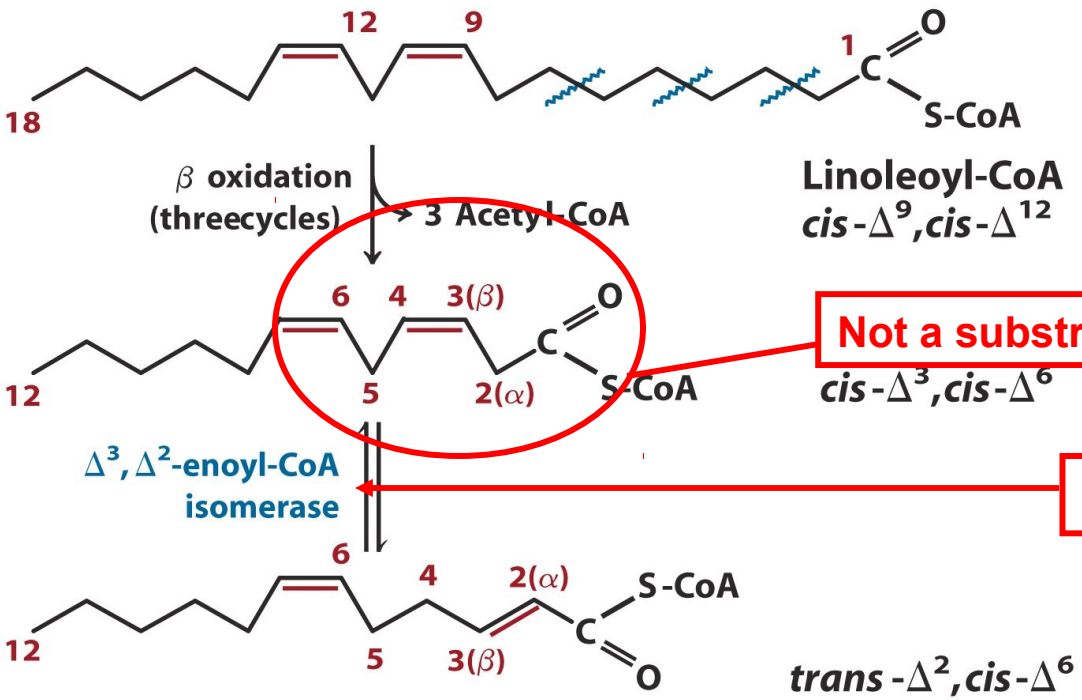
Problem 1

cis double bonds!

Not a substrate for enoyl-CoA hydratase

Enoyl-CoA isomerase converts the cis-Δ^3 double bond the the more stable ester conjugated trans-Δ^2 form.

Problems in the Oxidation of Unsaturated fatty acids.



Problem 2

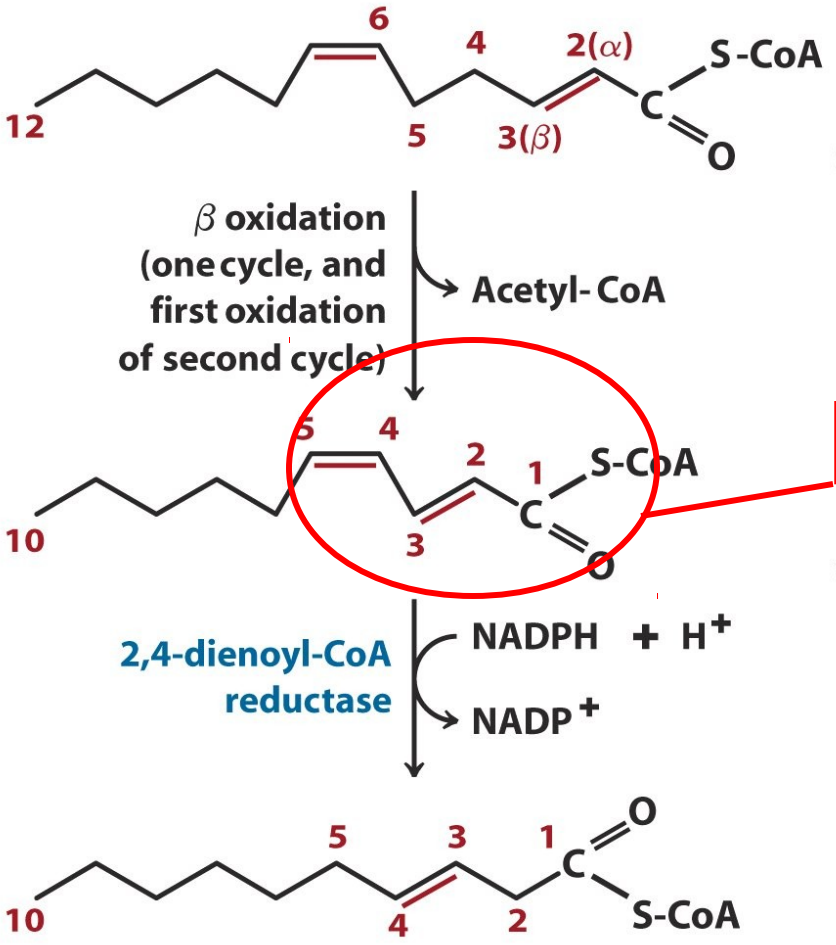
More cis double bonds!

Not a substrate for enoyl-CoA hydratase

But no problem!

Problems in the Oxidation of Unsaturated Fatty Acids.

Problem 2 cont.



trans-Δ^2,cis-Δ^4 double bond!

trans-Δ^2,*cis*-Δ^6

Poor substrate for enoyl-CoA hydratase

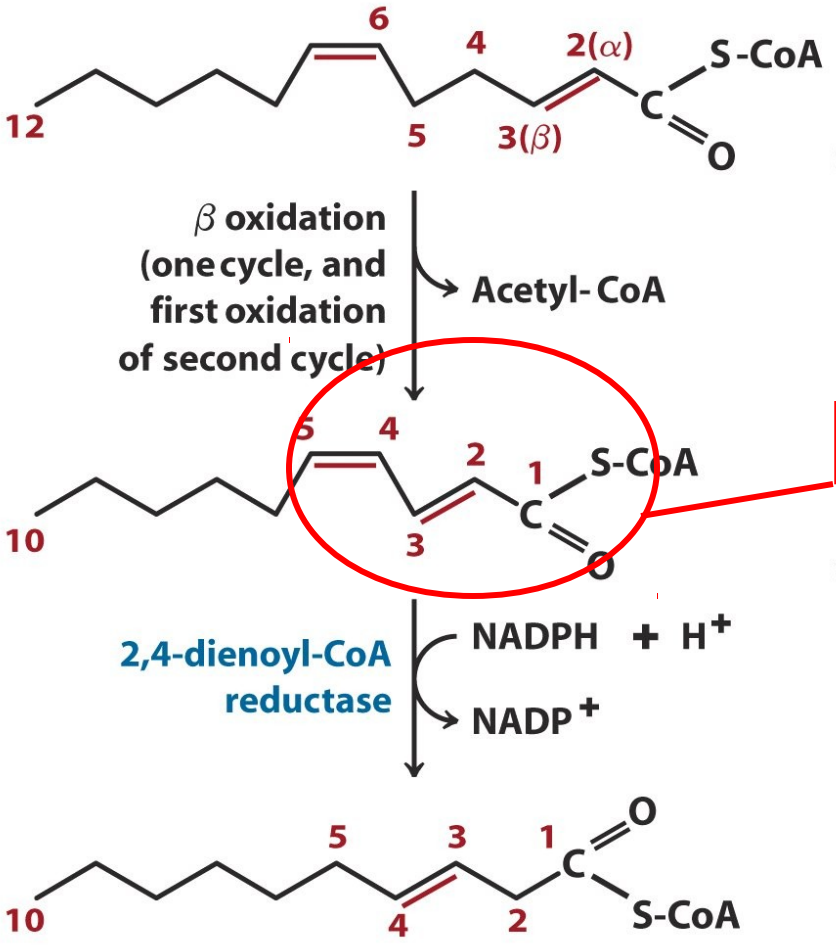
trans-Δ^2,*cis*-Δ^4

The 2,4-dienoyl-CoA reductase reduces the Δ^4 double bond

trans-Δ^3

Problems in the Oxidation of Unsaturated Fatty Acids.

