Bryan Krantz: University of California, Berkeley
MCB 102, Spring 2008, Metabolism Lecture 10
Reading: Ch. 18 of *Principles of Biochemistry*, "Amino Acid Oxidation and the Production of Urea."

Syllabus Adjustment.

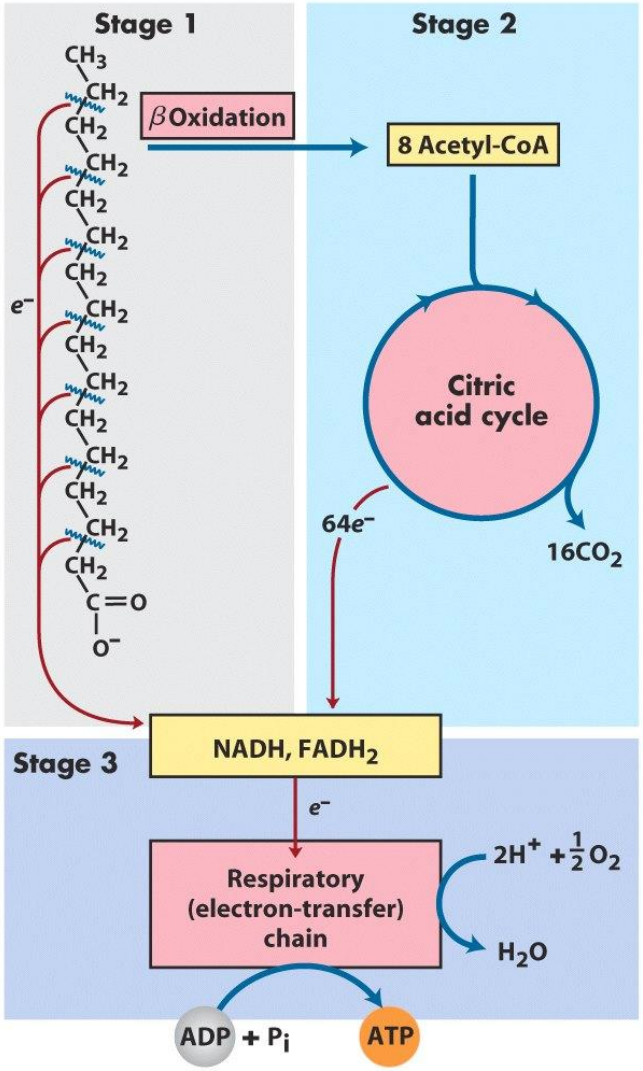
LECTURE 11	MON MAY 9	A.A. DEG & OX-PHOS	CHS. 18 & 19
LECTURE 12	WED MAY 7	OX-PHOS	CH. 19
LECTURE 13	FRI MAY 9	OX-PHOS & FATTY ACID SYNTH	CH. 19 & 21
LECTURE 14	MON MAY 5	NUCLEOTIDE DEG & SYNTH	CH. 22

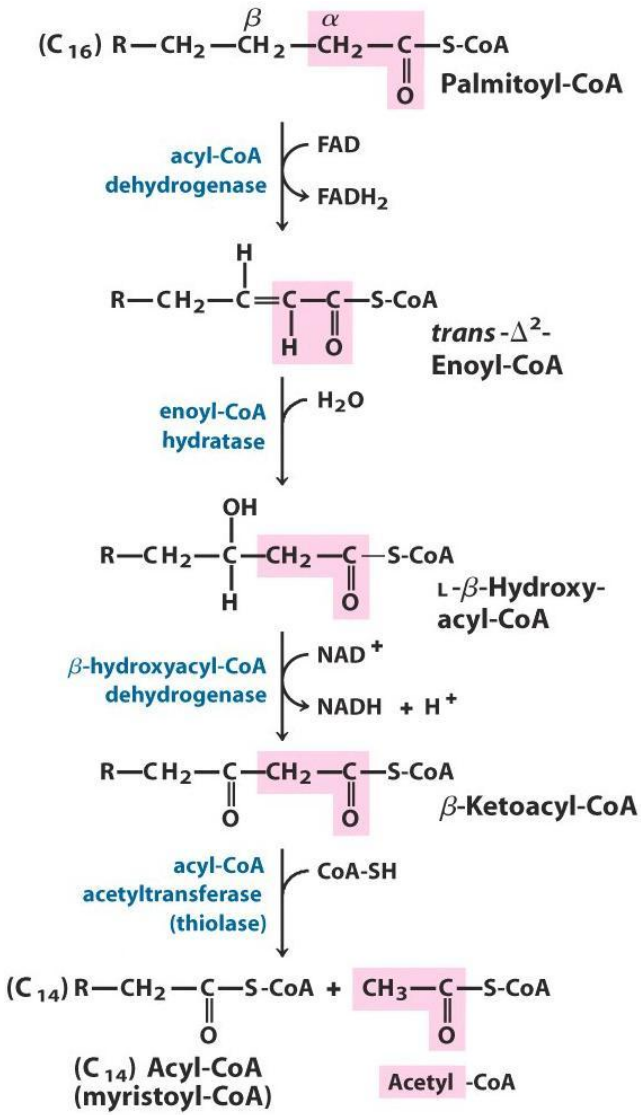
EXAM

[1] The room has changed for the final on Sat. May 17th from Wheel Aud to 100 Haas Pavil.

