**Problem Set 3** (Due February 4th)

1. In 1896, Christiaan Eijkman famously stated that "White rice can be poisonous!" Research this quote and determine how it relates to the material covered in this unit. Be specific – which enzymes are affected and how? Please note the date – this quote has nothing to do with the 2012 discovery that some rice has elevated arsenic concentrations. Eijkman spent some time in Jakarta, Indonesia and noted that many of the natives died from a disease we now know as beriberi. He made the connection that this was likely due to the white rice heavy diet. White rice has the husk stripped from it to make it easier to cook. By removing the husk, the nutritional content is also dramatically dampened (this is why we now fortify rice and other crops). Of particular note, the natives had a notable Vitamin B1 (also known as thiamin) deficiency. Thiamin is the precursor of the important cofactor TPP, which plays a critical role in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase.

2. We know that pyruvate is ultimately oxidized to 3 equivalents of CO_2.
   a. Starting with the average oxidation number on each carbon in pyruvate, determine how many electrons are produced during the oxidation to CO_2. Does this make sense based on the number of NADH and $FADH_2$ that are produced?
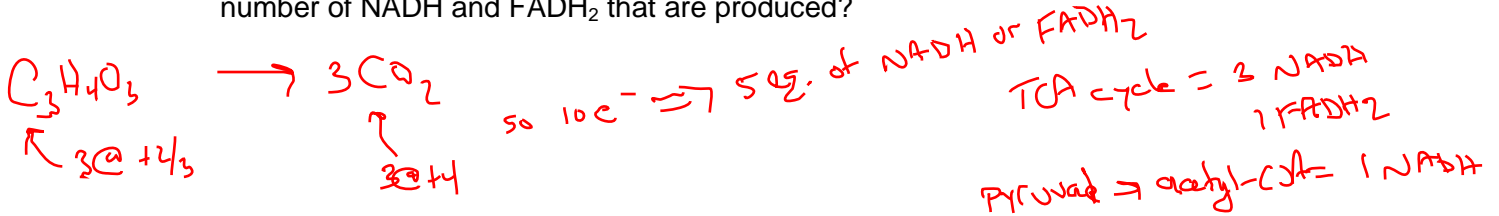
   b. Start with pyruvate. Number each carbon (C1, C2, C3). Trace the fate of each of these carbons through a single TCA cycle.
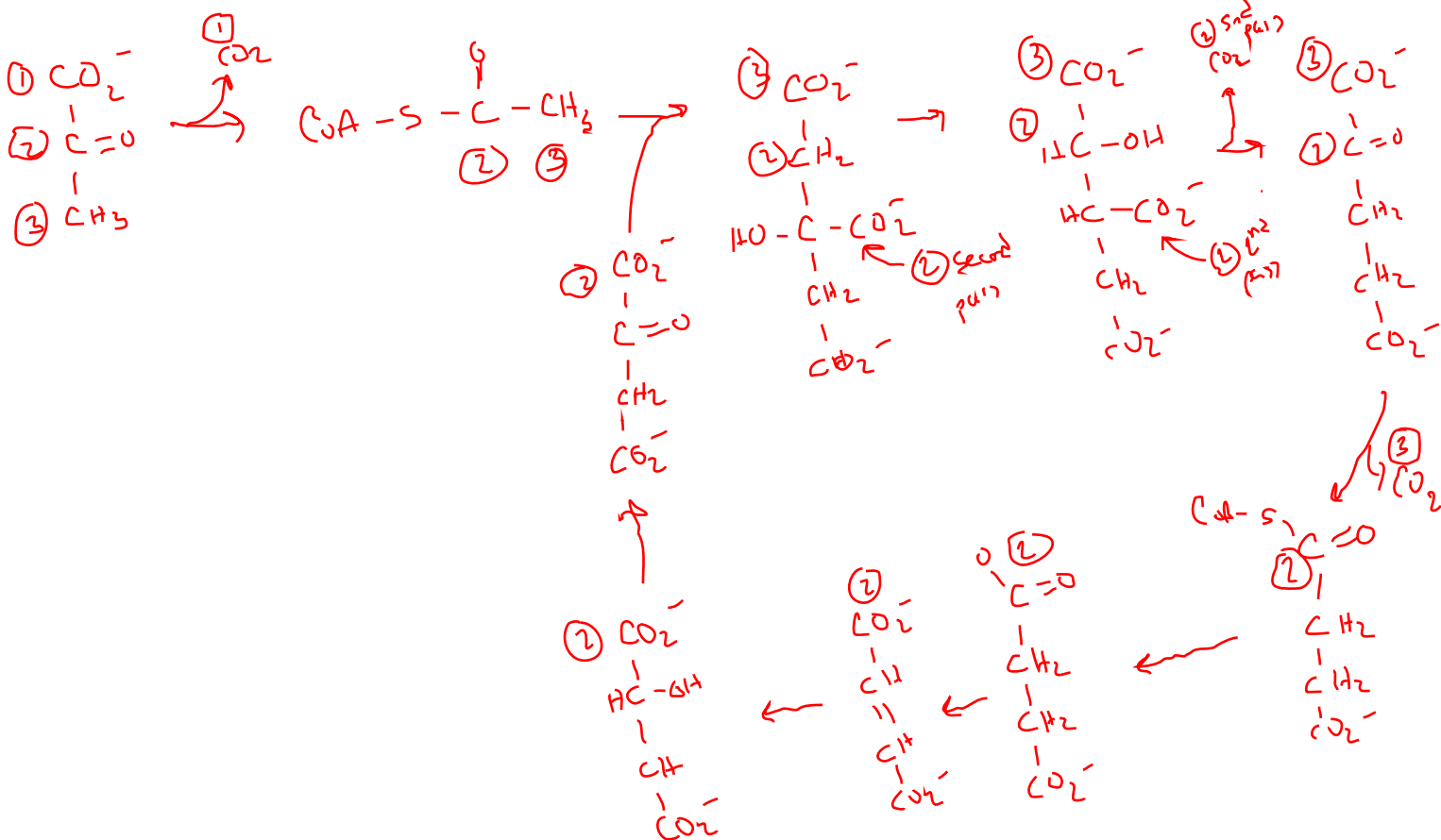
c. How many cycles does it take for these three carbons to be completely removed from the intermediate metabolites in the cycle (that is, at what point does C1 become CO_2, at what point does C2 become CO_2, etc.). Please state any assumptions that you make about symmetry or asymmetry in the intermediates/reactions.

