

Section 1: Reading guides (50 points)

**1. 20 pts. Fill in the blanks (2 points each.)**

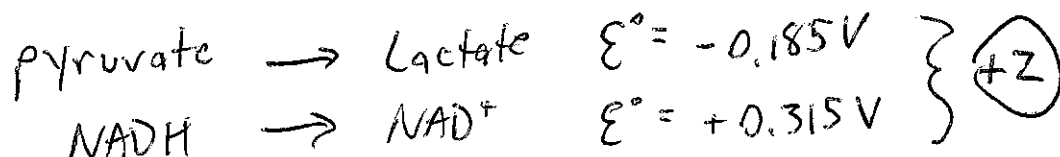
- A. Most NADH is made in the matrix of the mitochondria. NADH made in the cytosol are not taken directly into the mitochondria, but reducing equivalents are transported in through the malate-aspartate shuttle. (Glycerol-3-P)
- B. Complex II, also called Succinate DH from the citric acid cycle, adds to the pool of QH<sub>2</sub> but does not transport any protons.
- C. The ultimate electron acceptor for Complex IV is O<sub>2</sub>.
- D. In the Krebs's Cycle, formation of citrate is made spontaneous by the hydrolysis of the thioester functional group.
- E. In addition to transketalases and transaldolases, the non-oxidative phase of the pentose phosphate pathway utilizes epimerase or isomerase as enzymes.
- F. The pentose phosphate pathway is necessary to produce ribose for fast-dividing cells and NADPH for cells such as red blood cells, which need significant reducing potential.
- G. Glycogen is broken down into glucose-1-phosphate by the action of the enzyme glycogen phosphorylase.
- H. NADH is reoxidized to NAD<sup>+</sup> by yeast under anaerobic conditions by reaction with acetaldehyde. (accept pyruvate)
- I. The last step of glycolysis is upregulated through the feed-forward effect of the allosteric effector F-1,6-bP.
- J. In the glucolysis pathway, a total of 2 ATP are invested and 4 ATP are produced so that the overall net production in the pathway is 2 ATP.

2. 10 pts. True or false (1 point each)

- A. True A compound with a relatively higher reduction potential is more likely to accept electrons in a redox reaction.
- B. False The *in vivo* P:O ratio for NADH oxidation in mammals is about 1.5.
- C. False Chemically, the reaction catalyzed by  $\alpha$ -ketoglutarate dehydrogenase is more like isocitrate dehydrogenase than pyruvate dehydrogenase.
- D. False Although not possible in mammals, the glyoxylate pathway in plants allows pyruvate to be made into net glucose.
- E. True To run a molecule of glucose through glycolysis to pyruvate and then back through gluconeogenesis to glucose would cost the cell 4 ATP equivalents.
- F. True Fructose-2,6-bisphosphate is a potent inhibitor of phosphofructokinase in humans.
- G. True In the glycolysis pathway, the committed step is not the first irreversible step.
- H. False The reaction catalyzed by the pyruvate dehydrogenase complex requires TPP for the oxidation.
- I. False The citric acid cycle is down-regulated by the allosteric effectors NADH and ADP.
- J. True In the liver, glucose-6-phosphate can be made into glucose, but muscle tissue lacks the enzyme necessary for this transformation.

3. 20 pts. Short answer (5 points each)

A. What is the change in standard free energy for the reaction of the anaerobic reduction of pyruvate to lactate in humans? Write the overall reaction and calculate the free energy change.



$$\begin{aligned} (+1) \rightarrow \Delta G^{\circ} &= -n F \Delta \mathcal{E}^{\circ} \\ &= -2 \left( 96,485 \frac{\text{J}}{\text{V}\cdot\text{mol}} \right) (+0.13 \text{ V}) = \boxed{-25.1 \frac{\text{kJ}}{\text{mol}}} \end{aligned}$$

$\uparrow$   
(+1)