[2] The exam length is 1.5 hrs and covers just the last 3rd of the course on metabolism. (Prior concepts from earlier in the course may appear as part of the questions due to overlap with metabolism.)

β Oxidation of Fatty Acids. Analogous to the succinate → oxaloacetate steps in Citric Acid Cycle.





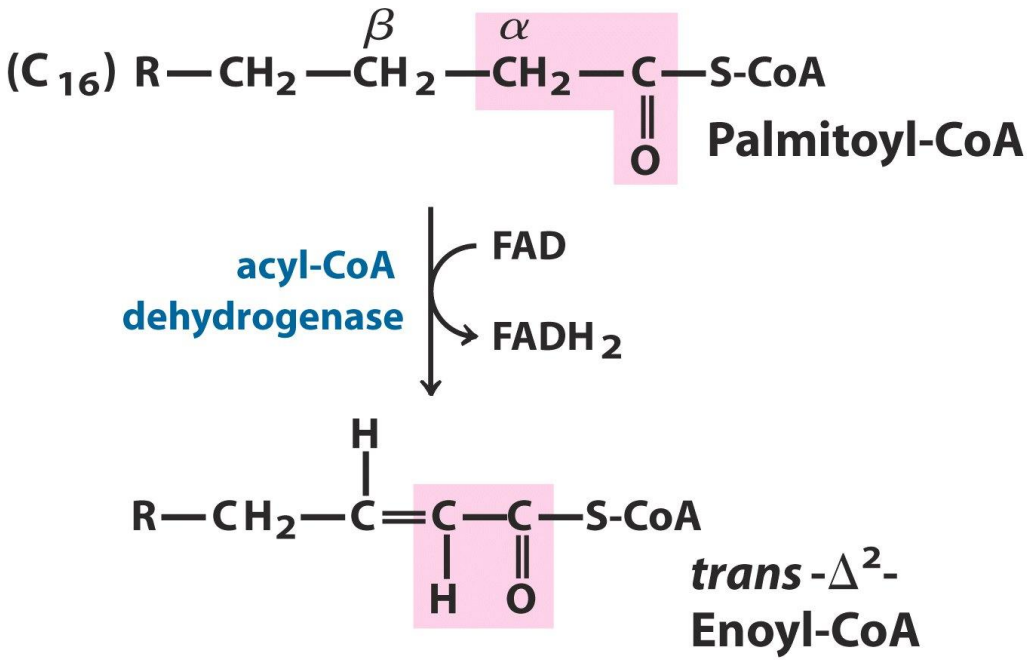
Acyl-CoA dehydrogenase.

Acyl-CoA + FAD → trans-Δ^2-enoyl-CoA + $FADH_2$

Mechanism. Hydrogen abstracted from the β carbon. If you take away hydrogen from this carbon, you end up producing trans-Δ^2-enoyl-CoA. You will notice that this reaction is pretty similar to what we have just seen in the citric acid cycle. FAD is the electron acceptor.

In the citric acid cycle, there is a very similar reaction. You start with succinate and end up with fumarate that has a trans double bond. When we talked about succinate dehydrogenase, we said that the succinate/fumarate pair is not strongly reducing, so you cannot reduce NAD^+ by using this reaction.

We use FAD, which is connected all the way to oxygen. This is precisely what happens here with acyl-CoA dehydrogenase. This enzyme is coupled to the reduction of FAD to $FADH_2$. A succession of electron carriers that are ultimately linked to O_2, which oxidizes $FADH_2$.

