

CHEM-643 Biochemistry
Final Examination
7-10 PM, Friday, 11 December 2009
Dr. H. White – Instructor

Name _____

There are 14 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 _____

Total points possible 140

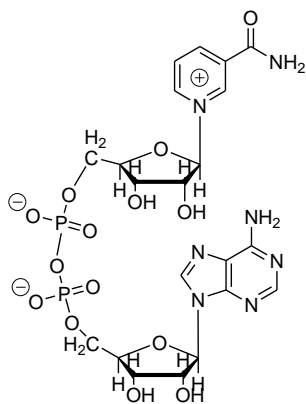
Class Mean _____

Your Score _____

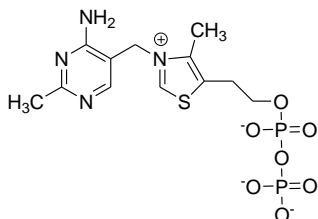
Your Rank in Class _____ out of 24

Course Grade _____

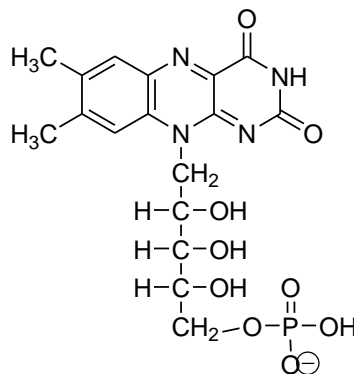
1. Structures (2 points each) **Identify** each of the cofactors shown below and **draw a circle** around the atom or atoms involved in the primary function of each.



