Metabolic Biochemistry Midterm
Thursday Oct 29, 2015; 7-10 pm

Happy Almost Halloween. One function of this exam is to solidify your knowledge of the commonly used ideas and terms that describe metabolism. The other is to gauge how much you know. We can best serve these two purposes by making the scoring of the midterm so that it can only help you: if including the midterm score helps you at the end of the quarter, we will use it in calculating the scoring. If it would not help (meaning you got a better percent grade on the final than the midterm)… it will not be used! So the course score it is more about what you end up learning rather than exactly when you learned it.

The only advice: Please please pretty please read the whole question before you start your answer. And please don’t hesitate to ask questions. That is why we are lumbering around like glycolytic crocodiles, ready to pounce on poor information and convert it to clarity… and then rest.

We are not about tricking you or trying to be crafty and cunning. This is a very straightforward exam, and what we ask for is what we want. Period. Enjoy, to the extent that this is possible in a midterm…

Summation

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Total  __________ (out of 100)
1 (4 pts) Dharles Carwin… It’s a science-y license plate for one letter code practice. This is one of those lucky plates where every letter is an amino acid. Below is a list of amino acid names. For each license plate letter, place it next to the correct full amino acid name below. If the full named amino acid is not represented on the plate, put NO next to that amino acid.

a) Isoleucine  
d) Proline  
g) Alanine  
b) Lysine  
e) Arginine  
h) Aspartate  
c) Serine  
f) Histidine Asparagine

j) Write the AAs on the plate with a net negative charge at pH 7 (one letter code)  
k) Which AAs on the plate that has a positive charge at pH 7 (one letter code)  
l) Which AAs on the plate have an aromatic ring (one letter code)  
m) Which amino acid on the plate functions in the chymotrypsin catalytic triad?

D (Asp) provides negative charge to facilitate His’s ability to accept H⁺ during catalytic cycle.

2 (2 pts) Explain how an enzyme changes the rate of a reaction without altering how far that reaction will proceed. Draw an energy diagram in the space to the right (→) to help with your explanation. Consider using the terms ΔG⁺ and ΔG to make your points clearly. 1-2 sentences

3 (2 pts) The Lineweaver-Burke plot on the right shows two lines. One is for an enzyme under study, and the other is for the same enzyme after it has been activated by phosphorylation.

a) Label the line representing the more active form of the enzyme as E-P, and the less active one as E. Make sure to be clear!

b) Label the two axes with what they represent.

c) Does activation of this enzyme alter Vmax, Km or both? Choose and explain:

Only Vmax. The common “x” axis intercept show km stays same. The two Vmax are different... E-P’s is larger.

3 (2 pts) Protein kinases add phosphate groups to other proteins. Using a simple representation of P-OH for an unphosphorylated protein, write the reaction by which phosphate is added to this enzyme by a protein kinase. Include all substrates and products. You can use –PO₄²⁻ for the phosphate group.

P-OH + ATP ⇌ P-OP₂O₃⁻ + ADP
4 (2 pts) Own More Shiny Gold! ... Dicarboxylic acids underlie the names of many metabolites. We have mentioned glutarate many times, and the Name Game tells you what its structure is.

a) Draw α-amino-glutarate in the box just to the right →

b) Hey! This is one of the amino acids used to make proteins! Which one is it (hint: starts with a G): GLUTAMATE

(c) What is this amino acid’s one letter code: E

d) Using what you learned from the Name Game, draw the structure of 2-OH-2-methyl-malic acid, which you have never seen or heard of before. Show all atoms, (example: CH3-CH2OH for ethanol). Use the space to the right →

5 (8 pts) Froo-co-co Cabaña! The picture shows fructose, in ring form. When fructose is ingested, it can be phosphorylated by hexokinase on the 6 carbon, allowing it to enter the glycolytic pathway.

a) Circle the OH that will be modified by the hexokinase reaction.

b) What substrate provides the added phosphate? ATP

c) On the fructose pictured, draw a small double line through the middle of the bond that will be broken by the action of aldolase during glycolysis of this molecule.

d) Write the reaction that uses Fr6P as a substrate in glycolysis, using a similar ring structure as the one pictured. Include structures of any fructose-derived metabolites, using the same level of detail as picture:

\[ \text{Fr}6\text{P} + \text{ATP} \xrightarrow{\text{PFK1}} \text{Fr6P-6P} + \text{ADP} \]

