CHEM-643 Biochemistry Name _____
Mid-term Examination
8:00 – 10:00 AM, Monday, 3 November 2014

Dr. H. White - Instructor

This examination will assess your learning, problem-solving skills, and ability to communicate clearly. Parts are intended to be challenging even to the best students in the class. Some of the questions will deal with material you have not seen before and is not in your text; however, those questions can be answered by applying basic principles discussed in the course.

There are 8 pages to this examination including this page. Metabolic pathway maps for glycolysis, gluconeogenesis, pentose phosphate pathway, citric acid cycle, β-oxidation, fatty acid synthesis, and isoprene biosynthesis are provided separately.

Write your name on each new page.

- Read every question so that you understand what is being asked. If you feel any question is unclear or ambiguous, clearly explain your answer or interpretation. Please call my attention to any errors you encounter.
- This is examination is closed book. You may refer to the metabolic pathway sheets provided.
- Do not expose your answers to the scrutiny of your neighbors. Please fold under each page before you go on to the next.

Breakdown of the examination by sections:

I. Multiple Choice-plus	28 Points
II. Problems	58 Points (+12 pt Bonus possible)
III. Essay	14 Points
Total	100 Points (112 Points max)

Exam Statistics

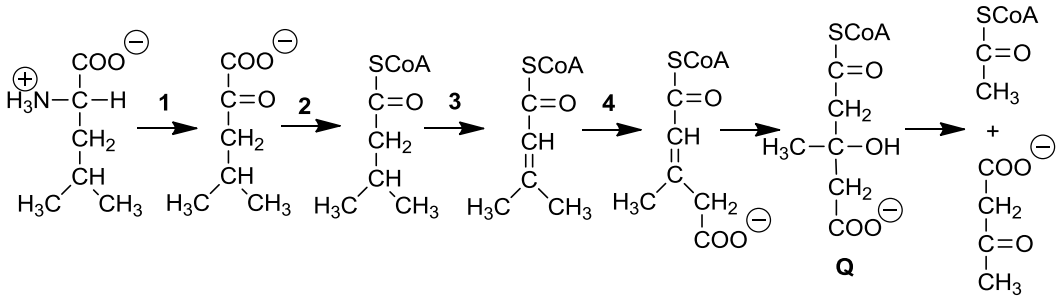
Class Range

Class Mean

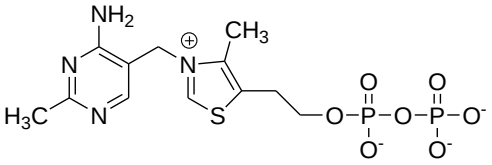
N =

Part I: Multiple Choice with follow-up (28 Points Total)

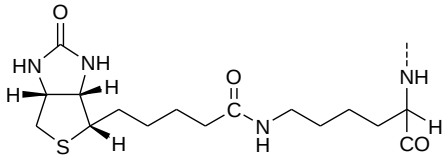
The catabolism of leucine to acetyl CoA and acetoacetate is shown below with the first four reactions labeled 1-4 to correspond to the questions that follow. The structures A-D are of various coenzymes/cofactors that may be involved in one or more of the numbered reactions.



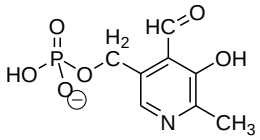
A



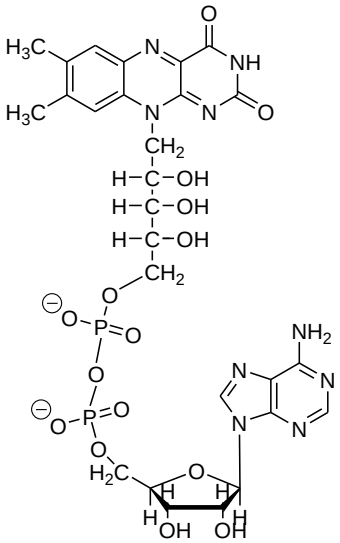
B



C



D



(2 Points each) Next to the structures above, identify *by full name* each of the coenzymes/cofactors. Half credit for abbreviation only.