Problem 2 cont.



trans-Δ^2,cis-Δ^4 double bond!

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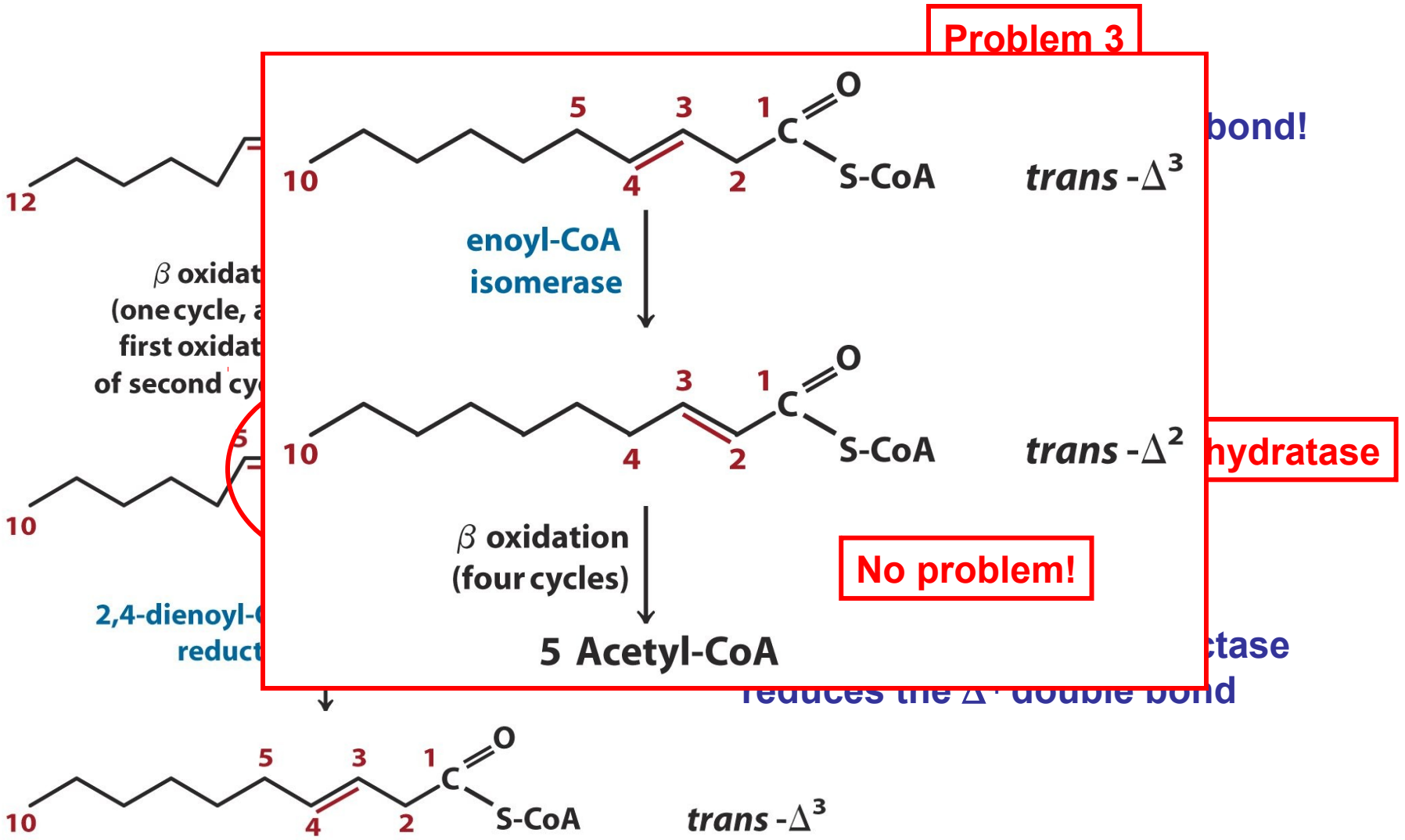
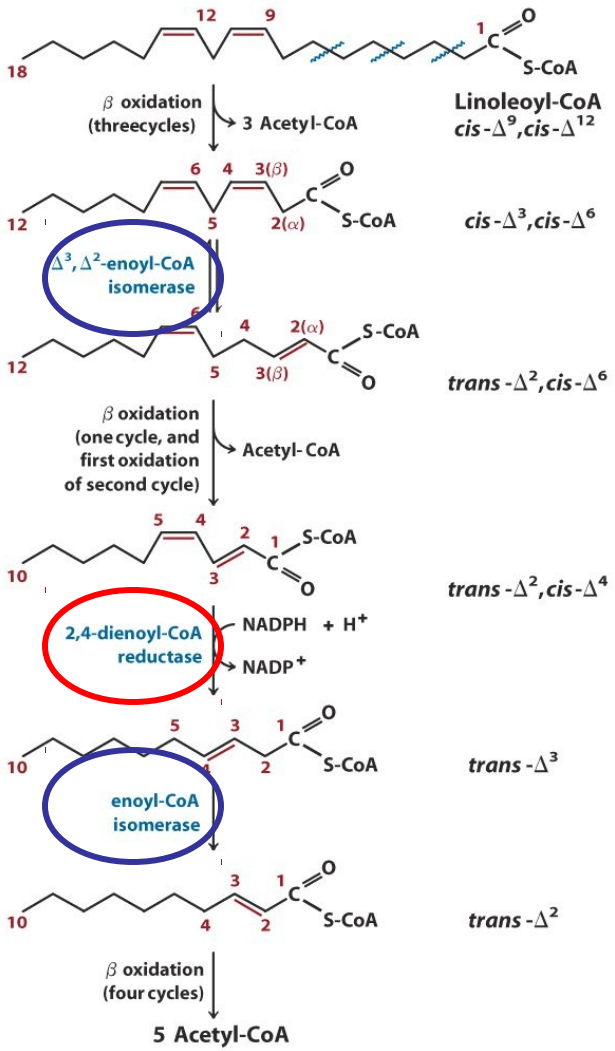
The 2,4-dienoyl-CoA reductase reduces the Δ^4 double bond

trans-Δ^3

Problems in the Oxidation of Unsaturated Fatty Acids.



Oxidation of a Polyunsaturated Fatty Acid



Oxidation of unsaturated fatty acids requires two additional reaction.

Δ^3,Δ^2 enoyl-CoA isomerase

2,4-dienoyl-CoA reductase

Oxidation of Odd-Number Fatty Acids.

Most naturally occurring lipids contain fatty acids with an even number of carbon atoms.

But many plants and some marine organisms have fatty acids with an odd number of carbon atoms.

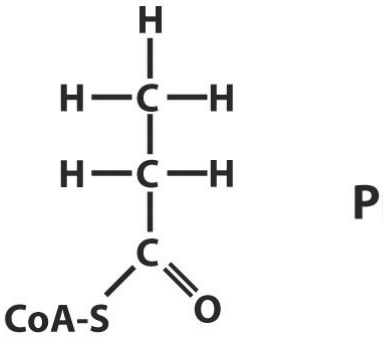
Cattle and other ruminant animals form large amounts of propionate in the rumen.