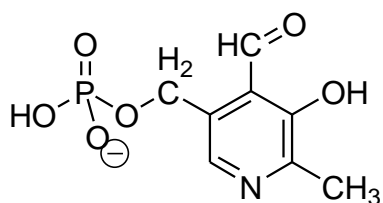
A. _____



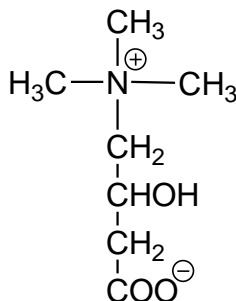
B. _____



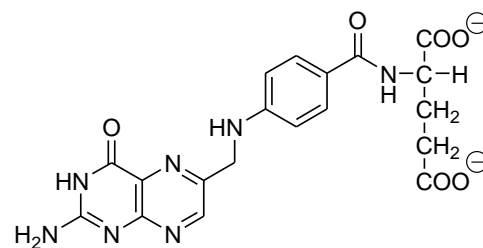
C. _____



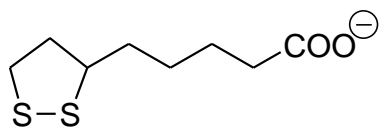
D. _____



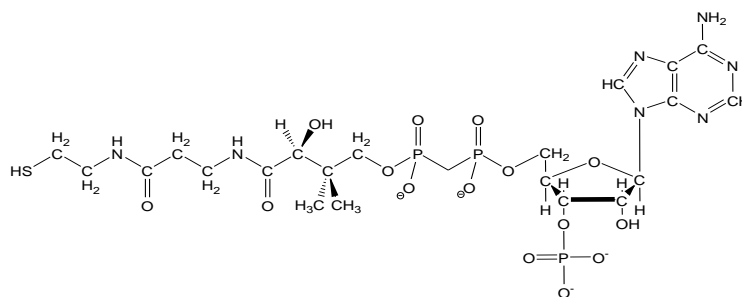
E. _____



F. _____

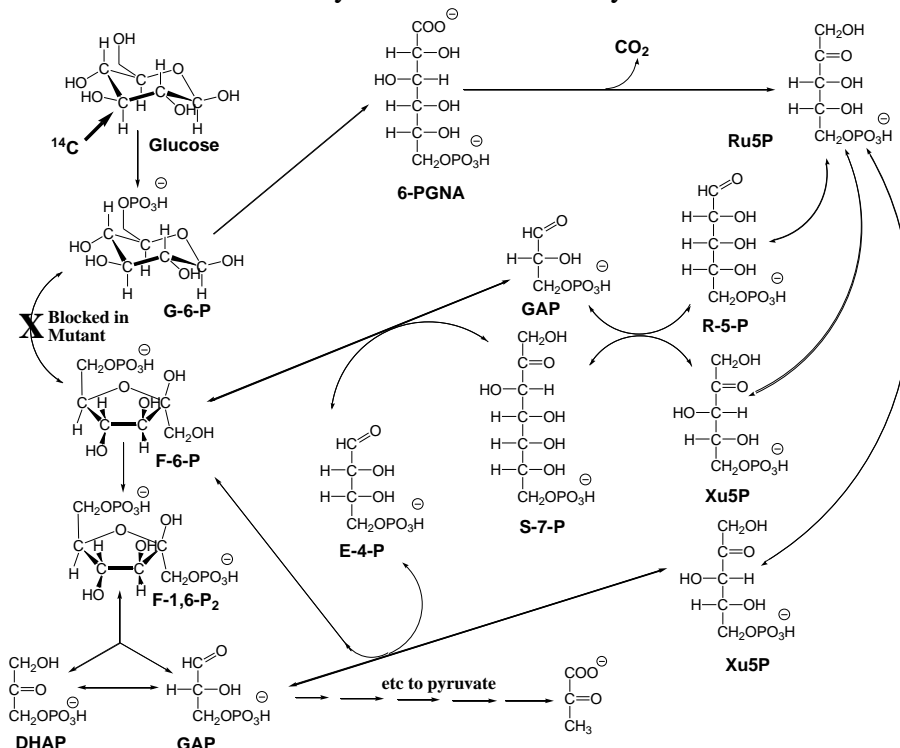


G. _____



H. _____

2. (13 Points) Consider the blue and gold bacteria, *Youdea hullihenii*, that normally oxidizes glucose by the Embden-Meyerhof Glycolytic pathway, but, due to a mutation in the gene for phosphoglucose isomerase (the aldo-keto isomerase that interconverts G6P and F6P), must use the pentose phosphate pathway as a detour around the metabolic block as shown below. The cofactors for the individual enzymes are omitted but you will need to know them.



___ ATP
 ___ NAD(P)H

A. (4 points) In this modified pathway for glucose oxidation, five, rather than six, glyceraldehyde-3-Ps (GAP) are produced per three glucoses. Calculate the net production of ATP and NAD(P)H per glucose in going from **glucose to pyruvate** in this pathway. (Use the back of this page as a work sheet if necessary for this and subsequent questions.)