(3 points each) Below, identify the correct cofactor(s) for each reaction.

____ 1. Reaction 1 requires this/these coenzyme(s)/cofactor(s).

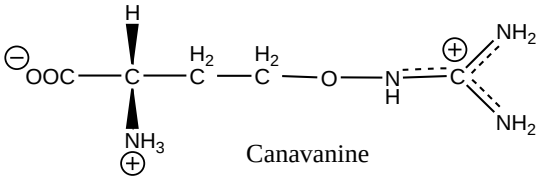
____ 2. Reaction 2 requires this/these coenzyme(s)/cofactor(s).

____ 3. Reaction 3 requires this/these coenzyme(s)/cofactor(s).

____ 4. Reaction 4 requires this/these coenzyme(s)/cofactor(s).

Bonus (2 Points) Name the next to last compound [Q] in the catabolism of leucine depicted above.

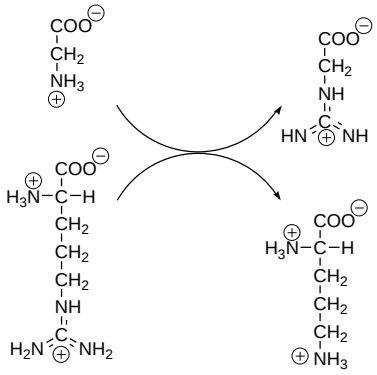
____ 5. (4 Points) Canavanine (below) is a toxic analog of arginine found in the seeds of certain legumes. It would make us sick because human enzymes cannot discriminate between canavanine and arginine. Canavanine is not toxic to germinating legume seeds because their enzymes can discriminate between arginine and canavanine.



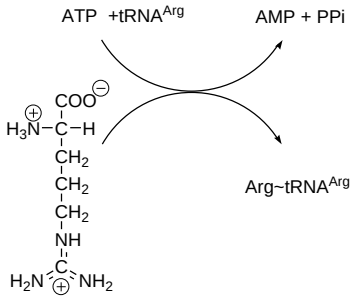
Canavanine

Several enzyme reactions involving arginine are shown below. Which one would be most responsible for the toxicity associated with canavanine?

