A :	______/16
B1:	______/ 8
B2:	______/12
B3:	______/ 8
B4:	______/ 9
B5:	______/ 7
B6:	______/ 8
B7:	______/12
B8:	______/12
B9:	______/ 8
Tot:	____/100

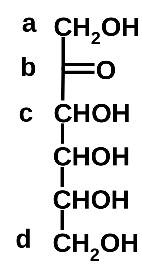
Part A: Multiple Choice. Please select the best single answer (2 pts, 16 total).

1. The following is usually calculated *directly* from X-ray diffraction data:
   a) The number of electrons in the crystal.
   b) The electron density at different locations in the crystal.
   c) The x, y, z coordinates of each atom.
   d) The size of the protein in the crystal.

2. Cholesterol is essential for normal membrane functions because it
   a) carries electrons in electron transport.
   b) spans the thickness of the bilayer.
   c) keeps membranes fluid.
   d) catalyzes lipid flip-flop in the bilayer.

3. The main difference between saturated and unsaturated fatty acids is
   a) the number of carbons.
   b) the presence of keto groups.
   c) the presence of double bonds.
   d) none of the above.

4. The sugar to the right is an ________ and the carbon labeled with ____ will become the anomeric carbon:
   a) ketose, d
   b) aldose, a
   c) ketose, b
   d) aldose, d

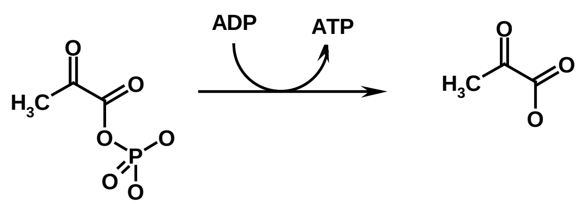


5. Glycogen is more highly branched than starch. Which of the following is the most important characteristic of glycogen?
   a) To provide more compact storage of glucose in animal cells.
   b) To provide more ends for the rapid release of glucose.
   c) To provide additional α (2-3) linkages.
   d) The original statement is false, neither glycogen or starch are branched.

6. In a eukaryotic cell, *activation* of fatty acids occurs in the _______ and *oxidation* occurs in the _________:
   a) cytosol, cytosol.
   b) cytosol, mitochondrial membrane.
   c) cytosol, mitochondrial matrix.
   d) mitochondrial matrix, mitochondrial matrix.

7. Mixing pure O_2 into a yeast culture growing on grape juice will cause the final product (wine) to:
   a) turn to vinegar.
   b) be a nearly alcohol-free 'beverage'.
   c) be no different than that obtained from yeast grown in air.
   d) have a higher ethanol level in the wine.

8. The reaction shown to the right is catalyzed by a:
   a) kinase
   b) phosphatase
   c) dehydrogenase
   d) hydratase



Part B: Short Answer. Please do all of the Questions. In most cases you have choices.

B1. Please do **one** of the following three questions (8 pts)

Choice A:

You work for a company that makes margarine (a common substitute for butter), which consists mostly of triglycerides. Your current formulation, or composition, of margarine melts at 70^o C, which is unsuitable for use in the summer (at least in Pittsburgh). How would you change the composition of the margarine such that it will remain *solid* at higher temperatures?

Either increase the length of the fatty acid chains or decrease the number of double bonds. (+7)

This will increase the degree of van der Waals interaction, raising the melting temperature (+1)

Choice B:

Electron transport within the mitochondrial membrane ceases to function as the temperature of the organism is decreased. Why does this occur and what component or step of the electron transport chain is most likely to be affected by the lower temperature.

At lower temperatures the membrane becomes less fluid, thus components cannot diffuse in the membrane as readily (+6)

This would have the largest effect on the electron carrier Q, since this must carry electrons from complex I and II to complex III. (+2)

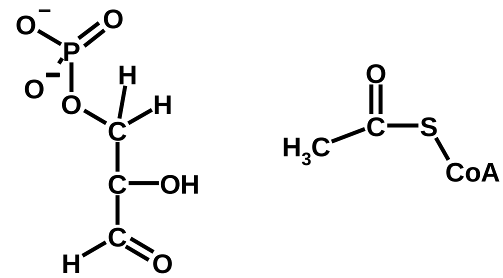
Choice C:

What major thermodynamic 'force', or interaction, drives the formation of phospholipid bilayers, as well as the tertiary structure of globular water soluble proteins.

The hydrophobic effect (+7).