(e) To the right is a re-draw of fructose. Use small arrows to indicate the carbon or carbons (s) on the fructose structure that will eventually become the –CH3 of pyruvate molecules produced by glycolysis of this molecule.

f) Circle the carbon or carbons that will be released as CO2 when each pyruvate made from this fructose becomes a substrate of pyruvate dehydrogenase PDH

g) In some organisms, pyruvate is converted into ethanol (yay!). Why is this step important for the energy metabolism of such organisms? One sentence will do

This process provides a way to **regenerate NAD^+** from the NADH produced in glycolysis, need for glycolysis to proceed.
6 (3 pts) Recycled Parts “Hey! This is the same parts list on a previous exam!” you say. Indeed it is. These are often how glycolytic intermediates are described in scientific talks and journal articles. Use these terms as the best answer(s) to the following questions about the glycolytic pathway:

a) The substrate of glyceraldehyde-3P dehydrogenase (GAPDH): $\text{G3P}$

b) The two molecules produced by the action of aldolase: $\text{G3P}, \text{DHAP}$

c) The two molecules that can spontaneously transfer Pi to ADP: $\text{1,3PG}, \text{PEP}$

d) This is produced when pyruvate can not be further oxidized by PDH: $\text{LAC}$

e) These two are interconverted by triose phosphate isomerase (TPI): $\text{G3P}, \text{DHAP}$

f) This metabolite undergoes dehydration by the action of enolase $\text{2PG}$

g) Each are products of reactions that CONSUME ATP in glycolysis $\text{2PG}, \text{Fru-1,6BP}$

h) Is produced by a glycolytic reaction that uses NAD$^+$ as a substrate $\text{1,3bPG}$

7 (7 pts) It takes a phosphate to make a phosphate We have heard the term substrate-level phosphorylation a number of times in class. There are two reactions in glycolysis that are examples of this process, the reactions catalyzed by pyruvate kinase, and phosphoglycerate kinase. These two reactions have similar features and are key reactions in the “payoff” phase of glycolysis

a) Why do I say these reactions are key in the “payoff” phase? One sentence:

They are the reactions that make ATP from ADP

b) Using letter abbreviations (no structure) like in the parts list above, write the two substrate level phosphorylation reactions of glycolysis. Include appropriate abbreviations for other substrates and products as well. Indicate which two enzyme is catalyzing each reaction.

\[
\begin{align*}
1,3\text{bPG} + \text{ADP} &\rightarrow \text{3PG} + \text{ATP} \\
\text{PEP} + \text{ADT} &\rightarrow \text{Pyr} + \text{ATP}
\end{align*}
\]

c) For ONE of the two reactions, (either pyruvate kinase or phosphoglycerate kinase) write that reaction including structures of the glucose-derived metabolites that are substrates or products. (Hint: each of these enzymes includes one reaction product in its name…)

\[
\begin{align*}
\text{1,3bPG} &\rightarrow \text{3PG} \\
\text{CO}_2 &\rightarrow \text{ADP} \rightarrow \text{Pi}
\end{align*}
\]

d) You hear a student say that “glycolysis is just a feeder reaction for mitochondrial oxidation”. You say: “NO, blasphemous metabolite! That isn’t true!” Describe an example or situation (we discussed three at least) where glycolysis is important in its own right as an energy source: 1 or 2 sentences:

1) high intensity athletic actions (sprint, shot put, attack)
2) anaerobic lifestyle or organisms
3) low O2 environment energy
8 (10 pts) **Lac-luster performance** The enzyme lactate dehydrogenase can play an important role in glucose metabolism. Lactate is shown on the right to help orient your thinking.

a) Write out the complete reaction by which lactate dehydrogenase produces lactate, including structures of the pathway metabolites. Include other substrates and products as needed.

\[
\text{CH}_3\text{C}(-\text{CO}_2^- + \text{NADH} \rightarrow \text{CH}_3\text{C}(-\text{H}-\text{CO}_2^- + \text{NAD}^+}
\]

b) Why is the lactate dehydrogenase reaction important in glycolysis? 1 sentence:

**TO RESTORE NAD FOR CONTINUED GLYCOLYSIS**

c) An anaerobic organism (grows in the absence of oxygen) is treated with a potent inhibitor of LDH, and the organism dies very quickly from lack of ATP. Why?