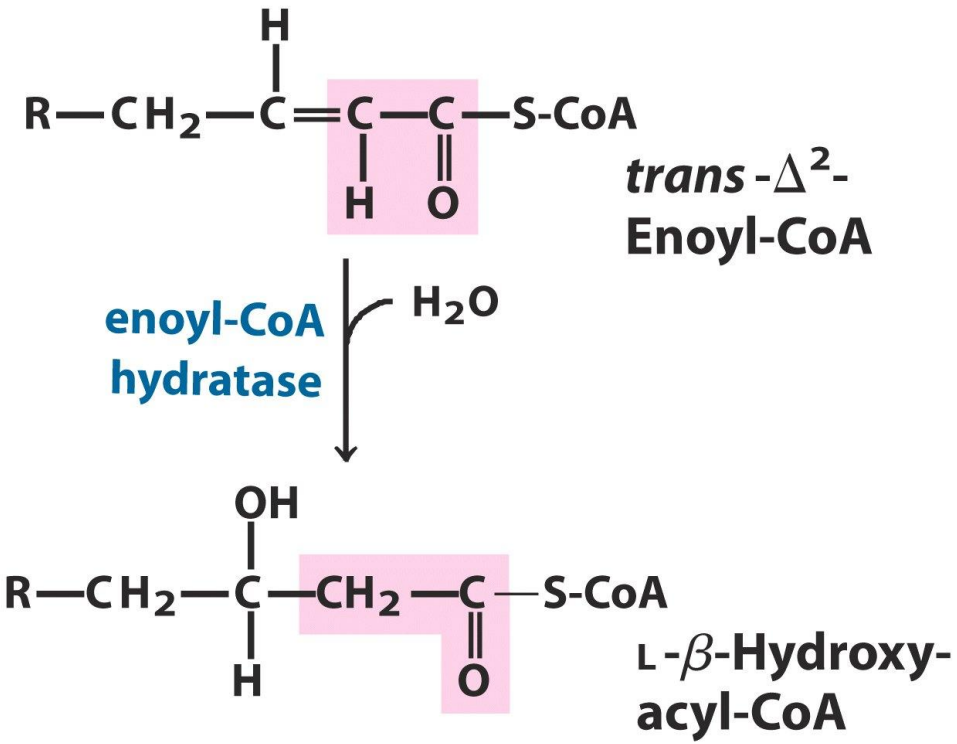


Enoyl-CoA hydratase.

Remaining parallel to the citric acid cycle, we do a hydratase reaction and add H_2O across the double bond.

trans-Δ^2-enoyl-CoA + H_2O → L- β-hydroxy-acyl-CoA

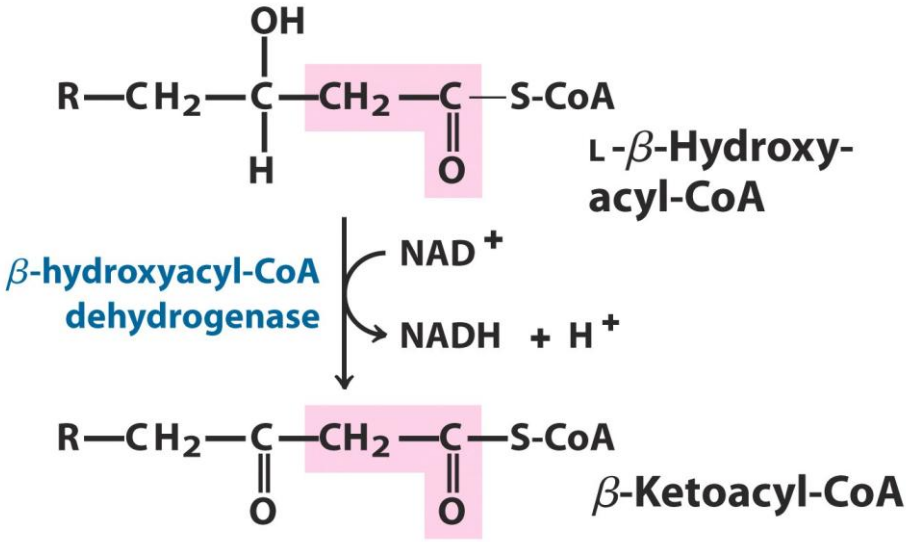
In the citric acid cycle, what happened to fumarate? There was an addition of water across the double bond. Here hydroxy-fatty acid is produced. This is L-β-hydroxy-acyl-CoA. This enzyme is called **enoyl-CoA hydratase** for catalyzing the addition of water across the double bond.



β-hydroxy acyl-CoA dehydrogenase.

L- β-hydroxy-acyl-CoA + NAD^+ →β-Ketoacyl-CoA + NADH

What happened to malate in the citric acid cycle? There was malate dehydrogenase and the same thing happens here. Oxidize malate to oxaloacetate by NAD^+ to generate NADH. The final product is like oxaloacetate and has a keto group. You will generate a β-keto-acyl-CoA. This is the final product that we get through the cycle of the β-oxidation pathway.

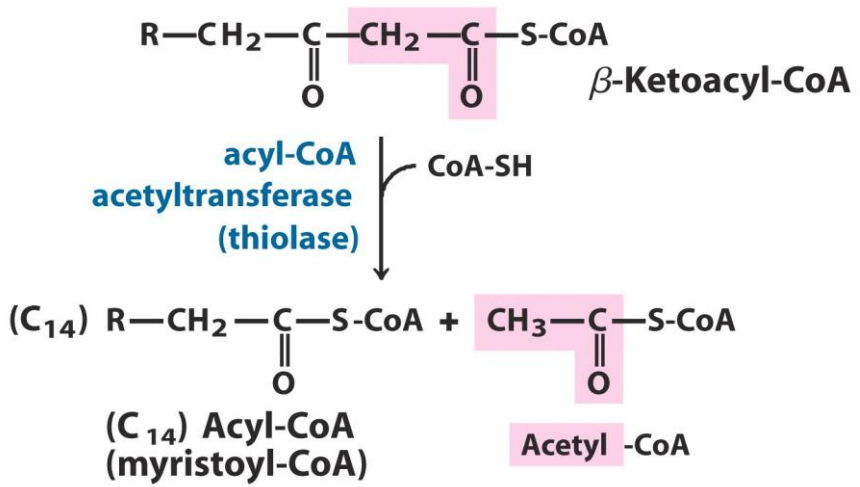


Thiolase

β-Ketoacyl(C_N)-CoA + CoA → β-Ketoacyl(C_{N-2})-CoA + Acetyl-CoA

Mechanism. The ketone functional group at the β-carbon makes the acyl chain susceptible to attack by an incoming HS-CoA.

Thus we get acetyl-CoA—the major product of the β-oxidation pathway.