Both bilayers and globular proteins have non-polar cores, that if exposed to the solvent will result in the ordering of water, which is unfavourable entropically. (+1)

B2. Please do **one** of the following two questions (12 pts):



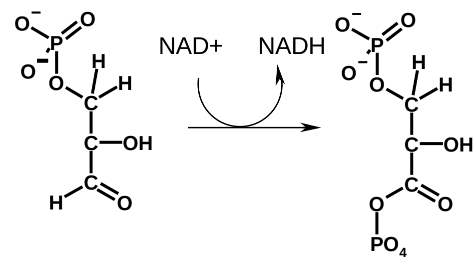
Choice A:

i) Describe, or illustrate with suitable chemical diagrams, the key energy producing step(s) in either glycolysis or the TCA cycle. (The chemical structures shown to the right may be of some use. The left-hand structure is found in glycolysis while the right-hand structure is found in the TCA cycle.).

ii) What is the general name for this type of reaction?

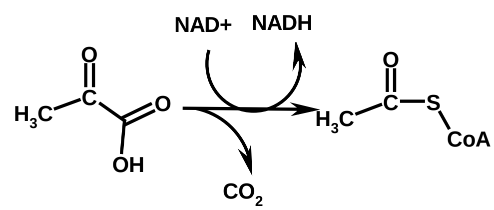
i)

Glycolysis:



- Oxidation of glyceraldehyde 3-P to 1,3 phosphoglycerate (+6).
- The energy associated with this oxidation is stored on NADH (+2).
- The enzyme that catalyzes this reaction is a dehydrogenase (+2)

TCA cycle:



- Oxidative decarboxylations occur at three steps. The reaction for the conversion of Pyruvate to AcetylCoA is shown to the right.(+6)
- The energy associated with this oxidation is stored on NADH (+2).
- The enzyme that catalyzes this reaction is a dehydrogenase (+2)

ii)Both of these steps are oxidations (+2) or more generally, redox reactions.

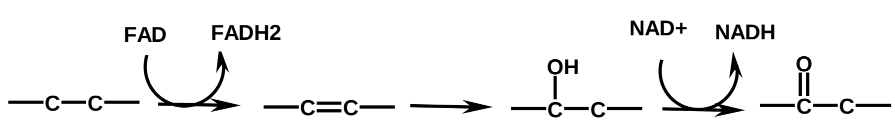
Choice B:

i) Describe, or illustrate with suitable chemical diagrams the chemical steps by which an alkane (C-C) is converted to a ketone (C=O) in biochemical pathways.

ii) What is the general name for this type of reaction?

iii) In what metabolic pathways does this overall reaction occur?

i) +4 for correct order of reactions. +4 for correct redox carriers (+3 if switched)



ii) An oxidation (+2) or more generally a redox reaction.

iii) Fatty acid oxidation (+1) and the latter part of the TCA cycle (+1)

B3. Many of the chemical changes in metabolic pathways involve large unfavorable changes in the standard free energy difference between the products and the reactants (ΔG^o). However, the reaction can be made favorable by either direct or indirect coupling. Briefly describe *either* direct or indirect coupling. Your answer should include an example from a biochemical pathway we have discussed in this course. You may find half-reactions useful in illustrating your answer in the case of direct coupling (8 pts).

Direct coupling occurs when the energy requiring reaction is coupled to an energy releasing reaction on the same enzyme (+4 pts). The overall ΔG^o becomes favorable, as illustrated by the following half-reactions for the phosphorylation of glucose by hexose kinase:

Glucose + $P_i \rightarrow$ G-6-P ΔG^o = +14 kJ/mol
ATP $\rightarrow$ ADP + P_i ΔG^o = -30 kJ/mol

Glucose + ATP $\rightarrow$ G-6-P + ADP ΔG^o = -16 kJ/mol

Any other kinase is a suitable example.

Indirect coupling is accomplished by changing the Gibbs free energy of a reaction to a negative value by reducing the concentration of the product. The reaction then becomes spontaneous. This occurs when a reaction following the energetically unfavourable reaction has a large negative ΔG^o. The coupling is indirect because two separate enzymes are involved.
Examples include the aldolase reaction in glycolysis and the conversion of fumarate to oxaloacetate in the TCA cycle.

B4. You are a track-and-field coach and you tell your sprinters (those who run short distances very fast) to go on a low carbohydrate/high protein diet. Your team begins to lose races. Why was your advice poor? Your answer should include a discussion of the metabolic pathways by which proteins are utilized by the body and the pathways for carbohydrate utilization and storage. (9 pts)

Your advice was poor because your sprinters will have low levels of glycogen stores because of their diet. In humans, it is not possible to convert acetyl-CoA into Pyruvate for the synthesis of glucose. Most amino acids enter metabolism through the TCA cycle, i.e. below acetyl-CoA. Consequently, it is difficult to make large amounts of glycogen. (+6).

