NAME:_____ Exam 1

The following exam questions are based on lab/class activities and the paper:

Yoannis Imbert-Fernandez, Brian F. Clem, Julie O'Neal, Danieal A. Kerr, Robert Spaulding, Lilibeth Lanceta, Amy L. Clem, Sucheta Telang, and Jason Chesney (2014). Estradiol Stimulates Glucose Metabolism via 6-Phosphofructo-2-kinase (PFKFB3). **JBC 289(13)**: 9440-9448.

For Your Information Only – DO NOT ANSWER

Pentose Phosphate Pathway Background:

1. Draw glucose.
2. React glucose with hexokinase.
3. Convert C1 to a carbonyl (i.e. ester). This is named 6-phosphoglucolactone.
    a. Suggest a name for this enzyme.
    b. Suggest any other reactants/products.
4. Linearize 6-phosphoglucolactone and convert C1 to a carboxylate. The enzyme that catalyzes this reaction is 6-phosphoglucolactonase, while the product is 6-phosphogluconate.
    a. Suggest any other reactants/products.
5. C1 is released as CO_2. This enzyme is named 6-phosphogluconate dehydrogenase. The final product is named ribulose-5 phosphate.
    a. A base abstracts the proton from the C3 hydroxyl group.
    b. One lone pair from the resulting oxyanion move to form double bond with C3, while the hydride attached to C3 is transferred to $NADP^+$. C3 should be a keto group.
    c. A lone pair from one of the C1 carboxylate oxyanions moves to form a double bond with C1. The electrons forming the C1 to C2 bond are withdrawn towards the ketone. These electrons form a double bond between C2 and C3. The carbonyl oxygen on C3 accepts an additional lone pair to form an oxyanion. C1 leaves as CO_2. How would you describe this intermediate?
    d. One lone pair of the oxyanion of C2 (note number change after CO_2 release) moves to reform the carbonyl, moving the pi-electrons towards C1, which picks up a proton.
    e. Suggest any other reactants/products.
6. Ribulose-5 phosphate undergoes a keto-enol tautomerization to the aldopentose to ribose-5-phosphate.

Exam Questions Begin Here

1. You are given a stock solution of glucose at 5.0 mg/mL. This stock solution needs to be diluted with phosphate buffer to result in 3 mL of solution at 0.520 mg/mL glucose. Determine the amount of glucose stock and phosphate buffer to mix.

_____ 5.0 mg/mL glucose stock

_____ phosphate buffer

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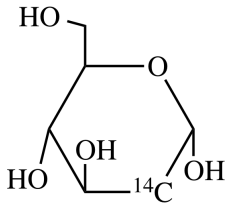
2. The authors use 2-[^{14}C]-dexoyglucose to track glucose uptake by cells. The method involves monitoring the activity of ^{14}C within cells. The ^{14}C must not be able to be metabolized completely. The structure of 2-[^{14}C]-dexoyglucose is below:



a. Track 2-[^{14}C]-dexoyglucose into/through glycolysis.
   i. Starting with 2-[^{14}C]-dexoyglucose, draw the chemical structure of each intermediate (metabolite) that is possible through the glycolytic pathway.

   ii. Is there a "dead end" intermediate that cannot continue to pyruvate?

b. Track 2-[^{14}C]-dexoyglucose into/through the pentose phosphate pathway.
   i. Starting with 2-[^{14}C]-dexoyglucose, draw the chemical structure of each intermediate (metabolite) that is possible through the pentose phosphate pathway.

   ii. Is there a "dead end" intermediate that cannot continue to ribose-5-phosphate?

c. Track 2-[^{14}C]-dexoyglucose into/through glycogen synthesis.
   i. Starting with 2-[^{14}C]-dexoyglucose, draw the chemical structure of each intermediate (metabolite) that is possible through the glycogen synthesis pathway.

   ii. Is ^{14}C incorporated into glycogen?

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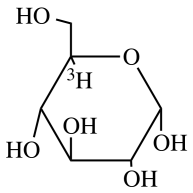
3. The authors use 5-[^{3}H]-glucose to track flux through glucose. The ^{3}H at position C5 is released through the process of glycolysis during the step catalyzed by enolase. To be an effective measure of glycolysis, the ^{3}H at position C5 must NOT be released through other known glucose metabolic pathways. The chemical structure of 5-[^{3}H]-glucose is below:



a. Draw a glycogen polymer and indicate where ^{3}H will be if 5-[^{3}H]-glucose is the starting reactant.

b. Draw ribose-5-phosphate and indicate where ^{3}H will be 5-[^{3}H]-glucose is the starting reactant for the pentose phosphate pathway.

4. Studies on several types of cancer cells (Bando *et al.* (2005). **Clin Cancer Res 11**: 5784-5792) indicate that PFKFB3 may be highly phosphorylated in malignant cells. If (*i*) as the title of the exam paper suggests "Estradiol Stimulates Glucose Metabolism via PFKFB3" and (*ii*) the PFKFB3 is phosphorylated as shown in some other cancer cells, which splice varient of PFKFB3 is present (e.g., hepatocyte, adipocyte, cardiac myocyte, and/or skeletal myocyte)? Explain your reasoning.