CHEM-643 Biochemistry Name _____
Final Examination
3:30 – 6:30 PM, Wednesday, 12 December 2012
Dr. H. White – Instructor

- There are 10 pages to this examination.
- **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 suspected errors you encounter.
- This is examination is closed book until 5:30PM when you may refer to your assignments and your lecture notes. Textbooks or electronic devises cannot be used. You may refer to the hand-drawn metabolic pathway sheets available ahead of time from the course website.
- This examination will assess your learning, problem-solving skills, and ability to communicate clearly. It is 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, the questions can be answered by applying basic principles discussed in the course.
- Do not expose your answers to the scrutiny of your neighbors. Please fold under each page before you go on to the next. You may use the backs of pages, if you need more space.
- The maximum possible score is 140. Graded Exams can be picked up starting Friday afternoon and will be held until Spring Semester.

Have a Safe and Happy Holiday!

Exam Statistics:

Class Range ___47 – 126/140 Total points possible 140

Class Mean ___94.15±22.5 (67.3%)__ N 33

Your Score _____

Your Rank in Class _____ out of 35

Course Grade _____

1) (24 Points Total) **Group Project**

a) (2 points) What organism and amino acid pathway did your group study?

b) (4 points) What is the reaction catalyzed by the enzyme you studied? (Provide structures of substrates and products and indicate any cofactor requirements.)

c) (5 points) In retrospect, what informative comparisons would you want to make now on your project that were not done by your group but were done by other groups??

d) (9 points) Your group project was a test of the "cognate-bias hypothesis"[1] which predicts that amino acid composition of enzymes involved in the biosynthesis of a particular amino acid will be relatively deficient in that amino acid as the result of natural selection. Would that selection be more or less strong for: (Explain reasoning for each)

i) A large vs a small enzyme in the pathway?

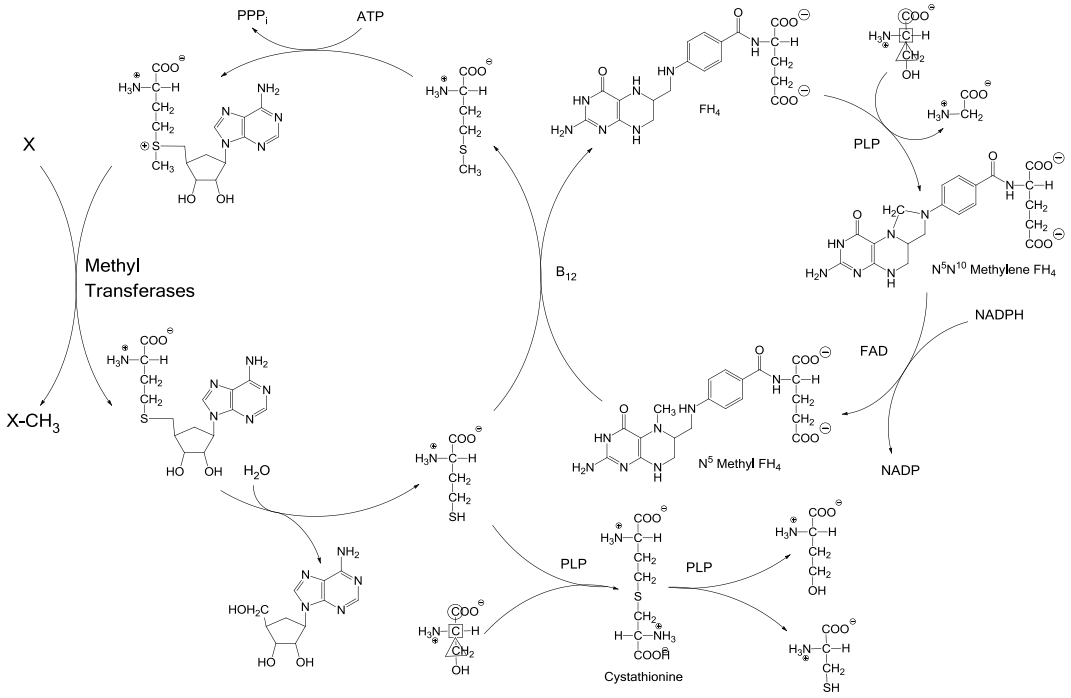
ii) A feedback regulated enzyme vs and unregulated enzyme in the pathway

iii) A multifunctional enzyme vs a single function enzyme

e) (4 points) Different proteins differ in their amino acid composition. As you learned, certain plant foods are nutritionally incomplete for humans because they are deficient in certain amino acids. Provide a one reason based on natural selection why beans or corn seeds are deficient in at least one amino acid.