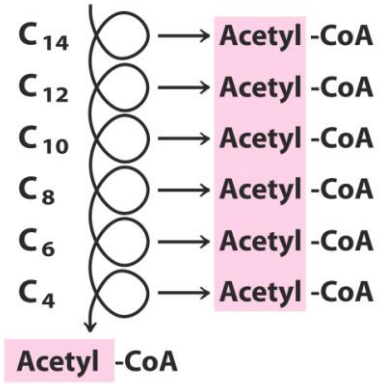
Energy Bookkeeping.

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

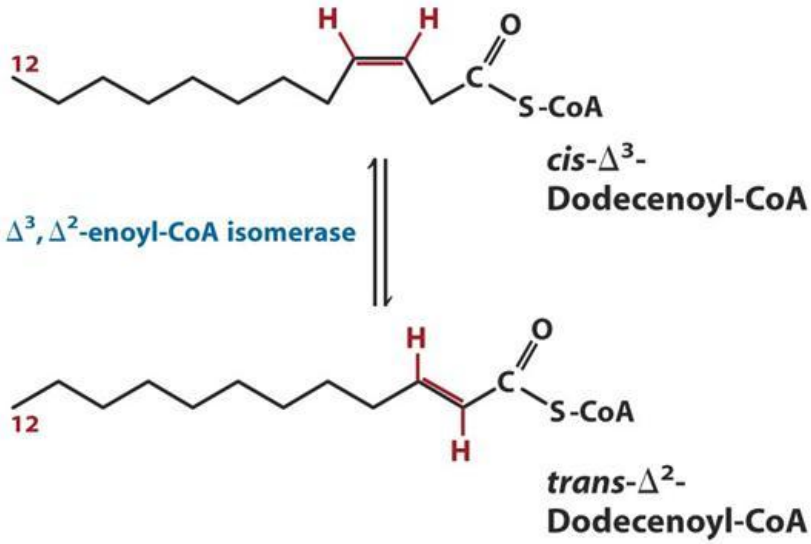
Saturated Fatty Acids are Broken Down in Pairs

Basically, the number of Acetyl-CoAs made is the chain length divided by two. This is how the body does it and all lipids made in humans are even-number fatty acid chain lengths.



Unsaturated, Branched & Other Odd Fatty Acids.

Unsaturated. For **unsaturated** sites, β-oxidation proceeds to the desaturation site, i.e., the double bond. Natural unsaturated fats are *cis* across the double bond; but β-oxidation uses *trans* double bonds after the acyl-CoA dehydrogenase step. Thus an isomerase enzyme is used, **Δ^3,Δ^2-enoyl-CoA Isomerase**.

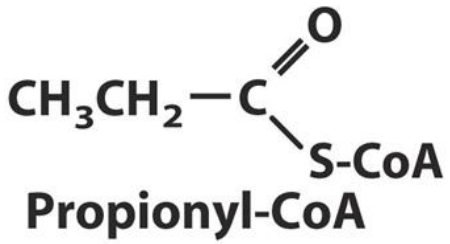


Branched. Oxidation is very modified for **branched** fatty acids. Here these branches prevent β-oxidation, and they require α-oxidation, which occurs in the peroxisome.

Phytic acid—is a precursor for fat soluble vitamins E and K_1 and is also a decomposition product of chlorophyll.

Refsum's Syndrome (a neurological disorder) may be caused by accumulation of phytanic acid due to a genetic deficiency in phytanoyl CoA hydroxylase.

Odd Carbons



While we cannot make odd-carbon-numbered, fatty acids, plants can. When you eat your vegetables, you can break down odd chains using β-oxidation, BUT at the end you will have **propionyl-CoA** instead of acetyl-CoA. So the math, for a C_N-length chain, when *N* is odd, is:

C_N-Fatty acid → (N-3)/2 Acetyl-CoA + 1 Propionyl-CoA

The math here is easy but the biochemistry is involved. How is propionyl-CoA broken down?

Propionyl-CoA carboxylase

At the beginning, you add the CO_2 to produce a four-carbon compound, **D-methyl-malonyl-CoA**. At the end of this conversion step. The enzyme uses biotin as a cofactor and activates bicarbonate to perform the carboxylation reaction. (Same as the pyruvate carboxylase enzyme basically.)

Propionyl-CoA + ATP + HCO_3^- →D-Methyl-malonyl-CoA + ATD + Pi

Epimerase. Remember sometimes a stereocenter needs to be flipped. Here a specific enzyme, **methyl-malonyl-CoA epimerase** changes the chirality from *D* to *L*.

