Questions 1-13 relate to human chorionic gonadotropin (hCG), a glycoprotein hormone important in the early stages of pregnancy. It is this molecule that is measured in home pregnancy tests and also at the doctor's office to confirm that a woman is pregnant. The crystal structure of hCG was first determined in 1994. Read the excerpts below from the original article describing this structure (Nature, 9 June 1994, 369:455) and answer the following questions. (Note that 'disulphide' is the British spelling of 'disulfide.')

Excerpt 1

"[Members of the family of glycoprotein hormones] consist of two subunits, α and β, which associate non-covalently to form a heterodimer; in a given species the α-subunits are identical and the β-subunits are different (but homologous) for the different hormones. Although the heterodimer is required for receptor binding, it is the β-subunit that determines the specific activity of each hormone. The hormones are all glycosylated with *N*-linked complex carbohydrates, which confer heterogeneity on a given hormone; hCG is the most heavily glycosylated with four additional *O*-linked carbohydrates on the serine-rich C-terminal extension of the β-subunit. In human glycoprotein hormones, the common α-subunit contains 92 amino acids, with 10 half-cystine residues which form five intramolecular disulphide linkages. The β-subunits vary in size from 114 … to 145 [residues] … and contain 12 half-cystines which form six conserved disulphide bridges."

1. Are any disulfide bonds formed between (connecting) the different subunits of hCG?

2. How many cysteine amino acids does hCG have?

3. Which other amino acid *must* hCG contain?
   a. threonine
   b. tyrosine
   c. histidine
   d. asparagine
   e. arginine
   f. glutamine

4. The β-subunits of different human glycoprotein hormones would be considered:
   a. orthologous
   b. paralogous
   c. both orthologous and paralogous
   d. neither orthologous nor paralogous

5. Consider the effects of changing temperature and solution conditions on a protein's structure.
   a. What effect would dramatic heating of hCG have on its structure? Why? Explain in 25 words or fewer.
   b. What additional type of treatment would be needed to break its disulfide bonds?
      A. organic solvent
      B. acid
      C. base
      D. reductant
      E. oxidant
      F. boiling (further heating)

Excerpt 2

"It was previously thought that heterogeneity of carbohydrate prevented any of the glycoprotein hormones from crystallizing. The bulk of the carbohydrate can be removed from hCG by treatment with anhydrous hydrofluoric acid, leaving the deglycosylated protein with the ability still to bind receptors, although post-translational effects are not triggered. Circular dichroism shows that the structures of the deglycosylated and native hormones are identical. Using the crystallization conditions for HF-treated hCG …, isomorphous crystals of neuraminidase-treated hCG have been grown in which only the terminal sialic acid residues have been removed."

6. Receptors are proteins that, on binding specific molecules, undergo a change that transmits a signal to another part of the cell.
    a. Which parts of hCG are necessary for binding to its receptor?
        A. protein only
        B. carbohydrate only
        C. both protein and carbohydrate
    b. Which parts of hCG are necessary for signaling by its receptor?
        A. protein only
        B. carbohydrate only
        C. both protein and carbohydrate

7. Regarding the sentence "Circular dichroism shows that the structures of the deglycosylated and native hormones are identical:"
    a. What kind of structural information does circular dichroism provide?
        A. Sequence (primary structure)
        B. Proportion of secondary structures
        C. 3D structure
        D. Protein-protein interactions (quaternary structure)
    b. In this sentence, what does "native hormone" refer to? (Be specific; 15 words or fewer).

8. Neuraminidase is an enzyme. Based on information in the excerpt, neuraminidase is what type of enzyme?
    a. glycosidase
    b. protease
    c. deaminase
    d. aminotransferase

Description & Excerpt 3

The structure of hCG revealed a unique arrangement of the disulfide bridges called a 'cystine knot.' This arrangement is found in each of the hCG subunits. The authors state, "Apart from the cystine-knot motif, there is no sequence similarity between the subunits, but when the cystine knots are superimposed, the similarity in structure is remarkable."

9. Based on this statement, why is it difficult to conclude whether the subunits arose through convergent or divergent evolution? Explain in 50 words or fewer.

Excerpt 4

"A number of residues important for the activity of hCG have been identified through chemical modification, the use of synthetic peptides in competitive inhibition studies, and site-directed mutagenesis… Much attention has been focused on the longest inter-cysteine loop β38-57. Synthetic peptides with this sequence stimulate steroidogenesis in rat Leydig cells and strongly inhibit binding of whole hCG. …Mutagenesis of Arg43 to Leu in hCG, or replacement of it by Ala or Asp in synthetic (38-57) peptides either significantly diminishes binding or eliminates it. … Residues 47-53 are predominantly hydrophobic and exposed at the surface, forming a wedge-shaped extrusion… likely to be important in receptor binding.

