CHEM-643 Biochemistry Name ______________________________

Final Examination

3:30– 6:30 PM, Wednesday, 10 December 2014

Dr. H. White – Instructor

- There are 12 pages to this examination. Additional metabolic pathway sheets will be available.
- **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 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. Remember, ***Problem-solving is what you do when you don't know the answer. If you know the answer, it is not a problem.***
- 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 including bonus points is 167 points. Graded Exams can be picked up starting Friday afternoon and will be held until Spring Semester.

Have a Safe and Happy Holiday!

Exam Statistics:

Total points possible 167

Class Range **___10-141_____**

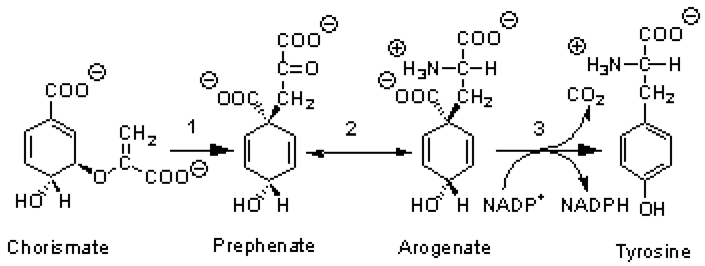
Class Mean **__96.3±28.1____**

Your Score _______

Your Rank in Class _____ out of 25

Course Grade _____

1. (18 Points) The final steps in tyrosine biosynthesis in plants and some microorganisms [*Z. Naturforsch* 41c, 69-78 (1986); *J. Bacteriol.* 144, 247-257 (1980)] is shown below. The questions that follow refer to this sequence of reactions.



a) (4 Points) Reaction 1 is a rare example in biochemistry of the Claisen rearrangement, a well-known reaction in organic chemistry. Reaction 3 is an oxidative decarboxylation. **Choose <u>one</u> of these two reactions** and show by pushing electrons how the reaction proceeds.

b) (2 Points) What coenzyme is required for Reaction 2? (Give its name rather than an abbreviation)

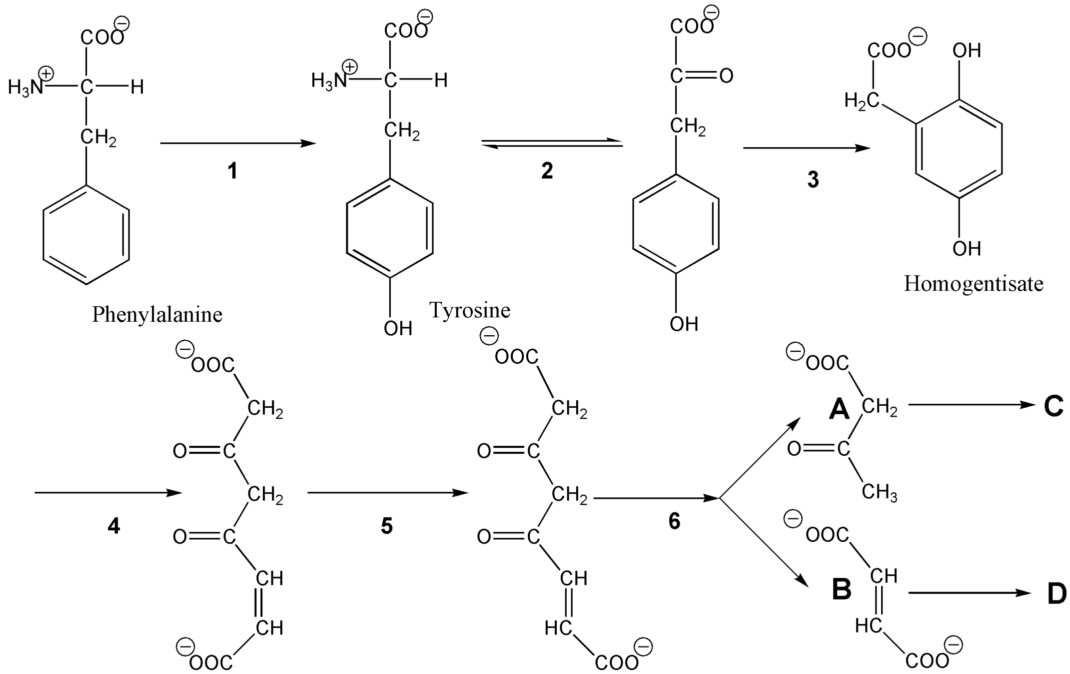
c) (4 Points) The coenzyme for Reaction 2 is normally associated with a particular amino acid in the enzyme's active site. How could one covalently link that amino acid residue irreversibly with the coenzyme? (Hint: Problem Set 2) Explain.

d) (3 Points) What amino acid cosubstrate would you expect to be the amino group donor in Reaction 2? Explain your reasoning?

e) (5 Points) NADPH is generated in the final step of this anabolic pathway. Does this make sense? Explain your answer.

Name ____________________________

2. The catabolic pathway for Phenylalanine and Tyrosine is shown below.

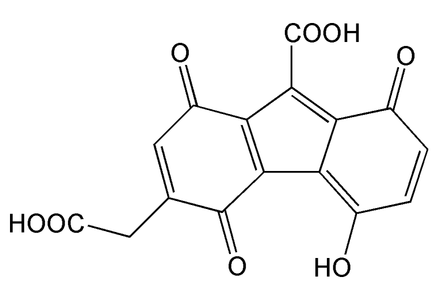


A. (8 Points) What are the names of compounds A and B? What would be the next compounds, C & D, in the metabolism of each?

