Problem Set 8 (**Due April 1st**)

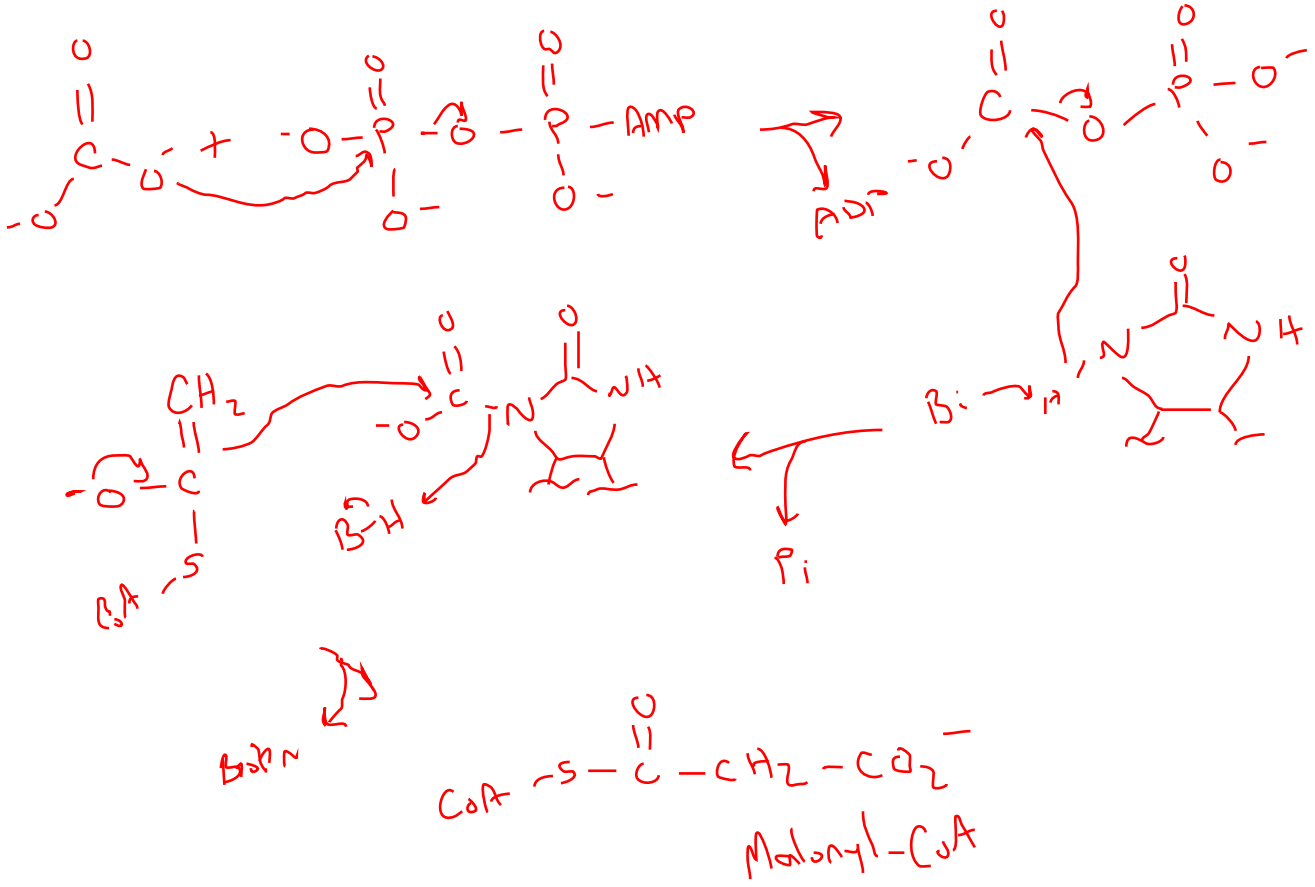
1. We have seen three different Carboxylase enzymes recently: pyruvate carboxylase, propionyl-CoA carboxylase, and acetyl-CoA carboxylase (ACC).
   a. What reaction does each of these enzymes catalyze and what process are they important in?