(+1) negative answer<sup>3</sup>

B. Write the name of each cofactor. In one case, write "none."

Q Organic redox reagent that carries two electrons but accepts and donates one at a time

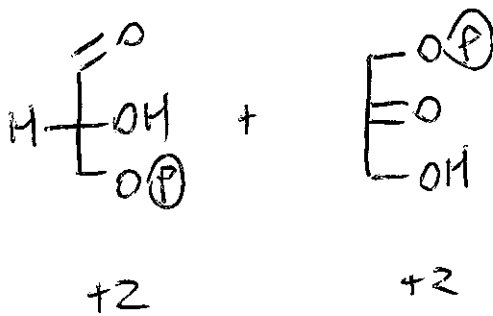
NO, NE Catalytic cofactor required in decarboxylation of  $\beta$ -ketoacids

NAD<sup>+</sup> Stoichiometric redox cofactor in the pyruvate dehydrogenase complex

HS-CoA Hydrolysis releases this cofactor in formation of citrate

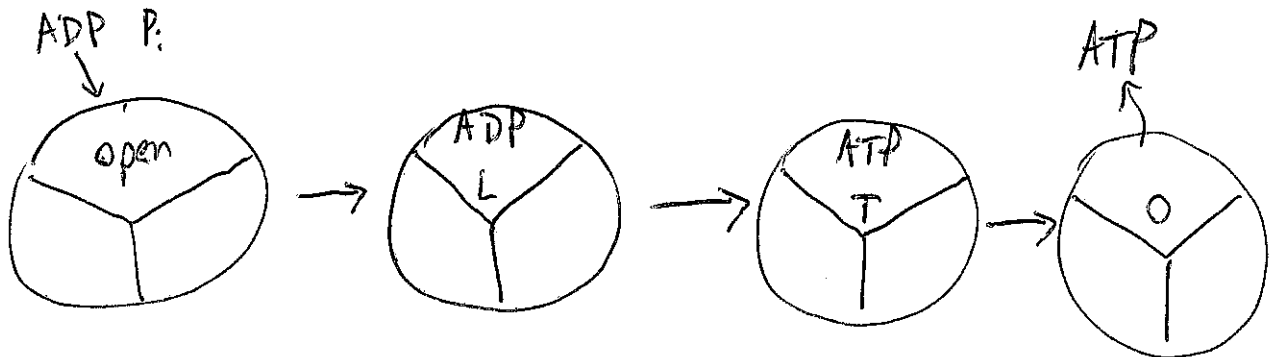
TPP Utilized by transketolase enzyme

C. Draw the structures of the two products produced when fructose-1,6-bisphosphate undergoes a retroaldol reaction catalyzed by aldolase. Explain why the reaction is spontaneous even though its standard free energy is a positive value.