[1] Alves and Savageau (2005) *Molecular Microbiology* **56**, 1017-154.

1. (45 Points Total**) Serine Metabolism.** Elevated plasma homocysteine concentration is a metabolic indicator of several pathologies in humans including cardiovascular disease, autoimmune diseases, cancer development, and neurodegenerative disease.[2] The pathways shown below display reactions associated with homocysteine metabolism. A series of questions will relate to this diagram.

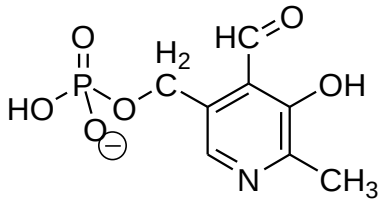


a) (2 Points) On the above metabolic diagram, draw a box around homocysteine.
b) (6 Points) The three carbons of serine are identified by different shapes surrounding them. Trace the fate of each these carbons by marking every carbon derived from them with the appropriate shape on the molecules in the diagram above. (One point deducted from 6 for each mistake.)
c) (5 Points) on the above metabolic diagram, write the name next to the structures of those amino acids that are among the twenty commonly found in proteins.
d) (6 Points) There are on the order of 500 genes encoding methyl transferases in the human genome. Give the names or structure of the substrate (X) or product (X-CH_3) of two methyltransferase enzymes that likely would contribute significantly to the flux in the reactions leading to homocysteine in tissues throughout your body.

[2] Schalinske and Smazal, *Advances in Nutrition* **3**, 755-762 (2012)

e) (8 points) In the cycle at the left hand side of the diagram on the previous page, ATP enters at the top and adenosine leaves at the bottom. Show with reactions, how adenosine would be converted back to ATP in your body.

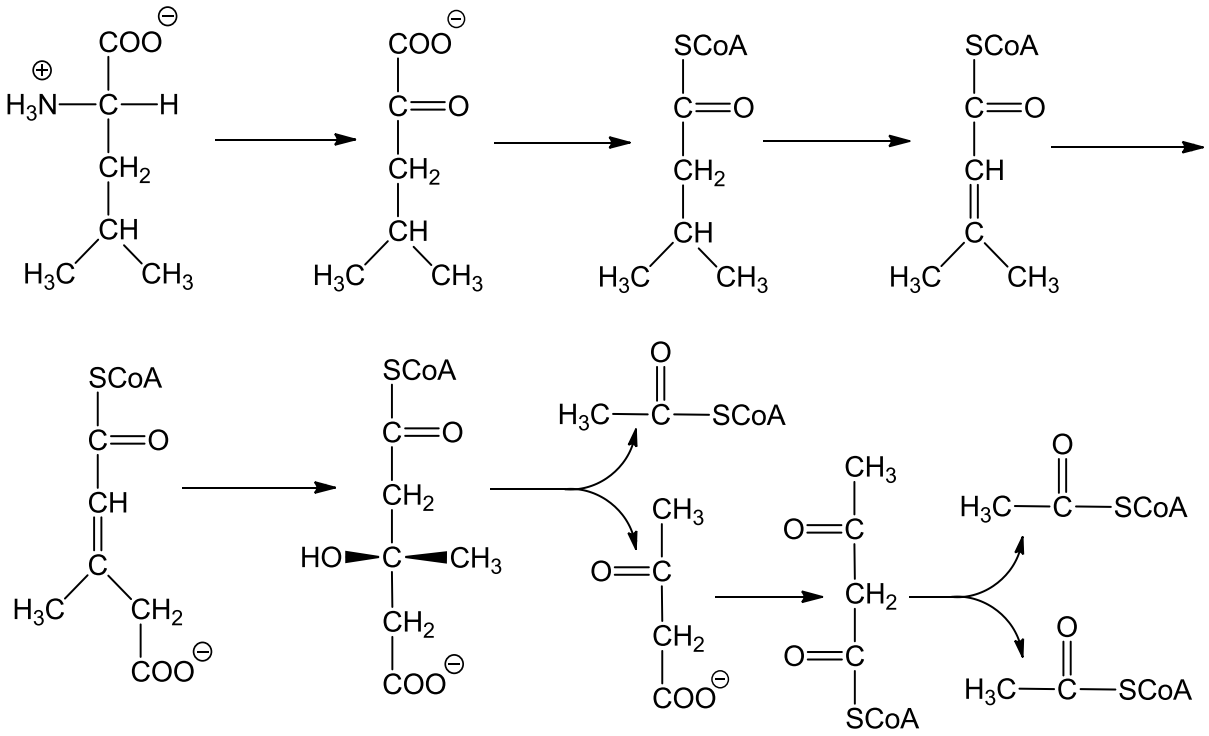
f) (8 Points) Cystathionine requires pyridoxal phosphate (PLP) as a coenzyme. Show mechanistically (arrow pushing) how PLP functions as a catalyst in that reaction.



