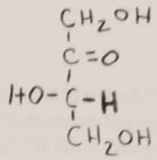
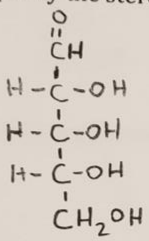
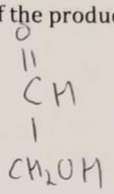
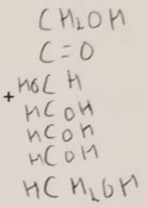
QUESTION 1 (15 points)

Consider the following sugar (L-erythrulose):

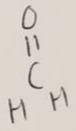
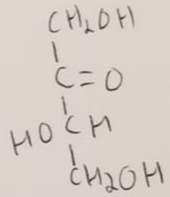


L-erythrulose

A. (4 points) Draw the products of the reaction catalyzed by Transketolase (TK) or Transaldolase (TA) if L-erythrulose is mixed with each of the following sugars (it is not necessary to specify the stereochemistry of the products):

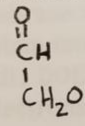
L-erythrulose +  ------- > TK  + 

1 pt. per correct structure

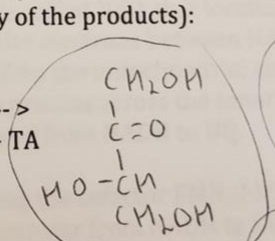
L-erythrulose +  ------- > TA  + 

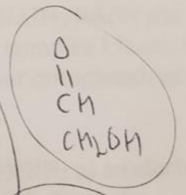
B. (5 points) Draw six products you expect to find if L-erythrulose is mixed with glycolaldehyde and BOTH Transketolase (TK) and Transaldolase (TA)(it is not necessary to specify the stereochemistry of the products):

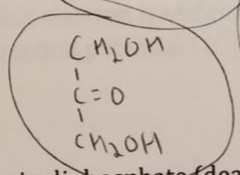
1 pt. per correct structure (only need 5 for full credit)

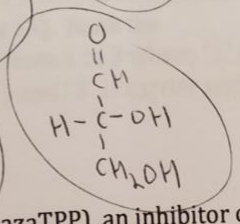
L-erythrulose +  ------- > TK + TA

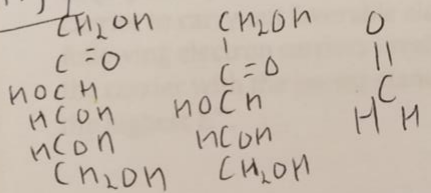
glycolaldehyde









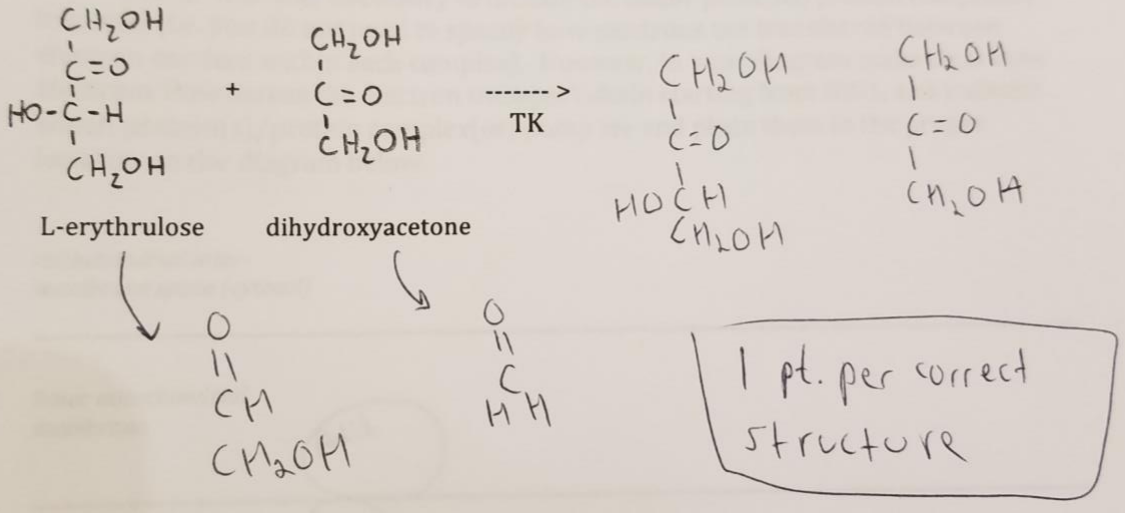


C. (2 points) If you include 3-deazathiamin diphosphate (deazaTPP), an inhibitor of all TPP+ dependent enzymes, in the reaction described in part B, circle the sugar(s) in you answer to part B that will NOT be produced.

