Exam 1 **NAME:**_____

CHEM/BIOL 260 (S13)

1.) Fructose-2,6-bisphosphate (FRU-2,6-P_2) was discovered in rat liver by Emile Van Schaftingen and coworkers in 1980. Working in the same laboratory as Van Schaftingen in 1981, Béatrice Lederer observed the following while working with *Saccharomyces cerevisiae* (baker's yeast, a single-celled eukaryote):

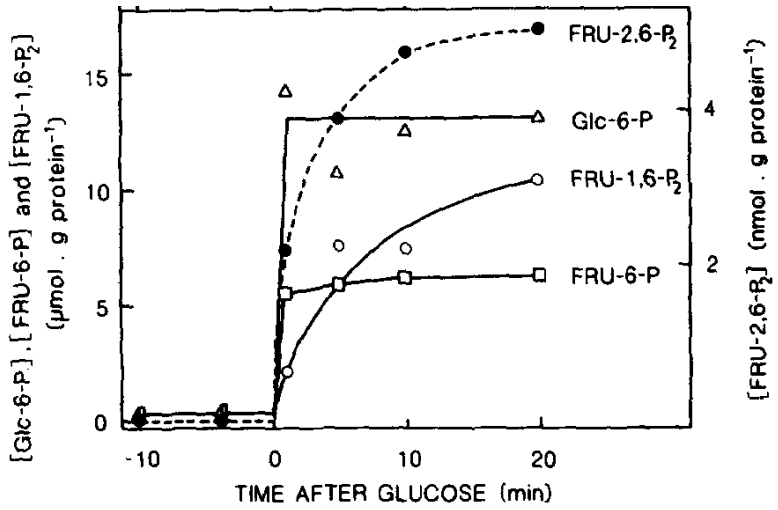

Figure 1: Concentration of hexose phosphates in starved *S. cerevisiae*. At time equal to zero, 2% glucose was added to the yeast cultures. [*Biochem. Biophys. Res. Commun.* (1981) **103**, 1281-1287]

a.) Twenty minutes after glucose stimulation, what are the relative observed concentrations of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosohate, and fructose-2,6-bisphosphate? Make sure to include units.

b.) Fructose-2,6-bosphsphate does not necessarily play the same metabolic role in yeast as is does in animals. **Are Lederer's observations consistent (not the same as conclusive) with fructose-2,6-bisphosphate being a regulatory molecule rather than a metabolic intermediate (metabolite)? Explain your reasoning.**

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Working in the same laboratory as Van Schaftingen in 1984, Jean François made the following observations in *S. cerevisiae*:

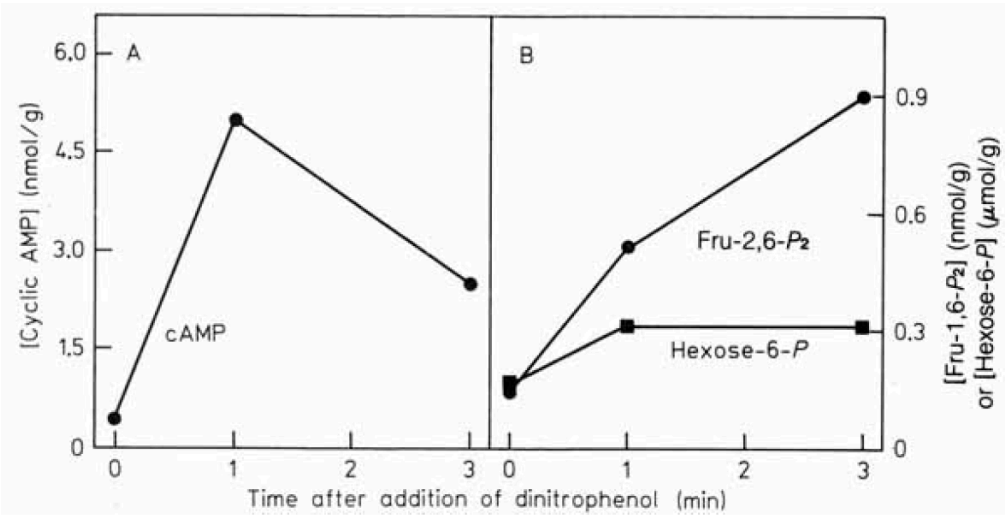

Figure 2: (A) Effect of 2,4-dinitrophenol on the concentration of cAMP and (B) hexose-6-P and Fru-2,6-P_2 in *S. cerevisiae.* Hexose-6-P is the sum of the concentrations of glucose-6-phosphate and fructose-6-phosphate. Culture media lacked glucose.

