Good Evening Inheritors of Tomorrow! Here is your final. The graded exams will be available for pickup at the Exam Depot, prob later next week. Stay tuned. The key will be posted on the website. The key will be posted tomorrow afternoon (Friday). Feel free to register comments, complaints, questions, or corrections to me by email. We will consider those suggestions or observations during the grading process. So thanks in advance to your vigilance...

I have enjoyed teaching this class. I always learn a lot, and appreciate the energy and dedication you all show. I hope you got something out of this class; I realize it is very hard, and perhaps not as intriguing when first confronted with evolution’s strange notion of minimalism. Happy Holidays!

Summation

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Total  (out of 150)
1) (5 pts) Branching Out

The diagram shows a branched pathway, with the letters representing metabolites. Each arrow represents an enzyme-catalyzed step. The indicated enzyme $\text{Enz}_{\text{reg}}$ is allosterically regulated. Each branch’s final product (D or G) is an allosteric regulator of $\text{Enz}_{\text{reg}}$. The regulation helps ensure that there is balanced synthesis of D and G. This system is a lot like chorismate mutase, which we studied in class.

a) Decide what kind of allosteric regulator of $\text{Enz}_{\text{reg}}$ each is, and say with one sentence why this makes sense:

D ________________: when D is high, need more production of G for balance
G ________________: when G is high, need more production of D for balance

b) Sketch in the provided box the effects of D and G on the $\text{Enz}_{\text{reg}}$ rate plot; Include the $\text{Enz}_{\text{reg}}$ rate plot without any added regulators. Label the axes.

c) What is a likely feature of the $\text{Enz}_{\text{reg}}$ structure?

2) (7 pts) PFK1: The Glycolysis Gateway

A key enzyme of glycolysis is called phosphofructokinase 1 (PFK1). Below are some PFK1 questions

a) Complete the PFK1 reaction, in the space provided, with the provided structure of its substrate. Include the structure of the resulting product and abbreviations for any other substrates or products (such as NAD, etc.). Also write name the two main metabolites.

b) The reverse reaction of PFK1 is NOT used gluconeogenesis. Write out the reaction that bypasses PFK1, allowing the product in a) to be converted into the substrate in a), during gluconeogenesis. You do not need to draw structures since you already did that in a):

$$\text{Fr}1,6\text{BP} + \text{H}_2\text{O} \rightarrow \text{Fr}6\text{P} + \text{P}_4\text{O}_4^-$$

c) What is the name of this gluconeogenic enzyme that bypasses of PFK1. Name or appropriate abbreviation?

Fructose 1,6 bisphosphatase; FBPase
3) (12 pts) And Now For Something Slightly Different...

Fructose 2,6 bisphosphate (Fr2,6bP) is an important molecule in mammalian metabolism, serving as a signal of glucose abundance in mammals.

a) In what organ does Fr2,6bP primarily function? : ____________

b) As we learned, PFK2 is responsible for producing Fr 2,6bP. Write the reaction of PFK2, using abbreviations for all substrates and products:

\[ \text{Fr}_6\text{P} + \text{ATP} \xrightarrow{\text{PFK2}} \text{Fr}_{2,6}\text{bP} + \text{ADP} \]

c) Fr2,6bP has regulatory effects on two different key metabolic enzymes. What are those enzymes and what are the effects on each enzyme?

Enz: PFK1 Phosphokinase 1 | Effect:stimulates activity, allosteric activator  
Enz: FBP1 Fructose bisphosphatase | Effect: inhibits activity, allosteric inhibitor

Fr2,6bP

d) now for a more global picture, what is the effect of high and low levels of the regulator that is produced by PFK2 on glycolysis and gluconeogenesis (one phrase):

**glycolysis:** high Fr2,6bP stimulates glycolysis, low Fr2,6bP inhibits glycolysis  
**gluconeogenesis:** high Fr2,6bP inhibits gluconeogenesis, low Fr2,6bP stimulates gluconeogenesis

e) The two main controllers of glycolysis and gluconeogenesis are insulin and glucagon. What are the effect of each of these on cellular levels of Fr2,6bP?

