1. Digitoxin is an inhibitor of the Na+/K+ ATPase, and is prescribed to patients with congestive heart disease. (a) Describe the effect that digitoxin would have on the intracellular [Na+] and [K+] concentrations in heart muscle cells. (b) The therapeutic benefit of digitoxin is to cause more powerful muscle contractions in heart due to increased cellular Ca++ concentrations. Speculate on how inhibiting the Na+/K+ ATPase would increase cellular calcium.

(a) Digitoxin inhibits the Na+/K+ ATPase, which pumps sodium ions out of the cell and potassium ions into the cell at the expense of ATP energy. If this pump is inhibited, then the intracellular [Na+] would increase and the intracellular [K+] would decrease.

(b) There is a Ca++/Na+ antiport system that will transport calcium ions into heart muscle cells while transporting sodium ions out of the heart muscle cells. If heart muscle intracellular sodium increases, this would allow for more activity of this antiporter and an increase in intracellular calcium ions.
2. Diabetics sometimes eat fructose instead of glucose to better regulate blood glucose levels.
(a) (10 points) Suppose that the diabetic needed to make glucose from fructose? Describe the pathway which a diabetic liver would use to make glucose from fructose?
(b) (5 points) Speculate on why fructose would be less harmful to a diabetic than would glucose.

Fructose would be less harmful to a diabetic than glucose because the pancreas reacts to increased glucose levels in the blood. It does not respond to increases in other sugars such as fructose.
3. Assume that a culture of yeast cells is grown on glycerol-3-phosphate as a sole carbon source in an aerobic medium.

(a) (5 pts.) Explain briefly how the yeast cells could incorporate glycerol-3-phosphate into a metabolic sequence (used by glucose) to derive metabolic energy.

(b) (10 pts.) How many moles of NADH and FADH$_2$ would be produced per mole of glycerol-3-phosphate under aerobic conditions (include the citric acid cycle in your calculations). How many moles of ATP (and GTP) would be formed per mole of glycerol-3-phosphate metabolized. **Explain your answer!** (i.e., tell me which enzymes encountered by the glycerol-3-phosphate make each of the ATP, NADH, etc.)

(c) (10 pts.) If the glycerol-3-phosphate were labeled in the 2 position with radioactive carbon, in which position would isocitrate be labeled in the first turn of the citric acid cycle? (Draw structures, indicating the position of the radiolabeled carbon with an asterisk (*)).

(a) Glycerol-3-phosphate could be oxidized by an enzyme (glycerol-3-phosphate dehydrogenase?) to make glyceraldehyde-3-phosphate. The electrons released in the oxidation would be collected by a carrier (probably NAD). The glyceraldehyde-3-phosphate is a glycolytic intermediate.

(b) Glycerol-3-phosphate DH – 1 mole of NADH
Glyceraldehyde-3-phosphate DH – 1 mole of NADH
Phosphoglycerate kinase – 1 mole of ATP
Pyruvate kinase – 1 mole of ATP
Pyruvate DH – 1 mole of NADH
Isocitrate DH – 1 mole of NADH
α-ketoglutarate DH – 1 mole of NADH
Succinyl-CoA synthase – 1 mole of GTP
Succinate DH – 1 mole of FADH2
Malate DH – 1 mole of NADH

Total: 6 NADH, 1 FADH2, 2 ATP, 1 GTP

(c) 

![Diagram of the glycolysis and Krebs cycle](image)

HO

COO^-
4. The conversion of phosphorylase b to phosphorylase a is mediated by the enzymes *phosphorylase kinase* and *protein phosphatase-1*.

(a) (5 pts.) Name the substrates and products of the reactions catalyzed by phosphorylase kinase and protein phosphatase-1.  