10. How would chemical modifications or mutations be able to show that certain residues are important for hCG activity? Choose the best answer.
    a. By disrupting the quaternary structure of hCG.
    b. By causing a conformational change in hCG.
    c. By changing the pI of hCG.
    d. By changing the K_d of hCG-receptor binding.

11. True or false? A peptide composed of residues 38-57 of the β-subunit acts as a competitive inhibitor of hCG binding to its receptor.

12. Based on the excerpt, which property of arginine 43 is likely to be most important for receptor binding?
    a. Positive charge
    b. Bulkiness
    c. Length
    d. Hydrophilicity

13. Other than an interaction involving arginine, name a type of interaction likely to be important in receptor binding by hCG.

14. You want to study the kinetics of hexokinase.

    a. Write a balanced equation for the reaction catalyzed by hexokinase.

To begin your kinetic experiments, you first need to make the buffer. You want to keep the reaction mixture at pH 7.2, and the buffers listed in the table to the right are available to you.

Buffer name	pK_a
Acetic acid	4.8
Citric acid	5.4
MES	6.2
Bis-Tris	6.5
PIPES	6.8
Sodium phosphate	7.2
HEPES	7.6
Glycylglycine	8.2
Tris	8.3
CHES	9.5

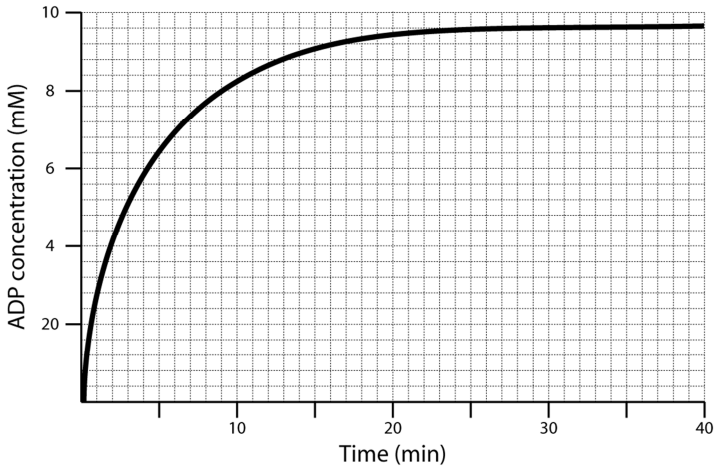
    b. Sodium phosphate is *not* a good buffer to use for this reaction, because it influences the kinetics of hexokinase. Which one of the other buffers listed above would be the best to use? Explain your selection in 25 words or fewer.

You make the buffer, to a final concentration of 100 mM. You add 1 mL of your buffer to a reaction vial, then add 10 mM glucose, 10 mM ATP, and 0.5 nM hexokinase.

    c. What are the initial concentrations of the protonated and deprotonated forms of the buffer? Show your work.

    d. What additional compound must you add in order for the reaction to begin?

You begin the reaction, taking measurements every minute. You produce the following curve:



    e. Which of the following can you determine based *only* on knowing the experimental setup and the data from this plot? There may be several correct answers.

A. [S]	F. k_{cat}
B. [ES]	G. K_{eq}
C. K_m	H. reaction rate
D. V_o	I. catalytic efficiency
E. V_{max}	J. Whether this enzyme exhibits sigmoidal kinetics

    f. You let the reaction run to completion. What is the final ratio of [ADP] to [ATP] in the reaction vial? Show your work. (The $\Delta G'^\circ$ for this reaction is -16.7 kJ/mol. Assume the experiment is carried out at 25°C.) *Hint: You don't need the quadratic formula.*

    g. What is the final concentration of ADP in the reaction vial? Calculate this from your answer in part f, and show your work.

    h. What is the final pH of the reaction solution? Assume that any effect of equilibration by water is negligible. (Assume all equilibration is done by the buffer.) Show your work.