Pyruvate carboxylase: pyruvate → oxaloacetate — gluconeogenesis

Propionyl-CoA carboxylase : propionyl-CoA → methylmalonyl-CoA — odd chain fatty acid oxidation

Acetyl-CoA carboxylase: acetyl-CoA → malonyl-CoA — fatty acid synthesis

   b. Draw a mechanism for the acetyl-coA carboxylase catalyzed reaction.

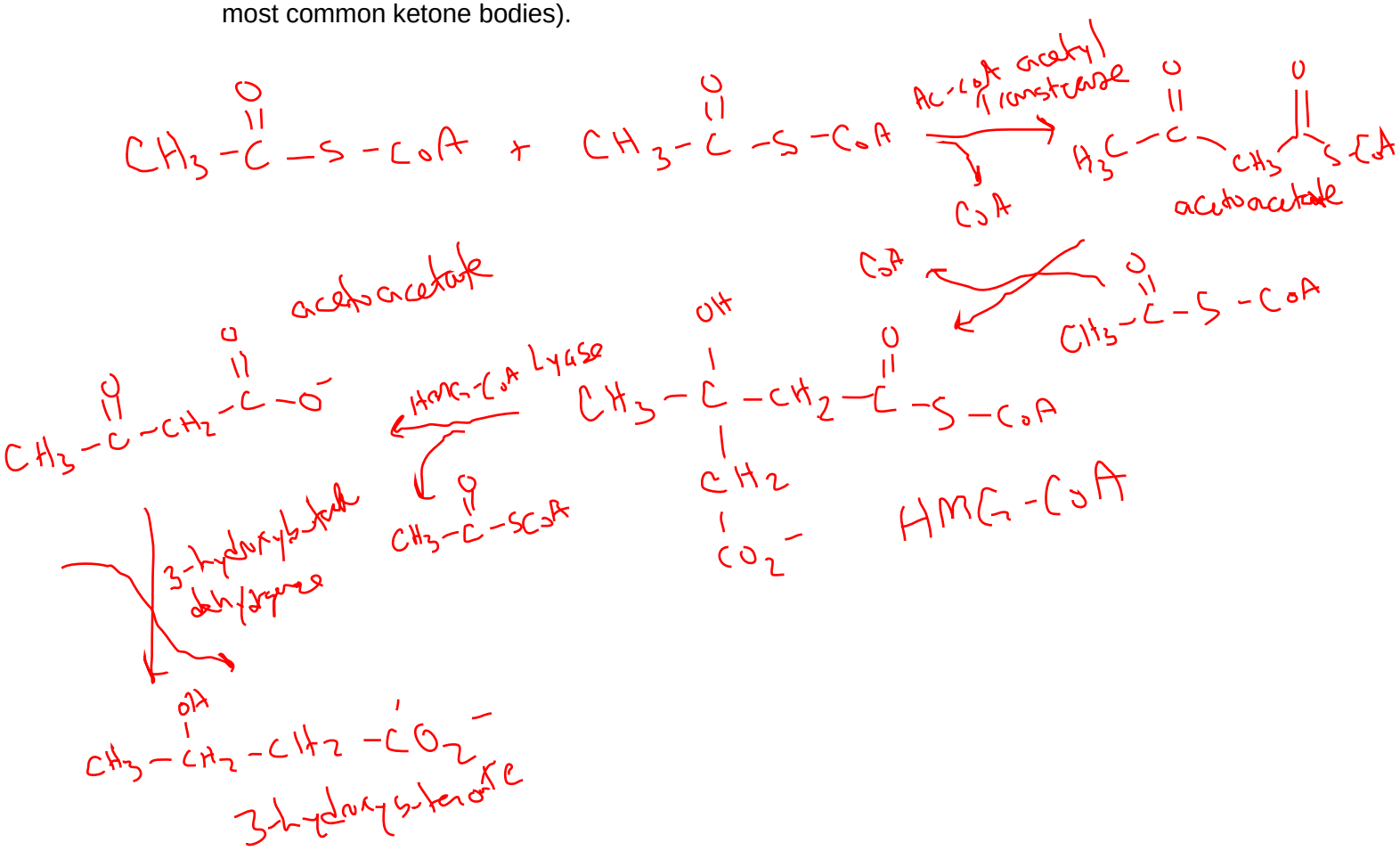


   c. Acetyl-CoA carboxylase and pyruvate carboxylase both put the CO_2 group at the end of a carbon chain, but propionyl-CoA puts the CO_2 on a central carbon. What makes propionyl-CoA different? Show a mechanism or reaction intermediate that supports your answer. Acetyl-CoA and pyruvate carboxylase both have the terminal carbon adjacent to a carbonyl, while propionyl-CoA does not. To stabilize the carbanion nucleophile (see mechanism above), it is necessary for this molecule to be in resonance with an enolate – the only way for this to happen with propionyl-CoA is to have the central carbon as the nucleophile.