Long-chain odd-number fatty acids are oxidized in the same pathway as the even-numbered acids.

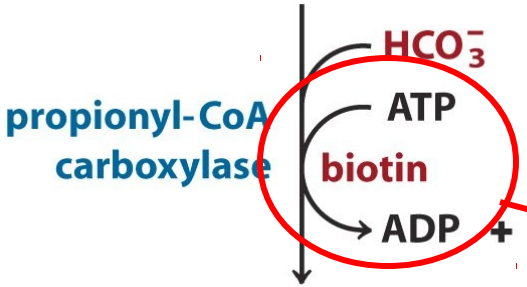
But the cyclic oxidation ends with propionyl-CoA

Oxidation of Propionyl-CoA

Step 1

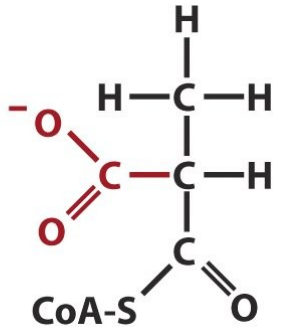


Propionyl-CoA



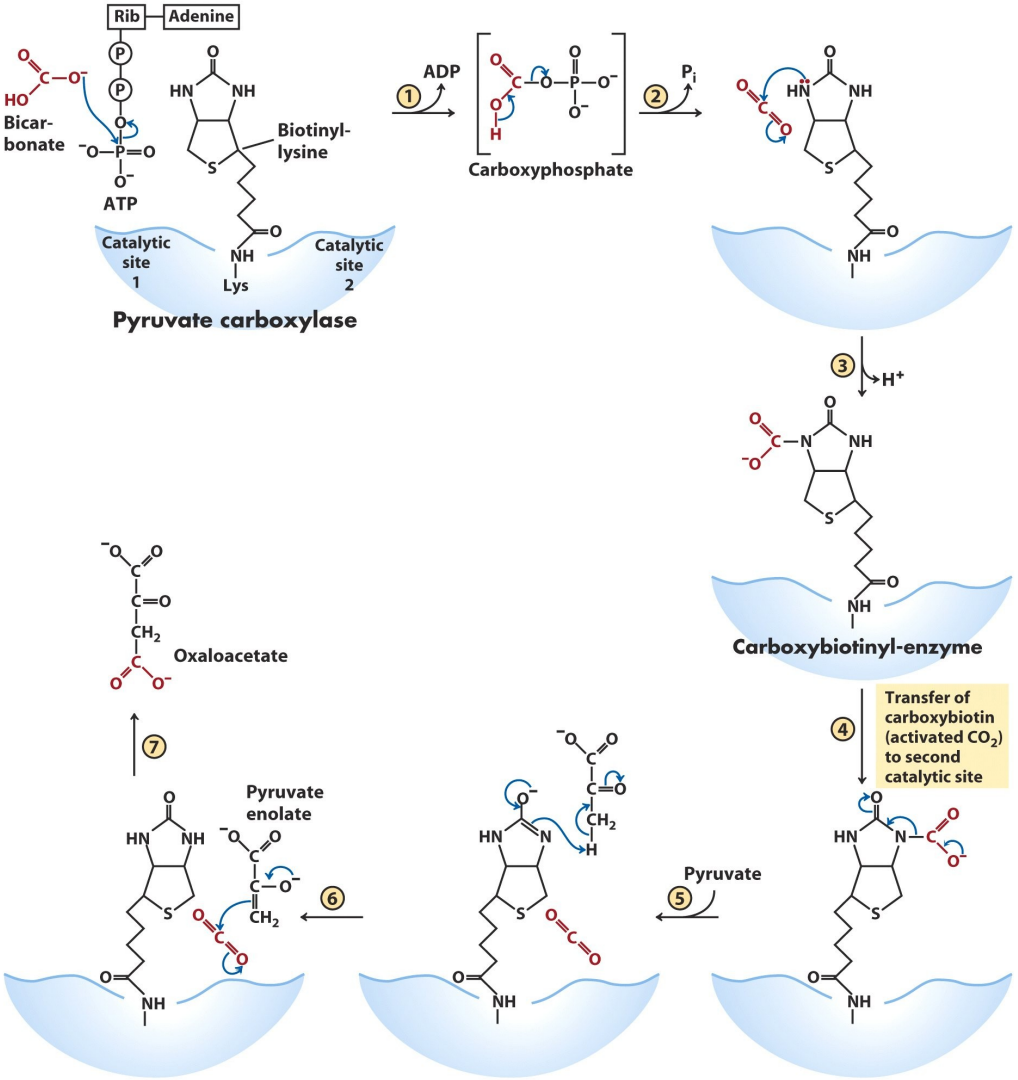
Propionyl-CoA is first carboxylated by propionyl-CoA carboxylase.

Remember that one?



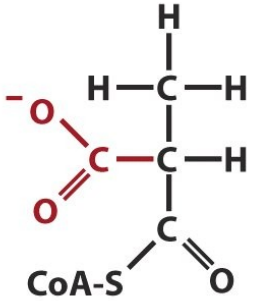
D-Methylmalonyl-CoA

Gluconeogenesis – Step 1



Oxidation of Propionyl-CoA

Step 2

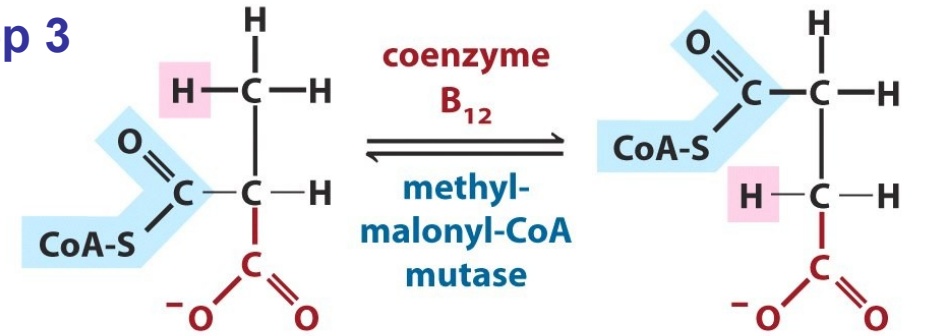


D-Methylmalonyl-CoA is epimerized to L-Methylmalonyl-CoA by methylmalonyl CoA epimerase

methylmalonyl-CoA epimerase

An intramolecular rearrangement results in the formation of Succinyl-CoA.