[*Eur. J. Biochem*. (1984) **145**, 187-193]

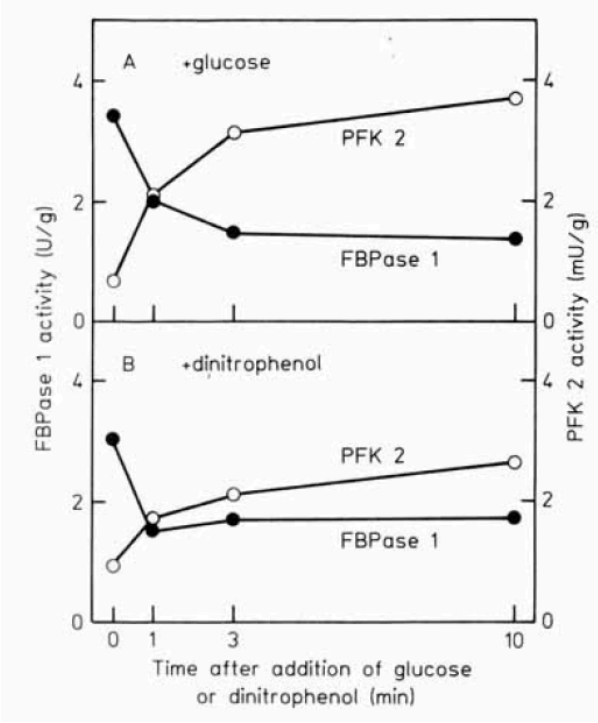

Figure 3: Effect of (A) glucose and (B) dinitrophenol on the activity of PFK-2 and FBPase-1 in *S. cerevisiae*. [*Eur. J. Biochem*. (1984) **145**, 187-193]

c.) Use Figure 2: **What effect does 2,4-dinitrophenol have on the level of cAMP in *S. cerevisiae* in the absence of a glucose stimulus*?*** Comment on the entire time interval.

d.) Using Figure 3: **What effect does 2,4-dinitrophenol have on the activity of PFK-2?**

e.) Does cAMP lead to activation or deactivation of PFK-2 in *S. cerevisiae?*

f.) What human cell type undergoes the same regulation of PFK-2 in response to cAMP as in *S. cerevisiae*? Explain your reasoning.

g.) If cAMP is the important second messenger in *S. cerevisiae* for the observed effects, why do the levels of fructose-2,6-bosphosphate continue to greatly increase between 1 and 3 minutes after dinitrophenol treatment even though the cAMP concentration drops during this interval?

cAMP regulation of PFK-2 evolved billions of years before humans came on the scene.....

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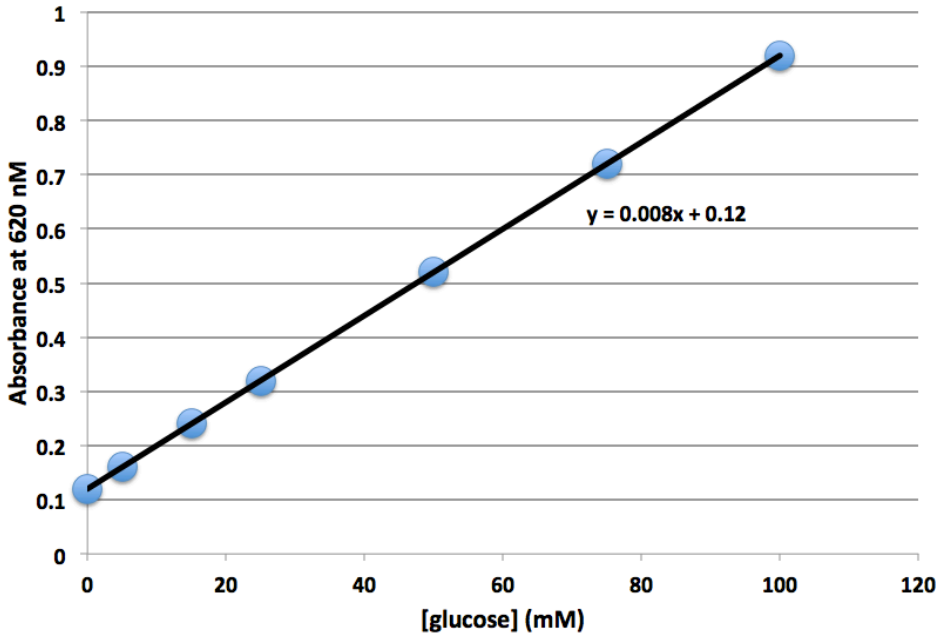
2.) Imagine that you collect the following standard curve with 3 mL samples:



You want to use this assay to determine the glucose concentration within the blood of a mouse.

a.) If you added 175 uL of blood to a 3 mL assay and found it to have an A_{620}=0.2104, what is the concentration of glucose in the original mouse blood? Show your work.

b.) If the mouse contained 1.46 mL of blood and had a mass of 25 g, what percentage of the mouse's body mass is blood glucose?

3.) The Entner-Doudoroff pathway is an alternate pathway used by some bacteria to convert glucose to pyruvate:

a.) Draw α-D-glucose.

b.) Put glucose through the hexokinase step of glycolysis.

c.) Convert C1 to a carbonyl (i.e. ester). This is named 6-phosphoglucolactone.
(i) Suggest a name for the enzyme for this step.
(ii) Suggest any other reactants/products.

d.) Linearize 6-phosphoglucolactone and convert C1 to a carboxylate. The enzyme that catalyzes this reaction is 6-phosphoglucolactonase, while the product is 6-phosphogluconate.
(i) Suggest any other reactants/products.

e.) Convert C2 to a ketone and C3 to a methylene (-CH_2-). Water leaves. The product is called 2-keto-3-deoxy-6-phosphogluconate.

(i) Suggest a name for the enzyme for this step.

(ii) Suggest any other reactants/products.

f.) Put 2-keto-3-deoxy-6-phosphogluconate through an aldolase reaction.

g.) Suggest a series of reactions by which these intermediates may be converted to pyruvate.