

CHEM-643 Biochemistry

Name _____

Final Examination

3:30 – 6:30 PM, Thursday, 13 December 2007

Dr. H. White – Instructor

There are 12 pages to this examination including this page. **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.

The first hour of this examination is with closed notes. You can use your notes thereafter. You may then 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 120. Graded Exams can be picked up starting Monday and will be held until Spring Semester.

Exam Statistics:

Class Range 43.5-107.5/120

Class Mean 75.3/120

Your Score _____

Your Rank in Class _____ out of 28

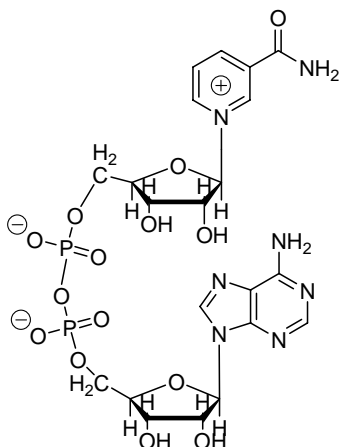
Course Grade _____

Part I - Short Answer Questions (1 point each)

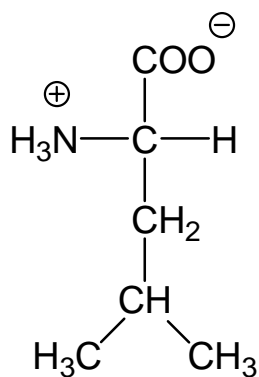
- _____ 1. Avidin, a protein in chicken egg white, binds this vitamin tightly.
- _____ 2. Instead of creatine, insects use this compound in their muscles.
- _____ 3. Analog of PABA used as an antibiotic
- _____ 4. Vegetarians may need to supplement their diet with this vitamin.
- _____ 5. Antitumor drug that inhibits dihydrofolate reductase.
- _____ 6. Amino acid that serves as a major source of carbon for folate-dependent reactions.
- _____ 7. Urea in mammals serves the same purpose as _____ in birds.
- _____ 8. Glyphosate (aka Roundup) inhibits this pathway.
- _____ 9. How long can an average human survive without food?
- _____ 10. Primary source of energy for a 100 meter race.
- _____ 11. The urea cycle occurs primarily in this organ.
- _____ 12. Product of transamination of oxaloacetate.
- _____ 13. Within an order of magnitude, how long does the average ATP molecule exist in your body before it is used?
- _____ 14. The prosthetic group of fatty acid synthase is also part

of this coenzyme.

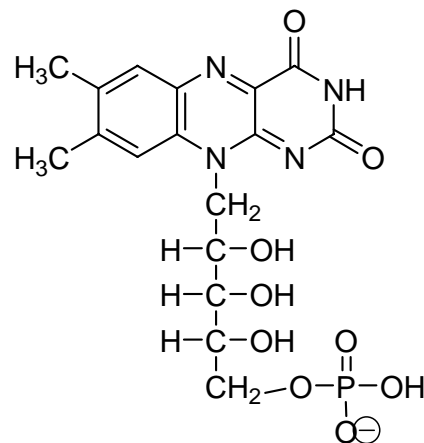
15-20 Identify the structures shown.