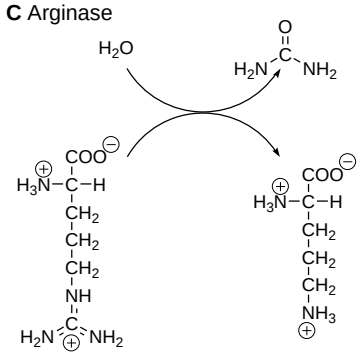
A Glycine amidinotransferase



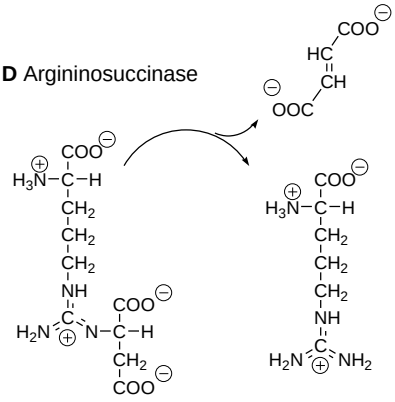
B Arginyl tRNA Synthetase



C Arginase



D Argininosuccinase



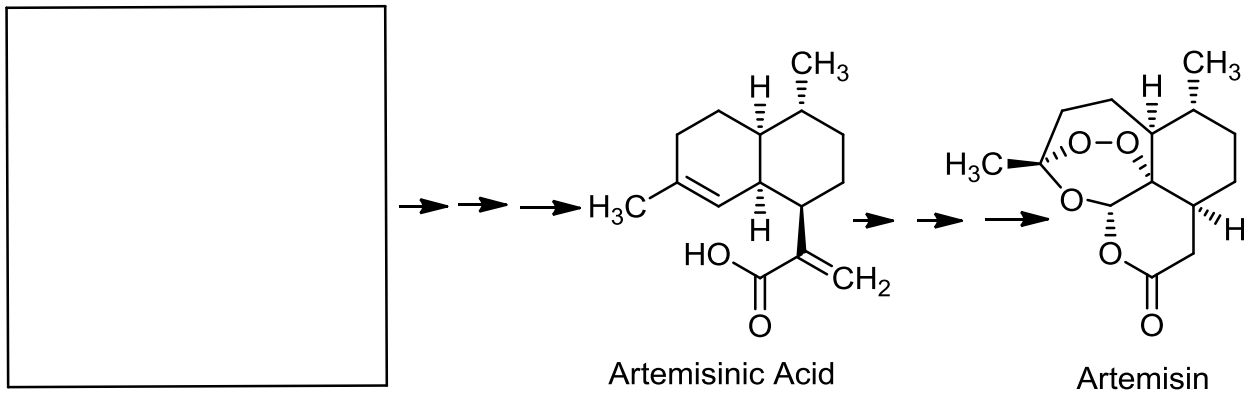
Explain your choice (4 points)

Part II: Problems and thought questions.

6. Artemisin is a plant sesquiterpene used as an antimalarial drug. A biosynthetic intermediate for it is artemisinic acid. Their structures are shown below.

   a. (5 points) In the box provided, draw the structure of the C_{15} precursor arranged such that the carbon back bone would superimpose on the structure of artemisinic acid.

   b. (4 points) Put an X on the carbon-carbon bonds in artemisinic acid that are not present in your precursor molecule.



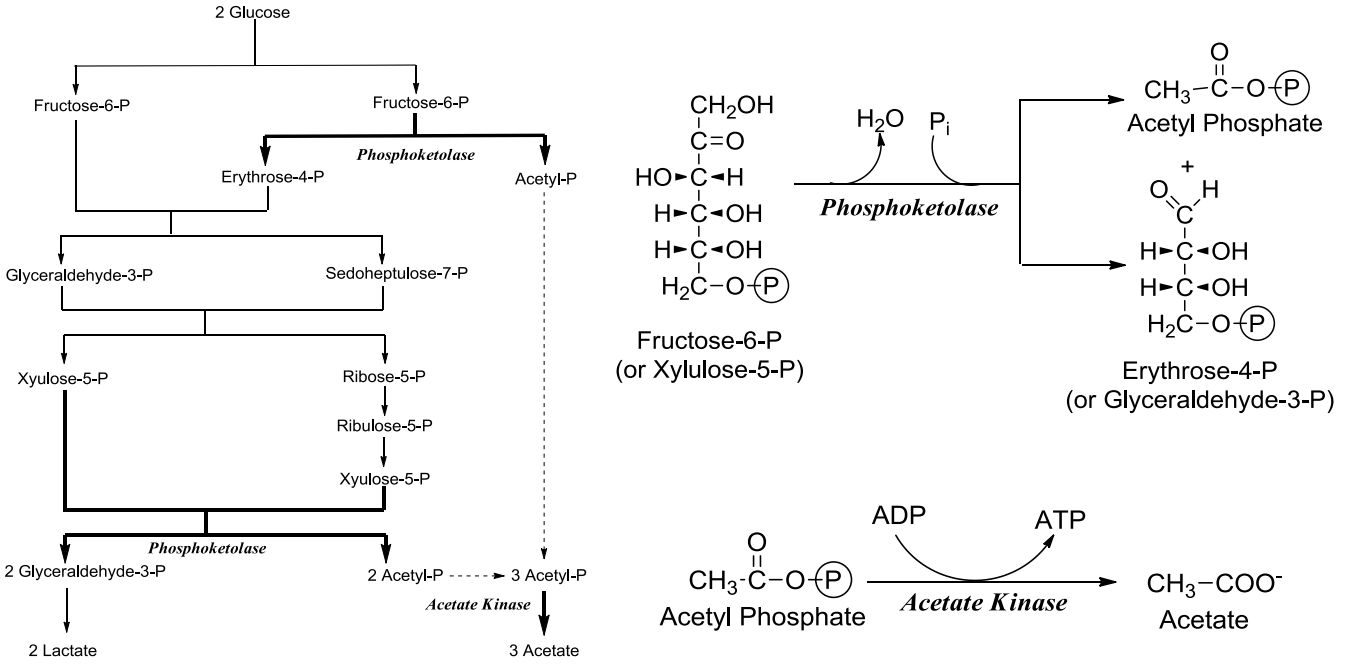
7. Ketone bodies produced in the liver are transported to peripheral tissues where they are metabolized for energy. Typically we think of ketone bodies being formed during starvation, but neonatal mammals apparently need the pathway due to the heavy energy demands during the period after birth when they are growing rapidly. Cotter et al. [*J. Biol. Chem.* 286, 6902 (2011)] genetically engineered a mouse lacking Succinyl CoA:Acetoacetate CoA Transferase. They found that suckling mice lacking this enzyme died and that providing supplemental glucose only slightly delayed death. They concluded that ketone body oxidation was essential for neonatal mice.

