7.05 Spring 2020 Problem Set 9

Question 1. Gluconeogenesis #1 (Luis)

Gluconeogenesis converts various products into glucose for the body to use.

A) Write the balanced equation for gluconeogenesis.
2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 6 H2O -> glucose + 4 ADP + 2 GDP + 6 Pi + 2 NAD+ 2 H+

B) Why is the equation for gluconeogenesis not the reverse of glycolysis?
Under cellular conditions, 3 reactions of glycolysis are essentially irreversible and will not happen in the reverse direction.

C) Provide the workarounds that gluconeogenesis takes to overcome the rate-limiting steps of glycolysis.

OAA → PEP via PEPCK
F-1,6-BP → F-6-P via F-1,6-BPase
G-6-P → Glucose via G6Pase

D) What is the net result of nucleotide triphosphate production/hydrolysis if gluconeogenesis and glycolysis occur at the same time? How do cells favor gluconeogenesis or glycolysis such that they are mutually exclusive if there are many steps that overlap between the two processes?

If gluconeogenesis and glycolysis take place at the same time in a cell, the net result is the hydrolysis of 2 ATP and 2 GTP molecules. The way cells ensure that these two processes are mutually exclusive is by inhibiting the enzymes involved in the 'rate-limiting' steps for each process.

E) Avidin, a 70kDa protein found in egg whites, binds biotin with a very high affinity. What step of gluconeogenesis would be inhibited if Avidin was introduced into the system?

Pyruvate carboxylase uses carboxybiotin to carboxylate pyruvate in the first step of gluconeogenesis. The presence of Avidin would inhibit this reaction. So technically all of gluconeogenesis is inhibited but mentioning this reaction is enough.

Question 2. Gluconeogenesis #2 (Sandhya)

A. Hyperglycemia, or high blood sugar levels, can lead to several physiological complications. As a researcher at a pharmaceutical company, you are searching for new drug targets to treat hyperglycemia. Which enzymes in gluconeogenesis would be good drug targets, and why?
PEPCK, FBPase, G6Pase

*Pyruvate carboxylase is important to gluconeogenesis but is not uniquely involved (inhibiting it will disturb other essential pathways)
These enzymes are key to the progress of gluconeogenesis because they catalyze the "irreversible" steps, which correspond to large changes in free energy. These enzymes are unique to gluconeogenesis and will not disrupt glycolysis or other essential pathways.

Two patients with severe hypoglycemia come into the ER. You suspect there are deficiencies in their gluconeogenesis pathways and do some tests.

	Cytosolic biotin levels	Mitochondrial biotin levels	Cytosolic GTP levels	Cytosolic ADP and AMP levels
Patient 1	Normal	Low	Slightly high	Slightly low
Patient 2	Low	Normal	Normal	Very high

B. Which key enzyme(s) in gluconeogenesis is/are likely affected in Patient 1? Explain how the data supports your conclusion.
PC; it converts pyruvate to OAA in the mitochondria and requires biotin as a cofactor. Low biotin levels in the mitochondria will prevent PC from catalyzing this reaction.

The slightly high GTP levels are likely a result of not enough OAA being generated. A phosphate from GTP is necessary in the conversion of OAA to PEP by PEPCK, so low OAA levels will correspond to higher GTP levels.

The slightly low ADP/AMP levels are likely due to the defective gluconeogenic pathway, which normally yields 4 ADP molecules.

C. Which key enzyme(s) in gluconeogenesis is/are likely affected in Patient 2? Explain how the data supports your conclusion.
The very high cytosolic ADP and AMP levels serve as feedback inhibitors to PC, PEPCK, and FBPase, which are key enzymes in gluconeogenesis.