0.5 pts per correct circled structure

(Question 1 continued)

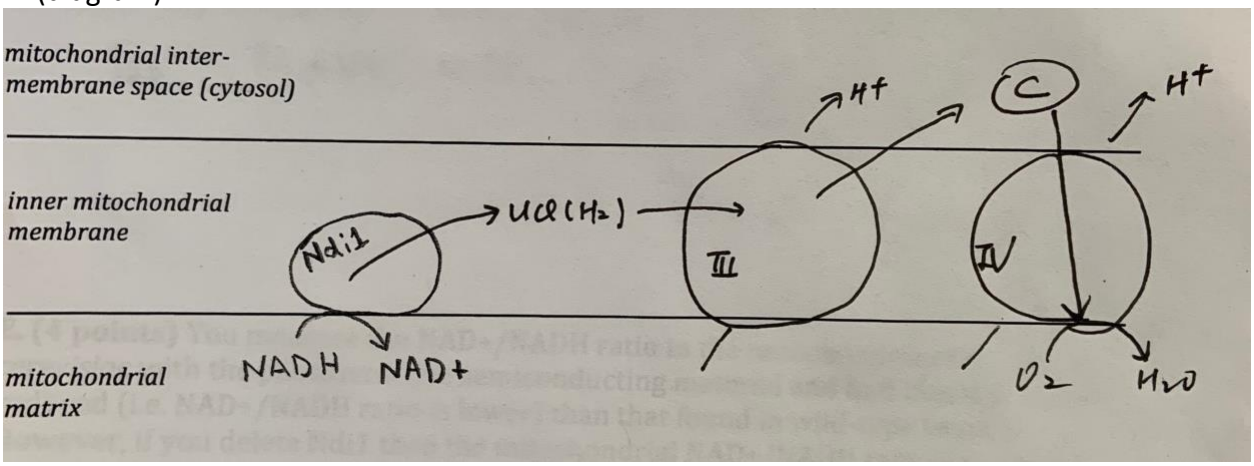
D. (4 points) If L-erythrulose is mixed with dihydroxyacetone and Transketolase (TK) what products do you expect to be produced? To answer the question, it will be important to consider the enzymatic mechanism of TK (it is not necessary to show the enzymatic mechanism or specify the stereochemistry of the products):



Question 2:

A. (3 points) 3: UQ/UQH2; 1: NAD+/NADH; 2: FMN/FMNH2 (1 point each)

B. (diagram):



Not mentioning proton flow: lose 1 point per complex; Not mentioning UQ(H2): lose 1 point; Not mentioning cytochrome c: lose 1 point.

B. (3 points): Less (1 point), because NdiI does not pump protons, but complex I does (2 points).

C. (2 points): Same (1 point), because energy release is the same regardless how many steps in the middle (1 point).

D. (4 points): Yes (2 points). UQH2->complex III->cytochrome c->complex IV->O2. And complex III and IV pump protons, so they will create delta potential / delta pH, which can drive ATP synthesis (2 points).

