A. Multiple Choice: Please circle the best answer (2 pts each, 14 points total)

1. In a eukaryotic cell, most of the enzymes of the citric acid (TCA) cycle are located in the _____, and the enzymes of involved in electron transport are located in the _____.
   a) mitochondrial matrix, inner mitochondrial membrane
   b) inner mitochondrial membrane, mitochondrial matrix
   c) intermembrane space, outer mitochondrial membrane.
   d) outer mitochondrial membrane, cytosol.

2. The TCA cycle is involved in:
   a) generation of energy from pyruvate
   b) synthesis of amino acids
   c) degradation of amino acids
   d) all of the above

3. Which of the following metal ions are often used in electron transport?
   a) Fe^{2+}.
   b) Mg^{2+}.
   c) Mn^{2+}
   d) all of the above.

4. Long-chain fatty acids are oxidized step-wise in ___ carbon units starting from the ___ end.
   a) 1, either
   b) 2, aliphatic (+1 pt)
   c) 2, carboxyl
   d) 3, carboxyl

5. Pyruvate, the end product of glycolysis, is converted to _____ in humans and to _____ in yeast under *anaerobic* conditions.
   a) acetaldehyde and methanol.
   b) lactic acid and ethanol.
   c) acetic acid and ethanol. **(+1/2)**.
   d) acetyl-CoA and ethanol **(+1/2)**

6. Epimers are carbohydrates with the same number of carbons but
   a) one is an aldose and one is a ketose.
   b) they differ in the configuration of their anomeric carbon.
   c) they differ in the configuration of one of their non-anomeric carbons
   d) they are mirror images of each other (+1/2)

7. The hormones glucagon, epinepherine, and insulin regulate glycogen levels by
   a) directly binding to the active site of glycogen synthase.
   b) by binding to glucose, preventing its incorporation into glycogen
   c) by affecting the phosphorylation level of enzymes.
   d) by elevating levels of Fructose 2,6 phosphate.

Bonus **(+1 pt**)
Caffeine is a stimulant because:
   a) it binds to receptors in the brain.
   b) it can be oxidized to produce lots of NADH.
   c) it inhibits the decay of cAMP.

A:	_____/14
1:	_____/ 4
2:	_____/ 6
3:	_____/ 6
4:	_____/ 9
5:	_____/ 8
6:	_____/ 8
7:	_____/ 8
8:	_____/10
9:	_____/ 5
10:	_____/10
11:	_____/12
Tot:	**_____/100**

1. *Briefly* describe how X-ray diffraction is used to determine the structure of a protein (4 pts).

- **The x-rays are scattered by the electrons surrounding atoms (1/2 pt)**
- **The position of the atoms affects the interference of the scattering (1/2 pt)**
- **An electron density map is produced from the scattered x-rays (1 pt)**
- **The atoms are placed into the electron density map, optimizing the fit between the structure and the electron density (2 pt)**

2. Please answer **one** of the following **two** questions (6 pts)

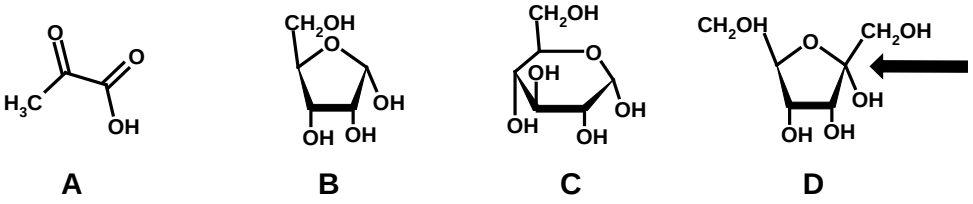
Choice A: Answer the following questions using the letter underneath each compound. Note that there may be more than one correct answer.

i) Which of the following compound(s) are aldoses? **B & C**

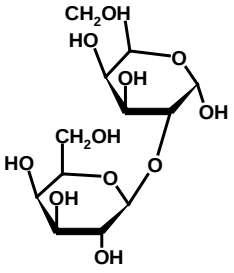
ii) Which of the following compound(s) are ketoses? **D**

iii) Draw an arrow that points to the anomeric carbon in compound 'D'

iv) What is the configuration of the anomeric carbon in compound 'D'? **α - the OH is pointing "down"**



Choice B: Name the disaccharide shown to the right, given that the monomeric units are both galactose.