Symmetry in the citric acid → isocitrate step is important

As I show above, it takes 1.5 cycles to get rid of all 3 carbons, BUT if you draw it with the -OH transferred to the C5 carbon, it will remain in the chain nearly indefinitely

3. Consider the conversion of Succinyl CoA to Succinate.
   a. What enzyme catalyzes this reaction? Succinyl-CoA Synthase
   b. Draw a mechanism for this reaction. Make sure to include the isolatable phosphohistidine residue.
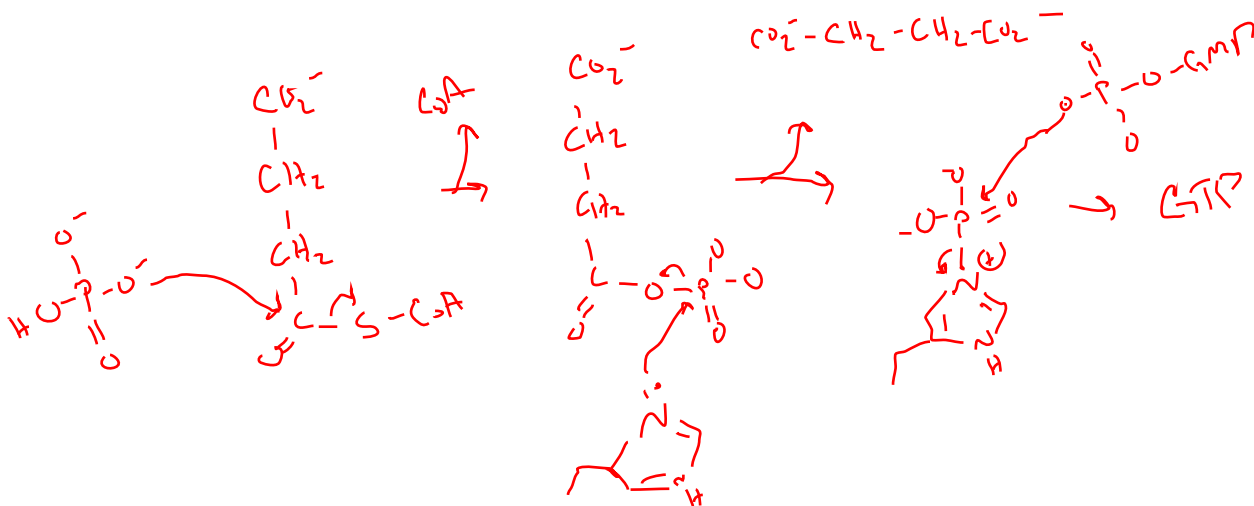
c. What provides the energy for GTP synthesis? Hydrolysis of the thioester
d. We often say that ATP is generated in this step. How can we make this statement when it is not part of the overall chemical reaction? Substrate Level Phoshporylation – GTP readily phosphorylates ADP

4. Oxaloacetate can by synthesized directly from pyruvate.
   a. This is an example of an anapleurotic reaction. What does this mean? Replineshing – It replenishes the intermediates of the TCA Cycle
   b. Why is this not the optimal route for carbons from pyruvate to enter into the TCA cycle? This route consumes an ATP and prevents the production of NADH; both are counterproductive to energy production.

c. Accumulation of Acetyl-CoA activates this enzyme. Why does this make sense in the metabolic scheme of the TCA cycle? *If acetyl-CoA is accumulating, that means that citrate synthase is not operating ideally. Perhaps this is because the [oxaloacetate] is below the Km, in which case making more oxaloacetate would enable citrate synthase to function normally*

d. Using the KEGG Pathway website, determine the name of the enzyme that catalyzes this reaction, the gene code in humans, and any cofactors that are necessary in humans.

Enzyme: Pyruvate Carboxylase
*Gene Code: 5091*
Cofactors: biotin and Mn or Zn

e. Propose a mechanism for this reaction (I encourage you to be creative and come up with a mechanism that makes sense based on your own chemical intuition – you will need to do this on exams).

The actual mechanism involves using the biotin cofactor to transfer CO_2, however we have not seen any biotin-dependent processes. A more simple mechanism can be predicted
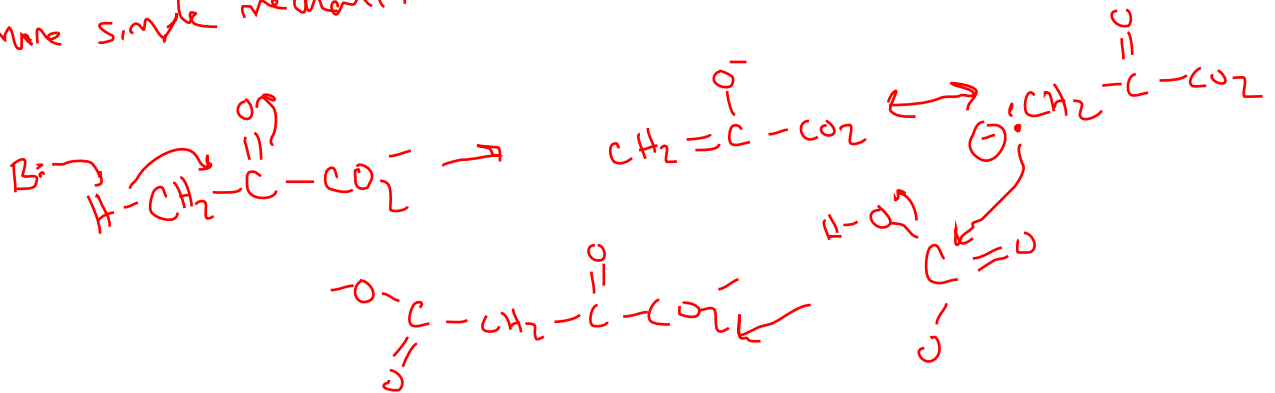
5. The standard free energies of hydrolysis for three high energy phosphate compounds are listed below. Based on the structure or each molecule, justify the trend.

