A LEARNER'S MANUAL:

PROBLEMS IN

CELL BIOLOGY, PHYSIOLOGY &

DEVELOPMENT



DR. DEBORAH MOWSHOWITZ & DR. ALICE HEICKLEN

13TH EDITION © 2021

DEPARTMENT OF BIOLOGICAL SCIENCES

COLUMBIA UNIVERSITY

NEW YORK, NEW YORK 10027

30^{00}

COURSEPACKS CANNOT BE RETURNED OR EXCHANGED

ACKNOWLEDGMENTS for the 13TH EDITION (2021)

The authors wish to thank Dr. Martin Chalfie, Dr. Judith Gibber & Dr. Mary Ann Price for permission to include some of their problems in this manual. Dr. Gibber is the author of many of the questions on physiology, and Dr. Price compiled the changes for this new edition.

We also wish to thank Sarah Kim & Evan Heller who helped with earlier editions, and Jaya Santosh who helped with the later editions.

Last but not least the authors also wish to thank all the students and teaching assistants who have struggled with these problems and their answers, and suggested ways to improve this manual.

HOW TO USE THIS MANUAL – ADVICE TO STUDENTS

This edition is substantially different from previous editions of the manual. We strongly recommend that you purchase the current (13th) edition.

The problems in this manual are intended to help you ***learn*** the material, not just to allow you to test yourself. Therefore it pays to go through the problems carefully and be sure that you understand the point of each problem, even if you think the answer is obvious. You will probably need to go over the course material before trying these problems, but you do NOT have to master all the material before doing the problems. However, by the time you have *finished* doing the problems, you should be on top of the material, and ready to ace the next exam. (See below & the course web site for Problem Solving Tips.)

Most of the questions in this manual are derived from old exam problems of biology UN2006 & UN2402 (previously called C2006 & F2402), or equivalent courses. Some of the problems have been rewritten to make them more useful for study purposes, and additional problems have been included to introduce and/or review important concepts.

In some problems, the answers to later parts depend on the answer (s) to earlier parts. For selected problems of this type, the answers to the earlier parts are given separately, as noted in the problem. In these cases, you are advised to look up the answers to the earlier parts before proceeding.

The starred problems and the problems in the "R" problem sets are taken directly from old exams. You are encouraged to treat these problems differently from the others. We suggest that you postpone doing the starred problems until AFTER you have done all the other problems (on that particular topic) and you feel confident that you understand the material. *Then* use the starred problems to test yourself. We suggest that you do the "R" problem sets later, for review.

Answers to all the problems are in the back, so you can check yourself, but be sure to work out each question carefully before checking the answer. Note that the explanations given in this manual are often quite long and detailed in order to help you get the most out of the problems. These explanations are more comprehensive than you would be expected to write on an exam. For more tips on what you should explain on an exam, see below, and the note on page 221.

Be sure you understand the vocabulary

In introductory biology at Columbia, you have to learn a new technical vocabulary (IFs, MTs, MHCs, autocrines, etc.) but the stress is on using the vocabulary, not just repeating it. This manual is designed to help you master the key terms and concepts, and to help you learn how to apply them.

If you can't solve a problem, you may be running into difficulty because you are confusing similar terms. If you don't distinguish between lamin and laminin, or chromosome and chromatid, you will not be able to solve problems using these terms. No one in this course will ask you for a definition, but you do have to know what the terms mean (in context) in order to decipher the questions. So be careful to master the vocabulary and try not to confuse similar terms with each other or to confuse technical meanings of words with their common sense meanings. If you find yourself with a long list of related terms, try to make a diagram or chart that illustrates their relationships. It is usually easier to grasp an overall visible pattern than to remember all the separate definitions.

Every effort has been made to match the terms used in class with the terms in this manual. However, there may be some technical terms used in these problems that were not (or not yet) covered in class, and not all scientists use exactly the same terms. So don't panic if you hit a term you don't recognize – look the term up in your text, ask a fellow student or go online and 'Google' it. You don't have to ask the TA or instructor right off, but don't hesitate to ask if you can't find an answer elsewhere.

Be sure you can explain your answers!

On tests you are usually asked to explain the reasoning behind your answers. It isn't enough to get the right answer -- you have to be able to explain how you got it. To be sure you get enough practice at explaining yourself, it pays to discuss the questions with your fellow students and/or to write out explanations of your reasoning. In order to get full credit for your explanations, you must both

(1) Include the right facts, principles, etc., AND

(2) Explain the logic that you used to solve the problem. How did you get from the facts to the answer?

It is not sufficient to pile up unselected facts (even if they are correct), OR just to state the facts (even if they are the right ones) without explaining how they relate to the problem at hand, OR to just explain the logical train of thought (even if it is correct) without any specifics.

That's what you shouldn't do. What should you aim for??

To get maximum credit, you should explain as if you were talking to a fellow student who is generally intelligent, prepared, etc., but can't figure out this particular question. In other words, explain your reasoning step by step. Don't just repeat all the related facts in the book or notes -- try to pick out the important, relevant points, put them in logical order, and explain (or diagram) how one leads to the next. (In other words, pretend you are writing a basic key.)

On some exam questions, you don't have to explain everything, but it is good practice to be sure you can explain all your answers.

The Problem Solving Tips on the next two pages may help you to solve the problems and to get the most out of them.

For a more complete set of problem solving tips and additional advice for succeeding in biology, check out the "advice" section on the course website. We continually update it with advice from former students, TA's, etc. Go to http://www.columbia.edu/cu/biology/courses/c2005/advice.html
The advice pages may refer to 'Bio C2005/F2401' but the same advice is applicable to both terms of Intro Bio, both to UN2005/UN2401 (previously C2005/F2401) and to UN2006/UN2402 (C2006/F2402).

Problem solving tips

Don't try to jump right to the answer – be sure you understand the question first. More time spent figuring out the question usually pays off in the end. As a successful student said, 'The issue is not to think outside the box, or inside the box, but to figure out ***what*** the box is.'

Draw a picture or diagram whenever possible. The relationships between the various parts and pieces of information are often not readily apparent until you get the words translated into pictures. Once you understand the relationships between the terms, facts, steps, etc., you will find the individual pieces are much easier to remember and recall. If a picture isn't appropriate, try a chart or diagram.

Break the problem down. If you have a complex problem to solve, don't try and get it in one shot. Read it through, and make an intelligent guess at the answer. Then go back and see if your solution explains all the data given. If it does, fine. If it doesn't, refine/adjust your solution and try again.

Try a different approach. The following list comes from a math class, and duplicates some of the suggestions above, but is a useful list of problem solving approaches to keep in mind:

Guess & check	Draw a picture
Look for a pattern	Make a model
Act it out	Use easier numbers
Write a number sentence	Make an organized list
Make a table or chart	Use logic
Work backwards	

Some Additional Problem Solving Tips from the 'advice' section of the course website.

1. How to use the Answers (so you get the most out of the problems):

A. Only read as much of the answer as you need to get on the right track. The problems are intended primarily for you to learn from, not just for you to test yourself. (Save a few to test yourself, but use the rest to learn from.). If you got the wrong answer, don't read the whole correct explanation and go on to the next problem. Only read as much of the correct explanation as you need to see where you went wrong. Then stop reading! Go back, and redo the problem from that point. That way you learn more.

B. Don't do a whole set of problems before looking up the answers. (This way you don't learn the point of earlier problems, and so you continue to make mistakes in the following problems.) For multipart questions, you may even want to look up the answers for some of the earlier parts (to see if you are on the right track) before finishing the rest of the problem. If you do too large a block at once, you won't learn from the earlier problems.

2. How to Approach the Problems. Students tend to think there are two options when faced with a question. Option 1 -- you'll know the answer immediately. Option 2 -- you don't know the answer. But there is a 3rd possibility. Option 3 (the "Blaer" option) -- you won't be able to answer the question right off the bat just by looking at it, but you CAN figure it out. I call this 3rd option the "Blaer option" because it was explained to me by Prof. Alan Blaer of physics. Most of the problems you encounter in college science courses require you to use the Blaer option. You have to learn to abandon hope of using option 1 most of the time, rule out option 2, and move on to option 3. Options 1 & 2 are unlikely to work, because we try to give you problems that are hard, but doable. They are unlikely to be impossible (option 2) or too trivial (option 1). We do make mistakes, so options 1 & 2 work sometimes, but you can count on taking the Blaer option almost every time. You may think that everyone else in the class is using option 1, but that's an illusion, and you shouldn't be ashamed of needing to think about each problem in order to solve it. You are not alone.

3. How to Review the Problems. It probably doesn't pay to do the problems over and over. (But see Method 4.) You want to review what you have learned, but doing the problems over multiple times may not be the most effective method. So how to review the problems effectively? There are (at least) four ways to deal with this:

Method 1. (Suggested by a TA.) Do the problems once, when they are assigned and/or you cover the topic. Then do them again, close to the exam. By that time, you will probably have forgotten the answer, so review is worth it. Don't do the problems twice in close succession, and don't do them over again if you can still remember the answers. So what to do if you remember the answers? See additional methods below.

Method 2. Do the problems once. After each problem, write down what you learned from doing the problem -- not the answer, but the principle or idea that you learned from doing it. Review the list of principles, not the problems themselves.

Method 3. Do each of the problems once. Check your answers after each problem. If you got a problem (or part of it) right the first time, don't bother to do it again. (It doesn't usually pay to do it again, but you might want to write down what you learned, as in Method 2.) When you review, just go over the problems or parts you missed the first time. Repeat the reviewing cycle as many times as needed, until there are no 'missed' parts left. (This method is recommended by some TAs and by Cal Newport in his book, 'How to be a Straight A Student.')

Method 4. Do the problem sets multiple times, but do it differently each time. Use the first time to learn the material, and the second time to test yourself and practice writing for an exam. (This was suggested by a student who wrote: 'These methods have helped me improve immensely!') The first time, do your best with a question, struggle with it, write an answer, and then read the solution at the back of the manual. (You may also want to write a 'learning log' as in method 2.) Student wrote, 'I think the problems actually help me learn the material better than just reading the lecture notes, so I don't think of this first pass through the problem sets as testing my knowledge, but rather as another way of studying.' After this first pass, re-read the lecture notes + handouts (&/or learning log), and then, in a few days, go back and test yourself -- make a neatly written final solution key to the problem set as if you were turning it in for a grade. In addition, you can do a 3rd pass closer to the exam to review the questions you had trouble with.

Whichever method or combination of methods you chose, do each problem carefully, looking up the answers only after you have thought long and hard about the problem. And if you do the problems more than once, be sure you are doing them from a different angle each time. Also don't be afraid to ask for help in office hours or recitation.

4. What if the problem has more than one solution? Many problems in biology do not have a simple, unique solution. They have many possible solutions, but only one or a few are very likely. That's why many of the problems in this class ask for what will ***probably*** happen. You are not asked for the most ingenious solution, or for any possible solution that fits, but for the ***most likely*** solution. You are not supposed to 'guess' (that is, make a stab in the dark), or to know the answer right off (because of advanced material or lab experience), but you are expected to be able to identify the most reasonable alternative(s), given the material you have learned in this course. If you pay close attention to the question, and consider what principle is being tested, you may find it easier to choose the best (most probable) answer, and to avoid possible, but improbable, solutions.

TABLE OF CONTENTS -- PROBLEMS & ANSWERS

Problem Set 1	OVERVIEW OF EUKARYOTIC CELL STRUCTURE AND MEMBRANES.....….	1
Problem Set 2	MEMBRANE FUNCTION -- TRANSPORT …….....……	9
Problem Set 2R	*REVIEW PROBLEMS ON CELL STRUCTURE & TRANSPORT*…….....	15
Problem Set 3	INTRACELLULAR COMPARTMENTS: STRUCTURE., FUNCTION & PROTEIN TRAFFICING…….….	23
Problem Set 3R	*REVIEW PROBLEMS ON PROTEIN STRUCTURE, SYNTHESIS & TRANSPORT*…	31
Problem Set 4	NUCLEI & EUKARYOTIC GENE REGULATION …….……..	37
Problem Set 4A	ALTERNATIVE SPLICING, RNA STABILITY & TRANSL REGULATION….	43
Problem Set 4R	*REVIEW QUESTIONS ON CHROMATIN, REGULATION, & PROTEIN SORTING*	49
Problem Set 5	HOMEOSTASIS & THERMOREGULATION…….....…….....……....	55
Problem Set 6	MECHANISM OF SIGNAL TRANSDUCTION & HORMONE ACTION……..	57
Problem Set 7	HORMONES…….……..…	65
Problem Set 7R	*REVIEW QUESTIONS ON HORMONES, SIGNALING, & HOMEOSTASIS*……	71
Problem Set 8	NERVOUS SYSTEM -- NEURONS, SYNAPSES, CIRCUITS & SENSORS……..	75
Problem Set 9	MUSCLE -- SKELETAL, SMOOTH & CARDIAC.....……..	85
Problem Set 9R	*REVIEW QUESTIONS ON NERVES & MUSCLE*……..……	89
Problem Set 10	RESPIRATORY SYSTEM (GAS EXCHANGE)……...	93
Problem Set 11	HEART FUNCTION & CIRCULATORY SYSTEM…….	97
Problem Set 12	URINARY SYSTEM.....……	99
Problem Set 12R	*REVIEW QUESTIONS ON PHYSIOLOGY*…….	105
Problem Set 13	IMMUNOLOGY.....….	109
Problem Set 14	DEVELOPMENT	115
Problem Set 15	CANCER & REGULATION OF THE CELL CYCLE…….……...	121
Problem Set 15R	*REVIEW QUESTIONS FOR THE FINAL* …….……...…....	127

SOLUTIONS TO ALL PROBLEM SETS ……. 133

****

SOLUTIONS & TO SELECTED PROBLEMS IN SET 2 …….…….. …….22

SOLUTIONS & HINTS TO SELECTED PROBLEMS IN SETS 3 & 4…….. …….42

SOLUTIONS & HINTS TO SELECTED PROBLEMS IN SETS 6, 7, & 8……..….70

Problem Set #1 -- Overview of Eukaryotic Cell Structure & Membranes

1-1. The limit of resolution of the light microscope is approximately 0.2 μm (micrometer) yet single microtubules (diameter = 25 nanometer) can be seen with immunofluorescence microscopy. Resolve this paradox. (What is a nanometer?)

1-2. Suppose red blood cells (RBCs) are subjected to a variety of experimental conditions (listed below) and then treated with a water soluble enzyme that degrades phospholipids (such enzymes are called phospholipases). After the enzymatic digestion, the percent remaining (in the RBC) of three phospholipids (A, B, and C) is determined. The results are given below:

Condition of the RBCs before Phospholipase Treatment *	% of Each Lipid Remaining in RBCs A	B	C
1. Intact	24	80	100
2. Lysed	0	0	0
3. Lysed and resealed	24	80	100
4. Lysed, disrupted, and resealed	50	50	50
5. Lysed, disrupted, resealed, and relysed	0	0	0

A. How much of each phospholipid is accessible to the enzyme when:
(1) the RBCs are intact and (2) when the RBCs are lysed?
B. What is the effect of disruption (why are all the values in line 4 the same)?
C. What is the normal distribution of the three phospholipids in the RBC membrane?
D. Why were RBCs a good choice for these experiments?

*Note: Lysed RBC have holes or cracks in their membranes, so that material can leak out (or enter the cells), but the membranes remain essentially intact. When RBC are disrupted, the membranes are broken into fragments. When RBCs are 'resealed' the membranes or fragments that are present form sealed vesicles.

1-3. Some membrane proteins do not move around in the plasma membrane but are found only in localized regions (e.g. at the specialized contact points between a nerve and a muscle [the neuromuscular junction]). Does this mean that the membrane is not fluid? Speculate on how a non-uniform distribution of membrane proteins might be maintained in a cell.

1-4. You have been asked to study the membrane of Martian Blue blood cells. Except for their color, these cells are remarkably similar to human red blood cells, e.g. they only have one membrane, a plasma membrane. You are surprised, however, to find that the total amount of lipids is enough to cover the outer surface of the cells three times, and that the lipids consist of both partially polar (amphipathic) molecules and completely nonpolar (neutral) lipids. Propose a model for the Martian blue cell membrane that fits the data.

1-5. A chamber is separated into two equal parts, 1 and 2, by a lipid bilayer.

A. What will be the steady state distribution (greater in 1, greater in 2, the same in both) of a weak acid (HA) that is initially added to chamber 1 under the following conditions? (Hint: Would the compound cross the membrane in an ionized or unionized form?)

i) Chamber 1 has a low pH; chamber 2 has a high pH.
ii) Chamber 1 has a high pH; chamber 2 has a low pH.
iii) Both chambers are acidic.
iv) Both chambers are basic.

B. Answer questions (i) to (iv) above for a weak base (BOH).

C. Over the summer, you are hired by the coroner's office. The police bring in the body of a person they believe has been poisoned by strychnine (an alkaloid that is a weak base). Where in the cell would you expect to find the evidence of such a poisoning?

1-6. You isolate all the membranes of the nuclear envelope (without any attached ER). You extract the lipid and make a monolayer. You measure the area of the monolayer (M), and calculate the area of the nuclear surface (N). What value do you expect for M/N?

1-7. Consider the 3 standard components of the cytoskeleton. Which are hollow? Which are made of tubulin and/or actin? Which are polymers of globular proteins?

1-8. Fill in the blanks with the appropriate component of the cytoskeleton and/or circle the best answer, and explain.

A. The component of the cytoskeleton with the largest diameter is _____ and the one(s) NOT involved in movement is (are) _____.

B. If you add fluorescent antibody against actin to normal cells in interphase, you will "light up" the component of the cytoskeleton called _____ AND you will see fluorescence in the (nucleus) (cytoplasm) (mitochondria) (nucleus & cytoplasm) (cytoplasm and mito.) (nucleus and mito.) (all of these). Note: Antibody is added to cells that have been "permeabilized" -- specially treated to allow passage of antibody (protein) through membranes.

C. If you add the same antibody (as in B) to a red blood cell, you will see (no fluorescence) (fluorescent staining over a larger area) (fluorescent staining over a more restricted area) (beats me). Explain this one briefly.

1-9. During mitosis both the Golgi and the ER break down into small vesicles which are spread out throughout the cell. After mitosis, the fragments reassemble and the organelles reform. When the ER comes back together, it appears to get stretched into place, as if it were anchored at one end (the nuclear envelope) and the other end were pulled or pushed <u>out</u> toward the edge of the cell. When the Golgi reforms, the pieces appear to be pulled <u>in</u> toward the center of the cell, so that all the pieces end up in one place near the middle of the cell. Colchicine inhibits reformation of both organelles after mitosis; cytochalasin B has no effect. *(For all problems using inhibitors of MF, MT, and IF, you can look up the effects of the drugs.)*

A. The simplest explanation is that reformation of both organelles requires
(microtubules) (microfilaments) (intermediate filaments)
(different cytoskeletal elements for the two organelles) (none of these).

B. It is likely that motor molecules are required for the reformation of the organelles. If motor molecules are involved, the ones involved in reformation of the two organelles are probably
(the same) (different for the two organelles) (can't predict).

C. Which motor molecule(s) is/are most likely to be involved in reformation of the Golgi? _____
Explain A to C.

1-10. In the embryo, sheets of cells fold into tubes. This process is blocked by cytochalasin B; cholchicine has no effect. Given these results, tube formation is probably dependent on the function of (MT) (MF) (IF) (MT or MF) (MF or IF) (MT or IF) (IF, MF, or MT) (none of these).
MT = microtubules; MF = microfilaments; IF = intermediate filaments.

1-11. You discover two new drugs that interact with components of the cytoskeleton. You treat animal skin cells *in vitro* (growing in a bottle) with drug A or drug B. If you expose the cells to drug A, you get large, multinucleate cells; each nucleus is normal (with the normal number of chromosomes) but there are many nuclei per cell. If you expose the cells to drug B, you get polyploid cells. The cells are normal size, and have only one nucleus, but the number of chromosomes is a multiple of what it should be (2X or 4X).

A. What is the <u>simplest</u> explanation for the effects of Drug A?

A-1. Drug A probably binds to (tubulin) (actin) (spectrin) (ankyrin) (clathrin) (actin or tubulin).

A-2. Drug A probably affects (chromosome separation) (cytoplasmic division) (endocytosis) (budding of vesicles from the ER) (attachment of integral membrane proteins to the cytoskeleton).

A-3. Drug A probably affects functioning of (MT) (MF) (IF) (MT or MF) (MF or IF) (any of these) (none of these). Explain all your answers to part A.

B. What is the <u>simplest</u> explanation for the effects of Drug B?

B-1. Drug B probably binds to (tubulin) (actin) (spectrin) (ankyrin) (clathrin) (actin or tubulin).

B-2. Drug B probably affects (chromosome separation) (cytoplasmic division) (endocytosis) (budding of vesicles from the ER) (attachment of integral membrane proteins to the cytoskeleton).

B-3. Drug B probably affects functioning of (MT) (MF) (IF) (MT or MF) (MF or IF) (any of these). Explain all your answers to part B.

1-12. Consider the standard types of cell-cell connections in animal cells. In which type(s), if any, do you find the following structures or features?

A. Connexons.

B. Fusion of neighboring cells along an entire surface.

C. Filaments (visible in EM) attached directly or indirectly to the inside surfaces of the cell membranes at the point of cell-cell connection.

D. Connects cytoplasm of adjacent cells.

E. Involves close apposition of bilayers from adjacent cells.

1-13. Fill in the blanks with "increase" or "decrease" or "stay the same," and explain. Assume you are looking at the layer of epithelial cells surrounding the intestine.

A. If you increase the number of gap junctions between two cells, the flow of ions <u>between</u> the two cells (under optimal conditions) should _____ , AND the likelihood of water passing <u>across</u> the layer through the extracellular space between the two cells should _____.

B. If you increase the number of spot desmosomes between 2 cells, the number of filaments (either kind) <u>in between</u> the two cells should _____ , AND the number of filaments attached to plaques <u>inside</u> the two cells should _____.

1-14. Connexons are made up of a protein called connexin. Six molecules of connexin make up one connexon. Suppose you freeze fracture some tissue containing gap junctions. When you look at sections of the freeze fractured membranes, you see lots of bumps on the P face but few on the E face. (Areas of the same tissue that lack gap junctions have a lot less bumps.)

A. The P and E face look different because you are looking at (2 different lipid bilayers) (2 different sides on the inside of a bilayer) (two different sides on the outside of a bilayer) (either 2 different bilayers or 2 different sides of the same bilayer).

B. When gap junctions are intact (before freeze fracture), connexin molecules should be bound to (connexin molecules from neighboring cells) (connexin molecules in the same cell) (the cytoskeleton beneath the membrane) (all of these) (none of these).
Circle one or more choices and explain your answers to A & B.

C. What do the bumps on the P face correspond to? The extracellular or intracellular domain(s) of connexin? How do you know?

1-15. You wish to study the proteins that bind to the cytoplasmic face of the plasma membrane from cells of a certain type, say liver carcinoma cells. You have found a method that gives a good yield of inside-out vesicles from the plasma membrane of the cells. However, your preparations of inside-out vesicles are not completely clean -- they are always contaminated with variable amounts of right-side-out vesicles. You decide to purify your preparation of vesicles by passing it over a column made of lectin (a carbohydrate binding protein) coupled to solid beads.

Note: these vesicles are NOT formed by invagination/RME -- they are formed by breaking up the plasma membrane into pieces and allowing the fragments to seal up into small vesicles. The fragments either reseal in the original orientation (= right-side-out) or the reverse orientation (= inside-out).

A. Why do you want inside-out vesicles?

B. How do you expect the column to help you purify inside-out vesicles?

1-16. A membrane protein is 130 amino acids long. Carbohydrates are attached to amino acids 2-4, 10-15, 22, 26, 37, 44, 47, & 50. Trypsin treatment of this protein produces 7 peptides. Six peptides are water soluble; one peptide, made of amino acids #73-95, is not. Freeze fracture of a membrane containing this protein shows particles in the exposed surface.

A. This is an (intrinsic) (extrinsic) (transmembrane protein). Chose as many as apply.

B. Draw a diagram of the likely orientation of this protein in the membrane and label it carefully.

1-17. Protein P is part of the plasma membrane. Here is a diagram of protein P. xxxx = hydrophobic region of peptide P; ------- = hydrophyllic region; A, B, C, D refer to 4 domains of the peptide.

(Amino) xxxxxx----------xxxxxx---------- (Carboxyl)
A B C D

Newly made protein P has all 4 domains; Mature protein P (in the plasma membrane) is missing domain A. For this problem, ignore domain A, and assume the protein is inserted in the plasma membrane with its amino end on the outside of the cell. (You need domain A for a later question.) Now suppose you freeze fracture some cells and look at the plasma membrane. You find protein P on the P (protoplasmic) face of the membrane.

A. The part of protein P you see in the electron micrograph is probably the (amino end)) (the middle) (the carboxyl end) (either end) of mature protein P, AND it probably corresponds to domain(s) _____.

B. When protein P is in the plasma membrane, it probably is
(an intrinsic protein) (an extrinsic protein on one side or the other) (an extrinsic protein on the outside) (an extrinsic protein on the inside) (any of these).

C. The number of transmembrane segments should be _____.

D. If there is carbohydrate attached to protein P, you would expect to find it on domain(s) _____.

The following 2 questions (and some more in 3R) were on exam #1 of '04. They are all about a family of proteins that we will call presidins (in honor of President's day). All members of the presidin family are single pass transmembrane proteins. Note that different questions are about different members of the family, and all the questions are independent. These problems are designed to review the various proteins and cell structures.

1-18. A. All members of the presidin family are best described as (soluble proteins) (intrinsic membrane proteins) (peripheral membrane proteins) (either type of membrane protein) (will depend on which presidin we are talking about).

B. Which of the following proteins could be presidins? (fibronectin) (clathrin) (spectrin) (collagen) (lamins) (cadherin) (mature acid hydrolase) (actin) (tubulin) (RBC anion exchanger) (catalase) (none of these). Circle all that fit the bill.

C. Fill in each blank below with the name of one appropriate protein from the list given above or "none of these." (The protein you chose does not have to be a presidin.) Do not use the same protein more than once. For each choice, give ONE sentence that explains why the protein you selected fits the criteria.

(1). Movement of vesicles using dynein requires _____ .

(2). _____ is a protein with a nuclear localization signal.

(3). _____ is a protein made on free ribosomes and then found in an organelle with a single membrane.

(4). _____ is a protein that is part of the ECM.

(5). When the protein _____ is defective, red blood cells are unusually fragile. (But other cells are normal.)

(6). When the protein _____ is defective, some epithelial cells can separate from their neighbors and cause severe blistering of the skin. (But attachment to ECM is normal.)

1-19. Suppose presidin C is an integrin in the plasma membrane of an epithelial cell. The cell is part of an epithelium surrounding the lumen of the intestine. (Presidin C has a cytoplasmic domain, an extracellular domain, and one transmembrane domain.)

A. Consider the following structures. Which of them is most likely to contain presidin C? (a gap junction) (an adherens junction) (a hemidesmosome) (a desmosome) (either type of junction) (a hemidesmosome or a desmosome) (any of these).

B. What should be connected (directly or through intermediary proteins) to presidin C?

(i) The cytoplasmic domain of presidin C should be connected to:
(MF) (MT) (IF) (MT or MF) (MF or IF) (MT or IF) (MT, MF, or IF) (MF or none of these) (none of these).

(ii) The extracellular domain of the presidin C should be connected to:
(a transmembrane protein from the next cell) (a protein in the ECM) (either one) (both) (neither).

C. Where should the presidin C be located? It should be (on the apical surface of the cell) (on the basal part of the BL surface) (on the lateral part of the BL surface) (on any part of the BL surface) (on any part of the plasma membrane).

Draw the cell, showing your chosen structure. Indicate where the presidin C is, and how it is connected to your choices in B.

1-20. Consider two transmembrane proteins. Protein A is a single pass protein. Protein B is a multipass protein with 4 transmembrane domains. Assume for this question that both proteins are found in the plasma membrane, and the carboxyl ends of both proteins interact with cytoplasmic proteins.

A. How many extracellular domain(s) does each protein have? Protein A has _____ extracellular domain(s), and protein B has _____ extracellular domain(s).

B. Draw a picture showing how both proteins fit into the plasma membrane.

(1). Label the P and E sides of the membrane,

(2). Label the ends of the proteins, and

(3). Show where the plane of fracture would be if the membrane were split by the freeze-fracture technique.

C. Which protein(s) could have oligosaccharides attached to two different domains of the protein? (A) (B) (both) (neither) (can't predict). Circle the best answer and then explain in a sentence or two.

1-21. Suppose proteins A & B described in the previous problem are the major proteins found in tight junctions.

A. Consider an epithelium – a layer of cells connected by the usual junctions. If protein A or B is defective, you would expect

A-1. The passage of ions from cell to cell in the epithelium to (increase) (decrease) (stay the same).

A-2. The passage of ions across the epithelium should (increase) (decrease) (stay the same).

B. Consider the polarized cells of an epithelium – the cells have an apical surface and a basolateral (BL) surface. What should be the final destination of normal protein A? (either the BL or apical membrane) (both the BL and apical membranes) (the soluble cytoplasm) (an internal membrane of some organelle) (the plasma membrane area at the border between the apical and BL regions) (can't predict). Explain each part of this question briefly.

*1-22. You have an antibody to a human protein called ENaC. ENaC is found only in the apical membrane of colon epithelial cells. It's function does not matter for this problem. You break open cells, solubilize the membranes, and use the antibody to precipitate the ENaC protein. You then analyze the proteins in the precipitate, and you find that it contains both ENaC and spectrin A. (Assume spectrin A is not the same as RBC spectrin, but is in the same protein family.)

A. The simplest interpretation of these results is that:

A-1. (Antibody to ENaC binds to spectrin A) (ENaC binds to spectrin A) (both of these occur) (something else is going on -- neither of these types of binding can explain this result).

A-2. In cells of the colon, ENaC is probably connected to (an actin cytoskeletal network) (a tubulin cytoskeletal network) (either one) (neither) (any of these -- can't tell from information given). Explain briefly.

B. You can alter epithelial cells (by genetic engineering) so that the cells produce protein segments fused to GFP. Suppose you can isolate 2 different fusion proteins and inject them into cells. Then you can precipitate either of the fusion proteins (see below) with antibodies to ENaC. The details, the results, and the questions, are on the next page.

Problem 1-22, cont.

Details of Experiments:
You inject cells with one of the following 2 fusion proteins. Each protein contains a 50 amino acid section of ENaC fused to GFP (green fluorescent protein). Both fusion proteins are soluble. Normal ENaC has both ends in the cytoplasm.

Fusion protein #1 = First 50 AA of ENaC fused to GFP. (Only first 50 AA of ENaC are included.)

Fusion protein #2 = Last 50 AA of ENaC fused to GFP. (Only last 50 AA ENaC are included.)

Results:
In cells injected with fusion protein 1, there is diffuse fluorescence all over the cytoplasm.

In cells injected with fusion protein 2, you 'light up' the apical portion of the cells. (That is, you detect intense fluorescence in the apical portion.)

Questions:

B. Given the results reported above:

(1). You will expect to find spectrin A in the precipitate if you add antibodies to fusion protein (#1) (#2) (either one) (neither).

(2). Normal ENaC is probably anchored to the cytoskeleton by its (amino end) (carboxyl end) (both) (neither).

(3). In colon epithelial cells, spectrin A is probably found (throughout the cytoplasm) (in the ECM) (on the apical side of the plasma membrane) (on the basolateral side of the plasma membrane) (all around the plasma membrane) (can't predict).

Explain how your answers follow from the results.

C. To detect the proteins in the precipitate you have four different types of antibodies. You have unlabeled antibodies to ENaC and to spectrin A. You also have labeled secondary antibodies. The labeled antibodies are either goat anti-rabbit (for detecting ENaC) or goat anti-mouse (for detecting spectrin A). I am not making this up – it comes from an actual paper.

C-1. Two different labeled antibodies are needed here because the two *unlabeled* antibodies are (directed against different proteins) (made in different species) (both) (neither).

C-2. Suppose you actually use the antibodies described above to analyze the precipitate. (See top of page for how you got the precipitate.) You dissolve the precipitate, and separate the proteins by SDS gel electrophoresis. Then you use the antibodies to visualize where the proteins are in the gel (really a blot of the gel). Suppose you add the unlabeled antibody to ENaC to the gel, wash off unattached antibody, and add the labeled goat anti-mouse antibody.