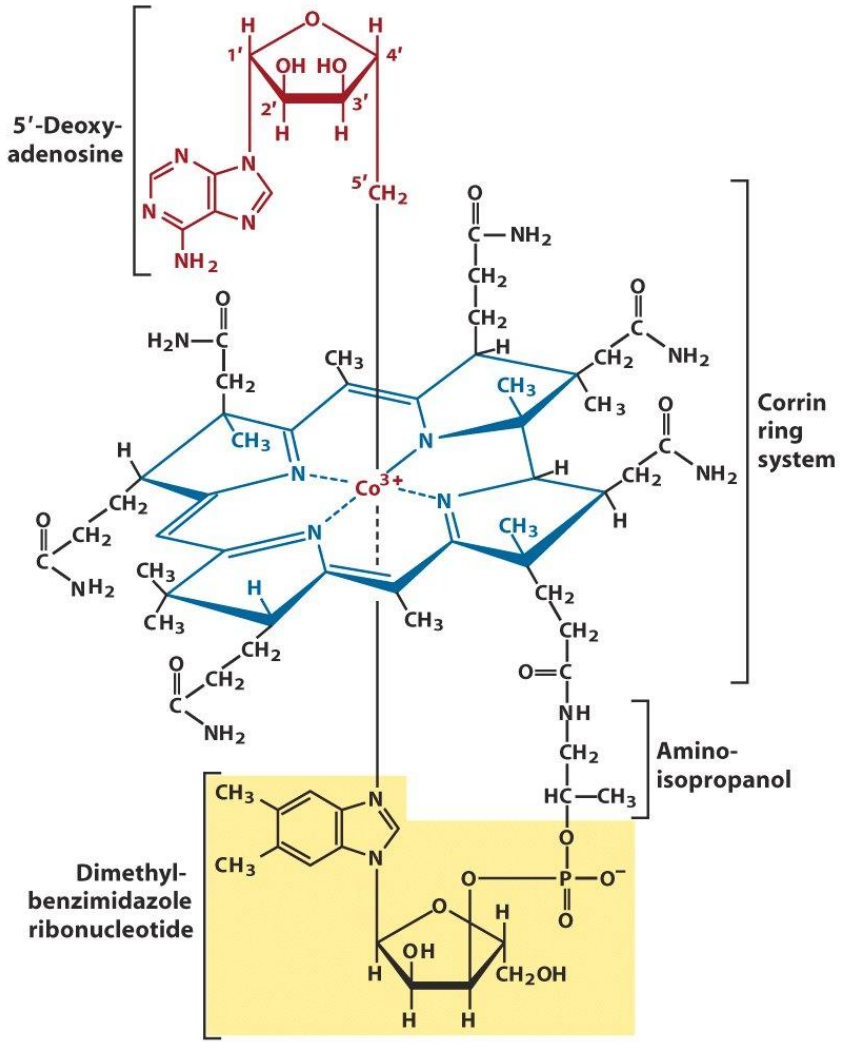
***D*-Methyl-malonyl-CoA ←→ *L*-Methyl-malonyl-CoA**

Methyl-malonyl-CoA mutase.

L-Methyl-malonyl-CoA → Succinyl-CoA

Dorothy Crowfoot Hodgkin
1910–1994

B_{12} co-enzyme. This enzyme requires vitamin B_{12} studied by the late **Prof. Barker**, after whom Barker Hall is named. Also **Dorothy Crowfoot Hodgkin** solved the X-ray crystal structure of vitamin B_{12} and received the Nobel Prize for her efforts. This cofactor contains Co^{3+}, heme, deoxyadenosine, and another usual nucleotide base (dimethyl-benzimidazole ribonucleotide.)