Phosphoenolpyruvate → pyruvate ($\Delta G'^o$ = -61.9 kJ mol^{-1}), ATP → ATP ($\Delta G'^o$ = -30.5 kJ mol^{-1}), Phosphocreatine →creatine ($\Delta G'^o$ = -43.1 kJ mol^{-1})
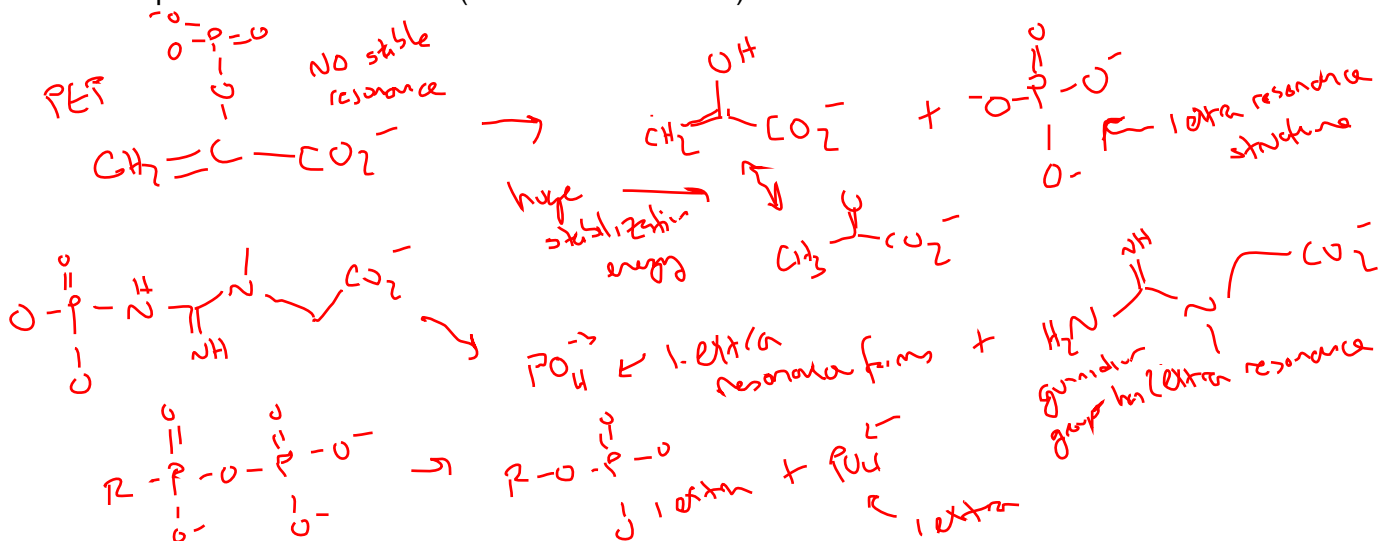
6. The typical intracellular concentrations of ATP, ADP, and Pi in muscles are 5.0, 0.5, 1.0 mM, respectively. Using the values in problem 5, and assuming standard biological conditions:

a. What is ΔG for the hydrolysis of ATP in these conditions?

