CHEM-643 Biochemistry Name _____
Final Examination
3:30 – 6:30 PM, Wednesday, 15 December 2004
Dr. H. White - Instructor

There are 13 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 errors you encounter.

This is an **open-notes examination**. You may refer to your assignments and your lecture notes, but not textbooks. You may also refer to the metabolic pathway sheets available 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.

Breakdown of the examination by sections:

I. Short Answer	15 Points
II. Problems	105 Points
III. Short Essays	20 Points
Total	140 Points
Additional bonus points possible	30 Points

Exam Statistics: Class Range 55-139 Class Mean 93.9

Your Score _____ Your Rank in class N=20

"...how slight is the twist of biochemical fate which determines that it should be lactate and not alcohol that is poured into the blood under stress. Were the enzyme make up of human muscle just that little bit different, hard physical exercise might be more popular than it is - the opposite of a sobering thought."

F. R. Jevons (1964) p. 67 in The Biochemical Approach to Life

Part I - Short Answer Questions (1 point each)

_____ 1. Folic acid is to pantothenic acid as tetrahydrofolate is to what?

_____ 2. Name of the 4 epimer of glucose.

_____ 3. Poisonous breakdown product of a compound in peach pits.

_____ 4. Human deficiency disease associated with diets based primarily on corn.

_____ 5. Approximate half-life of ^{14}C in years.

_____ 6. Transamination of pyruvate yields this amino acid.

_____ 7. What metabolic pathway in humans has many intermediates and reactions similar to those in the Calvin Cycle?

_____ 8. A pathway in green plants that produces ATP at night.

_____ 9. Protein amino acid with the longest biological half-life in rats. (Hint: Figure this out from the last case study problem)

_____ 10. Dihydrofolate reductase is inhibited by this antitumor drug.

_____ 11. Compound biosynthesized by plants, but not by humans.

_____ 12. Another compound biosynthesized by plants, but not by humans.

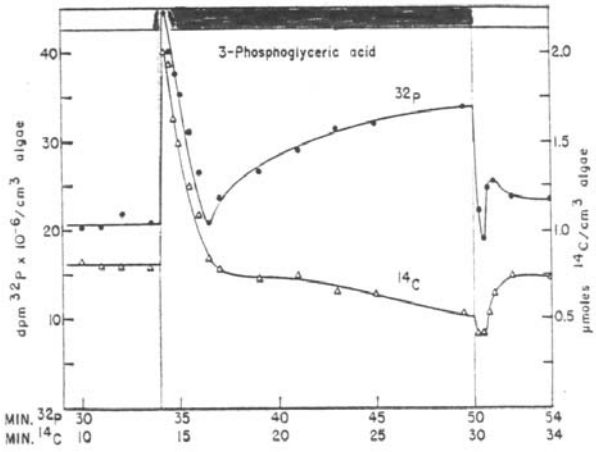
_____ 13. Compound biosynthesized by humans, but not by plants.

_____ 14. Another compound biosynthesized by humans, but not by plants.

_____ 15. Compound containing an N-glycosidic bond.

Part II Problems:

1. The following questions relate to the figure below that comes from the first Case Study Problem.



The effects of light and dark on the amounts of ^{14}C and ^{32}P labeling of 3-phosphoglycerate (3-PGA) in *Chlorella pyreniodosa*. After 30 minutes of photosynthesis under steady-state conditions with unlabeled CO_2, ^{32}P-labeled phosphate was added to the algae, and 20 minutes later, $^{14}CO_2$ was added. This was sufficient to achieve isotopic equilibration in the Calvin Cycle intermediates but not long enough to significantly label the pool of stored carbohydrate (starch). At the times indicated, and during the light, dark, and again in the light, samples were taken and cells were killed in ethanol. The radiolabeled metabolites were analyzed by two-dimensional chromatography and radioautography.

a. (4 pt) What causes the abrupt increase in ^{14}C and ^{32}P in 3-PGA when the light is turned off?

b. (4pt) Why is the rise in 3-PGA brief before it drops rapidly?

c. (4pt) Why do the lines corresponding to ^{14}C and ^{32}P in 3-PGA then diverge?

d. (4pt) Which isotope, if either, is proportional to 3-PGA concentration? Explain your reasoning.

e. (4pt) On the figure above, draw a line representing the ^{32}P label in ribulose 1,5 *bis* phosphate (RuDP). (The pattern is what is important, not the actual numbers. Use the space below the 3PGA ^{14}C line.)