This organism uses LDH to restore NAD^+ for continued glycolysis. Glycolysis is the only source of ATP in this circumstance... = death!

d) Some organisms use a different short pathway instead of lactate dehydrogenase to accomplish the same metabolic goal. The two enzymes used are pyruvate decarboxylase, and alcohol dehydrogenase. Using the structure of pyruvate provided (and labeled in Spanish: Piruvato!) write the reactions, and name the metabolites. Indicate the enzyme involved in each reaction. Include the structures of the pyruvate-derived metabolites, and reasonable abbreviations for other substrates or products involved.

\[
\text{CH}_3\text{C}(-\text{CO}_2^- \xrightarrow{\text{PD}} \text{CH}_3\text{C}(-\text{H}) + \text{NADH} \xrightarrow{\text{AD}} \text{CH}_3\text{C}(-\text{H} + \text{NAD}^+)
\]

Take a breath, and enjoy the minions dressed in costumes, and the pumpkins dressed as minions. Then proceed...
9 (6 pts) Pyruvate dehydrogenase: The pyruvate dehydrogenase (PDH) complex is a multi-activity enzyme that converts pyruvate into a useful source of carbon. Answer these questions about this remarkable nanomachine.

a) Write out the net reaction of the PDH. What goes in, what comes out. No need for structures or the PDH internal cofactors.

\[ \text{Py}^- + \text{CoASH} + \text{NAD}^+ \rightarrow \text{AcCoA} + \text{NADH} + \text{CO}_2 \]

Three cofactors that operate deep within the PDH to catalyze the net reaction you described in. They are called TPP, lipoic acid, and FAD.

b) Below are structures that depict key parts of each of these cofactors. First, match up the three cofactor names (using the spaces provided in the “cofactor” row) with the representative structures grabbed from the internet. One is not part of any of the three cofactors, and you can just write NOT in that space.

c) Each of the three PDH cofactors is associated with one of three separate enzymes that together make up the PDH. We refer to them as E1, E2 and E3. For each representative structure, write the PDH E that it is associated with in the spaces in the E1-3 row. Just put an X under NOT.

d) Where would you expect to find a pyruvate transporter in the cell? Location location! We have recently started to talk about cellular location and metabolism. For the metabolic pathways listed, write the best answer for the location in the cell where the process occurs. Designate sub compartments of the mitochondrion if a process occurs in that organelle.

9 (4 pts) Location location! We have recently started to talk about cellular location and metabolism. For the metabolic pathways listed, write the best answer for the location in the cell where the process occurs. Designate sub compartments of the mitochondrion if a process occurs in that organelle.

a) Krebs cycle: __________________________

b) Glycolysis: __________________________

c) Ketone body formation: __________________________

d) Fatty acid β-oxidation reactions: __________________________

e) Formation of acyl-CoA from free fatty acid: __________________________

f) Pentose phosphate pathway: __________________________

g) Conversion of pyruvate into lactate: __________________________

h) Conversion of pyruvate into AcCoA: __________________________
10 (6 pts) You’ll meet your fate as gluconate: Glucose is also metabolized by the pentose phosphate pathway (PPP). This pathway is not for energy so much as to make stuff.

**a)** We discussed two main products of the PPP that are used in many processes. Name them and briefly describe how they are important in metabolism. Don’t use CO₂...

1) **NADPH**: Major reducing agent of cell

2) **Ribose-5P**: Used in DNA, RNA synthesis; nucleotide synthesis

**c)** How many reduction reactions occur in the PPP pathway? **2**

**d)** Describe in one sentence and draw a generic ester, using R groups like in your organic chemistry classes to keep it simple. Example: carboxylic acid is R-COO⁻:

![Generic Ester Diagram]

**e)** Now back to the pentose phosphate pathway. Below is glucose-6-phosphate. The first enzyme of the PPP is called glucose-6P-dehydrogenase, and uses G6P molecule as a substrate. Complete the reaction, including the name and the structure of the resulting glucose-derived product, and any other substrates or products that participate. Why did I ask about esters above? Because the product of this enzyme is an internal ester called a lactone. Remember?