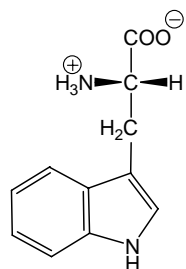
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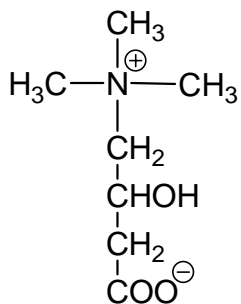
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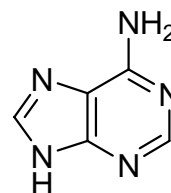
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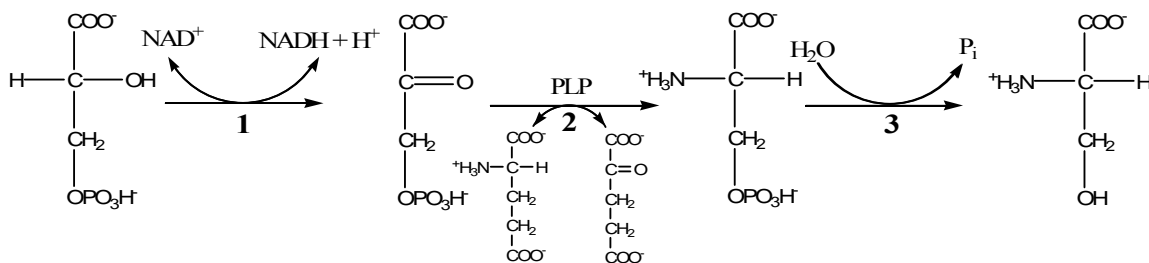
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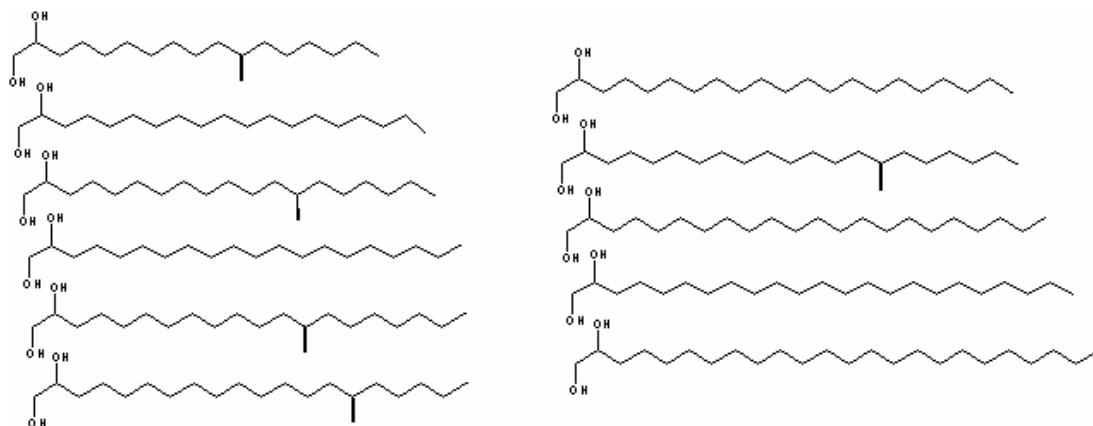
Part II Problems:

1. (8 Points) Thermodynamics strips away mechanism and tells us that the energy difference between initial and final states is independent of the path taken. There is nothing in biochemistry that violates that principle. However, the rate at which a reaction goes from starting materials to products is dependent on the mechanism (path). Consider the three enzymatic steps that convert 3-phosphoglycerate to serine as shown below.



- A. (4 points) Why must the dehydrogenation of Step 1 precede the transamination of Step 2?
- B. (4 points) One might think that it wouldn't matter where the hydrolysis of phosphate (Step 3) occurs in the sequence. However, there is a good explanation for Step 2 (transamination) to precede step 3. Provide a sensible explanation for the order of Steps 2 and 3. (Hint; Consider the consequences of reversing the order.)