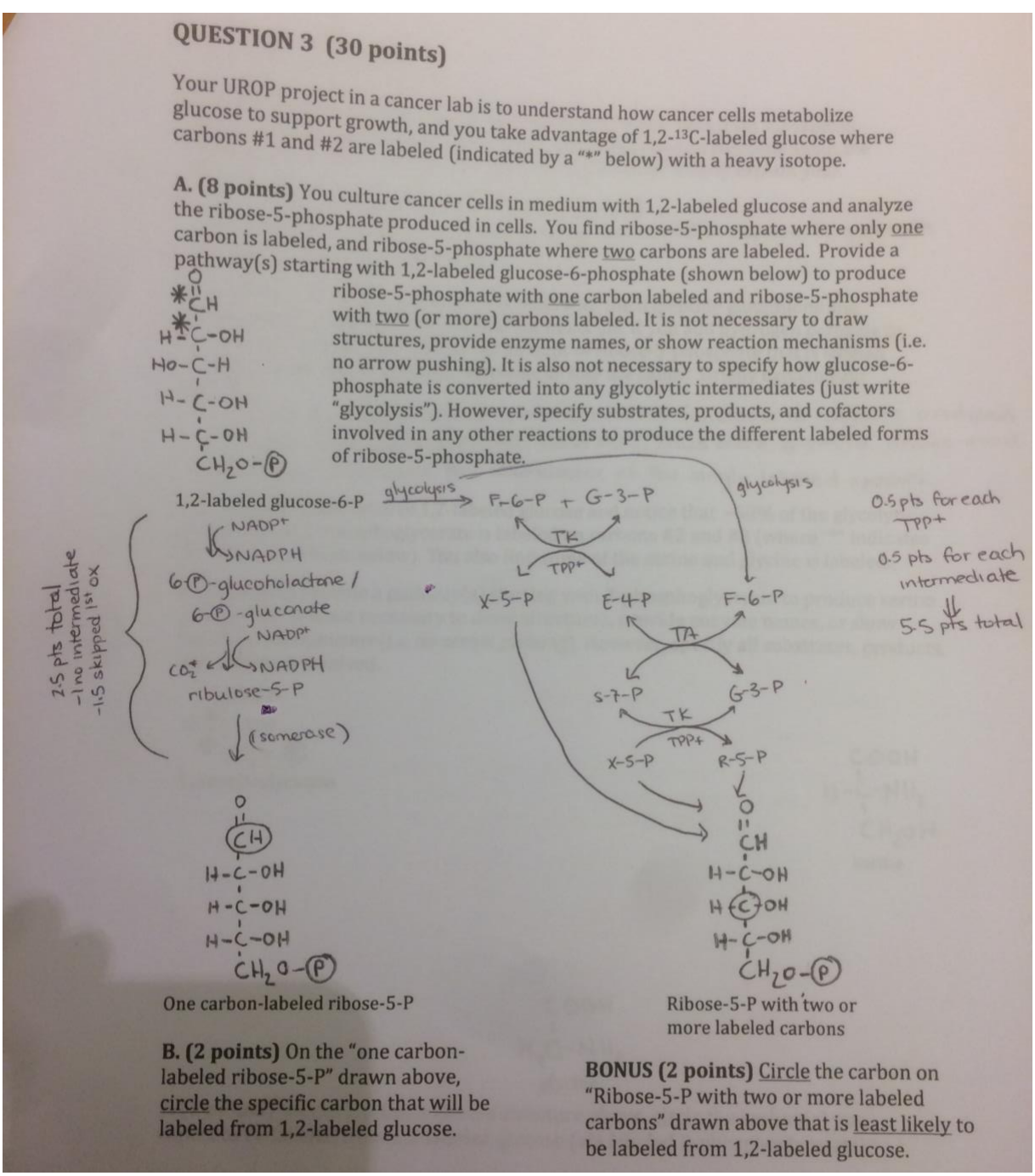
E. (4 points): UQH2 can reduce NAD+ -> NADH via NdiI only if light produces UQH2.

F. (4 points): No (2 points). Multiple reasons are accepted: no rubisco (generally mentioning no co factors lose 2 points); cannot generate NADH etc.

QUESTION 3 (30 points)

Your UROP project in a cancer lab is to understand how cancer cells metabolize glucose to support growth, and you take advantage of 1,2-^{13}C-labeled glucose where carbons #1 and #2 are labeled (indicated by a "*" below) with a heavy isotope.

A. (8 points) You culture cancer cells in medium with 1,2-labeled glucose and analyze the ribose-5-phosphate produced in cells. You find ribose-5-phosphate where only one carbon is labeled, and ribose-5-phosphate where two carbons are labeled. Provide a pathway(s) starting with 1,2-labeled glucose-6-phosphate (shown below) to produce ribose-5-phosphate with one carbon labeled and ribose-5-phosphate with two (or more) carbons labeled. It is not necessary to draw structures, provide enzyme names, or show reaction mechanisms (i.e. no arrow pushing). It is also not necessary to specify how glucose-6-phosphate is converted into any glycolytic intermediates (just write "glycolysis"). However, specify substrates, products, and cofactors involved in any other reactions to produce the different labeled forms of ribose-5-phosphate.



One carbon-labeled ribose-5-P

Ribose-5-P with two or more labeled carbons

B. (2 points) On the "one carbon-labeled ribose-5-P" drawn above, circle the specific carbon that will be labeled from 1,2-labeled glucose.

