

CHEM-643 Biochemistry

Name _____

Final Examination

3:30 – 6:30 PM, Monday, 13 December 2010

Dr. H. White – Instructor

There are 13 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 examination is open notes. You may refer to your assignments and your lecture notes, but not textbooks. You may also refer to the metabolic pathway sheets printed 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 Tuesday and will be held until Spring Semester.

Exam Statistics:

Class Range 34-131.5

Total points possible 140

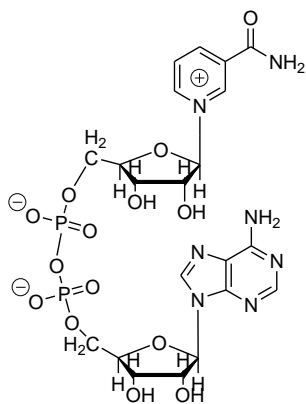
Class Mean 82.5

Your Score _____

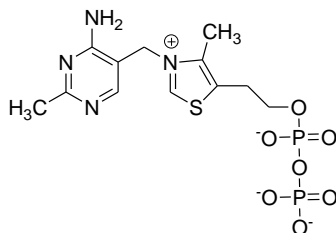
Your Rank in Class _____ out of 19

Course Grade _____

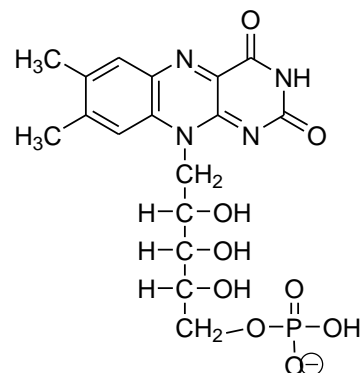
1. Structures (16 Points, 2 points each) ***Identify*** each of the cofactors shown below and ***draw a circle*** around the atom or atoms involved in the primary function of each.