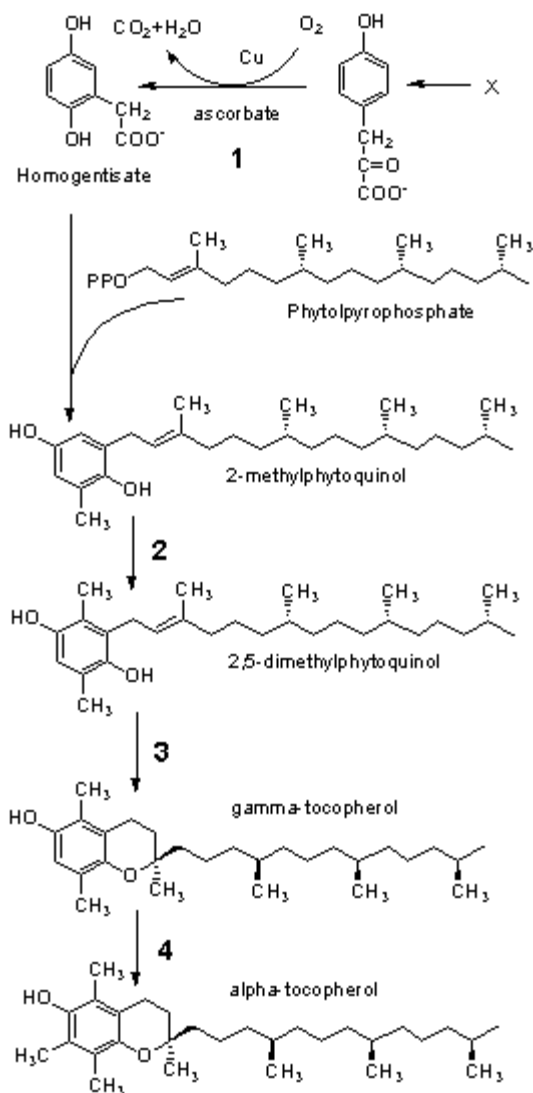
___ % COO^-
 ___ % C=O
 ___ % CH_3

B. (6 points) If carbon 3 of glucose were labeled with ^{14}C , how would the label be distributed in pyruvate? Total should equal 100%.

___ % COO^-
 ___ % C=O
 ___ % CH_3

C. (3 points) How would the ^{14}C label be distributed in pyruvate, if the normal glycolytic pathway were operating alone, i.e. phosphoglucose isomerase is fully functional and the pentose phosphate pathway were not involved.

3. (10 Points) α -Tocopherol, the most active form of Vitamin E, is biosynthesized by photosynthetic organisms. It is a lipid soluble antioxidant that appears to function by quenching the free radicals that form on polyunsaturated fats. There is some evidence that increased consumption of Vitamin E can reduce the incidence of atherosclerosis, however, the prospect of consuming 2.5 kg of spinach or 0.8 kg of soybean oil daily to obtain a therapeutic dose is not appetizing. Dean DellaPenna, a plant biochemist at Michigan State University, noted that many plant oils have large amounts of γ -tocopherol, a direct precursor of α -tocopherol which is only 10% as active. Using genetic engineering, he has introduced a cyanobacterial methyl transferase into *Arabidopsis* and demonstrated that the seed oil from the transgenic plant now contain more than 10 times the normal amounts of α -tocopherol. The metabolic pathway for α -tocopherol biosynthesis is shown in the following figure.



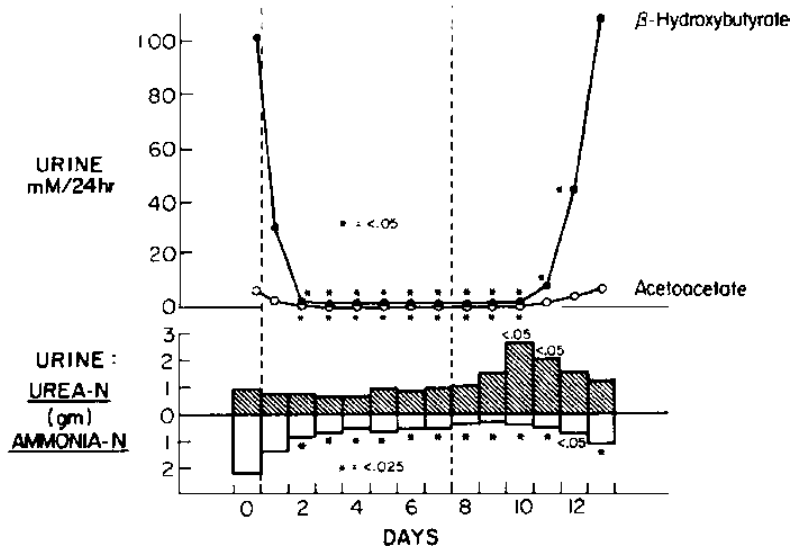
A. (2 point) Of the enzyme reactions shown, only those from "X" to homogentisate occurs in humans. They are part of the catabolic pathway for "X". What is "X"?

B. (2 point) Name a 5-carbon precursor of phytolpyrophosphate.

C. (2 points) What would be the expected methyl donor in reactions 2 and 4?

D. (4 points) Assuming that the same transgenic manipulations can be done in a commercial crop, what biochemical or nutritional concerns would you have before going into production for human consumption?

4. (16 points) In humans, significant changes in intermediary metabolism occur during starvation. The graph below displays data obtained for one individual during an experiment on starvation. Please answer the questions that follow pertaining to this graph.