$ATP \rightarrow ADT + Pi$

$$\Delta G = \Delta G^\circ + RT \ln \frac{ADP \cdot Pi}{ATP}$$

$$= -30500 \frac{J}{mol} + 8.314(298) \ln \frac{0.0005(0.001)}{0.005} = -53.3 \, kJ/mol$$

b. Calculate the equilibrium concentration ratio of phosphocreatine to creatine in the creatine kinase reaction

$$\text{Creatine + ATP} \rightleftharpoons \text{Phosphocreatine + ADP}$$

$Cr \rightleftharpoons PiCr \quad \Delta G^\circ = +43.1$

$ATP \rightleftharpoons ADP \quad \Delta G^\circ = -30.5$

$$\Delta G^\circ = 12,600 \frac{J}{mol} = -8.314 \frac{J}{mol \cdot K}(298 \, K) \ln \left(\frac{0.5 \cdot PCr}{Cr} \right)$$

$$0.00619 = 0.1 \frac{PCr}{Cr} \qquad \frac{PCr}{Cr} = 0.062$$

c. What concentration ratio of ATP to ADP would be required to yield equal concentrations of phosphocreatine and creatine?

$$\frac{12,600}{-8.314(294)} = \ln \frac{ADP}{ATP}$$

$$\frac{ADP}{ATP} = 0.0062$$

d. Assuming the concentration of Pi does not change, calculate the free energy of hydrolysis of ATP under the conditions determined in 6c.

$$\Delta G' = -30500 + 8.314(298) \ln 0.0062(0.001) = -60.21 \, kJ/mol$$

7. Recall that α-Ketoglutarate is converted to Succinyl-CoA in a reaction very similar to pyruvate decarboxylation. Please propose a mechanism for this reaction including important intermediate structures and all cofactors that are involved.
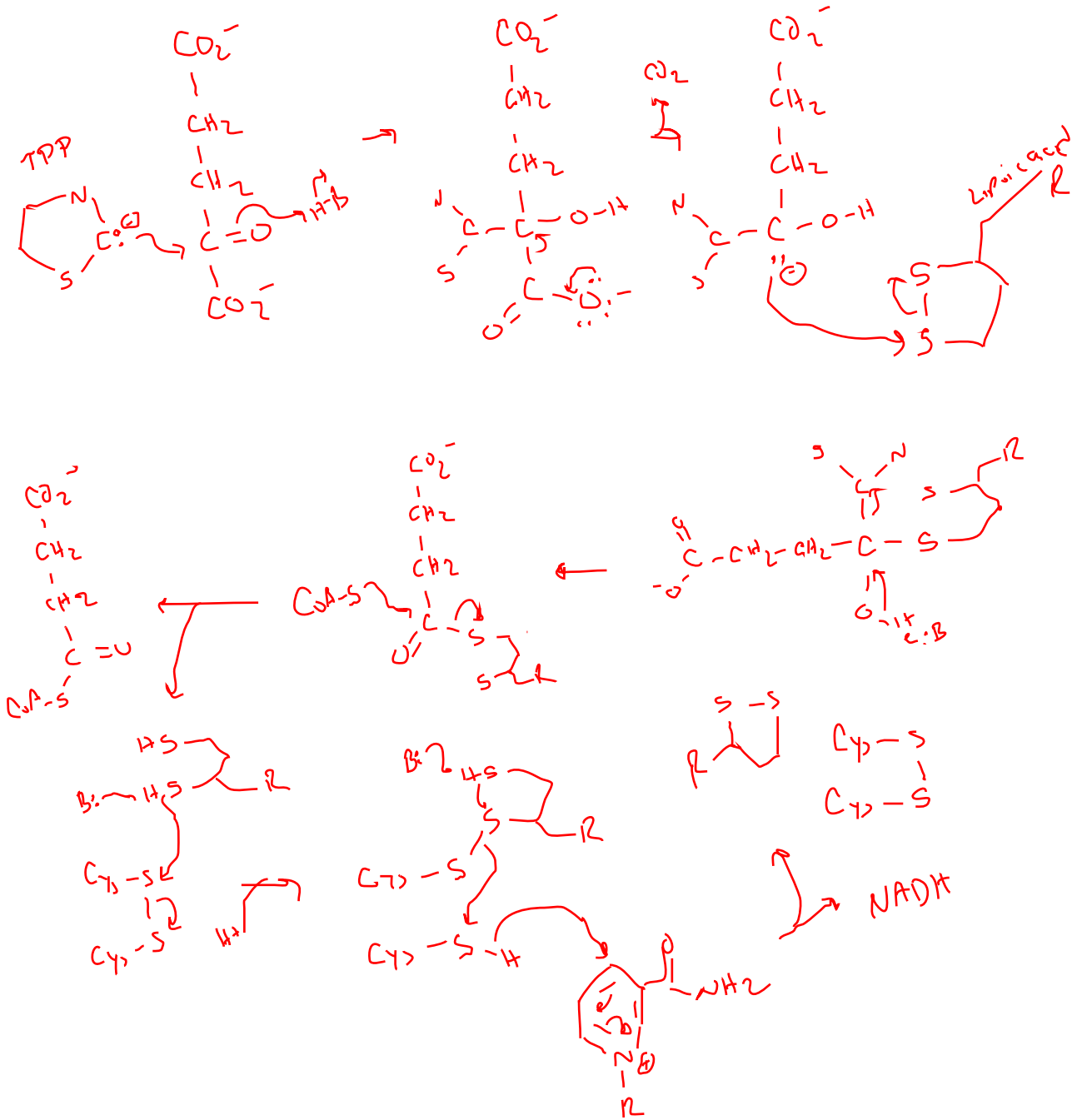
8. Noting that each equivalent of NADH produced in glycolysis and TCA Cycle leads to the production of 2.5 ATP and each $FADH_2$ produces 1.5 ATP (the topic of next week's lecture), determine how many equivalents of ATP are synthesized form one molecule of pyruvate.