DHAP is converted into GAP, which is consumed, disfavoring the back rxn  
+1

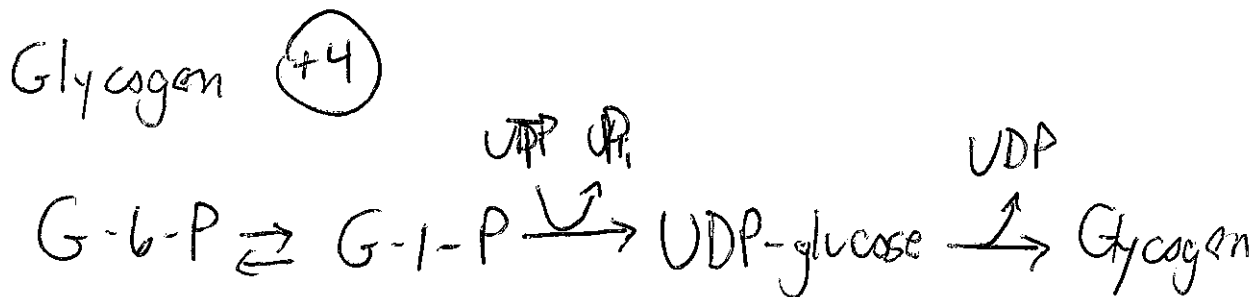
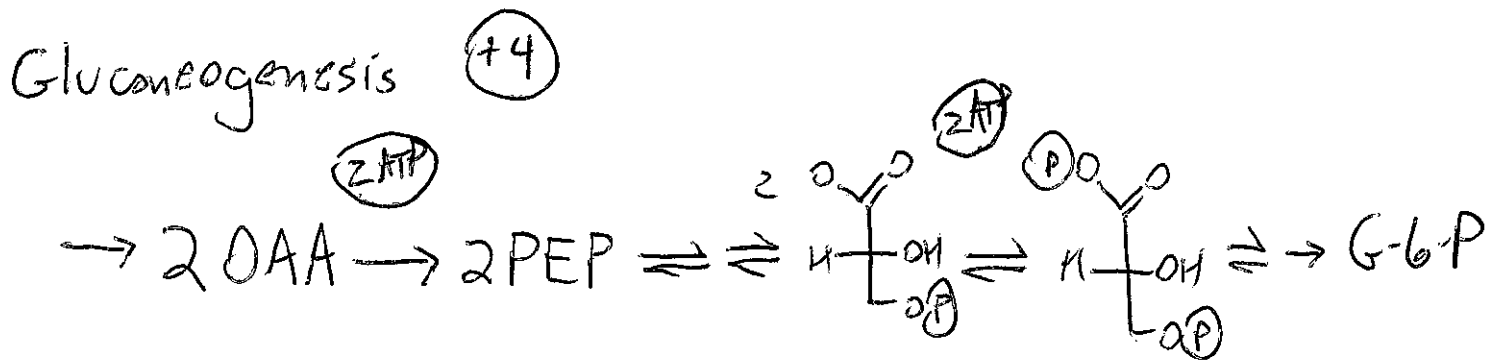
D. Draw a schematic of ATP synthesis through the bond change mechanism of ATP synthase.



+3 open, loose, tight  
+2 catalytic

**Section 2: Problems (10 points each)**

4. What is the minimum number of NTP required to transform two molecules of oxaloacetate into a glucose unit stored in the form of glycogen? For full credit, outline the pathway necessary for this transformation/storage and indicate which steps require NTP input.



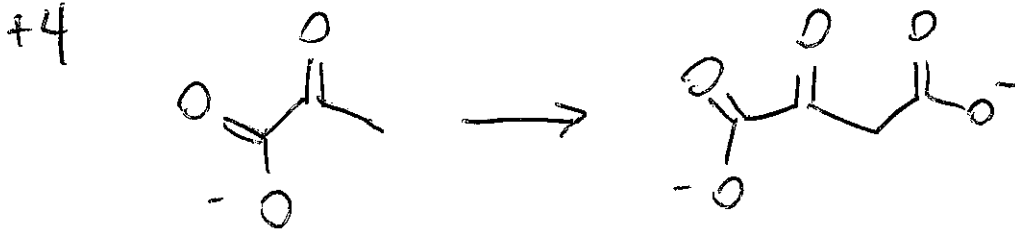
Total:

4 ATP	glycolysis	(+2)
1 UTP	glycogen	

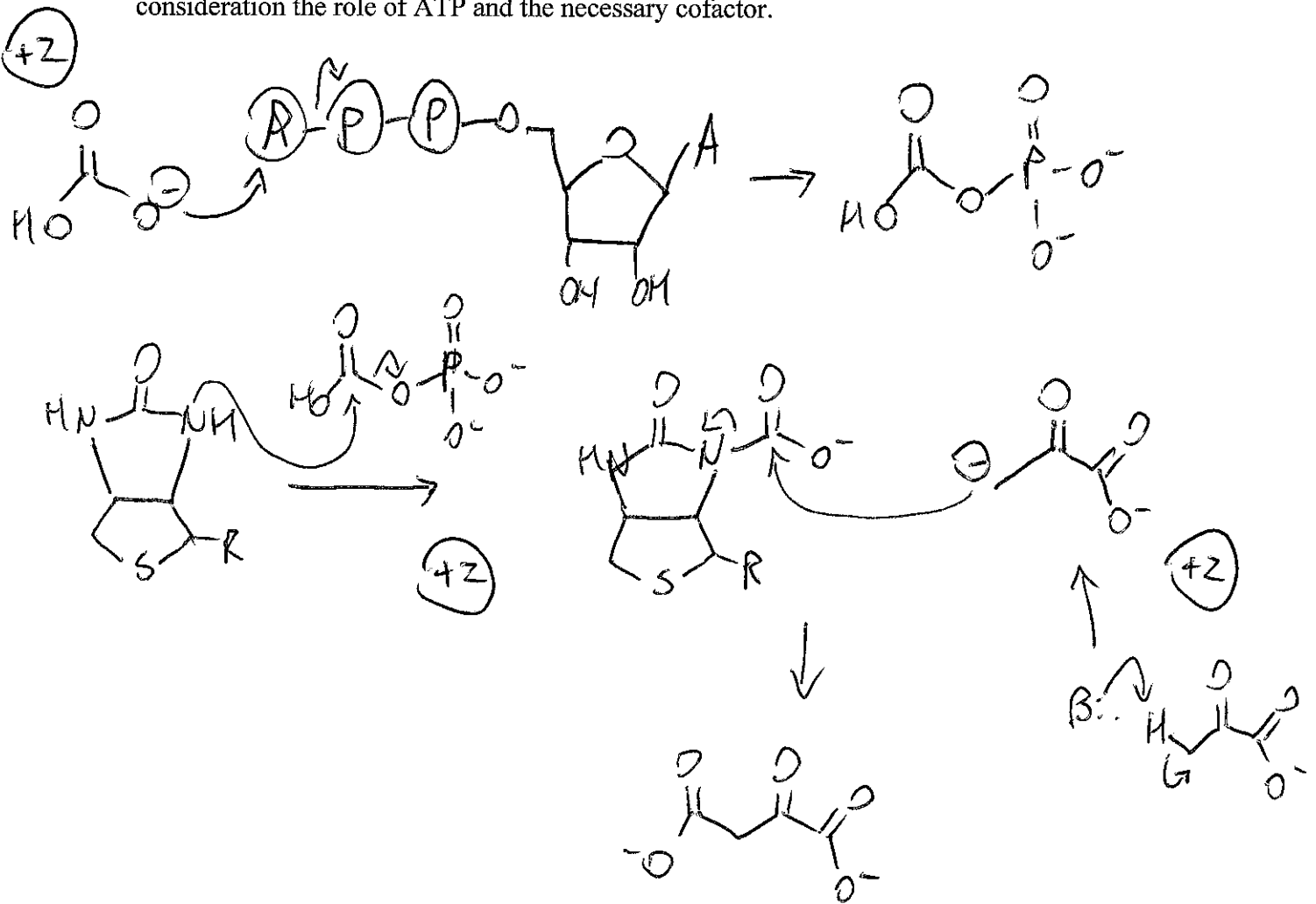
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5 NTP

