# 7.05 Spring 2020 Problem Set 9

**Question 1. Gluconeogenesis #1**

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

A) Write the balanced equation for gluconeogenesis.

B) Why is the equation for gluconeogenesis not the reverse of glycolysis?

C) Provide the workarounds that gluconeogenesis takes to overcome the rate-limiting steps of glycolysis (Write the reactions and enzymes).

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?

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?

**Question 2. Gluconeogenesis #2**

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?

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.

C. Which key enzyme(s) in gluconeogenesis is/are likely affected in Patient 2? Explain how the data supports your conclusion.

D. Describe a feedback mechanism within the gluconeogenesis pathway.

E. Why is compartmentalization necessary in gluconeogenesis?

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

**Question 3. Glycogen Synthesis and Degradation**

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?

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

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

2. High epinephrine levels

3. Drug that increases insulin receptor affinity for insulin

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.

**Question 4. Carbohydrate storage and utilization**

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.

A.3. Suggest why cellulose and amylose/amylopectin have such different chemical properties and cellular functions. (Be general.)

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?

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.

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?

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?

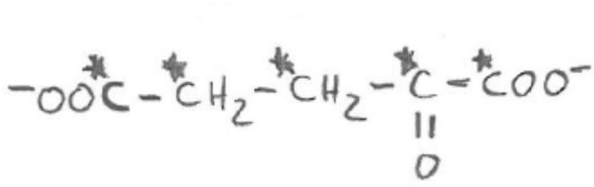
**Question 5: Adapted from 2016 Exam 3**

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?

**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.

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:

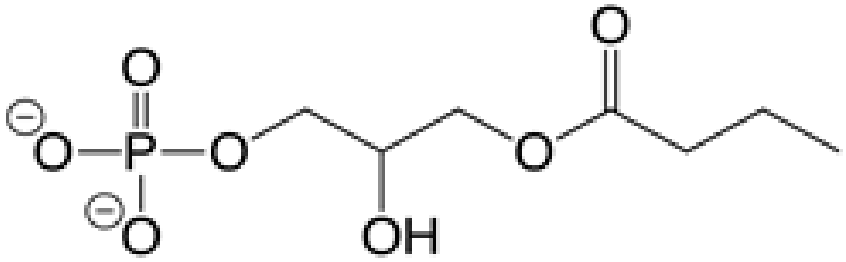


**C. (5 points)** Based on your knowledge of TCA cycle reactions, circle the carbons on citrate that you expect to be labeled if it is produced from labeled □-ketoglutarate.
For your answer, assume that glucose generates unlabeled acetyl-coA. It is not necessary to provide enzymes names or any reaction 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?

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

7.05 General Biochemistry
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