BONUS (2 points) Circle the carbon on "Ribose-5-P with two or more labeled carbons" drawn above that is least likely to be labeled from 1,2-labeled glucose.

(Question 3 continued)

C. (3 points) You find that most ribose labeled in cancer cells from 1,2-labeled glucose contains <u>two or more</u> labeled carbons. What can you conclude about the relative demand of these cells for NADPH versus ribose? Briefly explain your answer.

Ribose demand > NADPH demand

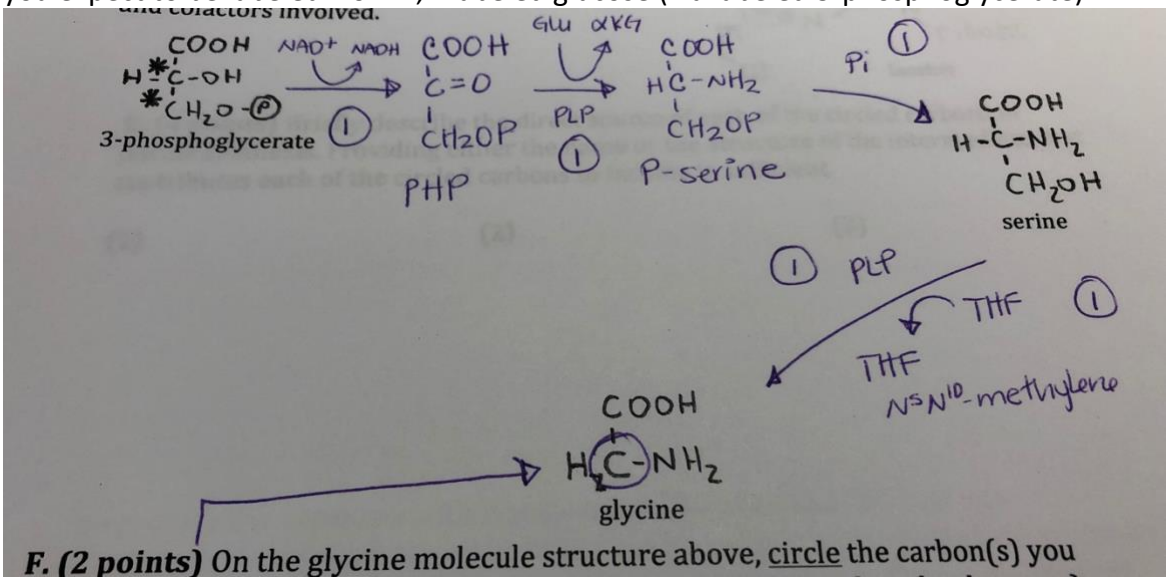
2+ labeled species come from running non-oxPPP in reverse to generate ribose w/o making NADPH

D. (2 points) Hydrogen peroxide will react with the tripeptide glutathione and promote the oxidized G-S-S-G form of the molecule. If you add hydrogen peroxide to the culture media, do you expect the amount of ribose-5-phosphate that is labeled on <u>only one carbon</u> to increase, decrease, or stay the same? Briefly explain your answer.

Increase singly labeled species. NADPH functions to reduce oxidized G-S-S-G and protect the cell from ROS. Thus more G-S-S-G drives more oxPPP and increases the abundance of the singly labeled species.

E (5points total – each labeled element in diagram is worth 1 point): Provide a pathway(s) starting with 3-phosphoglycerate to produce serine and glycine. It is not necessary to draw structures, provide enzyme names, or show reaction mechanisms (i.e. no arrow pushing). However, specify all substrates, products, and cofactors involved.

F (2 points total – all or nothing) : On the glycine molecule structure above, circle the carbon(s) you expect to be labeled from 1,2-labeled glucose (via labeled 3-phosphoglycerate).



F. (2 points) On the glycine molecule structure above, <u>circle</u> the carbon(s) you

G (4 points total – 2 for circling thymine only and 2 for the explanation: Considering ONLY labeled carbon that you could be incorporated from serine and glycine from 1,2-labeled glucose

via the pathway you provided in E, circle each pyrimidine base below that you expect to contain labeled carbon. Very briefly explain your answer.

Thymine only is the answer. One carbon is donated through THF that methylates dU to form dT.

H (4 points total – 1.3 each): Briefly describe the direct source of each of the circled carbons in the purine synthesis. Providing either the name or the structure of the intermediate that contributes each of the circled carbons to inosine is sufficient.