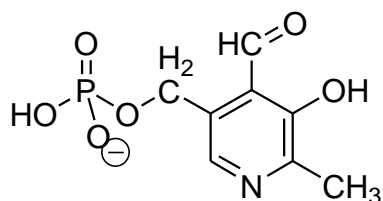
A. _____



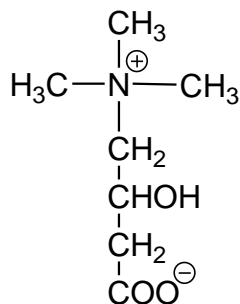
B. _____



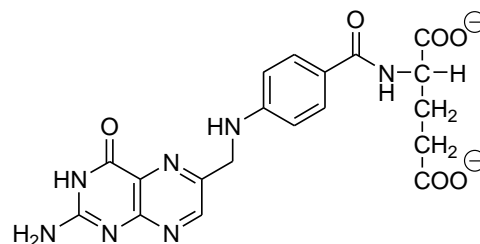
C. _____



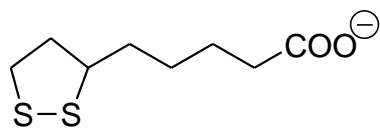
D. _____



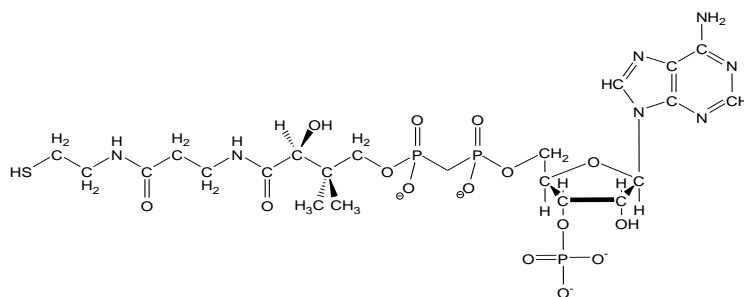
E. _____



F. _____



G. _____

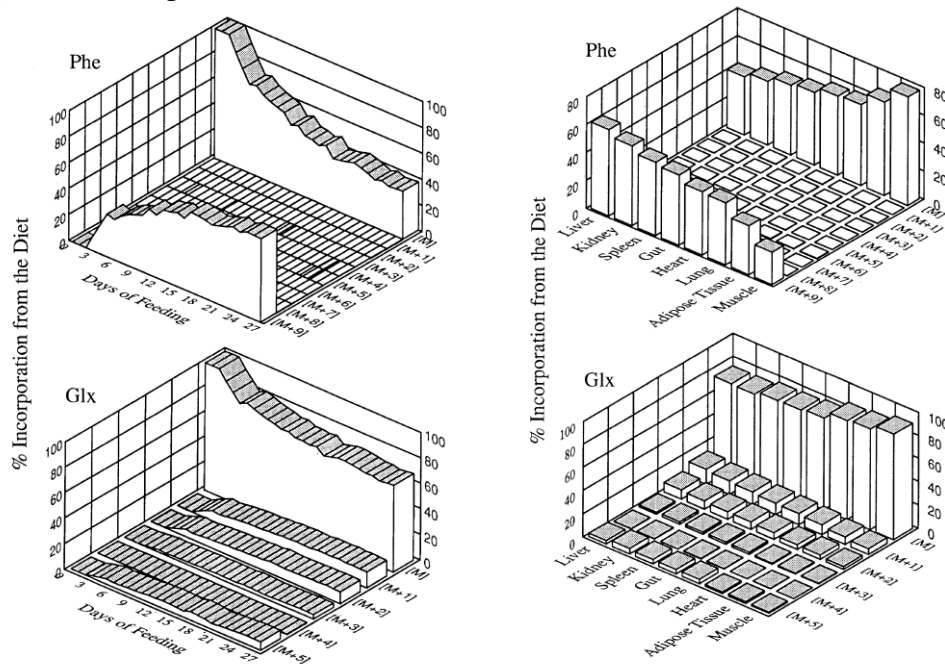


H. _____

2. (8 Points) Researchers studying the genetics of coat color in mice obtained two different pure-bred recessive strains of albino mice. When they mated individuals of one strain with individuals of the other strain, all of the offspring had normal brown coat color. When these siblings were mated with each other, slightly less than half ($\sim 7/16$) of the resulting offspring were albino.

What is a simple metabolic interpretation of these results relating to melanin biosynthesis? ***Explain clearly*** your thinking and how the results are expected. Use genetic diagrams if that will help.

3. (10 Points) *Spirulina platensis* is an edible cyanobacteria (blue green algae). By growing it in the presence of highly enriched (99%) ^{13}C CO₂, it is possible to obtain proteins, lipids, carbohydrates, and nucleic acids that are uniformly labeled with ^{13}C and contain minimal ^{12}C . This compares with a natural abundance of ^{13}C of ~1%. Berthold et al.¹ fed ^{13}C -enriched protein from these cyanobacteria to a laying hen for 27 days. They isolated protein from her eggs through the period and from various tissues at 27 days. The protein was hydrolyzed to free amino acids which were analyzed by mass spectrometry. The following are two figures from that article reporting on Phenylalanine (Phe) and Glutamate + Glutamine (Glx) in egg white (left) and various tissues (right). The axis with M, M+1, etc. represents results for each of the possible isotopomers.



- _____ (2 points) What is the percentage of the phenylalanine (Phe) in egg white at day 27 that came directly from dietary phenylalanine.
- _____ (2 Points) What is the percentage of the glutamate + glutamine in egg white at day 27 that came directly from dietary Glx?
- (6 Points) What would be a reasonable explanation for why the M+9 isotopomer of phenylalanine is not uniformly distributed among all the tissues by 27 days?

¹ Berthold, H.K., Hachey, D.L., Reeds, P.J., Thomas, O.P., Hoeksema, S. & Klein, P.D. (1995) *Proc. Natl. Acad. Sci. USA* 92, 10123-7.