2. (5 Points) Cats are obligate carnivores that require large amounts of protein in their diet. An article [*Science* 199:431-2 (1978)] reported that cats fed a single meal containing all the common amino acids found in proteins except arginine can die within several hours. Explain why the omission of arginine, but not other amino acids, could cause this effect.

3. (10 points) If you were to eat a gram of ^{13}C-enriched glycine, which of the following compounds would be become labeled significantly because glycine is a direct precursor? Draw a circle around those that would become labeled.

Adenine Creatine Carnitine Serine Heme Cytosine

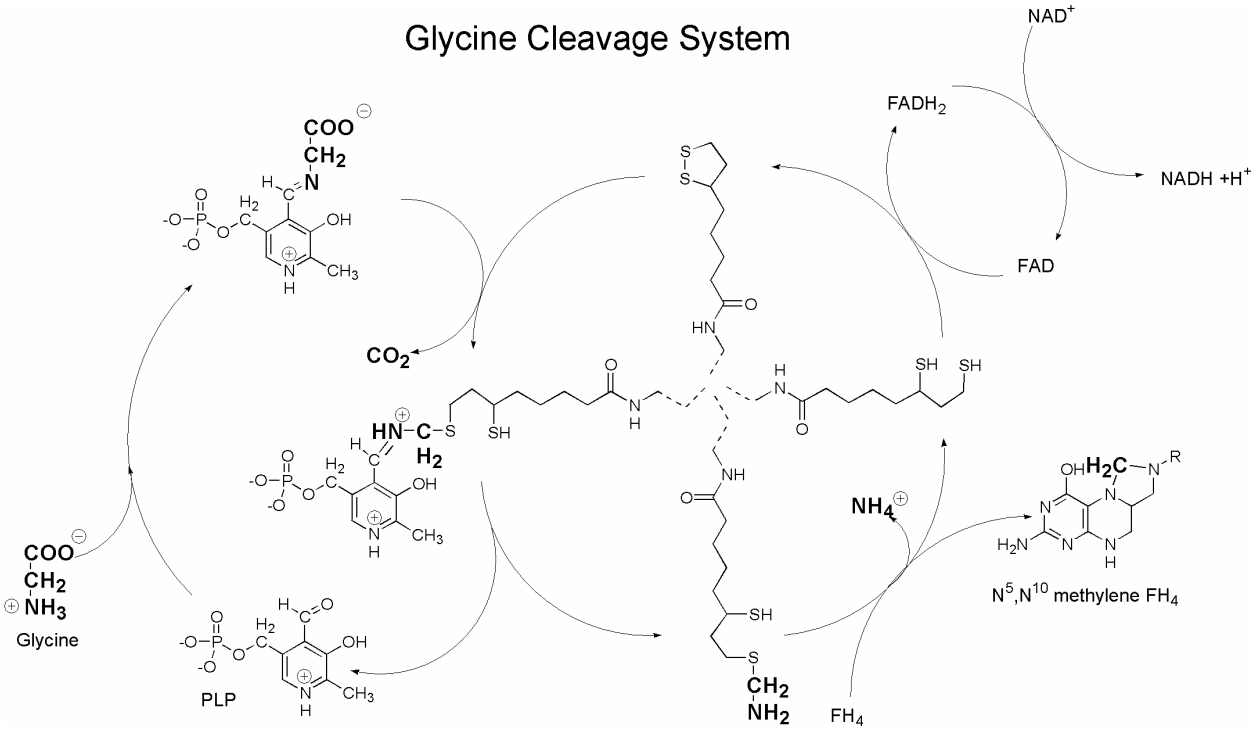
4. Over 90% of the threonine catabolized by our liver is converted to glycine and acetyl CoA in two steps. The first enzyme, threonine dehydrogenase, oxidizes threonine to 2-amino-3-ketobutyrate (AKB). In the second step, AKB reacts with Coenzyme A in a pyridoxal phosphate-dependent reaction.

a. (10 points) Create a figure appropriate for a biochemistry textbook that shows the two-step pathway and the chemical structures of the molecules involved. (Don't forget to include coenzymes as necessary.)

b. (5 points) AKB is chemically unstable and decomposes with a $t_{1/2}$ of about 10 minutes at pH 7.0 [*Biochem. Biophys. Res. Commun.* 190:1066-1072 (1993)]. Predict the products of this decomposition based on reasonable chemistry.

c. (10 points Bonus) AKB-CoA ligase catalyzes the PLP-dependent formation of glycine and acetyl CoA from AKB [*J. Biol. Chem*: 269, 4057-4064 (1994)]. Propose a reasonable mechanism for this reaction. Show how electrons move for each step of your mechanism.