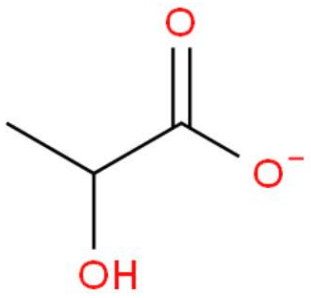
D. Describe a feedback mechanism within the gluconeogenesis pathway.
- High PEP levels activate FBPase. This is called feed-forward activation, as PEP is a product early on in gluconeogenesis
- AMP and ADP inhibit PC, PEPCK, FBPase as negative feedback

E. Why is compartmentalization necessary in gluconeogenesis?
In general, compartmentalization maintains different ratios of metabolites (like ATP/ADP or NADH/NAD+) to favor certain reactions other others
In the case of gluconeogenesis, the mitochondria has a high ATP/ADP ratio which favors the pyruvate carboxylase reaction to convert pyruvate to OAA. The ATP is required to phosphorylate bicarbonate to then interact and react with biotin so that pyruvate can be carboxylated.

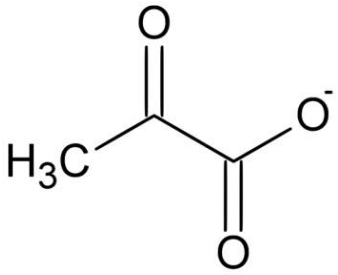
F. List and provide structures for 3 different products that can be used for gluconeogenesis.

Amino Acids

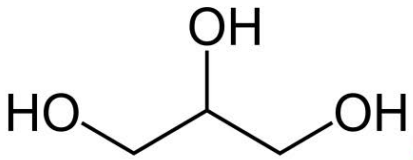
Lactate :



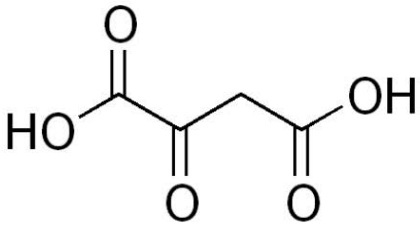
Pyruvate:



Glycerol:

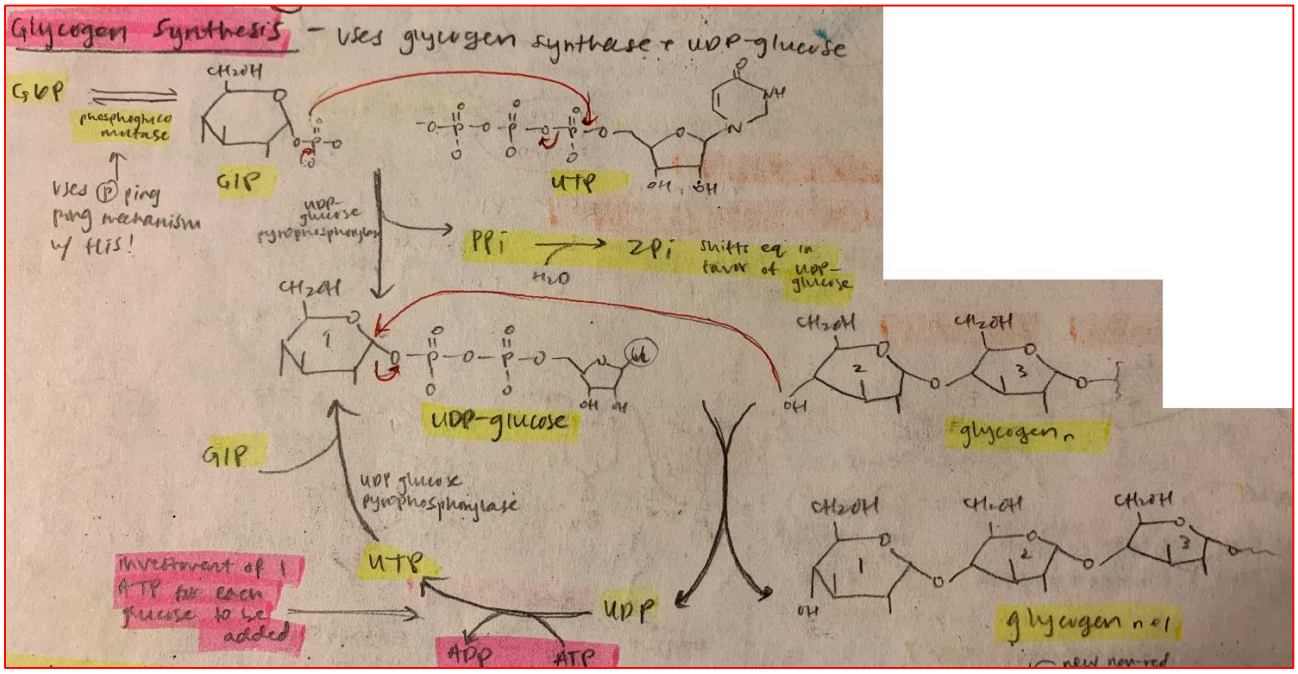


Oxaloacetate:



Question 3. Glycogen Synthesis and Degradation (Sandhya)

A. Draw the pathway for glycogen synthesis as catalyzed by glycogen synthase. Make sure to include any intermediates/cofactors/organic compounds that are necessary to drive this reaction.



B. How many net ATP are required to add 1 glucose molecule to a glycogen chain?

2 ATP (1 ATP to regenerate to UTP, and 1 ATP to phosphorylate glucose to get G1P (glucose—> G6P—> G1P))

C. Describe how phosphorylation regulates the active and inactive states of glycogen phosphorylase and glycogen synthase.

Phosphorylation of glycogen phosphorylase: active

Phosphorylation of glycogen synthase: inactive

D. How would each of the following affect an individual's blood glucose levels through glycogen synthesis or degradation? Give a brief explanation.

1. Overactive phosphodiesterase

   Lower blood glucose levels; PDE would deplete cAMP levels and prevent PKA activation. If PKA is not activated, it cannot phosphorylate glycogen phosphorylase to activate it. Glycogen phosphorylase releases G1P from glycogen, which can enter the bloodstream.

2. High epinephrine levels

   Higher blood glucose levels; epinephrine leads to the activation of PKA which phosphorylates glycogen synthase to inactivate it, and phosphorylates glycogen phosphorylase to activate it. This dual action promotes the release of glucose from glycogen and prevents other glucose molecules from being incoroporated into glycogen.

3. Drug that increases insulin receptor affinity for insulin

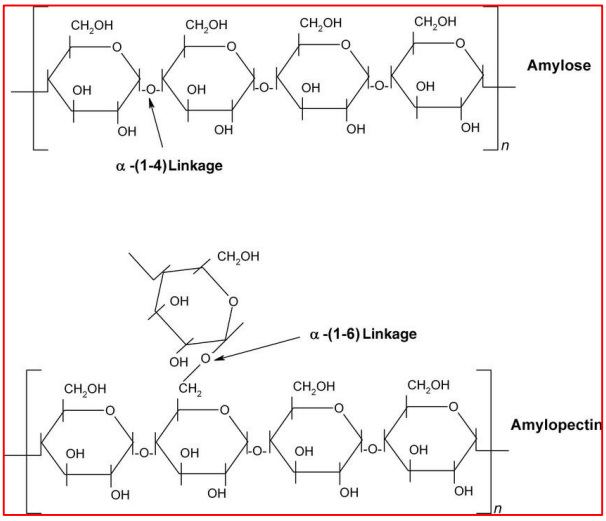
   Lower blood glucose levels; Insulin inhibits GSK. GSK normally promotes the inactivation of glycogen synthase by phosphorylating it. Inhibition of GSK thus prevents the inactivation of glycogen synthase and increases glycogen synthesis.