   a. (4 points) Based on this enzyme's name, draw the reaction catalyzed showing the chemical structures and providing the names of the substrates and products.

   b. (4 points) Considering the reaction catalyzed, predict whether the $\Delta G^{0'}$ for this reaction is negative, positive, or near zero. Explain your reasoning.

   c. (4 points) How would the absence of this enzyme interfere with ketone body metabolism?

8. While the Embden-Meterhoff glycolytic pathway gets prime billing in textbooks because it is widely distributed and we have it, there are a variety of other glycolytic pathways that can be seen as evolutionary variations. The addition of a few enzyme reactions and elimination of a few others while retaining many familiar reactions yield alternative ways of obtaining energy from glucose. Shortly after birth, the intestinal tract of breast-fed human infants is colonized by *Bifidobacteria,* These anaerobic bacteria metabolize lactose and other sugars. When they are grown on glucose in culture, they produce acetate and lactate in a 3:2 ratio by a pathway that is familiar in many respects, but different in others. Two enzymes in this pathway are Phosphoketolase and Acetate Kinase. The pathway is outlined below and the two new reactions shown with bold arrows. The questions that follow will explore this pathway, its relationship to the Embden-Meyerhoff and Pentose Phosphate Pathways, and the enzymes associated with the pathways. Use handouts displaying the glycolytic and pentose phosphate pathway provided. This question was inspired by Suzuki et al. *J. Biol. Chem.* **285**, 34279 (2010).



a. (2 points) What cofactor would you expect to be involved in the phosphoketolase reaction? [10 bonus points to anyone who proposes a reasonable mechanism for the reaction involving this cofactor. Use the back of this page.]

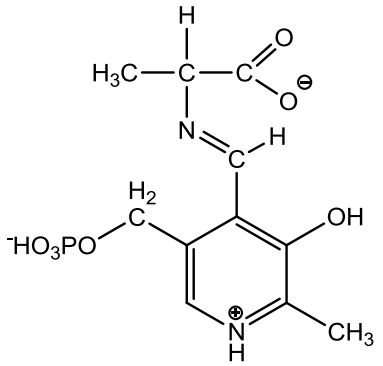
b. (4 points) Draw or name another metabolic intermediate that would have a similar $\Delta G^{0'}$ for hydrolysis as acetyl phosphate.

c. (5 points) *On the pathway outlined on the previous page*, indicate *where* and *how many ATPs* are used or generated.