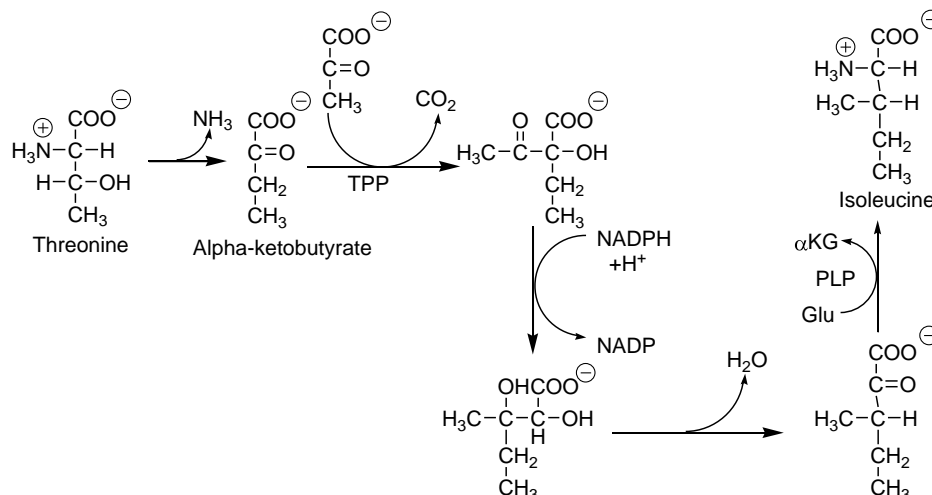
Effect of 150 grams of glucose daily for seven days (between dashed lines) on urinary metabolites following three weeks of starvation. The starvation regime was resumed after the 7th day of glucose.

From Cahill, *Annu. Rev. Nutr.* 26:1-22. (2006).

- A. (4 points) What does the drop in β -hydroxybutyrate indicate on day 1 of glucose feeding?
- B. (4 points) What does the decrease in total nitrogen excretion reflect in the first few days of glucose feeding?
- C. (4 points) Why is there a peak in urea excretion at day 10, three days after glucose feeding stopped?
- D. (4 points) Explain the four day lag before β -hydroxybutyrate increases after glucose feeding has stopped.

5. (12 Points) Threonine, an amino acid with two chiral centers, is the precursor of isoleucine, the only other common amino acid with two chiral centers, as is shown below.

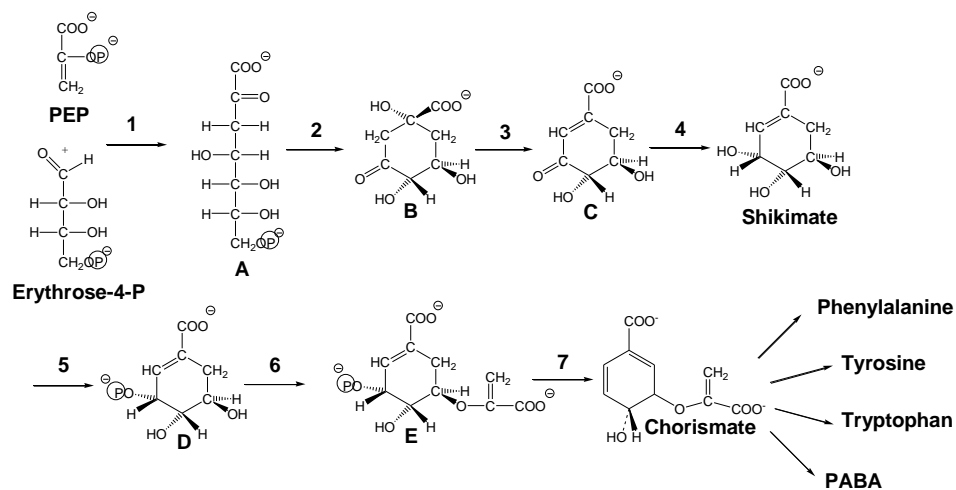
A. (4 points) Put a circle around the carbon atoms in isoleucine that are derived from pyruvate.