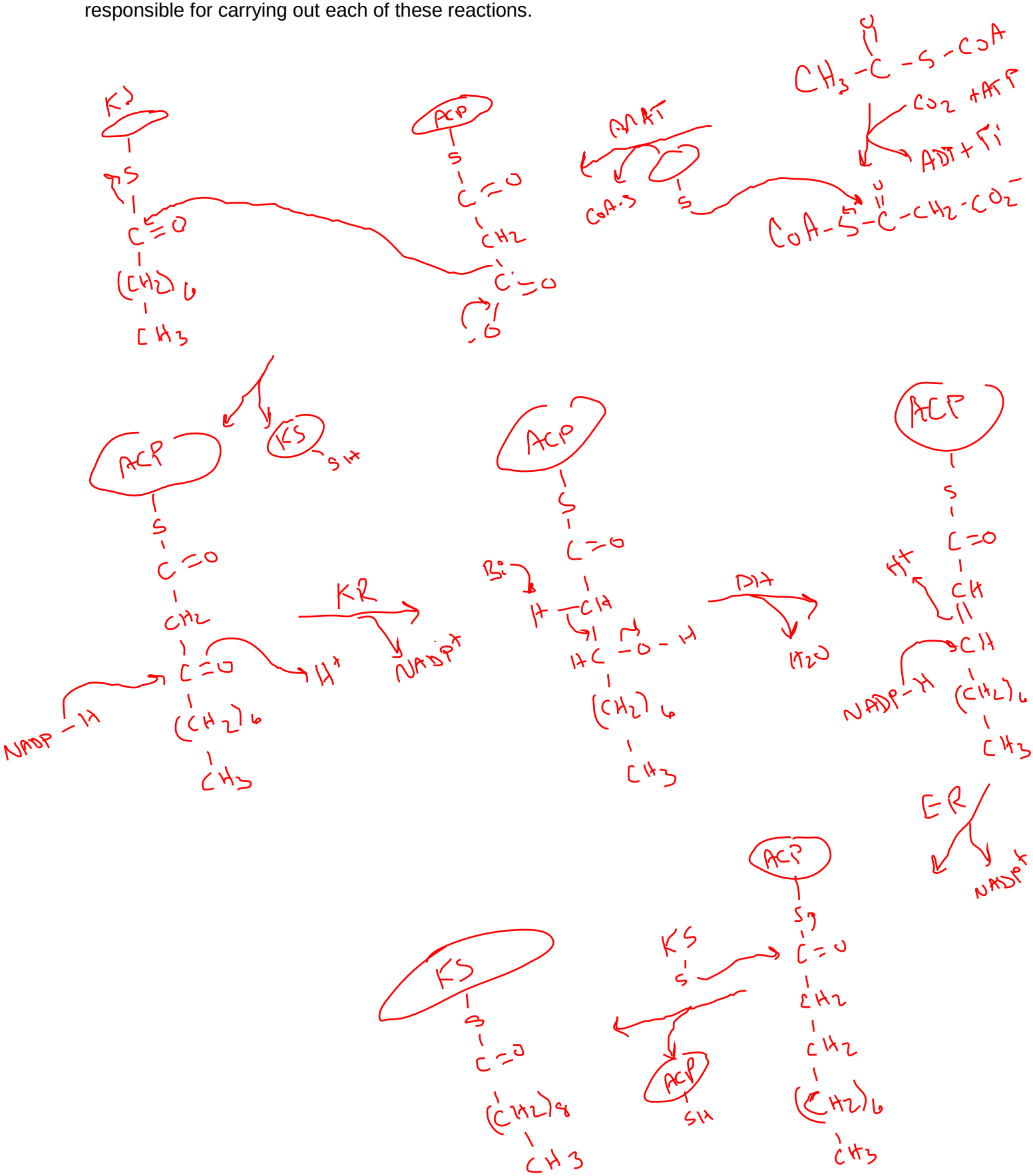
2. How are dietary triacylglycerides transported to myocytes or adipocytes? Dietary triacylglycerides are emulsified in the GI tract by bile salts. Intestinal lipases hydrolyze the triacylglycerols to free fatty acids which are taken up by the intestinal mucosa where they are repackaged as triacylglycerols. These are packaged with lipoporteins and cholesterol to form chlyomicrons and move through the lymphatic system. When they reach myocytes or adipocytes, they get hydrolyzed by lipases that are activated by the chylomicron surface proteins. The free fatty acids are transported across the plasma membrane for metabolism.