![Glucose-6-Phosphate (G6P) Diagram]

Glucose-6-Phosphate (G6P)
11 (12 pts) Krebne West & Kim Krebdashian: The first reaction in the Krebs cycle, that starts the whole thing going, is catalyzed by citrate synthase. The product, not surprisingly, is citrate, which is shown on the right.

a) Write the citrate synthase reaction below, using abbreviations for all substrates and products. No structures. We will get to structures in a minute...

\[
\text{OAA} + \text{AcCoA} + \text{H}_2\text{O} \rightarrow \text{citrate} + \text{CoA-SH}
\]

b) Now, draw the structure of oxaloacetate, or OAA, which is a substrate of citrate synthase, using the conveniently provided box to the right.

c) In the picture of citrate above, notice there are two methylene (-CH$_2$-) groups in the citrate molecule (the \( /\) bends in the drawing). When drawn this way, it is a fact that the left and only the left -CH$_2$- receives the OH by the action of aconitase. Why did this surprise chemists back in the early days of metabolism? One sentence:

These two methylenes are chemically identical; the enzyme provides the stereochemistry.

d) Now that you know it is the LEFT -CH$_2$- group that always gets the OH, which -CH$_2$- was most recently added to OAA by citrate synthase? left or right, and why?:

The RIGHT -CH$_2$- was part of the acetyl group, added in OAA formation. That -CH$_2$- is never used in the following aconitase Rxn.

c) Aconitase changes the citrate into isocitrate, shown to the right. This isocitrate undergoes a reaction catalyzed by isocitrate dehydrogenase (IDH). You can tell from the name what is going to happen. Anyway, write the reaction, including all substrates and products. Include the full name of the Krebs cycle metabolite produced by the isocitrate reaction: include structure of isocitrate and its product.

\[
-\text{Cit} + \text{NAD}^+ \rightarrow \text{Iso} + \text{CO}_2 + \text{NADH} + \text{H}^+ + \text{H}_2\text{O}
\]

d) Notice that isocitrate has 3 -CO$_2$- groups, which we will call left (L), middle (M) or right (R). Which one (L, M or R) is lost during the isocitrate dehydrogenase (IDH) reaction? Which one (L, M or R) stays on all the way through the Krebs cycle to be part of the next OAA that is produced?

L is lost during IDH reaction; M will be part of the OAA produced.

e) The “late stages” of the Krebs cycle include a series of three reactions that we see often in metabolism. They are: oxidation to produce a double bond, followed by addition of H$_2$O to make an alcohol, and then oxidation of the alcohol to produce a ketone. Represent the sequence of three Krebs reactions that are described in this way. No need for structures. Just abbreviations, like CIT \(\rightarrow\) ISO. You don’t even need to add cofactors. Just the Krebs molecule names.

\[
\text{SUC} \rightarrow \text{FUM} \rightarrow \text{MAL} \rightarrow \text{OAA}
\]
11 (8 pts) **The Phat of the Land**  This year we talked about fatty acid oxidation in the first set of lectures, so here it is, on the midterm. Give the best answer for each of the fat combustion questions.

**a)** What is the name of the molecule pictured to the right (hint: sounds like carnival) __________

**b)** Why is this molecule so important in β-oxidation of fatty acids? One sentence: 

*it is required for transport of fatty acids into the mitochondrion*

**c)** How are the enzymes CPT1 and CPT2 involved in the use of this key molecule? Once sentence:

*CPT1 transfers acyl groups from acyl-CoA to carnitine \( \text{CPT2 } \cdot \cdot \cdot \cdot \cdot \) acyl-carnitine back to CoA*