B. (4 Points) Interestingly, each of the enzymes above has dual substrate specificity such that a methyl group can replace the ethyl group. Thus, in contrast to isoleucine, all of the carbon atoms of valine synthesized by this pathway come from pyruvate. Acetolactate synthase, the TPP-dependent enzyme, is the target for the potent sulfonylurea herbicides, Oust® and Glean®, manufactured by Dupont [*Trends in Biotech.* 2(6), 158-161 (1984)]. In cells inhibited by these compounds, α -ketobutyrate accumulates but pyruvate does not. How do you explain this?

C. (4 Points) While Oust® and Glean®, with K_i values in the nM range, are toxic to plants at a few grams per hectare, they have low toxicity to animals. What is a reasonable explanation for this large difference in toxicity?

6. (28 Points) The biosynthesis of aromatic amino acids occurs via the shikimic acid pathway which branches after chorismate as depicted below. The following questions will refer to the shikimate pathway.

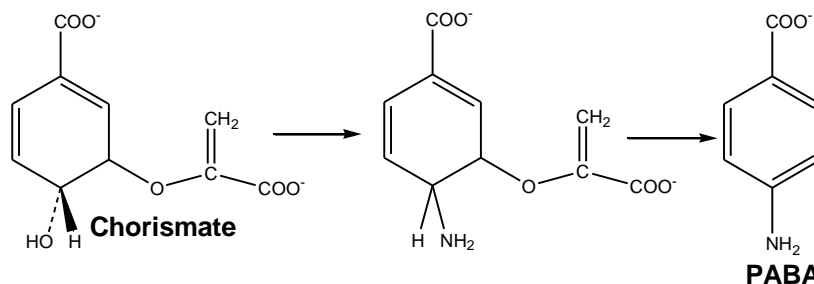


A. (6 points) In *E. coli*, There are three isoenzymes of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DHAP synthase) labeled as #1 above [*J. Biol. Chem.* **254**, 3761 (1979)]. Why would it make sense to have different isoenzymes for DHAP synthase? In what specific way would you expect their properties to differ?

B. (12 points) Complete the following table that identifies the type of reaction, coenzyme (if any) used and produced, and additional substrates and/or products (if any) for reaction 3-7.

Reaction number	Type of reaction	Expected coenzyme Requirement, if any.	Other substrates and/or products, if any.
3			
4			
5			
6			
7			

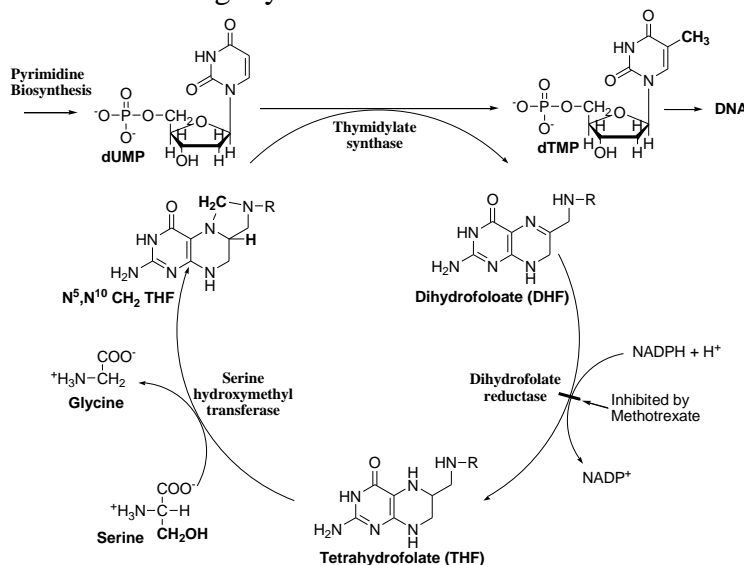
- C. (4 Points) Chorismate is converted to *para*-amino benzoic acid (PABA) in two steps as shown below.



If you wanted to label the ring carbon *para* to the carboxyl group of PABA with ¹⁴C, where should the label be placed in PEP or E-4-P? Draw structure with the labeled carbon atom clearly indicated.