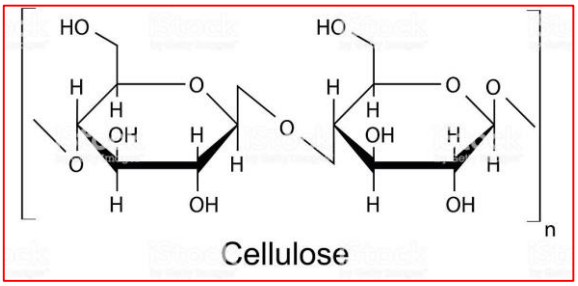
E. Glycogen is a polymer of glucose with both reducing and non-reducing end(s). Draw an end from a glycogen polymer where glucose monomers are added or removed (you only need to show two glucose monomers in your glycogen polymer, but clearly label the end and add a dashed line where to the bond would be to the next monomer in glycogen). State whether the end you draw is the reducing or the non-reducing end of glycogen and explain briefly why that end is either reducing or non-reducing.
Glucose monomers are added/removed from the non-reducing end + correct figure of a non-reducing end

Question 4. Carbohydrate storage and utilization (Luis)

A. Starch is a carbohydrate storage polymer synthesized from glucose in plants that can be linear (called amylose) or contain some degree of branching (called amylopectin — similar to glycogen). Plants also synthesize cellulose from glucose, which plays a more structural role and humans cannot digest.

A.2. Starch polymers are made from glucose monomers linked via alpha-(1,4)-glycosidic bonds, while cellulose is made from glucose monomers linked via beta-(1,4)-glycosidic bonds. Draw amylose and cellulose polymers, showing at least 3 monomers per structure. On your amylose structure, add a monomer that would be added if this was branched amylopectin. Describe this bond chemically.

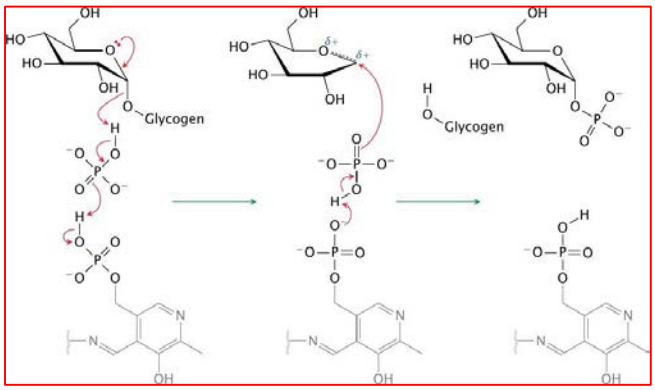




A.3. In words (and very generally), suggest why cellulose and amylose/amylopectin have such different chemical properties and cellular functions.

Different glycosidic linkages/orientations of the glucose monomers mean alcohol groups in the sugar molecules and are available for different hydrogen bonding arrangements.

A.4. You incubate rat glycogen phosphorylase, phosphate, and amylose *in vitro* and find that phosphorylated sugar monomers are produced. Draw the mechanism for this. Why does glycogen phosphorylase not require ATP to drive the reaction?



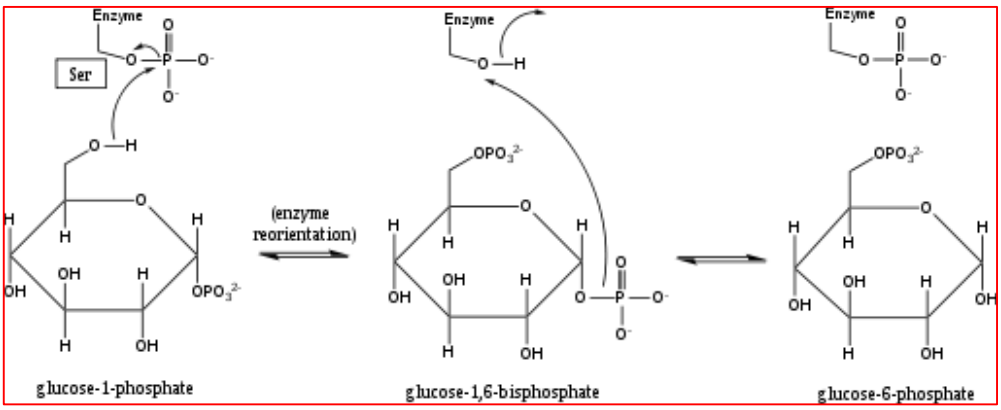
You are breaking a glycosidic linkage, which is favorable and high concentrations of inorganic phosphate in the cell drives reaction in the direction of glycogen breakdown.