A ______________________________ → C ______________________________

B ______________________________ → D ______________________________

3. (4 Points) A report in *Nature* [**429,** 363 (2004)] described the isolation and characterization of a bright red compound in hippopotamus sweat that serves as a sun screen for this virtually hairless tropical animal. The structure of hipposudoric acid is shown below. What compound would be a likely precursor to this hipposudoric acid and account for all of the carbon atoms in it? Show why you selected the compound you did.



4. (14 Points) Knoop in 1905 studied the oxidation of a variety of ω-phenyl acids in rabbits. He fed 2g of each and observed a limited variety in the metabolic products excreted in the urine. His results were as follows.

	Compound Fed	**Compound Excreted**
1	C_6H_5-COOH	C_6H_5-COOH
2	C_6H_5-CH_2-COOH	C_6H_5-CH_2-COOH
3	C_6H_5-CH(OH)-COOH	C_6H_5-CH(OH)-COOH
4	C_6H_5-CH_2-CH_2-COOH	C_6H_5-COOH
5	C_6H_5-CH(OH)-CH_2-COOH	C_6H_5-COOH
6	C_6H_5-CO-CH_2-COOH	C_6H_5-COOH
7	C_6H_5-CH=CH-CH_2-COOH	C_6H_5-CH_2-COOH
8	C_6H_5-CH_2-CH(NH_2)-COOH	None observed
9	C_6H_5-CH_2-CO-COOH	None observed
10	C_6H_5-CH_2-CH_2-CH_2-COOH	C_6H_5-CH_2-COOH
11	C_6H_5-CH_2-CH_2-CH_2-CH_2-COOH	C_6H_5-COOH

A. (4 Points) Ignoring compounds 8 & 9, explain these results in a coherent way?

B. (4 Points) Actually the metabolic products observed by Knoop were glycine amide derivatives of the acids shown above: hippuric acid (C_6H_5-CO-NH-CH_2-COOH) and phenylaceturic acid (C_6H_5-CH_2-CO-NH-CH_2-COOH). In the space below predict the enzyme reaction in which they are formed. (Hint: It is a way that a cell recycles CoA.)

C. (2 Points) What is the common name for compound 8?

D. (2 Points) How are compounds 8 and 9 interconverted metabolically?

E. (2 Points) Why didn't Knoop observe metabolic products from compounds 8 and 9?

5. (8 Points) When tadpoles undergo metamorphosis to become frogs the obvious

morphological changes are also accompanied by significant metabolic changes as shown in the figure and data below. These data come from an article by Philip P. Cohen entitled, "Biochemical differentiation during amphibian metamorphosis" *Science* **168**, 533 (1970). Cohen measured the activity of a number of enzymes in the liver of adult frogs and pre-metamorphic tadpoles and expressed them as a ratio.

Liver Enzyme Activity	Frog/Tadpole Activity Ratio
Carbamyl phosphate synthetase	30
Ornithine transcarbamylase	8
Argininosuccinate synthase	35
Argininosuccinase	20
Arginase	30
Glutamate dehydrogenase	10
Lactate dehydrogenase	0.4
Glucose 6-P dehydrogenase	0.5
Malate dehydrogenase	1.4
Glutamate-oxaloacetate transaminase	5

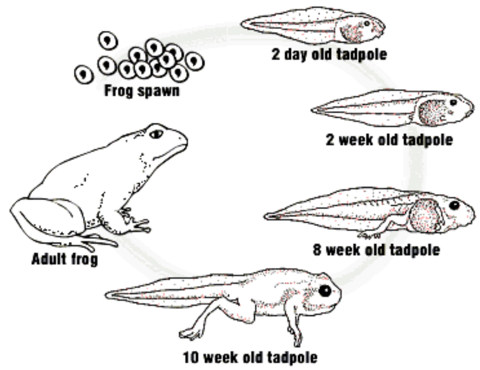


Image http://dj003.k12.sd.us/SCHOOL%20NOTES/chapter_12.htm

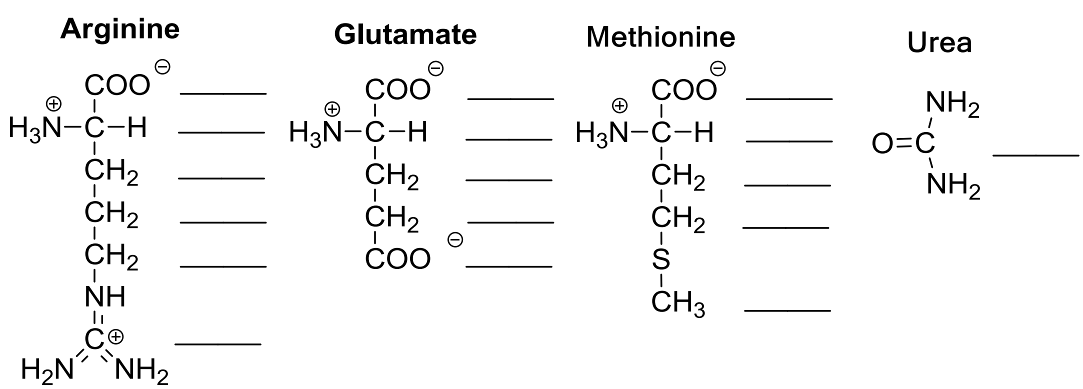
a. (4 Points) Based on the enzyme activity ratios reported, what conclusions can you make about the metabolic changes that occur during amphibian metamorphosis?

b. (4 Points) How do these metabolic changes make sense in terms of the metamorphosis of a frog?