Step 3

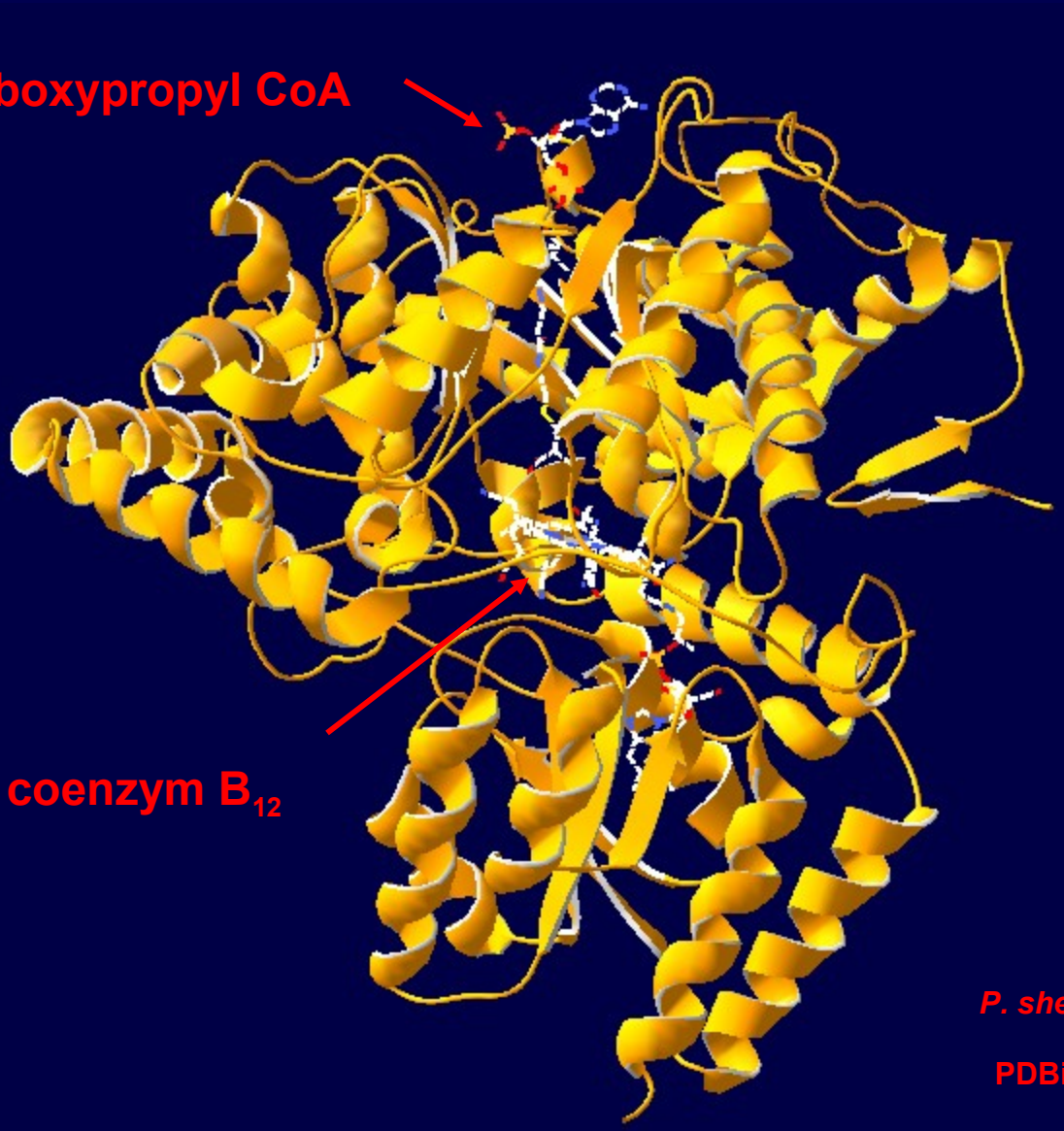


L-Methylmalonyl-CoA

Succinyl-CoA

Rearrangement is catayzed by methylmalonyl-CoA mutase, requiring coenzyme B_{12}

Methylmalonyl-Co A Mutase



P. shermanii

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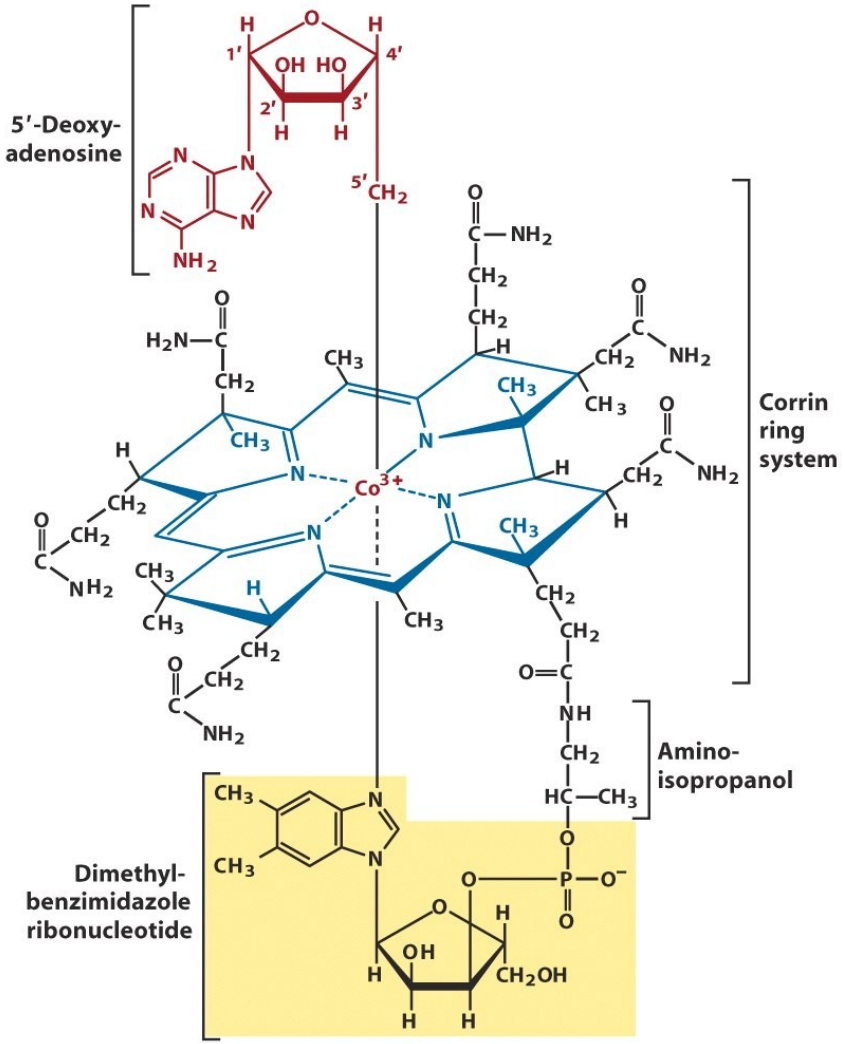
Coenzyme B_{12}

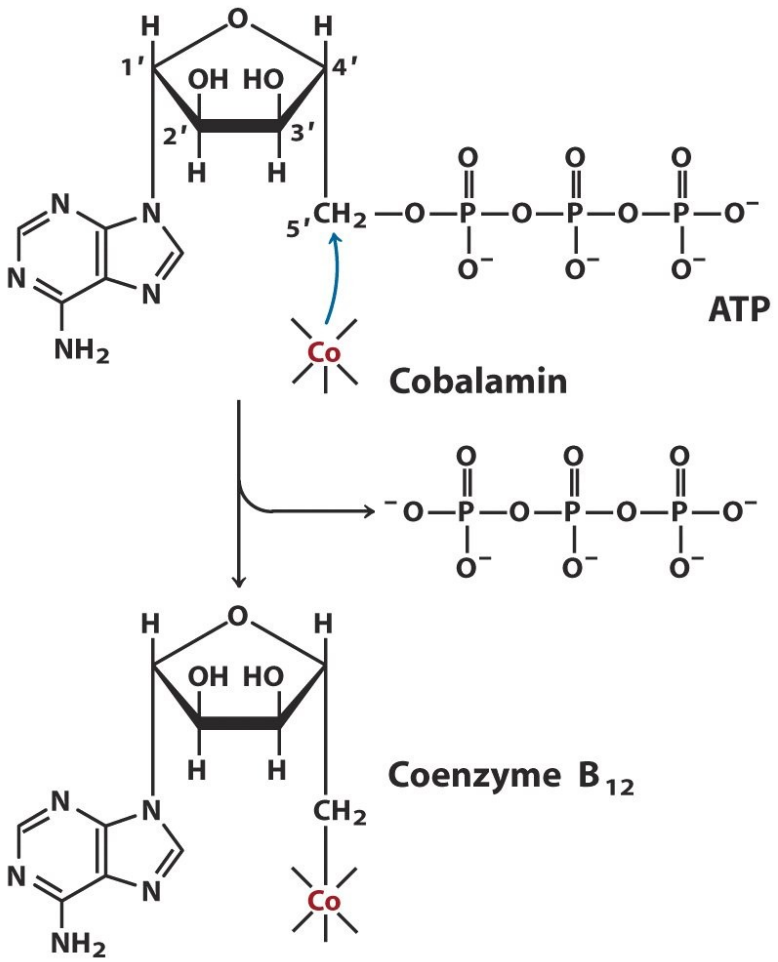
The complex corrin ring system is related to the porphyrin ring system of heme and coordinates Cobalt.