**Insulin:** increases cellular levels of Fr2,6bP  
**Glucagon:** decreases cellular levels of Fr2,6bP

f) What is the enzyme activity that causes the breakdown of Fr2,6bP ____________

g) Suppose someone made an animal that completely lacked the gene encoding the PFK2 enzyme activity. What would you expect the activity of the enzyme in f) to be, and why? (one sentence):

FBP2 activity would be absent, because both activities are part of the same protein; PFK2 and FBP2 are the two activities of a single bifunctional enzyme.
4) (12 pts) The Cat (and gluconeogenesis) Came Back...

Cats, like Carbon (shown on the right), eat almost no carbohydrates. In fact, cats cannot even taste sweets like sugar. They make most of their glucose by gluconeogenesis. We touched upon gluconeogenesis above. Let’s talk about this process in a bit more depth.

Pyruvate is one of the main sources of carbon for gluconeogenesis. In glycolysis, pyruvate is made by the last reaction of the pathway, in which PEP is used to make ATP, catalyzed by pyruvate kinase.

a) First, write out the **glycolytic reaction**, catalyzed by pyruvate kinase, that produces pyruvate from PEP, including the structures of PEP and pyruvate, which is \( \text{CH}_3\text{-CO-CO}_2 \). Include all other substrates and products:

\[
\begin{align*}
  \text{CH}_3\text{-CO-CO}_2^- & \rightarrow \text{ADP} \quad \text{PK} \\
  \text{CH}_3\text{-CO-CO}_2^- & \rightarrow \text{ADP} 
\end{align*}
\]

b) In gluconeogenesis, PEP is made from pyruvate, but not by the reverse reaction of pyruvate kinase. Instead, PEP is made by the sequential action of two enzymes, pyruvate carboxylase and PEP carboxykinase. Write that sequence of reactions, using abbreviations for the metabolites, and including any other substrates or products. No structures needed here.

\[
\begin{align*}
  \text{PYR} + \text{H}_2\text{O} + \text{ATP} & \rightarrow \text{OAA} + \text{GTP} \\
  \text{OAA} + \text{ADP} & \rightarrow \text{PEP} + \text{GDP} \\
  \text{PEP} & \rightarrow \text{Glu}
\end{align*}
\]

c) Gluconeogenesis depends on a source of carbon atoms. What are the two main sources of carbon atoms used for gluconeogenesis in mammals:

- **Glycolaldehyde**
- **Amino acids**

e) Gluconeogenesis happens mainly in two organs. What are those organs:

- **Liver**
- **Kidney**

f) The Cori Cycle describes cooperation between two tissues to help maintain blood glucose levels. Draw a schematic of the Cori Cycle, including the two tissues. No need for whole pathways, just the main features and the relationship between the two tissue types:

![Cori Cycle Diagram]
5) (13 points) That’s How the Krebs Cycle Rolls...
It’s a brand new Krebs graphic! Notice these features: The ENZYMES are indicated by NUMBERS, the METABOLITES are indicated by LETTERS. Answer the following questions, using the letters or numbers as needed. **An answer can have one, more than one or “none” as an answer.**