(1). How many labeled bands will you see on the gel? (0) (1) (2) (>2) (can't predict).

(2). Suppose you repeat the experiment, but use unlabeled antibody to spectrin A instead. All other conditions are the same. How many labeled bands will you see on the gel this time? (0) (1) (2) (>2) (can't predict).

***1-23.** There is a protein named emerin that is 254 amino acids long. The protein has 1 hydrophobic region 11 amino acids away from its carboxyl end. All the rest is hydrophilic. Emerin has many sites where phosphorylation is possible, and only one where glycosylation is possible. When cells are fractionated, emerin is in the membrane fraction. (See note at * below.)

A. If emerin is in the part of the membrane fraction containing the plasma membrane, then:

A-1. You expect emerin to be (a peripheral protein on the cytoplasmic side) (a peripheral protein on the extracellular side) (a single pass protein) (a multipass protein) (can't predict).

A-2. The amino end of emerin is probably (in the cytoplasm) (in the membrane) (extracellular) (cytoplasmic or extracellular) (any of these – can't predict). See hint if you can't decide.

A-3. If the protein is glycosylated, the sugars are probably attached to amino acids that are (near the amino end) (near the carboxyl end) (near one end or the other) (near both ends) (not necessarily near either end).

A-4. The largest section of emerin that is in the cytoplasm should contain approximately _____ amino acids.

Draw a labeled picture showing the major domains and features of emerin, including how it is oriented relative to the bilayer of the membrane and the cytoplasm. Explain briefly how you got your number in A-4.

Hint: Which parts of the protein are usually glycosylated? Phosphorylated?

*Note: 'Fractionated' does not mean 'freeze fractured.' The two terms are unrelated. 'Fractionated' means the cells were broken open and the parts were separated into various fractions by biochemical methods.

B. Defects in emerin can cause the muscle wasting syndrome called EDMD or Emery-Dreifuss muscular dystrophy. (That's how the protein got the name 'emerin.') Defects in lamin A can also cause EDMD. Defects in other proteins do not cause EDMD. Given this information,

B-1. The most likely cell localization for mature emerin is (the plasma membrane) (the ER or Golgi membrane) (the inner nuclear membrane) (the outer nuclear membrane) (either nuclear membrane) (any cell membrane) (any of these).

B-2. In a normal person, emerin is probably bound to: (MF) (MT) (IF) (IF or MF) (MF or MT) (any of these) (none of these).

C. Suppose you freeze fracture the membrane that normally contains emerin. In a person with EDMD, there are no particles observable on either face of the membrane. In a normal person, where would you expect to see particles? (primarily on the P face) (primarily on the E face) (about equally distributed on both faces of the membrane) (on neither face) (can't predict from information given). How do you know? (See note at **.)

**Note: Only relatively large protein domains containing many amino acids form particles large enough to see in freeze-fractured membranes.

Note: Problems 1-1 to 1-5 are courtesy of Dr. Martie Chalfie; problem 1-15 is based on a problem from the Alberts problem book.

Problem Set #2 -- Membrane Function: Transport

2-1. Using the following two experiments, deduce a mechanism for the uptake of compound C2406 into cells. (It is a good idea to look at these data graphically.) This question courtesy of M. Chalfie.

Experiment 1. C2406 is added to a suspension of cells at an initial concentration of 10 mM. At various times, samples of cells are removed and the concentration of C2406 within the cells is determined.*

Time (sec)	0	5	10	20	40	60	80	100	150
[C2406] (mM) in cells	0	3.6	5.3	7.0	8.2	9.0	9.5	9.8	9.9

Experiment 2. The initial rate of entry of C2406 (μmoles/sec) is determined at different concentrations of the compound.

[C2406] (mM)	0	0.5	1	2	4	6	8	10	15
Initial uptake rate	0	19	28	37	45	51	56	59	65

* The cell volume is much smaller than the volume of solution outside, so uptake does not significantly alter the concentration of C2406 in the solution surrounding the cells.

2-2. Substances F and G can get into cells. They are both hydrophilic and neutral. There are two known mutants, each of which has a defect in the ability to take up a single substance, either F or G.

A. Based on this information, which uptake mechanism(s) is (are) likely? Consider the usual list of suspects: diffusion -- simple, through ion channels, or facilitated; active transport, primary or secondary.

B. If the Na^+/K^+ pump is inhibited, substance F continues to enter the cell for a while, but then its rate of uptake decreases. Give the information so far, which uptake mechanism(s) is (are) likely?

C. If energy metabolism is inhibited, substance G continues to enter the cell for a much shorter time than in B, then the rate of uptake decreases. Which uptake mechanism(s) is (are) likely?

2-3. You have cells that normally take up amino acids. You do two different experiments with these cells -- either you add an ionophore or you inhibit the Na^+/K^+ pump.

If you add an ionophore that creates an ungated (always open) Na^+ channel in the plasma membrane, transport of amino acids stops almost immediately.

If you inhibit the Na^+/K^+ pump, amino acid transport continues for a while, and then stops.

A. The most likely mechanism for uptake of amino acids in these cells is (primary active transport) (secondary active transport) (simple diffusion) (facilitated diffusion) (diffusion through an ion channel) (any of these).

Before continuing to part B, please look up the answer to part A on p. 22 to be sure you are correct.

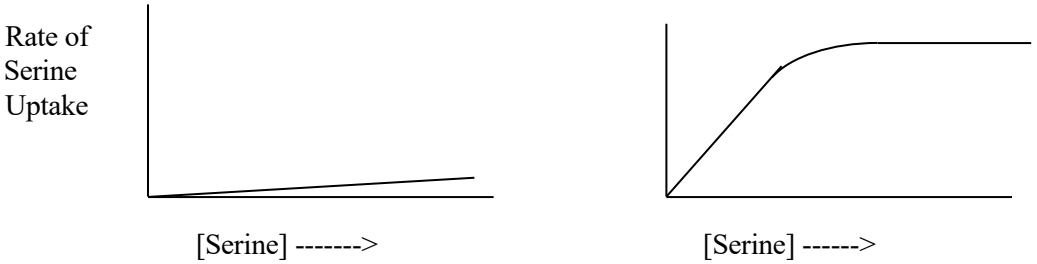
B. Amino acids should be able to enter the cell as long as ($[Na^+]$ inside is > outside) ($[Na^+]$ inside is < outside) ([amino acid] inside is > outside) ([amino acid] inside is < outside) ($[Na^+]$ inside is > $[K^+]$ inside) ($[Na^+]$ inside is < $[K^+]$ inside).

C. It is likely that the transport protein involved in amino acid uptake binds (Na^+) (K^+) (ATP) (amino acids) (none of these -- no transport protein should be required) on the outside of the cell.
For parts B & C, circle all appropriate answers and explain all your choices.

D. Suppose you measure the rate of uptake of amino acids. You vary the [amino acid] but use a fixed (relatively high) concentration of all other soluble components needed. When should the rate of amino acid transport be proportional to [amino acid]? (only at relatively low [AA]) (only at relatively high [AA]) (at all [AA]) (none of these -- it should be independent of [AA] at all concentrations). Explain.

2-4. You can make artificial vesicles with only a lipid bilayer (no protein at all). You can then add specific proteins to the bilayer and see how the properties of the vesicles change. (Assume you can get the protein properly inserted into or attached to the bilayer.) You decide to use this system to measure transport of the amino acid serine across a membrane. First you measure uptake in vesicles with no protein. (Graph #1.) Then you measure uptake using a second batch of vesicles that contain protein P inserted into the bilayer. (Graph #2.) In all experiments, the only molecule added to the solution outside the vesicles is serine, and the concentrations of all ions inside and outside of the vesicles is the same. No ATP is present.

Graph #1 **Graph #2**



A. Given the results shown in the graphs, how is serine being transported into the vesicles?

A-1. In vesicles containing protein P, uptake of serine most likely occurs by (primary active transport) (secondary active transport) (active transport, but can't tell which kind) (facilitated diffusion) (simple diffusion) (passage through a channel) (receptor mediated endocytosis) (beats me), AND

A-2. Protein P is best described as (a pump) (a channel protein) (a carrier or permease) (a receptor) (a kinase) (a phosphatase) (no one of these is best -- more than 2 of these terms are equally apt -- namely _____). How do you know?

Before continuing to part B, please look up the answer to part A on p. 22 to be sure you are correct.

B. Protein P is normally located in the plasma membrane. In its normal position, it is most likely to be (a peripheral protein on the cytoplasmic side) (a peripheral protein on the outside of the membrane) (a transmembrane protein) (a peripheral protein on one side or the other).

C. Now suppose you do another experiment and measure the concentration of serine inside and outside the vesicles as a function of time (in vesicles containing protein P). You get a curve of $[ser]_{in}$ vs time (min). Call this graph #3. The curve is the same shape as graph #2 above, but remember that the axes are different. (It probably pays to draw curve #3 for yourself.) Suppose there is an arrow pointing to the middle of the flat part of the curve. At the time indicated by the arrow, you would expect ($[ser]_{in} = [ser]_{out}$) ($[ser]_{in} > [ser]_{out}$) ($[ser]_{in} < [ser]_{out}$) (can't predict). Explain your answer. (Note: to get credit for your explanation, you had to explain the mechanism, not just name the process.)

D. You repeat all the experiments described so far with a series of different amino acids. With threonine you get results very similar to the ones with serine. With lysine, aspartic acid or valine, the uptake in vesicles with peptide P is identical to the uptake in vesicles without peptide P. Explain this result in terms of amino acid structure and the mechanism of uptake involved here. (Amino acid structures were provided; look them up in your text.)

E-1. Suppose you measure transport of serine through gap junctions from cell A to Cell B. You measure the rate of transport as a function of concentration of serine in cell A. In this case, the curve is likely to have the same shape as (graph #1) (#2) (either way) (neither of these), AND

E-2. If you measure transport through tight junctions, the curve should have the same shape as (graph #1) (#2) (either way) (neither of these). Explain both answers.

2-5. You lyse red blood cells and wash out all the cytoplasm. You put the washed membranes in a solution containing ATP, K^+, Na^+ (and negative ions) and reseal the ghosts **inside out**.

A. If ATP is split (hydrolyzed), what should happen to the concentration of K^+, Na^+ and ATP on the inside of the ghosts?

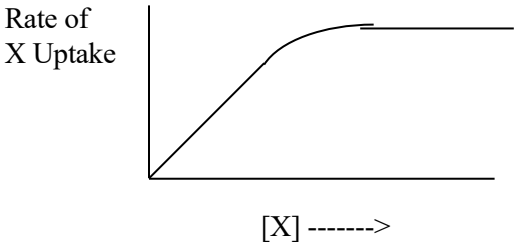
A-1. The Na^+ concentration inside should (rise) (fall) (stay the same), AND

A-2. The K^+ concentration inside should (rise) (fall) (stay the same), AND

A-3. The ATP concentration inside should (rise) (fall) (stay the same).

B. Suppose you can obtain sealed ghosts and the negative ions in the solution are Cl^- and HCO_3^-. You wait until the system reaches equilibrium for these anions. You then add additional HCO_3^- so the HCO_3^- concentration on the outside is higher than on the inside. In order for HCO_3^- to enter the ghosts, which of the following must occur? (membranes must be sealed right side out) (ATP must be hydrolyzed) (membranes must be sealed inside out) (Cl^- must leave the ghosts) (Cl^- must enter the ghosts) (HCO_3^- concentration outside must remain higher than the concentration inside) (none of these). To keep it simple, assume there is no potential (charge) difference across the membrane. Circle all appropriate answers and explain.

2-6. Here is a graph showing the rate of uptake of compound X vs concentration of X. (Assume the concentrations used are physiological.)



A. Given the observed results, which of the following mechanisms of transport can you rule out? (carrier mediated facilitated diffusion) (simple diffusion) (active transport) (Receptor mediated endocytosis) (pinocytosis -- no receptor) (ion channel) (more than one of these) (none of these).

B. You make antibody (in a mouse) to a cell surface protein that binds compound X. You also have fluorescent anti-mouse antibody (made in a goat), so you can do a double (indirect) immunofluorescent labeling experiment. First you treat the cells with the mouse antibody, then you add goat anti-mouse antibody, and then you wash off all unattached antibody and look for fluorescence. At first you see fluorescence distributed evenly but sparsely all over the cell. Then after about 30 minutes you see the fluorescence concentrated in clusters around the cell. Then after about 2 hours, no fluorescence is visible on the surface of the cell. What is the most likely mechanism of transport of compound X into the cell? (Assume that the goat anti-mouse doesn't cross link individual mouse antibody molecules.)

C. You have a mutant that makes abnormal clathrin -- the mutant clathrin works at 23ºC but does not function at 37ºC. You grow some cells at 23ºC, and do the indirect antibody procedure as in part B. After you add the second antibody and wash at 23ºC, you raise the temperature to 37ºC. After 2 hours, the fluorescence should be (sparse, all over the cell surface) (in clusters on the cell surface) (sparse or in clusters) (gone from the cell surface) (can't predict). Explain what you think is wrong with the clathrin, and how that could produce the observed results.

2-7. Suppose you obtain a mutant that cannot take up substance E, which is hydrophilic and neutral. (All other substances are taken up normally in the mutant.) When substance E enters normal cells, it is found in endosomes before it is found free in the cytoplasm. Based on this information, which uptake mechanism(s) is (are) likely (for compound E), and what protein is probably abnormal in the mutant?

2-8. You wish to follow the path of a ligand into a cell, so you add a pinch of labeled ligand. Ligand uptake requires energy and the presence of a specific protein in the cell membrane. You measure the amount of ligand in the appropriate cell compartments, and get the following results:

Radioactivity/Cell Fraction

Compartment	5 min	10 min	15 min	30 min
Endosomes	-	++	+	-
Lysosomes	-	+	++	-
Coated Vesicles	++	-	-	-
Peroxisomes	-	-	-	-
Cytoplasm (w/o organelles)	-	-	-	-

A. From the results shown, it seems most likely that your ligand is entering the cell by (facilitated diffusion) (simple diffusion) (active transport) (receptor mediated endocytosis) (active transport or RME) (any of these but simple diffusion) (any of these).
Before continuing to part B, please look up the answer to part A on p. 22 to be sure you are correct.

B. You would expect to find labeled ligand in coated pits (never) (in <5 min.) (in about 5 min.) (about 10 min.) (about 15 min.) (about 30 min.) (> 30 min.).

For the remaining parts of this question, circle all answers that are appropriate (& explain).

C. Suppose you can also label the specific membrane protein required for uptake (in a way that does not interfere with its function). If you follow the labeled membrane protein, you never find any in lysosomes. You would expect to find labeled membrane protein, at the appropriate times, in (endosomes) (peroxisomes) (coated vesicles) (none of these), AND the membrane protein is probably never found in lysosomes because the protein (stays on the plasma membrane) (is recycled back to the plasma membrane) (is degraded) (none of these).

D. Suppose the membrane bound organelles discussed in this problem all have the same protein/lipid ratio and about the same diameter. In that case, you could probably measure the amount of label in each organelle (after differential centrifugation) (after equilibrium centrifugation) (by autoradiography) (by none of these methods -- none of them would work).

2-9. Assume you are looking at the layer of epithelial cells surrounding the intestine.

A. If you eat a lot of candy (containing glucose), active transport of glucose across the basolateral surfaces of the epithelial cells should (increase) (decrease) (stay the same) (stay the same at zero) AND active transport of glucose across the apical surfaces should _____.

B. Suppose you measure the rate of transport of glucose in the tissues from a human. (You measure the rate of transport from one cell or sub-cellular compartment to another as a function of glucose concentration in the first compartment. You should get a curve that looks like #2 of problem 2-4 if you measure transport (into muscle cells) (into epithelial cells of the intestine) (into capillaries) (out of capillaries) (out of the intestinal epithelial cells) (none of these). Circle all that apply.

2-10. There are many different transport systems for hydrogen ions. Two of the systems for transporting hydrogen ions out of cells across the plasma membrane are as follows:

(a). Hydrogen ions are transported out; transport requires a Na^+ gradient.

(b). Hydrogen ions are transported out in exchange for K^+ (1:1); transport requires hydrolysis of ATP.

A. Transport system (a) is an example of (symport) (uniport) (antiport) (none of these).

B. Transport system (b) is an example of (primary active transport) (secondary active transport) (both) (neither).

C. Suppose a cell runs out of ATP.

(i). In the short run you would expect inhibition of (a) (b) (both) (neither).

(ii). In the long run you would expect inhibition of (a) (b) (both) (neither).

2-11. Consider two receptors, R-1 and R-2 that bind their corresponding ligands. Both receptors internalize their ligands by RME, and both ligands are degraded within the cell.

A. Suppose you follow the receptors during RME. Without additional information, you can predict that both receptors will sooner or later be found in (uncoated endocytic vesicles) (coated vesicles) (mitochondria) (peroxisomes) (free in the cytoplasm) (Golgi) (lysosomes) (endosomes) (none of these). Circle all that apply.

B. Suppose you follow the ligands during RME. The ligands should be found first in (uncoated endocytic vesicles) (ER) (lysosomes) (coated vesicles) (peroxisomes) (endosomes) (depends on the ligand) and last in (uncoated endocytic vesicles) (ER) (lysosomes) (coated vesicles) (peroxisomes) (endosomes) (depends on the ligand).
Outline the pathway(s) that the ligands and receptors follow, based on the information so far. No pictures are necessary, just words.

C. Most cell surface receptors have a half life of a few days. If ligand for receptor R-1 is present, the half life of the receptor is reduced to about one hour. From these results, you can conclude that

C-1. Receptor R-1 separates from its ligand in (uncoated endocytic vesicles) (coated vesicles) (endosomes) (none of these) (can't predict).

C-2. Receptor R-1 that has bound its ligand usually ends up at the end of RME (in lysosomes) (in peroxisomes) (recycled back to the plasma membrane) (recycled or in lysosomes) (any of these) (none of these). Explain how ligand reduces the half life of receptor.

D. The half life of R-2 is unaffected by the presence or absence of ligand. However, the amount of R-2 on the cell surface is reduced in the presence of ligand. From these results it seems that:

D-1. Receptor R-2 separates from its ligand in (uncoated endocytic vesicles) (coated vesicles) (endosomes) (none of these) (can't predict).

D-2. Receptor R-2 that has bound its ligand usually ends up at the end of RME (in lysosomes) (in peroxisomes) (recycled back to the plasma membrane) (recycled or in lysosomes) (any of these) (none of these).

D-3. Receptor R-2 is more likely to enter coated pits (if it is bound to ligand) (if ligand is not bound) (either way -- whether ligand is bound or not should make no difference) (either way – can't predict if binding of ligand will have any effect or not).

Explain how ligand reduces the amount of receptor R-2 on the surface.

***2-12.** Many epithelial cells have a Na^+ channel in their apical membrane. The protein that makes up the channel is called ENaC for epithelial sodium channel. The channel is found in the epithelial cells surrounding the colon (part of the large intestine); it is NOT found in the epithelium surrounding the small intestines and upper GI tract.

A. Na^+ is absorbed in the colon. (Absorption = transport of Na^+ from the lumen of the colon to the interstitial fluid.) Assume the epithelial cells surrounding the colon have no other Na^+ transport protein (other than ENaC) in their ***apical*** membrane. For each part of A-1 & A-2, circle one or more correct answers. Answer all parts of A, and then explain all your answers. (It may help to draw the set up.)

A-1. Na^+ absorption from the colon to the interstitial fluid should require transport of Na^+ through: (gap junctions) (adherens junctions) (desmosomes) (tight junctions) (the cytoplasm of epithelial cells) (the endo-membrane system of epithelial cells) (none of these).

A-2. Na^+ transport from the lumen to the interstitial fluid should require (primary active transport) (secondary active transport) (channel mediated facilitated diffusion) (transcytosis) (carrier mediated facilitated diffusion) (simple diffusion without a protein) (none of these).

A-3. Suppose the concentration of Na^+ in the colon varies considerably under normal circumstances. The rate of Na^+ absorption into the interstitial fluid will probably be:
(proportional to the $[Na^+]$ at lower levels of Na^+) (independent of the $[Na^+]$)
(proportional to the $[Na^+]$ at high levels of Na^+) (proportional to the $[Na^+]$ at all levels of Na^+).
Explain your answers to all parts of A briefly.

B. Suppose you have an analog of ATP that binds like ATP but can not be hydrolyzed. You inject the analog into colon epithelial cells. Assume the starting Na^+ & ATP concentrations in the cells and/or the medium are normal, and you add a large excess of the analog. (Ratio of [analog] / [ATP] is high.) After you add the analog, you measure Na^+ transport.

B-1. The rate of Na^+ leaving the lumen will (slow down immediately) (slow down after a while) (stay the same) (slow down only when the cell dies) (increase immediately) (increase after a while) AND

B-2. The rate of Na^+ reaching the interstitial fluid will _____. (same choices).

C. In the colon, the epithelial cells have a GLUT protein on the basolateral side. The cells do not have any other glucose transport proteins.

C-1. In a normal human, you would expect this protein to transport glucose (into the cells) (out of the cells) (both ways) (one way or the other, but can't predict which) (any of these), AND

C-2. If you add the ATP analog described above to the cells, the rate of glucose transport should: (increase) (decrease) (stay the same) (change direction) (stay the same at zero). Ignore any gross effects on overall energy metabolism, and stick to effects on transport using GLUT.

D. Suppose, for this part only, that the amount of ENaC in the apical membrane varies, depending on diet, and that the level is higher on a low sodium diet. (I am making this up for study purposes; it may or may not be true.) Assume that the amount of ENaC per cell and the amount of ENaC mRNA per cell is about the same, regardless of diet.

D-1. If you examine the ENaC in cells from rats grown on a high sodium diet, you should find some ENaC in (the soluble cytoplasm) (lysosomes) (peroxisomes) (nuclei) (vesicle lumens) (vesicle membranes) (lysosomes and some other type of vesicle) (none of these) (all of these).

D-2. Which of the following is most likely to happen if the rat is switched to a low sodium diet? (increase in synthesis of ENaC) (decrease in degradation of ENaC) (exocytosis) (endocytosis) (insertion of soluble ENaC into the plasma membrane) (transcytosis).

2R -- Review Questions on Problem Sets 1 & 2 – Cell Structure & Transport

The questions in the Review Problem sets are from exams of the last few years. On the actual exams, some of the parts had to be explained and some didn't. For study purposes, it is usually best to try and explain everything. That way you are more likely to arrive at a thorough understanding of the problems (& answers). You can write out the explanations for yourself, but it is usually even better to explain the answers to a study partner or exchange written answers with a partner. You and your partner can then check each other's answers. If you can't find a partner, try explaining everything out loud to yourself.

2R-1. Proteins are hydrolyzed (broken down) in the intestine. The hydrolysis produces some amino acids, but mostly it produces di- and tri-peptides. The peptides are transported from the lumen of the intestine into epithelial cells using a protein that is called pepT (for peptide transporter). PepT carries out peptide/H^+ co-transport – peptide transport is dependent on proton transport.

A-1. Transport of ***peptides*** into the epithelial cells by pepT is best described as:
(primary active transport) (secondary active transport) (either type of active transport)
(facilitated diffusion) (simple diffusion) (either type of diffusion) (any of these).

A-2. The peptides in the epithelial cell are hydrolyzed to amino acids and the amino acids are then transported out of the epithelial cells across the basolateral membrane. Transport of ***amino acids*** across the BL membrane probably uses:
(a channel protein) (a carrier protein) (a pump or co-transporter protein) (a channel or carrier protein)
(a carrier, pump, or co-transporter protein) (any of these) (none of these).

B. Researchers have cloned (isolated) the pepT gene and added the gene to oocytes (immature egg cells). The idea is to get the genetically engineered oocytes to make pepT so the researchers can study pepT function in a convenient system.

B-1. Why didn't the researchers put the gene into RBC? The researchers won't be able to study pepT function in genetically engineered RBC because: (it is difficult to measure transport in or out of RBC)
(RBC don't normally make pepT) (RBC won't be able to make pepT from the added gene)
(RBC are too hard to get) (all of these) (none of these – must be some other reason).

B-2. PepT is expressed in genetically engineered oocytes – that is, the added gene is transcribed, and the protein is made and inserted into the plasma membrane of the cells. The researchers put the genetically engineered oocytes into various solutions containing peptides and the usual concentrations of Na^+ and K^+ found in the extracellular (interstitial) fluid. Then they measured the transport of peptides into the cells. Their results are as follows:

Expt. #	Type of cells	Solution outside of Cells	Intracellular pH	Transport of peptides into cells?
1	Oocytes + pepT gene	pH 6	pH 7.2	Yes
2	Oocytes + pepT gene	pH 7.2	pH 7.2	?
3	Oocytes + pepT gene	pH 6	pH 6	?
4	Oocytes + pepT gene	pH 7.2	pH 6	?
5	Normal oocytes	pH 6	pH 7.2	No

(1) Why is there no transport of peptides in experiment #5? Explain in one sentence.

(2). What do you expect for experiments 2 & 3? You expect transport of peptides into the cells in (both) (only 2) (only 3) (neither).

(3). In experiment #4, if you could measure transport of peptides in either direction, you would expect: (no net transport of peptides) (net transport of peptides into the oocytes) (net transport of peptides out of the oocytes) (transport of peptides, but can't predict direction) (no idea).

2R-2. The structures of pepT, all GLUT proteins (glucose transporters), and the glucose/Na^+ co-transporter (SGLT for sodium glucose transporter) have been determined. All have 12 transmembrane domains. PepT can transport many different peptides, but it does not transport amino acids or sugars.

A. Based on the information so far, you would expect a greater similarity in 3D structure and/or amino acid sequence between pepT and (GLUT proteins) (SGLT protein)
(neither -- both should be equally similar to pepT) (either way -- can't predict). Explain why.

B. All GLUT's are glycosylated between transmembrane domains 1 & 2. Where do you expect to find the amino end of the protein? (in the cytoplasm) (outside the cell) (within the membrane)
(in the cytoplasm or outside the cell) (can't tell from information given).

C. SGLT is glycosylated between transmembrane domains 5 & 6. Could SGLT & GLUT's have the same orientation in the membrane? (yes) (no) (can't tell from information given).

D. In SGLT there is a site in the amino acid sequence hydrolyzed by the enzyme chymotrypsin. This site is located between transmembrane domains 11 & 12. You have some intact epithelial cells. If you add chymotrypsin to the outside of the cells, the protein would be:
(split) (unaffected) (split in half the cells) (can't predict from info given).

For B-D: Make a simple diagram of SGLT (not GLUT) that shows how the SGLT is located in the membrane. Label the inside and outside of the cell, and indicate where the protein is glycosylated, where it is split by chymotrypsin, and (if there is enough information) where the carboxyl and amino ends are (relative to inside and outside).

2R-3. Consider the following list of organelles and/or structures: Lysosomes (L), Peroxisomes (P), Microfilaments (MF), Mitochondria (Mito), Nuclei (N), Nucleoli (Ni), Desmosomes (D), Focal adhesions (FA).

A. Which structure(s) on the list is/are surrounded by a single membrane (one bilayer)? _____

B. Which structure(s) on the list is/are in the cytoplasm but are NOT part of the endomembrane system? _____

C. Which structure(s) on the list contain functional ribosomes? _____

D. Which structure(s) on the list contain or are connected to some type of intermediate filament? _____

E. Which structure(s) on the list contain or are connected to actin? _____

Draw a crude diagram of a cell that shows the relative positions of all the items on the list except MF. Include (& label) one of each of the items mentioned. Do not include any other structures except the plasma membrane. Artistic ability and fine detail are not important – we only want to know the relative positions of the structures.

2R- 4. Using genetic engineering you make an organism that has a fusion protein consisting of GFP (green fluorescent protein) attached to one end of pepT.

A-1. In normal epithelial cells, you would expect to detect fluorescence:
(all around the membrane of the cell) (only in the cytoplasm) (in the apical part of the membrane)
(in the basolateral part of the membrane) (either part of the membrane, but not both) (can't predict).

A-2. If you expose epithelia to certain conditions, such as low Ca^{++}, the tight junctions come apart. Suppose you put your layer of epithelial cells into a medium lacking Ca^{++}, and examine the amount of fluorescence. If the total amount of fluorescence per cell stays the same, the amount in the part of the membrane facing the lumen should: (increase) (decrease) (stay the same) (stay the same at zero).

Problem 2R-4, cont.

B. Epithelial cells exposed to certain hormones transport peptides faster than unexposed cells. The amount of pepT protein in the cells is unchanged by the presence of hormone, and the transport ability of each molecule of pepT is unaffected. If you add hormone to a cell containing pepT-GFP fusion protein, you see an increase in fluorescence in one surface (BL or Apical) of the membrane. Given these results, the most likely effect of the hormone is to promote:
(endocytosis) (exocytosis) (synthesis of more pepT) (change of state of phosphorylation of pepT). Explain briefly what is happening. (See note at end of problem.)

C. Suppose the hormone is a peptide, and is degraded inside its target cells. However the number of hormone receptors (and amount of pepT) per cell remains constant, whether hormone is present or not. (See note at end of problem.) In that case, after you add hormone to cells containing the fusion protein:

C-1. You would expect endosomes to contain (hormone) (hormone receptors) (both) (neither).

C-2. You would expect to find fluorescence in (lysosomes) (uncoated endocytotic vesicles) (both) (neither).

C-3. You would expect to find hormone ***or*** receptors, ***but not both***, in (lysosomes) (uncoated endocytotic vesicles) (coated endocytotic vesicles) (desmosomes) (mitochondria) (peroxisomes) (none of these). Explain your answer to C-3 only. Do not describe or reproduce the entire class handout – explain only the parts relevant to your answer to C-3.

Note: Hormones are transported by the blood. So where would the hormone and its receptors be found? On which surface of the epithelium? Same side as pep-T?

2R-5. The apical surface of the epithelial cells surrounding the intestinal lumen has a H^+/Na^+ exchanger. This exchanger is used to regulate intracellular pH.

A. If the intracellular Na^+ concentration is in the normal range (relative to the outside), then this exchanger could be used to (raise cell pH) (lower cell pH) (either way) (neither – this exchanger could not effect pH).

B. Under normal conditions, the intracellular pH is 7.2 and the lumen pH is 6. Suppose you start with normal cells under normal conditions and you inhibit the H^+/Na^+ exchanger. All other processes continue normally. Then you would expect the transport of peptides into epithelial cells to (stop immediately) (stop after a while) (continue without any change) (increase).

2R-6. Consider everything that you know about the action of pepT, the H^+/Na^+ exchanger, and the usual modes of transport. Suppose that you have some normal intestinal epithelial cells *in situ* (in their proper place) at normal conditions of pH. (Lumen of the intestine = pH 6; intracellular pH of the epithelial cells = 7.2.)

A. Suppose you add a Na^+ ionophore – a compound that creates open channels for Na^+ in the membrane. (All other processes are normal.)

A-1. Then you would expect the transport of **glucose** into epithelial cells to (stop immediately) (stop after a while) (continue without any change) (increase).

A-2.Then you would expect the transport of **peptides** into epithelial cells to (stop immediately) (stop after a while) (continue without any change) (increase). Explain very briefly the difference, if any, between the two cases.

B. Suppose you inhibit the Na^+/K^+ pump (but all other processes are normal.) What effect would you expect on the transport of **peptides** into epithelial cells?
(No effect at all) (Inhibition, and it would be faster than adding a Na^+ ionophore) (Inhibition, but it would be slower than adding a Na^+ ionophore) (Inhibition, at the same rate as adding a Na^+ ionophore) (Stimulation – transport would speed up) (can't predict from information given).
Explain (or outline) how the action of pepT, the H^+/Na^+ exchanger, and the Na^+/K^+ pump are (or are not) interrelated.

2R-7. If you freeze fracture normal oocyte membranes, both faces of the membrane are relatively smooth. You see only a few small bumps or particles on the inside faces of the membrane.

A. These results indicate that normal oocytes have relatively few (peripheral proteins on the cytoplasmic side) (peripheral proteins on the extracellular side) (transmembrane proteins) (peripheral proteins of either kind) (any of these).

B. Suppose you look directly at the E face, and see a few particles. In the normal, unfractured membrane, the part of the particles that you see was probably located (in the lipid bilayer) (inside the cell in the cytoplasm) (outside the cell) (either inside or outside, but not in the bilayer) (any of these).