5. Glycine is catabolized by the glycine cleavage system, a multienzyme complex with similarities to the pyruvate dehydrogenase complex. The overall reaction, shown below, includes five cofactors.

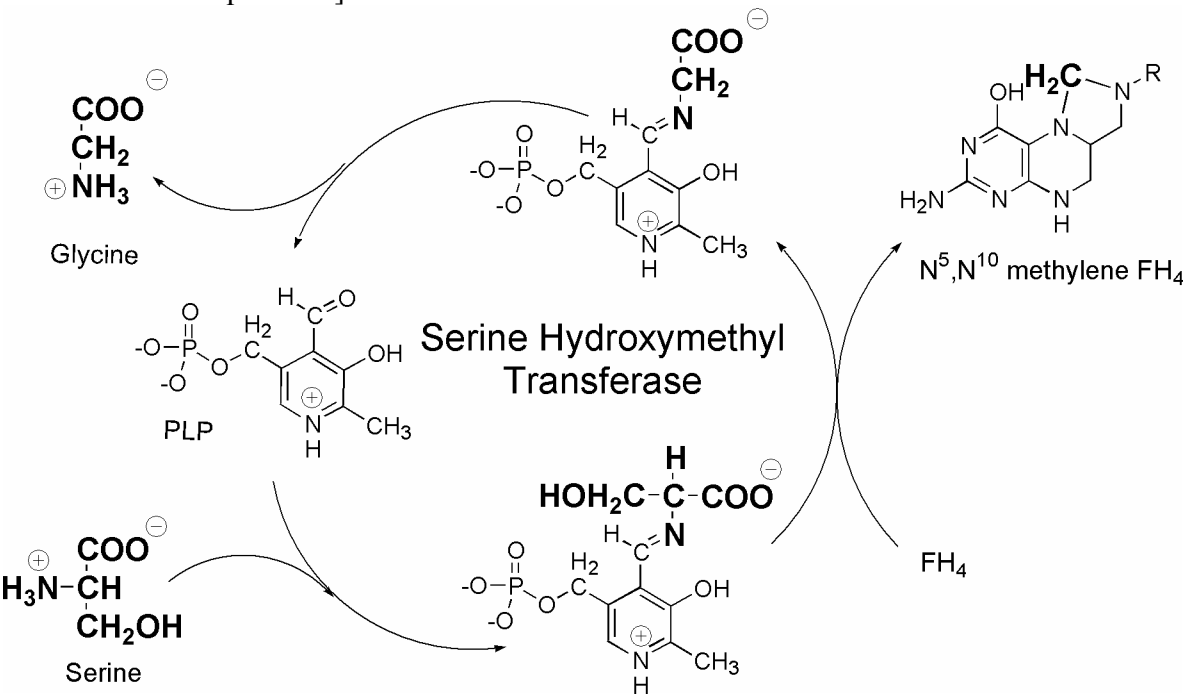


a. (5 points) The first step of the reaction pathway involves a PLP-dependent decarboxylation catalyzed by the P-protein which transfers an aminomethyl group to lipoamide of the H-protein. The amino acid sequence of the P-protein is known from *E. coli*, pea, chicken, and human. Near residue 700 of the P-protein, the following, highly conserved, 25-residue sequence occurs in all four species [*Eur. J. Biochem*. 216:539-548 (1993)].

H L N L H K T F C I P H G G G G P G M G P I G V K

Within this region pyridoxal phosphate binds covalently. To which amino acid would you expect PLP to be bound? Draw the structure of the adduct with that amino acid?

6. (10 points) Glycine is also a precursor of serine in mammalian cells by reversal of the serine hydroxymethyl transferase reaction shown below. When [2-^{14}C]-glycine is incubated with a liver extract, the synthesized serine has ***two*** labeled carbons. Explain clearly how this happens. [If you think you have no clue, for partial credit (≤ 5 points), make a list of things –learning issues- that you don't know but think would help you answer this question.]



7. Consider the preceding questions and examine the exchange of ^{15}N among various amino acids displayed below from a portion of Table 3 from the case study problem, "Plants vs Animals in the Dining Hall." The following questions relate to the numbers in the shaded cells.