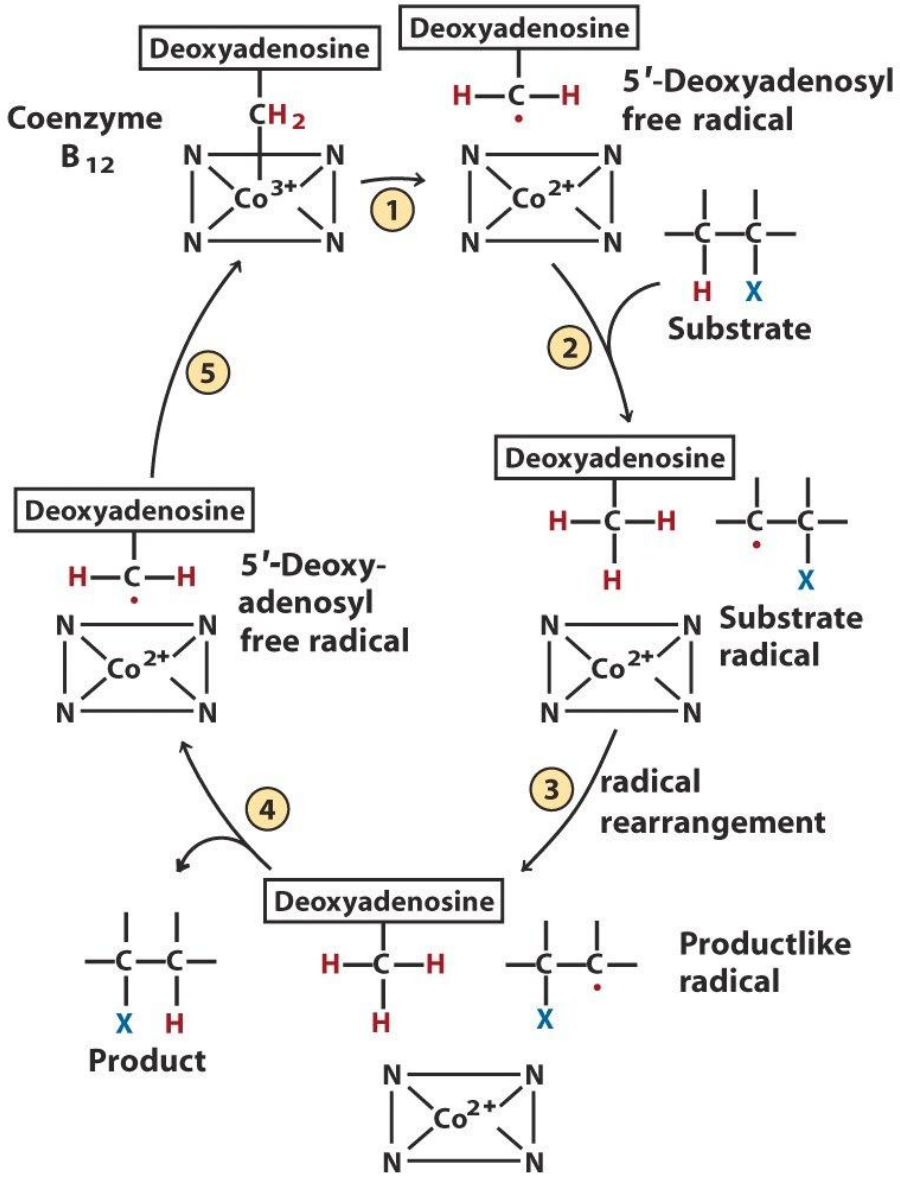


Something this complicated can only be made by microbes (and only a few can make it.)

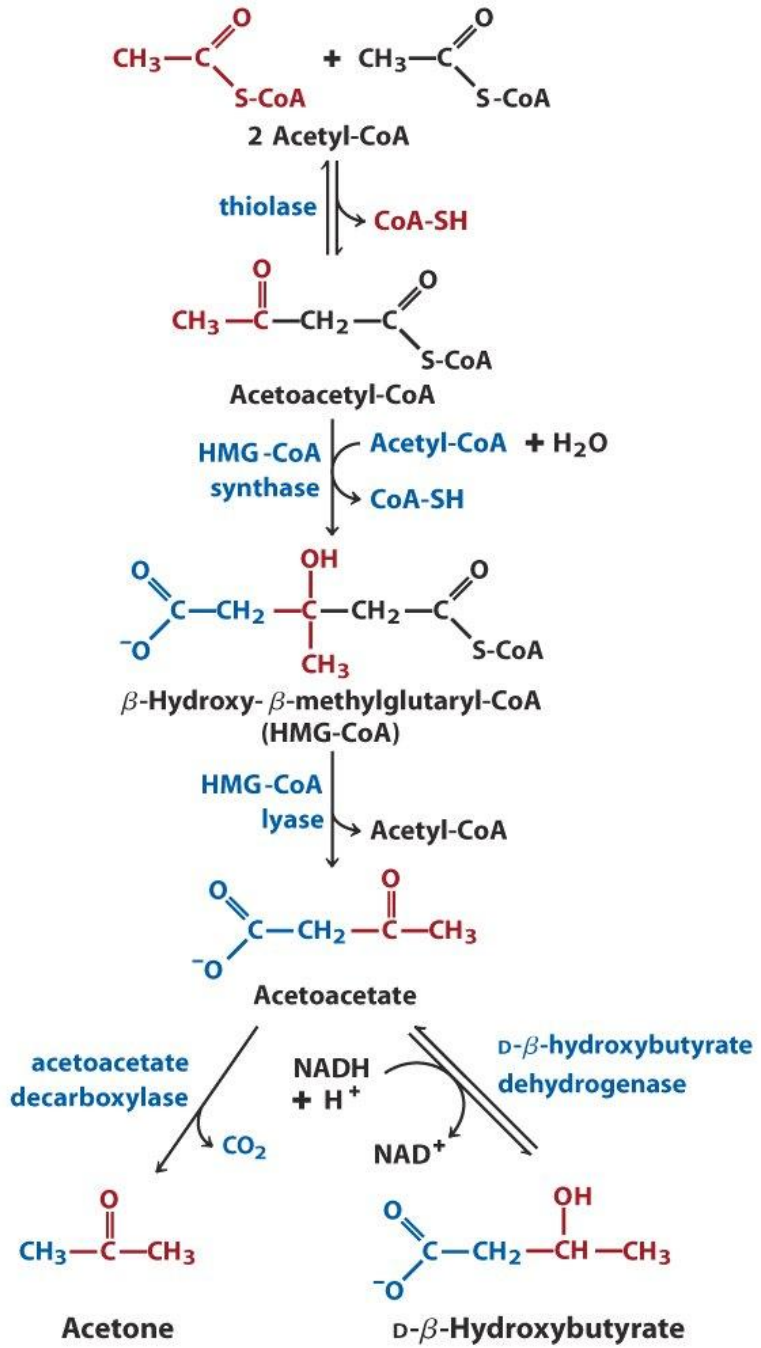
Relax, the first B_{12}-producing soil bacteria, *Clostridium tetanomorphum*, was isolated from the mud of San Francisco Bay in 1959. We can get our B_{12} supplement there. Plants do not make B_{12} perhaps, because it is light sensitive.

Pernicious anemia—a reduction in hemoglobin and red blood cell count attributed to the failure to absorb B_{12} in the intestine, reflecting the fact that this stuff needs to be adsorbed from the outside world.