3. Ketone bodies can be produced from fatty acid degradation.
   a. Why are ketone bodies made? Very few molecules are allowed to cross the blood-brain barrier to provide energy for the brain; glucose and ketone bodies can. When dietary or stored sugar is not available to be sent to the brain, fat is broken down to provide fuel. However, mammals do not have the ability to convert acetyl-CoA into glucose, so acetyl-CoA is converted to ketone bodies instead.

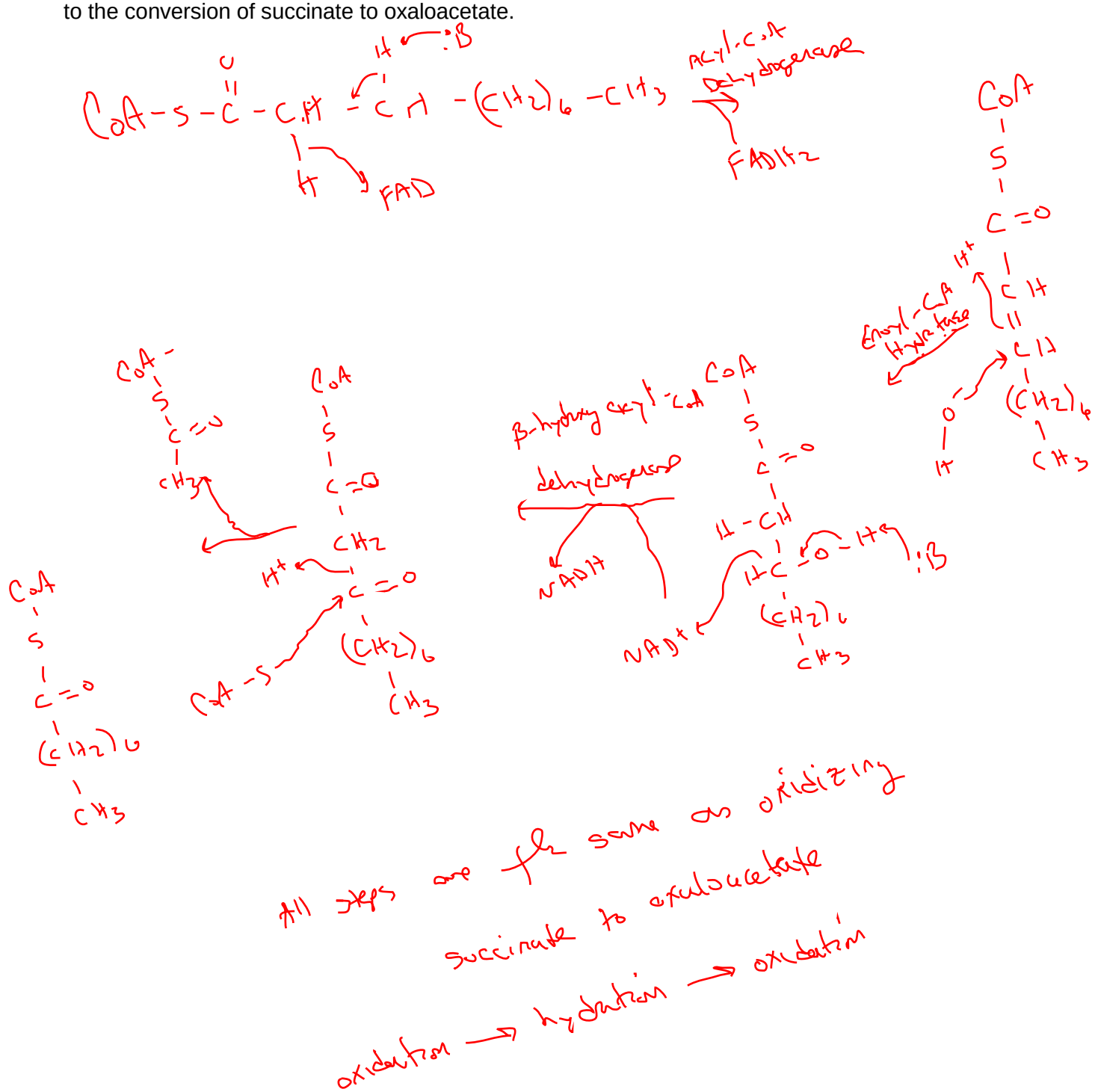
   b. Show a reaction scheme for the biosynthesis of Acetoacetate and β-hydroxybutyrate (the two most common ketone bodies).



