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 intracellular Ca++ concentrations. Speculate on how inhibiting the Na+/K+ ATPase would increase intracellular calcium (hint – describe a calcium transporter).
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.
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 (*)).
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.

(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).

(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?

(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.

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$_3$?

6. (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.
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? (b) How would the added DNP affect the rate of 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?