β-galactopyranosyl (1-2) α galactopyranose
+2 +2 +2

3. Answer **one** of the following **three** questions in the space provided, clearly indicate your selection (6 pts).

Choice A: How does starch differ from glycogen? How are they similar?

Similarities: Both composed of glucose polymers, in α(1-4) linkage with α(1-6) branches. (+3 pts.)

Difference: Glycogen is more highly branched.(+3 pts)

Choice B: How do bacterial cell walls differ from cellulose? How are they similar?

Similarities: Both are composed of saccharides linked by β(1-4) linkages.

Differences: Glucose is modified in bacterial cell walls, which also contain a protein crosslink.

Choice C: How does cellulose differ from starch? How are they similar?

Similarities: : Both composed of glucose polymers.

Differences: cellulose has β(1-4) linakages, starch α(1-4). cellulose is sheet like, starch (α(1-4)linked glucose) is helical (only two required for full points).

4. (9 pts)

i) Draw the structure of *any* phospholipid (4 pts).

ii) Using the structure of your molecule to illustrate concepts/features, briefly discuss the *major* energetic term that drives the formation of the lipid bilayer (5 pts).

i) Structure

- **two acyl chains (+1)**
- **ester linkage to glycerol (+1)**
- **glycerol (+1)**
- **any head group (+1)**

ii) Bilayer assembly is driven by the hydrophobic effect. The non-polar acyl chains are buried in the bilayer, releasing ordered water molecules. (+5)

5. Please answer **one** of the following **three** questions in the space provided. Clearly indicate your choice (8 pts).

Choice A: How does the presence of *cis* double bonds in unsaturated fatty acids affect the phase transition of the membrane? What intermolecular interaction is affected by the presence of these groups in the bilayer?

The cis double bond lowers the melting temperature (+2) because the kink introduced into the chain by the double bond reduces the close packing of the acyl chains (+2 pts), reducing van der Waals interactions (+4 pts).

Choice B: Compare and contrast the structure of a membrane protein (e.g. bacteriorhodopsin) to that of a soluble protein (e.g. myoglobin)?

- **Membrane proteins form extensive mainchain hydrogen bonds, forming α-helices and β-sheets. This is because there are no hydrogen bond donors or acceptors in the membrane, therefore all mainchain h-bonds have to be made (+4 pts).**
- **The surface of membrane proteins that contact the acyl chains will be non-polar.(+4 pts)**

Choice C: Explain why it is important for biological membranes to be fluid, and discuss the role of cholesterol in this property of the membrane.

- **Fluidity is important because many components in the membrane have to diffuse, such as the electron carrier Q. In addition, many membrane proteins need to undergo conformational changes during function, thus the membrane must be deform to allow this to happen.(+4 pts)**
- **Cholesterol causes the membrane to be more fluid (+3 pts), by interfering with van der Waals interactions (+1 pt)**

6. Select the purification scheme that will separate protein "C" from a mixture of the following three proteins. Justify your answer by showing that the scheme will actually work. (8 pts).

Protein	Molecular Weight	Solubility in ammonium sulfate (conc required to ppt 50% of the protein).	#Asp + Glu	#Lys & Arg
A	50,000 Da	4.0	5	10
B	100,000 Da	3.0	10	5
C	50,000 Da	4.0	10	5

Scheme 1: Gel filtration chromatography → precipitation with 4 M ammonium sulfate.

Scheme 2: Gel filtration chromatography → ion exchange chromatography at pH=7.

Scheme 2 is the one that will work. (+4 pts)

- **The gel filtration step will remove protein B from the mix, leaving A and C.(+2 pts)**
- **The charge on A and C will be clearly different because of the different number of neg and positive residue.(+2pts)**

7. (8 pts) Please do one of the following two questions. Note that in choice B, you have additional choices.

