1. The intensity of scattered x-rays depends on
   a) the number of electrons. (+1)
   b) the position of electrons. (+1)
   c) the number of neutrons.
   d) both a and b.(+3)

2. Cholesterol is essential for normal membrane function because it
   a) insures that a bilayer is formed.
   b) neutralizes harmful fatty acids.
   c) keeps membranes fluid.
   d) carries electrons during oxidative phosphorylation.

3. In a eukaryotic cell, the enzymes of glycolysis are located in the ____ and the enzymes of the TCA cycle are located in the ____:
   a) plasma membrane, cytosol.
   b) cytosol, mitochondrial matrix.(3 pts)
   c) cytosol, mitochondrial membrane.(1pt)
   d) nucleus, cytosol.

4. A kinase is an enzyme that:
   a) adds water to a double bond.
   b) uses $FADH_2$ to change the oxidation state of the substrate.
   c) uses ATP to add a phosphate group to the substrate.(3 pts)
   d) removes phosphate groups off of substrates.(1 pt)

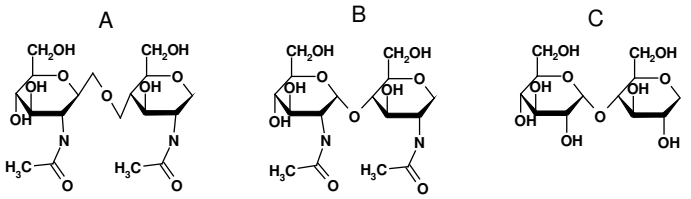
5. The common metabolic intermediate that is shared by both metabolism of glucose and fatty acids is:
   a) oxaloacetate.
   b) lactic acid.
   c) ethanol.
   d) acetyl-CoA

6. Which of the following compound is responsible for *coordinated* regulation of glycolysis and gluconeogenesis?
   a) NADH
   b) acetyl-CoA
   c) fructose 2,6 bis phosphate (3 pts)
   d) fructose 1,6 bis phosphate(1 pt)

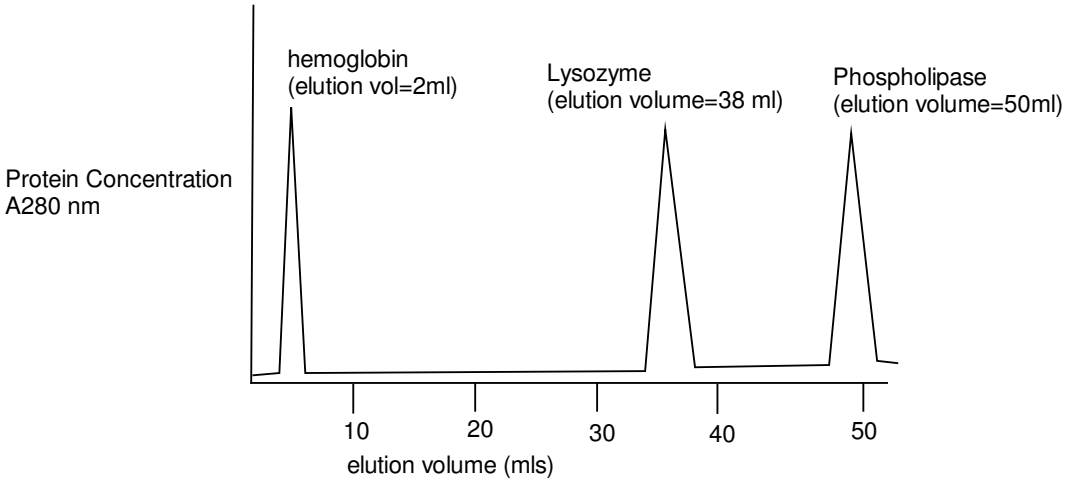
B1. (14 Pts) A solution contains a mixture of three proteins. You are trying to purify lysozyme from this mixture. Some of physical properties of these proteins are given in the table below (Recall that hemoglobin is a tetramer).

	Molecular Weight	log(MW)	Isoelectric pH
Phospholipase	10 kDa	1.00	5
Lysozyme	15 kDa	1.18	7
Hemoglobin	60 kDa	1.78	7

i) Which of the following three substrates would you use to assay for lysozyme, briefly justify your answer. [Hint: Lysozyme digests bacterial cell walls] (5 pts).