6. (15 Points + 4 Bonus Points) Sucrose is a readily metabolized carbon and energy source for most animals. The carbons from sucrose get redistributed through many metabolic pathways including those involving amino acids. Proteins extracted from livers of mice fed uniformly labeled ^{14}C-sucrose (Steele, 1952) were hydrolyzed, the amino acids separated, and their ^{14}C content determined and reported as nCi^{14}C/mgC. The table of results is given below:

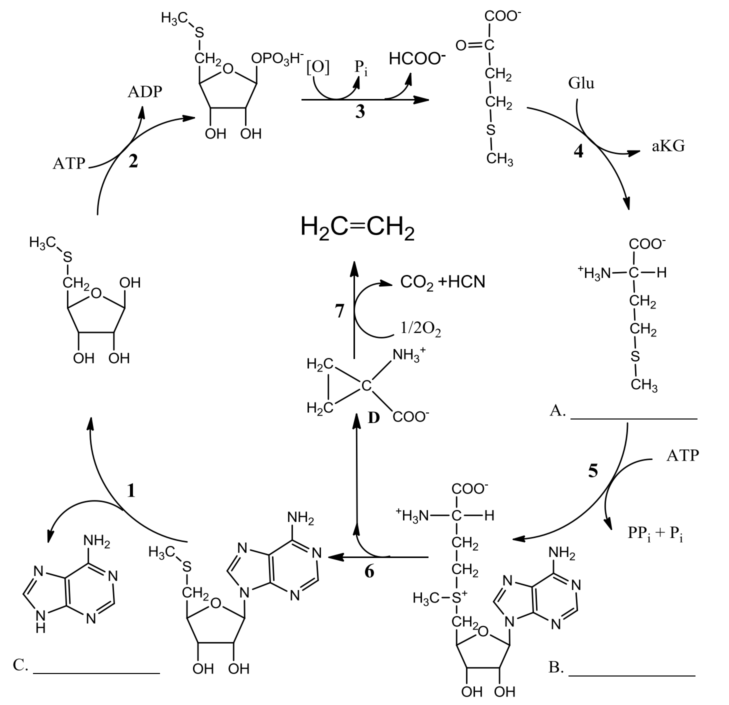
Amino Acid	nCi/mgC	Amino Acid	nCi/mgC	Amino Acid	nCi/mgC	Amino Acid	nCi/mgC
Glutamate	19.0±1.9	Threonine	0.09±0.02	Valine	0.02±0.01	Lysine	0.0±0.02
Aspartate	15.8±0.9	Serine	8.4±0.1	Phenylalanine	0.02±0.07	Histidine	0.07±0.08
Alanine	26.5±3.3	Glycine	5.1±0.2	Tyrosine	0.0±0.07	Cystine	3.3±0.3
Proline	3.1±0.1	Isoleucine	0.06±0.05	Arginine	3.0±0.2	Methionine	1.03±0.06

a. (15 Points + 4 Bonus Points) **The values given may or may not reflect the** nCi^{14}C/mgC for each carbon in the amino acid. The structures of three amino acids and urea are given below: Make a reasonable estimate of the nCi^{14}C/mgC **for each carbon** in each molecule. Urea prediction is a four point bonus.



b. (3 Points) Why would asparagine and glutamine not be included in the table above?

7. (22 Points Total) Plants produce ethylene as a gaseous hormone that induces ripening in fruit. The metabolic pathway for ethylene synthesis is shown below and the questions that follow refer to this figure.



a. (6 Points) Identify by name compounds A, B, and C. Write names on the figure.

b. (3 points) With carbons leaving the cycle as compounds such as C_2H_4, CO_2, HCN, and $HCOO^-$, the cycle's carbon must be replenished. Draw a circle around the source of all carbon coming into the cycle.

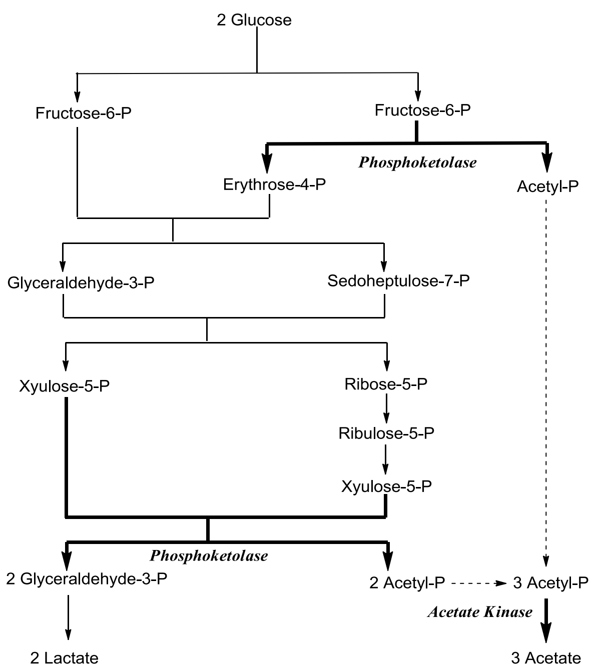
c. (4 Points) For reactions 2 and 4, write the generic name for enzymes catalyzing that type of reaction.