PLP

g) (10 Point essay) The concentration of metabolites vary and are dependent upon a number of factors. Assume you are embarking on a program to identify causes of increased plasma homocysteine levels in human disease. What factors would influence the levels? Conceptually frame the situation and generate at least three hypotheses that might explain the elevated levels.

2. (42 Points) **Leucine catabolism** is your professor's favorite pathway because it involves many different coenzymes. Below the intermediates in this pathway are shown without indicating any coenzymes.



a. (10 Points) For each of the enzymes represented by arrows above, write in on the diagram all missing coenzymes, reactants, and products that should be involved.

b. (6 Points) If one could equally label all six carbons of leucine and capture the three acetyl CoAs formed in its metabolism in a living animal, quantitatively, how would the amount of labeled carbon in the methyl and carbonyl groups of the acetyl group compare with each other? Explain your answer.

c. The enzyme (BCKD) catalyzing the second reaction in the leucine catabolic pathway is regulated by phosphorylation by a protein kinase. In its phosphorylated state, the BCKD's E1a subunit is inactivated. As reported in the recent 19 October issue of *Science*,[3] a genetic deficiency of the kinase is associated with a form of autism.

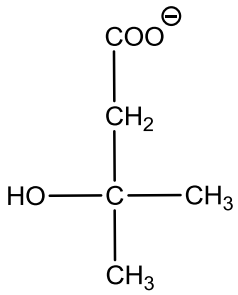
(i) (6 Points) Based on this information, draw a picture/diagram that represents the phosphorylation of BCKD by the kinase (and likely dephosphorylation by a phosphatase involved in this regulation). Indicate the active and inactive forms of BCKD and any additional substrates, reactants, or products.

(ii) (6 Points) What would be the effect of a deficiency of the kinase on the flux of metabolites through the leucine catabolic pathway? Explain

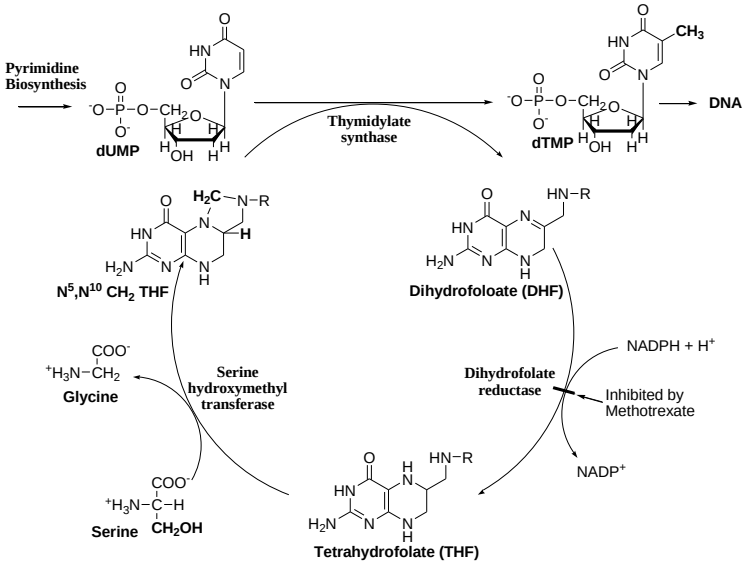
(iii) (6 points) What might the resulting metabolic consequences of this deficiency be? Explain

[3] Novarino et al. (2012) *Science* **338**, 394-397.