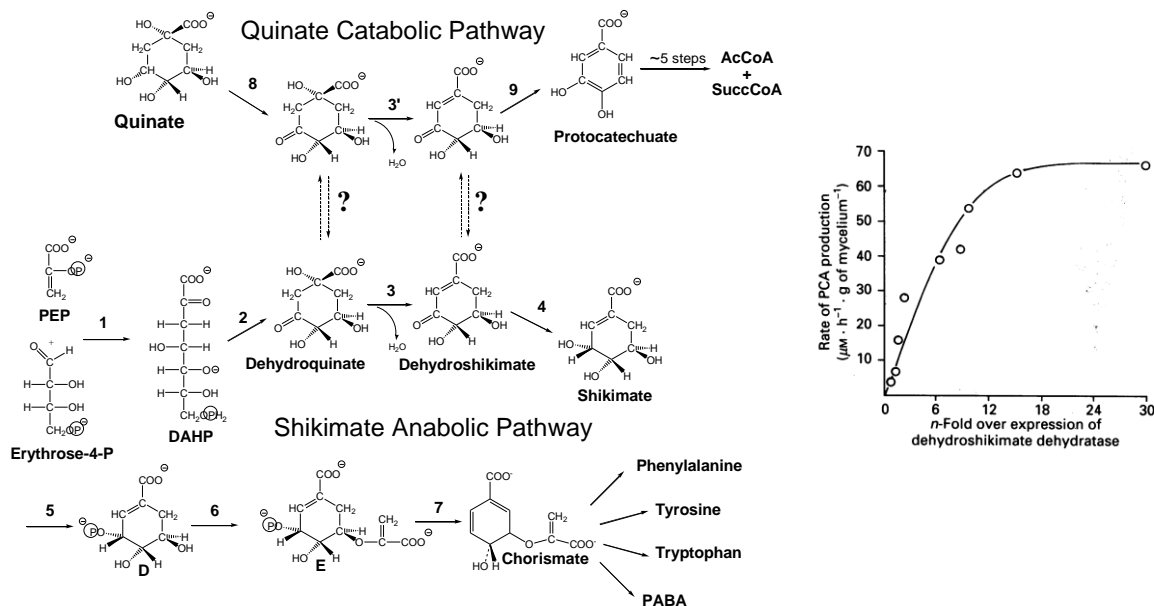
4. In addition to studying amino acids, Berthold et al.² in a separate report described their analysis of isolated RNA from the hen's liver and other tissues at 27 days. The RNA was hydrolyzed to mono nucleotides (AMP, GMP, CMP, and UMP) with RNAase, the phosphoryl groups removed with phosphatase, and the resulting nucleosides analyzed by mass spectrometry. The following table records the abundance of isotopomers for each in percent of total for the different nucleosides.

Isotopomer	Isotopomer Mass Relative to ¹² C Nucleoside (M)										
	M+0	M+1	M+2	M+3	M+4	M+5	M+6	M+7	M+8	M+9	M+10
Adenosine	50.7%	22.2%	13.3%	7.86%	3.32%	1.19%	0.39%	0.43%	0.36%	0.20%	0.12%
Guanosine	60.2%	25.1%	10.2%	3.05%	0.53%	0.39%	0.21%	0.10%	0.07%	0.06%	0.07%
Cytidine	57.4%	2.48%	4.71%	4.54%	1.12%	0.07%	0.46%	1.57%	5.67%	21.9%	
Uridine	48.2%	5.39%	2.51%	1.51%	2.00%	1.00%	0.96%	2.20%	7.38%	28.8%	

- A. (4 Points) Draw the structure for guanosine.
- B. (2 Points) Why is there no M+10 isotopomer for cytidine or uridine?
- C. (4 Points) What patterns do you see in these data? (Respond with clear complete sentences.)
- D. (4 points) What can you conclude about nucleotide metabolism in chickens from these data?

² Berthold ,H.K., Crain, P.F., Gouni, I., Reeds, P.J., & Klein, P.D. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 10123-10127.