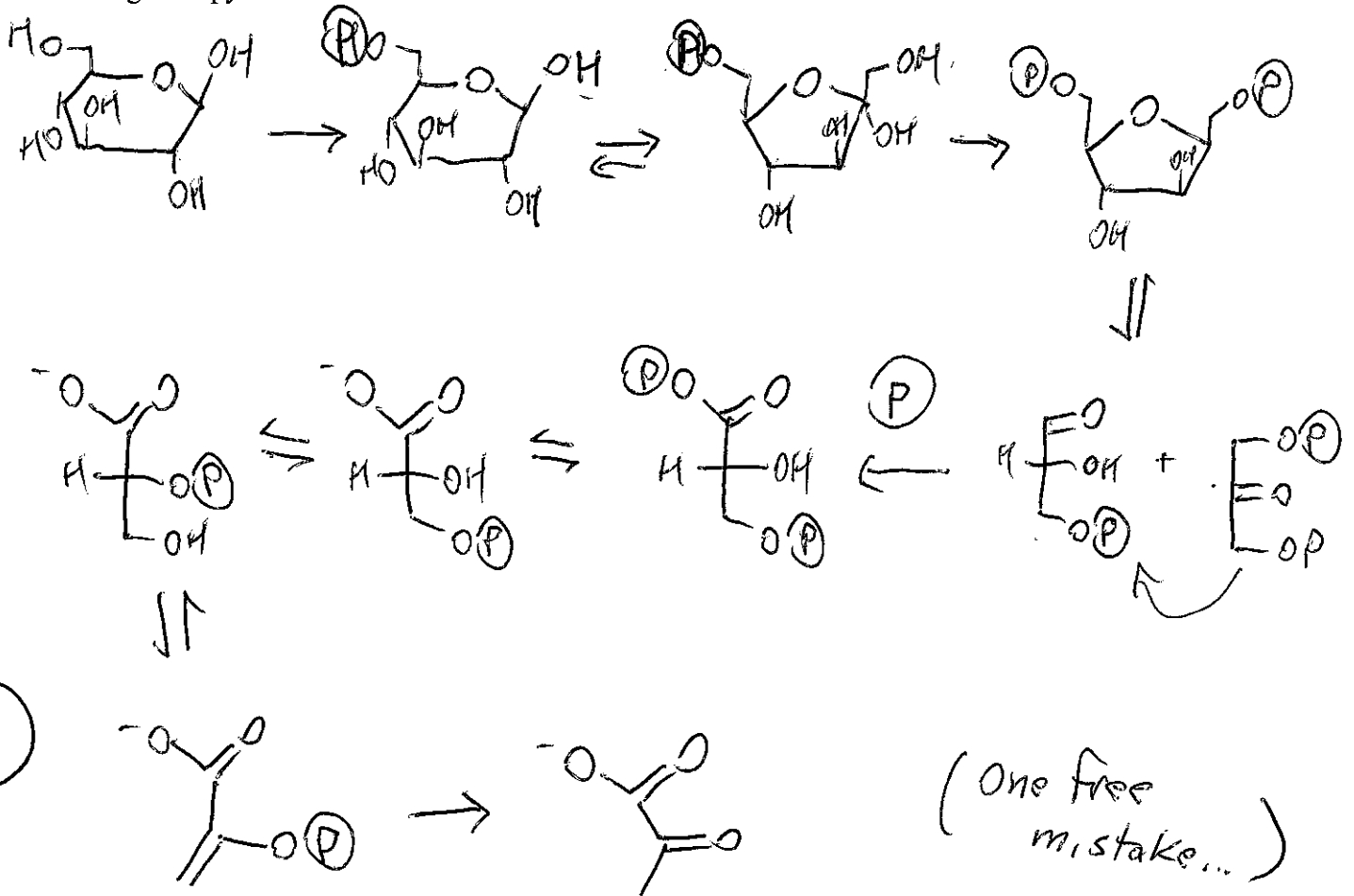
5. A. What reaction is catalyzed by pyruvate carboxylase? Draw the starting material and product.



B. Draw an arrow mechanism for this reaction starting from bicarbonate, taking into consideration the role of ATP and the necessary cofactor.