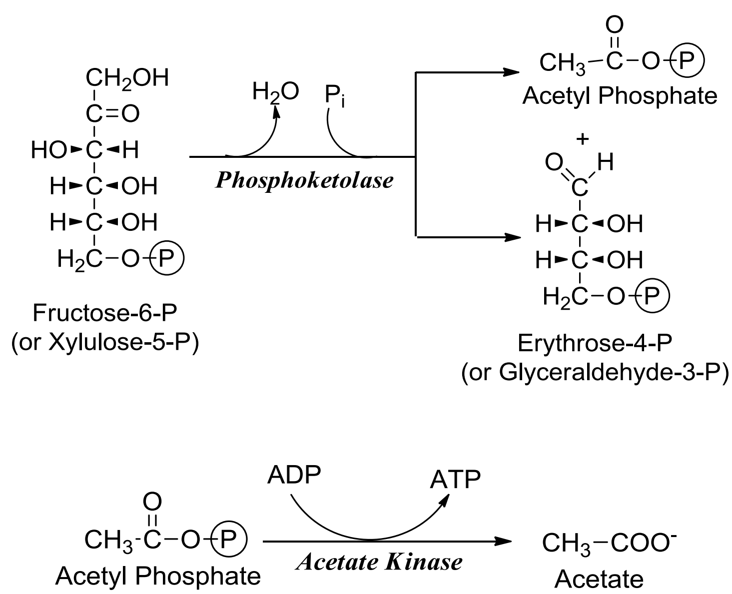
2. ____________________________, 4. ______________________________

d. (5 Points) The two adjacent methylene groups in compound B are converted in two steps to ethylene via D, a cyclopropanyl amino acid intermediate. Would an enzyme be able to distinguish between the methylene groups in compound D? Explain your answer.

e. (4 Points) Provide two good reasons why Reaction 7 would be thermodynamically favorable.

8. (24 Points +10 Bonus Points) 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.]

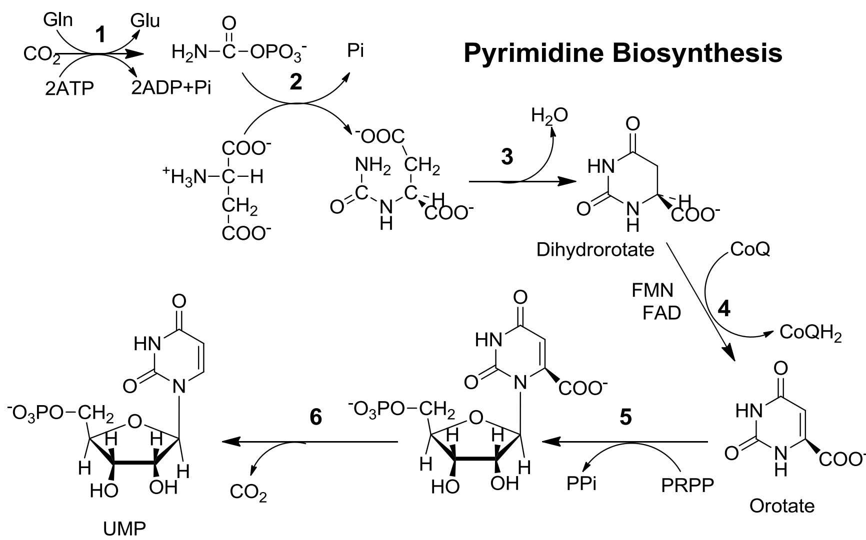
b. (4 Points) Draw or name another metabolic intermediate that would have a similar $\Delta G^{0'}$ for hydrolysis as acetyl phosphate.

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 **1-^{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. (10 Points) It was 3AM and a group of CHEM-643 students were discussing a homework problem dealing with the catabolic pathway for pyrimidine biosynthesis. Consider a mutant that had 10-fold excess of Enzyme 6 compared to wild type with all other enzymes at wild type levels. What would be the consequences?



Betsy: *I think the concentration of the substrate and product of the enzyme would also increase 10 fold.*

George: *That's not so, the concentration of reaction product would increase, but the substrate would stay about the same.*

Glenn: *If you ask me, you're both wrong. The concentrations of the substrate and product would change little, but the flux in the pathway would be increased several fold.*

Please help set the students straight on what to expect and the reasons in this example? Feel free to use an illustration.

Part IV - Essays (15 Points each)

10. (15 Points) The assignment of amino acids as either *essential* or *non-essential* was determined nutritionally based on whether or not animals continued to grow normally when fed different artificial diets each lacking in one of the 20 amino acids commonly found in proteins. While those terms are still used, our understanding of metabolism reveals a more complex categorization. In what ways does a metabolic perspective alter the meaning of essential and non-essential amino acids? A compete answer requires specific examples presented in a coherent way.