A is the correct choice (+3 pts). 2 points were given for B. You also needed to state something about the presence of the N-acetyl group (+1 pt) as well as cleavage of a β linkage (1 pt)



ii) **The expected elution profile is shown to the right. 3 pts were given for the correct order of the peaks (hemoglobin elutes 1st since it is the largest). 1/2 point for the y-axis label and 1 point for the x-axis label. 1/2 point for each elution volume.**

iii) Could you separate these three proteins using a Gel filtration column.? Justify your answer. If your answer is no, what additional purification step(s) would be necessary to separate these proteins?(3 pts)

Yes, in fact they would be clearly separated.

Alternatively, if you stated no, your answer was graded according to the following scheme:

+1 pt: for stating the peaks for lysozyme and phospholipase would overlap.
+1.5 pts: for stating a workable purification scheme (e.g. isoelectric focusing, affinity chromatography, ion exchange chromatography.
+0.5 pts: for stating how the purification scheme would work, eg different charge at different pHs, etc.

B2: (6 pts) Answer **ONE** of the following two questions, please indicate which one you are answering

i) The principle component of margarine or butter are triglycerides. Both margarine and butter melt on hot summer days. Suggest a way of altering the triglyceride composition to prevent melting in the summer. You answer should include some information on the underlying molecular forces involved.

Need to raise the melting point. This can be done by either reducing the number of cis double bonds or increasing the length of the acyl chains (3 pts). Either way, increasing Van der Waals forces will lead to an increase in the melting temperature (3 pts)

OR

ii) How does the structure of integral membrane proteins differ from that of normal water soluble proteins.

Nonpolar character (2 pts). Integral membrane proteins have nonpolar surfaces in contact with the lipid. Soluble proteins have more polar surfaces (1pt)

Hydrogen bonds(2pts): All H-bonds have to be satisfied in membrane proteins since there are no suitable donors or acceptors in the lipid. Soluble proteins can H-bond with water.(1 pt)

B3: (13 Pts) Answer **ONE** of the following two questions:

i) The aconitase reaction in the TCA cycle converts citrate to isocitrate. The standard free energy for this reaction, ΔG^0, is 0 kJ/mol.

a) Given that the Gibbs free energy for this reaction is -10 kJ/mol, what is the ratio of isocitrate to citrate during normal operation of the TCA cycle? (9 pts)

b) Provide a plausible explanation for the *non-equilibrium* concentration of isocitrate during the normal operation of the TCA cycle.(4 pts)

$\Delta G = \Delta G^o + RT \ln$ [isocitrate]/[citrate]
-10 kJ/mol = 0 + 2.5 ln [isocitrate]/[citrate
0.018 = [isocitrate]/[citrate]

Since ΔG^o is zero, the equilibrium concentration of isocitrate and citrate should be equal. The concentration of isocitrate is lower because the next step in the pathway has a large negative ΔG^o

OR

ii) In anaerobic metabolism pyruvate is reduced to lactate by NADH.

a) Using the 1/2 reactions on the formula page, write a balanced equation for the overall reaction.(3 pts)

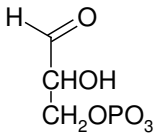
b) Calculate the overall standard free energy change, ΔG^o for this reaction.(5 pts)

$Pyr + 2H^+ + 2e^-$	**$\rightarrow$**	**lactate**	**+35.5 kJ/mol**
NADH	**$\rightarrow$**	**$NAD^+ + H^+ + 2e^-$**	**-60.5 kJ/mol**
$Pyr+NADH+H^+$	**$\rightarrow$**	**$Lactate + NAD^+$**	**-25 kJ/mol (overall reaction)**

c) If pyruvate is mixed with NADH (in the presence of a suitable enzyme) some of the pyruvate will be converted to lactate. At *equilibrium* will the concentration of pyruvate exceed that of the lactate or will there be more lactate than pyruvate. Briefly justify your answer. (5 pts)

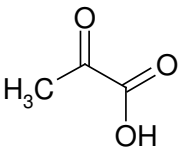
Since ΔG^o is less than zero, the products would be favored, [lactate]>[Pyr]

B4: (6 pts) Do **ONE** of the following three questions. Please indicate which question you are attempting. You may want to look at question B5 before attempting this question.



i) The key energy generating step in glycolysis is the conversion of glyceraldehyde-3-Phosphate to 1,3 bisphosphoglycerate. The substrate for this reaction is shown to the right. Draw the chemical structure of the product and indicate any other substrates and/or products (e.g. ATP) that are involved in this reaction.