6. Draw the structures of the intermediates of the glycolysis pathway, starting with glucose and ending with pyruvate.

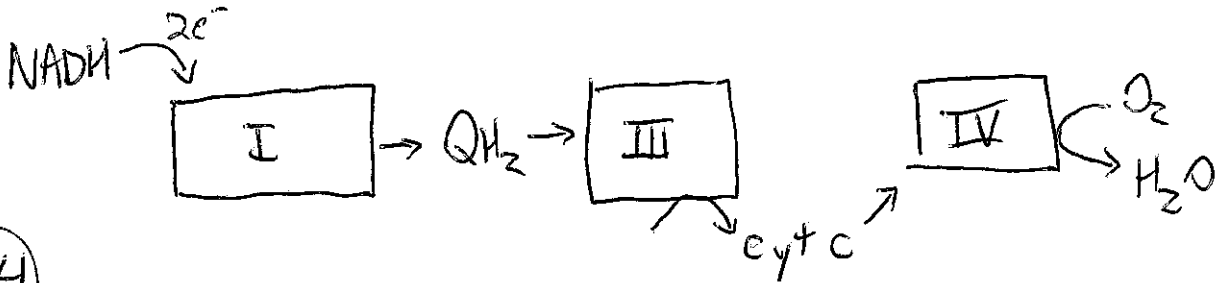


Arsenate ( $\text{AsO}_4^{3-}$ ) is a phosphate analog. It reacts in glycolysis just like phosphate does, but the anhydride it forms is unstable and hydrolyzes quickly. How much ATP can be produced per molecule of glucose under anaerobic conditions in the presence of arsenate? Explain.

Zero Loss of 2 ATP that would form in step 7.

(3) Under anaerobic conditions, 2 ATP input produces 2 ATP, for a net zero gain.

7. A. Draw a schematic of the electron transport chain starting from NADH and ending in formation of water. Include all complexes and the mobile carriers between the complexes.



(+4)

B. Cyanide acts as a respiratory poison by binding to Complex 4 in the place of oxygen. A newly developed antidote functions to bypass the problem by taking the place of cytochrome c in the chain. This compound accepts electrons just like cytochrome c, but then donates the electrons directly to oxygen without the aid of Complex 4. With reference to your figure from part A, explain why this antidote works.

IF the normal path is blocked at IV, this blocks the mechanism by which cytochrome C passes e<sup>-</sup> to O<sub>2</sub>, so this antidote does the work of IV.

(+2)

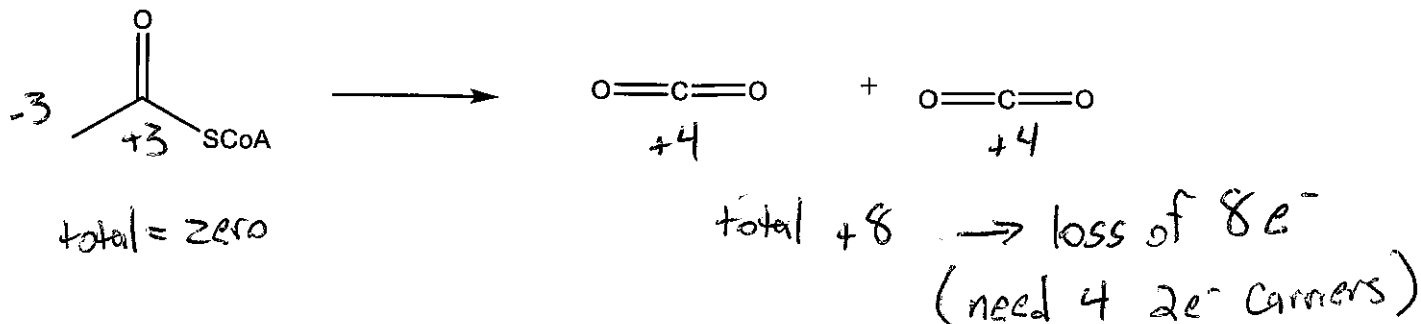
C. What is the P/O ratio for respiration using this antidote? Explain.