C. Suppose you freeze fracture membranes from genetically modified oocytes. Each type of genetically modified oocytes has lots of large particles on one or both faces of the membrane. The size of the particles depends on which gene was added to the cells. If you add a gene for a single pass protein, the cross section of the particles is about 1.5 nm^2. If you add a gene for SGLT, which has 12 transmembrane domains, the cross section of the particles is about 18 nm^2. If you add a gene for connexin (the protein of connexons) the cross section of the particles is about 36 nm^2. It is known that each molecule of connexin has 4 hydrophobic regions, each about 25 amino acids long. (These numbers are based on a real experiment, but have been modified slightly to make the calculations easier.)

C-1. A single molecule of connexin should have a cross section of about _____ nm^2.

C-2. Where would you expect to see particles of 36 nm^2 in freeze fractured membranes from normal somatic cells (no added genes)? You would find them in membranes from (tight junctions) (adherens junctions) (gap junctions) (focal adhesions) (desmosomes) (any of these) (none of these).

The following 4 questions (8-11) are about a type of epithelial cells called parietal cells. Parietal cells are part of the epithelium that lines the stomach. Parietal cells have the usual Na/K pump and additional transport proteins. One of the transport proteins is called the gastric H/K pump. The H/K pump is similar to the Na/K pump, but it moves H^+ instead of Na^+. Both the Na/K pump and the gastric H/K pump have similar structures and mechanisms; they are homologous (evolutionarily related). In active parietal cells, the gastric H/K pump is localized on the side of the cell that faces the lumen of the stomach. Its primary function is to keep the stomach acid (at low pH). You have to figure out where the Na/K pump is.
Note: In these problems, H/K or Na/K means H^+/K^+ or Na^+/K^+. Individual ions are written as Na^+, K^+ or H^+.

2R-8. Consider the gastric H/K pump.

A. The gastric pump carries out (antiport) (symport) (uniport) (antiport or symport) (any of these).

B. When the gastric pump is working, H^+ should move (into the lumen) (out of the lumen) (can't predict)

C. The energy to move the protons should come from: (movement of K^+ down its gradient) (hydrolysis of ATP) (the gradient of H^+ -- no additional energy is necessary) (movement of Na^+ down its gradient) (movement of Na^+ or ATP hydrolysis) (any of these).

D. The gastric pump is located on the (basal) (lateral) (apical) (basolateral) (apicolateral) side of the cell.

Draw a parietal cell. Indicate where *the lumen of the stomach* is, and where *tight junctions* are expected. Show where the gastric H/K pump is, and which way K^+ is moved by the gastric pump. (No additional details needed. Don't try to draw the structure of the pump – just assume it is an **oval** and show where it goes.)

2R-9. Omeprazole inhibits the gastric pump but not the Na/K pump. Ouabain is the opposite – it inhibits the Na/K pump but not the gastric pump. Both inhibitors combine with the **extracellular** domains of their respective target proteins.

A. Ouabain (Drug B) should bind to parietal cells on the side facing:
(the lumen of the stomach) (the interstitial fluid) (either one) (neither – it needs to enter cells to act).

B. Omeprazole (Drug A) should bind to the parietal cells on the side facing:
(the lumen of the stomach) (the interstitial fluid) (either one) (neither – it needs to enter cells to act).

For an explanation of A & B, add the Na/K pump to the picture you drew for the previous problem. (Just show where the pump is – don't draw the complete structure. Assume it is a **rectangle** and put it in the proper place.) Then indicate where drug A (omeprazole) and drug B(ouabain) bind. Be sure you have the proper side of the proper pump.

C. People with reflux disease or ulcers take drugs to reduce stomach acid. Which of the following should be the best treatment? (ouabain – drug B) (Omeprazole – drug A) (both) (neither –- try something else). Explain briefly.

2R-10. For each pump (H/K or Na/K), the α subunit contains the catalytic part of the protein and the part that binds the drugs. The α subunit is a single polypeptide with 10 transmembrane (TM) domains. Remember that the two pumps are homologous, and that the drugs bind to the extracellular domains of their respective pumps. A series of chimeric (recombinant) α subunits have been made that contain parts from each pump protein. Some chimeric α subunits are sensitive to ouabain (inhibited) and some are resistant (not inhibited). The results are shown below. Answer the questions based on the experimental results.

A. Which of the <u>following</u> part(s) of the normal **H/K** alpha subunit binds to ouabain? The part(s) between
(TM 3 & 4) (TM 2 & 3) (TM 1& 2) (TM 3 & 4 and TM 1 &2) (none of these)
(all of these) (can't tell from information given).

B. Which of the part(s) of the normal **Na/K** alpha subunit that are listed bind(s) to ouabain? The parts between _____. (Same choices as in A.)

C. Where is the ***amino*** end of the catalytic subunit that makes up the Na/K pump?
(in the cytoplasm) (extracellular) (either way -- can't predict from information given).

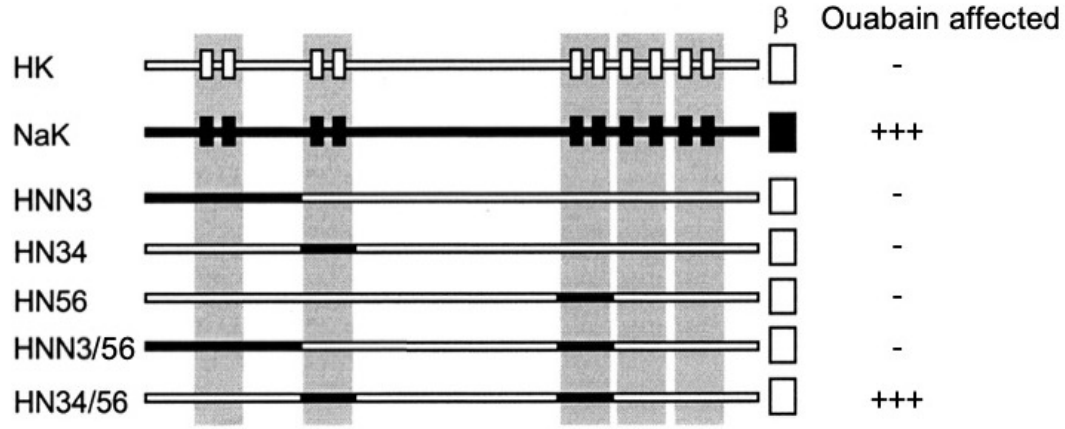
D. The carboxyl end of the Na/K pump should be (on the same side as the amino end)
(on the opposite side) (either way -- can't predict from information given).

E. The carboxyl end of the Na/K pump and the carboxyl end of the H/K pump should be:
(on the same side) (on opposite sides) (either way -- can't predict from information given).

Show how the alpha subunit polypeptide of the ***Na/K*** pump is oriented in the membrane, and where ouabain (Drug B) binds in the normal protein. Indicate the ends of the protein, the TM's that are at or near the spot(s) where the drug binds, and both sides of the membrane.

F. If the α subunit of the Na/K pump is glycosylated, you are more likely to find sugars attached to (TM 3) (TM 2) (TM 1) (the region between TM 2 & TM 3) (the region between TM1 & TM 2) (any of these TM regions) (the region between TM 2 & TM 3 **and/or** the region between TM 1 & TM 2) (can't predict from info given).

Below is a schematic representation of the chimeras and wild type α subunits. The top 2 bars represent the 2 normal α subunits. The remaining bars represent the chimeric proteins. The boxes represent the locations of the TM sequences. The protein is written in the usual convention as to which end is the amino end. The *open regions of the bars* represent sequences from the H/K pump, and the *solid regions* represent sequences from the Na/K pump. Inhibition by ouabain (+++), or lack of inhibition (-) is indicated at the right. The figure is adapted from fig. 1 in Koenderink et al, PNAS, <u>97</u>, 11209 -11214, (2000). The whole article, on which this problem is based, is at http://www.pnas.org/content/97/21/11209.full



2R-11. Parietal cells have additional transport proteins besides the pumps mentioned so far. Their membranes also contain the bicarb/Cl^- anion exchanger, a K^+ channel, and a Cl^- channel. The two channels are on the side of the cell facing the lumen and the anion exchanger is on the opposite side. The cells contain carbonic anhydrase. (Carbonic anhydrase catalyzes: $H_2O + CO_2 \leftrightarrow$ bicarb + H^+).
Bicarb = HCO_3^- .

For this question, consider *only* the processes and transporters mentioned so far about parietal cells. (There may be some other processes & transporters involved, but ignore them.)

A. The stomach contains HCl. Putting all the transport processes mentioned so far together, how does the Cl^- reach the lumen of the stomach?

A-1. Cl^- probably enters the lumen by (primary active transport) (secondary active transport) (passive transport) (primary or secondary active transport) (any of these).

A-2. When the parietal cells are secreting (releasing) Cl^- into the lumen, the anion exchanger should be transporting (both anions *into* the cells) (both anions *out of* the cells) (Cl^- in and bicarb out) (bicarb in and Cl^- out) (only Cl^-) (only bicarb) (the two anions in opposite directions, but can't predict which way) (none of these – the anion exchanger is not involved here).

A-3. When the parietal cells are secreting (releasing) Cl^- into the lumen, carbonic anhydrase is (unnecessary) (catalyzing formation of bicarb) (catalyzing formation of CO_2) (beats me).
Explain (or diagram) briefly how carbonic anhydrase, the anion exchanger, and/or the Cl^- channel are involved in movement of Cl^-. (Ignore movement of protons for now.)

B. When the H/K pump is activated, the pH of the parietal cells usually remains neutral.

B-1. The cells probably remain neutral because the effect of the pump on pH is balanced by (adding protons) (removing protons) (adding or removing protons as needed) (none of these – no balancing movement of protons is needed).

B-2. Which of the following is required to maintain a neutral pH when the H/K pump is running? (addition of CO_2) (removal of CO_2) (neither – CO_2 has nothing to do with it). Explain briefly how you expect the pH balance to be maintained when the H/K pump is running.

C. If the cell does not have the right amount of CO_2 (for balancing pH **or** any other reason), how will the imbalance be fixed? The most likely way is (conversion of CO_2 to bicarb and transport of bicarb) (simple diffusion of CO_2 in or out of the cell) (passive transport of CO_2 using a transport protein) (active transport of CO_2 using a transport protein) (passive transport with or without a transport protein) (any of these).

D. Suppose the H/K pump & the Na/K are working, and the K^+ channel on the lumen side of the membrane is open. What do you expect to happen?

D-1. Some K^+ ions will move from the parietal cells into (the interstitial fluid) (the lumen) (neither) (both).

D-2. The K^+ ions that exit the cells will probably be moved (passively) (actively) (not at all – no K^+ ions should leave) (actively or passively, but can't predict which).

D-3. Net flow of K^+ ions out of the cells will stop when the concentration of K^+ on both sides of the membrane(s) is (higher on the outside) (higher on the inside) (equal) (none of these – K^+ ions won't move) (can't predict). Explain briefly.

2R-12. The epithelial sodium channel, ENaC, is found in the epithelia of the kidney, as well as in the colon. In the kidney, ENaC is targeted to lysosomes by addition of a chemical group (we'll call it Q) to the side chains of certain lysines in ENaC. The enzyme Nedd4 catalyzes the addition of Q. In normal kidney epithelium, under ordinary conditions, ENaC is sent to lysosomes at a steady rate, but the amount of channel protein on the surface remains constant. When the need arises, regulatory factors change the amount of ENaC on the surface (& the rate of Na^+ transport) by altering the rates of production, removal, etc.

A-1. Inhibition of Nedd4 could be used for:
(up-regulation of Na^+ transport) (down-regulation of Na+ transport) (both) (neither).

A-2. Endocytosis of ENaC is probably triggered by:
(binding of ENaC to its receptor) (binding of ENaC to its ligand) (covalent modification of ENaC)
(binding of ENaC to its receptor or ligand) (any of these).

A-3. If Nedd4 activity increases, you would expect to find that the ENaC in the cell:
(increases) (decreases) (relocates, but total stays the same) (stays the same in the same location).

A-4. In a normal cell, the amount of ENaC on the surface probably remains constant because:
(ENaC is recycled by exocytosis) (new ENaC is constantly synthesized)
(ENaC protein is anchored to the cytoskeleton) (Lateral diffusion of ENaC is blocked by tight junctions)
(any of these).
Explain how the amount of ENaC on the surface remains constant, including the role (if any) of Nedd4.

B. Suppose the kidney cells have receptors for LDL, transferrin, & EGF. You isolate several kinds of vesicles from the cells, and suppose the vesicles carry ENaC.

B-1. If you isolate uncoated endocytic vesicles that carry ENaC, the vesicles could also contain
(LDL receptors) (transferrin receptors) (EGF receptors) (none of these) (all of these), AND

B-2. If you isolate vesicles that have budded off from endosomes and carry ENaC, the vesicles could also contain _____. (Same choices).
Pick one or more choices for each part, and explain briefly in a sentence or two.

C. In people with the genetic condition called Liddle's syndrome, there is too much Na^+ transport. Each individual channel transports Na^+ normally, but there is more overall transport than normal. (This causes high blood pressure.) People with Liddle's syndrome have ENaC that is missing the last 45-60 amino acids.

C-1. The most likely cause of the symptoms in Liddle's syndrome is: (too many channels)
(not enough total channels) (channels that fail to reach the apical surface)
(something else – number of channels in apical membrane wouldn't affect Na^+ transport).

C-2. The primary cause of Liddle's syndrome is probably: (a high rate of ENaC synthesis)
(a low rate of ENaC synthesis) (improper folding of ENaC) (a high rate of ENaC endocytosis)
(a slow rate of ENaC endocytosis) (an unusual rate of synthesis or rate of endocytosis) (any of these), AND

C-3. The section with the last 45-60 amino acids probably contains a site for
(binding of ENaC ligand) (binding of ENaC receptor) (enzymatic modification of ENaC)
(binding of ENaC ligand or receptor) (transport of Na^+).

C-4. What result would you expect from changing the activity of Nedd4?
(It should improve the symptoms.) (It should make the symptoms worse.)
(It should have no effect on the symptoms.)
(Effect on symptoms depends on whether you increase or decrease the activity of Nedd4.)
(Can't predict). Explain all your answers to C, and how they fit together.

ANSWERS TO SELECTED QUESTIONS FROM PROBLEM SET 2

For these questions, parts B, C, etc. depend on the answer to part A. Therefore the answer to part A is given here, so you can check it without accidentally seeing the answers to parts B, C, etc.

2-3. A. Secondary active transport.
Transport of amino acids is dependent on a Na^+ gradient because an ionophore which dissipates the gradient stops transport immediately. The gradient is built up by the Na^+/K^+ pump at the expense of ATP. ATP is not needed to transport amino acids directly; it is needed to set up (& replenish) the Na^+ gradient, and the chemical energy of the Na^+ gradient is used to drive amino acid transport (against an amino acid gradient). This is active transport because energy is needed; it is secondary active transport because the ATP is used to set up a gradient, and the gradient (not the ATP itself) drives transport.

2-4. A. Serine is being transported by facilitated diffusion using a carrier or permease.
Since ATP is not present, primary (but not secondary) active transport is ruled out. You know it is not secondary active transport because there is no ion gradient. (You don't need to have ATP present for secondary active transport to occur. In a real cell you might need ATP to set up an ion gradient, but once an ion gradient is established, by any means, it can drive secondary active transport for a while in the absence of ATP.) The only source of energy is the serine gradient itself, so serine must be flowing down its gradient, by either simple or facilitated diffusion. Since the rate of transport saturates as a function of serine concentration, there must be a carrier protein involved that binds to serine. This rules out simple diffusion, with or without a channel, and leaves transport using an enzyme-like carrier (one that binds to serine) as the only option. So this is carrier-mediated transport = facilitated diffusion using a permease. Passage through a protein channel is sometimes called facilitated diffusion, but transport through a channel does not use a protein that binds intimately to the material it transports, and so the rate of transport does not saturate (as a function of concentration) except at extraordinarily high concentrations. So this is not facilitated diffusion through a channel.

2-8. A. Receptor mediated endocytosis (RME). Since process needs a specific protein, you can rule out simple diffusion. Since it needs energy, you can rule out facilitated diffusion, and since it involves vesicles or endosomes, must be endocytosis. (To get full credit, when this was on an exam, you had to mention implications of needing energy, a protein, and going through vesicles.)

Problem Set #3 -- Intracellular Compartments: Structure, Function & Traffic Between Them

3-1. Suppose a plasma membrane protein has 5 transmembrane segments.

A. When the protein is made in normal cells, it should be made on ribosomes that are (attached to the ER) (free in the cytoplasm) (either way).

B. In a normal cell, you would expect to find some of this protein (in or attached to the Golgi membrane) (inside the Golgi lumen -- unattached to the membrane) (either way) (neither) (can't predict).

C. The protein probably has _____ signal peptides (SP). In addition to the SP, it probably has _____ start transfer sequences, _____ stop transfer sequences. The SP (should) (should not) be removed, and the COOH end of the finished peptide should be on the (cytoplasmic) (extracellular) side of the membrane. NOTE: There is more than one possible solution to C. Pick one solution, make sure that all your answers fit, and be sure you can explain how the protein is put in place.

3-2. Insig is an integral membrane protein in the ER. Mature Insig is 277 amino acids long. The gene for Insig encodes 6 hydrophobic sections. Two different structures have been proposed for mature Insig.
Model A: In one of the basic cell biology texts, Insig is shown with 5 transmembrane domains. One end of the protein is in the lumen and the other end is in the cytoplasm.
Model B: A different source says that Insig has 6 transmembrane domains, that both ends of the protein are hydrophilic, and that the amino end is in the cytoplasm.

A-1. You would expect the carboxyl end of the protein to be in the cytoplasm in (A) (B) (both) (neither).

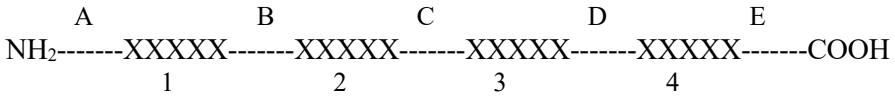
A-2. You would expect the gene for this protein to code for a sequence recognized by signal peptidase in ____. (Same choices for all parts of A.)

A-3. The signal recognition particle should bind to a stretch of amino acids included in the 277 in _____.

A-4. The first transmembrane sequence in the mature protein should be a stop transfer sequence in _____. Explain how either protein structure is possible, given the gene structure. (A picture will help.)

B. Insig works by inhibiting binding of other proteins to the cytoplasmic side (outside) of the ER membrane. The first transmembrane sequence in mature Insig starts at amino acid #84. Mutations that affect amino acids 40-60 of mature Insig do not affect insertion into the ER membrane, but they abolish the inhibitory effects of Insig. Given this information, mature Insig probably has (5 transmembrane domains) (6 transmembrane domains) (either way). Explain your reasoning briefly in terms of structure and function.

3-3. The glutamate receptor is located in the plasma membrane. The protein contains 4 hydrophobic segments (XXXXX) numbered 1-4, 5 hydrophilic segments (-------) labeled A-E plus a signal peptide that is removed. The mature protein looks like this:



A. In the simplest, most straightforward case, the glutamate receptor would have _____ transmembrane domains, _____ extracellular domains, the COOH end should be on the (inside) (outside) (either side) of the plasma membrane, and the amino end should be on the _____. Draw the membrane and show how the protein and all its parts (A-E, 1-4, COOH and NH_2 ends) fit in; explain briefly why you think the protein will look this way.

B. The following results have been recently obtained (about the glutamate receptor):

(1) In a very similar protein from bacteria, the parts of the protein that bind glutamate are sections A and D.

(2). There are chemical reagents that react with the COOH end of proteins. These reagents can not react with the COOH end of the glutamate receptor when they are added to the outside of intact cells.

(3). You can make vesicles from plasma membrane and treat them with proteases. If you use right side out vesicles, region #2 is not degraded. If you use wrong side out vesicles, both ends of region #2 are degraded.

See next page for questions & definition of 'right side out.'.

Problem 3-3, cont.

Vesicles that are 'right side out' have the same orientation as whole cells – what is outside of the vesicle is what would be outside of the cell. These are NOT vesicles formed by RME.

Given all the recent results described on the previous page, what is the most reasonable structure for the glutamate receptor? The receptor probably has _____ transmembrane domains, _____ extracellular domains, the COOH end should be on the (inside) (outside) (either side) of the plasma membrane, and the amino end should be on the _____. Draw the membrane and show how the protein and all its parts fit in, and then explain how your model fits each of the results given.

3-4. Consider Protein P, the protein described in problem 1-17.

A. How can you tell (with out being told outright) from the information given, that the protein should be inserted in the membrane with its amino end on the outside of the plasma membrane?

B. Suppose you can make copies of the gene for peptide P (by genetic engineering) that lack the section(s) that code for domain A and/or C.

B-1. If you delete section A of the gene, the protein should end up (in the plasma membrane) (in the cytoplasm) (secreted) (in the ER or Golgi) (can't predict), AND

B-2. If you delete section C of the gene, the protein should end up (in the plasma membrane) (in the cytoplasm) (secreted) (in the ER or Golgi) (can't predict), AND

B-3. If you delete sections A and C of the gene, the protein should end up (in the plasma membrane) (in the cytoplasm) (secreted) (in the ER or Golgi) (can't predict).

C. Suppose protein P has carbohydrates attached to it, and the glycosylated protein is found in a vesicle inside the cell.

C-1. The carbohydrates should be found on the (outside) (inside) (either side) (both) of the vesicle, AND

C-2. The carbohydrates are most likely to be on section(s) (A) (B) (C) (D) (more than one of these) (can't predict), AND

C-3. The carboxyl end of protein P should be on the (outside) (inside) (either side) (both) of the vesicle.

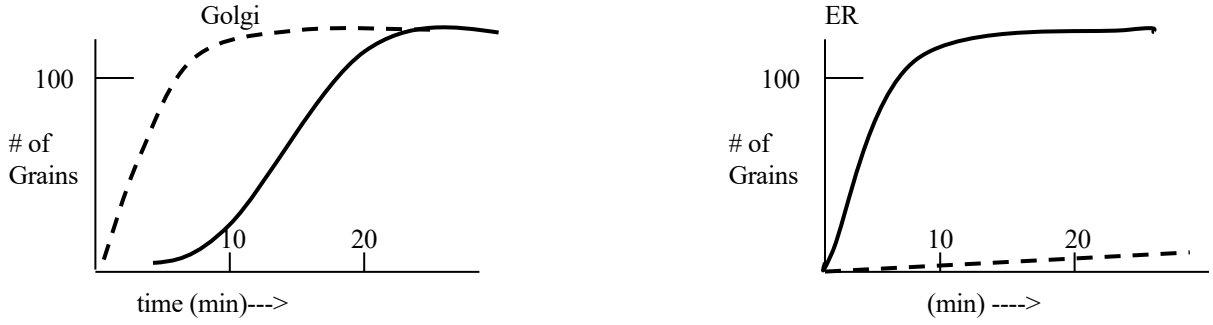
D. You discover that protein P has a complex carbohydrate attached to it that contains (at least) two different sugars, S and T. In one experiment, you feed some cells a radioactive sugar that is a precursor to S; in a second experiment you use a radioactive precursor to T. At 5, 10, 15 .... minutes after adding each precursor, you take samples of the cells for autoradiography. After you wash (to remove unused precursor), fix, expose, and develop the samples, you count grains over the ER and the Golgi. You get the results shown below. (Note: Each graph shows the results of two separate experiments using different precursors.) In each graph, dashed line = precursor to S; solid line = precursor to T.

Given these results,

D-1. Sugar S is added in the (Golgi) (ER) (Both) (Golgi or ER & Golgi) (ER or ER & Golgi),

D-2. Sugar T is added in the _____ , AND

D-3. The first sugar added to each molecule of protein is (S) (T) (can't tell).



3-5. A. How many membranes separate a protein in the mitochondrial matrix from a protein in the nucleolus?

B. If a protein moves from the nucleolus to the mitochondrial matrix, how many separate times must it pass through membranes?

3-6. Suppose protein P (containing a single peptide) is an integral protein in the inner mitochondrial membrane. The gene for protein P is in the nucleus. Assume that in this case, protein P enters the mitochondrial matrix and is then targeted to the inner membrane.

A. How many localization signals would you expect the gene for protein P to code for?

B. How many localization signals should the finished protein P have once it has reached its final destination?

C. Suppose you make radioactive protein P *in vitro* and add it to isolated mitochondria. After a few minutes you isolate proteins from (a) complete reaction mix = mitochondria + added protein P; (b) mitochondrial matrix (c) inner mitochondrial membrane. You run the proteins on gels, and look for radioactive bands.
In (a) you find 3 radioactive bands corresponding to 3 different sized peptides.

C-1. Given your answers to parts A & B, explain the results in (a).

C-2. What should the gels look like for (b) and (c)?

3-7. Suppose protein Q (containing a single peptide) is found in the lumen of peroxisomes.

A. Where do you expect to find the gene for peptide Q?

B. How many localization signals/sequences (in the protein Q) would you expect the gene to code for?

C. How many localization signals/sequences would you expect to find in finished protein Q, once the protein has reached its final destination?

D. Suppose you make radioactive protein *in vitro* and add it to isolated peroxisomes. You wait a few minutes, long enough for some (but not all) of the protein to enter peroxisomes. Then you isolate proteins from your complete reaction mix = peroxisomes + added protein Q. You then run the proteins on gels, and look for radioactive bands (as in problem 3-6). In this experiment, how many radioactive bands do you expect to find on the gel?

3-8. You can inhibit cytoplasmic protein synthesis with the drug cycloheximide. The drug stops elongation, so growing peptide chains cannot be finished and remain attached to ribosomes (the incomplete chains are not released). If you treat cells with cycloheximide, you will find ribosomes attached to the outside of mitochondria, although this is not the usual state of affairs. However you will not find any ribosomes attached to the outside of peroxisomes.

A. Why does drug treatment cause ribosomes to become attached to the mitochondria?

B. Why doesn't drug treatment cause ribosome attachment to peroxisomes?

3-9. There are patients with many different lysosomal storage diseases. Each of these patients lacks a single lysosomal enzyme, and so stores the corresponding undigested macromolecule. People with Hunter's and Hurler's syndromes cannot degrade mucopolysaccharides (= glycosaminoglycans). If you fuse cells from individuals with one syndrome with cells from an individual with the other syndrome, the hybrid cells can break down mucopolysaccharides normally. If you just culture the two cell types together in the same bottle, or add medium from a culture of one type to cells of the other, the mixed or treated cells can break down mucopolysaccharides.

A. Why do hybrid cells have a normal phenotype?

B. Why is it <u>not</u> necessary to fuse the two cell types -- why can extracellular factors from either cell type correct the defect in the other?

C. Should a similar co-culturing scheme work to "cure" complementary defects in enzymes of the cytoplasm, mitochondria, etc.?

D. If you take medium from Hurler's cells and treat it with nucleases, proteases, phosphatases or periodate (which destroys carbohydrate) will it still "cure" Hunter's cells?

Note: Problems 3-6c & 3-7 to 3-9 are based on problems from Alberts *et al*'s problem book.

3-10. Researchers have tested Golgi function *in vitro* to see how material is transferred through the Golgi. There are two classic models for Golgi function, the stationary model and the progression (or maturation) model. Note that these terms refer to what happens to the saccules, not the vesicles.

According to the stationary model, saccules (with their characteristic enzymes) stay put, while vesicles shuttle material (substrate) from one saccule to the next. This is also known as the "vesicle transport model."

According to the progression model, each saccule moves through the stack (carrying its contents) as it matures and modifies its contents. According to this model, new saccules would be constantly formed on the cis side and old ones would constantly break up on the trans side.

How are the models tested? The basic idea is to mix a Golgi stack from mutant cells (that make a specific viral protein but can't modify it properly in the trans Golgi) with a Golgi stack from normal uninfected cells (that make no virus protein but have good Golgi modification enzymes) and follow the fate of the viral protein in the mixture. (You have to add cytosol and ATP along with the isolated Golgi stacks.) In these experiments (originally carried out by Rothman), viral protein is modified.

A. Historically, it was assumed that the movement of vesicles was anterograde (forwards, **towards** the trans face). If vesicles move anterograde, which model of the two listed above does the experimental result support? Explain the sequence of events that would produce modified protein *in vitro* according to this model..

B. The original models all assumed that vesicles carry material anterograde. However, recent evidence suggests that vesicles move in a retrograde direction (backwards, **away from** the trans face). This has led researchers to favor a modified progression model. In this model

(1). What must be carried by the vesicles?

(2). How are the results of the Rothman experiment explained?

3-11. You decide to identify and/or isolate lysosomes, peroxisomes, Golgi, ER, and mitochondria from cultured mammalian cells. You want a "marker enzyme" to characterize each fraction or organelle, that is, you want an enzyme that is unique to each compartment.

A. What is a reasonable collection of marker enzymes to use? (There is more than one possible list.)

B. Suppose you want to look at structures *in situ* in the light microscope, or in the EM, or you want to isolate structures (by centrifugation, etc.) from the cells. How will that affect your choice of enzymes and/or substrates?

3-12. You are studying an enzyme, call it enzyme W, that catalyzes the reaction A ↔ B. Compound B is insoluble and electron dense. (A and B are not the normal substrates and products of this enzyme, but they are similar enough for the enzyme to work on them.) You provide cells with compound A and look in the electron microscope to see where compound B is found. Compound B is inside vesicles surrounded by a single membrane. None of the vesicles containing B appear to be located near (or fusing with) the plasma membrane. You then supply radioactive sugars to the cells, and see where the radioactivity ends up. A lot of it ends up in vesicles, but none of the vesicles in the cell contain both radioactivity and compound B.

Note: A vesicle must be enclosed by a membrane, but it does not have to be part of the EMS.

A. Given all the information supplied so far, what organelle probably contains enzyme W? (Lysosomes) (nuclei) (mitochondria) (peroxisomes) (ER) (Golgi) (secretory vesicles) (none of these).

Before continuing to part B, please look up the answer to part A on p. 42 to be sure you are correct.

B. Given your answer to A, what is the most reasonable choice for enzyme W? (acid phosphatase) (catalase) (DNA polymerase) (sugar adding or modifying enzyme) (signal peptidase) (cytochrome oxidase) (phospholipid synthesizing enzyme) (none of these are possible).

If more than one choice is equally likely, circle all equally likely answers and explain.

C. By recombinant DNA techniques you can make (and introduce into the cell) a recombinant gene that is like the gene for enzyme W except that its 5' end has been altered. The recombinant gene has the 5' end of the gene for an extracellular matrix protein (like collagen) instead of the 5' end of the gene for enzyme W. The rest of the recombinant gene is normal. (3' and 5' refer to the sense strand of the DNA.) Where will your recombinant protein end up? (outside of the cell) (in the cytoplasm) (in the organelle enzyme W belongs in) (none of these) (can't predict).

3-13. Consider the two sides of the ER membrane, the lumen side (L) and the cytoplasmic side (C). Fill in the blanks with L, C, both or neither.

A. The E face is part of the ____ of the membrane bilayer. (Is the E face on the inside or outside of the bilayer?)

B. If no flipase for lipid X is present, lipid X will probably be found in the ____ of the membrane bilayer.

C. If carbohydrates are added to a section of a peptide in the Golgi or the ER, the site for carbohydrate addition should be on the _____.

D. SRP should bind on the _____.

E. After a peptide has entered the ER about halfway, the NH_2 end of the signal peptide (assuming it has not yet been removed) should be closer to the _____.

F. An integral plasma membrane protein that is a receptor for LDL should have an LDL binding site on the _____.

G. Consider an enzyme for catalyzing fatty acid synthesis. The domain containing the active site of the enzyme should be found on the _____.

H. The band 3 protein/anion exchanger should be <u>made</u> by ribosomes on the _____, and once protein synthesis is complete, the protein will be found on _____ side(s) of the ER.

3-14. Consider the following list of proteins**:

(1). Acid phosphatase (AP)
(2). Catalase (Cat)
(3). An extracellular matrix protein (Extra)
(4). An enzyme of glycolysis, such as hexokinase (Hexo)
(5). Histone H1
(6). Enzyme that catalyzes the last step in mannose-6-phosphate addition to proteins (M)
(7). An enzyme of the Krebs cycle (K)

Answer each question below, and explain briefly. Stick to the proteins on the list.