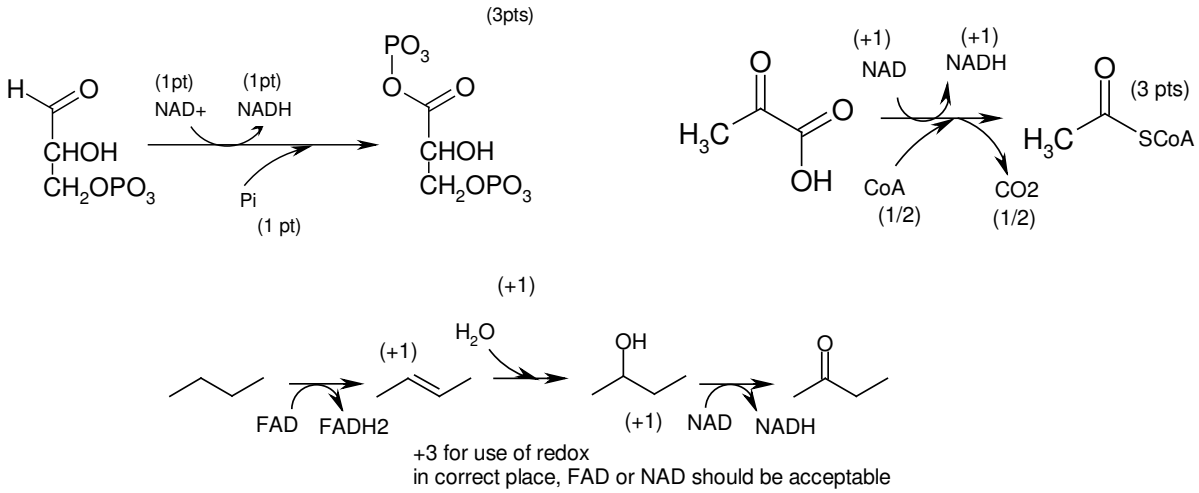
OR



ii) Most of the key energy generating steps in the TCA cycle generate energy with an identical biochemical mechanism. The substrate for one of these reactions, Pyruvate, is shown to the right. Draw the chemical structure of the product and indicate any other substrates and/or products that are involved in the reaction.

OR

iii) An energy generating scheme that is common to both the TCA cycle and β-oxidation of fatty acids is the conversion of an alkane to a ketone. Beginning from the structure of the alkane, draw the chemical changes that would transform this into a ketone. Indicate any other substrates and/or products that are involved at each step of the reaction.



B5: (12 Pts)

Regardless of your choice for problem B4, the answer to the following questions are the *same*.

i) What is the *general* name for the reactions described in question B4 (Hint, what is the ultimate fate of carbon in metabolism.) (2 pt)?

Oxidation (+2 pts), if you stated REDOX you should have gotten 1.5 pts.

ii) All of the above transformations release energy. In what form is this energy stored after the reaction is complete (2 pts)?

As 'high-energy' electrons (1.5 pts), carried on either $NADH_2$ or $FADH_2$ (1/2 pt).
Alternatively you should have gotten full credit for stating a proton gradient.

iii) *Briefly* describe the final steps of metabolism that converts this stored energy to ATP (8 pts).

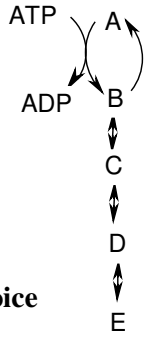
The grading for this section was divided into three parts:

4 pts: You needed to mention that electrons were passed to CoenzymeQ (1pt) and ended up on water (1pt). Some indication of the general path (e.g. Complex I→Complex II→Complex III→Complex IV) was necessary (2 pts).

2 Pts: The establishment of a proton gradient across the membrane by proton pumping (1.5 pts) from complexes I,III, and IV (1/2 pt).