11. (15 Points) Your group project was to test the hypothesis that enzymes in the biosynthesis of a particular amino acid would experience selection against incorporation of that amino acid in preference to other amino acids in non-essential positions. Below are two figures taken from a group's paper in 2012 about the selection against proline in the enzymes of proline biosynthesis in *Methylobacterium marinus.*

Assume these data are included in a manuscript submitted for publication and I, as Editor in Chief of the journal, have selected you as a reviewer. Please evaluate figures below based on your expertise. State the conclusions warranted for each figure and whether or not they support the hypothesis. Elaborate any questions you have about the figures. Are there other comparisons or things you would want the authors to do before you would accept their manuscript for publication. I am looking for a through and reasoned response.

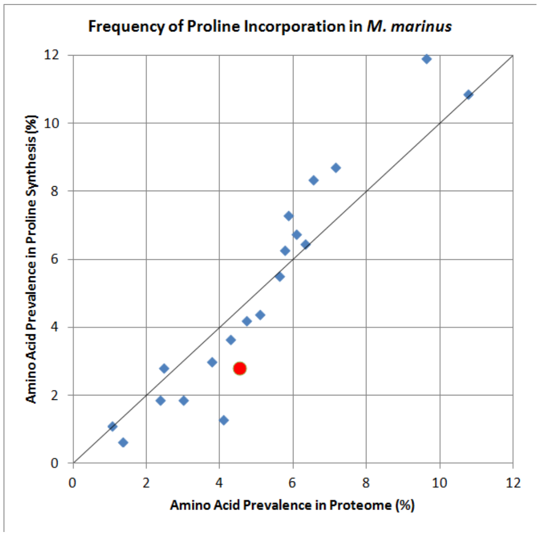


Figure 2. Prevalence of proline in the enzymes of proline synthesis as compared to the *M. marinus* proteome. Points below the line indicate lower abundance in proline synthesis than predicted by the proteome; points above indicate the opposite. Proline is shown as a large dot.