1) *N^{10} formyl-THF*
2) *Glycine*
3) *N^{10} formyl-THF*

Question 4:

A (7 points): See below diagrams. The schematic/drawing of the enzyme complexes was not required; however, the reactions and their intermediates were. Written descriptions were also accepted. The inclusion of the following co-factors and substrates was awarded a point: ACC; Biotin; malonyl CoA, ACP, NADP+/NADPH. An additional two points were allocated and were deducted for incorrect statements about the pathway.

Malonyl-CoA Synthesis by ACC:

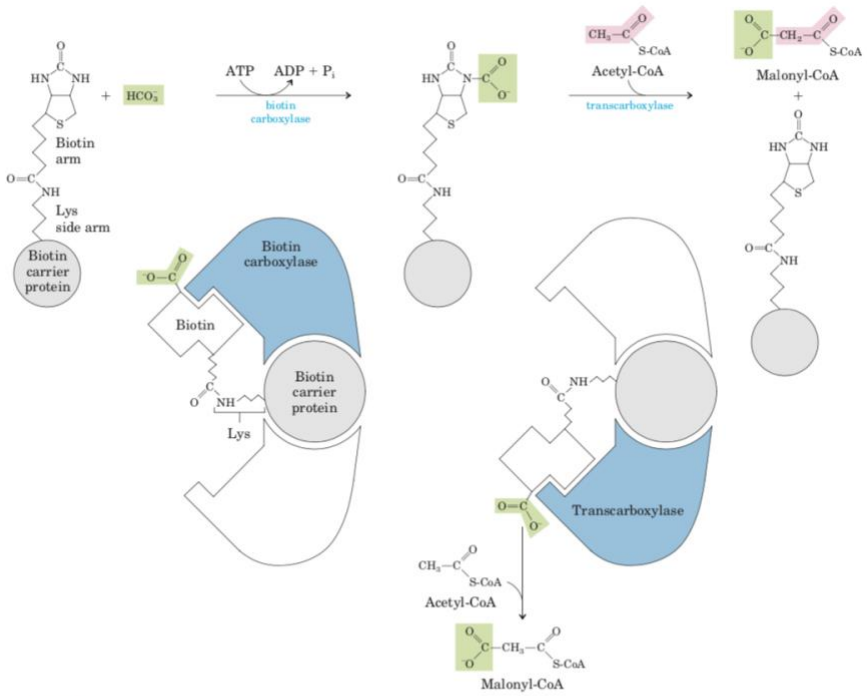


FIGURE 21–1 The acetyl-CoA carboxylase reaction. Acetyl-CoA carboxylase has three functional regions: biotin carrier protein (gray); biotin carboxylase, which activates CO_2 by attaching it to a nitrogen in the biotin ring in an ATP-dependent reaction (see Fig. 16–16); and transcarboxylase, which transfers activated CO_2 (shaded green) from biotin to acetyl-CoA, producing malonyl-CoA. The long, flexible biotin arm carries the activated CO_2 from the biotin carboxylase region to the transcarboxylase active site, as shown in the diagrams below the reaction arrows. The active enzyme in each step is shaded blue.

4 carbon fatty acyl-Coa synthesis by ACC:

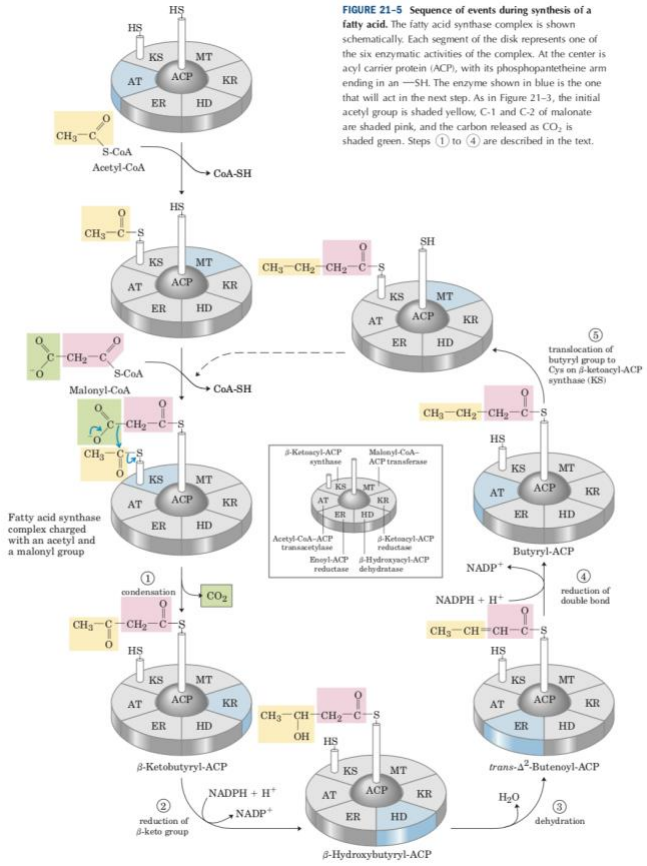


FIGURE 21–5 Sequence of events during synthesis of a fatty acid. The fatty acid synthase complex is shown schematically. Each segment of the disk represents one of the six enzymatic activities of the complex. At the center is acyl carrier protein (ACP), with its phosphopantetheine arm ending in an —SH. The enzyme shown in blue is the one that will act in the next step. As in Figure 21–3, the initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. Steps ① to ④ are described in the text.

B (3 points): High oxygen content in the water would compete for CO2 in Rubisco and lead to photorespiration. By generating CO2 in this step, this organism can keep CO2 levels higher to compete with oxygen. Some statement about how a high O2 concentration could interfere with carbon fixation was awarded partial credit. The mechanism including some description of Rubisco or photorespiration was awarded full points.

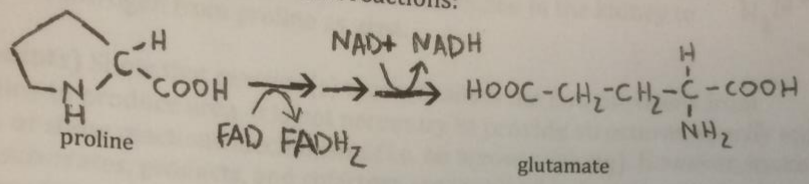
C (2 points): ACC is a critical regulatory step for fatty acid synthesis in humans. Without ACC, regulation must occur through a different set of enzymatic steps in this organism. Partial credit was given for some description that the regulation might be different because the pathway was different (ie no mention of ACC specifically).

D (3 points): Glucogenic amino acids can be broken down to OAA which is required for malonyl-CoA and fatty acid synthesis in this organism. Ketogenic amino acids could contribute via an acetyl-CoA to OAA conversion if there existed a glycoxlyate pathway in this organism — which does exist in many plants, bacteria, protists, and fungi. A correct description of glucogenic/ketogenic amino acids and their products was awarded partial credit, further credit was awarded for the understanding glucogenic amino acids would be required for fatty acid

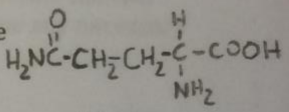
synthesis in this organism without the glycoxylate pathway, and the inclusion of the glycoxylate pathway for the use of ketogenic amino acids was required for full credit.

QUESTION 5 (15 points)

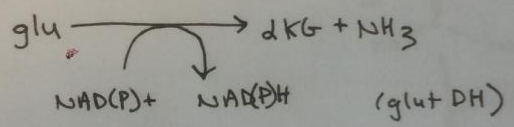
Recently it was discovered that some cancer cells can catabolize proline (derived from breakdown of collagen) as a source of energy to survive. Proline is converted to glutamate via a series of oxidation reactions:



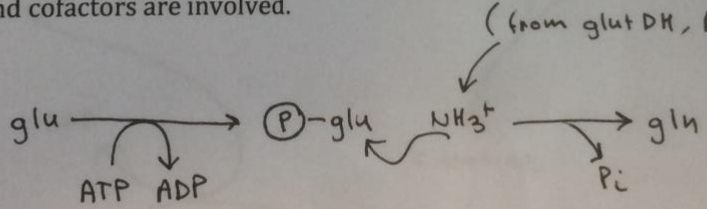
You add proline that contains isotopically labeled nitrogen to the culture media under conditions where the cells catabolize proline, that find that the cells excrete both ammonia (NH_3) and glutamine (shown at right) that contain labeled nitrogen.



A. (2 points) Show the reaction that will allow glutamate with nitrogen derived from proline to produce ammonia. It is not necessary to specify enzyme names or show reactions mechanisms (i.e. no arrow pushing). However, make clear which substrates, products, and cofactors are involved.