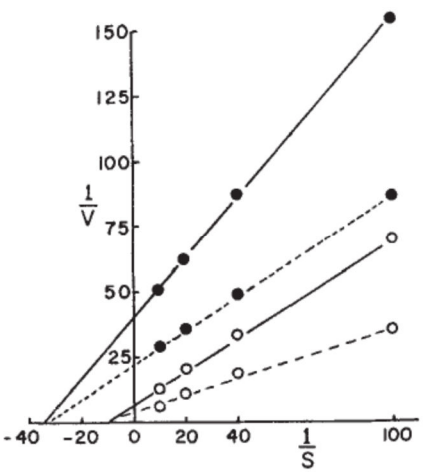
15. The following is the first paragraph of a scientific publication from 1966:

> The brain in sheep, as in non-ruminant mammals, appears dependent on glucose as the substrate for oxidation, yet the blood glucose concentration of adult sheep is only about 2.5 mM (50 mg/100 ml.) compared with about 5 mM (100 mg/100 ml.) in rats, dogs, men and pigs. If the availability of a substrate is less in one species than in another, and a tissue were to utilize the substrate at comparable rates in both species, then one might look for an inter-species difference in enzyme affinity for this substrate. So we have estimated the apparent *Km* for glucose of hexokinase in brain, also liver, muscle and adiposa, of sheep and rats.

In the paper, the authors describe how they purify hexokinase from the listed tissues of sheep and rats, and then they analyze the kinetics of the enzymes. They are able to purify hexokinase from each tissue, and they purify glucokinase from rat liver. The sheep liver contains no glucokinase.

a. What does the absence of glucokinase from the sheep's liver mean for energy storage in sheep? Explain in 15 words or fewer.

Below is the author's kinetic plot for the brain hexokinase of sheep (filled circles) and rat (open circles). The solid lines reflect the initially purified enzymes. The dashed lines reflect the enzymes after additional purification. 'S' represents [glucose].



b. What kind of plot is this?

c. What does the plot tell you about the difference(s) between sheep and rat hexokinase? Explain in 15 words or fewer.

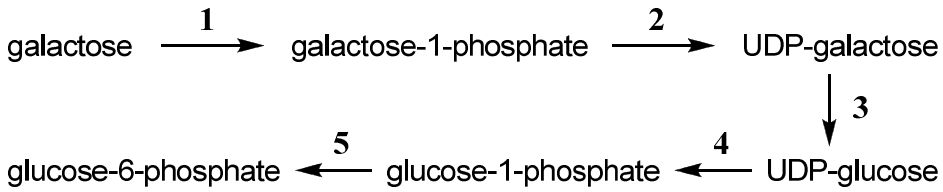
d. Would you expect this result based on the blood glucose concentrations given in the first paragraph in the paper? Explain why in 20 words or fewer.

e. What is different between the two samples (original and higher purity = solid and dashed lines) that results in the change shown in the plot. Be specific. (10 words or fewer).

f. Why do the authors report "apparent *Km*" values for the enzymes, instead of the actual Km values? Explain in 15 words or fewer.

g. True or False? Sheep hexokinase and rat hexokinase are isozymes.

16. Lactose is the principal sugar found in milk, the sole diet of newborn babies. In the catabolism of lactose, the sugar is first broken down into its component monosaccharides. We've learned about the catabolism of glucose. Galactose breakdown requires a few additional steps, through which it is converted into glucose-6-phosphate:



UDP is a nucleoside diphosphate, like ADP or GDP, but with a different base – uracil instead of adenine or guanine.

In the first step of the conversion, galactose is phosphorylated:

$$\text{galactose} + \text{ATP} \rightarrow \text{galactose-1-phosphate} + \text{ADP} + H^+$$

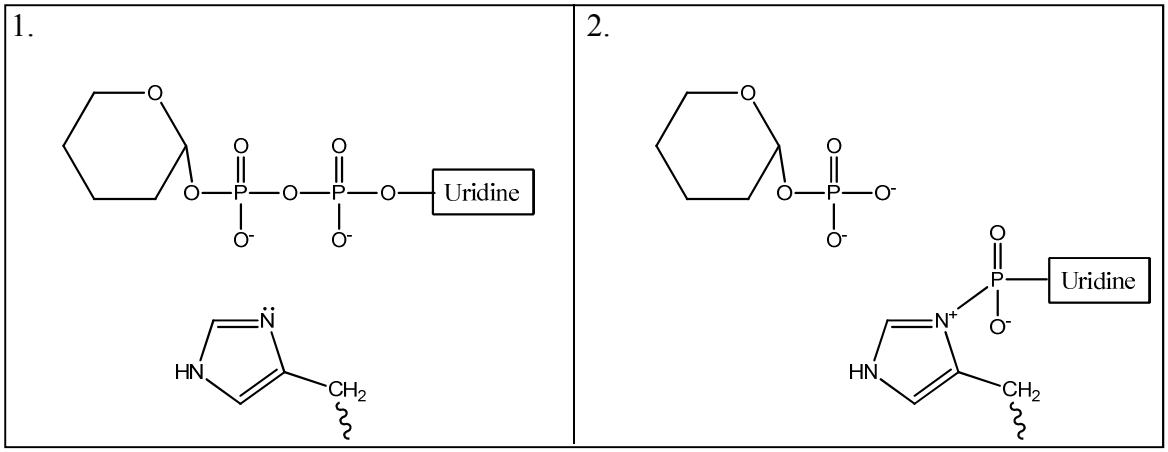
e. What *class* and *subclass* of enzyme catalyzes this reaction?