The Radical Mechanism of Methyl-malonyl-CoA Mutase. B_{12} coenzyme works totally different from any other enzyme. This movement of the carbonyl-CoA group takes place through a **free radical** mechanism.



Ketone Bodies. If acetyl-CoA is overproduced by fat degradation, you do not have too much of three-carbon or four-carbon compounds. You have an oxaloacetate deficiency. All of the acetyl-CoA cannot enter the citric acid cycle for further oxidation. There will be a super-abundance of acetyl-CoA that will result in the generation of **ketone bodies**. Ketone bodies are only formed in the liver. Ketone bodies are not insoluble as the word, "body," implies, but they are highly soluble and can be delivered to **extrahepatic tissues**—those outside the liver.



● Ketone Bodies are fuels for the extrahepatic tissues: Brain, heart, kidney and skeletal muscle.

● The production of ketone bodies starts with the generation of acetoacetate, which you can imagine as occurring by the condensation of the two acetyl-CoA molecules. But this is a bit more complex.

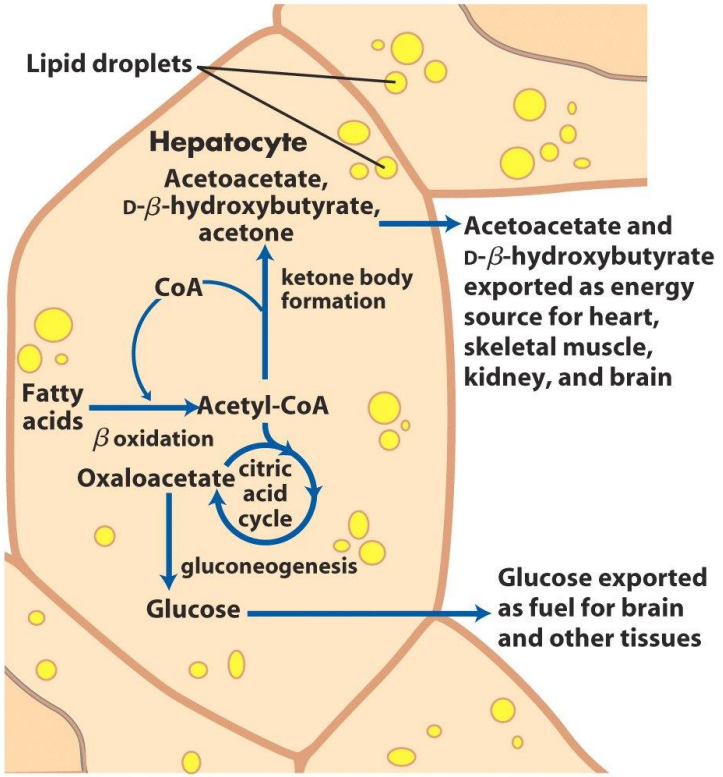
● The reverse process (catalyzed by some new enzymes) occurs in the extrahepatic tissues to regenerate the Acetyl-CoAs to be used as a fuel source.

● By this pathway the liver can continue to degrade fatty acids to acetyl-CoAs, recycling the CoA, and exporting a fuels to tissues that need energy.

Starvation and Untreated Diabetes. These conditions leads to the formation of **ketone bodies**. When people suffer from the generation of ketone bodies, or **ketosis**, their blood contains enough acetone so you can smell acetone on the breath and urine. Acetone comes from the spontaneous decarboxylation of acetoacetate to form acetone. The general release of a lot of β-hydroxy butyrate in the bloodstream changes the blood pH in extreme cases, which is called **acidosis**.

Starvation →Gluconeogenesis depletes CAC intermediates→Acetyl-CoA →Ketone bodies

Low insulin → Liver cannot get glucose from blood required to start fatty acid biosynethesis→
Increasing production of ketone bodies due to accumulation of Acetyl-CoA→
Extrahepatic tissue energy demand falls →accumulation of ketone bodies in blood →
ketosis and acidosis ensue