Choice A:

i) Compare and contrast the reaction catalyzed by a kinase and a phosphatase (3 pts)

ii) In what metabolic pathway(s) are reactions of this type prevalent? (2 pt)

iii) Explain how direct coupling in kinase reactions reduces the overall ΔG^o of phosphorylation of compounds, provide an example (3 pts).

i)

- **A kinase adds a phosphate to a compound from ATP (+1 1/2)**
- **A phosphatase removes a phosphate, no ATP is involved.(+1 1/2)**

ii)

- **Glycolysis.(+2), TCA cycle (+1)**

iii)

- **Phosphorylation of compounds is energetically unfavorable, if this is coupled to direct transfer of the phosphate from ATP, then the energy of hydrolysis of ATP can be used to aid in the phosphorylation. (+2 pts)**

Examples are (+1 pt)

1. **Hexosekinase**
2. **Phosphofructokinase.**

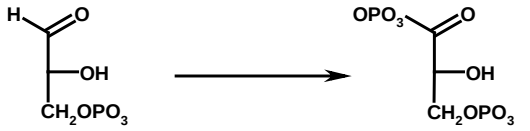
Choice B: Selection **one** of the following **three** reactions. Note that some of the transitions between substrate and product may involve more than one step, but only the first and last compounds are given below.

i) State the *general* nature of the chemical changes that occur to produce the shown product and discuss how the energy associated with this change is captured (3 pts).

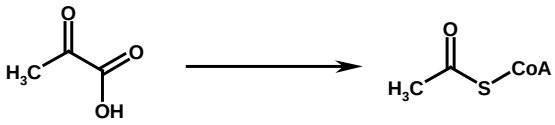
ii) Indicate any missing co-substrates/co-factors, products, etc. (3 pts).

iii) State the metabolic pathway in which the reaction occurs (2 pts).

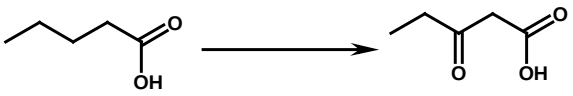
A



B



C



i) These are all oxidations, or redox reactions (1 1/2 pts.) The energy associated with the oxidation is stored as high energy electrons on electron carriers (1 1/2 pts)

ii) In all cases, electron acceptors would be required, usually NAD^+, but FAD is also used. In 'C', both are used. (2 1/2 pts). In 'A' an inorganic phosphate is required, In 'B' CO_2 is given off (1/2 pt).

iii) A: glycolysis, B: entry to, and in, TCA cycle. C: Fatty acid oxidation, TCA cycle. (2 pts)

8. Select **one** of the following **two** questions. A *well labeled* diagram is an acceptable answer. Please indicate your choice (10 pts).

Choice A: *Briefly* discuss the electron transport process, beginning with NADH and ending with the production of H_2O. You should *not* discuss ATP synthesis.

- **In the case of NADH: Electrons flow from complex I → III via coenzyme Q, then via cytochrome C to complex IV, where they are deposited on water.**
- **4 protons are pumped in complex I, 4 in complex III, and 1 in complex IV.**

- **In the case of $FADH_2$: Electrons flow from complex II → III via coenzyme Q, then via cytochrome C to complex IV, where they are deposited on water.**
- **0 protons are pumped in complex II, 4 in complex III, and 1 in complex IV**

+ 6 for having correct description of overall complex, O_2 electron acceptor.

+ 1 for a discussion of NAD path.

+ 1 for a discussion of $FADH_2$ path.

+ 2 for proton pumping, stoichemetry not important, except that complex II does not pump protons.

Choice B: *Briefly* discuss the mechanism of ATP synthesis in the mitochondria. Your answer should indicate the source of energy for ATP synthesis. You should *not* discuss electron transport.

ATP synthase consists of three β-subunits that have different affinity for ATP. The affinity of each subunit is changed as protons pass through the enzyme, due to a 120 degree rotation of the γ-subunit. (5 pts). The proton gradient provides the energy (1 pt)

- **Initially the β-subunit has low affinity for ADP+P_i and ATP. (1 pt)**
- **It then becomes a high affinity site for ADP+P_i (1 pt)**
- **It then becomes a hight affinity site for ATP, forcing the conversion of ADP+P_i to ATP (1 pt)**

9. Please do **one** of the following **two** choices. Please indicate your choice (5 pts).

Choice A: Compare and contrast direct versus indirect coupling in metabolic pathways. Provide one example of indirect coupling.

Both use a favorable interaction to drive an unfavorable one. In the case of direct coupling, both reactions occur on the same enzyme. In the case of indirect coupling, the reactions are catalyzed by separate enzymes and the favourable reaction follows the unfavorable and reduces the concentration of its product, hence lowering the Gibbs free energy (+4 pts).

Examples : aldolase in glycolysis, conversion of malate ot oxaloacetate (malate dehydrogenase) in the TCA cycle.(+1 pt).