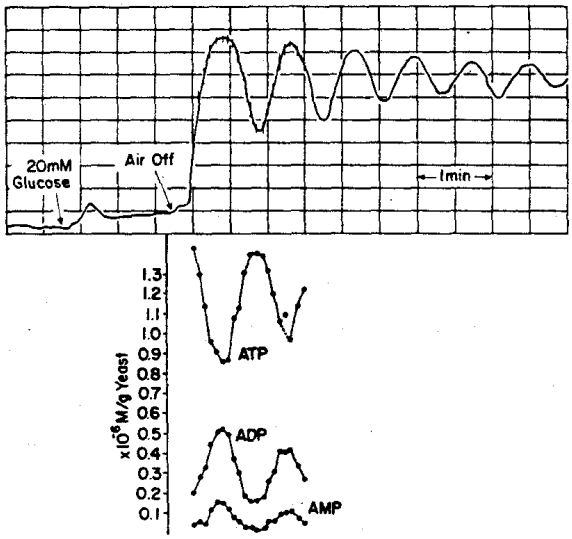
Table 3. Distribution of ^{15}N among the amino acids of liver proteins 8 hours after intravenous injection of various amino acid sources of. Values are normalized to the ^{15}N content of the source amino acid (100) incorporated into protein [*Acta Chem. Scand*. 5:1046-1064 (1951)].

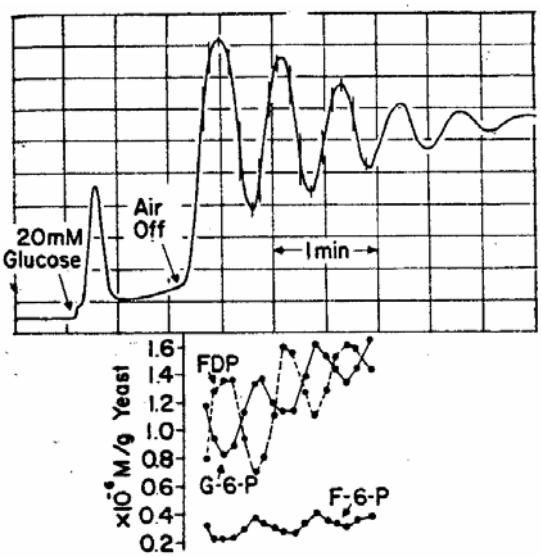
	Amino acids incorporated into rat liver proteins														
^{15}N-enriched Amino Acid	**Glu**	**Asp**	**Ala**	**Pro**	**Thr**	**Ser**	**Gly**	**Leu**	**Ile**	**Val**	**Phe**	**Tyr**	**Arg**	**Lys**	**His**
Threonine	6	5	5	2	100	20	14	1	2	4	2	5	5	1	<1
Serine	9	9	12	2	14	100	50	3	2	2	2	6	9	1	1
Glycine	19	12	16	1	0	88	100	nd	nd	nd	3	nd	16	<1	2

a. (5 points) Is the similarity between the ^{15}N-content values for Glu (or Asp and Ala, for that matter) and Arg significant? Explain.

b. (10 points) Are the three pairs of reciprocal relationships among Thr, Ser, and Gly reasonable? (e.g. Thr→Gly vs Gly→Thr, Thr→Ser vs Ser→Thr, Gly→Ser vs Ser→Gly) Explain.

8. While our arms and legs can survive oxygen deprivation for an hour or more, our brains are absolutely dependent on oxygen. Within less than 10 minutes, irreversible damage occurs. Unlike us, yeast thrives with or without oxygen. However, some metabolic switching must occur in the transition from one to the other. The figures below shows the changes in concentration of NADH (fluorescence trace), adenine nucleotides, F-6-P, G-6-P, and FDP for an aerobic suspension of yeast after suddenly being deprived of oxygen [Betz and Chance, *Arch Biochem Biophys*. 109:585-94 (1965)].





a. (10 pt) In the space below, draw a diagram (semi-quantitative representation) of the change in **Energy Charge** as a function of time. (label axes)

b. (10 pt) In the space below, draw a plot (qualitative representation) of the **carbon flux** in the yeast glycolytic pathway as a function of time in this experiment.

c. (5pt) How are parts a and b mechanistically connected?

III. Essay Questions

Writing reflects how you think. Among the "right answers" I will read for the following questions, some will be better than others because they show greater depth of understanding, avoid extraneous or inaccurate information, 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.

1. (10 Points) Fix your attention on a **particular carbon atom in a glucose molecule** that you consumed at your last meal. In a narrative (no structures), **generate a reasonable story** of the fate of that carbon atom that involves muscle, liver, brain, and adipose tissue, and several different metabolic pathways before exiting as carbon dioxide.