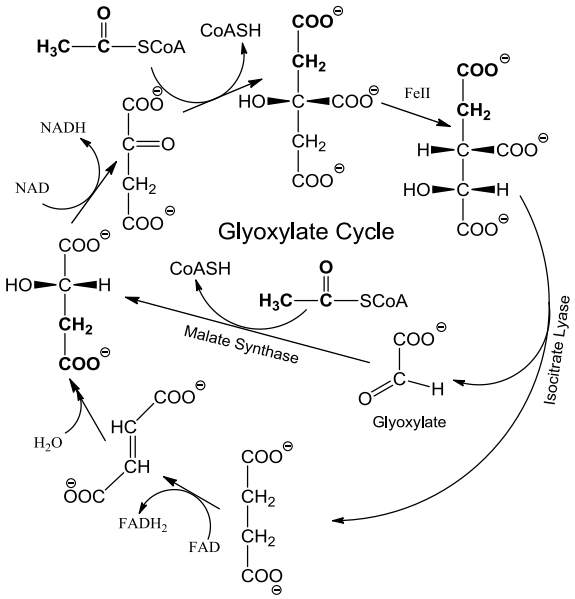
d. (5 points) This pathway produces **less**, **more**, or the **same** amount of ATP per glucose as the Embden-Metyerhoff glycolytic pathway? Circle one answer. Explain.

e. (8 Points) If *Bifidobacterium* were grown with 3-^{14}C-glucose as an energy source, where would the labeled carbon end up in lactate and/or acetate? Be specific about which carbon(s) would be labeled in the products.

9. (5 Points) The figure below shows a Schiff base enzyme intermediate of an amino acid with pyridoxal phosphate poised for a decarboxylation. On the figure, how this would happen by showing how the electrons would move (arrows). **Name** the amino acid.



10. Germinating seeds do not photosynthesize. They rely on stored lipids for energy. Unlike animals, germinating seeds can convert acetyl CoA generated by fatty acid oxidation into glucose and other carbohydrates using the glyoxylate cycle, a modification of the citric acid cycle that, as shown to the left, doesn't involve the loss of carbon as CO_2. Canvin and Beevens [*J. Biol. Chem.* **236**, 988 (1981)] studied the synthesis of glucose from ^{14}C-methyl labeled acetate in germinating castor beans. The table (below) from their experiments shows the distribution of ^{14}C in the six carbons of glucose as percent of the total at various time periods.



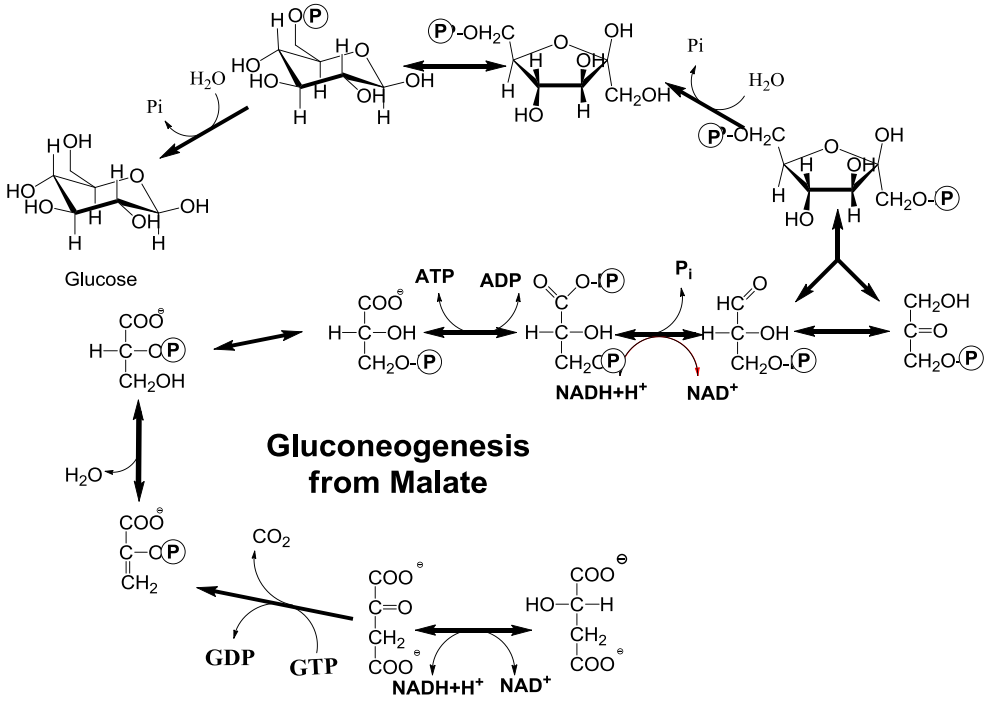
Carbon in glucose	Time of incubation (min)			
	15	30	60	120
1 CHO	22.5	21.5	21.9	22.5
2 H-C-OH	23.8	24.5	23.8	24.0
3 HO-C-H	2.5	4.7	6.0	3.6
4 H-C-OH	3.7	5.3	8.8	9.6
5 H-C-OH	23.0	21.1	20.8	16.6
6 CH_2OH	24.5	22.7	18.8	23.4