A. Which protein(s) must pass through (but not be retained in) the Golgi? _____

B. Which protein(s) should NOT be made on ribosomes attached to the ER? _____

C. Which protein(s) should NOT have a localization signal of any kind (at any point in their synthesis)? _____

D. Which protein(s) should end up in an organelle surrounded by a single membrane? _____.

3-15. Consider the following proteins**, which are all associated (at least part of the time) with a cellular membrane.

(1). Clathrin
(2). Docking protein -- domain that binds SRP
(3). EGF receptor -- domain that binds EGF
(4). EGF = epidermal growth factor
(5). Lamin A, B, or C
(6). Signal Peptidase

Answer each question below, for the proteins listed, and explain briefly.

A. What membrane are they associated with, and on which side(s)?

B. Which of the proteins listed should be made on ribosomes bound to the ER?

C. Which of these protein(s) definitely do <u>not</u> have a signal peptide in <u>mature</u> form?

**You should be familiar with most of the proteins listed. If any of the proteins listed in 3-14 or 3-15 were not discussed yet in class, look them up in the texts or online, or wait, and consider those proteins later.

3-16. Consider the two plasma membrane proteins described in problem 1-20. Protein A is a single pass protein & Protein B is a multipass protein with 4 transmembrane domains. The carboxyl ends of both proteins interact with cytoplasmic proteins.

A. Suppose cells contain vesicles that are carrying these proteins to the plasma membrane. In the lumen of the vesicles, you will find the amino end of (A) (B) (both) (neither).

B. Which protein(s) were made on ribosomes attached to the ER? (A) (B) (both) (neither) (can't predict).

C. When the proteins are being made, which protein(s) should have a sequence recognized by signal peptidase? (A) (B) (both) (neither) (can't predict).

3-17. Protein IP1 (imaginary protein #1) has two forms; one is phosphorylated and one is not. The phosphorylated form sticks to the plasma membrane and to clathrin; the unphosphorylated form doesn't stick to either (membrane or clathrin). Clathrin alone doesn't stick to the plasma membrane (without IP1). Note: this scheme is hypothetical but similar to what is thought to happen.

A-1. IP1 should be made on ribosomes that are (attached to the ER) (in mitochondria) (free in the cytoplasm) (attached to the plasma membrane) (none of these) (can't predict) AND

A-2. Do you expect newly made IP1 to have a signal peptide? (yes) (no) (beats me).

B-1. IP1 should be found associated with (clathrin coated vesicles) (coated pits) (endosomes) (lysosomes) (none of these) (all of these) (some but not all of these -- specify which) AND

B-2. For a clathrin coated vesicle to uncoat, you should need a (protein kinase) (protein phosphatase) (protease) (all of these) (none of these) (some but not all of these -- specify which).

*3-18. Ceramidase is a typical soluble acid hydrolase found in lysosomes. SAPs are soluble proteins found in lysosomes that help in degradation of some complex lipids.

ICD (I cell disease) is caused by a mutation in the gene for the phosphotransferase that adds mannose 6 phosphate (M6P) to hydrolases. In people with ICD, ceramidase does not reach the lysosome. However the transmembrane proteins and several soluble proteins, including the SAPs, do reach the lysosomes in people with ICD. See note at bottom of page.

A. There are cells that have normal phosphotransferase, but the M6P receptor is missing. In these cells,

A-1. Ceramidase should end up (secreted) (in endosomes) (in lysosomes) (in the cytoplasm) (in the cis Golgi) (in the trans Golgi) (in the ER) (can't predict) **AND**

A-2. The SAPs should end up _____ (same choices).

B. Suppose you add normal ceramidase to the outside of some cells. (Normal = isolated from cells of normal person.) Some of the ceramidase will end up in lysosomes if the cells are from (a person with ICD) (a person or cell culture lacking the M6P receptor) (a normal person) (none of these). Circle one or more choices and explain your reasoning briefly.

C. Sortillin is a transmembrane protein first identified in the Golgi. If sortillin is lacking its cytoplasmic domain (the 'tail'), the SAPs do not reach lysosomes. The SAPs have 3 domains, A, B & C. If sortillin is normal, but the SAPs are lacking the C domain, the SAPs do not reach lysosomes.

C-1. Sortillin was first discovered in the Golgi. In a normal cell, you would expect most of the sortillin to be in the (trans) (medial) (cis) part(s) of the Golgi.

C-2. You would expect to find sortillin in vesicles going from (the Golgi to secretory vesicles) (lysosomes to the Golgi) (endosomes to the lysosomes) (endosomes to the Golgi) (endosomes to the ER) (the Golgi to endosomes) (secretory vesicles to the Golgi) (none of these). Circle one or more choices for each part and explain briefly.

D. You use recombinant DNA methods to make a gene for a chimeric protein. All the human DNA used to make the chimeric gene was from normal people. The gene codes for a protein that has the luminal & transmembrane domains of the M6P receptor and the tail (cytoplasmic domain) of sortillin. This protein could be used to direct newly made (SAPs) (ceramidase) (both) (neither) from the trans Golgi to lysosomes. Explain briefly.

E. You use recombinant DNA methods to make a different chimeric gene that codes for a chimeric protein – this gene codes for a protein that has the amino end (and localization signals) of a mitochondrial matrix protein and has the carboxyl end of the SAPs, including domain C, on the carboxyl end. If the recombinant gene is added to normal cells, where should the chimeric protein end up? (in the cytoplasm) (secreted) (in mitochondria and/or in lysosomes) (in lysosomes) (in mitochondria) (in the Golgi) (can't predict). Explain your reasoning briefly.

Note: A lack of ceramidase causes Farber disease. A lack of glucosylceramidase, usually called glucocerebrosidase, causes Gaucher disease. Each of these diseases is a standard lysosomal storage diseases caused by a genetic defect in the gene for the respective enzyme.

*3-19. The enzyme luciferase (what makes fire flies light up) is found in peroxisomes (in normal insects). If you use immunofluorescent antibody to locate the luciferase in a cell, you see 'punctate staining.' This means you see many small bright (fluorescent) areas in the cytoplasm in the light microscope. If you use immunofluorescent antibody to locate an enzyme in the cytosol, such as PKA, you see diffuse staining -- fluorescence is spread out thinly all over the cytosol, not concentrated in many small spots. (The cells are treated to permeabilize all cell membranes before the antibodies are added.)

A-1.Which enzyme(s) should be made on ribosomes that are free in the cytoplasm? (luciferase) (PKA) (both) (neither).

A-2. Suppose you could also stain for other proteins, using immunofluorescence or *in situ* assays. The punctuate staining for luciferase should co-localize with staining for: (acid phosphatase) (catalase) (RNA polymerase II) (RNA polymerase I) (a glycosylation enzyme) (tubulin) (actin) (lamins) (a mitochondrial matrix enzyme). Circle one or more.

A-3. Suppose you can tag luciferase with an electron dense label. If you look at cell sections in the electron microscope, you expect to see (small dark areas surrounded by a double membrane – 2 bilayers) (small dark areas surrounded by a single membrane – 1 bilayer) (small dark areas surrounded by a monolayer) (small dark areas without a membrane around them) (no small dark areas -- the dense material should be spread evenly throughout the cytoplasm).

B. You can make recombinant genes that code for recombinant luciferase. You can inject the genes into cells that don't normally make luciferase, and use immunofluorescence to locate the enzyme. You get the results shown below.

B-1. These results indicate that in a normal insect, luciferase has a localization signal on its (amino end) (carboxyl end) (both) (either one).

B-2. Suppose a recombinant gene codes for a protein that has a signal peptide added to the amino end of luciferase. If this recombinant gene is injected, luciferase will probably end up (in peroxisomes) (in mitochondria) (in the cytosol) (as part of the plasma membrane or outside the cell) (in the ER or Golgi)..

C. Thiolase is another enzyme normally found in peroxisomes. You can detect thiolase with immunofluorescent antibody. If you stain for thiolase in normal cells, you get punctuate staining that co-localizes with luciferase.

You have cells from many people with defective peroxisomes. Each person is homozygous for one mutation, and has one abnormal gene/protein.

You inject the genes for normal thiolase &/or normal luciferase into these abnormal cells and measure staining. Based on the results, you group the abnormal cells into 4 types, A-D. (See table below.)

C-1. From these results, you would expect thiolase and luciferase to have (the same localization signal) (different localization signals) (either way).

C-2. In cells of type B, which of the following proteins is most likely to be the one that is abnormal? (a receptor for the thiolase localization signal) (a receptor for the luciferase localization signal) (a receptor used by the localization signals of both enzymes) (thiolase) (luciferase) (some other peroxisomal protein).

Experimental Results:

For Part B. (1). If you inject a normal gene for luciferase, staining is punctuate (normal).

(2). If you inject a recombinant gene that is missing the first 2 exons, staining for luciferase is punctuate.

(3). If you inject a recombinant gene that is missing the last 2 exons, staining for luciferase is diffuse.

For Part C.

Genetic Defect	Thiolase staining	Luciferase staining
Type A	Diffuse	Diffuse
Type B	Punctuate	Diffuse
Type C	Diffuse	Punctate
Type D	Punctate	Punctate

3-20. Consider a large ribosomal subunit that is used during synthesis of α-tubulin. Can the same ribosomal subunit be used to synthesize an enzyme responsible for O-glycosylation? (yes, at the same time) (yes, at a different time) (no, never) (maybe – beats me).

3-21. Enzyme X and enzyme Z are both normally found in lysosomes. People with PX disease have no enzyme X in their lysosomes, because of a mutation in the gene encoding enzyme X.

A. Suppose you can isolate lysosomes from a person with PX disease. Enzymes from these lysosomes should be able to degrade the substrate(s) of (enzyme X) (both enzyme X & enzyme Z) (enzyme Z) (neither enzyme).

B. In a normal person, which enzyme(s) should be enzymatically active, but only at low pH? (enzyme X) (enzyme Z) (both enzymes) (enzyme X or enzyme Z but not both) (neither enzyme).

*3-22. Enzymes E, F, G, P & W are all normally found in lysosomes. People with ICD have no enzyme E, F, G, or P in their lysosomes, but enzyme W is normal in both function and location.

A. In a normal person, which enzyme(s) should separate from M6P receptors in endosomes? (enzyme W) (enzyme P or enzyme W but not both) (enzyme P) (neither) (both).

B. Suppose normal enzyme W can be obtained from mammalian cells grown in culture. (The cells are grown in bottles, in a drug factory.) You want to use the cultured enzyme to treat people who are unable to make their own enzyme W. (These people have enzymes E, F, G, & P in their lysosomes, but no enzyme W.) The cultured enzyme W from the factory has all the carbohydrates found on normal enzyme W from normal people. Which of the following is most likely to help people without enzyme W? Cultured enzyme W that is
(heated to unfold the enzyme) (treated to remove or cover up M6P) (treated to reduce S-S bonds)
(treated to add or uncover M6P) (untreated – no modifications should be necessary)
(treated to remove all attached carbohydrate).

Explain your answers to both A & B.

Problem Set 3R – Problems on Protein Structure, Synthesis, and Transport

3R-1. In mature lysosomes, the enzyme acid phosphatase (AP) is normally found as a soluble enzyme inside the lumen of the organelle. Newly made AP is a transmembrane protein that includes a cytoplasmic domain. Once AP reaches its final destination, an enzyme catalyzes release of soluble AP into the lysosomal lumen. A mutation on one side of the transmembrane domain causes the AP to remain permanently in the lysosomal membrane. A (different) mutation in the cytoplasmic domain of AP causes AP to accumulate in the plasma membrane instead of in lysosomes.

A. Assume that AP has an amino terminal SP and enters the ER in the usual way.

A-1. The transmembrane sequence that anchors AP in the lysosomal membrane is probably (the amino terminal SP) (a separate stop transfer sequence) (either way), AND

A-2. The enzyme that releases AP from the lysosomal membrane is probably found (on the lumen side of the lysosomal membrane) (on the cytoplasmic side of the lysosomal membrane) (either way) AND

A-3. The enzyme probably cuts (after the amino terminal signal peptide) (on the amino side of a stop transfer sequence) (on the carboxyl side of a stop transfer sequence) (on either side of a stop transfer sequence).

Explain all your answers -- a picture might help.

B. People with I cell disease have normal AP. Most of their lysosomal enzymes are misdirected to the extracellular space, but the proteins of the lysosomal membrane and AP are directed to their proper destination . However there are other mutations that cause AP to end up in the wrong place as described above.

B-1. The sequence that targets AP to the lysosome should be (in luminal domain of AP -- part that ends up in the lumen of the lysosome) (in cytoplasmic domain of AP) (in a transmembrane sequence) (can't predict), AND

B-2. The sequence that targets AP to the lysosome should be (the sequence for addition of M-6-P) (the amino terminal SP) (an NLS) (a transit peptide) (some other sequence) (can't predict), AND

B-3. If the transmembrane segment of AP were deleted, then AP should end up in (lysosomes) (plasma membrane) (extracellular space outside the plasma membrane) (ER or Golgi) (cytoplasm) (can't predict).

Be sure your answers are consistent with your answers to part A. Explain all your answers.

C. You discover a new drug; call it drug C. When cells are treated with drug C, the mannose-6-phosphate receptors fail to separate from their ligands.

C-1. After treatment with drug C, most of the acid hydrolases normally found in lysosomes will probably end up (in the ER) (in the cytoplasm) (secreted outside the cell) (in the Golgi) (in the lysosomes) AND

C-2. This drug is most likely to work by inhibiting a proton pump in the membrane of (endosomes) (Golgi) (cytoplasm) (lysosomes) (mitochondria) (ER) (any of these). Explain your answers.

C-3. Do you expect drug C to effect the localization of acid phosphatase?

3R-2. You have some cells that have protein X receptors on their surface.

A. You have two batches of vesicles. Batch A was made from ER. Batch B was made from plasma membrane. In both cases (batches A & B), the appropriate organelle or cell fraction was isolated, the membrane was broken into small fragments, and the fragments resealed (in their usual orientation) into small vesicles. You add fluorescent tagged protein X to the solution surrounding the vesicles.
You would expect to find protein X receptors associated with (A) (B) (both) (neither), AND
You would expect to see binding of the fluorescent protein X to vesicles from (A) (B) (both) (neither).

B. You supply some cells with radioactive amino acids. (AA are small molecules that enter cells using carrier or transport proteins; they don't enter by RME.) You also add fluorescent protein X to the cells. Then you examine the various kinds of vesicles from the treated cells. These are normal, intracellular vesicles, not fragments of something else. You look at the vesicles at increasing intervals of time after adding the labels (radioactivity and fluorescence) and measure radioactivity in receptors. (You don't measure the total radioactivity in the cells – only the radioactivity made into receptors.)

B-1. You are likely to find radioactivity first in (lysosomes) (coated endocytic vesicles)
(exocytotic vesicles -- vesicles going to the plasma membrane on their way out)
(acidified endocytic vesicles = endosomes) (none of these will ever have label) (can't predict), AND
You are likely to find fluorescence first in _____. (same choices).

B-2. If you expose the cells long enough to both labels, you find both radioactivity and fluorescence together in all the various vesicles mentioned, except there is no radioactivity in the lysosomes and no fluorescence in the exocytotic vesicles. From these results, you can conclude that in the case of protein X --
(ligand) (receptor) (both) (neither) (can't tell which) is/are recycled to the cell surface.

3R-3. The peptide chain of connexin (see problem 1-14) has the same number and order of hydrophobic and hydrophilic segments as the glutamate receptor (see problem 3-3). Assume the segments of connexin are labeled as in problem 3-3. However, connexin is not necessarily inserted into the membrane in the same way as the glutamate receptor. When connexin is in a default vesicle on its way to the plasma membrane, the protein has 4 transmembrane domains, and both ends of the protein (corresponding to segments A & E shown in 3-3) are in the cytoplasm. Note: the vesicle shown is a normal intracellular vesicle in a normal cell; it is not some artificial vesicle made in an *in vitro* experiment.

A. Suppose the default vesicle reaches the plasma membrane and exocytosis occurs.

A-1. The extracellular domain(s) of the protein will be (A) (B) (C) (D) (E) (1) (2) (3) (4) (none of these) AND the cytoplasmic domain(s) will be _____. Indicate all choices that apply.

A-2. Which of the following should NOT have carbohydrates attached? (A) (B) (1). Circle as many choices as apply.

A-3. When connexin was in the Golgi or ER, domain A was (in the lumen) (in the cytoplasm) (transmembrane) (in the lumen or the cytoplasm) (depends on which organelle it was in -- orientation is not necessarily the same for both).

B. Suppose connexin is being made. When the SRP binds to the growing peptide, it should bind to domain (1) (2) (3) (4) (A) (B) (C) (D) (E) (none of these). Assume domain A is at the amino end of the protein.

C. When you freeze fracture tissues containing gap junctions, you see more bumps on the P face than on the E face. Suppose the bumps on the P face correspond to parts of domains B and/or C. In that case, the bumps probably correspond to parts of domain(s) (B) (C) (either one but can't tell which) (both) (beats me). Explain your answer.

3R-4. When the stomach is empty, people stop secreting acid – their parietal cells become inactive. In inactive parietal cells, only small amounts of the H/K pump are found on the side of the cell surface facing the lumen. When the stomach fills up again, the parietal cells become active, and large amounts of the H/K pump are found on the cell surface facing the lumen. No protein synthesis is required to activate the cells.

A. When parietal cells switch from active ***to inactive***, the H/K pump protein is most likely to be transported to (lysosomes) (peroxisomes)(mitochondria) (endosomes) (lysosomes &/or peroxisomes) (endosomes &/or lysosomes) (ribosomes) (the nucleolus) (can't predict – any of these are equally likely).

B. When parietal cells switch from active ***to inactive***, removal of the H/K pump from the luminal cell surface should require (integrin) (clathrin) (spectrin) (ankyrin) (kinesin) (any of these) (none of these) .

C. When parietal cells get the signal to switch from inactive ***to active***, most of the H/K pump protein is most likely to be in: (a part of the cell plasma membrane away from the lumen) (the lumen of the stomach) (the interstitial fluid) (the lumen of a vesicle) (the membrane of a vesicle) (the soluble cytoplasm – not in an organelle) (none of these – it should be degraded) (beats me).

D. Suppose active parietal cells ***become inactive***. The <u>first step</u> in the process should involve (the organelle chosen in A) (the protein chosen in B) (the location chosen in C) (choices in A & B) (all three choices) (can't predict).

Answer the questions, and explain in one or two sentences what is going on here. Do not put in all the steps and details – just explain the big picture of how the parietal cells are activated and inactivated. What process(es) are involved here?

Here are some more questions (5-7) about presidins. (See Questions1-18 & 1-19.) All presidins are single pass transmembrane proteins, and all presidins are members of the same protein/gene family.

3R-5. Consider the protein presidin A. Presidin A is a receptor (for something or other) and presidin A is found entirely within the cell. No part of it is extracellular. Given this info, answer A & B.

A. What is a reasonable function for presidin A? Of the following choices, the best choice is: (receptor for SRP) (receptor for LDL) (receptor for M6P = Mannose-6-phosphate) (M6P or SRP receptor) (LDL or SRP receptor) (M6P or LDL receptor) (any of these receptors).

Explain briefly in a sentence or two why your choice is best and how you ruled out the receptors you did NOT circle.

Before continuing to part B, please look up the answer to part A on page 42 to be sure you are correct. Then base your answers to B on the best answer to A.

B. (i) Mature presidin A (doing its normal job) is ***most likely*** to be found in which of the following? (in some part of the ER) (in some part of the Golgi) (completely in the cytoplasm) (inserted in the plasma membrane) (in lysosomes) (in peroxisomes) (in mitochondria).

(ii) The domain of presidin A that binds ligand should be found (in the ER lumen) (inside a Golgi sac) (in the cytoplasm) (inside lysosomes) (protruding out of the cell).

Show where the binding domain of presidin A should be – sketch the correct cell compartment or location, put presidin A in the right place, and label the binding domain as "B."

3R-6. Suppose multiple molecules of ubiquitin get attached to one of the presidins using the appropriate enzyme.

A. Then the presidin will probably end up: (in a lysosome) (in a peroxisome) (in the nucleus) (bound to a ribosome) (in a proteasome) (bound to a chaperone) (depends which presidin it is).

B. The presidin that gets ubiquitin added will be: (glycosylated) (phosphorylated) (degraded) (dephosphorylated) (elongated) (folded).

3R-7. Consider the protein presidin B. Mature presidin B is located permanently in the plasma membrane. It has one transmembrane domain like all presidins, and both an extracellular and a cytoplasmic domain.

A. Consider how presidin B reaches the plasma membrane.

(i) Where are you likely to find newly made molecules of presidin B with the complete amino acid sequence? Newly made molecules with all the AA (but not necessarily modified) are likely to be found ***first*** (in the lumen of the ER) (inserted in the membrane of the ER) (in the trans Golgi) (in the cis Golgi) (in endosomes) (inserted in the plasma membrane) (completely in the cytoplasm) (in peroxisomes) (in mitochondria).

(ii). Which of the following vesicles should be the ***last*** to get newly made molecules of presidin B? (vesicles that bud from the ER) (vesicles that bud from the trans Golgi) (vesicles that bud from the cis Golgi) (vesicles en route to peroxisomes) (vesicles en route to mitochondria).

(iii). Consider the domain X – the domain of presidin B that is extracellular when the protein is located in the plasma membrane. Now suppose you look at a vesicle ferrying presidin B around the cell. Where should domain X be found? (inside the lumen of the vesicle) (outside the lumen of the vesicle, in the cytoplasm) (in the membrane of the vesicle) (can't predict -- depends on how presidin B is made) (can't predict -- depends on which vesicle it is).

Explain part (iii) only. Show how presidin B should be located in the vesicle, and label domain X. (If you chose "can't predict" explain why not.)

B. Suppose that presidin B in the plasma membrane is 425 amino acids long and is oriented with its ***amino end*** in the cytoplasm. Its cytoplasmic domain contains about 200 amino acids, and the transmembrane domain is an alpha helix of 25 amino acids.

(i). Consider the first 100 amino acids on the amino end of presidin B. You would expect these first 100 amino acids to include sequences recognized by the enzymes of (O or N glycosylation) (formation of disulfide bonds) (both) (neither) (either one or both – can't tell without more information).

(ii). Consider newly made presidin B that has not been processed at all.

(a). The new protein should include:

(a signal peptide in addition to the 425 amino acids)
(a signal peptide that is included in the 425 amino acids) (no signal peptide at all)
(a signal peptide, but could be included in the 425 amino acids or not).

(b). Signal peptidase should cut the newly made protein:

(near the amino end of the SP)
(near the carboxyl end of the SP)
(near one end or the other of the SP)
(nowhere – signal peptidase should not cut presidin B at all).

(iii). Suppose you use genetic engineering to construct a new, altered version of the gene for presidin B. The new gene is missing the part that codes for amino acids 201 through 226. Suppose a cell uses the altered version of the gene to make presidin B. The presidin B made from the altered gene should be located primarily (in the plasma membrane, with the normal orientation – amino end in the cytoplasm) (entirely in the cytoplasm) (in the plasma membrane, but with the ends reversed – carboxyl end in the cytoplasm) (outside the cell) (in the ER or Golgi – it should get stuck there).

Explain parts (ii) & (iii). Show or explain why the presidin B normally ends up with its amino end in the cytoplasm, and what happens when you alter the gene.

3R-8. Enzyme X is a marker enzyme for the trans Golgi. Enzyme X has a short, cytoplasmic domain (or "tail") at the amino end, a single transmembrane domain, and a large luminal domain at the carboxyl end. Assume that in normal cells enzyme X is made and reaches its destination using the usual mechanisms.

A. Suppose a cell is making new enzyme X. *(See note at the bottom of the page for definition of 'new' and 'newest' enzyme X.)*

A-1. You would expect the ribosomes making enzyme X to be (attached to the Golgi) (attached to the ER) (attached to the ER or Golgi) (attached to mitochondria) (free in the cytoplasm) (free in the cytoplasm or the matrix of the mitochondria) (attached to an organelle, but can't predict which one).

A-2. Suppose you follow some newly made enzyme X through the Golgi. Of the choices given, where would you expect to find the ***newest*** enzyme X? (In the membrane of the cis Golgi) (In the membrane of the trans Golgi) (In the plasma membrane) (In the lumen of the cis Golgi) (In the lumen of the trans Golgi) (In an endosome).

A-3. Suppose you find some of the new enzyme X in a vesicle near the trans portion of the Golgi. The "tail" part of the enzyme should be found (inside the vesicle) (in the cytoplasm) (depends on which way the vesicle is moving). Explain what you mean by "newest" enzyme X.

B. Suppose the ***newly made*** enzyme X (*see note below*) is in a vesicle near the trans portion of the Golgi.

B-1. This fits best with a (stationary cisternal model) (cisternal progression model) (either one – fits equally well with both)

B-2. The vesicle should be moving (toward the trans side) (toward the cis side) (can't predict).

B-3. Suppose enzyme X reaches its final destination. Then according to the model you have chosen, the "old" enzyme X will (never enter a vesicle again) (eventually enter an anterograde vesicle = forward vesicle) (eventually enter a retrograde vesicle) (enter one type of vesicle sometimes and the other sometimes). Explain all your answers to B. A picture might help.

C. You add an inhibitor to normal cells. The cells make the normal amount of enzyme X, but about 1/2 the molecules of enzyme X ends up in the cytoplasm and half the molecules end up in the Golgi. (Where the inhibitor acts is part 2 of this question.)

C-1. If the mis-localized protein is degraded, it is most likely to be degraded in (lysosomes) (peroxisomes) (proteasomes) (ribosomes) (spliceosomes) (endosomes) (any of these).

C-2. Which protein did you probably inhibit? Given the following choices, the most likely candidate is: (Signal peptidase) (A protein in the SRP – signal recognition particle) (IF = initiation factor required to bind met-$tRNA_i$ to ribosome) (coat protein needed to form vesicles transporting proteins between parts of EMS) (SRP or signal peptidase) (IF or SRP) (signal peptidase or coat protein) (coat protein, SRP, or signal peptidase) (any of these – they're all equally likely). Explain why your choice is best. (Rule out the other choices very briefly.)

D. Consider an ordinary acid hydrolase and catalase, and assume your answer to C-2 is correct. Then when you add the inhibitor, you expect to find a change in the localization of (the acid hydrolase) (catalase) (both) (neither). Explain briefly.

Note: The enzyme is still called 'enzyme X' whether it is completely modified or in its final position or not. Therefore the 'new' enzyme molecules should be the ones whose amino acid chains were just made, and the 'newest' enzyme molecules are the ones whose amino acid chains were made the most recently. This is the way the term 'newest' or 'new' enzyme X is generally used. (If you wanted to specify 'the newest completely modified enzyme molecules that have reached their final destination,' you would say that, instead of 'newest enzyme X.'.)

3R-9. Enzymes Y & Z are transmembrane proteins found in the Golgi. They all have the same basic structure as enzyme X (see previous problem). All three enzymes have a short cytoplasmic tail, a transmembrane sequence, and a large luminal domain. The locations of the 3 enzymes and their enzymatic roles are as follows:

Enzyme	***Location***	***What enzyme does***
Enzyme X	trans Golgi	Adds sugar M
Enzyme Y	medial Golgi	Adds sugar N
Enzyme Z	cis Golgi	Adds sugar Q

Enzymes X, Y and Z are all involved in O-glycosylation. In a normal cell, the result is a trisaccharide chain with all three sugars attached to the side chain of serine.

A-1. In a fully glycosylated protein, in a normal cell, which trisaccaride would you expect to find added to the side chain of serine? (Q-M-N-serine) (Q-N-M-serine) (N-M-Q-serine) (N-Q-M-serine) (M-Q-N-serine) (M-N-Q-serine) (any order of M, N & Q should be possible). Explain at end of A-2.

You can make recombinant genes that produce hybrid proteins. The recombinant genes code for hybrid proteins that have the luminal domain of one enzyme attached to the amino end and/or transmembrane domain from one of the other enzymes (or a different protein). The results of several such experiments are listed in Table 1 below. Each of the remaining parts refers to one or more of the experiments in the table.

A-2. Consider cells that have the hybrid enzyme described in experiment 3 instead of enzyme Z. (The other 2 enzymes are normal, but there is no normal enzyme Z.) When glycosylation is complete, what sugars should be attached to the side chain of serine? (same as in normal case) (only one sugar) (different order of three sugars) (two sugars instead of three) (none -- no sugars should be added). Explain what happens normally, and what is (or isn't) different with the hybrid enzyme.

B. In experiment 5, the hybrid protein should end up in (Cis Golgi) (Trans Golgi) (Medial Golgi) (beats me). Explain how you know in one sentence.

C. Consider experiment 6. Glycophorin is a single pass plasma membrane protein with a large extracellular domain (several 100 amino acids) at the amino end. The hybrid protein is made from a recombinant gene that includes the normal 5' end of the glycophorin gene. The resulting protein has the amino end of glycophorin -- first 100 amino acids -- attached to the luminal domain of enzyme X. Where is the hybrid protein likely to end up? (in the cytoplasm) (in some part of the Golgi) (in the plasma membrane) (in the ER or Golgi) (outside the cell). Explain the mechanism (or show the steps) that will determine where the protein ends up.

Table 1: Results for Problem 3R-9.

Expt.	***Amino end***	***Transmembrane Domain***	***Luminal Domain***	***Location of hybrid Enzyme***
1	Enz. X	Enz. Z	Enz. Z	Cis Golgi
2	Enz. Z	Enz. X	Enz. X	Trans Golgi
3	Enz. X	Enz. X	Enz. Z	Trans Golgi
4	Enz. Z	Enz. Z	Enz. X	Cis Golgi
5	Enz. X	Enz. Y	Enz. Z	?
6	Glycophorin	---	Enz. X	?

Note: Each protein is made from a recombinant gene. The recombinant genes are made by splicing together sections of the normal genes for enzymes X, Y, Z and glycophorin.

Problem Set 4 -- Nuclei & Eukaryotic Gene Regulation

4-1. The primate virus SV40 has a circular "mini-chromosome." If the mini-chromosome is extracted from infected cells and examined in the electron microscope, it has a circumference of about .2 microns and a distinctly beaded appearance. The purified DNA from the mini-chromosome forms a circle with a circumference of about 1.48 microns.

A. Why is the mini-chromosome shorter than the DNA?

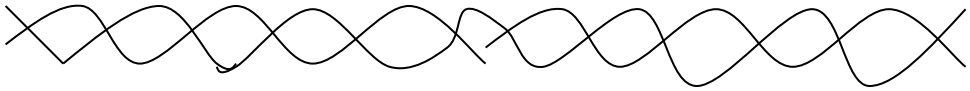
B. The DNA is about 5.2 kb (kilobases) long. About how many beads should you see along the length of the mini-chromosome?

C. You can digest the SV40 mini-chromosome briefly with DNase, isolate the DNA, and run the digested DNA on a gel. Alternatively, you can isolate the DNA first, treat it with DNase, and run the digest on a gel. Will the two gels look the same? Draw the expected results.

4-2. Psoralen is a chemical that crosslinks DNA -- it covalently links the two strands in a double helix. If you denature cross linked DNA, the two strands will not completely separate -- they will remain connected at the points where the cross links are. You can look at the cross linked and denatured DNA in the electron microscope and see where the two strands are connected. If you treat isolated DNA with psoralen, and denature it, the DNA is cross linked in an irregular pattern and looks like this in the electron microscope:



If you treat most regions of chromatin with psoralen, and then isolate and denature the DNA, the DNA is cross linked in a regular pattern about every 200 bases and looks more like this in the electron microscope:



A. The DNA from chromatin probably shows the observed regular pattern of crosslinking because of (binding of transcription factors) (nucleosome formation) (heterochromatin formation) (phosphorylation of histones) (none of these) (any of these).

B. Suppose you treat some actively transcribed chromatin with psoralen, isolate the DNA, and examine it in the electron microscope. If you look at the entire transcribed region of a gene from this chromatin, you will probably see (a random pattern) (a regular pattern as above) (a regular pattern with larger distances between the crosslinks) (a regular pattern with shorter distances between the crosslinks) (a regular pattern in introns, but not exons) (a regular pattern in exons, but not introns) (any of these).