Co^{3+} forms a covalent bond with C-5' of the deoxyadenosyl group.

The Co-C bond dissociation energy is about 110 kJ/mol



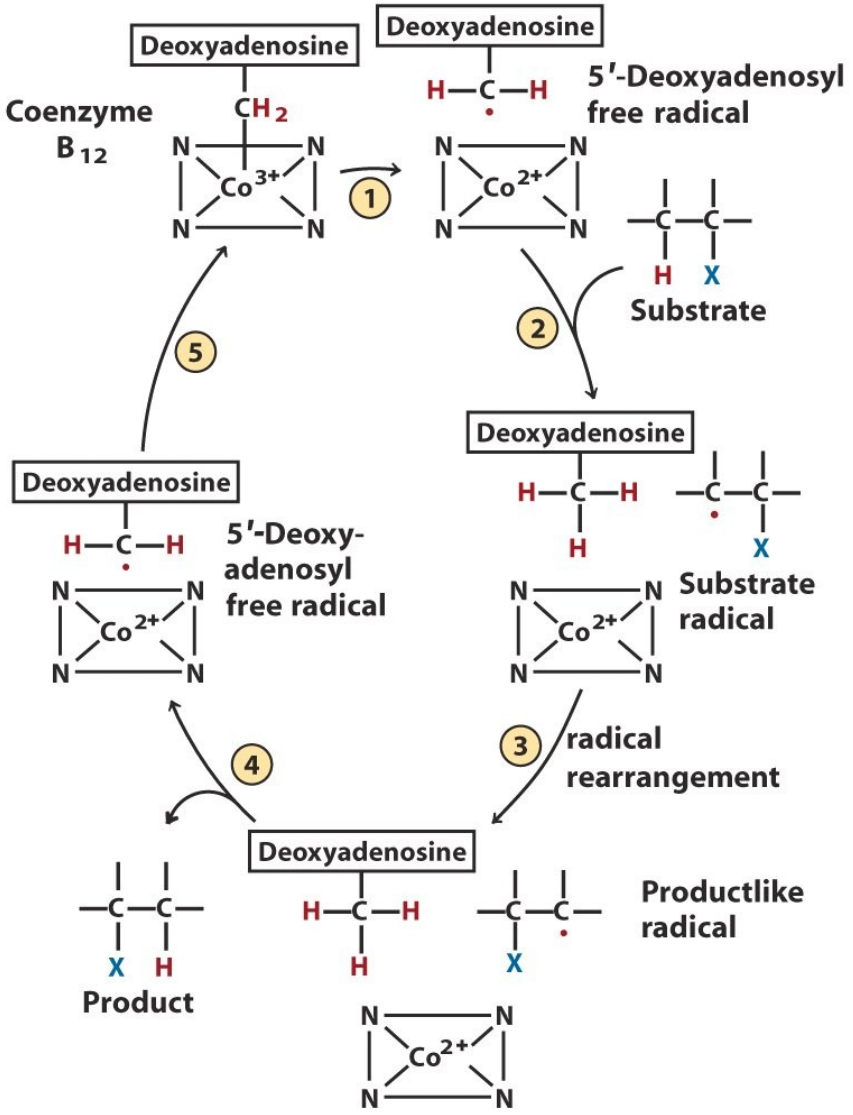
Coenzyme B_{12}



Duing the formation of the factor a triphosphate is cleaved from ATP.

Only two reactions are know that involve the formation of a triphosphate from ATP.
→ S-adenosylmethionine formation (Amino acid metabolism)

Mechanism Methylmalonyl-CoA Mutase

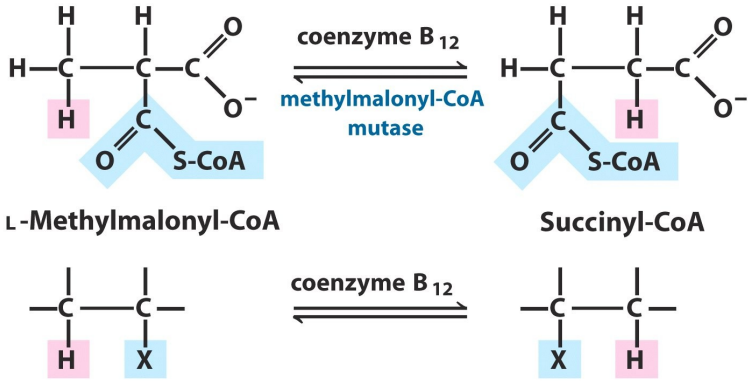


The Co-C bond is broken resulting in a Co^{2+} and a free radical at 5'-deoxyadenosyl moity.

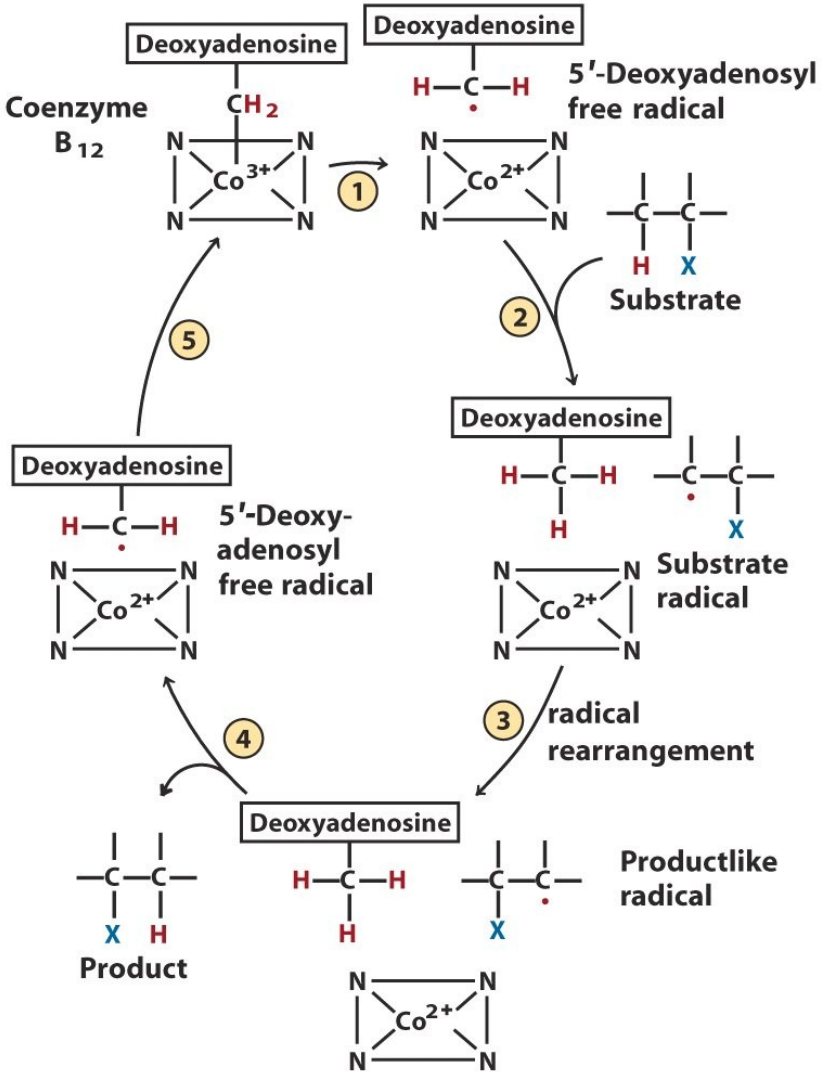
The radical abstracts the hydrogen from the substrate.