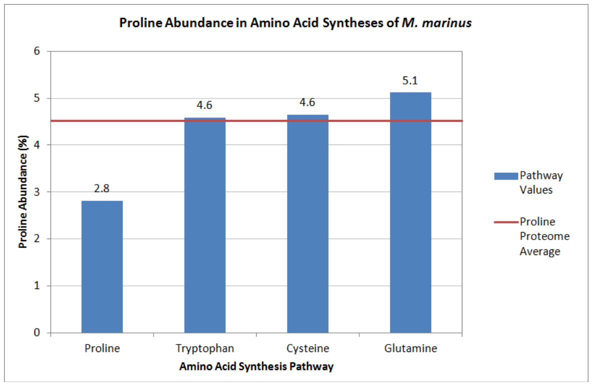
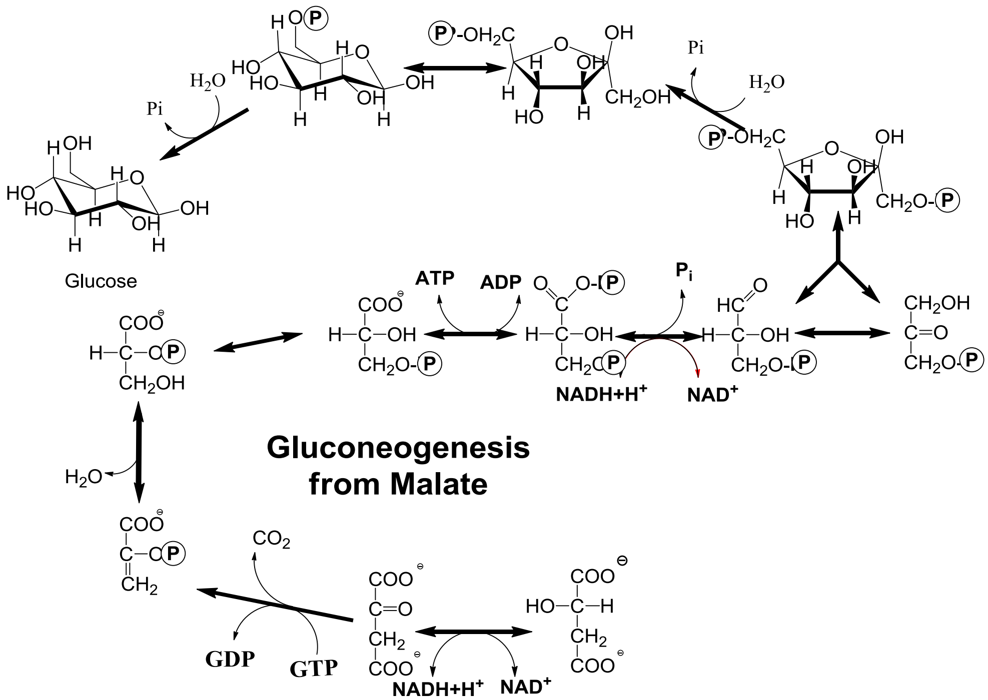
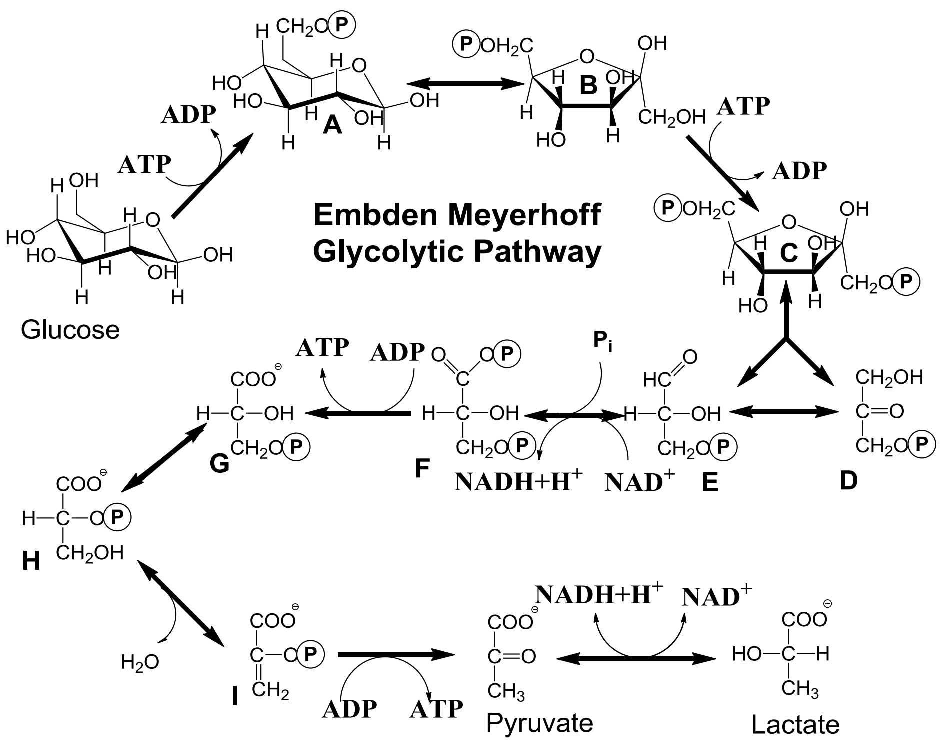
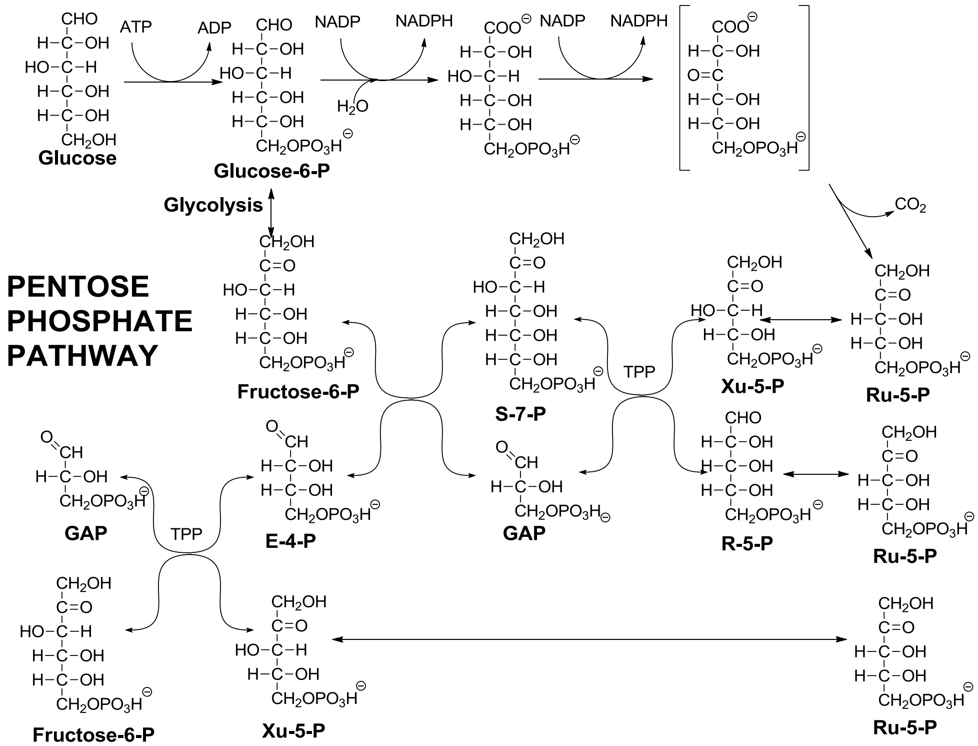
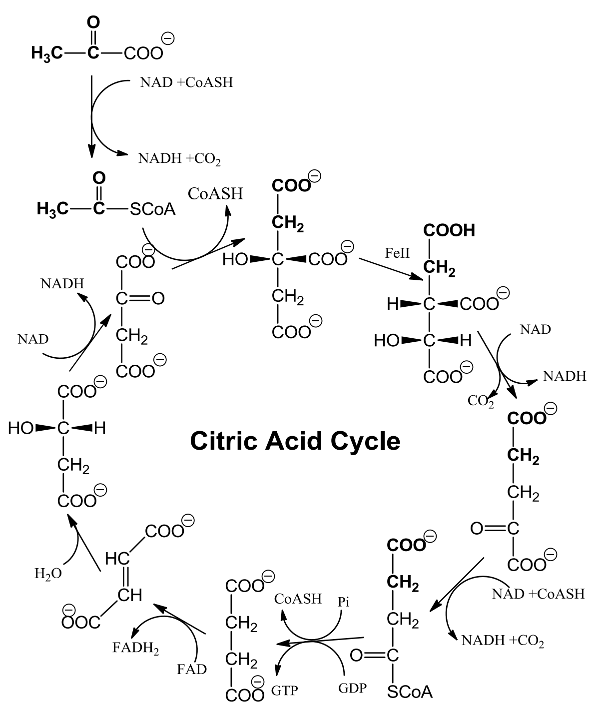