High energy demands, such as during sprinting, are provided almost entirely by glycolysis. The glucose levels are rapidly depleted and glycogen must be hydrolyzed to glucose to provide additional glucose for glycolysis (+3)

B5. (7 pts) The properties of the three proteins are listed below.

	Molecular Weight	Solubility in Ammonium Sulfate[1]	# of Asp and Glu residues. ($pK_a = 4.0$)	# Lys and Arg Residues ($pK_a = 9.0$)
A	12,000 Da	2.0M	5	10
B	12,000 Da	2.0M	5	8
C	34,000 Da	2.0M	0	10

[1]This is the concentration of ammonium sulfate that will precipitate 50% of the protein, 75% will precipitate when the concentration is 0.5M higher than this value.

Which one of the following three purification schemes will provide pure protein A? You answer should briefly explain which proteins are separated at *each* step in the purification scheme.

Scheme A: Separation by gel filtration, followed by the addition of 1.5 M ammonium sulfate.

Scheme B: Separation by gel filtration, followed by anion exchange chromatography at pH 7.0.

Scheme C: Separation by gel filtration, followed by cation exchange chromatography at pH 7.0

Scheme A cannot work since all three proteins have the same solubility in Amm. sulfate.

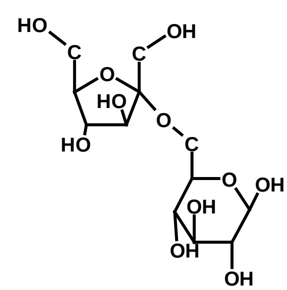
Both scheme B and C will separate proteins A and B from C on the basis of the difference in their molecular weights. (+3 pts)

To see which ion exchange chromatography to use, calculate the net charge on protein A and B at pH 7.0. In this calculation you can assume the acidic residues are fully ionized and the basic residues are fully protonated.

$q_A = -5 + 10 = +5$ $q_B = -5 + 8 = +3$

Therefore, you should use cation exchange chromatography since both proteins have a positive charge, Scheme C is correct. (+4 pts)

B6. Please answer one of the following four choices in the space below. (8 pts)



Choice A: What are the differences and similarities between glycogen and cellulose?

Choice B: What are the two major differences between cellulose and bacterial cell walls?

Choice C: The disaccharide to the right is composed of the monosaccharide glucose and fructose. Name the sugar.

Choice D: Draw *either* of the following two lipids (hexane=C_6, butane=C_4):

- 1-hexanoyl 2-butanoyl 3-phosphatidylcholine
- 1-hexanoyl 2-butanoyl 3-phosphatidylserine

Choice A: Both contain glucose as the monosaccharide (+2), both have (1-4 linkages) (+2). Glycogen is branched containing both α(1-6) and α(1-4) linkages. (+2) Cellulose is not and contains β(1-4) linkages (+2).

Choice B: Both contain glucose in β 1-4 linkages, bacterial cell walls contain N-acetyl glucose (NAG) (+2) and N-acetyl glucose with muramic acid (NAM), (+2) and are crosslinked by polypeptide chains (+4).

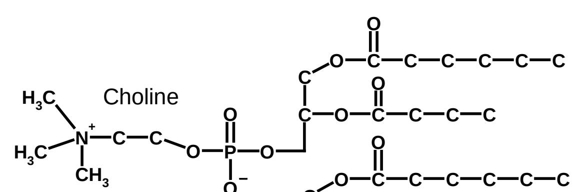
Choice C: O-α-fructofuranosyl (2-6) β-glucopyranose

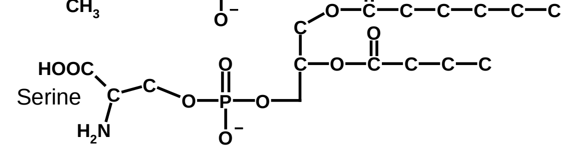
+1 +1 +1 +2 +1 +1 +1

Choice D: +2 for correct acyl length & ester link.

+2 for phosphate group

+4 for head group





B7. Please answer **one** of the following two questions (12 pts:)

Choice A: Describe how high energy electrons are processed by the electron transport chain. You answer should include the names of the organic electron carriers, a brief description of the complexes (including their location) that carry the electrons, including some examples of non-organic electron transport mechanisms. Finally, you should describe where the electrons reside at the end of the process and how the energy from electron transport is stored. You should not discuss ATP synthesis (see choice B).