*Phosphorylase a and ATP are the substrates for phosphorylase kinase; phosphorylase b and ADP are the products.*  
*Phosphorylase b and water are the substrates for protein phosphatase-1; phosphorylase a and phosphate are the products.*

(b) (5 pts.) Draw the pseudocycle created using the two enzymes, and write out the net metabolic reaction performed by the pseudocycle (we did this in class for phosphofructokinase and fructose-1,6-bisphosphatase).

\[
\begin{array}{c}
\text{ATP} \\
\text{ADP} \\
\text{phosphorylase a} \\
\text{Pi} \\
\text{H}_2\text{O} \\
\text{phosphorylase b}
\end{array}
\]

*The net reaction: ATP + H2O \rightarrow ADP + Pi*

(c) (5 pts.) A protein inhibitor of protein phosphatase-1, *Inhibitor-2*, has been discovered. How will the addition of inhibitor-2 to the pseudocycle described in (b) effect the ratio of phosphorylase a/phosphorylase b?

*If the phosphatase is inhibited, more phosphorylase will be phosphorylated (that is, in the form of phosphorylase b)*

(d) (The tough 10 pts.) The regulation of the conversion of phosphorylase a and phosphorylase b in muscle is proposed to exhibit *zero order ultrasensitivity*. In a nutshell, the relative concentrations of phosphorylase a and phosphorylase b are dependent only upon the *ratio* of active phosphorylase kinase and protein phosphatase-1, and independent of the concentrations of the two individual enzymes. Use your knowledge of enzyme kinetics to explain the concept of zero order ultrasensitivity.
The Michaelis-Menton equation says that

\[ v = k_2 [E]_i [S] / (K_m + [S]) \]

That is, the measured velocity is proportional to the total active enzyme concentration. If you have two enzymes, one catalyzing the forward reaction and one the reverse reaction, the relative amounts of reactants and products will depend upon the relative catalytic rate constants and the relative enzyme concentrations. It won’t depend upon the total concentrations of reactants and products, because the system will come to a steady state. Therefore, if you change the ratio of concentrations of active enzymes, you will change the relative concentrations of reactants and products.
5. (10 pts.) Cyanide inhibits electron transport by binding tightly to the ferric form (Fe\(^{3+}\)) of cytochrome a\(_3\). Cyanide poisoning can be overcome by the immediate addition of sodium nitrite (NaNO\(_2\)) to the patient; the treatment does not work if delayed, or if sodium nitrate (NaNO\(_3\)) is added instead. How does sodium nitrite treatment overcome cyanide poisoning of cytochrome a\(_3\)?

*Sodium nitrite will reduce the iron in cytochrome a\(_3\) to its +2 oxidation state:*

\[
2 \text{Fe}^{3+} + \text{NO}_2^- \rightarrow 2 \text{Fe}^{2+} + \text{NO}_3^-
\]

*The heme iron cannot bind cyanide in its +2 oxidation state. Nitrate will not reduce the iron, and if nitrite is added too late, the cyanide will irreversibly bind and the iron will not be available for the reduction.*

5. (15 pts.) Describe the effect on the citric acid cycle of (a) increasing the concentration of NAD+, (b) reducing the concentration of ATP, and (c) increasing the concentration of isocitrate.

*Increasing NAD+ will stimulate the cycle by activating pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.*

*Reducing [ATP] will stimulate the cycle by activating pyruvate dehydrogenase and α-ketoglutarate dehydrogenase.*

*Increasing the concentration of isocitrate will stimulate the cycle by increasing the concentration of citric acid cycle intermediates (oxaloacetate) which will increase the amount of acetyl-CoA allowed into the cycle.*
7. (20 pts.) 2,4-Dinitrophenol (DNP) is an uncoupler of mitochondrial respiration. (a) If DNP is added to isolated rat liver mitochondria fed with pyruvate, what would be the effect of DNP on the rate of pyruvate utilization? *It would greatly increase, since the proton gradient will be dissipated and any NADH made will quickly be reoxidized to NAD+, stimulating the citric acid cycle.*

(b) How would the added DNP affect the rate of ATP synthesis. *ATP synthesis would stop, since there would not be a proton gradient that is required for ATP synthesis.*

(c) DNP was once given as a diet drug, with disastrous effects (see p. 591). However, brown adipose mitochondria use the uncoupling protein in the same manner, without adverse effect. What are the differences between the uncoupling protein in brown adipose mitochondria and DNP in rat liver mitochondria?

*Regulation – the uncoupler protein in brown adipose is regulated by the concentration of free fatty acids, which in turn are hormonally controlled.*