Glucose-1-phosphate monomers are produced.

A.5. What conversion is needed to prepare these liberated monomers for glycolysis in a cell? What enzyme could you add *in vitro* to achieve this? Using mechanisms you know for glycolytic enzymes, propose a reaction mechanism for the enzyme needed to prepare glycogen breakdown products for glycolysis.

Glucose-6-phosphate is required for glycolysis to proceed. Phosphoglucomutase can perform this conversion from glucose-1-phosphate. Its reaction mechanism is

analogous to phosphoglycerate mutase (has a 2x phosphorylated intermediate).



A.6. You treat the chemical products from your *in-vitro* reaction with the enzyme(s) proposed that prepare them for glycolysis. You then incubate this entire extract with a suspension of human cells in a buffered solution. You find that none of the metabolites in this mixture are uptaken by the cells. What prevents this uptake?

Phosphorylated sugars like these are not transported across cell membranes. Instead, dephosphorylated sugars are transported and subsequently phosphorylated inside a cell.

A.7. How does the liver prepare sugar monomers released from glycogenolysis so they can be uptaken by cells in other tissues? If cells could transport the phosphorylated sugars from your experiment freely back and forth how would this complicate the regulation of intracellular and blood glucose levels?

The liver dephosphorylates glucose monomers before secreting them into the blood. If phosphorylated sugars could freely diffuse between the blood and cells, 'trapping' mechanisms like the phosphorylation of glucose by hexokinase would not function. New inputs would have to be devised to regulate and drive sugar uptake from the blood.

Question 5: Adapted from 2016 Exam 3

You are characterizing a new cancer cell line that you generated as a part of your UROP project. You notice that these cells consume glucose, but generate very little lactate under standard culture conditions where oxygen is abundant. However, when you culture these cells in very low oxygen, you notice that they continue to consume glucose but now produce lactate.

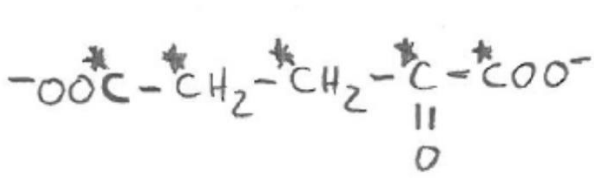
A. (5 points) What role does lactate production play in glycolysis? How does having oxygen around alleviate the need to produce lactate?

Lactate enables the regeneration of NAD^+ from NADH, which is required for glycolysis to continue. When oxygen is present, the reduction of oxygen to H2O by the electron transport chain regenerates NAD^+ from NADH instead, making lactate production unnecessary.

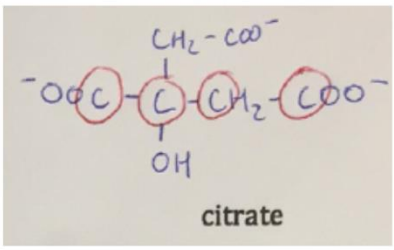
B. (5 points) The signaling pathway in mammalian cells that senses low oxygen inhibits the activity of the pyruvate dehydrogenase complex (PDH), which in turn affects citrate levels in cells. How will the expected change in citrate levels in response to low oxygen affect the rate of glycolysis? In your answer please specify the enzyme(s) in glycolysis that are sensitive to citrate levels.

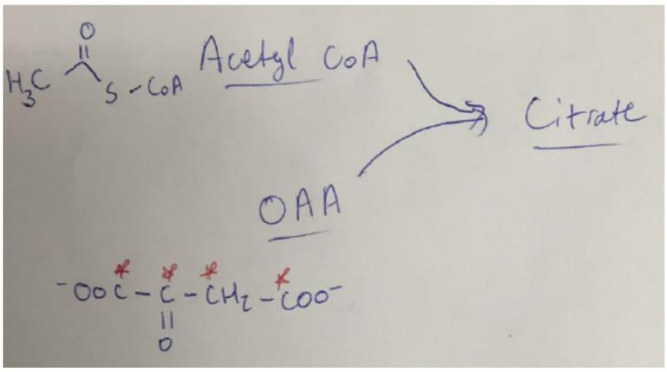
Inhibition of PDH will **reduce** citrate levels. Because citrate normally inhibits PFK, low citrate levels will effectively activate PFK and therefore **increase** the rate of glycolysis.