Four complexes are located in the inner mitochondrial membrane (+2)

Complex I: Oxidizes NADH to NAD+, 2 electrons are transferred to coenzyme Q, protons are pumped (+2)

Complex II: Oxidizes FADH2 to FAD, 2 electrons are transferred to coenzyme Q, no protons are pumped (+2)

Complex III: Receives electrons from CoQ, and transfers them one at a time to cytochrome C. This process also pumps protons (+2)

Complex IV: Receives one electron at a time from cytochrome C, when it has four electrons it reduces Oxygen to water. This process pumps protons.(+2)

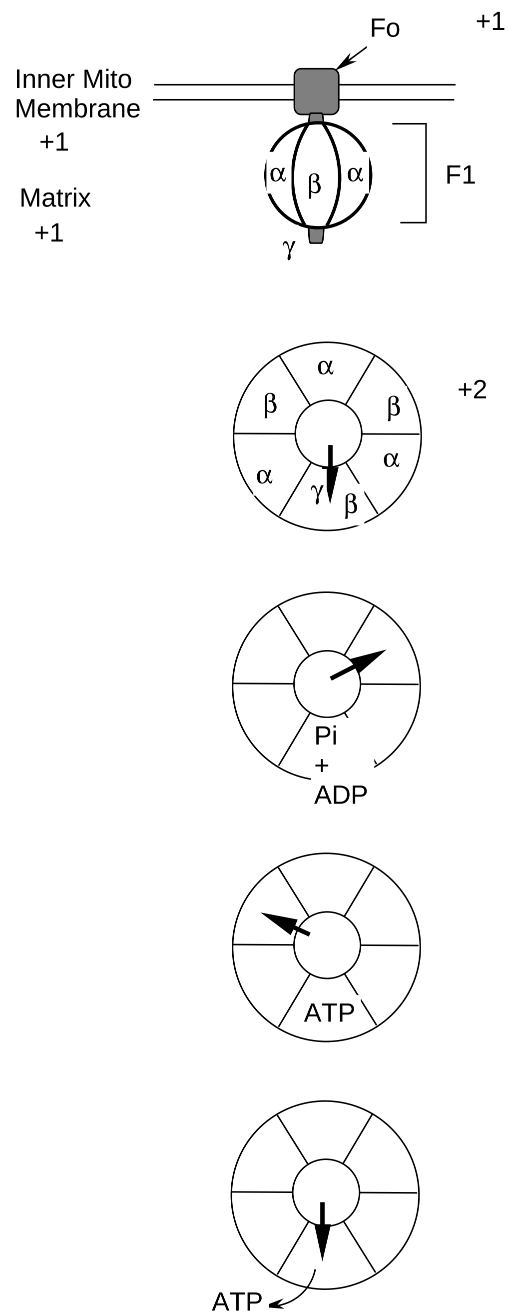
Non-organic electron carriers include any one of the following (+1)

- Iron-sulfur centers
- Heme-iron on cytochrome C.
- Copper

The energy released by electron transport is stored as a proton gradient across the inner mitochondrial membrane (+1).

Choice B: Describe how the energy released by electron transport is converted to ATP. You answer should include a diagram of the enzyme that catalyzes this reaction. You should also describe the cellular location of this process and the mechanistic details of ATP synthesis.

+5 for the diagram shown to the right.



+7 for a description of the mechanism:

1. Position of gamma subunit affects binding state of β subunit. Initially, doesn't bind anything.
2. Transport of three protons causes 120° rotation of gamma subunit.
3. ADP+Pi binds
4. Transport of three protons causes addtional rotation, conformational change generates ATP
5. Transport of three more protons coverts β subunit back to low affinity, ATP is released.

B8. Select **one** of the following four choices and indicate your choice (12 pts)

Choice A: Describe or define feedback inhibition and discuss how this process is used to regulate glycolysis or the TCA cycle.

Feedback inhibition is when a compound later on in a metabolic pathway inhibits a step higher up in the pathway. The idea is that if intermediates are building up, then the pathway should be shut down (+6).

Examples include:

- PFK (+3) which is inhibited by both PEP and Citrate (+3).
- Citrate Synthase (+3) which is inhibited by citrate as well as succinyl-CoA (+3)
- Hexose kinase (+3) which is inhibited by its product, Glucose-6-P.(+3)

Choice B: Both glycolysis and the TCA cycle are regulated by energy sensing. How does this form of regulation insure efficient use of the celluar resources? Your answer should include an example of a regulated step in either pathway and a discussion of how that step is regulated.