4. (22 Points) In a number of instances, enzymes catalyzing consecutive reactions in a metabolic pathway are part of a single polypeptide chain. Biochemists have reasoned that this makes good sense because the product of one enzyme would be produced in close proximity to the next enzyme thereby reducing or eliminating the need for much or any diffusion. Channeling, as this is called, would seem to increase the efficiency of a pathway. However neat this channeling hypothesis is, it is difficult to test. An experiment was designed to test the hypothesis in a particular case involving the synthesis of aromatic amino acids. In the fungi *Aspergillus nidulans*, enzymes 2-6 in the diagram below are part of a single polypeptide chain. A single polypeptide isozyme (3') exists in the same organism as part of a catabolic pathway for quinate, also shown. The researchers generated strains with a constitutive quinate pathway that varied the activity of enzyme 9 over a 30 fold range³. They grew the strains on a minimal media in the absence of added aromatic amino acids and in the absence of quinate and determined the production of protocatechuate (graph at the right).



A. (6 Points) Show mechanistically (arrow pushing) with an intermediate step how Dihydroshikimate could be converted to Protocatechuate.



³ Lamb et al. (1992) *Biochemistry Journal* 284, 181-7.

B. (4 Points) Is this reaction thermodynamically favorable? Why, or why not? Provide an analysis.

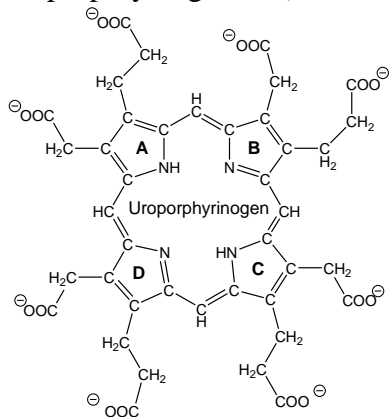
C. (6 Points) Chemically, Reaction 4 and Reaction 8 on the previous page look virtually identical but with arrows in opposite directions. What coenzymes would you expect for each reaction? Why?

Reaction 4
Why?

Reaction 8
Why?

D. (6 Points) Interpret the graph. Would you conclude that intermediates in the shikimate pathway to aromatic amino acids are channeled? Explain why, why not, or why one can't be sure.

5. (8 Points, 2 points each) The clockwise hypothesis for heme biosynthesis⁴ postulates an ordered decarboxylation sequence in which the acetate side chains on rings D, A, B, & C of uroporphyrinogen III (shown below) are decarboxylated successively.

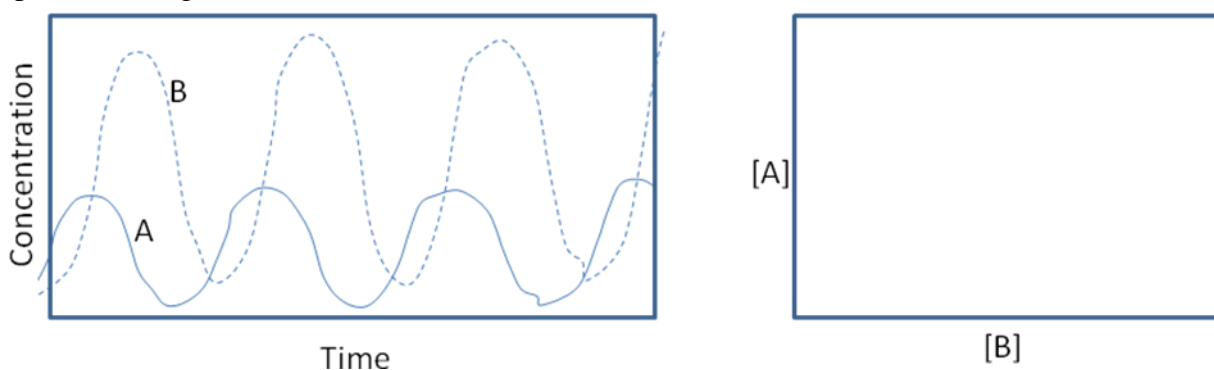


Respond to each of the following facts as to whether it supports, does not support, or is inconclusive with respect to the clockwise hypothesis.