B. (3 points) Show the reaction(s) that will allow labeled nitrogen from proline to produce glutamine with <u>two</u> labeled nitrogens. You do not need to show how proline is turned into glutamate with one labeled nitrogen (it occurs via the reactions shown above). It is also not necessary to provide structures, specify enzyme names, or show reactions mechanisms (i.e. no arrow pushing). However, make clear which substrates, products, and cofactors are involved.

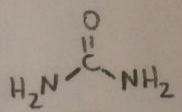


C. (2 points) Will the oxidation of proline in the mitochondria to derive energy require access to oxygen? Briefly explain your reasoning.

yes. need e⁻ acceptor for $FADH_2$ + NADH

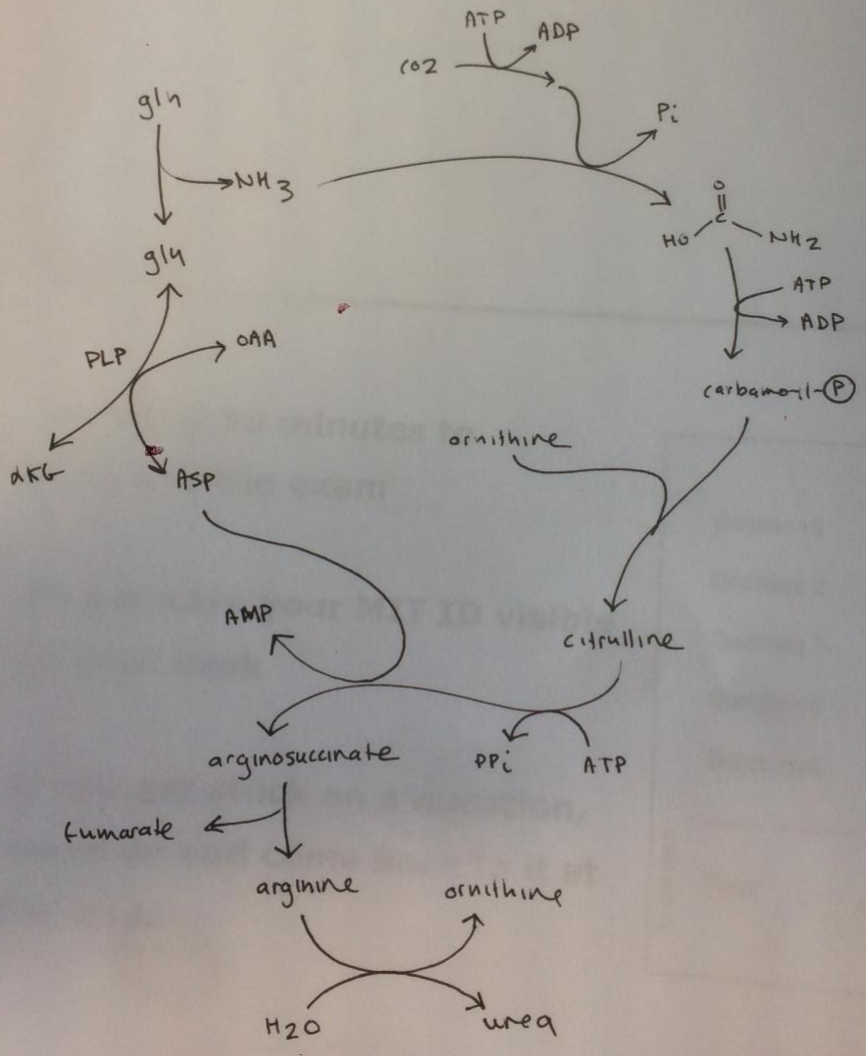
(Question 5 continued)

You analyze what is excreted into the blood from a proline-consuming tumor, and find that here is a large amount of glutamine. You reason that this glutamine must be metabolized in the kidney to excrete the nitrogen from proline as urea.



urea

D. (8 points) Show that reaction(s) that will allow the two nitrogens from glutamine to produce urea. It is not necessary to provide structures, specify enzyme names, or show reactions mechanisms (i.e. no arrow pushing). However, make clear which substrates, products, and cofactors are involved to incorporate the two nitrogens from glutamine into the same molecule of urea. Assume any necessary metabolites and cofactors are freely available.



7.05 General Biochemistry
Spring 2020