a) Enzyme that produces CO₂ as a product __________

b) Enzyme most similar to PDH __________

c) Substrate of the malic enzyme __________

d) Called succinate __________

e) Produces NADH as a product __________

f) Enzyme that produces CoA-SH as a product __________

g) Enzyme adds water to create OH in its product __________

h) Metabolite also found in malate aspartate shuttle __________

i) Metabolite also found in glycerol-3-phosphate shuttle __________

j) Called malate dehydrogenase __________

k) Transported to cytosol to provide carbon for fatty acid synthesis __________

l) Substrate of citrate lyase __________

m) Product of citrate lyase __________

n) Produced by pyruvate carboxylase __________

o) Substrate of PEP carboxykinase __________

p) Is an optically active molecule (can have R or S forms) __________

q) Enzyme produces GTP as a product __________

r) Enzyme produces FADH₂ as a product __________

s) One of the complexes of the ETC __________

t) Six carbon molecule __________

u) Its cognate amino acid is abbreviated “D” __________

v) Also metabolites of the glyoxylate cycle __________

w) Enzyme that catalyzes an oxidation __________

x) Substrate in reaction used to metabolize ketone bodies __________

y) Uses O₂ in its reaction __________

z) Uses CoA-SH as a substrate __________
6) (12 pts) Macrophage Metabolic Magic

Macrophages are a type of white blood cell that can engulf and kill bacteria. It turns out that when macrophages are in the presence of dangerous bacteria, they produce a protein that until recently was poorly understood. Recently it was shown that this mystery protein is an enzyme and that enzyme converts citrate into the compound shown on the right, that we have never seen before. It is called itaconic acid. So what? Well it all starts to make sense when someone realized that itaconic is an potent inhibitor of the enzyme isocitrate lyase, an enzyme of the glyoxylate cycle. Hmmm. Lets try to make sense of that

a) What is the function of the glyoxylate cycle? (one sentence)

b) What is the main product of the glyoxylate cycle _______________________

c) What substrate molecule provides the carbon for the product in b)? __________

d) Given the structure of isocitrate, write the reaction of isocitrate lyase. Include the structures of any products. Name the products as well.

\[ \text{isocitrate lyase: } \\
\text{IL: } \text{IL} \rightarrow \text{CO}_2 + \text{CH}_3\text{CO}_2\text{H} + \text{glyoxylate} \]

\[ \text{products: } \text{CO}_2, \text{CH}_3\text{CO}_2\text{H}, \text{glyoxylate} \]

e) The crazy (or great) thing about this discovery is that mammals do not produce isocitrate lyase. What organisms DO have glyoxylate cycle? Bacteria do! Name another class of organisms that also have the glyoxylate cycle: _______________________

f) It turns out that bacteria need the glyoxylate cycle to grow well. So why does it make sense that macrophages would produce itaconic acid when they are near invading bacteria? (One sentence)

\[ \text{It inhibits the growth of the invading bacteria helping the macrophage do its job} \]

g) Most of the enzymes of the glyoxylate cycle are shared by another central metabolic process. What is that other central metabolic process _______________________

h) The other enzyme that is unique to the glyoxylate cycle is called malate synthase. Write the reaction of malate synthase, using just abbreviations for the products and reactants.

\[ \text{glyoxylate + AcCoA } \text{ms} \rightarrow \text{malate + CoASH} \]

i) How many acetyl-CoA molecules are used with each turn of the glyoxylate cycle? ______

j) How many CO₂ are produced with each turn of the glyoxylate cycle? ______
7) (12 pts) Mal-icious or delicious?

A challenge for the medical world is how to alter appetite safely and effectively. Accordingly, it is important to understand how the brain controls appetite. One hypothesis for appetite control concerns our old friend malonyl-CoA! It turns out that levels of malonyl-CoA in the hypothalamus (a brain region) correlates with satiety, which means feeling full: high levels of hypothalamic malonyl-CoA are associated with feeling full, while low levels of malonyl-CoA are associated with feeling hungry. The assumption of this model is that the malonyl-CoA responsible for this effect is made by enzymes in the hypothalamic cells.

Wait! We studied malonyl-CoA! We learned about it in this very class! Answer these questions about malonyl-CoA:

a) First, Once More inStead of GueSSing, draw the structure of malonyl-CoA in the space provided. You can use CoA-SH for the carrier.

b) What biochemical pathway centrally features malonyl-CoA? (One sentence):

Fatty acid synthesis


c) What is the connection between the enzyme acetyl-CoA carboxylase (ACC) and malonyl-CoA? (one sentence)

ACC produces malonyl-CoA from acetyl CoA

d) Write the reaction of ACC. You don’t need to draw any structures, but include all substrates and products:

\[
\text{acyl-CoA} + \text{NAD}^+ + \text{ATP} \rightarrow \text{malonyl-CoA} + \text{ADP} + \text{Pi}
\]

The “malonyl-CoA hypothesis” states that high levels of malonyl-CoA in cells of the hypothalamus inhibit eating behavior in the whole animal. One piece of evidence consistent with this is the observation that inhibition of hypothalamic fatty acid synthase (FAS) in animals causes decreased feeding. Lets think about this observation

d) What is the connection between fatty acid synthase and malonyl-CoA? One sentence:

FAS uses malonyl-CoA as a substrate

e) So why would inhibiting FAS in hypothalamic cells increase levels of malonyl-CoA? One sentence:

Less FAS activity would mean less consumption of malonyl-CoA and thus higher levels in the cell

f) According to this hypothesis, what would you expect to happen to mouse eating behavior if you could lower malonyl-CoA levels in their hypothalamic cells? (One sentence)

↓ malonyl-CoA → ↓ Feeding; the low levels of malonyl-CoA would cause increased feeding behavior
8) (12 points) A Rheostat for Fat...

Speaking of malonyl-CoA, there is another interesting feature of this molecule. It turns out that malonyl-CoA is a potent allosteric inhibitor of carnitine palmitoyl transferase I (CPS1), which you may recall is important in fatty acid oxidation:

a) Using Car-OH for carnitine, and R-CO-SCoA for a fatty acyl CoA, write the reaction of CPT1 below. Include all products or substrates:

\[
\text{Car-OH} + \text{R-CoSCoA} \xrightarrow{\text{CPT1}} \text{Car-O-C-R} + \text{CoASH}
\]

c) How is the product of the reaction in b) important for fatty acid metabolism? (One sentence)

The acyl carnitine molecule can cross the mitochondrial inner membrane, allowing delivery of the acyl group to the matrix where oxidation occurs.

d) There are actually two CPT enzymes, CPT1, and CPT2. Where does CPS2 function, and what does it do?

CPT2 catalyzes the reverse reaction inside the mitochondrial matrix, regenerating acyl-CoA.

e) If you compare type I and type II muscle fibers, there are higher levels of CPS1 in type I fibers compared to type II fibers. Why does this make sense? (One sentence)

Type I fibers are more able to oxidize fatty acids, and CPS1 is a critical enzyme of this process.

f) What would you expect to happen to levels of CPT1 in muscles after endurance training, and why?

Expect to see increased CPT1 levels, because endurance training increases FA oxidation capacity.

g) Let's go back to the original observation of this question: malonyl-CoA is a potent inhibitor of CPT1. What does this make physiological sense? (One sentence)

Malonyl-CoA is made during FA synthesis, so this inhibition blocks fatty acid breakdown when synthesis is occurring, preventing destruction of when the cell is trying to create FA.
9) (12 pts) Fat Burners Anonymous

Since we are on the subject of fat catabolism, let's dig a little deeper. One of the largest sources of energy in your body is stored triglycerides.

a) Draw a generic triglyceride in the space to the right, using squiggly fatty acids like I draw (including the carbonyl O).

b) Fatty acid oxidation is an “iterated” pathway with a few steps that happen in repeated cycles. The list below describes the four main steps of the iterated pathway by which an acyl-CoA undergoes oxidation. Put them in order:

1. __________ oxidation to produce a C=C double bond
2. __________ reaction with CoA-SH to form acetyl-CoA
3. __________ oxidation to form to an β-keto group
4. __________ addition of H₂O to form a β-OH

c) Fatty acid oxidation is a great source of energy. Describe the ways that products of fatty acid oxidation are used to make ATP: list them, and describe in simple terms what happens to them to produce energy:

\[ \text{NADH} \rightarrow \text{ETC (at complex I)} \rightarrow \text{ATP} \]
\[ \text{FADH}_2 \rightarrow \text{ETC (at complex II)} \rightarrow \text{ATP} \]
\[ \text{Ac-CoA} \rightarrow \text{KREBS to produce NADH, FADH}_2 \text{ (and GTP)} \]

d) Why can triglycerides contribute to new glucose production in mammals (such as Carbon or Polar Bears) while fatty acids can not? (One sentence)

The glycerol that is released in TG catabolism can be used for glyconeogenesis.

e) Ketone bodies are also possible products of fatty acid oxidation. What organ produces them, and what are their function in the mammal?

The liver makes KBS to provide alternate glucose-sparing fuel.