f. Draw a mechanism for this reaction.

The second and fourth steps of this conversion are the same. UMP is removed from a molecule of UDP-glucose and given to galactose-1-phosphate:

$$\text{galactose-1-phosphate} + \text{UDP-glucose} \rightarrow \text{UDP-galactose} + \text{glucose-1-phosphate}$$

g. A histidine of the enzyme temporarily holds the UMP during the reaction. Given portions of the sugar structures below, complete the structures and draw in the electron motions involved in this reaction.



The third step converts UDP-galactose to UDP-glucose.

h. Complete this sentence: UDP-galactose and UDP-glucose are _____.

A. epimers
B. enantiomers
C. isozymes
D. homologs

(Continued from the previous page)

In the fifth and final step of the conversion, glucose-1-phosphate is converted to glucose-6-phosphate.

i. What *class* and *subclass* of enzyme catalyzes this reaction?

j. In this fifth step, the enzyme catalyzes the reaction using a mechanism very similar to that of an enzyme of glycolysis. Name this enzyme.

k. Which enzyme (1-5) used in the conversion of galactose to glucose-6-phosphate is most likely to be regulated? Why? Explain in 20 words or fewer.

l. Suggest a negative effector for this enzyme (from part g).

m. Suggest a positive effector for this enzyme (from part g).

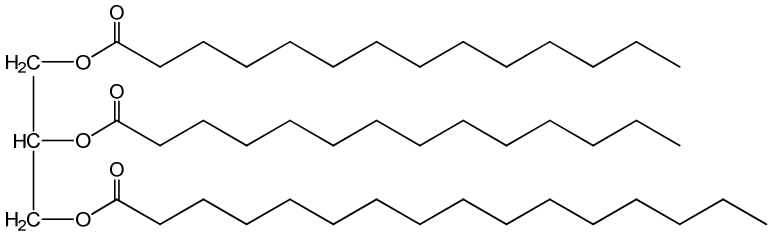
n. How many ATP equivalents would be produced through the complete oxidative catabolism of *galactose*? Select the *net* number produced assuming the malate-aspartate shuttle is present.

A. 29
B. 30
C. 31
D. 32
E. 33
F. none of the above

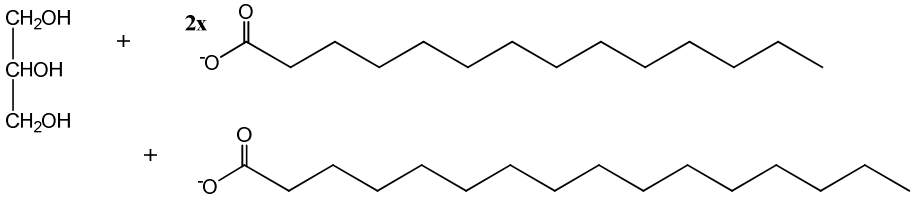
17. Like the breakdown of glucose, the breakdown of lipids provides energy for the cell.

    a. In what form are lipids stored in the body? (Name the lipid class.)

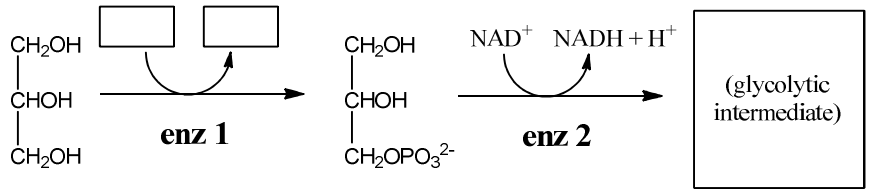
    b. A specific example of a lipid is shown below. Name this lipid.