4. Draw a mechanism for the conversion of an 8 carbon fatty acid to a 10 carbon fatty acid. Please make sure to show what the fatty acid is anchored to at all times. Please identify what domain of FAS is responsible for carrying out each of these reactions.



5. Show a mechanism for the conversion of a 10 carbon fatty acid to an 8 carbon fatty acid. Please identify the enzyme that catalyzes each reaction. Please also explain how these reactions are similar to the conversion of succinate to oxaloacetate.

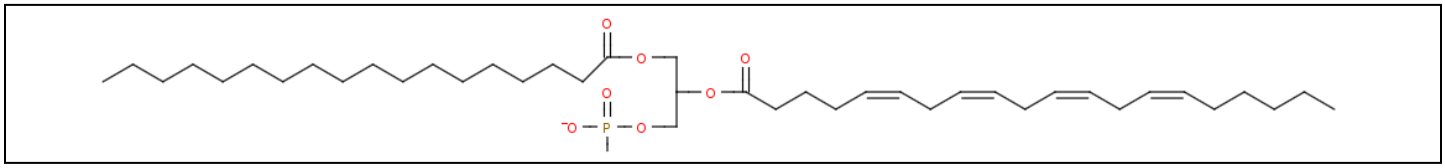


all steps are the same as oxidizing succinate to oxaloacetate

oxidation → hydration → oxidation

6. Consider Phosphatidylinositol-4,5-triphosphate (PIP_2).
   a. Where have we seen PIP_2 before? Please describe the role it played. PIP_2 is the lipid anchored form of IP_3 that we saw in the phosphoinositide cascade. An external signal stimulate the GPCR, which causes the dissociation of the G protein and the alpha subunit activates PLC. This enzyme, in turn, hyrdolyzes PIP_2 to DAG and the secondary messenger IP_3. IP_3 activates the Ca^{2+} channel in the ER and results in Ca^{2+} release into the cytosol. This activates a number of proteins including Protein Kinase C which phosphorylates lots of substrate proteins.

   b. Draw this molecule. Note that this molecule includes arachidonic acid and stearic acid at the appropriate positions on the glycerol backbone.