C. Suppose you discover a region of chromatin which does not give a regular pattern. This region probably gives an irregular pattern because of (binding of transcription factors) (nucleosome formation) (heterochromatin formation) (phosphorylation of histones) (none of these) (any of these),
AND its sensitivity to DNase is probably (very high) (very low) (same as for average transcribable chromatin) (same as for average nontranscribable chromatin) (any of these).

4-3. A. You isolate chromatin from an interphase cell and treat it with a small amount of DNase. You spread out the treated chromatin, stain it, and look at it in the electron microscope. You see a structure that looks like a piece of a necklace, with 4 beads.

A-1. The number of molecules of double stranded DNA in the structure should be:
(1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (0) (>10).

A-2. The longest double stranded DNA molecule in the structure should be:
(200 bp) (400 bp) (600 bp) (800 bp) (1000 bp) (1200 bp) (1600 bp) (2000 bp) (<200 bp) (> 2000 bp)
(none of these – there's no DNA in the structure).

Problem 4-3, cont.

A-3. The number of molecules of H2A in the structure should be:
(1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (12) (15) (16) (20) (24)(0) (>24).
Explain your answers to part A. (A labeled picture is fine.) Show how you arrived at all 3 answers.

B. Suppose you could examine the equivalent section of the chromatin when the cell is in mitosis. (Equivalent = contains same DNA as that in the chromatin described above.)

B-1. Then this section of the chromatin would be (shorter) (about the same length) (shorter or longer) (longer) (same or shorter) (same or longer) (any of these).

B-2. This section of the chromatin in mitosis should contain _____ molecules of H1.
Explain briefly how this section of chromatin in mitosis compares to the equivalent section in interphase.

C. Suppose the section of interphase chromatin described above came from an active gene in a muscle cell. This section (with its necklace like structure) is most likely to be derived from: (the basal promoter) (an enhancer of the gene) (an exon in the gene) (an intron in the gene) (any transcribed region of the gene) (a regulatory element of the gene other than an enhancer or basal promoter) (any of the cis-acting elements listed) (any of these) (none of these). Explain briefly how you arrived at your choice.

4-4. Suppose protein H is made by nerve cells but not by muscle cells

A. You examine the chromatin containing base pairs +1 to + 4200 of the gene for protein H.

A-1. What is the maximum number of nucleosomes that should be found in that region of the chromatin? ___

A-2. You expect base pairs +1 to + 4000 (**region A**) to be transcribed into the 1° transcript in (muscle cells) (nerve cells) (both types of cells) (neither type of cells).

A-3. You examine the number of nucleosomes in this region of the chromatin in nerve cells and in muscle cells. You expect the number of nucleosomes in region A to be
(lower in muscle) (higher in muscle) (about the same in both cell types) (can't predict).

B. You examine the chromatin containing the DNA from **region B** of the protein H gene. R**egion B** = The first 100 base pairs on the upstream side of region A.

B-1. You treat the chromatin with DNase, remove the protein, and measure the state of the DNA that is left from region B. The DNA is more likely to be degraded if the chromatin came from (muscle cells) (nerve cells) (one of the other but can't tell which) (either type of cells – degradation should be about the same in both cases).

Draw the ***sense strand*** of the gene. Label the ends, regions A & B, and the start (& direction) of transcription.

4-5. Circle one or more correct answers for A – C. Consider a ribosome that is translating mRNA, and making a protein say, protein P.

A. The ribosome could be in (the lumen of the ER) (a mitochondrion) (a peroxisome) (the nucleus) (the nucleolus) (the perinuclear space) (the cytoplasm) (none of these).

B. Suppose protein P will be a cytoplasmic ribosomal protein, i.e. become a part of a cytoplasmic ribosome.

B-1. When the synthesis of protein P is finished, protein P could pass through (the nucleolar membrane) (a nuclear pore) (a translocon) (the plasma membrane) (the mitochondrial membranes at a contact point) (none of these).

B-2. Under normal conditions, a ribosome making protein P could be attached to (the ER membrane) (the inner membrane of the nuclear envelope) (the outer membrane of the nuclear envelope) (the nucleolar membrane) (the nuclear lamina) (the outer mitochondrial membrane) (none of these).

C. The gene for rRNA is 13,000 base pairs long. Human cells contain 200 copies of this gene per haploid genome.

C-1. If all the rRNA genes are in nucleosomes, how many such nucleosomes are there in each of your ordinary (diploid) body cells? _____

C-2. Suppose these nucleosomes are part of a 30nm fiber. Then all these nucleosomes should contain a total of _____ molecules of histone H1, AND _____ molecules of histone H2A. Show your calculations

4-6. Fill in the blank with (increase) (decrease) (stay the same) (stay the same -- at zero) (can't predict) unless other choices are given.

A. Suppose a cell increases the rate of ribosome production.

A-1. The rate of protein synthesis inside the nucleus should _____ ,
AND the rate of ribosomal proteins moving **out of** the nucleus should _____.

A-2. The rate of ribosomal proteins moving **in to** the nucleus should _____ ,
AND the rate of rRNA crossing the nucleolar membrane should _____.

B. Consider the lamins.

B-1. During the transition from G2 to M, the rate of depolymerization of lamins should _____,
AND during the transition from G1 to S the rate of depolymerization of lamins should _____.

B-2. Lamins are (IF) (MT) (MF) (any of these) (MF or IF) (none of these), AND
Newly made lamins should have a (signal peptide = SP) (nuclear localization signal = NLS) (transit peptide = TP) (transmembrane domain) (SP & NLS) (SP or TP) (transcription activating domain) (none of these).

4-7. You have a protein that is involved in regulation of gene expression in yeast. It is thought to act by modifying histones. Would you expect this protein to be involved in gene regulation in *E. Coli*?

4-8. You have two samples, X and Y, of identical cells. All cells are of the same type, from the same tissue & the same organism, all grown in the same way. You isolate chromatin from each sample, using a different procedure and buffer for each one.

Expt.1: You look at the two chromatin preparations in the electron microscope. Chromatin from X appears mostly as 30 nm fibers. Chromatin from Y appears as lots of thinner fibers with a regular beaded appearance; no 30 nm fibers are visible.

Expt. 2: You isolate proteins from each chromatin preparation, separate the proteins on gels and stain for protein. The gel for one sample has 5 bands and the other one has 4 bands.

A-1. You notice that in experiment 2 you forgot to record which sample went on each gel! By putting the results of both experiments together, you decide that the sample with 5 bands probably came from (sample X) (sample Y) (can't tell from data given).

A-2. In the sample with 5 bands, the amount of stain (i.e. the amount of protein) in each band should be (about the same in 4 bands, and darker for one) (about the same in 4 bands, and lighter for one) (no predictable pattern -- very different amounts of staining for each band) (about the same in all bands) (beats me).

A-3. For the sample with 4 bands, the number of molecules of protein detected on the gel per 2000 base pairs of DNA should be _____.

B. Suppose you are able to isolate chromatin from an actively transcribed region of the DNA and you use the procedure described above to examine the chromatin. If you look in the electron microscope, you would probably get chromatin that looks like (X) (Y) (neither) (can't predict). Explain your answer.

4-9. "Fibronectin" is really a class of proteins -- all the fibronectins made in an organism are similar but they are not all the same. The fibronectins made by different cell types vary in amino acid sequence and function, although they are all very similar. There is only one gene (per haploid cell) for fibronectin. You have 2 different cell types. Type #1 makes fibronectin type A (forms dimers) and type #2 makes fibronectin type B (forms large polymeric complexes). The two types of fibronectin have differences in amino acid sequence.

A. In this case, what is the most likely explanation for the difference between the 2 cell types in the type of fibronectin? (DNA replication) (transcription) (splicing) (initiation of translation) (degradation of mRNA) (modification of protein after translation) (none of these) (all of these) (can't predict -- two or more of these choices are equally likely). ***Before continuing to part B (on next page), please look up the answer to part A on p. 42 to be sure you are correct.***

B. You would expect to find differences between the 2 cell types in the sequences of their (DNA) (mRNA's) (primary transcripts) (two or more of these) (none of these).

*4-10. You have a plasma membrane protein; call it protein M. Protein M has two different forms that are found in different cell types. The two forms are made by alternative splicing of the same primary transcript. Both forms of protein M have a single extracellular domain. The extracellular domain is identical in both forms, but the way the protein is anchored in the membrane is different. Form #1 is anchored in the membrane by a hydrophobic transmembrane domain near its COOH end. Form #2 is missing the hydrophobic transmembrane domain; it is anchored in the membrane by a covalently attached lipid. (The lipid is added in the ER to the COOH end of the protein.) You have 2 different probes to the (sense strand of) gene for protein M.

Probe	Region of Gene M it hybridizes to
Probe B	part that codes for extracellular domain of form #2.
Probe C	part that codes for the transmembrane domain of form #1.

A. You look at newly made nuclear RNA from cells making form #1 or form #2 of protein M. Assume both cell types are making equal amounts of protein M. The amount of new nuclear RNA hybridizing to probe C should be (the same in both cell types) (higher in the cell type making form #1) (lower in the cell type making form #1) (can't predict).

B. You look at the chromatin from cells making form #2. You measure the sensitivity to DNase of the regions in gene M that hybridize to probes B & C. Which region do you expect to more sensitive to DNase? (the region that hybridizes to B) (the region that hybridizes to C) (either one -- can't predict) (neither -- both regions should be equally sensitive).

C. You look at the cytoplasmic RNA from cells making from #1 or #2. Assume both cell types are making equal amounts of protein M.

C-1. The amount of cytoplasmic RNA hybridizing to probe B should be (the same in both cell types) (higher in the cell type making form #1) (higher in the cell type making form #2) (can't predict) AND

C-2. The amount of cytoplasmic RNA hybridizing to probe C should be (the same in both cell types) (higher in the cell type making form #1) (higher in the cell type making form #2) (can't predict)

4-11. You are interested in identifying the structure of XYC, a gene involved in the inactivation of the X chromosome.

A. What information does the location of DNase hypersensitive sites (HSSs) within XYC reveal? (heterochromatin) (euchromatin) (loose DNA) (looser DNA) (loosest DNA) (transcribed region of the DNA) (basal promoter) (enhancers) (the gene is actively being transcribed) (the gene isn't being transcribed).

B. The consensus sequence for the binding of Oct4, a transcription factor (TF), is identified in a regulatory region of XYC. Oct4 would most likely bind to a XYC regulatory region when it is in what chromatin configuration? (Heterochromatin) (loose) (looser) (loosest) (all euchromatin).

Explain A & B

4-12. Pax6, which is expressed in pancreas, retina, lens, cornea and brain, is one of the TFs that activates crystallin in the lens.

A. The most direct way to decrease the level of transcription of Pax6 only in the lens is to mutate the: (Pax6 binding site on crystallin) (pancreas activator) (lens enhancer on the Pax6 gene) (lens activator) (basal promoter of the Pax6 gene) (basal promoter of the crystallin gene)

B. The most direct way to decrease the level of transcription of Pax6 in all cell types where Pax6 is normally expressed is to mutate the: (Pax6 binding site on crystallin) (pancreas activator) (lens enhancer on the Pax6 gene) (lens activator) (basal promoter of the crystallin gene) (basal promoter of the Pax6 gene). Explain A & B

4-13. The DNA encoding human NFATc1 is shown below. The gene is expressed only in the heart and in the immune system and contains 11 exons. This gene contains two basal promoters, one just upstream of exon 1, used in the heart & one just upstream of exon 2, used in the immune system. An enhancer which drives NFAT expression in the immune system is located in intron 10 & an enhancer that drives expression in the heart is located in intron 1.

A. Which region(s) (or most of the region) of the NFAT gene would you expect to be in HSSs in the heart? (Just 5' of exon 1) (Exon 1) (Intron 1) (Exon 2) (Intron 2) (exon 9) (intron 9) (exon 10) (intron 10) (exon 11) (intron 11) (none)

B. Which region(s) (or most of the region) of the NFAT gene would you expect to be in HSSs in the immune system? (Just 5' of exon 1) (Exon 1) (Intron 1) (Exon 2) (Intron 2) (exon 9) (intron 9) (exon 10) (intron 10) (exon 11) (intron 11) (none)

C. Which region(s) (or most of the region) of the NFAT gene would you expect to be in HSSs in the lens? (Just 5' of exon 1) (Exon 1) (Intron 1) (Exon 2) (Intron 2) (exon 9) (intron 9) (exon 10) (intron 10) (exon 11) (intron 11) (none).
Explain A, B & C

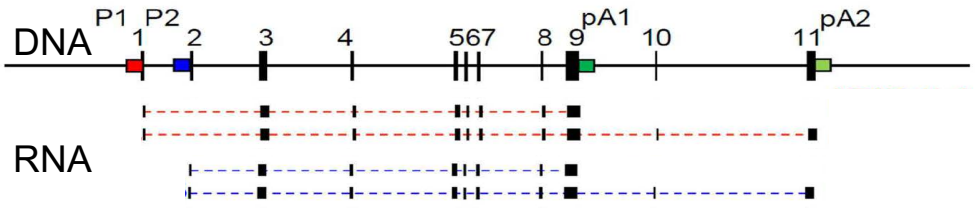
D. Which regions of the DNA would loop around and interact to drive expression in the heart? (Just 5' of exon 1) (Exon 1) (Intron 1) (Exon 2) (Intron 2) (exon 9) (intron 9) (exon 10) (intron 10) (exon 11) (intron 11)

E. Which of the following proteins would be present in the protein complex driving transcription in the heart? (TBP) (clathrin) (basal TFs) (catalase) (regulatory TFs) (mediator) (TFIID)
Explain D & E

F. Deletion of the 5' basal promoter (P1) would result in loss of expression in the: (heart) (immune system) (both) (neither)

G. Deletion of intron 10 would result in loss of expression in the: (heart) (immune system) (both) (neither)
Explain F & G

NFAT gene & RNA structure – for Question 4-13.
The gene encoding human NFATc1 is expressed only in the heart and in the immune system. The gene contains 11 exons (exons are symbolized by rectangles or vertical lines numbered 1-11; the smallest exon is 100bp). This gene contains two basal promoters, one just upstream of exon 1 (small box denoted P1) that is used in the heart & one just upstream of exon 2 (small box denoted P2) that is used in the immune system. NFAT also contains two poly addition A sites (small boxes denoted pA1 & pA2). An enhancer which drives NFAT expression in the immune system is located in intron 10 and an enhancer that drives expression in the heart is located in intron 1. RNAs that result from this gene are depicted below the DNA. Dashed lines denoted regions which are removed from the RNA during processing to produce mRNA.



HINTS & ANSWERS TO SELECTED QUESTIONS FROM PROBLEM SETS 3, 3R & 4

For these questions, parts B, C, etc. depend on the answer to part A. Therefore a hint or answer to part A (or 4R-4D) is given here, so you can check it without accidentally seeing the answers to parts B, C, etc.

3-12. A. Peroxisomes. There are no carbohydrates attached to the proteins in the vesicle, so that rules out secretory vesicles and lysosomes. (The enzymes that add sugars are inside the ER & Golgi, and the proteins that pass through the EMS are virtually all glycosylated.) ER and Golgi are not considered vesicles; they may contain vesicles ("be vesicular") but they have a more extensive and complex structure (although they contain carbohydrates being added to proteins). Nuclei and mitochondria have double membranes, so they are ruled out too. The vesicles are also not involved in endo- or exocytosis, as they are not found near the plasma membrane. Peroxisomes fit the bill; the rest do not.

3R-5. A. SRP receptor is the best answer; M6P or SRP receptor is second best. The LDL receptor is a transmembrane protein of the plasma membrane with an extracellular domain, so it is ruled out. SRP receptor is located permanently in the ER membrane, so it is always entirely within the cell. M6P receptors are located primarily in the Golgi, where the receptors traps hydrolases (with M6P) destined for the lysosome. Some M6P receptors would be found in endosomes and in vesicles recycling back to the Golgi as well. All of these M6P receptors would be entirely intracellular. However some of the M6P receptors are found on the plasma membrane, where they participate in the salvage pathway -- their extracellular receptor domains can bind escaped hydrolases and return them to the lysosomes by RME.

4-9. A. Splicing. This looks like a case of alternative splicing, because there is only one copy of the gene per haploid set of chromosomes.

Hint for 4R-4 Part D. In cells with mutant SREBP (no TM domains), cellular levels of cholesterol are abnormally high, not abnormally low. Now that you know this, try to come up with a reasonable mechanism.

Problem Set 4A – Problems on Alternative Splicing, RNA stability and Translational Regulation

4A-1. Suppose gene A has 5 exons.

A. How many different lariat structures will form during the splicing of its primary transcript? (For this part assume no alternative splicing – assume there is only one way to splice the transcript.)

B. Suppose in liver cells, gene A transcripts are alternatively spliced so that exon 3 is skipped. In the course of splicing in liver cells, exon 3 will be found in: (mRNA) (the primary transcript) (a lariat structure) (the nucleus) (the cytoplasm) (nowhere, not transcribed). Circle all that apply.

4A-2. Protein P is synthesized in many tissues, including the kidney. Gene P, the gene that codes for protein P, has 11 exons. In most tissues, including kidney, protein P is 150 amino acids long, and exon #6 is skipped.

A. In normal kidney cells, splicing should connect the 5' donor site of intron #5 to the 3' acceptor site of intron (#4) (#5) (#6) (#7) (#8) (#4 or #5) (#5 or #6) (none of these) (any of these). Draw the relevant introns & exons and show how the splicing should go in the kidney.

B. In the photoreceptor cells of the eye, exon #6 is not skipped. Exon #6 is 30 nucleotides long, and is in frame.

B-1. In normal people, how long should the protein P be in the photoreceptor cells? The number of amino acids should be (< 150) (150) (156) (160) (166) (165) (180) (>180) (can't predict).

B-2. Consider the primary transcript of gene P in eye cells. In normal people, the primary transcript of gene P in kidney cells should be (longer) (shorter) (the same length) (can't predict).

B-3. Consider the ***number*** of lariats formed during splicing of the primary transcript in eye cells. In normal people, the number of lariats in kidney cells should be (larger) (smaller) (the same) (can't predict).

Show how you get protein P of the length in B-1 in the eye.

C. You take mRNA from normal photoreceptor cells and from normal kidney cells. You run the mRNAs in separate lanes on a gel, blot, and hybridize to two labeled probes – one for exon 5 of gene P (probe 5) and one for exon 6 (probe 6). What will you see on the blot?

C-1. If you use probe 5, you will see one or more labeled bands if you use mRNA from (eye cells) (kidney cells) (both) (neither) (can't predict).

C-2. If you use probe 6, you will see one or more labeled bands if you use mRNA from (eye cells) (kidney cells) (both) (neither) (can't predict). Explain briefly.

C-3. Suppose in at least one case, you find a labeled band corresponding to a size of 900 nucleotides. This means that 900 nt is the length of (the probe) (the mRNA) (both) (neither). Explain briefly.

C-4. If you compare lanes on the blot, which of the following will get you the same results --same # of labeled bands, in the same place? You will get the same results if you use (probe 5 with either mRNA) (probe 6 with either mRNA) (mRNA from kidney cells with either probe) (mRNA from eye cells with either probe) (none of these). Explain briefly. You don't need to repeat anything you said above, but it might help to draw all relevant blots.

4A-3. *This question is an extension of problem 13R-2 in the Fall Learners'Manual.*
A double stranded DNA fragment is shown below (at the end of the problem). All the parts of this question refer to this fragment. The DNA fragment includes a single gene (which codes for peptide X) and some of the surrounding nontranscribed DNA.

A. In the DNA shown, how many of each of the following would you expect to find?

A-1. Expected # of promoters is_____.

A-2. Expected # of stop codons is _____.

A-3. Expected # of 5' (donor) splice sites is _____.

A-4. Expected # of exons is _____ and first exon should be _____kb long.

Going along the sense strand from 5' to 3', in what order should the sites, codons, etc. occur? (Consider only the first one of each, if there is more than one.) The order should be _____.

B. If this DNA fragment comes from a normal cell that has never been genetically altered in a lab, then the DNA fragment is probably from a (eukaryotic cell) (prokaryotic cell) (either one) (neither one is possible.)

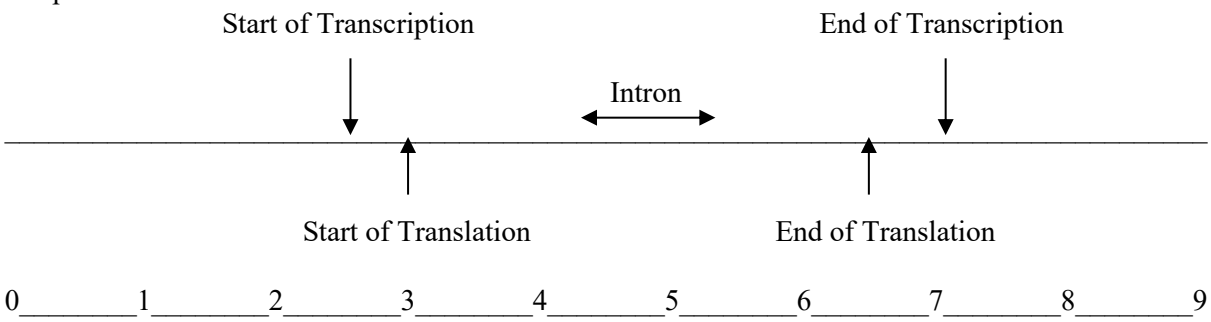
C. You isolate mRNA from this gene and use the mRNA to make a radioactive cDNA probe. The probe should be approximately _____ kb long. (Assume no poly A.)

D. You have cells in a bottle that are making protein X.

D-1. You isolate RNA from the cytoplasm of the cells, run it on a gel, make a blot of the gel, and hybridize the blot to the radioactive cDNA probe that you made. The number of radioactive bands on the blot should be _____, AND the band containing the longest RNA should contain an RNA that is approx. _____ kb long.

D-2. You isolate RNA from the nucleus of the cells, run it on a gel, make a blot, and hybridize to your radioactive cDNA probe as before. You compare your results to the previous experiment (D-1). This time you should get (more) (less) (the same #) of radioactive bands, AND the band containing the longest RNA should contain an RNA that is approx_____ kb long.

For problem 4A-3:



"Ruler" gives length of DNA in kb (kilobases) starting from 5' end of sense strand.
Transcription goes left to right.

4A-4. Look at the picture in question 4-13 showing the DNA region including the gene for NFATc1. Use the picture to answer the questions below. Remember that the relative sizes and distances in this picture are accurate, and that the smallest exon is about 100 BP long. Explain each part.

A. Consider the regions that should be spliced out of the primary transcript to make mRNAs (using pA2).

A-1. The region labeled 'exon 2' should be spliced out in (immune system) (heart) (both) (neither) (one or the other, but can't tell which) (beats me).

A-2. The region labeled 'exon 1' should be spliced out of the primary transcript in (immune system) (heart) (both) (neither) (one of the other, but can't tell which) (beats me).

B. Suppose mRNA A is made in the heart, and mRNA B is made in the immune system. Both mRNAs use pA1.

B-1. These two mRNAs should differ in sequence
(at the 5' end) (at the 3' end) (at both ends) (at the 5' or 3' end, but can't predict which)
(none of these – both mRNAs should have the same sequence).

B-2. Which mRNA should be longer? (mRNA A) (mRNA B)
(neither – both should be about the same length) (can't predict at all)
(mRNA A or mRNA B but can't predict which).

C. Suppose an additional mRNA is made by alternative splicing of the shorter transcript in heart. To make this mRNA, exon 7 is skipped.

C-1. The 5' donor site used for alternative splicing must be at one end of intron (5) (6) (7) (8) (9) (5 or 6) (5 or 7) (6 or 7) (7 or 8) (6 or 8) (7 or 9) (any of these).

C-2. During alternative splicing, which of the following should be joined?
(5' end of exon 6 to 3' end of exon 8) (3' end of exon 6 to 5' end of exon 8) (both 3' ends) (both 5' ends) (either 5' end to either 3' end) (none of these – you need some other combination).

Draw a picture including exons 5 to 9 (& relevant introns) that supports your answers. A well labeled diagram is sufficient.

C-3. Suppose you get a working protein from the alternatively spliced mRNA, one that uses the normal stop codon for translation. Assume exon 7 is approximately 3X as long as the shortest exon. In that case, which of the following is the most reasonable estimate for the length of exon 7? (3 BP) (9 BP) (99 BP) (150 BP) (293 BP) (299 BP) (301 BP) (304 BP) (306 BP) (360 BP) (363 BP) (>363 BP).

Explain briefly how you made your estimate.

4A-5. TOR is a soluble protein kinase. One substrate of the TOR kinase is eXBP (eXT binding protein). eXBP binds to the protein eXT. eXT is required to bind to the mRNA Cap. If eXT is not present, protein synthesis of capped mRNA does not occur. eXBP prevents eXT from binding to the Cap. eXT is not a substrate of TOR. Activation of TOR kinase activity stimulates protein synthesis. (I have changed the names of the proteins, but I am NOT making this up.)

A. TOR action is most likely to increase binding of (eXBP to the 5' end of mRNA)
(eXBP to the 3' end of mRNA) (eXBP to eXT) (eXT to the 5' end of mRNA)
(eXT to the 3' end of mRNA) (none of these) (more than one of these, namely _____).

B. You would expect unphosphorylated eXBP to (stimulate protein synthesis) (inhibit protein synthesis) (have no significant effect on protein synthesis) (inhibit or stimulate, but can't predict which) (do any of these).

C. Phosphorylation is most likely to directly (activate eXT) (inactivate eXT) (activate eXBP) (inactivate eXBP) (activate eXBP or inactivate eXT) (inactivate eXBP or activate eXT) (beats me).

Show or explain how activation of TOR should stimulate protein synthesis.

4A-6. The LDLR gene contains 18 exons and 17 introns. There is a deletion that goes from the middle of intron 15 to the middle of exon 18 (it deletes the normal stop codon). In people with this mutation, the LDLR protein is shorter than normal, but contains a sequence of 55 AA that are not found in the normal protein.

A. When the transcript from people with a normal LDLR gene is spliced, how many lariats (looped out sections) could be formed? (13) (14) (15) (16) (17) (18) (>18).

B. When the transcripts from people with this deletion are spliced, how many lariats (looped out sections) could be formed? (13) (14) (15) (16) (17) (18) (>18).

C. The sequence of 55 AA not found in normal people could be encoded in (intron 14) (exon 15) (intron 15) (intron 16) (exon 16) (exon 17) (can't tell).

Draw the part of the gene involved here, and indicate why the deletion produces the LDLR protein described above. Show the mRNA & protein produced by people with the deletion. (No individual explanations required for A-C.)

4A-7. Protein Q is a cell surface receptor; it is a single pass protein in the PM with both an extracellular and an intracellular domain. When the protein is in a vesicle inside the cell, the amino end is in the lumen.

A. The normal version of the gene for protein Q should encode [circle all that are correct]
(a NLS) (a transit peptide) (a PLS) (an amino terminal SP) (a site recognized by SPase) (an internal SP)
Explain how you know.

B. Protein Q is a receptor for a paracrine growth factor (a paracrine that stimulates growth). The region that binds the paracrine is more likely to be (near the carboxyl end) (near one end or the other) (in the TM segment)(near the amino end).
Show how protein Q fits in the PM, and where its ligand binds. (Label both ends of the protein.)

Additional info for parts C-E: The gene for protein Q has many exons, and two possible sites of polyA addition. The major site is at the end of exon 12 (the last exon) and an alternative polyA addition site is in intron 6. If the alternative site is used, the protein is secreted.

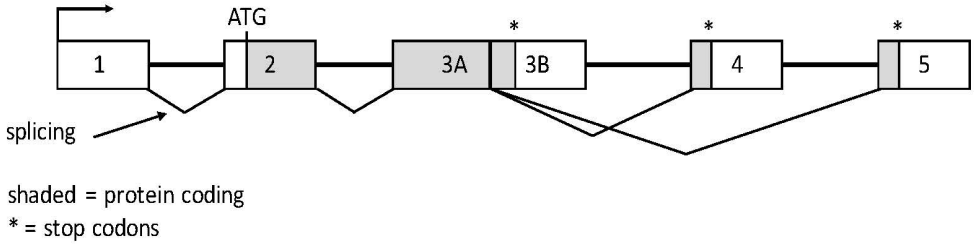
C. Suppose the TM segment of protein Q is encoded in a single exon. Then the TM segment is most likely to be encoded in (exon 5 or 6) (exon 6 or 7) (exon 7 or 8)
(any of the exons listed – all are equally likely) (none of the exons listed – must be in a different exon).

D. Suppose you can grow cells in vitro. All the cells transcribe the gene for protein Q. You have two batches of cells. One batch is normal – these cells use primarily the major polyA addition site in the transcript. You have a 2nd batch of cells (treated with some drug) – these treated cells primarily use the alternative polyA addition site in the transcript. Both normal and drug-treated batches are grown in the presence of the same (normal) amount of paracrine growth factor. You expect the drug-treated batch to grow (faster than normal) (at about the normal rate) (slower than normal).

Problem 4A-7, cont.

E. Suppose you have 3 types of cells, type R, type G #1, and type G #2. Suppose you can grow mixed cultures (in vitro) with two of the cell types. Each mixed culture contains R type cells and either #1 or #2 type G cells. All the cells (see below) have the gene for protein Q. R and G type cells are distinguishable because one type (G) makes a green fluorescent protein and the other type (R) makes a red fluorescent protein. (Neither fluorescent protein is protein Q.) You grow your mixed cultures with the same amount of paracrine growth factor. The R type cells make normal protein Q, using the major polyA site, but the G cells are as follows:
G type #1 cells use mostly the alternative polyA site in the transcript of the gene for protein Q.
G type #2 cells don't make any protein Q at all.
You compare the growth rates of the R type cells in the two mixed cultures (R + G #1 OR R + G #2. NOT G #1 + G #2.) The R type cells should grow (about the same in both cases) (faster with G #1) (faster with G #2).

4A-8. The structure of the IASH gene = Imaginary, Alternatively Spliced, Hormone gene is shown below. (This is a simplified version of a real gene).



Not all the splice sites shown are necessarily used to make any one mRNA. However,
(i) the only possible splices are the ones shown – no others are found.
(ii) translatable mRNAs must not contain introns – in normal cells, transcripts with introns are retained in the nucleus and never reach the cytoplasm.
(iii) Note that there is no intron between the exons labeled 3A and 3B. (The darker line divides the two exons. All of 3A is protein coding, but only the start of 3B is protein coding.) 3A and 3B are considered separate exons because of possible alternative splicing of section 3A to either exon 4 or exon 5.

A. Consider a translatable mRNA from the IASH gene that contains exon 4.
A-1. How many different translatable mRNAs can contain exon 4? _____
A-2. If a translatable mRNA contains exon 4,
(i) the polyA addition site is probably in exon (1) (2) (3A) (3B) (3A or 3B) (4) (5) (4 or 5) (any of these), *and*
(ii) whichever exon contains the poly A addition site, the site could be at (the 5' end) (the 3' end) (either end) of the chosen exon(s).

B. Consider all the translatable mRNAs that can be made from this gene. Assume that the splices shown are the only ones possible.
B-1. How many different translatable mRNAs can be made from the IASH gene? _____
B-2. There must be no acceptor splice junction at the start of exon (2) (3A) (3B) (3A & 3B) (3B & 4) (4) (5) (any of these) (all of these).
B-3. There must be no donor splice junction at the end of exon (1) (2) (3A) (3B) (3A & 3B) (3B & 4) (4) (any of these) (all of these).
B-4. Consider the minimum # of splice events needed to make a functional mRNA.
(i) The minimum # is _____, *and*
(ii) If the min. # of splice sites is used, the number of exons that will be skipped (spliced out along with introns) will be (0) (1) (2) (3) (1 or 2) (1, 2, or 3).

C-1. All proteins made from the IASH gene will probably have the same (COOH end) (5' end) (amino end) (3' end) (none of these). Circle all correct answers.

C-2. Consider N to be the # of amino acids encoded in any one of the translatable mRNAs from the IASH gene. The number of AA in the mature protein hormone made from that mRNA should be (>N) (<N) (=N) (≤N) (can't predict -- depends on which splice sites are used).

4A-9. Some mRNAs have a sequence of nucleotides in their 3' UTR called an ARE (AU-rich element), which binds to the protein AUF1. The β-adrenergic receptor (β-AR) is a GPCR that binds to epinephrine and couples with the G protein that activates adenylyl cyclase. β-AR mRNA has an ARE in its 3' end. After epinephrine treatment, β-AR protein levels decrease. β-AR protein turns over, but for this problem, assume there is no change in protein half-life upon epinephrine treatment. Upon epinephrine treatment, there is no change in transcription of the β-AR gene, but the half-life of β-AR mRNA is 5h, relative to 12h in untreated cells.