Rearrangement of the radical → migration of the group X

→ product like C-skeleton



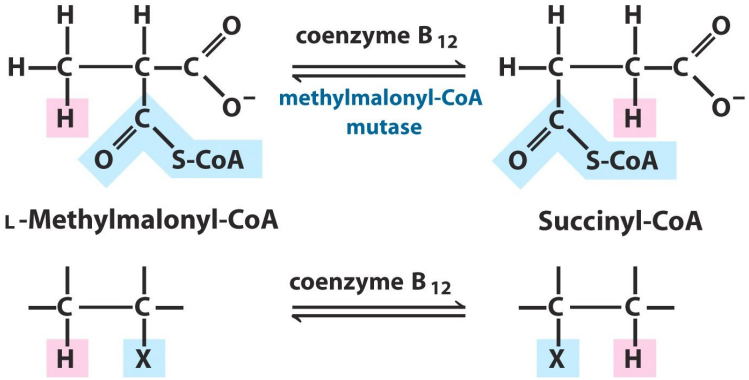
Mechanism Methylmalonyl-CoA Mutase



Rearrangement of the radical
→ migration of the group X
→ product like C-skeleton

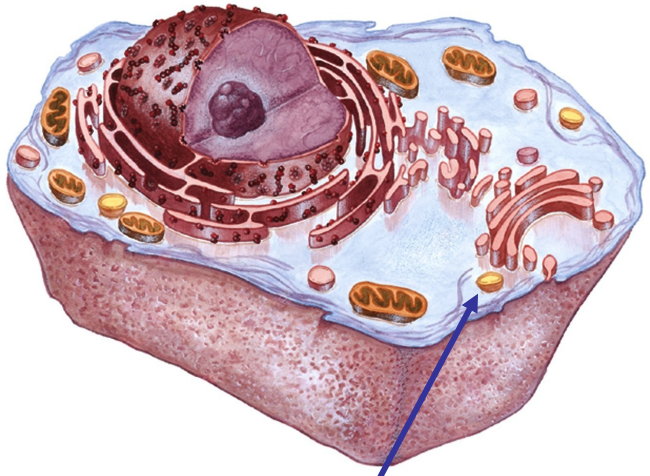
One of the CH_3-group of the deoxyadenosyl moiety is returned to the product-like radical.
→ formation of product

The Co-C bond reforms.
→ destruction of the free radical
→ Co^{2+} is regenerated



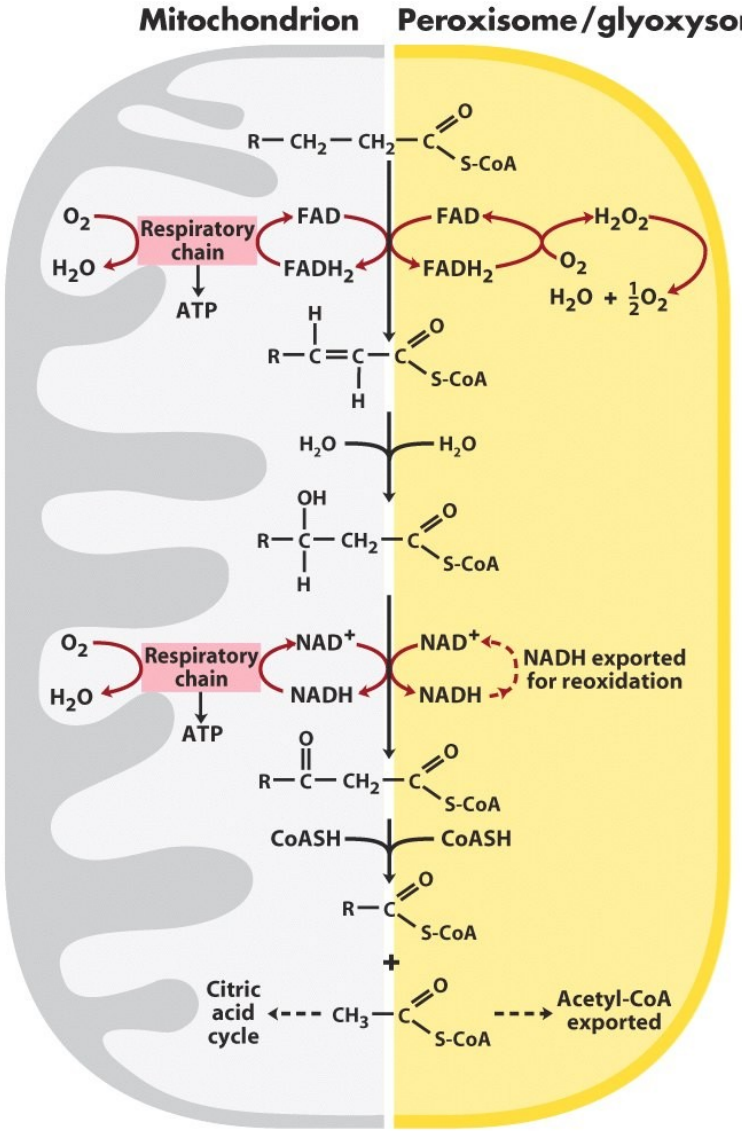
Peroxisomal β Oxidation

The β-oxidation of fatty acids also occurs in peroxisomes. Peroxisomal β-oxidation in animals functions to shorten very long chain fatty acids (> 22 C atoms).



Peroxisomes (microbodies) are membrane-enclosed organelles (0.5 µm)

Peroxisomal β Oxidation



The intermediates are coenzyme A derivatives.

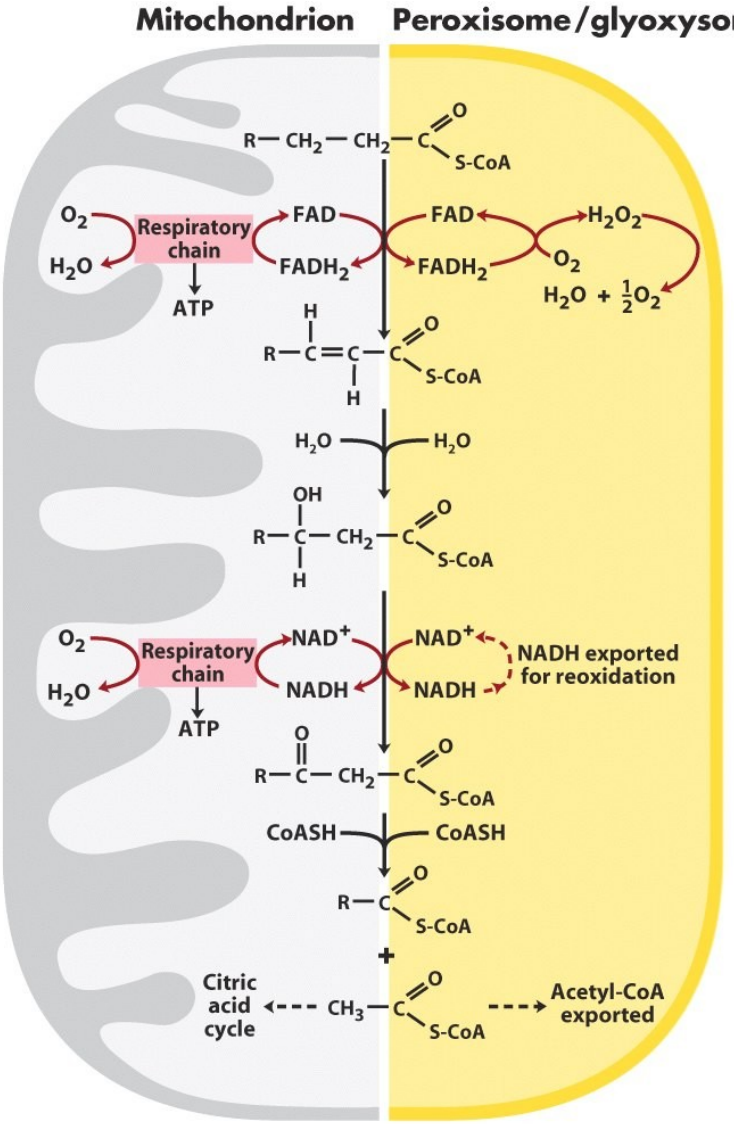
The process consists of four steps:

1) **dehydrogenation**
2) **addition of H_2O**
3) **oxidation of β-hydroxyacyl-CoA**
4) **thiolytic cleavage by CoA**

Differences:
Flavoprotein acyl-CoA oxidase passes electrons directly to O_2 producing H_2O_2

H_2O_2 is immediately cleaved by catalase $\rightarrow H_2O + O_2$

Peroxisomal β Oxidation



Differences:

Flavoprotein acyl-CoA oxidase passes electrons derectly to O_2 producing H_2O_2

H_2O_2 is immediately cleaved by Catalase → $H_2O + O_2$

The import of very long chain fatty acids does not require carnitine and its more active for very long chain fatty acids.

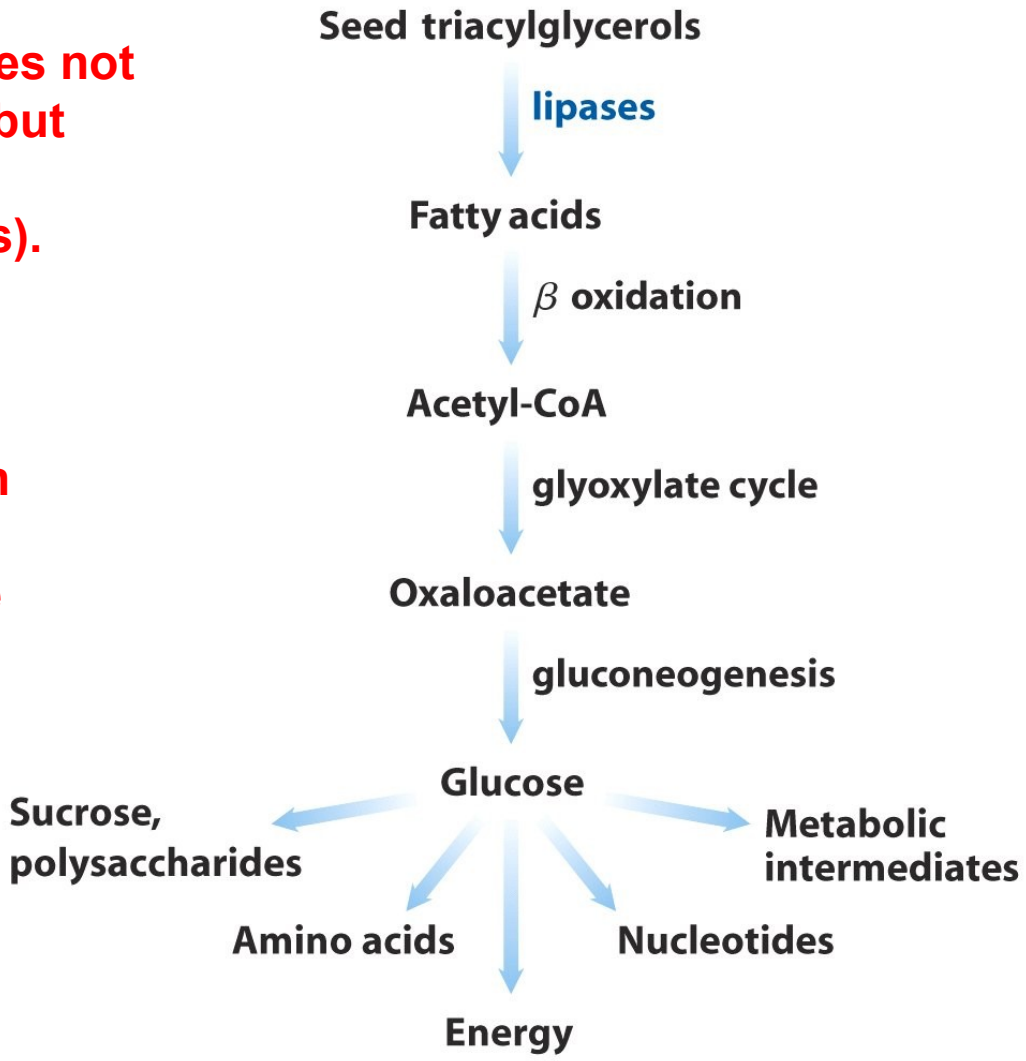
Peroxisomal enoyl-CoA hydratase and 3-L-hydroxyacyl-CoA DH activties occur on a single polypeptide.

Thiolase has a different chain length specificity than the mito counterpart. → almost inactive for C_8 or less.

β Oxidation in Plants

Fatty acid oxidation in Plants does not primarily occur in mitochondria but in peroxisomes (leafs) and in glyoxysomes (germinating seeds).

The biological role of β oxidation in these organelles is to used stored lipids primarily to provide biosynthetic precursors, not energy.



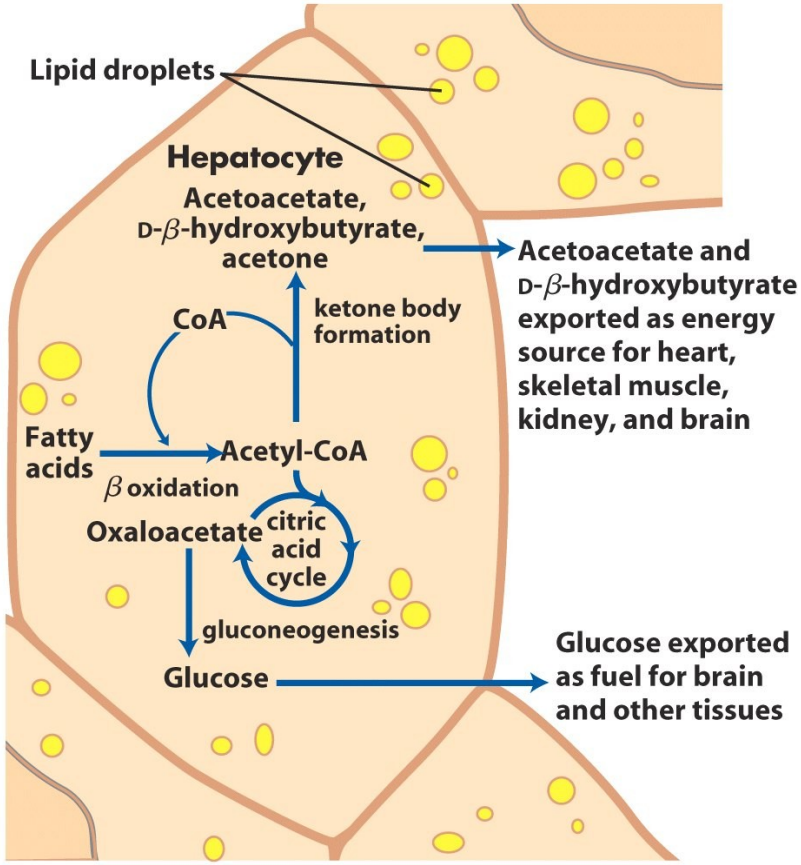
Ketone Bodies – An Alternative Fuel

In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can:

- enter the citric acid cycle

or

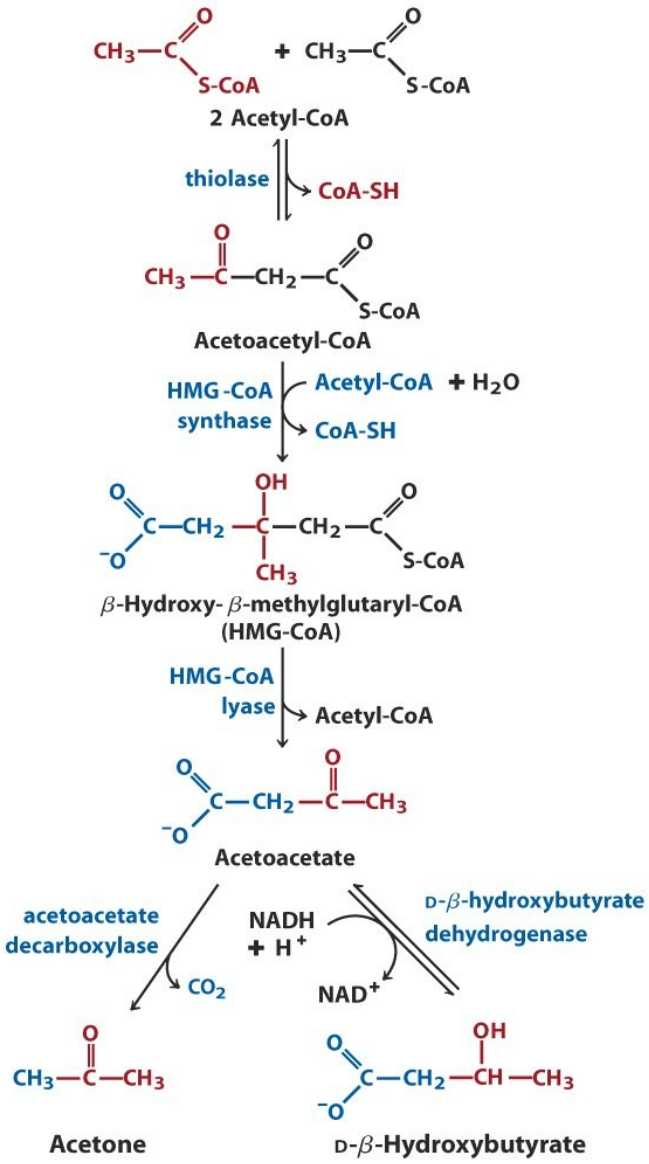
- be converted to ketone bodies (acteone, acetoacetate, and D-β-hydroxybutyrate)



Ketone bodies are exported to extra-hepatic tissues (e.g. brain)
→ conversion to acetyl CoA

Ketone bodies allow continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle

Ketone Bodies – An Alternative Fuel



Step 1: Formation of acetoacetate
→ condensation of to acetyl-CoA
→ Thiolase

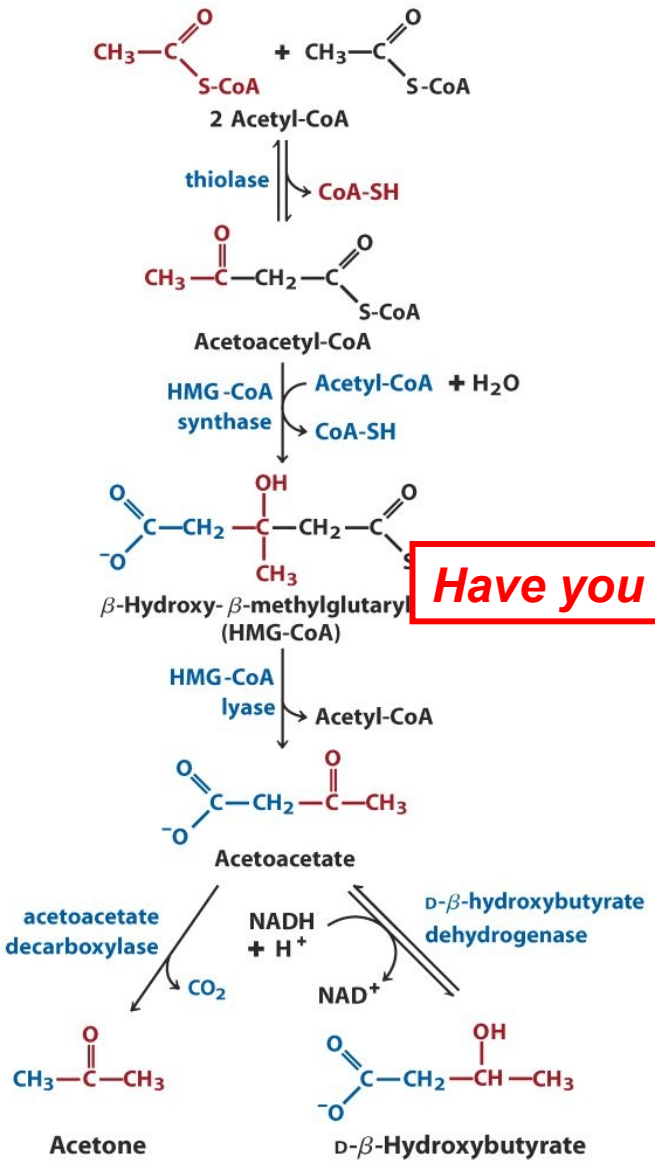
Step 2: β-hydroxy- β-methylglutaryl-CoA (HMG-CoA) synthase catalyses the condensation of acetyl-CoA with acetoacetyl-CoA.

Step 3: β-hydroxy- β-methylglutaryl-CoA is cleaved by HMG-CoA lyase to form acetyl-CoA and Acetoacetate

Step 4a: Acetoacetate is decarboxylated to form Acetone

Step 4b: Acetoacetate is reduced to D-β-hydroxybutyrate.
→ the DH is specific for the D form

Ketone Bodies – An Alternative Fuel



Step 1: Formation of acetoacetate
→ condensation of to acetyl-CoA
→ Thiolase

Step 2: β-hydroxy- β-methylglutaryl-CoA (HMG-CoA) synthetase catalyses the condensation of acetyl-CoA with acetoacetyl-CoA.

Have you seen them before ?

Hydroxy- β-methylglutaryl-CoA is cleaved by HMG-CoA lyase to form acetyl-CoA and Acetoacetate

Step 4a: Acetoacetate is decarboxylated to form Acetone

Step 4b: Acetoacetate is reduced to D-β-hydroxybutyrate.
→ the DH is specific for the D form

Ketone Bodies – An Alternative Fuel

D-β-Hydroxybutyrate as a fuel:

In extrahepatic tissue
D-β-Hydroxybutyrate is oxidized to acetoacetate.

Acetoacetate is activated to Acetoacetyl-CoA by transfer from succinyl-CoA.
→ β-ketoacyl-CoA transferase.

Cleavage by thiolase yields two acetyl-CoAs → citric acid cycle