- D. (6 points) on the figure above, show with electron arrow-pushing, how PABA is formed in the last step. What is the three carbon product of this reaction?

7. (17 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.

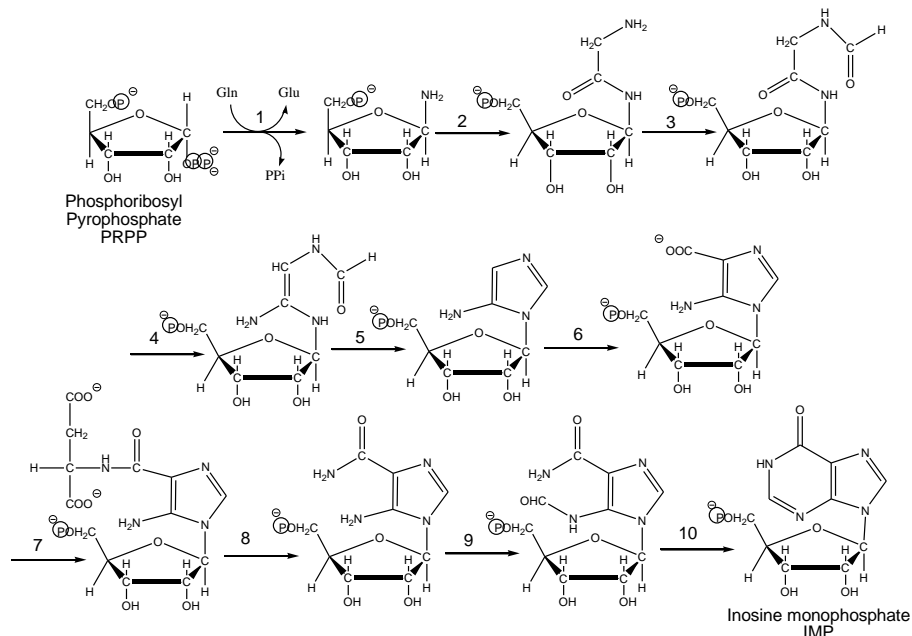


- A. (2 Points) Why is MTx an effective antitumor drug?**
- B. (5 Points) Conceptually, draw a graph and label the axes for the rate of dTMP production as a function of [MTx] in a tumor cell.**
- C. (5 Points) Over time tumors become resistant to MTx inhibition. From a biochemical perspective, list three ways (mechanisms) that a cell could develop resistance to MTx.**
- i.
 - ii.
 - iii.

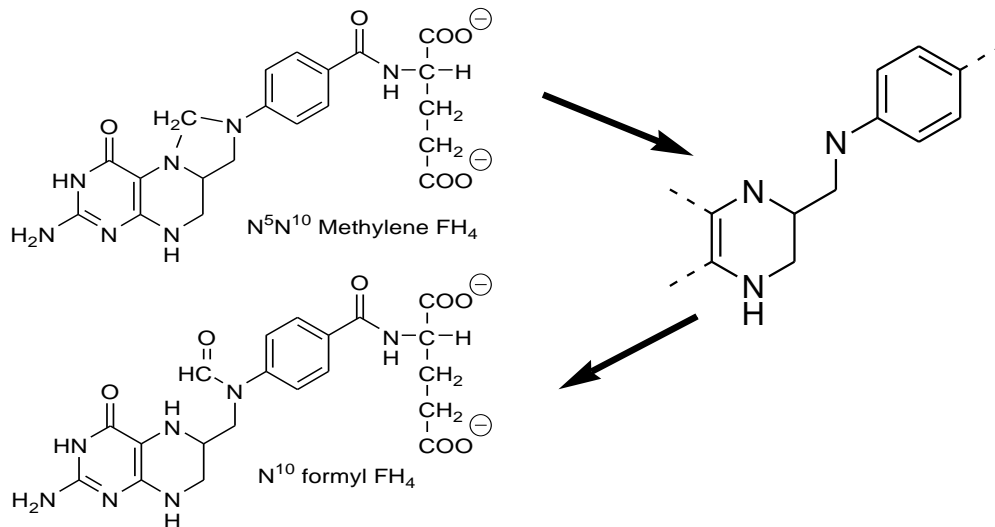
- D. (5 Points) Robert Schimke's laboratory at Stanford studied the development of resistance to MTx in cultured mouse tumor cells by adding more and more MTx to the culture medium as the cells became more and more resistant. In the end, they had selected for tumor cells (AT-3000) that could grow in the presence of MTx 3000 times more concentrated than would normally kill the parental Murine Sarcoma 180 cells [Alt et al. *J. Biol. Chem.* **253**, 1357 (1978)]. 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

8. (18 Points) The synthesis of the purine ring system is shown below without the required cofactors or atom donors.