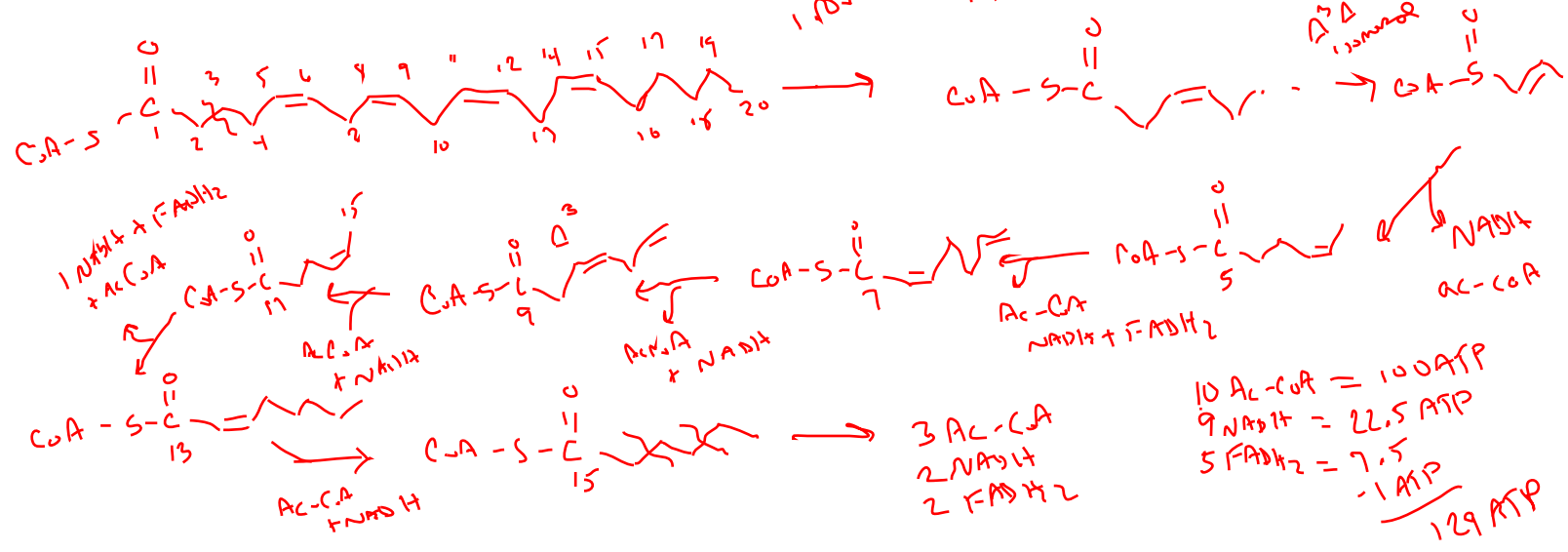
   c. What class of proteins can hydrolyze this molecule to produce free fatty acids? Phospholipase
   d. Describe the process of converting these free fatty acids to CO_2 and ATP. Note that the hydrolysis event (part c) occurs in the cytosol and β oxidation is in mitochondrial matrix.

Once phospholipase releases the two fatty acids, they are modified to acyl-CoA molecules by **Acyl-CoA Synthase.** Once modified, they can be transported to the mitochondria where they are taken into the matrix in a carnitine mediated fashion. Inside the matrix, they can be degraded to acetyl-CoA, NADH and $FADH_2$. Each of these can produce ATP energy. The electrons on NADH and $FADH_2$ can be directly moved into the Mito Electron Transport Chain, while Acetyl CoA will enter the TCA cycle.

   e. How many molecules of ATP can be generated from the complete oxidation of this molecule? Assume that the polar head group is recycled (so it does not get metabolized). Makes sure to consider the glycerol 3-phosphate backbone.

Backbone: 3-phosphoglycerol →DHAP (**+1 NADH**) → Pyruvate (**+2 ATP + 1 NADH**) → PDH and TCA (**+4 NADH + 1 $FADH_2$ + 1 ATP**) → **Total 19.5 ATP**

Stearic Acid: Activation – (**-1 ATP**) → 8 round of b-oxidation (**+8 NADH**) → 9 Acetyl-CoA → TCA Cycle (9x3 NADH = **27 NADH + 9 ATP + 9 $FADH_2$**) → **121 ATP**



7. Odd chain fatty acids result in a product other than Acetyl-CoA. What is this product and how does it get metabolized? How many ATP are produced from oxidation of propionyl-CoA? Propionyl-CoA is the product. This molecule gets carboxylated (**-1 ATP**) to form a 4 carbon molecule (methyl malonyl-CoA), racemerized and isomerized to get succinyl-CoA, a TCA cycle intermediate. Since we don't want to accumulate TCA cycle intermediates, the succinyl CoA needs to exit the cycle to be metabolized. This occurs after conversion to malate **(+1 ATP and +1 $FADH_2$)** using the Malic Enzyme, which generates pyruvate while producing 1 NADPH (in problem 8, we determine that 2.667 ATP are sacrificed per NADPH, so in this case, we save those ATP → **+2.67 ATP**). The pyruvate can now be shuttled through the PDH complex (**+1 NADH**) and TCA cycle (**+3 NADH +1 $FADH_2$ +1 ATP).**