Choice B: Compare and contrast a feedback versus a product inhibitor. Provide an example of one.

Both are intermediates in a metabolic pathway. A product inhibitor inhibits the enzyme that created it, a feed-back inhibitor will inhibit another enzyme in the pathway (+4 pts).

Example: Product inhibitor: G-6-P inhibits hexose kinase. (+1 pt).

Feedback inhibitor: PEP and citrate inhibit PFK.

10. (10 pts) Select **one** of the following **two** questions. Please indicate your choice T=300 K, RT=2.5 kJ/mol

Choice A: The conversion of 2-phosphoglycerate to 3-phosphoglycerate is a step in glycolysis that is reversed in gluconeogenesis. In a cell, the concentration of 2-phosphoglycerate is 0.1 mM and the concentration of 3-phosphoglycerate is 1 mM. Assuming ΔG^o for this reaction is 0 kJ/mol, is this cell undergoing glycolysis or gluconeogenesis? Justify your answer using the Gibbs free energy..

Correction: The correct direction in glycolysis is from 3-phosphoglycerate to 2-phosphoglycerate, i.e. the wording above should have been:

"The conversion of 2-phosphoglycerate from 3-phosphoglycerate....."

Assuming the reaction direction is 3-P to 2-P

ΔG=RT ln [2-P]/[3-P] = 2.5 kJ/mol ln (0.1 mM)/(1mM) = 2.5 x ln (.1) = 2.5 x (2.3) = -5.76 kJ/mol

(+6 pts for calculation)

The Gibbs energy is negative, so the spontaneous flow will be from 3-P to 2-P. Glycolysis is active.

(+4 pts for conclusion)

Choice B: The cells in the lining of your stomach pump hydrogen ions into your stomach in order to acidify the contents of your stomach. Assume that the hydrogen ion concentration is 10^{-2} M (pH = 2) in your stomach and 10^{-7} M (pH = 7) inside the cell, how many moles of ATP are required to pump one mole of protons. The free energy of hydrolysis of ATP is -30 kJ/mol. You can assume $\Delta\psi$ =0.

($\Delta G = \Delta G^o + RT \ln[B]/[A] + ZF\Delta\psi$)

Assume that protons in the stomach are the products, and protons in the cell are the reactants, therefore:

ΔG = RT ln $(10^{-2})/(10^{-7})$ = RT ln 10^5 = 2.5 x 11.51 = +28.78 kJ/mol.

Note that ΔG^o is zero, since the product and reactant are the same chemical species and therefore the energy required to convert one to the other is zero.

(+6 pts for calculation)

Since the energy released by one ATP hydrolysis is 30 kJ/mol (ΔG^o = -30 kJ/mol), it would take one ATP to pump one proton - assuming the pump was 100% efficient.

(+4 pts for conclusion)

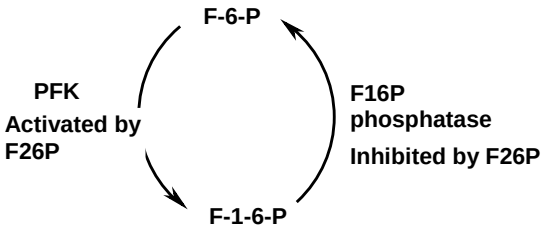
11. Please select **one** of the following **four** questions. Please indicate your choice (12 pts).

Choice A: Discuss the general concept of *coordinate* regulation of pathways using *either* the regulation of glycolysis/gluconeogenesis *or* glycogen metabolism as an example. Be sure to discuss the compounds(s) that regulate either of these pathways and how the compounds regulate the pathways in a useful manner.

General concept: In synthetic or degradative pathways, the regulated step is performed by two different enzymes, regulated in a fashion such that when one is activated the other is inhibited. This insures that only one pathway is on at a given time. (+6 pts)

Any one of the following three examples is just fine. (+ 3 pts for details on regulation, +3 as to why it is useful.)

Example I: Regulation of glycolysis and gluconeogenesis by F26P.

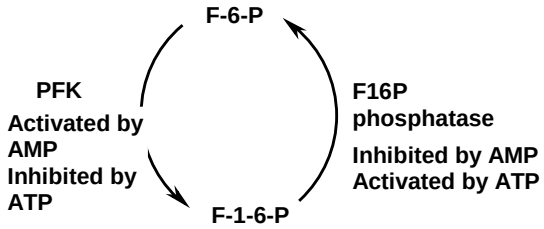


The coordinately regulated enzymes are PFK (glycolysis) and fructose 1,6 bisphosphatase (gluconeogenesis)

- PFK is activated by F26P
- F16 bisphosphatase is inhibited by F26P.