3. (8 Points) Recent television ads have touted a nutritional body-building supplement identified as HMB, hydroxymethyl butyrate (shown to the right). It is also called 3-hydroxyisovalerate. This compound is not a normal metabolite but accumulates in the urine of children with multiple carboxylase deficiency[4] and is considered a breakdown product of leucine. Show how this compound could be formed in two steps from one of the catabolic intermediates of leucine using common enzyme reactions.



4. (19 points) **Thymidylate Synthase** catalyzes a methylene tetrahydrofolate-dependent reaction that, in addition to transferring a one-carbon unit, involves reduction of the substrate. The resulting oxidized dihydrofolate (DHF) needs to be recycled. It is reduced by Dihydrofolate Reductase (DHFR) as shown below. DHFR is the target of Methotrexate (MTx), an antitumor drug that binds to mammalian DHFR very tightly, 10,000-50,000 times more tightly than DHF binds.



a. (2 Points) Why is MTx an effective antitumor drug?

[4] Sweetman, L., Bates, S. P., Hull, D. & Nyhan, W. L. (1977 *Pediatric Res*. **11**, 1144-1147.

b. (5 Points) Conceptually, draw a graph and label the axes for the rate of dTMP production as a function of [MTx] in a tumor cell.

c. (6 Points) Over time tumors become resistant to MTx inhibition. From a biochemical perspective, list three ways (mechanisms) that a cell could develop resistance to MTx.
i.

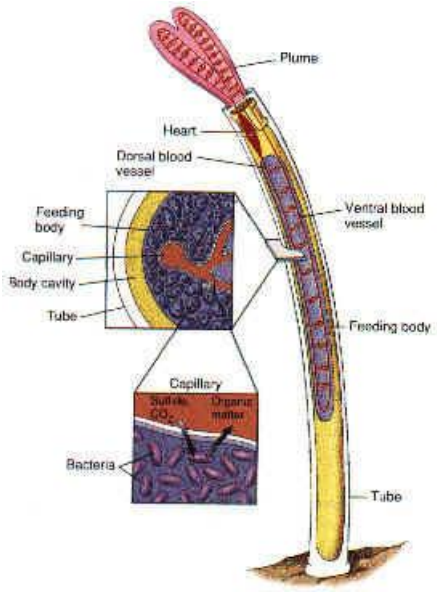
ii.

iii.

d. (6 Points) Robert Schimke's laboratory at Stanford studied the development of resistance to MTx in cultured mouse tumor cells by adding more and more MTx to the culture medium as the cells became more and more resistant. In the end, they had selected for tumor cells (AT-3000) that could grow in the presence of MTx 3000 times more concentrated than would normally kill the parental Murine Sarcoma 180 cells.[5] The amino acid sequence of DHFR in the resistant cells was identical to that in the parental (S-3) tumor cell line. Based on the following normalized data, what has happened to the cells to make them MTx resistant?

S-180 Cell line	DHFR activity	DHFR mRNA levels	DHFR Gene copies
S-3	1	1	1
AT-3000	250	220	180

[5] Alt et al. *J. Biol. Chem*. **253**, 1357 (1978)



5. (10 Points) Living organisms do not violate the laws of thermodynamics. Within terrestrial and shallow water ecosystems, the energy that maintains the living organisms can be directly attributed to photosynthetic organisms, the primary producers. It was a great surprise in the late 1970's when a whole communities of organisms were found on the ocean floor where no light penetrates.[6] The energy input for these organisms apparently comes from H_2S which comes out of nearby hydrothermal springs. One rather large organism there is *Riftia pachyptila*, a type of worm several inches in diameter and up to 10 feet long. These worms lack a mouth, digestive tract, and anus. Certain parts of their body contain high densities of endosymbiotic bacteria presumed to be capable of coupling the aerobic oxidation of sulfide to sulfate to the fixation of CO_2 via the Calvin Cycle.[7,8,9]

Based on your knowledge of metabolism and the information above, suggest ***at least*** two specific pieces of biochemical information that you would want to know before you would accept the above hypothesis as possibly valid and not just a science fiction story. Indicate why this additional information would help your evaluation.

[6] *Natl. Geog.* **156**, 680 (1979)
[7] *Nature* **293**, 616 (1981), *TIBS* **7**, 201 (1982)
[8] *TIBS* **7**, 201 (1982)
[9] *Science* **219**, 297 (1983)