(+4) 
$$\frac{3 \text{ ATP}}{8 \text{ H}^+} (8 \text{ H}^+) = \frac{3 \text{ ATP}}{2 \text{ e}^-} \quad \text{P/O} = 3$$

Only 8 H<sup>+</sup> are pumped with Complex I and III active.

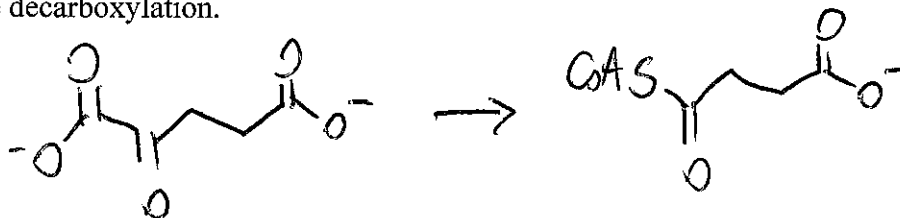
8. A. The citric acid cycle (CAC) can be used to oxidize acetyl CoA into 2 CO<sub>2</sub> molecules, while reducing 4 cofactors. Using the oxidation states of the atoms in the starting material and final products, show that this redox stoichiometry makes sense.

(+2)



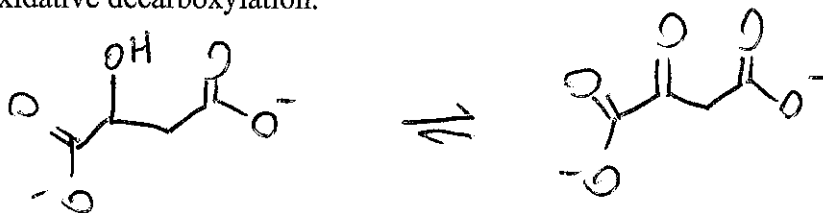
B. Draw the starting material and products of the CAC redox reaction that is a TPP-mediated oxidative decarboxylation.

(+2)



C. Draw the starting material and products of the CAC redox reaction that utilizes NADH but is not an oxidative decarboxylation.

(+2)



D. Draw the starting material and products of the CAC redox reaction that utilizes Q as a cofactor.

(+2)



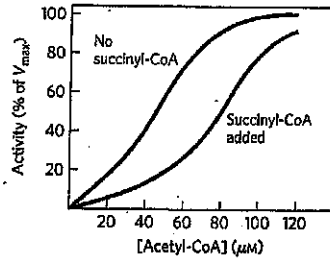
E. Draw the starting material and products of the remaining CAC redox reaction.

(+2)



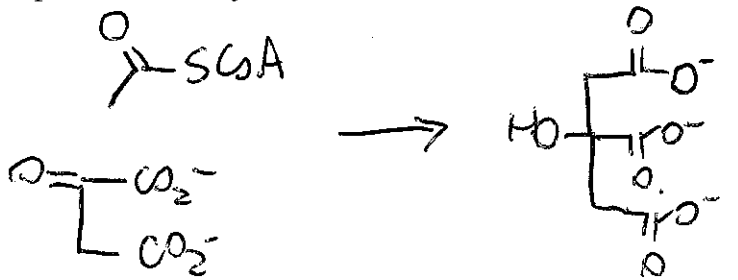


**Section 3: Case study (10pts)** When the enzyme citrate synthase is treated with saturating amounts of oxaloacetate, its activity demonstrates a sigmoidal dependence on the concentration of acetyl CoA, shown below. Furthermore, succinyl-CoA is an allosteric effector of citrate synthase.



A. Draw the structures of the reactants and products of the reaction catalyzed by citrate synthase. Does it require ATP or any cofactors?

(x3)



No.

B. Is succinyl-CoA a positive or negative effector of citrate synthase? Explain why this makes sense physiologically.

(x2) - Negative effector

(x3) - If succinyl CoA is building up, it is because flux through CAC is slowed, which shuts down entrance of acetyl CoA into the cycle via feed back inhibition.