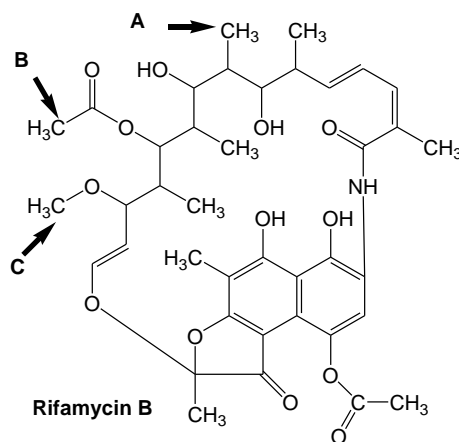
- A. Feces of hexachlorobenzene poisoned rats accumulate 7d, 6da, and 5dab tetra pyrroles. (Numbers refer to the number of carboxyl groups. Small letters refer to the rings in which the carboxyl group has been removed.)
- B. All 14 possible decarboxylation intermediates are found in human urine.
- C. All 14 possible decarboxylation intermediates were synthesized chemically and all were metabolized to protoporphrin IX by chicken red blood cells.
- D. A patient suffering from *porphyria cutanea tarda* accumulated 7d.

⁴ Lash, T. D. (1991) *Biochemistry Journal* 248, 901-903.

6. (6 Points) On November 16, less than a month ago, Britton Chance died at age 97. Among other things, he was one of the outstanding biophysical chemists of the 20th century. (He also received a gold medal in sailing at the 1952 Helsinki Olympics and had 12 children.) You are familiar with some of his work because he was a coauthor of the article on which the *Life without Oxygen* case study problem was based. In that article⁵, Chance introduced the use of phase-plane plots as a way to visualize and analyze relationships among oscillations in the concentrations of glycolytic metabolites in yeast after an aerobic to anaerobic transition. In honor of Britton Chance's contribution to our knowledge of biochemistry, please transform the hypothetical oscillations of two metabolites, A & B, shown on the left to a phase-plane plot on the right.



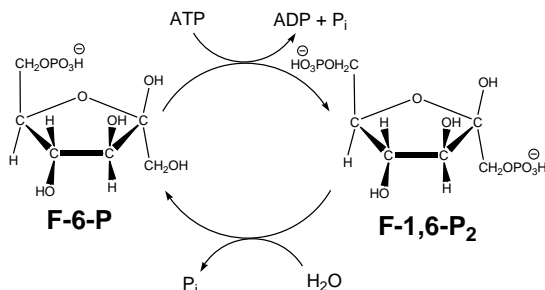
7. (6 Points, 2 Points each) The structure of Rifamycin B, an antimicrobial produced by *Streptomyces mediterranei* that inhibits DNA synthesis, is shown below. It contains methyl groups derived from three different precursors. Examine the structure and predict the precursors.



A. _____ B. _____ C. _____

⁵ Augustin Betz and Britton Chance (1965) Phase Relationship of Glycolytic Intermediates in Yeast Cells with Oscillatory Metabolic Control, *Arch. Biochem. Biophys.* **109**, 585-594

8. (12 Points) Bumble bees are frequently observed foraging among flowers on cool mornings when the temperature is in the low 40's (~5°C)⁶. A measurement of their body temperature is much higher, near 36°C. This is also observed with large sphinx moths which often fly on cool nights. These "cold blooded" animals warm up before flying by substrate cycling. Futile substrate cycles are permitted to run uncontrolled with the net hydrolysis of ATP and the generation of heat. The best understood futile cycle is that involving fructose phosphates.



- A. (4 Points) What are the names of the two enzymes involved in this futile cycle?