2. (10 Points) A recent article entitled, "UK policy on folate fortification of foods" [*J. Biol. Educ.* 38(3):106-107 (2004)], leads with, "The UK Food Standards Agency has decided not to recommend fortification of foods with folate." This recommendation, which differs from the practice in the US, was not unanimous; however, it was based on the input of experts.

   Write a short essay on the pros and cons of folate supplementation and **speculate** on the basis for the UK decision.

Bonus Questions

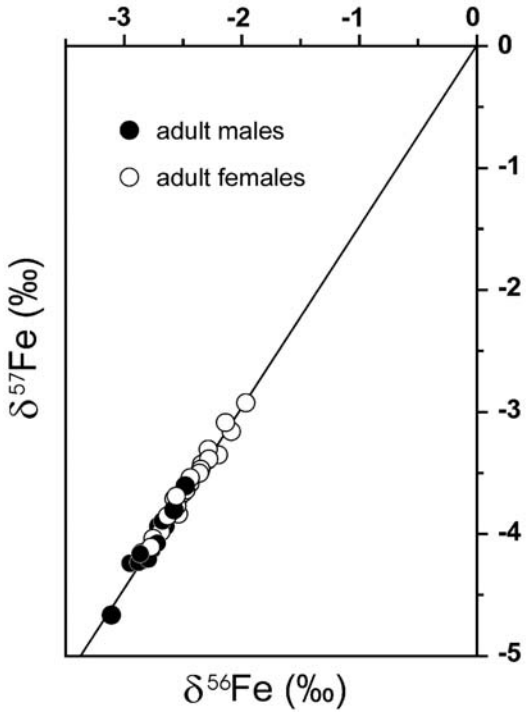
Much of intermediary metabolism focuses on the flow of carbon and nitrogen. However, one can discuss the metabolism of every other element that is important to life such as Sulfur and Phosphorus, which are bound covalently, to Sodium and Calcium, which are ions, to transition metals such as Iron.

1. (10 points) Consider a Fe atom/ion in your most recent meal that is destined to become incorporated into the heme group of a hemoglobin molecule in a red blood cell. Recognizing that organisms mediate metabolism with proteins that provide specificity and channel the flux in particular ways using membrane transporters, carrier molecules, effector molecules, and enzymes that are genetically encoded, propose a pathway that takes the iron atom/ion from your intestinal tract to the center of a heme group in hemoglobin. What are the necessary steps for this process that accounts for about 70% of the Fe in your body? **Draw a diagram** that includes the necessary protein-dependent steps. You may annotate your diagram to explain things that may be unclear. (This is a hypothesis/prediction of your part and will be evaluated on whether it is reasonable.)

2. (10 points) The atomic mass of iron is the weighted average of the masses of its isotopes of which the four common non-radioactive ones are listed below from the 77th Edition of the CRC Handbook of Chemistry and Physics.

Isotope	% Natural Abundance	Mass
^{54}Fe	5.845	53.939615
^{56}Fe	91.754	55.934942
^{57}Fe	2.119	56.935398
^{58}Fe	0.282	57.933280

Mass spectrometry can measure the relative abundance of each isotope in various samples with great accuracy and precision. Thus, differences in isotope abundance among samples of as little as 0.01% can be detected. The figure below was published about two and a half years ago [*Science* 295: 2065-2066 (2002)]. In a single graph it depicts the relative abundance of the three most common iron isotopes in blood samples taken from 15 men and 29 women. For example, a δ^{57}Fe (‰) value of -3 on the y-axis means that there is a 3 part per thousand (0.3%) deficit of ^{57}Fe in an iron sample relative to ^{54}Fe. A reference standard with the natural abundances listed above would be a point at the origin in the upper right hand corner.



$$\delta^{56}\text{Fe (‰)} = \{ [(^{56}\text{Fe}/^{54}\text{Fe})_{\text{sample}}/(^{56}\text{Fe}/^{54}\text{Fe})_{\text{std}}] - 1 \} \times 1000$$

$$\delta^{57}\text{Fe (‰)} = \{ [(^{57}\text{Fe}/^{54}\text{Fe})_{\text{sample}}/(^{57}\text{Fe}/^{54}\text{Fe})_{\text{std}}] - 1 \} \times 1000$$

a. (3 pt) Place an "X" on this graph to show where Fe in the diet would fall.

b. (7 pt) This is the largest isotope fractionation for iron observed in nature. Given what you know about the factors that lead to isotope fractionation, where on your proposed pathway for iron assimilation in your body would the fractionation likely occur? Explain your reasoning.