C. What effect, if any, would high [succinyl-CoA] have on the reactions of the electron transport chain? Explain.

(x2)

It shuts down formation of NADH, which would slow ETC reactions.

## Data Tables

### Data Tables and scratch work

#### Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ}$ (kJ · mol <sup>-1</sup> )
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
ATP → AMP + PP <sub>i</sub>	-45.6
Phosphocreatine	-43.1
ATP → ADP + P <sub>i</sub>	-30.5
Glucose-1-phosphate	-20.9
PP <sub>i</sub> → 2 P <sub>i</sub>	-19.2
Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

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**[ TABLE 15-1 ]** Standard Reduction Potentials of Some Biological Substances

Half-Reaction	$E^{\circ}$ (V)
	<b>0.815</b>
	<b>0.48</b>
	<b>0.42</b>
Cytochrome a <sub>3</sub> (Fe <sup>3+</sup> ) + e <sup>-</sup> ⇌ cytochrome a <sub>3</sub> (Fe <sup>2+</sup> )	<b>0.385</b>
Cytochrome a (Fe <sup>3+</sup> ) + e <sup>-</sup> ⇌ cytochrome a (Fe <sup>2+</sup> )	<b>0.29</b>
Cytochrome c (Fe <sup>3+</sup> ) + e <sup>-</sup> ⇌ cytochrome c (Fe <sup>2+</sup> )	<b>0.235</b>
Cytochrome c <sub>1</sub> (Fe <sup>3+</sup> ) + e <sup>-</sup> ⇌ cytochrome c <sub>1</sub> (Fe <sup>2+</sup> )	<b>0.22</b>
Cytochrome b (Fe <sup>3+</sup> ) + e <sup>-</sup> ⇌ cytochrome b (Fe <sup>2+</sup> ) (mitochondrial)	<b>0.077</b>
Ubiquinone + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ ubiquinol	<b>0.045</b>
Fumarate <sup>-</sup> + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ succinate <sup>-</sup>	<b>0.031</b>
FAD + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ FADH <sub>2</sub> (in flavoproteins)	<b>~ 0.</b>
Oxaloacetate <sup>-</sup> + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ malate <sup>-</sup>	<b>- 0.166</b>
Pyruvate <sup>-</sup> + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ lactate <sup>-</sup>	<b>- 0.185</b>
Acetaldehyde + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ ethanol	<b>- 0.197</b>
S + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ H <sub>2</sub> S	<b>- 0.23</b>
Lipoic acid + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ dihydrolipoic acid	<b>- 0.29</b>
NAD <sup>+</sup> + H <sup>+</sup> + 2 e <sup>-</sup> ⇌ NADH	<b>- 0.315</b>
NADP <sup>+</sup> + H <sup>+</sup> + 2 e <sup>-</sup> ⇌ NADPH	<b>- 0.320</b>
Acetoacetate <sup>-</sup> + 2 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ 3-hydroxybutyrate <sup>-</sup>	<b>- 0.346</b>
Acetate <sup>-</sup> + 3 H <sup>+</sup> + 2 e <sup>-</sup> ⇌ acetaldehyde + H <sub>2</sub> O	<b>- 0.581</b>

Source: Mostly from Loach, P. A., in Fasman, G. D. (ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. 1, pp. 123-130, CRC Press (1976).

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$$\Delta G = RT \ln \frac{[X]_{final}}{[X]_{initial}} + ZF\Delta\psi \qquad \Delta G^{\circ} = -nF\Delta E^{\circ}$$

$$R = 8.314 \text{ J/mol} \cdot \text{K}$$

$$F = 96,485 \text{ J/V} \cdot \text{mol}$$

$$\Delta G^{\circ} = -RT \ln K_{eq}$$

$$\Delta G_{\text{reaction}} = \Delta G^{\circ}_{\text{reaction}} + RT \ln \frac{[\text{products}]}{[\text{reactants}]}$$

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