This lipid is initially broken down into the following compounds:



The parent compound (left) is converted in two steps into an intermediate of glycolysis, then broken down by the subsequent enzymes of glucose catabolism. The initial two steps are shown:



    c. Based on what you know about similar reactions in biochemistry, suggest additional reactant(s) and product(s) of enzyme 1 (indicated by the boxes), and name the class and subclass of enzyme 1.

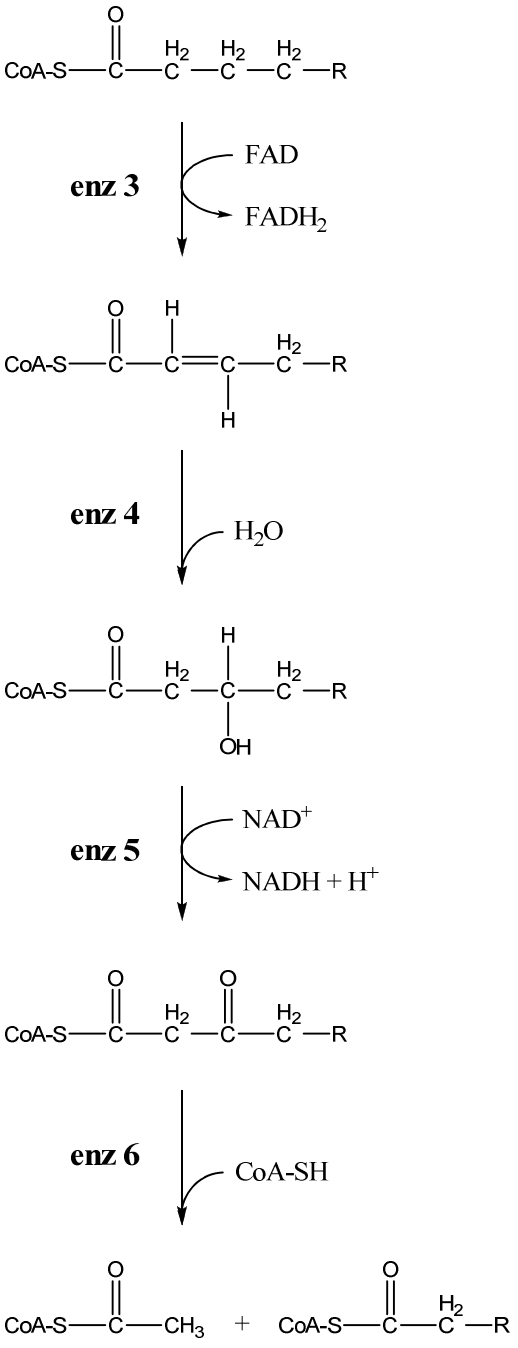
    d. Name all possible glycolytic intermediates produced by enzyme 2, and name the class and subclass of enzyme 2.

    e. What is the net production of energy carriers by the complete, aerobic catabolism of the parent compound (including the steps catalyzed by enzymes 1 and 2 above)? Show your reasoning, and then list the net numbers of ATP+GTP, NADH, and $FADH_2$ produced.

(Problem continued on next page.)

(Problem continued)

The remaining portions of the original lipid are transported into the mitochondrial matrix, yielding a CoA-derivative; this transport requires the hydrolysis of one ATP *per molecule*. Once in the matrix, these compounds are broken down in a process called β-oxidation, yielding acetyl-CoA. The process is repeated again and again until all of the original molecule has been broken down into acetyl-CoA.



f. Name the enzyme class and subclass (if applicable) for enzymes 3, 4, 5, and 6.

g. Why is this pathway called β-oxidation? Explain in 10 words or fewer.

h. How many acetyl-CoA molecules are produced from the complete β-oxidation of the three substituent components of the original lipid? Show your reasoning.

i. What is the net production of energy carriers by the complete, aerobic catabolism of these three lipid substituents, including their transport into the matrix, the rounds of β-oxidation, and the catabolism of acetyl-CoA? Show your reasoning, and then list the net numbers of ATP+GTP, NADH, and $FADH_2$ produced.

j. Combining your values for parts **e** and **i**, calculate the total (net) number of ATP molecules (or ATP equivalents) produced by the catabolism of the original lipid molecule. Assume that the malate-aspartate shuttle is used.

k. The molecular weight of this lipid is roughly 750; that of glucose is 180. How much more ATP can be made, per unit mass, through the catabolism of this particular lipid versus the catabolism of glucose? Show your reasoning, and express your answer as a factor. (For example, *5x more ATP* per unit mass from the catabolism of this lipid).