To further characterize metabolism of your cell line in culture with normal oxygen, you provide the cells with alpha-ketoglutarate that is labeled with the isotope ^{13}C on all five carbons:

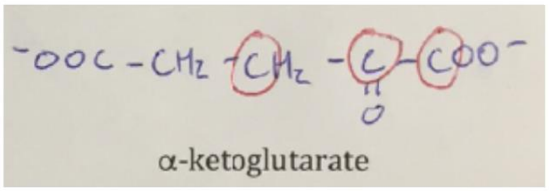


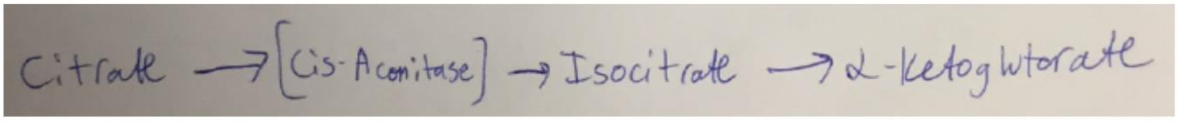
C. (5 points) Based on your knowledge of TCA cycle reactions, circle the carbons on citrate that you expect to be labeled if it is produced from labeled □-ketoglutarate.
For your answer, assume that glucose generates unlabeled acetyl-coA. It is not necessary to provide enzymes names or any reaction mechanisms, but in addition to circling the carbons, show the substrates and products of any enzyme catalyzed steps involved in converting □-ketoglutarate and acetyl-coA that allowed you to arrive at your answer.





D. (5 points) If the citrate that is labeled as shown in your answer to part C above, is further metabolized via the TCA cycle to alpha-ketoglutarate, circle the carbons that you would expect to be labeled on alpha -ketoglutarate.
Include the enzyme-catalyzed steps involved in converting citrate to alpha -ketoglutarate that allowed you to arrive at your answer. It is not necessary to provide enzymes names or any reaction mechanisms.