When the cell has high energy levels, as indicated by high ATP and NADH levels, the energy producing pathways (glycolysis, TCA) should be inhibited because the cell does not have to produce any more energy. Conversely, when AMP and ADP levels are high due to ATP hydrolysis, then energy levels are low and more energy (ATP) needs to be made. (+6)

- PFK (+2) , the regulated enzyme in glycolysis (+1), is activated by AMP and ADP, but inhibited by ATP. (+3)
- Pyruvate dehydrogenase and citrate synthase (+2) in the TCA cycle) (+1) are inhibited by high levels of ATP and NADH.(+3).

Choice C: Explain how the regulation of glycogen metabolism by protein phosphorylation/dephosphorylation maintains constant levels of blood glucose. Your answer should include a description of the hormones involved and how the hormones regulate protein phosphorylation. You should *not* discuss G-protein coupled receptors or adenyl cyclase production, etc.

- Under conditions of glucose demand, as signaled by the hormones epinepherine and/or glucagon the signaling cascade leads to phosphorylation of enzymes. (+3) When glucose is plentiful, as signaled by the presence of insulin, enzymes become dephosphorylated (+3).
- Glycogen phosphorylase, which releases glucose from glycogen is active when phosphorylated , therefore releasing glucose from glycogen when it is needed (+3).
- Glycogen synthase, which stores glucose in glycogen, is active when dephosphorylated, therefore storing glucose when there is plenty. (+3)

Choice D: Under conditions of high glucose levels, the levels of fructose 2,6-bisphosphatase are elevated. Explain how this key regulatory compound regulates glycolysis and gluconeogenesis in a coordinated fashion. You need not explain the regulation of F-2.6-bisphosphate levels by protein phosphorylation.

- When glucose levels are high, the cell can afford to convert it to ATP, so glycolysis should be turned on and gluconeogenesis should be turned off. (+3).
- When glucose levels are low, the cell should make glucose from pyruvate and ATP, so gluconeogenesis should be turned on and glycolysis should be turned off.(+3)
- PKF, in glycolysis is activated by F2,6P and therefore will be active when glucose is plentiful.(+3)
- Fructose 1,6 bisphosphatase, the key regulated step in gluconeogensis, is inhibited by F2,6P and will be inactive when glucose levels are high. However, when F2,6P levels drop as the glucose levels drop, this enzyme will become active, leading to the syn of glucose.(+3)

B9. Please do **one** of the following two choices (8 pts):

Choice A: The reaction of A → B in a metabolic pathway has a standard free energy change, ΔG^o, or + 10 kJ/mol. Assuming that the concentration of [A] is 1 mM, what concentration of [B] will insure that this reaction is spontaneous in the direction written.

If the reaction is to be spontaneous, the Gibbs free energy, ΔG, should be negative. First calculate the concentration of B at equilibrium ($\Delta G=0$), any concentration of [B] below this level will lead to a negative ΔG and therefore a spontaneous reaction.

$\Delta G=\Delta G^o + RT \ln[B]/[A]$

$0= +10 + 2.5 \ln [B]/[A]$ (Assume T=300K, RT=2.5 kJ/mol)

$e^{-10/2.5}=[B]/[A]$

$[A]e^{-4} = [B]$

$(0.001)(0.0183) =[B]$

1.8×10^{-5} M = [B]

18 μM =[B]

Therefore [B] must be lower than 18 μM

Choice B: Phosphate ions (PO_4^{3-}) have to transported across the mitochondrial membrane for use in ATP synthesis. If the concentration of phosphate outside the mitochondria is 10 mM, while that inside the mitochondria is 0.001 mM. Given a membrane potential of -100 mV (inside negative), is the transport of phosphate into the mitochondria spontaneous or not? Justify your answer with a numerical calculation.

$\Delta G = RT \ln [X]_{IN}/[X]_{OUT} + Z F \Delta\psi$
$\Delta G = (2.5) \ln (1\times10^{-6})/(1\times10^{-2}) + (-3)(96)(-0.1)$
$\Delta G = (2.5) \ln (10^{-4}) + (-3)(96)(-0.1)$
$\Delta G = -23$ kJ/mol + 28.8 kJ/mol
$\Delta G = +5.8$ kJ/mol

Although the concentration gradient is favourable for spontaneous transport ($\Delta G=-23$ kJ/mol), the negative charges on the phosphate make it unfavourable to move the ions to a region of negative potential ($\Delta G=+28.8$), therefore the net ΔG is positive (not spontaneous), energy would have to be provided to transport the ions.