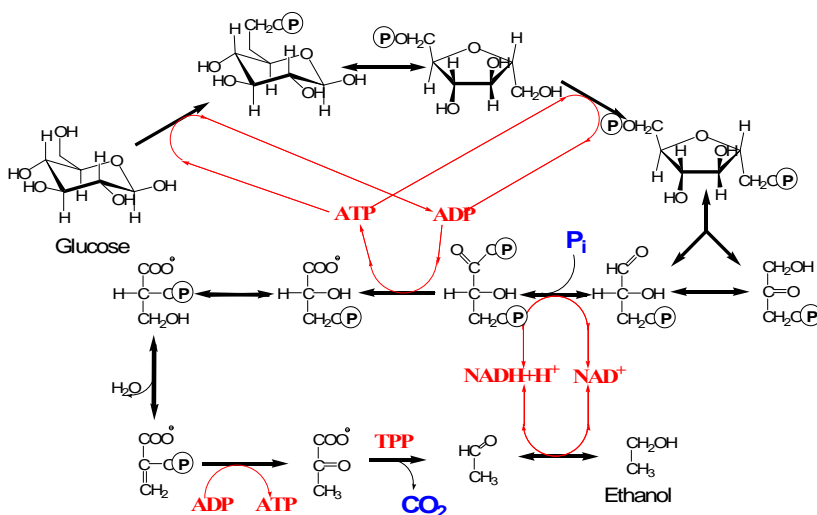
2. (8 Points) An article in *Science* [231, 1134-1136 (1986)] entitled, "Long-chain diols: A new class of membrane lipids from a thermophilic bacterium," reports the structures of the lipids shown below. There was no glycerol among the hydrolysis products of the membranes of *Thermomicrobium roseum*, an organism that grows at 75°C, but there were fatty acids and phosphate. Stearic acid and 12 methylstearic acid together account for 84% of the fatty acids.



Examine the structures and considering the structure of typical glycerophospholipids, postulate the structure of membrane lipids of this organism. How does your structure differ from a typical glycerophospholipid?

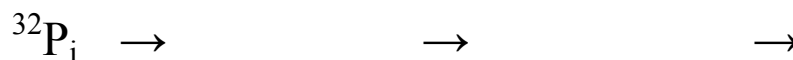
3. (16 Points) In 1906 very little was known about glycolysis. The structures of most of the intermediates in the pathway displayed below were unknown. In a classic experiment, Harden and Young broke open yeast cells in buffer and removed the cell debris to make a cell-free yeast extract. They added glucose to fuel the fermentation pathway and reported the following observations.

- Inorganic phosphate added to the extract stimulated the production of CO₂.
- The extra CO₂ evolved was stoichiometric with the amount of phosphate added and,
- The phosphate became organically bound during this process.

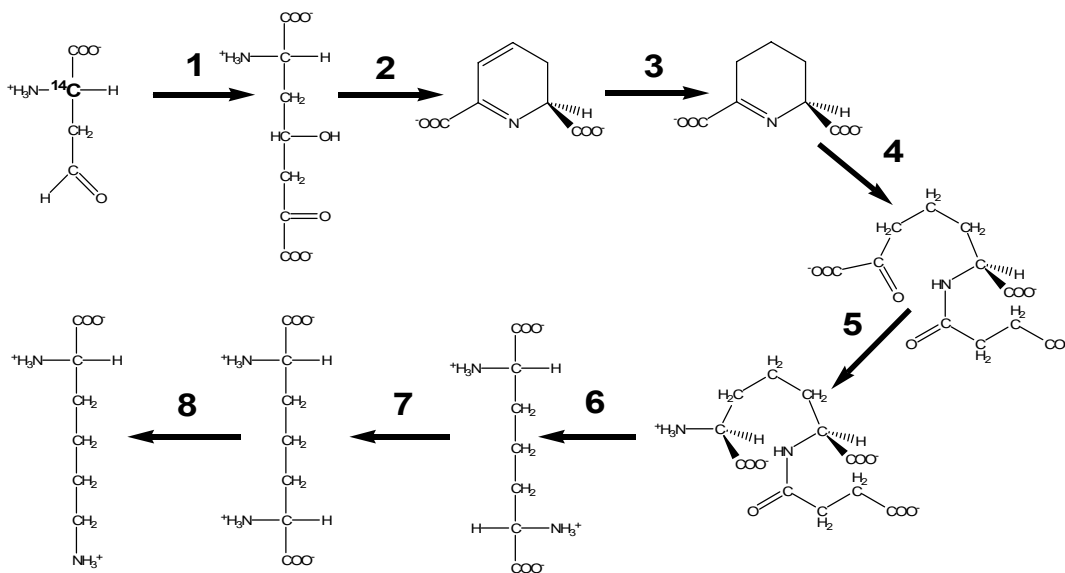


A. (8 points) Examine the glycolytic fermentation pathway above and explain why there is a burst of CO₂ production with the addition of phosphate and why there should be or could be a one to one stoichiometric relationship between added phosphate and CO₂ produced.