3-Hydroxy-3-methylgluraryl-CoA is a product formed in ketone body synthesis and in lipid anabolism. Sometimes people use “b” instead of “3” in the name. Same thing.

f) Using Only Mneonics that Sound Good, draw HMG-CoA in the box. Include the “S” of CoA so we know what kind of a bond is there...

3-Hydroxy-3-methylgluraryl-CoA.

______ STEROL SYNTHESIS; MEVALONATE PATHWAY
10) (13 pts) Funnel-mental Nitrogen Metabolism

When an amino acid is metabolized, the nitrogen is removed and the resulting α-keto acid is then used in a variety of ways. The nitrogen from that amino acid is transferred to our old friend α-ketoglutarate (αKG). You can use the name game (or the Krebs cycle above) if you cant remember the structure of αKG...

a) Using the structure of alanine (A) below as prototype amino acid, write the reaction catalyzed by transaminase, that uses α-KG as an acceptor substrate to produce glutamate. Name the reactants and products of the reaction, and include structures of products and reactants. (If you can’t draw a structure, employ an abbreviation).

\[
\begin{align*}
\text{COO}^- & \quad + \quad \text{CO}_2 = 0 \\
\text{H}_3\text{N}^-\text{C} - \text{H} & \quad \text{transaminase} \quad \rightarrow \\
\text{CH}_3 & \quad \text{CH}_3\text{N} = \text{CH} - \text{NH}_3^+ \\
\text{CO}_2 & \quad \text{CH}_3\text{N} = \text{CH} - \text{NH}_3^+ \\
\text{pyruvate} & \quad \text{Glutamate} \\
\end{align*}
\]

b) What cofactor is used by transaminases to do this chemistry?

c) After that αKG by a separate enzyme called glutamate dehydrogenase, and free ammonia is produced. Write the reaction for this enzyme. No structures needed here. But include all substrates and products. “Dehydrogenase” is a strong hint about one of the substrates...

\[
\begin{align*}
\text{Glutamate} + \text{NAD}^+ + \text{H}_2\text{O} & \rightarrow \alpha\text{KGL} + \text{NH}_4^+ + \text{NAD}^+ \\
\end{align*}
\]

d) Draw and name the product of that key nitrogen removal cycle, in the box provided.

**Carbamoyl phosphate synthetase, CPS1,** is a critical enzyme in nitrogen catabolism.

e) Write the reaction for CPS1, including the structure of the main products and substrates. Indicate other substrates and products as well.

\[
\begin{align*}
\text{HCO}_3^- + 2\text{ATP} + \text{NH}_3 & \rightarrow 2\text{ADP} + \text{P}_{\text{i}}^+ + \text{O}_3\text{P} - \text{C} - \text{N}_2 \text{H}_4 \\
\end{align*}
\]

f) Patients with a genetic defect in CPS1 have severe symptoms, including elevated blood ammonia levels. Why does this make sense? (One sentence)

g) **CPS2** is another form of CPS that functions in anabolism. What metabolic pathway is CPS2 involved in?
11) (12 pts) It is, after all, and Electron Year

The picture shows a schematic of electron flow in the ETC like we have drawn it many times. This picture is directly from the problem set. The letters are each electron acceptors or donors that we have discussed many times. Use the letter or numeral that gives the best answer. You can use the letters, the roman numeral, or the brief abbreviations (such as “Q”) in your choice. One per space only.

a) _______ This is reduced cytochrome c
b) _______ This complex does not pump protons across the inner membrane
c) _______ The reduced form of a cofactor that has a long lipid tail
d) _______ The final acceptor of electrons that can come all the way from glucose
e) _______ oxidizes cyt c to pump protons
f) _______ is also a substrate of GAPDH
g) _______ Is a Krebs cycle enzyme
h) _______ Uses a reduced quinone as an electron donor
i) _______ Uses oxidized cyt c as a substrate
j) _______ Produces reduced cytochrome c as a product

k) One of the things we hear about a lot are reactive oxygen species, or ROS. They are considered the possible cause of important cellular stresses and perhaps have a role in aging. How is the ECT important in the biology of ROS?