Figure 3. Comparison of proline composition of the enzymes of various amino acid synthesis pathways in *M. marinus*. The average abundance of proline in the *M. marinus* proteome is given as a horizontal line at 4.5%

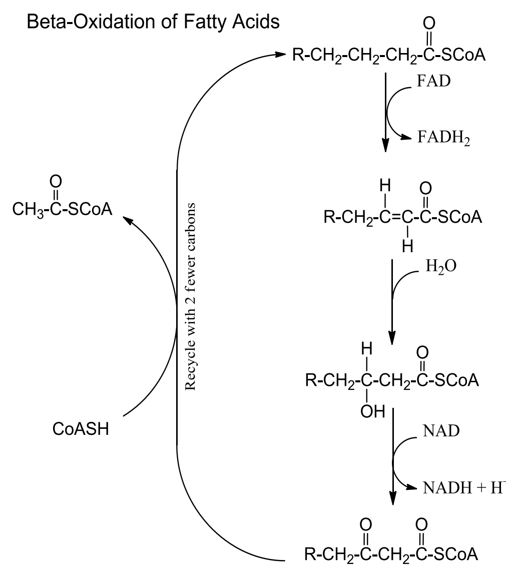

Gluconeogenesis
from Malate
Glucose
H₂O
Pi
ATP
ADP
NADH+H⁺
NAD⁺
CO₂
GDP
GTP


Embden Meyerhoff
Glycolytic Pathway
Glucose
A
B
C
D
E
F
G
H
I
ATP
ADP
NADH+H⁺
NAD⁺
H₂O
Pyruvate
Lactate





Beta-Oxidation of Fatty Acids



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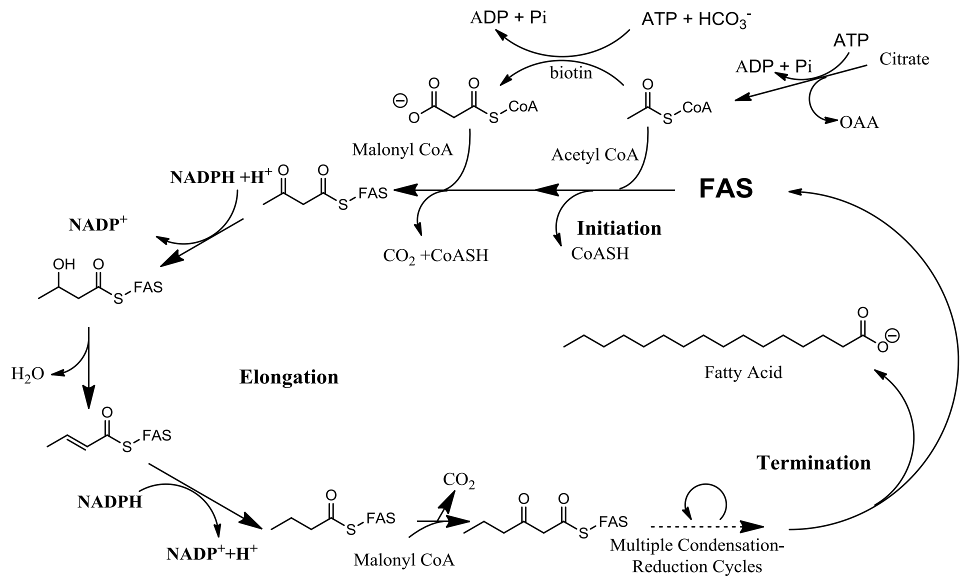
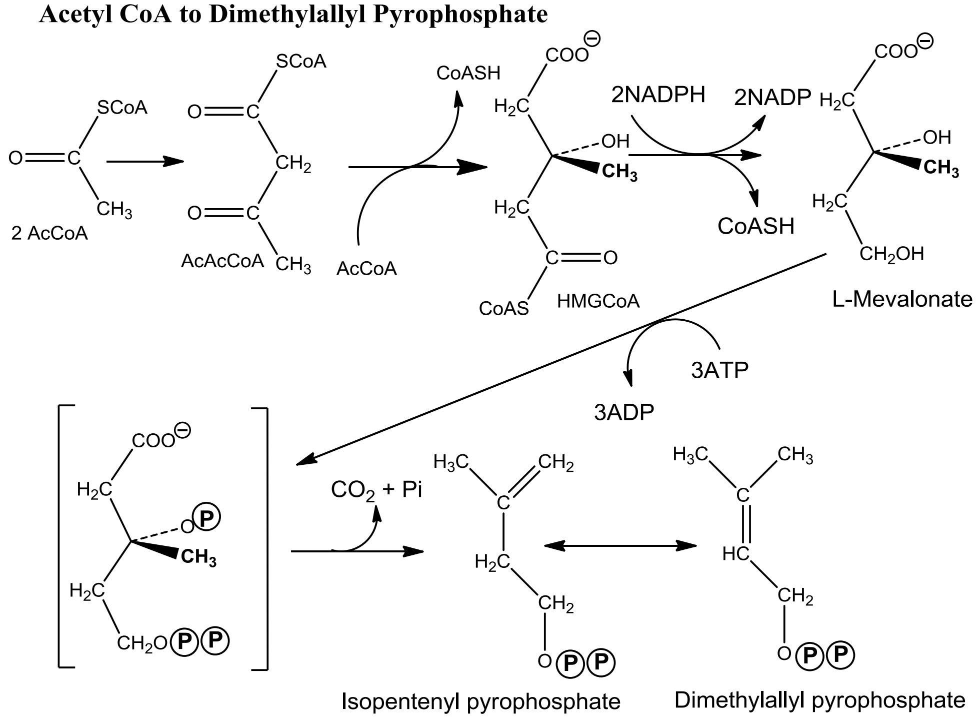
Fatty Acid Synthesis