F26P levels are high when blood glucose levels are high. Thus glycolysis is turned on when there is glucose to burn. Conversely, when glucose levels are low, F26P levels are low, glycolysis is no longer activated, and gluconeogenesis is no longer inhibited. (only one case needed to be given)

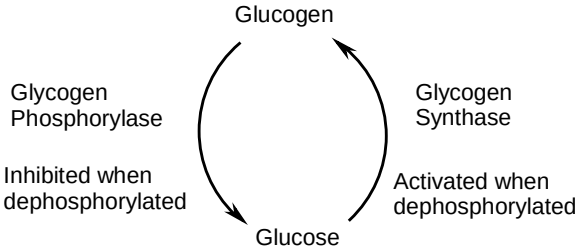
Example II: Regulation of glycolysis and gluconeogenesis by energy sensing.



When cellular energy resources are high, as indicated by high ATP levels, PFK is inhibited, turning off glycolysis - why make more ATP when there is lots. Gluconeogenesis is turned on in this case because F16bis phosphatase is activated by ATP, i.e. the ATP is used to make glucose.

When cellular energy resources are low, as indicated by low ATP and high AMP/ADP, then glycolysis is activated because AMP activates PFK. AMP inhibits the bisphosphatase, turning off gluconeogenesis. (only one case needed to be given)

Example III: Glycogen Metabolism:



The activity of glycogen synthase and glycogen phosphorylase, which breaks down glycogen to glucose, are controlled by protein phosphorylation. (only one case needed to be given)

- When blood glucose levels are high:
  Enzymes are dephosphorylated, glycogen synthase is activated so that the glucose is stored in glycogen. Glycogen phosphorylase is inhibited by dephosphorylation.
- When blood glucose levels are low:
  Enzymes are phosphorylated. This activates glycogen phosphorylase, such that glucose is released from glycogen. The phosphorylated form of glycogen synthase is, of course, inactivated.

Choice B: You recently consumed a candy bar, elevating your blood glucose levels. Briefly describe the steps that would lead to storage of that glucose into glycogen in the liver. Your answer should include a brief discussion of hormonal and other regulatory signals that are important to this process.

1. **High blood glucose leads to release of the hormone insulin from the pancreas (+3 pts)**
2. **Insulin binds to the insulin receptor on the surface of the liver cell (+3 pts)**
3. **Protein phosphatases are activated, leading to dephosphorylation of enzymes. (+3 pts)**
4. **Glycogen synthase is active when dephosphorylated, glucose is stored in glycogen. (+3 pts).**

Choice C: You have not eaten in 8 hours, yet the concentration of glucose in your blood remains constant because of the release of glucose from glycogen. Explain how this occurs. Your answer should include a brief discussion of hormonal and other regulatory signals that are important to this process.

1. **Low blood glucose leads to release of the hormone glucagon from the pancreas (+3 pts)**
2. **Glucagon binds to the glucagon receptor on the surface of the liver cell (+3 pts)**
3. **Adenyl cyclase is activated by G-protein, protein kinase are activated, leading to phorylation of enzymes. (+3 pts, not necessary to give all steps)**
4. **Glycogen phosphorylase is active when phosphorylated, glucose is released from glycogen. (+3 pts)**

Choice D: You are a long-distance runner. One hour into the race your glycogen supplies in the liver and muscle have become depleted, yet your body is still able to produce ATP to drive your muscles. What is the major source of this ATP? Your answer should include a brief description of the major pathways that are being used to generate ATP, in the order that they would occur.

1. **The main source of energy is from fat (+6 pts)**
2. **Triglycerides would be broken down to fatty acids (+1pt)**
3. **Fatty acids would be activated to acyl-CoA (+1 pt)**
4. **Fatty acids would be oxidized in β-oxidation, producing acetyl-CoA (+1 pt)**
5. **Acetyl-CoA would be oxidized to CO_2 , producing NADH (+1 pt)**
6. **Electrons from NADH go through electron transport. (+1 pt)**
7. **ATP synthesized by ATP synthase (+1 pt)**

[There are more details above than necessary]