2 Pts: Protons return across the membrane via ATPsynthase (1/2 pt), causing a conformational change that results in the synthesis of ATP from ADP and P_i (1.5 pts).

B6 Choice 1: A *hypothetical* metabolic pathway is shown to the right. In this pathway compound A is ultimately degraded to E. Compound E can also be used to synthesize A using most of the same enzymes in the pathway. However, it is necessary to perform the conversion of B to A using a different enzyme. The conversion of A to B and B to A is the regulated step in this pathway.



i) Which of the compounds (A-E) would be a product inhibitor for the ***biosynthetic*** pathway (E→A)? Why?(2 pts)

A since B→A is the regulated step and A is the product of this step.

ii) Which of the compounds (A, B, C, D, or E) would function as a feedback inhibitor for the *degradative* pathway? Briefly justify your answer.(3 pts)

Either C, D, or E (+1 pt) since they are all below the regulated step (1.5 pts). E is the most likely choice since it is the final product (1/2 pt)

iii) Why is it necessary to use one enzyme for the degradation of A to B and a different enzyme for the synthesis of A from B?(3 pts)

Since A→B requires energy the conversion of A→B must be thermodynamically unfavorable unless it is coupled to the hydrolysis of ATP. Therefore the reverse reaction would be spontaneous and need not use the same enzyme. In addition, the coordinated regulation of the opposing pathways would require two enzymes

iv) Using your knowledge of either the regulation of glycolysis/gluconeogenesis or the regulation of glycogen degradation/synthesis, describe a method to coordinately regulate this metabolic pathway such that both degradation and synthesis did not occur at the same time.(2 pts).

You would need to have an allosteric compound that would activate one direction and inhibit the other. This would be similar to the regulation of glycolysis/gluconeogenesis by F2,6P. Alternatively, protein phosphorylation could be used to activate on pathway and inhibit the other. This would be similar to the method used to regulate glycogen metabolism.

v) If this pathway was involved in the generation of energy in the direction of A→E what additional regulatory features would be desirable? Justify your answer and give an example from either glycolysis or the TCA cycle.(5 pts)

Inhibition by high levels of ATP or NADH or activation by ADP and NAD. The presence of the 1st two compounds indicate high energy reserves while the presence of the latter two indicate low energy reserves. Suitable examples are activation of PFK in glycolysis by ADP and its inhibition by ATP. Alternatively, most of the oxidatidative decarboxylation steps in the TCA cycle are inhibited by NADH and ATP.

B6 Choice 2:

i) When blood glucose levels are low, which enzyme is active, glycogen phosphorylase or glycogen synthase? Justify your answer in terms of the metabolic needs of the body under condition of low glucose.(4 pts)

When glucose is low it is necessary to release glucose from glycogen, therefore glycogen phosphorylase would be active.

ii) Briefly describe *how* this coordinated regulation occurs.(4 pts)

Protein phosphorylation is used to coordinately regulate glycogen synthesis and degradation. Glycogen phosphorylase is active when phosphorylated since the phosphorylation state of proteins is high when glucose levels are low. (2 pts for statements along these lines). Therefore glycogen synthase should be inhibited when phosphorylated. Dephosphorylation should inhibit glycogen phosphorylase and activate glycogen synthase.(2 pts)

iii) Glycogen storage diseases are often fatal if untreated. Assume that the enzyme for glycogen degradation was missing in an individual due to a genetic mutation. Give two metabolic consequences of this mutation? (3 pts)

Many answers were accepted. Including: Can't release glucose from glycogen, will continue to make glycogen, taking glucose out of circulation, glycogen levels will build up, eventually filling the cell full of glycogen, energy production from glycolysis will be impaired.

iv) How would you modify the diet of an affected individual to reduce the severity of this disease. Clearly state which alternative metabolic pathways would be used to satisfy the energy needs of the individual.(3 pts)

Amino acids, which will enter at the TCA cycle.

Fatty acids, which will be oxidized by β-oxidation, the resultant acetyl-CoA will enter the TCA cycle

v) What defect(s) in energy production could *not* be corrected by a change in diet?(1 pt)

There were several good examples:

Energy production under hormonal control will be impaired since glucose cannot be released from glycogen.

It will be difficult to generate energy quickly, since glycolysis cannot be used because glucose is not in the diet.