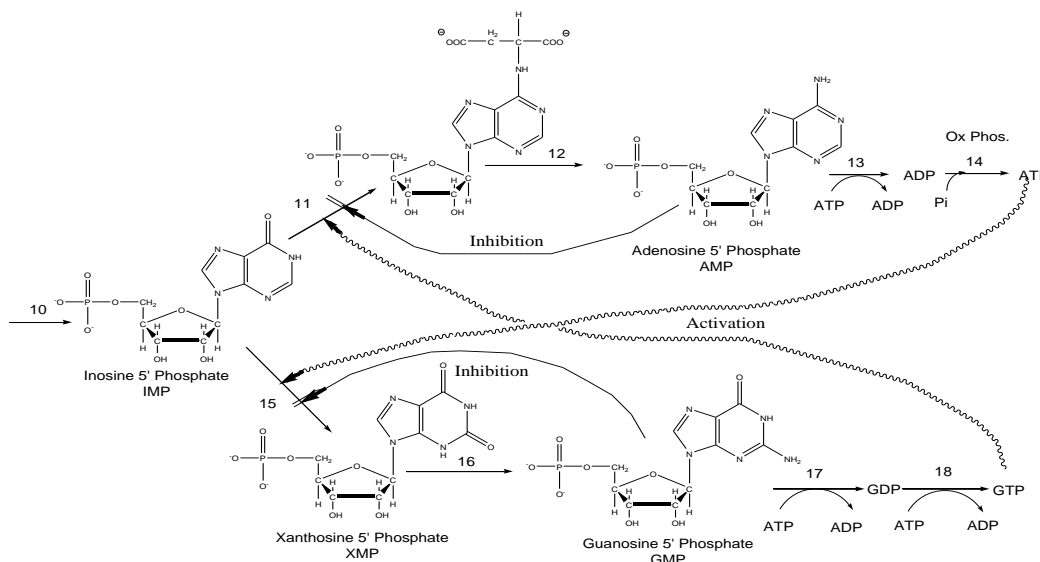


A. (6 points) There are indications that this pathway operates as a coordinated functional complex of enzymes. For example, enzymes catalyzing reactions 2, 3 and 5 are part of a single polypeptide chain [*Biochemistry* **24**, 7059 (1985)]. This multifunctional protein in turn forms a complex with several other enzymes—Serine transhydroxymethylase and the enzymes that convert N^5,N^{10} methylene tetrahydrofolate to N^10 formyl tetrahydrofolate. On the diagram below, fill in the missing intermediate in this process and indicate any coenzymes or other molecules involved in the two reactions.