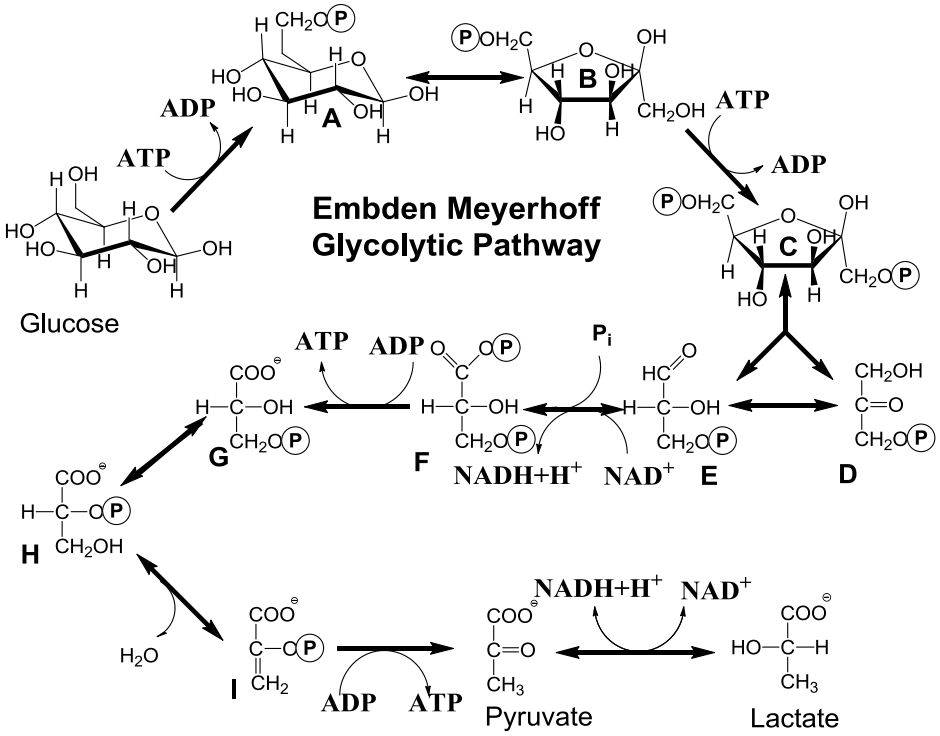
a. (8 Points) How would you explain the % labeling of the 6 carbon atoms in glucose from ^{14}C-methyl-acetylCoA given the glyoxylate pathway and the gluconeogenic pathway? (Recognize that measurement errors of a few percent can occur in these experiments.)