**d)** β-oxidation of fatty acids proceeds by a set of four repeated, or iterated, reactions. Below is an acyl-CoA, with most of the hydrocarbon chain shown only as “R”. Draw the sequence of reactions representing one cycle of β-oxidation. Include all substrates, and show the changes to the α and β carbons which are included in the starting structure and important in understanding this oxidation process. No need for enzyme names. No need for intermediate names.

```
RCH₂CH₂C O S-CoA
acyl-CoA
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11 (3 pts) **The Ketone Kops**: Ketone bodies are an antiquated name for a group of three molecules. One is acetone, and the other two are four carbon molecules, called acetoacetate, and β-OH-butyrate.

**a)** What substrate molecule is the carbon source for these ketone bodies? __________

**b)** Where are ketone bodies made in the human body? __________

**c)** What happens to these molecule when they are taken up by other tissues (just words, no reactions needed):

*converted back to AcCoA for fuel oxidation*

**d)** Draw your choice of one of the two four-carbon ketone bodies mentioned above, in the box, and indicate its name as well.

![Diagram of acetyl-CoA]
12) (11 pts) **The Electron Subway** - The electron transport chain ETC consists of four complexes, I, II, III, and IV that shuttle electrons gradually from high energy starting molecules like NADH, to their final resting place in H₂O.

**a)** Sketch a representation of the ETC showing how NADH, Succinate, Q, cyt C and O₂ all fit in to this grand redox system. Include the mitochondrial membrane in your drawing, indicate which membrane it is, and how the membrane is oriented (eg. which side is matrix and IMS). Make sure to include the direction of the proton pumping that is the critical part of this process. **No structures needed**

**b)** Complexes I-IV, comprise the Electron Transport Chain (ETC). The biochemistry of each complex can be described by a simple net chemical reaction involving an electron donor and an electron acceptor, such as NADH, Q, QH₂, or cytC, as examples. **PICK ONE COMPLEX**, and next to the complex name (I, II, III, or IV) **write the balanced NET reaction** that your complex catalyzes (no need for Q cycles and such nonsense). Don't worry about the proton gradient in this question.

\[
\begin{align*}
\text{Complex I:} & \quad \text{NADH} + H^+ + Q \rightarrow \text{NAD}^+ + \text{QH}_2 \\
\text{Complex II:} & \quad \text{Succinate} + Q \rightarrow \text{Fumarate} + \text{QH}_2 \\
\text{Complex III:} & \quad \text{QH}_2 + 2\text{cytC}_{red} \rightarrow \text{Q} + 2\text{cytC}_{ox} + 2H^+ \\
\text{Complex IV:} & \quad 2\text{cytC}_{red} + \frac{1}{2} \text{O}_2 + 2H^+ \rightarrow 2\text{cytC}_{ox} + H_2O
\end{align*}
\]

c) One of the odd aspects of the early study of oxidative phosphorylation was that the purification of the complexes I, II, III, and IV, was required to figure out the enzymology of each complex (substrates used, products made), but also **removed any possibility of understanding where the energy for ATP production comes from**. What do I mean by that? Why would solubilizing and purifying the enzyme complexes mask the key energetic features? Just a sentence or two will do:

The soluble enzyme complexes allowed detailed study of the enzymology of the isolated complexes, that is, how they converted substrate into products. But since they were no longer in the inner membrane, there is no ability to form or observe proton gradient formation.
13 (after all its Halloween; 10 pts) The Truth Will Freak You Out (T/F)
Write T or F for each question as the best answer for each.

a) ____ Some of the reactions of glycolysis are endergonic

b) ____ The glycolytic pathway as a whole is exergonic

c) ____ Ketone bodies can only be made in the liver

d) ____ The Krebs cycle includes reactions that use O$_2$ as a substrate

e) ____ The Krebs cycle includes a number of oxidation reactions

f) ____ Each acetyl group that enters the Krebs cycle is converted to two CO$_2$ during one turn

g) ____ For each acetyl group that enters the Krebs cycle, 2 CO$_2$ are produced

h) ____ the β oxidation pathway uses O$_2$ during its reactions

i) ____ It is reasonable to assume that an allosteric enzyme has quaternary structure

j) ____ pyruvate carboxylase directly converts pyruvate to a Krebs cycle intermediate

k) ____ When the liver makes more acetyl-CoA than can be consumed by the Krebs cycle, ketone body synthesis is inhibited

l) ____ An allosteric enzyme's rate behavior can be described with a single Km and a single kcat that are constant

m) ____ Changing the concentration of a reactant will alter the equilibrium constant

n) ____ A negative half cell potential means the reduction half reaction is spontaneous

o) ____ The B$_{12}$ cobalamin cofactor is used to metabolize propionyl-CoA

p) ____ Donuts are beyond delicious...