A. Binding of epinephrine to β-AR leads to an increase in AUF1 transcription. Which of the following are plausible explanations for this observation? **circle or check all that seem plausible**
(epinephrine binds to an intracellular receptor that upregulates AUF1 transcription)
(PKA phosphorylation activates a transcription factor that upregulates AUF1 transcription)
(cAMP binds to and activates a transcription factor that upregulates AUF1 transcription)
(Gαs binds to a transcription factor that upregulates AUF1 transcription)

B. Propose how AUF1 acts on β-AR mRNA. AUF1 is most likely to
(increase mRNA stability) (cause mRNA degradation) (activate translation) (inhibit translation)
(increase mRNA stability and activate translation) (cause mRNA degradation and inhibit translation).

Problem Set 4R – Problems on Chromatin, Regulation and Protein Sorting

4R-1. Answer each part of this question as indicated and explain briefly.

A. Suppose a gene which was previously not transcribed is now transcribed. The average number of nucleosomes per enhancer (of the gene) should (increase) (decrease) (stay the same) AND the number of enhancers per gene should _____.

B. The number of different tissue types is (larger than) (smaller than) (the same as) the number of different types of RNA polymerase(s) in the eukaryotic nucleus.

C. In normal cellular chromatin, the number of types of histones is (larger than) (smaller than) (the same as) the number of molecules of histone per nucleosome.

D. Which of the following proteins ***should not*** be made on ribosomes attached to the rough endoplasmic reticulum? (insulin receptors) (estrogen receptors) (insulin) (glut 4 = glucose transporter that responds to insulin) (Protein kinase A). Circle one or more choices.

E. Which of the protein (s) listed in D must have a signal peptide that is removed? _____.

4R-2. The region of the DNA that codes for the protein called Insig has binding sites for STAT at -1000 and for AP-1 at -685. STAT and AP-1 are two important transcription factors. For each part of this question, circle ***all correct answers***. There may be one or more.

A. The binding site that is farthest upstream of the start of transcription (for Insig) is the one for (STAT) (AP-1) (basal transcription factors) (none of these – all are downstream of the start of transcription) (can't tell – depends on whether STAT and AP-1 are regulatory or basal TF's).

B. Binding of STAT promotes transcription of the Insig gene. The binding site for STAT should be considered a (part of basal promoter) (enhancer) (silencer) (co-activator) (cis acting element) (trans acting element).

C. A paper about Insig says: 'The region from -153 bp to +18 is sufficient to drive expression of a reporter gene.' This means that they made a recombinant plasmid containing base pairs -153 to +18 of the Insig locus joined to the transcribed region of gene R. When they added the recombinant plasmid to cells, the cells made protein R. If they added a plasmid without the Insig part, no protein R was made.

These results indicate that the region from -153 to +18 of the Insig locus definitely includes the (basal promoter) (start of transcription) (start of translation) (polyA addition site) (first splice site) (none of these).

4R-3. A. You want to isolate mRNA for GLUT 4 (the insulin-responsive glucose transporter) so you can make cDNA. Insulin is a protein hormone made and secreted by pancreatic cells.

A-1. You should be able to get mRNA for GLUT 4 from cells of (liver) (brain) (muscle) (pancreas) (2 or more of these) (none of these) AND

A-2. Insulin has no effect on the transcription or translation of the glut 4 gene/mRNA. However, insulin increases the amount of GLUT 4 protein in the membrane (in the appropriate cell type). How is this possible?

B. You isolate DNA from the cell types listed above (in A). You extract the cell DNA, cut it up with restriction enzymes, run a gel, blot, and hybridize to a labeled glut 4 cDNA probe. (In other words, you do a Southern.) If you use DNA from the same cell type used to make the cDNA probe, you get three labeled bands on the blot. If you repeat the experiment using genomic DNA from the other cell types, you should get the same result with (some, but not all, of the other cell types) (all the other cell types) (none of the other cell types) (can't predict).

C. Suppose you take chromatin, and treat it with a DNase that cuts at random. Then you isolate DNA from the chromatin and do a Southern blot (as in B) except that this time you use insulin cDNA as a probe. If you start with chromatin from brain cells, and do a limited DNase treatment of the chromatin, you get one labeled band on the blot. Then you repeat the experiment, starting with chromatin from the other cell types. If you get a different result,

C-1. You would expect to get the different pattern from (liver) (muscle) (pancreas) (liver and/or muscle) (liver and/or pancreas) (muscle and/or pancreas) (any of these) AND

C-2. The "different pattern" you would expect is (one band but with more label -- darker) (one band but with less label -- lighter) (multiple distinct smaller bands) (can't predict).

D. You measure the levels of insulin in the blood AND the levels of insulin mRNA inside cells. You look at insulin and mRNA values in a fasting animal and in an animal that has just eaten. The insulin blood levels are different, but the insulin producing cells have the same level of insulin mRNA in both cases.

D-1. The most likely explanation is that blood insulin levels are adjusted by altering the rate of (transcription) (RNA splicing) (degradation of mRNA) (translation of mRNA) (modification of insulin) (exocytosis) (endocytosis), AND

[#]D-2. In the fasting animals, the levels of insulin in the blood should be (higher) (lower) (can't predict).

[#]If we haven't got to this yet, look up the function of insulin.

4R-4. SREBP = SRE binding protein. SRE = sterol response element. An SRE affects production of the proteins needed for uptake and synthesis of cholesterol. An SRE is found on the 5' side of the promoter of the gene for the LDL receptor, and the 5' side of the promoter of the gene for enzyme H (the enzyme catalyzing the first step in cholesterol synthesis). There are no other SRE's in or near these genes.

A. Assume for this part that SREBP is a soluble protein that binds to the SRE, and that SREBP is inactivated when the level of membrane cholesterol is high.

A-1. For enzyme H, the SRE should affect (transcription of the gene) (translation of the mRNA) (both) (neither)

A-2. A mutant that lacks SREBP should have levels of cholesterol that are (normal) (unusually high) (unusually low) (can't predict.) Explain your answers briefly.

B. SREBP is not really a soluble protein. It is an integral protein in the ER membrane. SREBP has two transmembrane segments and both ends of the protein are in the cytoplasm. The cytoplasmic domain on the carboxyl end can be detached from the rest by proteolysis (hydrolysis of the peptide chain). The detached part (called nSREBP) acts as a transcription factor.
A group of amino acids making up a nuclear localization signal is likely to be found in (SREBP) (nSREBP) (both) (neither). To clarify, SREBP = whole protein; nSREBP = the detached part.

C. SREBP is hydrolyzed to generate nSREBP by protease 1, which is located in the Golgi.

C-1. When SREBP is on its way from its site of synthesis to the Golgi, it should be (entirely in the lumen of a vesicle) (entirely in the cytoplasm) (in the membrane of a vesicle) (either in the lumen or membrane of a vesicle) (any of these).

C-2. When SREBP is in the Golgi, its carboxyl end should be (in the lumen of the Golgi) (in the cytoplasm) (either way).

D. In normal cells, high levels of cholesterol in the ER membrane prevent transport of SREBP to the Golgi. By genetic engineering you can get cells that make a mutant version of SREBP that lacks both transmembrane domains. In these cells, high levels of cholesterol have no effect on the synthesis of enzyme H or LDL receptor.

D-1. A sterol binding domain is likely to be found in (normal SREBP) (normal nSREBP) (both) (neither).

D-2. Mutant SREBP should be made on (ribosomes attached to the ER) (made on free ribosomes in the cytoplasm) AND newly made mutant SREBP should be found (in the ER membrane) (in the cytoplasm) (inside the ER lumen) (beats me).

D-3. In cells with the mutant SREBP (no transmembrane domains) you would expect cellular levels of cholesterol that are (normal) (unusually high) (unusually low) (can't predict). Explain your answers to D briefly.

This (part D) is a very hard question, so don't despair if you can't figure it out. If you get stuck, try the hint on page 42.

4R-5. Enzyme M is normally found in mitochondria, in the intermembrane space. You want to study how synthesis and regulation of enzyme M is controlled. You use genetic engineering to produce cells with deletions in different parts of the enzyme M gene. The structure of the gene and the results of the experiments are as follows. Note that all directions refer to the sense strand (& it is the one shown) – this is the standard convention.

Arrangement of Exons in enzyme M gene: 5' 1----2----3----4----5----6----7----8----9----10 3'

Expt.	Gene used	Enzyme M activity?	Where found?	Amount of Enzyme M
1	Normal (no deletions)	yes	Intermembrane space	normal
2	Delete exon 2	yes	not in mitochondria	normal
3	Delete exon 3	yes	Mitochondrial matrix	normal
4	Delete exon 5	no	Intermembrane space	normal
5	Delete intron between exons 9 &10	yes	Intermembrane space	low
6	Delete exons 2 & 3	yes	?	normal

A-1. Where would you expect to find the basal promoter for the enzyme M gene? (within an exon) (within an intron) (within either one) (overlapping an intron/exon boundary) (none of these) (can't predict), AND

A-2. Relative to the basal promoter, control elements, if any, should be found (5') (3') (either way).

B-1. From the data given, exon 2 codes for (a transit peptide) (a SP needed to enter the ER) (a localization signal needed to enter the mito. inner membrane) (some other localization signal) (none of these) AND exon 3 codes for _____ (same choices.)

B-2. Amino acids that are not found in the mature enzyme are coded for by (exon 2) (exon3) (exon 5) (2 & 3) (2 & 5) (3 & 5) (all 3 exons) AND in expt. 6, enzyme M should end up (outside the cell) (in the cytoplasm) (in the plasma membrane) (in peroxisomes) (in lysosomes) (in some other organelle).

C-1. The low levels of enzyme M made in experiment 5 are probably due to changes in (DNA replication) (rate of transcription) (accuracy or efficiency of splicing) (initiation of translation) (degradation of mRNA) (translation, but can't tell at which step) (post translational modification) AND

C-2. The low enzyme levels are caused by alteration or removal of a (cis acting control element) (a trans acting control element) (either one). Explain your answers.

D. Suppose you discover that enzyme M is localized to different organelles in different tissues of a multicellular organism. In most cells it's in the mitochondria as described above, but in liver cells it's in peroxisomes and in the lens cells of the eye it's in the cytoplasm. (I made this up, but it is possible.) Assume there is only one copy of the gene for enzyme M per haploid nucleus, and you are studying a normal organism -- no deletions, genetic engineering, etc.

D-1. The most likely explanation is that the various cell types differ in (DNA sequence) (levels of transcription) (number of DNA copies) (splicing) (transport of mRNA to cytoplasm) (translation) (beats me).

D-2. Consider the newly made form of enzyme M in liver cells that has just been released from ribosomes. This enzyme should contain (0) (1) (2) (3) (>3) (can't predict how many) localization signal(s) on its amino end AND _____ localization signal (s) on its carboxyl end. Describe or diagram out as specifically as possible how you could get these results.

E. Suppose (whether this fits your answers above or not) that if you omit exon 2, not all the enzyme M activity ends up in the same place. Some is outside the cell and some is in the cytoplasm. How could this be explained? (This question was not on the exam, but is included for study purposes.)

4R-6. Mature T cells (part of the immune system) have high enzyme Z activity. Immature T cells have very low enzyme Z activity. You wish to figure out the basis of the difference, because abnormal levels of enzyme Z activity are associated with disease states. (The function & localization of enzyme Z do not matter for this problem.)

A. You have a probe to an exon of gene Z (gene for enzyme Z). Mature T cells contain large amounts of cytoplasmic RNA that hybridize to the probe. Immature T cells contain low amounts of cytoplasmic RNA that hybridize to the probe. You repeat your experiment using several different probes to different exons, and the result is the same with all the probes. The cytoplasmic RNA is the same size (about 3,000 bases) in both types of cells.

Given the results above, what is a reasonable explanation for the change in enzyme Z activity when T cells mature? Could be a difference in (efficiency of translation of mRNA for enzyme Z) (covalent modification of enzyme Z) (transcription of gene Z) (splicing of transcript from gene Z) (stability of enzyme Z) (any of these).
Briefly explain your choice.

B. Mature and immature T cells should differ in
(basal transcription factors) (regulatory transcription factors) (enhancers) (basal promoters) (splicing factors) (regulatory proteins that bind to the mRNA) (response elements in the mRNA) (activity of protein kinases and/or phosphatases that act on enzyme Z, not on histones).

C. Suppose you treat the chromatin of the two cells with DNase, and then examine the DNA that remains after proteins are removed. You get a "ladder" pattern from some sections of the DNA – a regular pattern of bands of sizes 200 BP, 400 BP, etc.

C-1. What did the DNase do? In the section that gave the regular pattern, the enzyme must have cut or digested the DNA that is (in the middle of nucleosome cores) (in exons) (in introns) (in between nucleosome cores) (not in nucleosomes) (somewhere else – none of these would explain these results.)

C-2. Suppose you find a section of the DNA that gives a large fragment in immature T cells, but no distinct bands at all from mature T cells (under the same conditions of digestion, etc.). This section of DNA is not incorporated into the primary transcript in immature ***or*** mature T cells.

(1). Which sample of chromatin had ***more*** molecules of H2B on this section (before digestion)? There was more H2B in (sample from mature T cells) (sample from immature T cells) (neither – both have the same number) (neither – neither one had any H2B).

(2). Where is this section of DNA, relative to the nearest start of transcription? It could be (close to the start) (upstream, more than 200 bases away) (downstream, more than 200 bases away) (more than 200 bases away on either side) (any of these) (none of these).
Explain your answers to (1) & (2) briefly.

D. Suppose the section described in part C is part of the DNA that includes gene Z (and no other transcribed regions). Then the section described in C is most likely to be from (an exon) (an intron) (an enhancer) (silencer) (an exon or intron) (an enhancer or a silencer) (any of these) (none of these).

Problem Set 5 Homeostasis & Thermoregulation

5-1. A, B, and C represent three different chemicals or processes. Which of the following situations represents negative feedback?

a. A stimulates B, then B inhibits C.
b. A inhibits B, then B inhibits C.
c. A inhibits B, then B inhibits A.
d. A stimulates B, then B inhibits A.
e. A stimulates B, then B stimulates A.

5-2. T/F, Correct if false:

a. Blood is a type of epithelial tissue.
b. In an organism that maintains homeostasis, blood glucose levels stay constant.
c. On a hot day, blood vessels in the skin constrict to maintain body temperature.

5-3. There are thermoreceptors both near the surface of the body, in the skin, and deep inside the body, in the abdominal cavity and brain. What is the advantage of having thermoreceptors in both places?

5-4. The temperature of the hypothalamus always remains relatively constant, even when an animal moves from a very hot to a very cold environment. Explain.

5-5. While observing rats in a room that is slowly heating up, you notice that when the temperature reaches 30 C the animals begin to show a new behavior - licking themselves and spreading the saliva over the body, particularly on hairless areas such as the paws, tail and nose. What might be the function of this behavior?

5-6. The cells at the base of a sweat gland secrete large amounts of Na^+ into the lumen of the gland. The lumen fills with fluid which moves through the ducts towards the skin. Cells in the duct actively transport the sodium ions out of the lumen and back into the body tissues.

A. The tissue involved in sodium transport is _____ tissue. (what type?)

B. If the sodium is going to be reabsorbed, why does the gland expend energy on secreting it in the first place?

5-7. A football fan notices that a drink of alcohol makes him feel a sudden flush of warmth, so he drinks a few cans of beer to warm himself at a chilly football game. Surprisingly, he ends up feeling *colder* than his non-drinking companions. Suggest an effect alcohol might have on the circulatory system that would explain both these phenomena: an initial feeling of warmth, followed by cooling of the extremities.

5-8. Mary and Ann are sitting in a room that is quite cold. Mary has a normal basal metabolic rate (BMR) and is shivering. Ann has some pathological condition that results in a higher than average BMR, and she is not shivering.
Note: BMR is a measure of the basic rate of energy metabolism. It is calculated from the rate of uptake of oxygen for a person at rest.

a. The temperature in Mary's and Ann's hypothalamus is the same. Explain why both women have the same hypothalamic temperature.

b. Why isn't Ann shivering?

c. Ann's rate of oxygen consumption is probably (higher than) (lower than) (the same as) Mary's. (There is more than one possible answer here; it's the reasoning that counts.)

5-9. On a warm day, Guinevere gaily traipses along for 8 hours, producing 50 ml of perspiration each hour. Arthur loses an equal amount of fluid (400 ml), but he loses it all in half an hour of heavy sweating while strenuously jousting with his knights. Why is Arthur's sweat saltier than Guinevere's? (Hint: Transporters are used to recover salt from perspiration. The answer is not a male/female difference – it's about intensive vs. light exertion.)

5-10. Hot flashes (or flushes) are very common in menopausal women. They are triggered by falling levels of estrogen, but it's not clear how. (Falling estrogen is necessary, but not sufficient, to trigger them.) During a 'HF' episode, the person suddenly feels very hot. In response, she flushes – her face gets red, and she sweats. The HF only lasts a short time. The average core body temperature of people with hot flashes and those without is the same – 37°C.

A-1. In the short run, the response to a HF should cause the core body temperature to (increase) (decrease) (stay the same) (can't predict).

A-2. In the short run, the response to a HF should make the face feel (hotter) (cooler) (the same) (can't predict).

A-3. After the response to a HF, the core body temperature should (rise) (fall) (stay wherever it is) (can't predict).

A-4. The response to a HF is similar to what happens when someone (first starts developing a fever) (finishes with a fever) (is in the middle of a fever) (is at the start or end of a fever) (beats me).

B-1. The effectors causing the flush -- redness of face -- are (skeletal muscle) (smooth muscle) (liver) (sweat glands) (pancreas) (red blood cells). Circle all correct choices.

B-2. The regulated variable(s) here is/are (extent of vasoconstriction) (redness of face) (core body temperature) (face surface temperature) (all of these). Circle all correct choices.

B-3. Consider menopausal women who do or do not have HF episodes. The set point for the regulated variable should be (higher in women with symptoms) (lower in women with symptoms) (the same for both types of women) (beats me).

C. In the past, hormone replacement therapy (with estrogen) was widely prescribed to treat hot flashes. It is very effective, but has unfortunate side effects. Therefore people are trying to figure out the mechanism of hot flashes and looking for other ways (besides estrogen treatment) to reduce their frequency. Two drugs that affect the frequency of HF episodes are clonidine and yohimbine. Clonidine reduces hot flashes. Yohimbine does the opposite. Measurements indicate that these substances affect the body's thermo-neutral zone – defined here as the range of temperatures that induce neither sweating nor shivering in the body.

C-1. In women who do not have hot flashes, the thermo-neutral zone is about 0.4°C. In these women, the maximum core body temperature should be about _____.

C-2. The thermo-neutral zone should be wider in (women without hot flashes) (women with hot flashes) (either – can't predict).

C-3. How should yohimbine affect the critical point for sweating? It should (raise) (lower) (have no effect on) the temperature at which sweating occurs.

C-4. You have two women in a room; one has hot flashes (sometimes) and the other doesn't. If you cool down a room, who will probably start shivering first? (The one who doesn't have hot flashes) (The one who does have hot flashes) (neither – both will start shivering at the same time) (can't predict).

Note: Yohimbine and clonidine both affect the levels of the neurotransmitter NE in the brain, but it is not clear exactly how the drugs work.

Problem Set 6 – Mechanism of Signal Transduction & Hormone Action

6-1. Consider protein P, described in problem 1-17 (& 3-4). Suppose protein P is a receptor in the plasma membrane.

A. Which domain would you expect to be the ligand binding domain?

B. Is it likely that this receptor is linked to a trimeric G protein?

6-2. Consider the properties of G proteins.

A. A G protein should be able to catalyze (phosphorylation of GDP → GTP) (hydrolysis of GTP → GDP) (both) (either one).

B. When bound to GTP, a G protein (inhibits) (activates) (either inhibits or activates) its target protein(s).

C. The active form of the G protein should (phosphorylate) (de-phosphorylate) (either phosphorylates or dephosphorylates) (neither phosphorylates nor dephosphorylates) its target protein(s).

6-3. Most protein kinases add phosphate to ser, thr and/or tyr. Why these particular amino acids?

For more on G proteins, see problems 6-24 & 6-25 (added in 10th edition).

6-4. During larval development of a toad, epithelial cells invaginate inward to form a gland. Thyroid hormone stimulates more rapid development of this gland. Corticosterone, a hormone produced by adrenal glands of some animals, inhibits development of the gland, even if thyroid hormone is present. Two possibilities have been suggested to explain how corticosterone might inhibit gland development:

A. Corticosterone might act on the skin cells, to cause (up regulation) (down regulation) of receptors for (corticosterone) (thyroid hormone)

B. Corticosterone might act on the anterior pituitary gland, to (stimulate) (inhibit) its secretion of (TRH) (TSH) (TH) (ACTH) (CRH).

6-5. Phospholipase C (PLC) and Protein Kinase C (PKC) both act in the same signal transduction pathway. What is the pathway, and what are their respective roles in it?

For problems 6-6 to 6-9, consult your texts or handouts for the relevant pathways of glycogen metabolism. You are not expected to do this problem without the relevant pathways in front of you. A handout is at http://www.columbia.edu/cu/biology/courses/c2006/handouts/glycogen09.pdf

6-6. How will the conditions described below affect glycogen synthesis and breakdown in a cell in the absence of any hormone?

A. Protein kinase A becomes permanently active.

B. Phosphorylase kinase becomes permanently active.

6-7. Suppose one molecule of epinephrine contacts a liver cell. Estimate how many molecules of glucose would be released to the blood.

6-8. Suppose you add a hormone or a drug to a skeletal muscle cell and the activity of glycogen phosphorylase increases. If the hormone receptor is inactivated, neither the drug nor the hormone have any effect. The drug is acting as a/an _____ of the hormone. Choices: (antagonist) (agonist) (antagonist or agonist) (co-activator) (repressor) (allosteric effector).

6-9. To liver cells growing *in vitro* you add drugs that have the following effects. How will liver cell glycogen be affected by each? Explain your answers.

A. a drug that inhibits phosphodiesterase

B. a drug that prevents activation of protein kinase A

C. a drug that is an epinephrine agonist

6-10. Calcium ionophores are drugs that can be inserted into membranes, causing them to leak calcium.

A. Ca^{++} ionophores can be used to mimic the effect of _____

a. phosphodiesterase b. protein kinase A c. IP_3 d. DAG

B. If you want the ionophores to mimic the compound(s) you have selected above, the best place to insert the ionophores would be into membranes of the

a. ER b. Golgi c. nucleus d. cell (plasma membrane)

6-11. A particular hormone elicits six different responses in its target cell, all by way of cAMP. A certain drug eliminates one of these responses, but not the other five. Which of the following, if any, could the drug be blocking: the hormone's receptors, G protein, adenylyl cyclase, cyclic AMP?

6-12. Radio-labeled hormones A and B are used in binding studies. The hormones are added to intact cells, or cells which were treated with trypsin (which destroys proteins exposed to the outside of the cell), or cytosol, or membrane fractions. The amount of labeled hormone bound to each fraction is measured. Here are the results:

	Labeled hormone (counts per minute)	
	A	B
intact cells	9000	11000
trypsin treated cells	200	11000
cytosol	200	11000
membrane fragments	9000	200

A. Which hormone (A or B) is a steroid hormone, which a peptide?

B. How do you know?

C. One of these hormones is found to circulate free in the aqueous blood, the other is usually carried in association with a protein. Which is which? Explain.

6-13. The addition of either cortisol (a steroid hormone) or epinephrine (an amine) to liver cells *in vitro* stimulates the release of glucose into the medium, but the time required for this response is different in the two situations. Which hormone will induce the more rapid response? Explain.

6-14. For centuries, farmers had known that if a male's testes are removed, he loses interest in females. In the 1890's, Brown-Sequard, a prominent neuroscientist, wondered whether the reverse was also true: Was there something in a male's testes that could reawaken his sexual desire as he passed his 70th birthday? He used classical techniques to separate and purify the chemicals from the testes of a dog. By the end of this procedure, he had a dirty, oily phase floating on top of a clear, watery fluid. He injected himself with this clear liquid, and experienced a sudden rejuvenation of sexual fervor, attested to by his 20 year-old girlfriend. Could this experiment be used to support the notion that testicular hormone stimulates sexual desire? (Hint: what is the chemical nature of testicular hormone? If you don't know, look it up in your text.)

6-15. A cell with a radius of 50 microns has 100,000 hormone receptors in its membrane, each a perfect sphere with a radius of 10 A. Would you expect this large number of receptors to completely cover the surface of the cell? Calculate the percentage of the cell surface covered by the receptors.

Note: surface area of sphere = $4 \pi r^2$; 1 micron = 10^{-6} m; 1 A = 10^{-10} m

6-16. Cells growing in culture are found to produce enzyme A, which is neutral. When compound F is added to the growth medium, enzyme A rapidly acquires a negative charge, and the activity of enzyme A is increased, but the amount of mRNA/cell for enzyme A is not greater than before F was added. If, when compound F is added to the medium, the cells are simultaneously treated with an inhibitor of adenylyl cyclase, then enzyme A remains neutral.

Fill in the blanks with appropriate choices from this list, and explain both parts: (a transcription factor) (a cell surface receptor) (a receptor on the ER or Golgi) (a protein kinase in the cytoplasm) (a G protein) (calmodulin) (a protein phosphatase in the cytoplasm) (none of these).

A. The most likely explanation is that compound F is a ligand for _____ .

B. When cells are exposed to compound F, _____ should be activated.

6-17. Gelsolin is an ABP (actin binding protein) – it is actually an enzyme that catalyzes breakdown of actin polymers. Gelsolin is activated by Ca^{++}. (Its function is not important for this problem.)

A. When the Ca^{++} concentration in the cytoplasm rises, the activity of the gelsolin goes up, but gelsolin does not bind Ca^{++} itself, or any Ca^{++}-binding protein. The most likely explanation is that Ca^{++} acts <u>primarily</u> by affecting (adenyl cyclase) (guanyl cyclase) (phosphodiesterase) (protein kinase C) (protein kinase A) (any of these) (none of these).

B. Suppose some hormone activates gelsolin in its target cells by raising the $[Ca^{++}]$ in the soluble cytoplasm without changing the total intracellular Ca^{++}. In that case, the hormone probably opens Ca^{++} channels in the (plasma membrane) (ER) (Golgi) (nucleus) (mitochondria) AND the channels are probably gated directly by (voltage) (cAMP) (cGMP) (DAG) (IP_3) (the hormone itself) (G proteins). Outline the series of events that should allow the hormone to raise cytoplasmic Ca^{++} levels.

6-18. When intact cells are treated with a peptide hormone, a calcium-dependent enzyme, CDE, is activated in the cells. This enzyme is the target of a signal transduction system, which you would like to analyze.

You make *in vitro* systems by mixing various cellular fractions, treat with the peptide hormone, and then measure CDE activity. With the following *in vitro* systems, you get these results:

a. Cytosol (which includes CDE but no organelles) + ER + membranes: CDE is activated

b. Cytosol + membranes: CDE is not activated

c. Cytosol + ER: CDE is not activated

Inhibitors of adenylyl cyclase have no effect on activation of CDE in either intact cells or the *in vitro* systems.

Before trying to answer this question, draw out the signaling system involved. (If you get stuck, see p. 70.) Before continuing to part B, please look up the answer to part A on p. 70 to be sure you are correct.

A-1. When you add hormone, it probably binds to protein(s) in the (cytosol fraction) (ER fraction) (membrane fraction) (none of these) AND

A-2. The fraction(s) to which the <u>hormone itself</u> binds should release (DAG) (IP_3) (cAMP) (Ca^{++}) (GTP) (GDP) (none of these) into the soluble part of the mixture. For both parts of A, chose one or more answers and explain.

B. You have a mutant cell that lacks phospholipase C. You make an *in vitro* system with the three fractions mentioned above.

B-1. If you want to activate CDE *in vitro*, which fraction(s) should NOT come from the mutant? (cytosol) (membranes) (ER) (none of these -- all of these can come from the mutant and activation of CDE will still work).

B-2. If you do use all three fractions from the mutant, you would get activation of CDE if you add (IP_3) (Ca^{++}) (cAMP) (GTP) (GDP) (none of these will work) (none of these are necessary). For both parts of B, chose one or more answers and explain. You don't need to repeat anything you said in part A: just refer to it.

C. You have a mutant cell that lacks IP_3 receptors. You make an *in vitro* system with the three fractions mentioned above. The membrane and ER fractions come from this IP_3 receptor mutant and the cytosol fraction comes from normal cells. Which of the following should be added to get the maximal activation of CDE using this *in vitro* mix? (hormone) (IP_3) (Ca^{++}) (cAMP) (none of these will work). If two or more choices are needed, or two are equally good, circle all of them and explain. You don't need to repeat anything you said in parts A & B; just refer to it.

6-19. The receptor for cortisol, a steroid hormone, is found in the cytoplasm.

A-1. Cortisol receptor should be made on ribosomes that are (in mitochondria) (attached to the ER) (in the nucleus) (attached to the outer membrane of the nuclear envelope) (free in the cytoplasm) (can't predict) AND

A-2. Binding of cortisol to its receptor probably (causes removal of a localization signal) (unmasks a localization signal) (masks a localization signal) (masks or unmasks a localization signal) (none of these) (any of these).

B. Consider the region of the chromatin that is transcribed when the cortisol receptor binds to the DNA. Consider the histones in this region of the chromatin.

B-1. Before cortisol is added, the number of molecules of histone H2B per 100 base pairs of DNA should be _____ AND

B-2. After cortisol is added, the number of H2B molecules bound to DNA will probably (increase) (decrease) (stay about the same) AND the sensitivity to DNase should (increase) (decrease) (stay the same) (can't predict).

C. Consider the region of the chromatin that cortisol receptor binds to (called the cortisol response element). After cortisol is added, the number of H2B molecules bound to this region of the DNA will probably (increase) (decrease) (stay about the same) AND the sensitivity to DNase should (increase) (decrease) (stay the same) (can't predict).

D. The cortisol receptor has three sections, X, Y and Z (in that order). Using recombinant DNA technology, it is possible to make altered receptor genes that code for only part of the receptor; it is also possible to add normal or altered receptor genes to cells that don't normally respond to hormones. If you do it right, the added genes are expressed and make normal or altered receptors. Suppose you do some experiments with altered cortisol receptor genes/proteins and get the following results:

(i). You get a cell to make a shortened receptor, consisting of only section Z. This shortened receptor will move to the nucleus in response to added cortisol. Shortened receptors consisting of just X, just Y, or both, do not respond to hormone. Presence of this shortened receptor in a cell has no effect on transcription.

(ii). A shortened receptor consisting of Y plus Z responds to cortisol by moving to the nucleus and binding to DNA. Binding of this shortened receptor has no effect on transcription.

(iii). In a test tube containing ground-up cells (but not whole cells), receptor consisting of X + Y stimulates transcription from chromatin (without cortisol). In whole cells, production of a receptor with only X & Y has no effect at all (+ or - cortisol). Given all these results, answer the following questions:

D-1. Which part of the cortisol receptor probably contains a localization signal? (X) (Y) (Z) (X or Y) (Y or Z) (any part) (nowhere) (somewhere, but can't predict).

D-2. Which part of the cortisol receptor probably binds to a cis-acting regulatory sequence in the DNA?

D-3. Which part of the receptor is most likely to bind to other transcription factors or co-activators?

D-4. What is the standard terminology for each part (X, Y & Z) of the cortisol receptor?

Explain how you figured all this out from the results.

E. How can the addition of cortisol turn on transcription of many different genes?

6-20. Suppose protein kinase K has 2 regulatory subunits. Subunit A is calmodulin; subunit B is phosphorylated by protein kinase A. (This example is made up, but there are enzymes like this.)

A. PKK activity should be altered in response to changing levels of (cAMP) (DAG) (IP3) (cAMP & DAG) (cAMP & IP3) (IP3 & DAG) (all three) (none of these). Explain.

B. Suppose 2 different hormones activate PKK. Call them H1 and H2 (hormones 1 & 2). You get the following experimental results:

(1). Elevated levels of phosphodiesterase reduce the cellular response to H1 (but have no effect on response to H2).