Pyruvate $\Rightarrow$ acetyl CoA   1 NADH

TCA $\Rightarrow$ 3 NADH
    1 FADH$_2$

$4(2.5) + 1.5 = 11.5$ ATP

9. Mitochondrial Isocitrate Dehydrogenase relies on allosteric regulation through a regulatory subunit to activate or inhibit enzyme activity (the RCSB molecule of the month page does a good job at explaining this). *E. coli*, which lack mitochondria, rely on a different mechanism to regulate enzyme activity. Please read the attached paper and discuss how this *E. coli* enzyme is regulated and how this mechanism relates to the 'dietary' carbon source. E. coli Isocitrate Dehydrogenase (IDH) can be inhibited by the activity of another enzyme, IDH kinase/phosphorylase (gene aceK). This enzyme, which is part of an operon containing genes for Malate Synthase and Isocitrate Lyase (the two enzymes of the glyoxylate bypass), can inactivate IDH through phosphorylation. This inactivation of IDH decreases flux through the Krebs Cycle and, in turn, increases flux through the Glyoxylate Bypass. This bypass is particularly important because, when C_2 units (e.g. acetate) are the primary carbon source, the Krebs Cycle would result in an immediate conversion of both carbons to CO_2; this alternate pathway allows *E. coli* a way to ensure that some of the dietary carbon is reserved for anabolic pathways (instead of being converted to CO_2). This observation is reinforced by the fact that E. coli strains that lack *aceK* are not able to grow on acetate.

Perhaps you spent some time investigating the Glyoxylate Pathway in your book. If so, you would note that this cycle incorporates acetate in two reactions. The four carbon atoms exit the pathway as the C_4 intermediate succinate, which can be readily converted to other Krebs Cycle intermediates and serve as building blocks of other metabolites.