Acetyl CoA to Dimethylallyl Pyrophosphate



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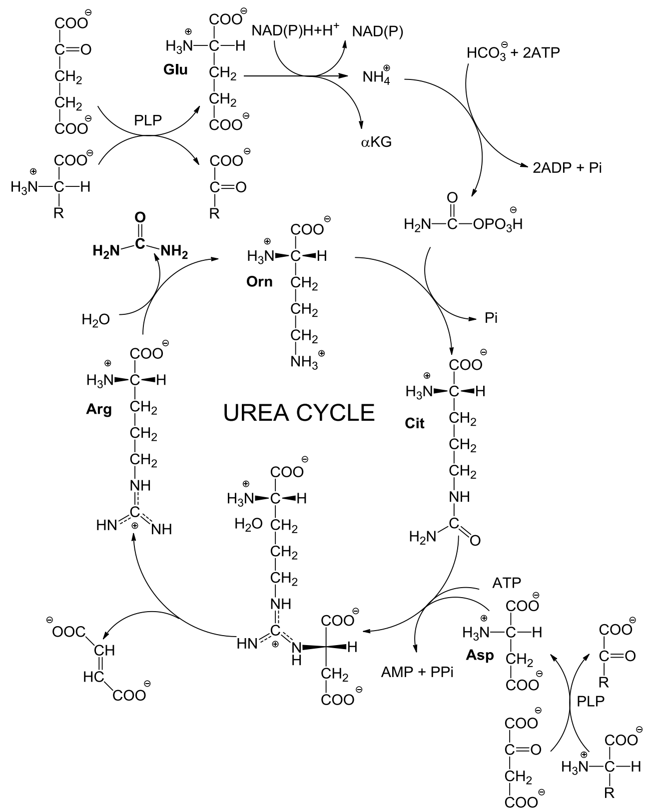
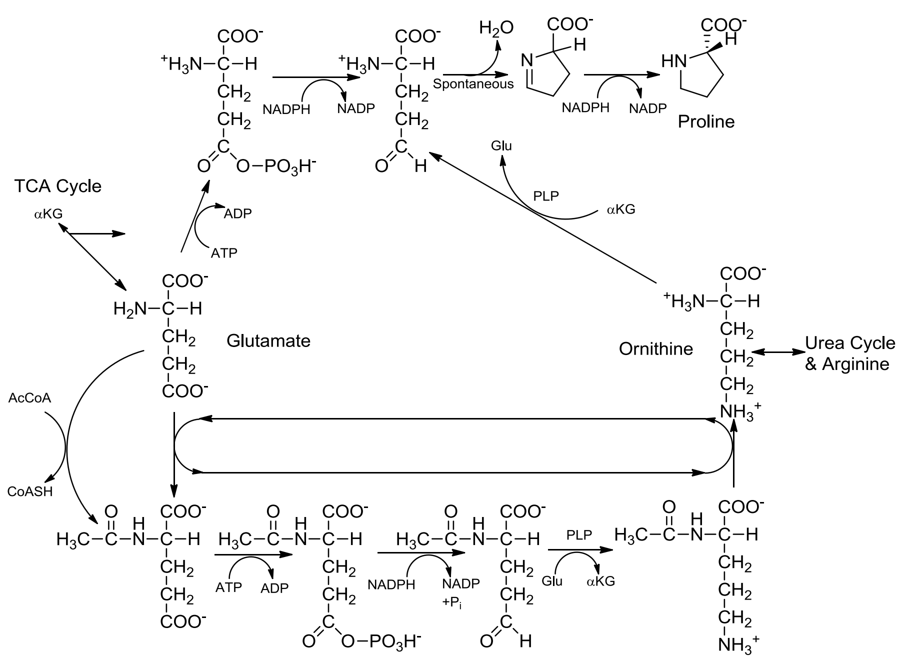

UREA CYCLE
Glu
PLP
NAD(P)H+H+
NAD(P)
NH4+
αKG
HCO3- + 2ATP
2ADP + Pi
Pi
Orn
Cit
Arg
Asp
ATP
AMP + PPi
H2O
PLP


TCA Cycle
αKG
ATP
ADP
NADPH
NADP
Spontaneous
H2O
Proline
Glu
PLP
αKG
Glutamate
Ornithine
Urea Cycle
& Arginine
AcCoA
CoASH
ATP
ADP
NADPH
NADP
+Pi
PLP
Glu
αKG