B. (8 points) Assume these experiments were done today with ³²P-labeled phosphate, trace the fate of the labeled phosphate in the yeast extract giving the order in which the first few compounds become labeled showing their structures and the position of the label.

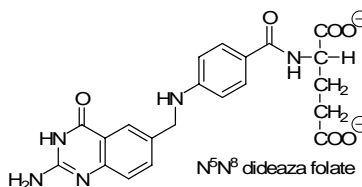
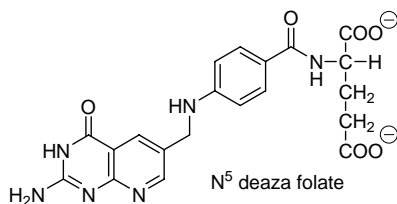
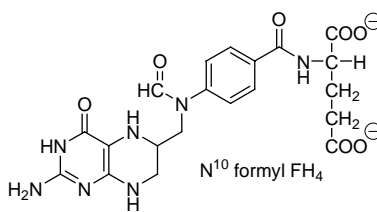
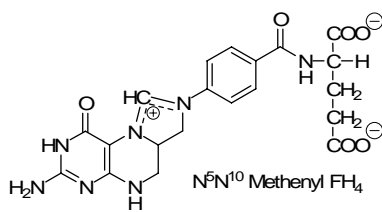
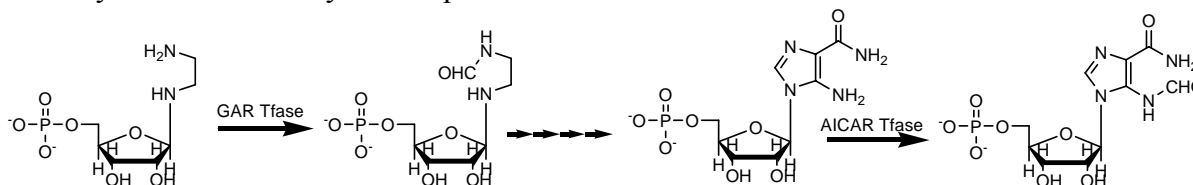


4. (25 Points) The pathway from aspartate semialdehyde to lysine as it occurs in *E. coli* is depicted below without cofactors, entering substrates, or departing products. Please answer the questions that follow. (2 points each except for part K)



- What three carbon compound is added in Step 1?
- What type of reaction occurs in Step 1?
- The product of Step 2 has a carbon-nitrogen double bond known as a:
- What would be a likely cofactor for Step 3?
- What type of reaction is Step 3?
- What is the source of the four carbon unit added in Step 4?
- What is expected coenzyme for Step 5?
- The four carbon unit added in Step 4 departs in Step 6. What role did it serve?
- What type of reaction is Step 7?
- What is the expected coenzyme for Step 8?
- (5 points) Consider the fate of the α -carbon in aspartate semialdehyde as it gets converted to lysine. On the above pathway, indicate clearly which carbon(s) of lysine would be ^{14}C labeled?

5. (10 Points) There are two formylation reactions in the biosynthesis of purines that involve folate coenzymes (see below). When these reactions were worked out originally by Buchanan, N^5N^{10} methenyl FH_4 was thought to be the donor form of the coenzyme. Later work suggested that N^5N^{10} methenyl FH_4 was converted to N^{10} formyl FH_4 by a separate enzyme in a multienzyme complex before transfer occurred.