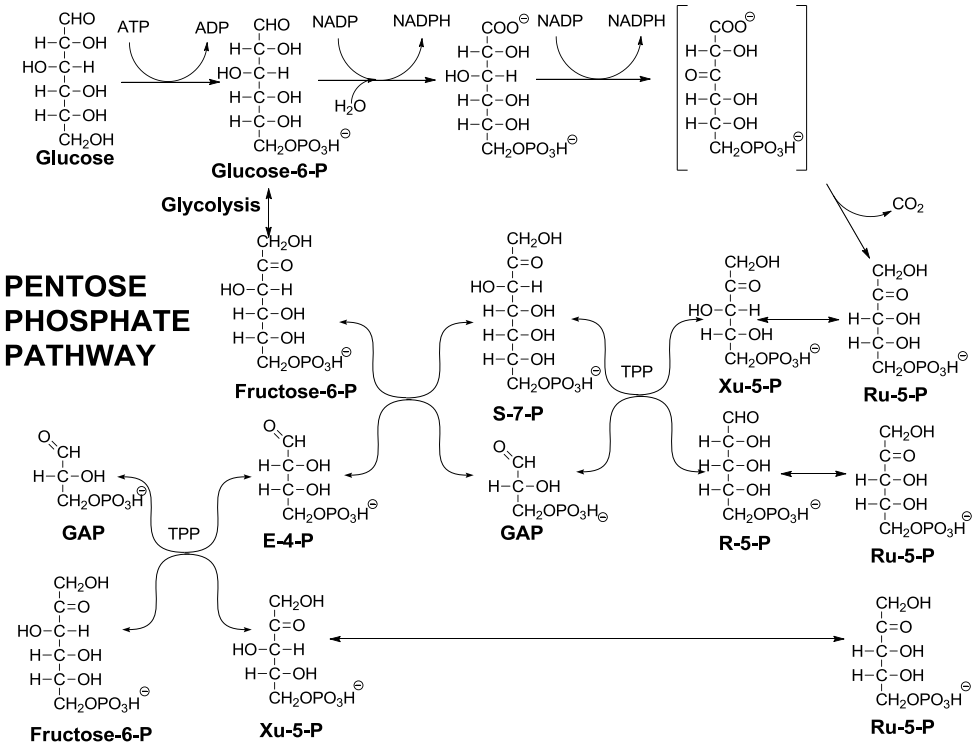
Part III: Essay Questions. (14 Points) Choose ***one*** of the following two questions to answer. Be thorough in your explanation and use equations and/or diagrams to illustrate your points as needed.

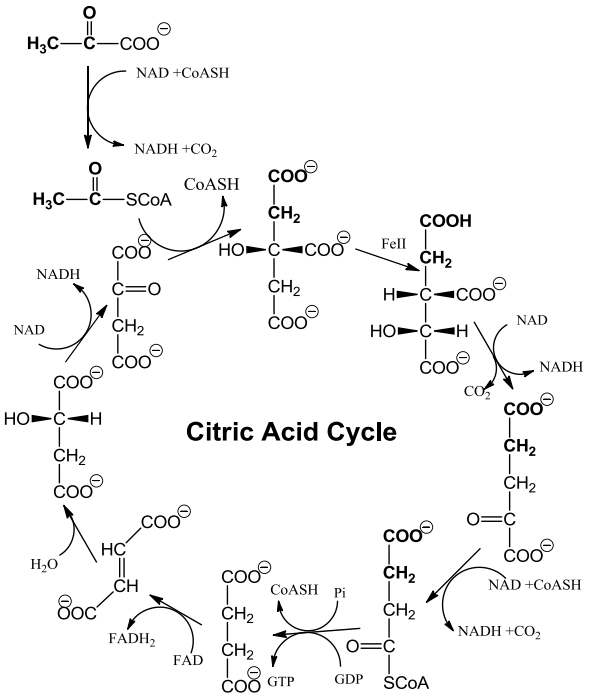
a. So far this semester, we have encountered two examples of isotope fractionation (kinetic isotope effects)—one in photosynthetic fixation of CO_2 and in the formation of chiral methyl groups. Describe the observations in each of those examples and explain the basis for the unequal reactivity of different isotopes. Or,