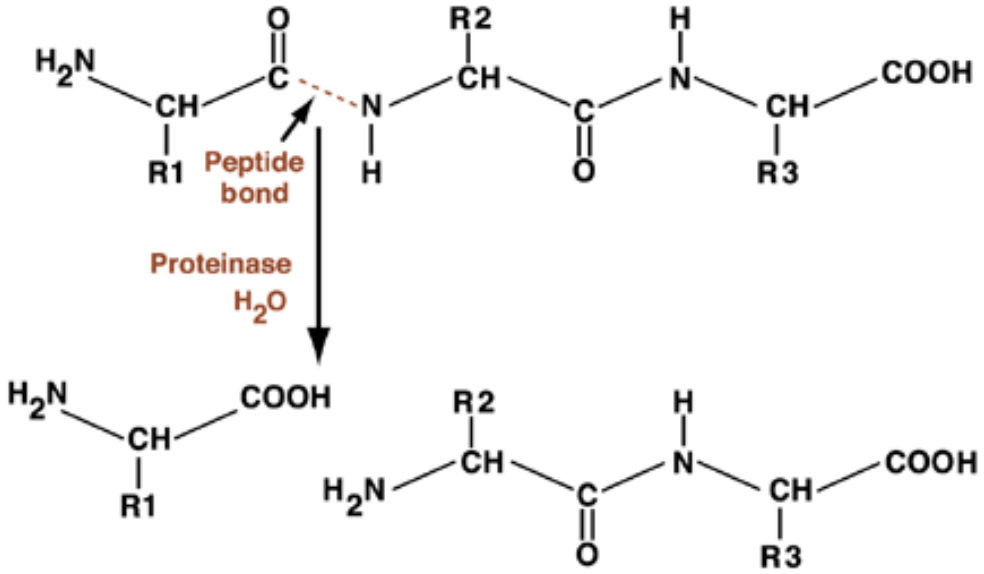
AMINO ACID DEGRADATION

● We utilize sugars, fat, *and proteins* as a source of energy.

● When proteins are used for energy, they first have to be degraded into amino acids, a hydrolysis reaction called **proteolysis**.

● Hormones stimulate the prancrease to release digestive enzymes. These degredative enzymes, called **proteinases** or **proteases**, can be digestive enzymes, like **pepsin**, **trypsin**, **carboxypeptidases**, and **chymotrypsin**. They act on the amide bonds in proteins via a hydrolysis reaction to produce smaller peptides and single amino acids.

● Remember proteases are dangerous to the cell, so these nasty digestive enzymes are secreted as **zymogens**, (trysinogen, pepsinogen, or any –ogen of a protease); zymogens are inactive forms, which then become active proteases after a piece of the protein is cleaved off. There are also inhibitors of these enzymes, like **basic pancreatic trypsin inhibitor**.



Transaminase

Amino acids are then utilized. The first step in utilization takes place with an enzyme called **transaminase** or **aminotransferase**. Transaminase starts with an amino acid and reacts it with a keto acid. The enzyme transfers the amino group from the amino acid to the keto acid to generate a keto acid from the original amino acid:

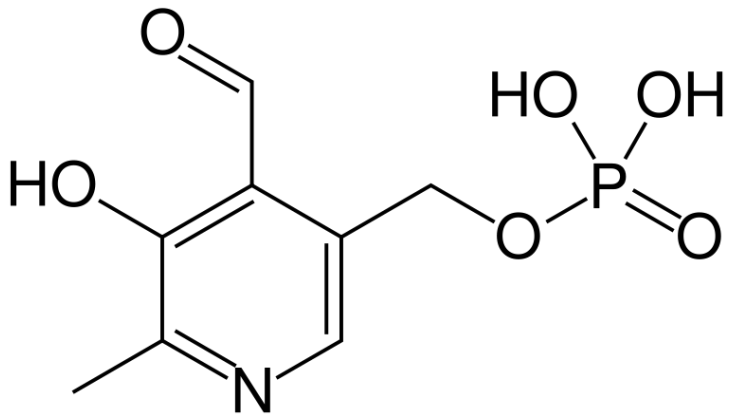
α-Ketoglutarate + Any amino acid ←→ Glutamate + Keto acid

The acceptor group now becomes an amino acid. *E.g.*,

α-Ketoglutarate + Alanine ←→ Glutamate + Pyruvate

The keto acid produced gets degraded into smaller parts and new >2-carbon skeletons are available for glycolytic intermediates; though in other cases, greasier amino acids generate acetyl-CoAs.

Pyridoxal phosphate cofactor. Transaminase works by using a coenzyme, **pyridoxal phosphate**, from vitamin B_6. Pyridoxal-phosphate has an aldehyde group.



Mechanism. When you have an aldehyde, as we have seen with aldolase, the carbonyl carbon tends to get attacked by the amino group of anything. In this case, the amino group of the free amino acid attacks this carbon. You produce a Schiff base with the aldehyde and a Lys residue in the active site; the amino acid substrate transaminates and exchanges with this Schiff base to make **pyridoxamine phosphate**.

Transaminase Mechanism.

Urea Cycle.

Overall energy requirement:

- **$NH_3 + CO_2$ + Aspartate + 3 ATP + 2 H_2O → Urea + Fumarate + 2 ADP + 4 P_i + AMP**

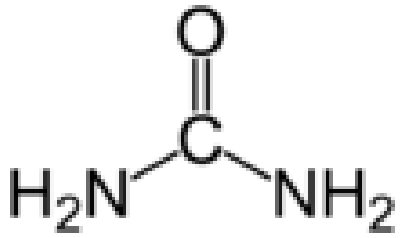
Overall equation of the urea cycle:

- **2 $NH_3 + CO_2$ + 3 ATP + H_2O → Urea + 2 ADP + 4 P_i + AMP + 2 H**

Why do it?

● Amino acids were degraded and excess ammonium is present.

● Ammonia is toxin to cells and must be excreted from the body.



Why urea?

● Urea is neither acidic nor basic, so it is a perfect vehicle for getting rid of nitrogen waste, also used an osmolyte by thekidney to readsorb water and useful ions.

● Other organisms can secrete ammonium directly or make less soluble solid forms (like uric acid) to reduce overall weight (important for birds for example).

Where?

● In the liver.

● Some steps occur in the liver mitochondria and others in the cytosol.

● Kidneys deal with the removal of excess urea from the blood.