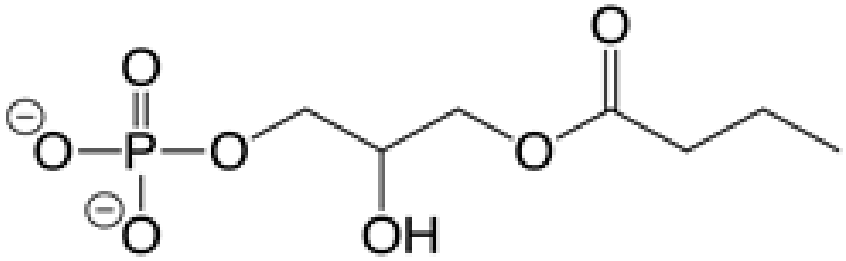




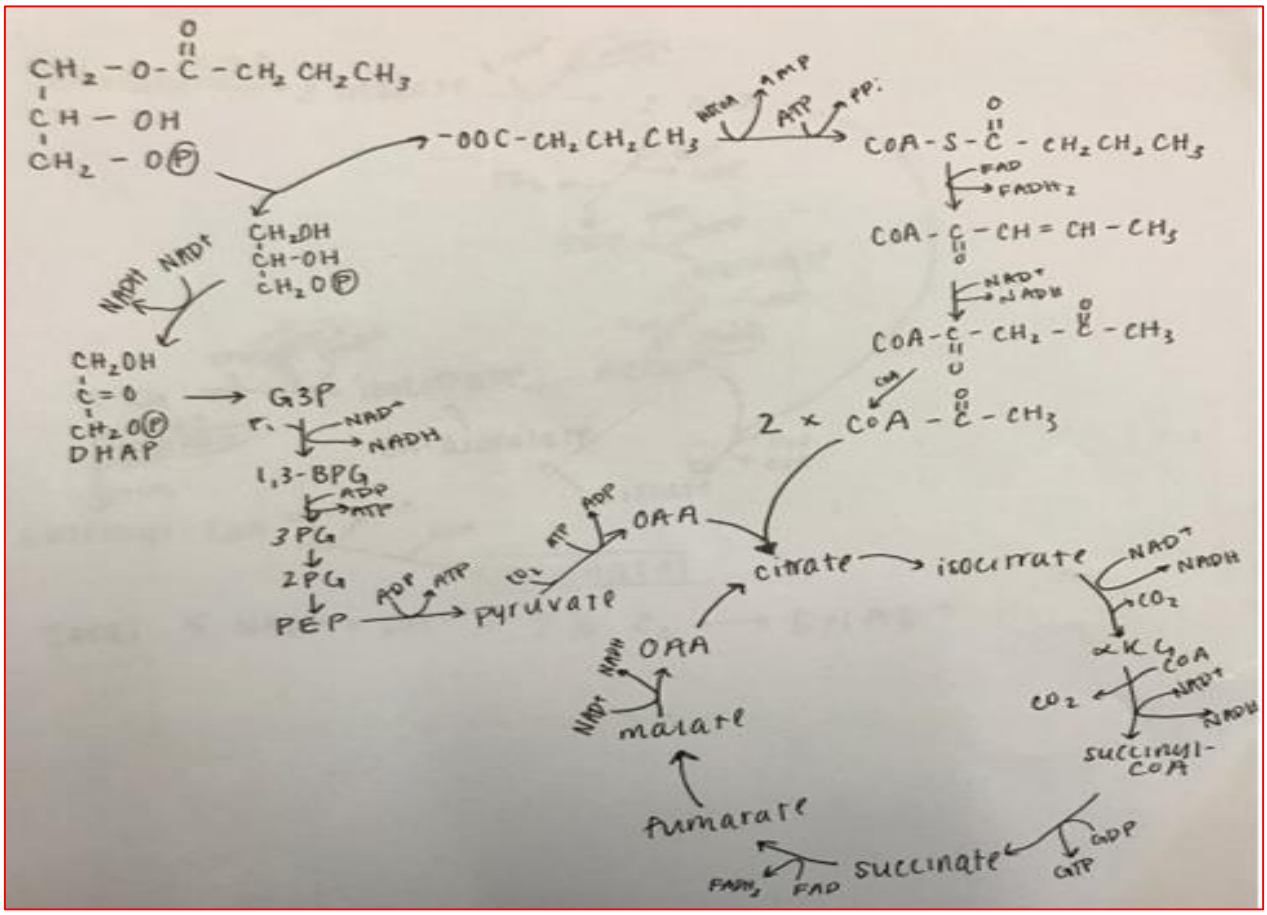
Question 6.

a) Show how the molecule shown below can be metabolized to generate **as many** NADH, $FADH_2$, and ATP equivalents as possible. Use reactions described in class and explicitly show

each step in the pathway you provide as your answer. Avoid introducing any glycolytic or TCA cycle intermediates unless they are a direct product of this molecule. Include any cofactors that are necessary, but enzyme names and enzymatic mechanisms are not necessary.



From your scheme, how many of the following are produced? CO_2, ATP (or GTP) equivalents, NADH, $FADH_2$



B) How would you adjust the diagram if the molecule shown above was being metabolized in the absence of oxygen? Why?

The citric-acid cycle portion of the diagram can be eliminated. NAD+ and FAD have to be regenerated in order for the TCA cycle to take place.

C) Certain anaerobic microorganisms have modified versions of the TCA cycle. How can the TCA cycle be accommodated under anaerobic conditions?

To undergo TCA cycle, you need a way to regenerate NAD and FAD so there has to be an electron acceptor different from molecular oxygen in these microorganisms.

7.05 General Biochemistry
Spring 2020