   Total ATP: 4 NADH x 2.5 + 2 $FADH_2$ x 1.5 + 3.67 ATP -1 ATP **= 15.67 ATP**

8. Fatty acids are synthesized in hepatocytes using glucose as a carbon and fuel source.
   a. Describe how glucose can be used to synthesize palmitic acid. Please be clear with the steps that you show (i.e. if you use acetyl-CoA in your process, which you should, make sure to state where it comes from. Acetyl-CoA gets carboxylated by ACC to produce malonyl-CoA; this is the C_2 substrate for fatty acid synthesis. The Acetyl-CoA is produced via glycolysis and pyruvate dehydrogenase.
   b. Oleic acid can be condensed with glycerol-3-phosphate to make a lysophosphatidic acid. The glycerol-3-phosphate also derives from glucose. Please describe how. Glycerol-3-phosphate can be made from DHAP through a reduction of the C2 carbonyl to an alcohol .
   c. Determine how much ATP energy is sacrificed to make this lysophosphatidic acid. Make sure to account for the energy that could be made from the glucose that are consumed, any ATP that is directly consumed in the process, as well as the ATP that is sacrificed to make the NADPH that gets consumed.

For glycerol-3-phosphate backbone: DHAP → glycerol-3-phosphate: 1 NADH used, Sacrificed: 5 NADH, 1 FADH, 3 ATP → Total of **19.5 ATP** sacrificed

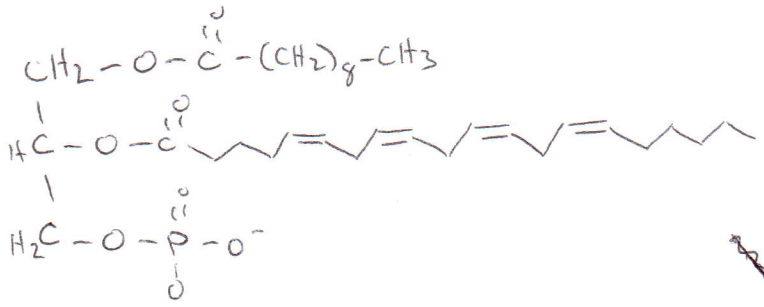
Palmitic acid is 16 carbons, so 8 Acetyl CoA are required. Each Acetyl-CoA would normally go into the TCA cycle and generate 3 NADH + 1 FADH2 + 1 ATP → total 10 ATP sacrificed per Acetyl-CoA x 8 = **80 ATP**

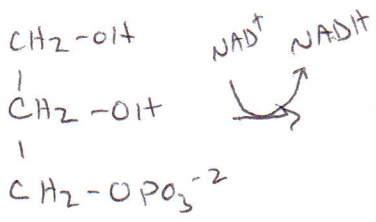
1st Acetyl-CoA does not need to be converted to Malonyl-CoA because it is directly transferred onto the KS domain. The other 7 are carboxylated by ACC, which requires 1 ATP each → **7 ATP**

Each round of elongation requires 2 NADPH, so 14 NADPH are needed. These are made from the Pentose Phosphate Pathway (reaction that matters: G6P + 6 $NADP^+$ ⇌ GAP + 3CO_2 + 6 NADPH). G6P would normally generate 33 ATP (10 NADH + 2 FADH2 -1 ATP + 6 ATP), but we lose out on 17 ATP from GAP (5 NADH + $FADH_2$ + 3 ATP) → 16 ATP per 6 NADPH = 2.667 ATP per NADPH *14 = **37.33 ATP**

I think that covers everything: **144 ATP** sacrificed to make 1-palmitate phosphatidic acid.