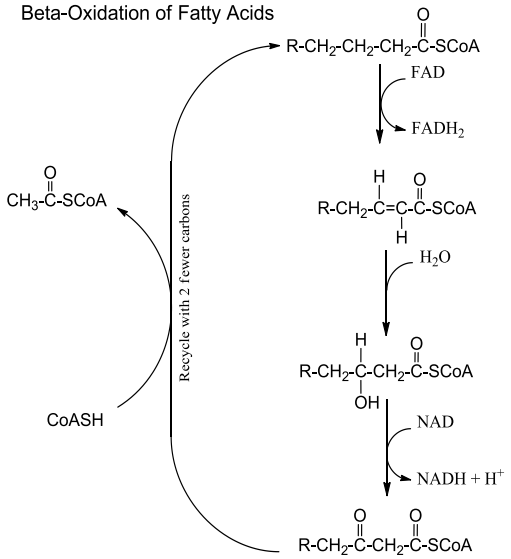
b. The average human adult can survive without food for two months which is longer than one would predict based on metabolic processes occurring while well-fed. *Specifically*, what metabolic changes/adaptations occur in humans and how do they contribute to prolong life during starvation?


Glucose
ATP
ADP
Pi
NADH+H+
NAD+
Gluconeogenesis
from Malate
CO2
GDP
GTP

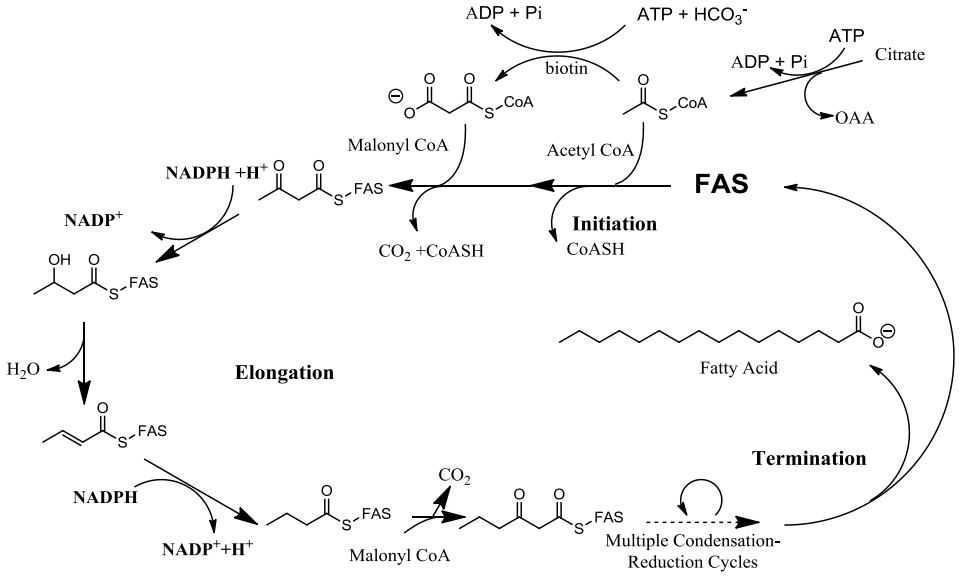

ADP
ATP
A
B
C
Glucose
Embden Meyerhoff
Glycolytic Pathway
ATP
ADP
Pi
G
F
NADH+H+
NAD+
E
D
H
I
NADH+H+
NAD+
ADP
ATP
Pyruvate
Lactate


Glucose
ATP
ADP
Glucose-6-P
NADP
NADPH
H2O
NADP
NADPH
Glycolysis
PENTOSE PHOSPHATE PATHWAY
Fructose-6-P
S-7-P
TPP
Xu-5-P
CO2
Ru-5-P
GAP
TPP
E-4-P
GAP
R-5-P
Ru-5-P
Fructose-6-P
Xu-5-P
Ru-5-P


NAD +CoASH
NADH +CO2
CoASH
FeII
NADH
NAD
NAD
NADH
CO2
Citric Acid Cycle
H2O
FADH2
FAD
CoASH
Pi
GTP
GDP
SCoA
NAD +CoASH
NADH +CO2


Beta-Oxidation of Fatty Acids
FAD
FADH2
H2O
NAD
NADH + H+
CoASH
Recycle with 2 fewer carbons

Fatty Acid Synthesis



Acetyl CoA to Dimethylallyl Pyrophosphate