Top = _____

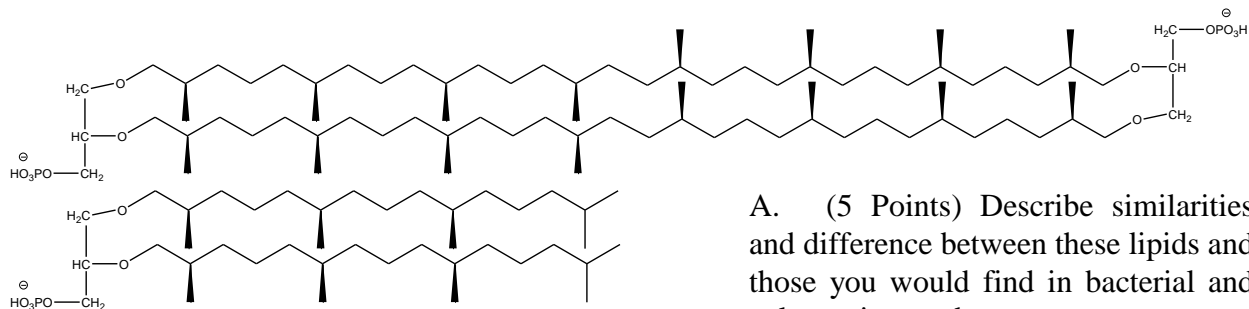
Bottom = _____

- B. (4 Points) What important roles do these enzymes play in human carbohydrate metabolism?

- C. (6 Points) To measure substrate cycling, bees were injected simultaneously with D-[5-³H] glucose and D-[6-¹⁴C] glucose. After a period of time, fructose phosphates were isolated and the ³H/¹⁴C ratio measured. The lower the ratio, the more substrate cycling had occurred. Explain how substrate cycling would cause the loss of ³H to water in these experiments while retaining ¹⁴C. During warm up the bees cycle FDP >9 times per time it is oxidized to pyruvate by glycolysis. In flight this occurs about 24% of the time.

⁶ Surholt, B., Greine, H. Baal, T., and Bertsch, A. (1991) Warm up and futile cycling in the flight muscles of male bumble bees. *Comp. Biochem. Physiol* 98A, 299-303.

9. (10 Points) The membrane lipids of Archaeobacteria are quite different than ours.⁷ Two examples are shown below.



- A. (5 Points) Describe similarities and difference between these lipids and those you would find in bacterial and eukaryotic membranes

- B. (5 Points) Based on these structure, identify two enzymes you would ***not*** expect to find in a cell extract of Archaeobacteria with these lipids.

⁷ De Rosa, M, Gambacorta, A., and Gliozzi, A. (1986) Structure, Biosynthesis, and Physiochemical Properties of Archaeobacterial Lipids, *Microbial Reviews* **50**, 70-80.

- 10.** (10 Points) Published on line by *Science* only a week and a half ago is an article entitled, “A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus”. Its authors are NASA-funded scientist who held a press conference that received attention the national news. Within days there were scientists questioning the claim and saying the article should not have been published.

As a budding scientist who has taken a course in Intermediary Metabolism and using the skeptical hat that scientist wear in evaluating new claims, make a ***list of five things*** you would like to know about the biochemistry of this organism that would convince you one way or the other of the veracity of the claim in the title. If you know anything about the arsenic in biology, please feel free to use that information.

Essay

Writing reflects how you think. Among the “right answers” I will read for the following question, some will be better than others because they show greater depth of understanding, avoid extraneous or inaccurate information, use knowledge from previous learning, provide a more logical structure, use appropriate examples, and choose words with precision. Better quality answers will receive higher marks. Therefore organize your thoughts before you write. Strive to write not that you may be understood, but rather that you cannot possibly be misunderstood. Stream of consciousness answers are rarely well organized or clearly presented.

11. (16 Points) In the first week of the course, several important concepts about metabolism were described. They are listed below. Pick one for a short informative essay that explains the concept and illustrates it with relevant examples.
- i. ATP and NAD(P)H are key components of metabolic systems.
 - ii. Anabolic and catabolic pathways are not the reverse of each other.
 - iii. All pathways are regulated.
 - iv. Pathways are coupled by common intermediates
 - v. Pathways are often compartmentalized.
 - vi. Metabolism is the product of $>3 \times 10^9$ years of evolution.
 - vii. Chemically similar enzyme reaction sequences recur in different pathways.
 - viii. A large proportion of metabolism depends of the chemistry of carbonyls.