Ros are thought to originate from e− that escape (or leak from) the ETC and modify things like O₂ to make highly reactive ROSs.

l) ROS production underlies one hypothesis for how caloric restriction (CR) slows the aging process. Describe (one sentence) how CRs protective effects might involve ROS?

The idea is that less flow through the ETC could lead to lower levels of ROS and thus less cellular damage and stress.
12) (12 pts) The Liver is Glyco-generous

We have called the liver a “glucostat” for the body, since it is so important in keeping blood glucose in a safe range.

a) When we have had a meal, and insulin is abundant while glucagon is low, what happens to the following LIVER processes (a word, phrase, or clause is fine)
glycogen breakdown: decreased; glycogen phosphorylase ↑
glycogen synthesis: increased; glycogen synthase ↓
glycolysis: F2,6Bp↑, PFK1↑
gluconeogenesis: decreased; F2,6Bp↓, FBP1↓

b) When we have fasted for awhile, and insulin is low and glucagon is high, what happens to the following LIVER processes (a word, phrase, or clause is fine)
glycogen breakdown: increased; glycogen phosphorylase ↑
glycogen synthesis: decreased; glycogen synthase ↓
glycolysis: decreased; F2,6Bp↓, PFK1↑
gluconeogenesis: increased; F2,6Bp↑, FBP1↑

When glycogen is broken down, glucose monomers are removed from the storage polymer by glycogen phosphorylase.

c) Using Glycₙ and the staring product, and Glycₙ₋₁, write the action glycogen phosphorylase, using appropriate abbreviations for all substrates and products.

\[
\text{Glycₙ + P₂} \xrightarrow{\text{Glycₙ phosphorylase}} \text{Glycₙ₋₁ + Glu₁P↑}
\]

e) What has to happen to the glucose monomer produced by glycogen phosphorylase in order for it to enter the glycolytic pathway? (one sentence)

The Glu₁P is converted to Glu₆P by a mutase...

d) What is the other enzyme that works along with glycogen phosphorylase to liberate glucose monomers from glycogen?

debranching enzyme

f) Why is glucose produced from glycogen in liver released into the bloodstream, while that produced in muscle is not? Include in your explanation the name of the enzyme that underlies this difference (one sentence)

The liver expressed Glu₆P phosphatase, that produces free Glu which can exit via a glucor-specific transporter; muscle does not express G6Pase

g) What are names of the two enzymes involved in adding glucose to the glycogen?

Glycogen synthase and Branching enzyme
True False (16 points) Answer T or F for each; best answer.

a) _____ gout is caused by excess production of uric acid
b) _____ the enzyme blocked in the treatment of gout uses PRPP as a substrate
c) _____ phosphoglycerate kinase runs backwards in gluconeogenesis
d) _____ muscles produce alanine from pyruvate to facilitate removal of ammonia
e) _____ insulin signaling is hypothesized to promote aging
f) _____ there are genes whose actions directly cause aging
g) _____ there are genes whose actions directly inhibit aging
h) _____ gluconeogenesis is only regulated by glucagon
i) _____ Gout is caused by excess xanthine oxidase activity
j) _____ arachidonic acid is an essential fatty acid
k) _____ arachidonic acid is a substrate of cyclooxygenase
l) _____ Keq is unaffected by concentrations of reactants or products
m) _____ $\Delta G^\circ$ determines the spontaneity of a reaction at all conditions
n) _____ The pentose phosphate pathway is important for fatty acid oxidation
o) _____ Type II muscle fibers are more abundant in sprinters than marathoners
p) _____ The phosphagen system is the first source rapidly required muscle ATP
q) _____ The inner membrane of the mitochondria is permeable to ATP and ADP
r) _____ The Warburg effect states that cancer cells must make their own fatty acids
s) _____ Type I diabetes is due to autoimmune attack of pancreatic cells
t) _____ Both glycolysis and gluconeogenesis are exergonic
u) _____ Sir2 action has been proposed to extend lifespan
v) _____ The malate-aspartate shuttle uses transamination enzymes
w) _____ After several hours of endurance exercise, insulin is high and glucagon is low
x) _____ (Fill in your own nutty question): It will be a most interesting year!