(2). Antagonists of DAG have no effect on the response to either hormone.

(3). Inhibitors of phospholipase C inhibit the response to H2 (but have no effect on response to H1).

B-1. Given these results, H1 probably activates PKK using (adenylyl cyclase) (protein kinase C) (Ca^{++}) (PKC and/or Ca^{++}) (adenylyl cyclase and/or PKC) (adenylyl cyclase and/or Ca^{++}) (any of these) (none of these), AND

B-2. H2 probably activates PKK using _____ , AND

B-3. H1 probably affects the state of (subunit A) (subunit B) (both) (neither) (can't predict), AND

B-4. H2 probably affects the state of _____, AND

Outline the pathway of action of each hormone; show how effects of inhibitors etc. fit in.

C-1. G-proteins could be involved in the action of (H1) (H2) (both) (one or the other but not both) (neither) (can't predict).

C-2. A single G-protein could be involved in the action of _____ (same choices as C-1).

C-3. A receptor that is a single pass protein could be involved in the action of _____ (same choices as C-1).

6-21. There are many different adrenergic receptors. All the adrenergic receptors are transmembrane proteins coupled to G proteins. This question is about the α1 & α2 (adrenergic) receptors.
If you add epinephrine to cells with the α1 receptor, phospholipase C (PLC) is ***activated***.
If you add epinephrine to cells with the α2 receptor, adenyl cyclase (AC) is ***inhibited***.
For this question, assume that there are no additional complications.

A-1. The G proteins used by the α1 and α2 receptors should (be the same) (be different) (be the same or different -- can't predict without additional information).

A-2. If you add an agonist of epinephrine to cells with the α 1 receptors (no other adrenergic receptors) you would expect a change in the cellular concentration of :
(IP3) (cAMP) (IP3 or cAMP, but not both) (either of these) (neither of these).

B-1. Suppose cells with the α2 receptor (no other adrenergic receptors) contain active PKA. If you add epinephrine to the cells, the amount of active PKA in the cells should (increase) (decrease) (stay the same) (can't predict).

B-2. Suppose you add an inhibitor of phosphodiesterase (PDE) at the same time that you add the epinephrine. How will this change your result? Compared to the effect in B-1, the change in the amount of active PKA should be (greater) (less) (in the opposite direction) (about the same). Draw out the relevant parts of the signaling pathway and show how it explains your answers. Please OMIT any factors or steps that are not involved.

6-22. If you add epinephrine to cells with the α1 or α2 receptor, the enzymes PLC or AC are affected as explained in the previous problem. In addition, if you add epinephrine to cells with either type of receptors, the Ca^{++} in the cytosol is rapidly increased.

A. This result is expected in (cells with α1 receptors) (cells with α2 receptors) (both) (neither).

Before continuing to part B, please look up the answer to part A on p. 70 to be sure you are correct.

B. Pertussis toxin binds to and modifies the G protein (G_i) used by the α2 receptors. (See table below, expt. 3, for effects of pertussis toxin). Pertussis toxin is most likely to inhibit (binding of GTP) (hydrolysis of GTP) (binding of GDP) (phosphorylation of GDP to GTP) (binding of GTP or GDP) (binding or hydrolysis of GTP). Explain briefly.

C. You decide to figure out more about the mechanism of Ca^{++} release in response to epinephrine. The experiments and the results are given in detail & summarized in Table 2 below. Given all the results,

C-1. An activated G protein is probably activating PLC in cells with (α1 receptors) (α2 receptors) (both) (neither).

C-2. The Ca^{++} is probably entering the cytosol from (the Golgi) (endosomes) (the ER) (the ER or Golgi) (lysosomes) (peroxisomes) (extracellular fluid) (extracellular fluid or one of the organelles) (depends on which type of receptor is triggering Ca^{++} entry).

C-3. The alpha subunit of a G protein is probably activating PLC in cells with (α1 receptors) (α2 receptors) (both) (neither).

Diagram how a normal cell with α2 receptors responds to epinephrine. Show the steps that produce both the Ca^{++} response and the appropriate PKA response. Show where the pertussis toxin and the β/γ binding proteins act to produce their effect.

Information for Question 22: Description of Experiments

(1). You add epinephrine to normal cells, and check the levels of IP3. The level of IP3 in the cytosol goes up in both cell types. (No inhibitors or toxins present.)

(2). You block the channels in the plasma membrane, add epinephrine, and check the level of Ca^{++} in the cytosol. The Ca^{++} in the cytosol still goes up in both cell types.

(3). You treat the cells first with pertussis toxin and then with epinephrine, and measure the levels of Ca^{++} and IP3 in the cytosol. The results are different for the two cell types. See table.

(4). There are proteins that bind to the β/γ subunits of all G proteins. (All the β/γ subunits have at least one domain in common.) You use bioengineering to make the two cell types produce these proteins. Then you measure the levels of Ca^{++} in the cytosol in response to epinephrine. The results are different for the two cell types. See table.

Table: Summary of Results:

		Cells with α1 receptors (only)		Cells with α2 receptors (only)		
		Does Ca^{++} level rise?	Does IP3 level rise?	Does Ca^{++} level rise?	Does IP3 level rise?	Is AC inhibited?
1	Add epi. alone	yes	yes	yes	yes	yes
2	Epi + Block channels	yes	nt (not tested)	yes	nt	yes
3	Epi + pertussis	yes	yes	no	no	no
4	Epi + β/γ binding prot.	yes	nt	no	nt	yes

*6-23. The ligand acetyl choline (ACh) triggers immediate secretion of digestive enzymes from pancreatic exocrine cells, using a G protein coupled receptor (GPCR). In cells lacking the IP3 Receptor IP3R-2, there is no secretion in response to acetyl choline. (Note that there are two different receptors mentioned here – The acetyl choline receptor and the IP3 receptor. Both are transmembrane proteins.)

A-1. Consider the IP3 receptor IP3R-2. The domain of the IP3 receptor that binds its ligand should be (extracellular) (in the lumen of an organelle) (in the cytoplasm) (in the nucleus) (in the nucleus or cytoplasm).

A-2. Consider the acetyl choline receptor in these cells. The domain of the ACh receptor that binds the ligand should be (extracellular) (in the lumen of an organelle) (in the cytoplasm) (in the nucleus) (in the nucleus or cytoplasm).

Explain where in the cell you would find these two receptors.

B. Suppose you treat normal pancreatic exocrine cells with ACh, and digestive enzymes are secreted.

B-1. In these cells treated with ACh, what enzyme should be activated first? (AC) (PLC) (PKA) (PKC) (PDE) (ACh esterase) (ACh kinase) (AC or PLC) (PLC or PKC) (PKA or PKC).

B-2. The ***cytosol (soluble cytoplasm)*** of the treated cells should contain: (DAG) (IP3) (cAMP) (Ca^{++}) (PIP2) (Ac Ch) (none of these). Circle all that apply.

Explain how the activation in B-1 leads to the results in B-2. (Please omit any steps that are not involved in the answer to B-2.)

B-3. In normal cells, what step is most likely to be affected in response to ACh? (transcription) (protein synthesis) (degradation of mRNA) (transcription or translation) (transport of proteins from ER to Golgi) (exocytosis) (endocytosis) (sorting of proteins in endosomes) (modification of proteins in the ER & Golgi) (budding of vesicles from the trans Golgi network = trans side of Golgi).

B-4. In panc. exocrine cells that lack IP3R-2, which of the following is most likely to trigger secretion of digestive enzymes? You'll probably get the best response from adding (an activator of PLC) (an inhibitor of PLC) (an activator of AC) (an inhibitor of AC) (a Ca^{++} ionophore – makes a channel for Ca^{++} in the plasma membrane) (IP3) (DAG) (cAMP) (cAMP or IP3) (IP3 or a Ca^{++} ionophore) (beats me).

Briefly explain how ACh triggers secretion in normal cells. Show where the block is in cells lacking IP3R-2, and how you might be able to fix it. (You don't have to repeat the steps explained above.)

C. In salivary exocrine cells, exposure to ACh causes secretion of water, presumably by opening of appropriate channels. Salivary cells that lack IP3R-2 do not secrete water in response to ACh. Which of the following is the most likely to be responsible for the difference in response to ACh between salivary and pancreatic exocrine cells?

(different ACh receptors) (different G proteins) (different targets of G protein) (different targets of Ca^{++}) (different proteins phosphorylated by PKA) (different 2nd messengers). Circle the best choice and explain briefly.

6-24. Some G proteins are not involved in signaling. These G proteins are activated by GPAFs (GPAFs = G protein activating factors; also called GEFs = guanine nucleotide exchange factors). You have an *in vitro* system with a G protein and a GPAF plus ATP and GTP. (No other proteins present.)

A. To activate the G protein, you need the GPAF and (ATP) (GTP) (both) (neither) (either one).

B. Suppose you use labeled nucleotides (either ATP and/or GTP) and the G protein becomes radioactive when it is activated. (The labeled nucleotides have radioactivity in all 3 phosphates.) You wait until the G protein is inactivated. Then you isolate the inactivated G protein, and put it into fresh solution with unlabeled GTP and ATP. Then you add GPAF and (re)activate the G protein. When the G protein is (re)activated, what radioactive material(s) will be released into the solution?) (GTP) (GDP) (GMP) (phosphate) (PPi) (ATP) (AMP) (cAMP) (ADP) (none -- nothing radioactive will be released). Circle all correct answers, and explain.

6-25. In normal fat cells, protein Q binds to lipid droplets and prevents the fats in the droplet from being hydrolyzed. Epinephrine, acting through a GPCR, activates PKA and triggers phosphorylation of protein Q. This allows HSL (hormone sensitive lipase) to bind to protein Q and start hydrolyzing the lipid.
You have an *in vitro* system with HSL, protein Q and PKA. No other proteins are present. You measure the binding of HSL to protein Q. You get binding if you add epinephrine, ATP, GTP, and cAMP. You should get just as much binding if you omit (epi) (GTP) (ATP) (cAMP) (none of these – you need them all). Circle all correct answers and explain your choices.

*6-26. A. A few years ago, scientists were surprised to discover that when sperm were treated with progesterone (a steroid hormone), there was an increase in Ca^{++} influx into the sperm cell within seconds. They interpreted this to mean that in addition to activating an intracellular receptor, progesterone also must activate a membrane-bound receptor. Explain why a very rapid response is more likely to imply involvement of a membrane receptor rather than an intracellular receptor.

B. A sexually aroused female rat will curve her back in a posture called lordosis. Rats normally show this behavior during only one night of their estrous cycle (a cycle controlled by hormones &/or neuroendocrines, like our menstrual cycle). If you stimulate her brain with either progesterone or with dopamine, you can induce her to show lordosis at other times. (Dopamine is a neurotransmitter, made of a modified amino acid). Do both P and DA use the same signal transduction pathway? The results of studies conducted to answer these questions are listed below. Use these results to draw a diagram that indicates the pathways used by P and DA to stimulate the neurons involved in lordosis. Show all components involved and where they are in the cell or outside it. Base your answer on all of these results:

1. If a phosphodiesterase inhibitor is injected into the brain, females show lordosis more than usual.

2. Rats with a mutation in the intracellular progesterone-receptor don't show lordosis at all, not in response to DA or to P.

3. DA has a strong affinity for a 7-pass protein found in the cell membrane.

4. DA receptor agonist leads to an increase in cAMP in the brain.

5. DA receptor agonist leads to activation of protein kinase A.

6. Progesterone treatment leads to an increase in cAMP in the brain, and an activation of protein kinase A.

7. When a DA receptor agonist and a progesterone receptor antagonist are given together, lordosis does not occur.

8. When phosphorylated, the protein DARPP-32 inhibits the activity of Protein Phosphatase-1

9. When DA receptor agonist and an antisense oligonucleotide to DARPP-32 are given together, lordosis does not occur.

10. When the nuclear progesterone-receptor is phosphorylated, it can bind to DNA, even if progesterone is absent.

C. Using your diagram for reference, explain the mechanism by which injecting a phosphodiesterase inhibitor into the brain (result 1) stimulates lordosis.

D. The enhancer region of the gene for the intracellular progesterone receptor contains Estrogen Response Elements. Which of these treatments would be most effective in inducing lordosis: (progesterone) (estrogen) (dopamine) (estrogen and progesterone simultaneously) (progesterone 24 hours before estrogen) (estrogen 24 hours before progesterone). Explain your answer.

Problem Set 7 – Hormones

7-1. Which of the following statements is/are incorrect?

a. A single endocrine gland may produce more than one hormone.
b. A single target cell may be influenced by more than one hormone.
c. A single organ may produce both endocrine and exocrine secretions.
d. A single hormone may be secreted by more than one endocrine gland.
e. A single hormone can influence only one type of target cell.

7-2. Which of the following statements is/are incorrect? Assume "hormone" = "endocrine."

a. Hormones are secreted through ducts into the blood.
b. Hormones are secreted at a constant rate to maintain homeostasis.
c. Hormones always exert tropic effects.
d. Hormones are always released from endocrine cells by exocytosis.
e. Hormones are always secreted into the blood.

7-3. There are three types of stimuli that can signal an endocrine or neuroendocrine cell to secrete its hormones:

a. nervous stimulation,
b. hormonal stimulation, or
c. a change in the concentration of a chemical in the fluid that surrounds the cell.

For each of the following structures, indicate which type of stimulation is the signal for that structure to secrete its hormones:

Follicle cells of thyroid	Adrenal medulla
Hypothalamus	Adrenal cortex
Anterior pituitary	Posterior pituitary

7-4. Read each of the statements 1-6 below. Indicate which glands (a-e) each statement refers to.

a. anterior pituitary
b. posterior pituitary
c. hypothalamus
d. follicle cells of thyroid
e. None of these

1. Hormones produced here are released into the blood stream through a duct.
2. This is derived from nervous tissue in the developing embryo.
3. This is made mostly of connective tissue.
4. Hormones produced here combine with intracellular receptors in their target cells.
5. Hormones produced here are modified amino acids.
6. This gland receives hormones through a portal vessel.

7-5. For each of the conditions below, indicate whether TH, TSH, and TRH would increase, decrease, or not change, and whether goiter would occur:

Condition	TH	TRH	TSH	Goiter?
Thyroid gland doesn't produce hormone				
Thyroid tumor: overactive in producing hormone				
Anterior pituitary doesn't produce hormone				
Anterior pituitary tumor: produces too much hormone				
Lack of dietary iodine				
Graves' disease				

7-6. Hypothyroidism sometimes is a result of damage to the pituitary gland, while in other cases it results from damage to the thyroid gland. How could you determine which of these two glands is damaged in a particular individual with hypothyroidism, based on measurements made on a blood sample? Explain the substance(s) you would measure in the blood and how it/they will be different in the two types of hypothyroidism.

7-7. In Graves' Disease, antibodies are produced to TSH receptors. These antibodies act as TSH agonists, stimulating the TSH receptors. If Graves' disease is untreated, what would you expect to happen to each of the following? Fill in the blank with "increase", "decrease", "not change" or "can't predict" and explain.

a. the size of the thyroid gland will_____.
b. the level of thyroxine in the blood will _____.
c. the level of thyrotropin will _____.
d. the cells that are targets of thyroxine will show a(n)___in level of cAMP.
e. the body weight will probably _____.
f. the temperature of the hypothalamus will probably_____.
g. blood levels of thyroglobulin will_____.

7-8. Suppose that in an adult, thyrotropin receptors fail to work. What consequences are expected? Consider all steps in production and action of thyrotropin.
a. What hormones should be abnormally high or low? b. Should goiter occur?
c. How should the condition be treated?

7-9. Where in the cells of the anterior pituitary gland would you expect to find hormone receptors? Explain why.
a. on the plasma membrane b. intracellular (in the nucleus or cytoplasm) c. both a. and b.

7-10. Goiter can be caused by eating large quantities of turnips, which contain substances that (stimulate) (inhibit) iodine uptake.

7-11. The anterior pituitary gland releases gonadotropins, hormones that stimulate the gonads (ovaries) to secrete ovarian hormones (estrogen and progesterone) and to release an egg. Gonadotropin secretion is controlled in a manner similar to TSH secretion (See Figure 1 at end of problem set.). Some women are infertile because they produce insufficient gonadotropins. They can be treated by injection of a substance isolated from the blood or urine of elderly Italian nuns. These women are post-menopausal, meaning that their ovaries are no longer producing hormone.
a. What substance would you expect is in their blood in such high levels?
b. Why is it high in these women?

7-12. T/F
a. Steroid hormones are released from endocrine cells by exocytosis.
b. Thyrotropin combines with membrane receptors on its target cells.
c. All hormones are synthesized first as pre-pro-hormones. (pre-pro = in inactive form, plus signal sequence)

7-13. Suppose the portal blood vessel from the HT to the AP is damaged in an accident, and there is no more blood flow through the vessel. (Assume you are provided with a list of acronyms such as HT, AP etc. You can look them up.) For A & B, circle all correct answers; explain each part..

A. Which of the following should be produced/released in abnormally low amounts?
(GnRH) (FSH & LH) (oxytocin) (PIH) (Prolactin) (none of these).

Before continuing to part B, please look up the answer to part A on p. 70 to be sure you are correct.

B. Which of the hormones listed should be produced/released in abnormally high amounts?
(GnRH) (FSH & LH) (oxytocin) (PIH) (Prolactin) (none of these).

C. Will the injured individual develop a goiter? (yes) (no) (only if iodine is low) (can't predict).

D. Will the injured individual need thyroxine pills? (yes) (no) (only if iodine is low) (can't predict).

7-14. A scientist wants to know how prolactin is controlled. In one group of rats (the control), she measures prolactin in the blood. From rats in a second group, she removes the prolactin secreting gland from its usual location and transplants it to the surface of the kidney, where it soon develops connections with the existing blood vessels. She measures prolactin in the blood, and finds that it is much higher than in the control animals. What does this imply regarding the usual control of prolactin secretion in rats?

7-15. After childbirth, the placenta detaches from the uterus and for the next couple of weeks, the uterus bleeds much as it does during a menstrual period. The uterus, which has enlarged to be big enough to hold a fetus, shrinks back to the size of a fist. This process can be accelerated and less bleeding will occur if the mother breastfeeds her baby. Explain how breastfeeding can have an affect on the uterus. Include the hormone(s) involved and the control pathway.

7-16. For each of the following, indicate whether it is true sometimes, always or never, and explain your answer.
a. Paracrine secretions are released through a duct into the blood stream.
b. Monoiodotyrosine is released into the blood in response to thyroid stimulating hormone (thyrotropin).
c. The integrator in homeostatic control systems is located in the hypothalamus.

7-17. Which of the following is/are neuroendocrine secretion(s)?
a. TRH b. TSH c. thyroxine d. vasopressin

7-18. Your little brother "doesn't have to make", so you tell him to go outside and play with his new bike. He gets very excited and runs around for just a few minutes, before coming back to tell you that now he "has to go". Emotion, exercise, and even just standing up, can all stimulate the production of urine through an effect on ADH. Exercise must (increase) (decrease) ADH. Explain.

7-19. A woman who broke her leg needed to use crutches for walking. After using them for several months, she began to lactate, apparently due to pressure put on a nerve while she leaned on the crutches.
Describe the neuronal and/or hormonal pathway that is responsible for lactation under normal circumstances. Then explain how the pressure from the crutch triggered this pathway.

7-20. Name
A. a hormone that is hydrophilic and is stored in the gland that produces it.
B. a hormone that is hydrophobic and is not stored in the gland that produces it.
C. a hormone that is hydrophobic and IS stored in the gland that produces it. Explain how it is stored.

D. Which ONE of the following best explains why it is necessary to store the hormone in part C? Explain your answer.
a) The pituitary gland lies just below the hypothalamus.
b) The supply of salts and minerals in the diet changes from day to day.
c) The mammary gland is an exocrine gland.
d) Several hours may elapse between meals.
e) All cell membranes contain some cholesterol.
f) Both nerves and muscles are dependent on calcium.

7-21. Out on the plains of Africa, some male baboons (dominants) always get their own way, and look healthy. Other male baboons (subordinates) living in the same troop always give in and look weak and sickly. Sapolsky suspected that cortisol was involved, since long-term secretion of cortisol can cause muscle degeneration and a decreased immune response. Control of cortisol secretion is similar to that of thyroid hormone. (See Figure 2 at end of problem set). Sapolsky measured cortisol in both types of males, and found that the subordinate males do have higher levels of cortisol even when not stressed. (It would have stressed the baboon to be chased and caught to get blood, so he sat calmly in the nearby grass and used a blowgun to shoot an anesthetic into the baboon, and then conducted these experiments on the anesthetized animals.)

To understand the physiological cause of the higher cortisol in subordinates, he asked a series of questions, and got answers from the following series of experiments. For each experiment,
i) explain what question he was asking, and
ii) describe what conclusion he could draw from the results.

A. Experiment A. He injected the same amount of (labeled) cortisol into dominant and subordinate males, and measured how much of the labeled cortisol remained in the blood several hours later.
RESULT: The same amount of added cortisol was found in the blood of dominant and subordinate animals.

B. Experiment B. He injected the same amount of ACTH into dominant and subordinate males and then measured blood cortisol.
RESULTS: The same amount of cortisol was found in the blood of dominant and subordinate animals.

C. Experiment C. He injected CRF (= CRH) into dominant and subordinate males and measured ACTH in the blood.
RESULTS: ACTH was higher in the blood of dominant males than subordinates.

D. Experiment D. He injected dexamethasone, a cortisol-receptor agonist (ie, a substance that mimics the effect of the ligand on the receptor), into dominant and subordinate males and measured cortisol levels.
RESULTS: Cortisol was lower in the blood of dominants than in subordinates.

7-22. One man has a thick, bushy beard, while a second has very little facial hair. Facial hair growth in men is stimulated by androgen hormones, and you guess that the men differ in how much of these hormones they each secrete. You sit them down just after class, at 7:00 pm, rapidly draw one sample of blood from each, and measure the concentration of blood androgens. This test shows that the men actually have identical levels of the hormones. Give at least three possible explanations that could explain why they have different beard growth, despite the similarity you measured in the hormones.

7-23. A certain hormone is added to two types of cells with the following results:

liver cells -- activates glucokinase, which phosphorylates glucose
muscle cells -- inserts glucose transporters into cell membrane

When this hormone affects muscle cells, glucose should (enter the cells) (leave the cells) (go in either direction) (can't predict)? Which hormone is this, and under what circumstances is it normally secreted?

7-24. Glucose uptake in the liver is not dependent on insulin (directly). Yet when insulin is present, glucose is (usually) taken up by liver and when insulin is absent, glucose is (usually) not taken up by liver. Explain why.

7-25. A. Name 2 target tissues for insulin, and specify <u>two</u> effects insulin has on each target.
B. Name one hormone that acts antagonistically to insulin -- specify the name, target, and one effect.

7-26. Why is failure to produce insulin worse than failure to produce glucagon?

Figure 1:

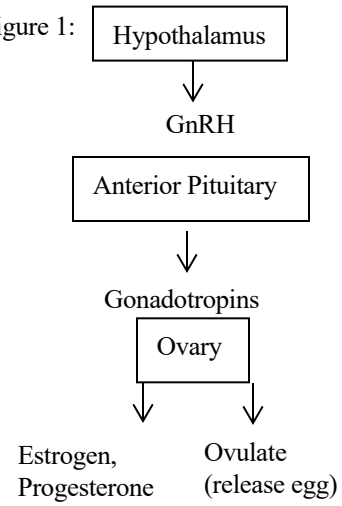
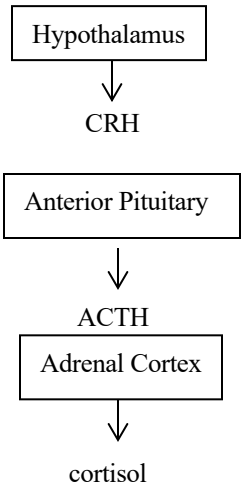


Figure 2:



ANSWERS TO SELECTED QUESTIONS FROM PROBLEM SET 6, 7 & 8

6-18. Here is the set up: This is a second messenger system since peptide hormones do not enter the cell but bind to the extracellular domain of membrane receptors. You should conclude that IP3 is involved, because a) inhibitors of adenylyl cyclase have no effect, which rules out cAMP, b) the in vitro system must include ER, which is the site of IP3 receptors, and c) the enzyme that is activated is dependent on calcium, which is increased when the IP3 pathway is activated. The series of events from hormone to release of IP3 should go as follows:
Hormone binds to extracellular receptor (in membrane fraction) → activates G protein bound to membrane (G protein binds GTP and releases GDP into soluble fraction) → activates phospholipase C (in membrane) → splits PIP2 → IP3 (soluble) and DAG (water insoluble; stays in membrane).

Answers to A:

A-1. Membrane fraction. Peptide hormones combine with membrane receptors.

A-2. IP3 and GDP (assuming receptor is coupled to a G protein).

Why GDP? G proteins are activated by exchanging GDP for GTP, not by phosphorylating the GDP which is already bound to the G protein. The G proteins are inactivated by hydrolysis of the bound GTP to GDP. The GDP is then released next time the protein is activated.

6-22. A. Cells with α1 receptors would be expected to respond to epi by increasing the Ca^{++} in the cytosol (since α1 receptors activate PLC and the IP3/Ca^{++} pathway, and IP3 triggers release of Ca^{++} from the ER).

No rapid Ca^{++} response would be expected in cells with α2 receptors, since α2 receptors affect the AC/PKA pathway.*

In this problem, you have to figure out how this unexpected response could be generated. To do this, you have to analyze the results of the experiments summarized in the table. This problem is included to give you some idea of how things are figured out, and to give you some practice at interpreting experimental results.

*Note: The PKA pathway may affect removal of Ca^{++} from the cytosol, but it doesn't normally trigger rapid release of Ca^{++} into the cytosol. If inhibiting AC blocks activation of a Ca^{++} pump in the ER membrane, that might allow a build up over time of Ca^{++} that is released in response to other signals and not returned to the ER. However, inhibiting AC should not by itself cause a rapid increase in cytosolic Ca^{++}.

7-13. A. If the portal vessel is severed, the releasing hormones (both inhibitory and stimulatory) from the HT won't reach the AP. If no GnRH reaches the AP, FSH and LH will not be released. Therefore FSH and LH release from the AP will decrease. Prolactin synthesis is primarily under control of an inhibitory factor, PIH, not a releasing factor. PIH will not reach the AP, so the level of prolactin release should increase, not decrease. Oxytocin release will not be affected by severing the portal vessel, as oxytocin is released directly into the general circulation from the PP.

For part B, don't forget to take negative feedback into account.

8-15. C. #1 -- Acetylcholine. ACh from preganglionic cell should produce EPSP.

#2 -- cAMP. Odorant binds to receptor on outside of cell and triggers second messenger cascade inside the cell. Odorant binding starts the whole process, but cAMP binding opens the channels (and generates potentials) as follows:
Odorant binds to receptors → activates G protein → activates adenyl cyclase → cAMP → opens * cAMP gated Na^+ channels → graded receptor potential (depolarizes toward threshold; extent of depolarization proportional to amount of stimulus) → opens voltage gated cation channels (if depolarization exceeds threshold) → Na^+ rushes in →action potential.

*Note: cAMP does not always work through PKA. It can bind to and affect some other proteins, as in this case.

Problem Set 7R – Questions on Hormones, Signaling, & Homeostasis

7R-1. TRH and TSH (thyrotropin) both use G protein linked receptors. TRH works by the IP3 pathway; TSH works by the cAMP pathway. For each part, circle all choices that apply and explain briefly how you arrived at your answer

A. The receptor for TRH should be (a transcription factor) (a kinase) (a single pass membrane protein) (a multipass membrane protein) (an intracellular protein) (either type of membrane protein) (any of these).

B. When TRH binds to its receptor, you expect activation of (adenyl cyclase) (PKC) (PLC) (PKA) (Ca^{++}) (ras) AND you expect an increase in (total cell Ca^{++}) (total cytoplasmic Ca^{++}) (Ca^{++} in the cytosol) (none of these).

C. Repeated stimulation by a hormone can lead to downregulation of receptors for that hormone. Receptor mediated endocytosis could be used to achieve downregulation of receptors for (TRH) (TSH) (TH) (none of these).

D. TRH acts in the pathway leading to TH, but it also directly stimulates secretion of at least one additional hormone. The most likely additional hormone secreted in response to TRH is (prolactin) (oxytocin) (ADH) (insulin) (cortisol) (epinephrine).

7R-2. Gs is the G protein that stimulates adenyl cyclase. For each part of this question, circle as many choices as apply, and explain briefly. (For parts C & D see problem 7R-1 above for mode of action of TRH and TSH.)

A. Activation of Gs should lead to activation -- or increased levels -- of (PKC) (PLC) (PKA) (MAP kinases) (PUKE) (CDK) (Ca^{++}).

B. Activation of Gs normally involves (hydrolysis of ATP) (hydrolysis of GTP) (binding of ATP) (binding of GTP).

C. You would expect Gs to be involved in response to (TRH) (TSH) (TH) (none of these). Explain very briefly why your choice(s) are right and why the other(s) are wrong.

D. In the pathway controlling levels of TH, you expect Gs to be involved in the (hypothalamus) (anterior pituitary) (posterior pituitary) (thyroid) (adrenal) (pancreas).

7R-3. Consider a tropic hormone that is **not** involved in production of TH.

A. What is one reasonable candidate for this hormone? _____.

B. Secretion of this tropic hormone should be ***decreased*** in response to high levels of _____ and ***increased*** in response to high levels of _____.

Draw the circuit showing how all your choices fit together to produce the changes described. If you don't know the name of a specific candidate hormone, then use the general names (for each category) and draw the generalized circuit.

7R-4. There are two hormones primarily responsible for maintaining glucose homeostasis.

A. What is the name of one of these hormones? _____.

Given your choice of hormone, circle as many choices as apply in the statements below, and explain at the end.*

B. Secretion of this hormone should ***decrease*** in response to (high) (low) levels of glucose in (the blood) (the liver).

C. Conditions that ***increase*** secretion of this hormone normally occur during (the post-absorptive state) (the absorptive state) (stress).

D. This hormone stimulates insertion of glucose transporters into the membrane of (liver cells) (muscle cells) (adipose cells) (none of these).

Explain briefly how these responses help maintain glucose homeostasis and a suitable response to stress.

*The exam only asked for answers for one hormone; for study purposes, you should try to answer this question for ***both*** hormones.

7R-5. Pituitary tumors (benign ones) are very common. Autopsy results indicate that 25% of "normal" adults have them. Many pituitary tumors have a mutant form of the gene for the alpha subunit of Gs. (The gene for Gs is called gsp.) Gs is the G protein that stimulates adenyl cyclase. The mutant gene codes for an alpha subunit with very low GTPase activity. For each part, circle all choices that apply and explain briefly.

A-1. How should the mutant form of Gs compare to the normal version? The mutant version should be (harder to activate) (harder to inactivate) (easier to activate) (easier to inactivate).

A-2. If this gsp mutation affects phenotype, it should cause cells to have abnormal levels of (IP3) (cAMP) (Ca^{++}) (DAG) AND the abnormal levels should be (too high) (too low) (too high for some components listed and too low for others). Explain briefly how the changes in Gs should lead to the changes in phenotype.

B-1. The normal version of the gsp gene should be considered a (tumor suppressor) (oncogene) (proto-oncogene) (inactive tumor suppressor or oncogene) (tumor suppressor or proto-oncogene) (inactive tumor suppressor).

B-2. GH (Growth hormone) is produced by the pituitary in response to stimulation by GRH (GH releasing hormone). GRH stimulates Gs. When a normal pituitary cell is stimulated, using a standard amount of GRH, it produces 100 units of GH . Suppose you add the standard amount of GRH to a heterozygous cell. The heterozygous cell has one copy of the mutant gsp described above, and the other copy of the gsp gene is normal. How much secretion of GH do you expect from the heterozygous cell? (>100 units) (about 100 units) (<100 units).

7R-6. Sam often feels cold. He wears coats and sweaters indoors when everyone else is in shirt sleeves.
His best friend Joe suggests he go to the doctor and get a hormone test. The doctor takes a blood sample and orders some hormone tests.

A-1. Suppose the doctor orders a test for TH. The most likely result is that the TH level in the blood will be (normal) (elevated) (below normal) (elevated or below normal, but can't predict which) (can't predict at all).