Smith et al. [*Biochemistry* **20**, 1241 (1981)] formylated (at the N^{10} position) the two deaza folate analogs shown below at the left and used them in place of N^{10} formyl FH_4 in assays of the GAR and AICAR transferases. Please examine the relative maximal velocities and K_m values in the table below. What observations and conclusions can you make?

Enzyme	Substrate	Relative V_{max}	K_m (μM)
GAR Tfase	N^{10} formyl FH_4	1	8.9
	N^{10} formyl dideazafolate	0.77	1.9
	N^{10} formyl N^5 deazafolate	0.002	-
AICAR Tfase	N^{10} formyl FH_4	1	68
	N^{10} formyl dideazafolate	0.0007	-
	N^{10} formyl N^5 deazafolate	0.47	102

6. (13 Points) Biotinidase deficiency disrupts the normal recycling of biotin. As a consequence, the activities of biotin-dependent carboxylase enzymes are greatly reduced which in turn causes the accumulation of their substrates. These metabolites then become substrates for enzymes in other pathways and ultimately show up in the urine as unusual organic acids. Methylcitrate and 3-hydroxypropionate are two such unusual organic acids that derive from the same carboxylase substrate.

A. (10 points) Select either one of these unusual acids. Show and describe how it is formed metabolically.

B. (3 points) Biotinidase deficiency is incurable, but it is treatable. What would you recommend as a rational treatment of this metabolic disease?

Part III Short Essays. Answer **two** of the three questions.

1. (10 Points) By having two copies of genes encoding each enzyme, diploid organisms like us are cushioned from the effects of mutations that generate non-functional enzymes. Interestingly, genetic carriers for devastating metabolic diseases (persons heterozygous for a recessive detrimental mutation) produce about half the normal amount of the affected enzyme and yet rarely show any detrimental effects. Basically they are normal. In terms of metabolic flux in pathways, how can this be rationalized? Please feel free to illustrate your answer.



2. (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 [*Natl. Geog.* **156**, 680 (1979)] The energy input for these organisms apparently comes from H₂S which comes out of

nearby hydrothermal springs. One rather large organism there is *Riftia pachyptila* (Image from www.unbsj.ca), 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 CO₂ via the Calvin Cycle [*Nature* **293**, 616 (1981), *TIBS* **7**, 201 (1982), and *Science* **219**, 297 (1983)].

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.

3. (10 Points) The following are descriptions of human metabolic defects in creatine metabolism as it relates to the brain in particular. Based on these descriptions, draw a diagram for creatine metabolism in the body showing showing the location of each defect.
- A. This infant boy had extremely low creatine concentrations in serum and urine as well as in muscle. *In vivo* magnetic resonance spectroscopy analysis showed a generalized depletion of creatine in his brain as well. Feeding arginine resulted in an increase in brain guanidinoacetate, but not creatine. Dietary creatine resulted in increased creatine and decreased guanidinoacetate in the brain, Stöckler et al. (1994) [*Pediatric Research* 36, 409-413]. A four year old girl with similar symptoms excreted large amounts of guanidinoacetate (~10X normal) in her urine, Schulze et al. (1997) [*Journal of Pediatrics* 131, 626-631].
 - B. These two young mentally retarded sisters had severe language delay. Magnetic resonance showed no detectable creatine or guanidinoacetate in their brains and an intracellular pH of 7.24 compared to 7.07 in normal brain cells. They had normal levels of creatine in their serum and in muscles. They excreted normal amounts of creatine and very low amounts of guanidinoacetate. Feeding large amounts of creatine eventually resulted in near normal brain creatine levels, Bianchi et al. (2000) [*Annals of Neurology* 47, 511-513].
 - C. This male child had elevated serum creatine levels (75 $\mu\text{mol/L}$ vs 15-44 $\mu\text{mol/L}$) and elevated excretion of creatine but no detectable creatine in his brain. Creatinine excretion was normal. Dietary supplementation with creatine had no effect on brain creatine levels, Cecil et al. (2001) [*Annals of Neurology* 49, 401-404].