9. Fatty acid metabolism is controlled in a similar way that sugar metabolism is.
    a. Would you expect flux through each of the major pathways we've discussed (glycolysis, gluconeogenesis, glycogen synthesis, glycogen degradation, β oxidation, fatty acid synthesis) to increase or decrease when the ATP/ADP ratio is low? For each pathway, please justify why flux increases or decreases. What the ratio is high, there is an abundance of cellular energy; anabolic pathways (glycogen synthesis, gluconeogenesis, fatty acid synthesis), should be upregulated and catabolic pathways (glycolysis, β oxidation, glycogen degradation) are down regulated.
    b. Fatty acid synthesis and oxidation are regulated by hormone levels. In Chapter your book describes how glucagon and insulin are able to influence the activity of ACC. Please summarize these mechanisms. Glucagon activates PKA, which will phosphorylate ACC; this results in the inhibition of ACC and decreased synthesis of malonyl-CoA (hence decreased flux through fatty acid synthesis). Insulin activates a phosphatase that will dephosphorylate ACC and reactivate fat synthesis.
    c. Please comment on how ACC activity influences fatty acid oxidation. The product of the ACC catalyzed reaction is malonyl-CoA; this molecule is a potent inhibitor of carnitine-acyltransferase. Inhibition of this enzyme prevents fatty acids from be transported to the mitochondrial matrix and therefore prevents fatty acid oxidation.



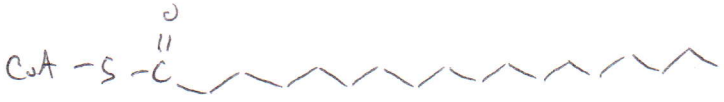


+ Stearic acid + arachodnic acid

6 NADH → 15
$FADH_2$ → 1.5
ATP → 3
19.5

Acetyl-CoA → TCA → 3 NADH, 1 $FADH_2$, 1 ATP

stearic acid: → activation (-1 ATP)

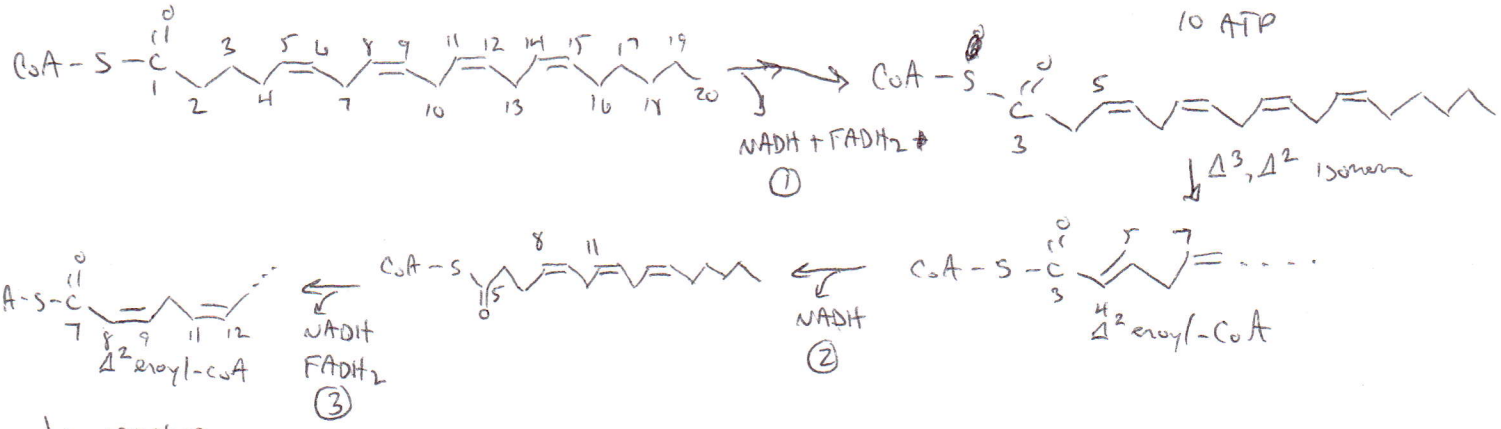


8 rounds of β-oxidation

8 NADH x2.5
8 $FADH_2$ x1.5
9 Acetyl CoA → TCA → 9x3 = 27 NADH x2.5
9 $FADH_2$ x1.5
9 ATP -1 ATP

87.5
25.5
8
121

arachodnic acid: activation (-1 ATP) → 10 Acetyl CoA produced → 30 NADH, 10 $FADH_2$, 10 ATP



Total from Arach. Acid

9 NADH
5 $FADH_2$
TCA: 30 NADH
10 $FADH_2$
10 ATP -1 ATP (activation)

39(2.5) + 15(1.5) + 9 = 129 ATP

2 NADH
2 $FADH_2$
2 rounds of β oxidation
⑧ ⑨ ⑩