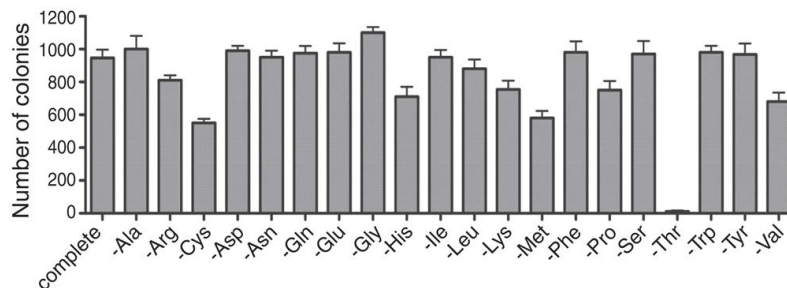
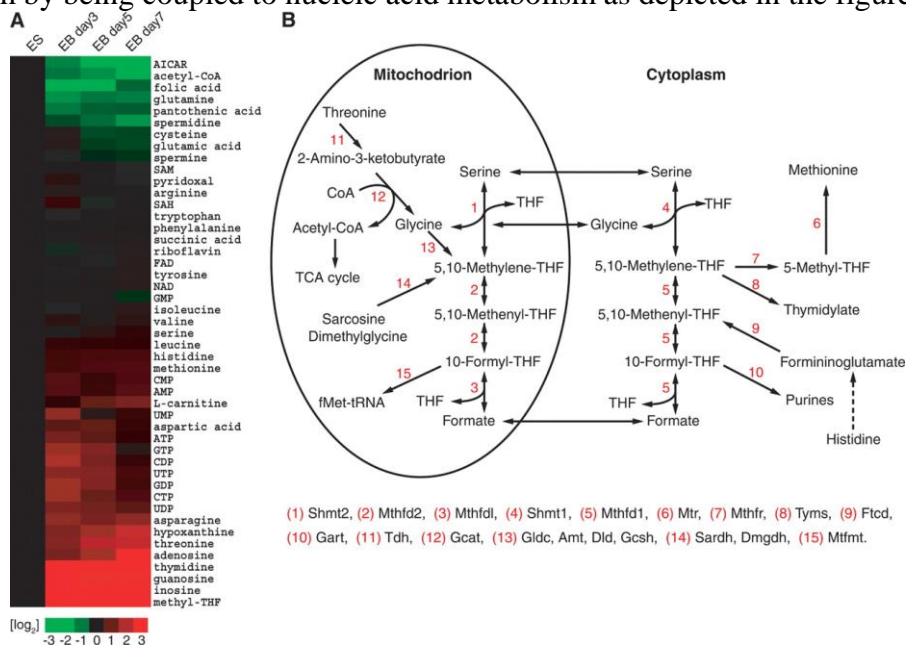


B. (4 points) In cultured human cells, An et al. [*Science* **320**, 103 (2008)] showed that all of the enzymes of purine biosynthesis form a multienzyme complex (the “purinosome”) in a culture media low in purines but are dispersed at high purine concentrations. Give two reasons that this aggregation-disaggregation behavior might be advantageous.

C. (8 points) The flux of IMP to ATP through Reaction 11 and to GTP through Reaction 15 is exquisitely regulated. Reaction 11 on the way to ATP requires GTP and reactions 17 & 18 on the way to GTP require ATP. Both ATP and GTP inhibit reaction 1 (previous page), the first committed step in purine biosynthesis. Based on the information in the figure below, what does all this accomplish?



9. Mouse embryonic stem cells are much smaller than normal differentiated cells (<5µm vs 20-30 µm) and divide more rapidly (~once every 5 h) than the fastest growing cultured tumor cells. If a particular growth factor is removed from the culture medium, the stem cells grow bigger, divide more slowly, and differentiate. An article in *Science* [325, 435 (2009)] this past summer reported the levels of various metabolites in cultured embryonic stem cells (ES) and in differentiating stem cells (EB) 3, 5, & 7 days after removal of the growth factor. As one might expect some metabolites dropped in concentration, some remained relatively unchanged, and others increased in concentration. While changes in some metabolites were readily interpreted in terms of changes in growth rate, others were enigmatic. The concentration of threonine rose approximately 6-fold during differentiation, while the amount of threonine dehydrogenase (Enzyme 11 below) and its mRNA dropped dramatically, more than for any other enzyme and mRNA examined. Furthermore, the threonine dehydrogenase mRNA in stem cells was three orders of magnitude higher than any adult tissue tested. The authors also found that the embryonic stem cell growth was more sensitive to the depletion of threonine in the culture medium than to depletion any other amino acid. They postulated that threonine had a special role in stem cell growth and metabolism by being coupled to nucleic acid metabolism as depicted in the figure below.



- A. (10 Points) Essay. Reflect on the information provided. You may critique the data, question the interpretation, suggest experiments, speculate on its significance, whatever, but select only **one** issue to explore or develop thoughtfully.