NAME:	

1. Imagine that you create a stock solution of the dye DCPIP. You need to know the absorbance of the stock solution, but it is too concentrated to read in the OceanOptic. The following dilution scheme allows you to determine the needed absorbance value. Fill in all the blank cells in the table below:

Tube	DCPIP	dH ₂ O	Dilution Factor from stock	Absorbance
1	stock	0 uL	1	
2	500 uL of tube #1	500 uL	1:2	
3	200 uL of tube #2		1:10	
4	200 uL of tube #3	800 uL		
5	100 uL of tube #4		1:500	0.68

2	17 - 2		1
2.	You ve	just eaten	lunch

a. Circle the metabolic pathways that are active in your hepatocytes.

Glycolysis	Gluconeogenesis
Glycogenesis	Glycogenolysis
Pentose phosphate pathway	Fructose breakdown

Lactate synthesis

b. List the hormonally-regulated enzymes that lead to the metabolic pathway pattern that you indicated in *part a* and indicate whether each is phosphorylated or dephosphorylated

Enzyme	Phosphorylated	Dephosphorylated

- 3. Chesney and coworkers concluded that estradiol stimulates glycolysis by increasing expression of PFK-2 in breast cancer cells with estrogen receptors in their recent publication [Imbert-Fernandez et al. (2014) **JBC**: in press].
 - a. Explain how the presence of increased amounts of PFK-2, which is not an enzyme involved in the glycolysis pathway, could be responsible for stimulating flux through glycolysis.

b. Chesney and coworkers used 2-deoxyglucose labeled with radioactive ¹⁴C at C1 to monitor glucose uptake by breast cancer cells. They found that ¹⁴C increased 12,000-fold within the cells in the presence of estradiol.

- i. What are the metabolic fates of glucose?
- ii. Of these pathways, which can fully utilize 2-deoxyglucose? Neglect enzyme specificity and focus on the organic chemistry of each pathway.
- iii. Does 2-deoxyglucose labeled with radioactive ¹⁴C at C1 accurately monitor glucose uptake or is it an over- or under- estimate? Explain.
- iv. Is it correct to conclude that glucose is accumulated in breast cancer cells in the presence of estradiol?

c. Chesney and coworkers used glucose labeled with radioactive ³H at C5 to monitor flux through glycolysis. They found a 1000-fold increase in H₂O containing ³H within cells in the presence of estradiol.

i. Explain why the accumulation of H₂O containing ³H is a measure of flux through glycolysis.

ii. Could any other position in glucose be labeled with ³H to yield a similar result? Explain why or why not.

4. Chen and coworkers created a transgenic mouse line that had significantly higher levels of a variant of Phosphoprotein phosphatase 1 (PP1) within liver cells. The authors found 8-fold higher levels of glycogen in cells over-producing PP1 than wild-type cells after an overnight fast. Explain this abnormal fasting-state result. [Zhang et al. (2014). Mol. Endocrinol 28(1): 116]