A-2. The set point (for internal body temperature) should be (higher for Joe) (higher for Sam) (about the same in both).

A-3. Suppose Joe and Sam are both sitting in a room with a normal indoor temperature (according to Joe). Contraction of smooth muscle surrounding the peripheral blood vessels is probably (higher for Joe) (higher for Sam) (about the same in both). Explain your answers briefly.

B. The doctor decides to do ***one*** additional test to pinpoint the problem. She takes another regular blood sample and sends it off to the lab. Given what you know so far,

B-1. The doctor is most likely to order a blood test for (TSH = thyrotropin) (ACTH = corticotropin) (TRH) (CRH = corticotrophin RH) (TSH or TRH) (CRH or ACTH) (TRH or CRH) (TSH or ACTH) (insulin) (glucagon) (insulin or glucagon) (any of these).
Explain briefly how you narrowed it down to one.

B-2. One possible explanation of Sam's symptoms is that his pituitary is not responding to the normal signals***. If this is the case,*** then the hormone that is probably at abnormal levels is _____ (pick one of the choices in B-1) and the levels of the hormone should be (elevated) (below normal) (elevated or below normal, but can't predict which). Explain briefly.

C. Suppose Sam has been taking lithium for a bipolar condition. Lithium is concentrated in the thyroid, where it affects production of TH. Lithium interferes with endocytosis on the side of the follicle cells facing the lumen. Given all the information so far, what is the most likely explanation of Sam's symptoms?

C-1. The TSH level in Sam's blood is probably (normal) (elevated) (below normal) (elevated or below normal, but can't predict which) (can't predict at all).

C-2. Sam's primary problem is probably with (production of TRH) (response to TRH) (synthesis of thyroglobulin) (proper modification of thyroglobulin) (transport of thyroglobulin to the lumen) (release of free TH to the blood) (production of TSH) (binding of TSH to its receptors) (the cAMP pathway) (the IP3 pathway) (either the cAMP or IP3 pathway).
Explain both your answers. Be as specific as possible about what is wrong with Sam.

7R-7. Both epinephrine and cortisol are produced by the adrenal gland in response to stress. Answer all 4 parts, and explain each answer in one or two sentences. (Even if we did not cover the stress response you should still be able to answer this question. It is about the properties of the two hormones, not about the stress response itself.)

A. If the adrenal receives signal(s) to release both at the same time, which hormone is likely to be released first? (cortisol) (epinephrine) (either one).

B. Suppose both hormones are being produced, and each hormone reaches its target tissue at the same time. Which hormone is likely to produce an effect first? (cortisol) (epinephrine) (either one).

C. Suppose you block all neuronal input to the adrenal gland. Which hormone(s) will be affected? Compared to the normal response to stress, you'll find a difference in release of (cortisol) (epinephrine) (either one but not both) (both) (neither – neuronal input doesn't affect release of either one).

D. Suppose both hormones are produced in large amounts. Which hormone is more likely to feedback inhibit its own production? (cortisol) (epinephrine) (both are equally likely) (neither is likely to have a feedback effect).

7R-8. Growth hormone (GH) has long term effects on growth, but it also has short term effects on glucose metabolism. GH is secreted in response to stress, exercise or hypoglycemia, and affects energy metabolism in skeletal muscle, adipocytes (fat cells) and liver.

A. When GH is secreted in response to hypoglycemia, you would also expect secretion of (insulin) (glucagon) (both) (neither) (either one or the other, but not both).

B. Which ***one*** of the following is the most likely direct (short term) response of <u>skeletal muscle</u> to GH? (glucose uptake increases) (glucose uptake decreases) (glucose release increases) (glucose release decreases) (glycogen synthesis increases) (both glucose uptake and glycogen synthesis increase) (glycogen breakdown increases) (both glycogen breakdown and glucose release increase)

C. People who have tumors that secrete GH have abnormalities of growth, and they often have abnormal blood sugar levels. From the short term effects of GH, they are likely to have episodes of (hyperglycemia) (hypoglycemia) (either way) (neither – only long term effects of GH would matter). Explain B & C briefly.

7R-9. GH is made as a pre-pro-hormone and released (in mature form) by the anterior pituitary.

A. Synthesis and release of mature hormone should require (cleavage of peptide bonds) (endocytosis) (exocytosis) (modification of tyrosine(s)) (transport down a long axon) (all of these) (none of these).

B. GH secretion is regulated by two proteins, a releasing hormone (GH-RH) and an inhibiting factor (somatostatin). GH-RH and somatostatin both affect the cellular level of cAMP. GH activates the MAP kinase pathway.

B-1. From this information, which of the following could use a TK-linked receptor? (GH-RH) (somatostatin) (GH) (cortisol) (all of these) (none of these).

B-2. Which of the following could activate the same G protein? (GH-RH & somatostatin) (GH & GH-RH) (GH & somatostatin) (all of these) (none of these -- no two of these could activate the same G protein). Explain B-2 briefly.

7R-10. GH has long term effects on growth that require synthesis of IGF (insulin-like growth factor).

A. IGF stimulates the growth of many cells. It uses a tyrosine kinase receptor, and stimulates the MAPK pathway. When IGF binds to its receptor, the receptor itself (without any other proteins) should be able to (activate a trimeric G protein) (dimerize) (bind to DNA) (phosphorylate its extracellular domain) (activate ras) (catalyze hydrolysis of ATP).

B. GH stimulates liver and bone (& other tissues) to produce IGF. The IGF from the liver cells acts as an endocrine, and stimulates growth of many cell types, including bone. The IGF from the bone cells acts as a paracrine and stimulates bone growth. Both the paracrine and endocrine effects of IGF are required for maximum growth.

Some very short children have low levels of IGF, and don't respond to added GH. Researchers have suggested treating the children with IGF instead of GH. It is possible that IGF treatment will inhibit their growth. Why?

B-1. Injecting IGF could inhibit growth by inhibition of (protein synthesis in bone) (GH synthesis) (IGF synthesis in liver) (GH-RH release) (somatostatin release) (all of these).

B-2. If growth inhibition occurs, it will be as a result of interfering with the (paracrine) (endocrine) (both) (neither) effects of IGF. Explain B briefly.

7R-11. You remove the anterior pituitary gland from a normal (but stressed) experimental animal. The animal is producing cortisol & epinephrine before you remove the anterior pituitary.

A. How would you expect each of the following to be affected (by removal of anterior pituitary.)?

A-1. Release of CRH (corticotropin releasing hormone) to (decrease) (increase) (stay the same) (stay the same at zero).

A-2. Release of ACTH (corticotropin) should (decrease) (increase) (stay the same) (stay the same at zero).

A-3. Release of cortisol should (decrease) (increase) (stay the same) (stay the same at zero).

A-4. Suppose you remove the anterior pituitary, as above, and let cortisol blood levels reach a steady state. Then you add a high level of dexamethasone, a cortisol agonist. You would expect a change in the rate of release of (ACTH) (CRH) (both) (neither). Explain A-4 briefly.

B. When you remove the anterior pituitary, epinephrine release decreases. If you then inject ACTH into the blood of the animal (with no anterior pituitary) epinephrine release from the adrenal increases. The investigators who found this out were surprised. They probably expected epinephrine release to be

__ (unaffected by removal of anterior pituitary)
__ (unaffected by adding ACTH)
__ (affected in the opposite direction by removal of anterior pituitary)
__ (affected in the opposite direction by addition of ACTH). Check as many as apply; no explanation required.

C. Consider an animal with no anterior pituitary. You inject ACTH into the blood of the animal. As explained above, epinephrine (epi) release from the adrenal increases. Metyrapone inhibits steroid synthesis. If you add ACTH and metyrapone, ACTH has no effect on epi release. (Some additional results are given in C-3. Please read the whole question first, and then answer the 3 parts separately.)

C-1. Given these results, the simplest explanation is:

a. ACTH directly stimulates synthesis of (epinephrine) (cortisol) (one or the other) (both) (neither), AND

b. The adrenal medulla has receptors for (cortisol) (ACTH) (one or the other) (both) (neither).

C-2. When epi release increases, the levels of the enzymes for making epi, & the synthesis of epi, also increase. Given your answers so far, increases in activity of these enzymes (the ones for making epi) & epi synthesis are probably due to (activation of pre-existing enzymes) (synthesis of new enzymes) (either one) (both). Explain how your answers fit the experimental results.

C-3. If you add normal levels of cortisol to an animal without an anterior pituitary, there is no effect on the release of epi. The scientists who did this work attribute these results (& those above) to the existence of a portal circulation between the adrenal cortex and the adrenal medulla. Consider an animal producing cortisol & epinephrine. If there is a portal vessel involved here,

a. The blood must flow (from cortex to medulla) (from medulla to cortex) (either way), AND

b. In the portal vessel you would expect to find that the concentration of (epi) (cortisol) (cortisol or epi) (both) (neither) is/are higher than the concentration in the general circulation. Explain why a portal circulation is invoked here.

PROBLEM SET #8 – Nervous System

8-0. A container is divided by artificial membrane. On the left side is a solution of 10 mM NaCl, 1 mM glucose. On the right side is an equal volume of a solution of 10 mM glucose, 1 mM NaCl.

A. Suppose the artificial membrane is permeable to water only. The volume of water will on left side will (decrease) (increase) (stay the same) (can't predict).

B. Suppose the artificial membrane is permeable to glucose only. At equilibrium, the glucose on the left side will (increase to be equal to the final concentration on the right side) (stay the same at 1mM) (increase, but will remain less than the final concentration on the right side).

C. Suppose the artificial membrane is permeable to Cl^- only. Cl^- will initially (move from the left to the right side) (move from the right to the left side) (have no net movement).

D. The concentration of Cl- on the left side after equilibrium is reached will be (~5.5 mM) (~10 mM) (~0 mM) (significantly <10 mM, but significantly >5 mM).

E. The membrane potential on the left side after equilibrium is reached is _____. Show your work.

8-1. Instead of referring to the endocrine and nervous system as two separate organ systems, one might speak of a single "regulatory system" that includes parts of both. Discuss at least two arguments in favor of considering these a single organ system.

8-2. Circle whichever is greater, x or y. If x=y, circle both.

A. x. permeability of a neuronal membrane to Na^+ during the rising phase of the action potential
y. permeability to K^+ at the same time

B. x. permeability of the resting membrane to K^+
y. permeability of the membrane to K^+ during the falling phase of the action potential

C. x. concentration of K^+ in the intracellular fluid before the action potential
y. concentration of K^+ in the intracellular fluid immediately after the action potential

D. x. speed of conduction of nerve impulse in neuron with diameter of 10 micron
y. speed of conduction of nerve impulse in neuron with diameter of 5 micron

E. x. number of directions action potential will travel if neuron stimulated at axon hillock
y. number of directions action potential will travel if neuron stimulated at dendrite

F. x. Na^+ ions moved out by Na/K pump
y. K^+ ions moved in by Na/K pump

8-3. Suppose a nerve cell membrane is made **less** permeable to passive diffusion of K^+ ions, without affecting the Na^+/K^+ ATPase pump.

A. What will happen to the amount of K^+ within the cell?
B. What will happen to the resting potential of the cell?
C. Will it be harder or easier to elicit an action potential?
D. How could a substance produce such an effect on the cell?

8-4. Suppose a nerve cell membrane is made **more** permeable to passive diffusion of Na^+ ions, without affecting the Na^+/K^+ ATPase pump.

A. What will happen to the amount of Na^+ and the amount of K^+ within the cell?
B. What will happen to the resting potential of the cell?
C. Will it be harder or easier to elicit an action potential?
D. How could a substance produce such an effect on the cell?

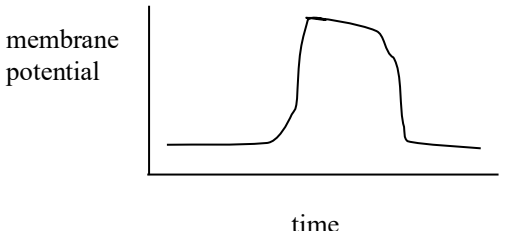
8-5. Hodgkin and Huxley conducted an experiment in which they removed sodium from the fluid surrounding a neuron, and replaced it with choline, a positively charged molecule that doesn't penetrate the membrane. They found that when the neuron was stimulated electrically to threshold, there was no action potential, but after a small delay (about 3 msec) an increase in positive charge could be measured on the outside of the stimulated membrane. Explain these findings in terms of our current knowledge of neuron function.

8-6. Which would occur if a neuron were electrically stimulated at both ends simultaneously? Explain your answer.

a. Action potentials would pass in the middle and travel to opposite ends
b. Action potentials would meet in the middle and be propagated back to their starting side
c. Action potentials would meet in the middle and stop there

8-7. The membrane of the heart develops a resting potential just like a neuron does. The action potential that occurs is sketched below. The membrane depolarizes, as a neuron does, due to movement of Na^+, but then the heart membrane <u>stays</u> at this potential for a while, due to changes in membrane permeability.

A. Assume that Ca^{++} is responsible for this plateau. How would a change in membrane permeability to Ca^{++} account for this plateau?
B. Assume that K^+ is responsible for this plateau. How would a change in membrane permeability to K^+ account for this plateau?



8-8. Fill in the blanks below, based on the nerve cell and synapses drawn on the next page. Assume that these are the only synapses that end on the neuron. Note: On the actual test, no explanations were required for this question, but you should always be prepared to explain your reasoning.

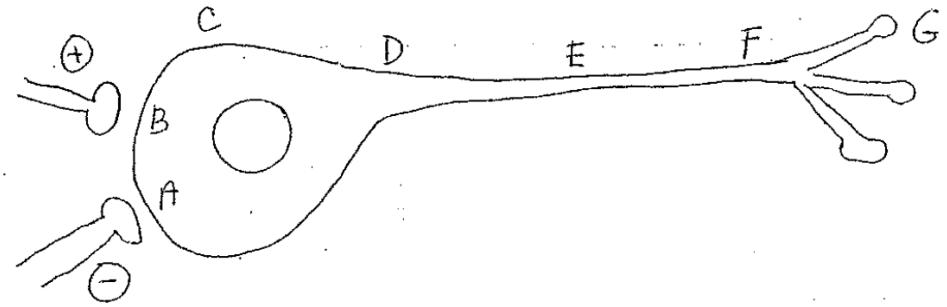
Fill in all letters that apply for each case.

A. Ligand gated ion channels should be found at _____.
B. Voltage gated ion channels should be found at _____.
C. Graded local potentials should be found at _____.
D. Action potentials should (normally) be initiated at _____.
E. IPSP expected at _____.
F. If you electrically stimulate the cell at (E), and initiate an action potential, the action potential should be propagated to _____.
G. Synaptic vesicles should be found mostly at _____.
H. Gated ion channels that generate potentials without a refractory period should be found at _____.

I. If the cell shown is a motor neuron, then it is (efferent) (afferent) (either way) AND a synapse to the right of point G should be (excitatory) (inhibitory) (either one).

Problem 8-8 continued on next page

J. If the cell shown is a postganglionic neuron from the sympathetic branch of the autonomic nervous system, then a synapse to the right of G should be (excitatory) (inhibitory) (either one) AND the transmitter at G should be (epinephrine) (norepinephrine) (acetylcholine) (any of these).



8-9. Circle whichever is greater in nerve cells, x or y. If x = y, circle both.

A. x. time it takes for voltage-gated potassium channels to open after threshold is reached
y. time it takes for voltage-gated sodium channels to open after threshold is reached

B. x. force pushing sodium into neuron at resting potential
y. force pushing potassium out of neuron at resting potential

C. x. voltage-gated Ca^{++} channels in axon hillock
y. voltage-gated Ca^{++} channels in nerve terminals

D. x. number of axon K^+ channels open at the threshold potential
y. number of axon K^+ channels open at the resting potential

E. x. chance of finding a graded potential at the presynaptic membrane
y. chance of finding a graded potential at the postsynaptic membrane

F. x. number of neurotransmitter receptors on postsynaptic membrane
y. number of neurotransmitter receptors on presynaptic membrane

8-10. Serotonin is a neurotransmitter that can be either stimulatory or inhibitory, depending on the particular receptor on the postsynaptic membrane. For each of these serotonin receptors, indicate whether it will be inhibitory (that is, lead to an IPSP) or stimulatory (lead to an EPSP).

Stimulation of the receptor….
A. increases levels of cAMP, leading to an increase in K^+ permeability
B. increases level of IP3, leading to a decrease in K^+ permeability
C. increases level of IP3, leading to an increase in Cl^- permeability
D. is directly coupled to ion channel that leads to an increase in permeability to both Na^+ and K^+

8-11. You are studying a synapse in a newly discovered worm species, and you want to determine whether it is a chemical or an electrical synapse. You don't have an electron microscope, so you can't visualize the synapse directly. Describe a different experimental technique that you could use to determine which kind of synapse is involved here.

8-12. Fill in this table to reflect the similarities and differences between the sympathetic and parasympathetic nervous systems.

	Sympathetic	Parasympathetic
Parameters on which they're similar		
Parameters on which they differ		

8-13. Make a table to reflect the similarities and differences between the autonomic and somatic nervous systems. (Use the headings below, but you'll probably need more space, so feel free to use a separate piece of paper.)

	Autonomic	Somatic
Parameters on which they're similar		
Parameters on which they differ		

8-14. Circle whichever is greater, x or y. If x = y, then circle both.

A. x. potassium channels open during an EPSP
   y. potassium channels open during an IPSP

B. x. nicotinic receptors on skeletal muscles
   y. muscarinic receptors on skeletal muscles

C. x. distance from spinal cord to parasympathetic ganglion
   y. distance from spinal cord to sympathetic ganglion

D. x. length of parasympathetic neurons that stimulate nicotinic receptors
   y. length of parasympathetic neurons that stimulate muscarinic receptors

E. x. rate of substance moving down axon <u>though the cytoplasm</u> (not AP in membrane) by simple diffusion
   y. rate of substance moving down axon by vesicular transport

F. x. sodium ions crossing the membrane during an EPSP
   y. sodium ions crossing the membrane during an IPSP

8-15. Consider two nerve cells that produce action potentials:
Cell #1 is a postganglionic cell of the parasympathetic nervous system.
Cell #2 is a sensory receptor cell in the olfactory (smell) system. (See hint below* &/or text for steps in olfactory system.)

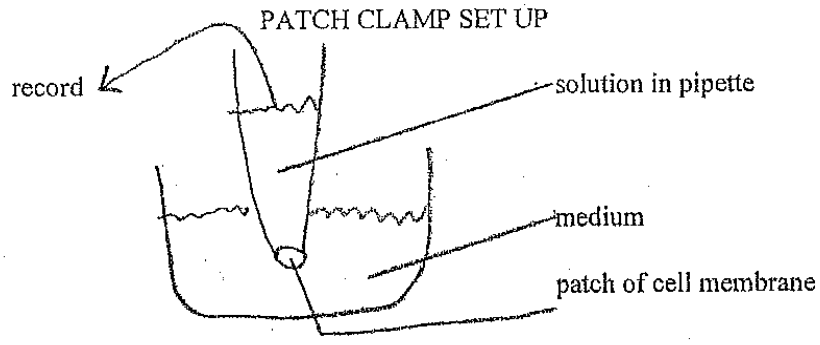
A. Which cell(s) are afferent? (#1) (#2) (both) (neither).
B. Which cell(s) should have voltage gated Na^+ channels? (#1) (#2) (both) (neither).
C. Both cell(s) should have ligand gated channels that allow passage of Na^+. Name the ligand(s) that should open the channels in each case.

Before continuing to part D, please look up the answer to part C on p. 70 to be sure you are correct.

D. Suppose the receptors of both cells are stimulated with the appropriate ligand(s); enough ligand is used so that both cells will respond by firing one action potential. If both cells are stimulated at the same time, which will fire the AP first? (#1) (#2) (both will fire at same time) (can't predict).
E. Suppose the receptors of both cells are stimulated with the same ligand(s) as in D, but less ligand is used.
   1. This time you would expect ligand gated Na^+ channels to open in cell (#1) (#2) (both) (neither)
   2. The voltage gated channels should open in cell (#1) (#2) (both) (neither).
   3. In cell #1 you would expect to see (an action potential) (a receptor potential) (an EPSP) (IPSP) (EPP = End plate potential) (none of these) (more than one of these -- specify which)
   4. In cell #2 you should see _____.

**Hint: Olfactory system is a one cell system; odorant receptors are metabotropic & 2nd messenger is cAMP.*

F. You set up a voltage (patch) clamp on cell #1 or #2 and make some recordings. The voltage is kept at the usual resting potential. Suppose you remove the patch from the cell, stick the end of the electrode (micropipette) with the patch into some medium and record some more. During your experiment, you put various ligands into the pipette and/or medium to see if they will cause the Na^+ channels to open.
   1. Suppose you have an isolated patch from cell #1 or #2. If you want the ligand gated Na^+ channels to open, you should put the ligand (in the pipette) (in the medium)
      (depends on which cell the patch is from) (either way).
   2. When the Na^+ channels open, the Na^+ ions should go (from medium to pipette) (pipette to medium) (either way -- depends on concentration gradient) (depends on which cell the patch is from)
      (can't predict).



8-16. The little green people from Mars arrive, and you are given the task of analyzing their nervous systems. You discover that they operate more or less as we do, but they can sense magnetic fields using a type of sensor on their antennae, which we do not have. You find out the following about their magnetic sensor system:
(1). The magnetic field receptor cells are not neurons, but they synapse with afferent neurons.
(2). When the receptor cells are stimulated through their cell surface receptor proteins, cAMP rises and Ca^{++} channels open.
(3). If the receptor cells are stimulated electrically by an electrode inserted inside the cell, no transmission occurs.
(4). If you drip acetylcholine on the proper spot on the afferent neuron, and record from a spot close by, you record a depolarization.

Given all this info, answer the following questions, and explain briefly.

A. Outline how the system works.

B. If you increase the intensity of the stimulus to the antennae, what would happen to each of the following? Write in "increase," "decrease," or "stay the same." (AP = action potential)
1. Activity of adenyl cyclase in the neuron would _____
2. Activity of adenyl cyclase in the receptor cell would _____
3. Opening of voltage-gated channels in receptor cell would _____
4. Height of AP would _____
5. Number of AP's per second in receptor cell would _____
6. Number of AP's per second in neuron would _____
7. Width of AP would _____
8. Threshold for AP would _____
9. Flow of Na^{+} into the post-synaptic membrane of the neuron would _____
10. Polarization of the post-synaptic membrane of the neuron would _____.

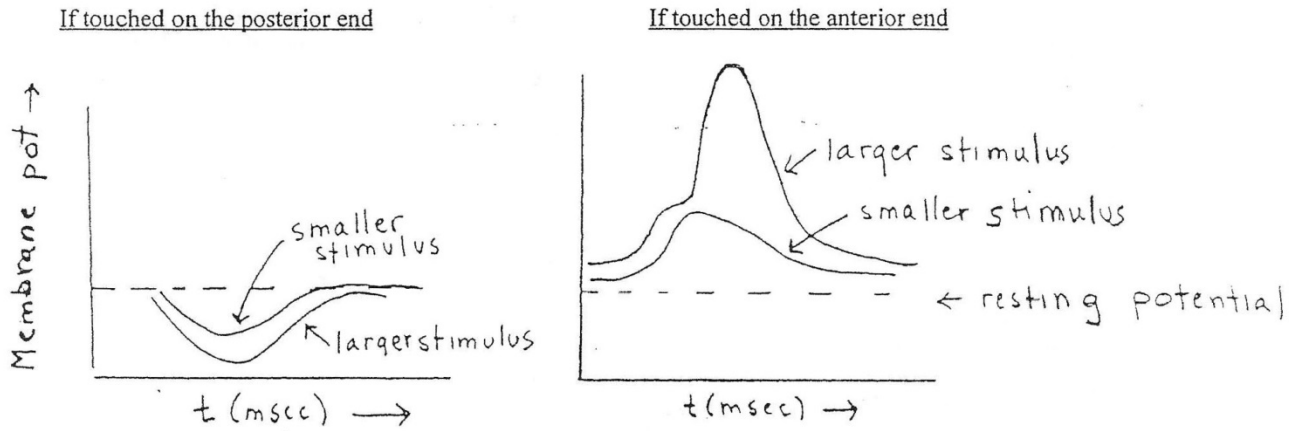
C. One of the Martians tells you that living on earth is driving her crazy because there are too many magnetic fields and she is bombarded with stimuli all the time. (On Mars, the fields are shielded.) She asks for your help. What will each of the following do for her hypersensitive condition? Write in "worse," "better," or "no effect" and explain. Assume you can restrict the action of the chemical to the synapse between the two cells discussed here.
1. An agonist of acetyl choline _____
2. An antagonist of acetyl choline _____
3. An inhibitor of acetyl choline esterase _____
4. A blocker of presynaptic Na^{+} channels _____
5. A blocker of Ca^{++} channels _____

Explain your answers briefly.

8-17. “Double, double, toil and trouble, Fire burn and cauldron bubble..." In mixing up their poisonous brew, witches made sure to include some "eye of newt and toe of frog". When frightened, frogs and newts release certain chemicals onto their skin, which have a toxic effect on an unfortunate predator. One of these chemicals, batrachotoxin, attaches to open voltage-gated sodium channels and prevents them from closing. Suppose you are studying a particular neuron whose normal threshold is -55 mV. You depolarize the neuron to -55mV in the presence of batrachotoxin.

Draw a graph showing the membrane potential you would expect to see. Label the x and y axis. Explain why you drew the graph this way.

8-18. Paramecia (one celled organisms with cilia) move around by waving their cilia to "swim" through the water.
If touched on the posterior end, the cilia beat more quickly as the paramecium escapes.
If touched on the anterior end, the cilia beat in the opposite direction, so the paramecium swims backwards and avoids the obstacle it bumped into.
Touching the paramecia causes ion channels to open, leading to changes in membrane potential. The changes in potential look like this:



Assume that a paramecium has three kinds of ion channels, each of which can be described as
either a Ca^{++} channel or a K^{+} channel
either ligand-gated or voltage-gated or mechanically-gated
either on the anterior end, or on the posterior end, or all over the membrane

Describe each of the three channels with regard to the three characteristics (specify ion, type of gating, and locations) and explain how you arrived at your answers.

*8-19. A. GABA is an inhibitory transmitter in the CNS, and the GABA receptor is a Cl^- channel. Suppose GABA binds to its receptor in the plasma membrane.

A-1. The permeability for Cl^- should (increase) (decrease) (stay the same) (stay the same at zero).

A-2. Assuming the RMP is less negative than the equilibrium potential of Cl^- in this cell, Cl^- should flow (into the cell) (out of the cell) (in or out -- either way is equally likely) (not at all – there will be no net flow of Cl^-).

A-3. The voltage difference across the membrane will probably become (more negative) (more positive) (less negative) (less positive) (zero). Explain briefly.

For the rest of this question, consider a neuron (#1) that synapses on one of the dendrites of a second neuron (#2). Assume GABA is the transmitter at this synapse.

B. Suppose there is a voltage gated Ca^{++} channel at this synapse. This channel is most likely to occur (in cell 1) (in cell 2) (in either cell – it's equally likely on either side of the synapse). Briefly explain the role of the Ca^{++} channel at this synapse.

C. Suppose you stimulate cell #1electrically and measure the changes in the membrane of cell #2. (Assume, to keep it simple, that there is no other input to cell #2.) The first time you stimulate cell #1 so that it fires exactly one action potential, and you measure the potential in cell #2. The second time you stimulate cell #1 so it fires several action potentials in rapid succession, and you measure the potential in cell #2.

C-1. Suppose you measure the change in potential in cell #2 at the synapse. What do you expect the second time?

C-1-A. The change in potential should be (larger*) (smaller) (the same) (the same, namely zero).

C-1-B. There should be an increase in the opening of (ligand gated channels) (voltage gated channels) (both) (neither) (depends on value of threshold).

C-1-C. The change in potential is best described as (EPSP) (IPSP) (IPSP or EPSP) (AP) (RMP) (any of these) (none of these).

C-2. Suppose you measure the potential in cell #2 in the middle of the axon. What do you expect the second time?

C-2-A. The change in potential should be (larger**) (smaller) (the same) (the same, namely zero).

C-2-B. There should be an increase in the opening of (ligand gated channels) (voltage gated channels) (both) (neither) (depends on value of threshold).

** "larger" or "smaller" here means larger or smaller in absolute magnitude.

Explain briefly how the events at the two spots (synapse vs axon) differ.

The question above is the way it was stated on the exam. For study purposes, answer the questions again if the transmitter is glutamate.

*8-20. You have two isolated neurons sitting in a solution; the axon of cell A synapses on a dendrite of cell B. Draw the two cells and the synapse between them. Show the relative locations of the dendrites, cell bodies, and axons of both cells. Label one dendrite, one axon, and the synapse.

A. The two isolated neurons are sitting in a medium with the usual ion concentrations (same as in usual extracellular fluid). You stimulate the dendrite of **cell A** by dripping a normal neurotransmitter (call it transmitter T) on the dendrite; this generates one action potential in axon of **cell B.**

A-1. For cell A, transmitter T is (excitatory) (inhibitory) (either way). No explanation required.

A-2. In the normal organism, where are the channels affected by transmitter T? (at a synapse) (not at a synapse) (at a synapse ***or*** elsewhere on the cell membrane) (none of these -- transmitter T does not open or close channels).

A-3. Suppose you stimulate a dendrite of cell B directly with a neurotransmitter. (Not necessarily transmitter T.) If you use enough transmitter, will you get an action potential in ***cell A***? (yes, if transmitter is excitatory) (yes, if transmitter is inhibitory) (yes, with either type of transmitter) (no) (yes, but can't predict which type(s) of transmitter will do it) (maybe -- can't predict without additional information). Explain A-2 & A-3 briefly.

B. Suppose you make some recordings of the membrane potentials from various parts of the two cells.

B-1. If you record from ***cell B*** at the synapse, what will the recording (trace) look like? See below at * for shapes of traces.
It will have the same shape as (a) (b) (c) (a or b) (a or c) (b or c) (none of these – trace will be flat).

B-2. If you record from the middle of the axon of ***cell A***, what will the recording look like? It will have the same shape as (a) (b) (c) (a or b) (a or c) (b or c) (none of these – trace will be flat). Explain briefly how you knew what the trace would look like in B-1, and why the recording in B-2 is, or is not, different.

C. You put your neurons into a Ca^{++} free medium. The Na^{+}, K^{+} and Cl^{-} ions are at their usual concentrations in the medium (same as in the normal ***extra***cellular fluid). You replace Ca^{++} with a different divalent cation to keep the charges the same as usual. Then you stimulate cell A in the same way as before, and record from the two cells as in the previous experiment. Compared to the experiment in part B, you expect to find a difference in the recording (from cell B at the synapse) (the axon of cell A) (both) (neither).
Explain briefly why the results here are, or are not, different from the experiments in part B.

D. You try your experiment one more time. This time you put your cells into medium that has a higher Na^{+} than usual. Everything else is normal. Then you stimulate cell A in the same way as before, and record from the two cells as in the previous experiments. Compared to the experiment in part B, you expect to find a difference in the recording (from cell B at the synapse) (the axon of cell A) (both) (neither).
Explain briefly why the results here are, or are not, different from the experiments in part B.

---

*Shapes of Traces:

Exam had 3 traces -- (a), (b) & (c). Only two, (a) & (b), are drawn below.
Trace (c) = standard action potential shape.
All traces are drawn on a scale of mV with more negative towards the bottom. Value of V for flat part = RMP.



(a)



(b)

PROBLEM SET 9 -- Muscle

9-1. Circle whichever is greater, x or y. If x = y, then circle both.

A. x. length of actin filament in skeletal muscle
y. length of tropomyosin filament in skeletal muscle

B. x. length of the actin filament in a relaxed skeletal muscle
y. length of the actin filament in a contracted skeletal muscle

C. x. troponin bound to actin in uterine muscle when oxytocin is present
y. troponin bound to actin in uterine muscle when oxytocin is absent

D. x. calcium-calmodulin in muscle of skin arteriole on hot day
y. calcium-calmodulin in muscle of skin arteriole on cold day

E. x. affinity of myosin for actin when ADP is bound to the myosin head
y. affinity of myosin for actin when ATP is bound to the myosin head

9-2. Skeletal muscle contains a protein called calsequestrin, which can reversibly bind up to 43 calcium ions. Where in the muscle cell would you expect to find this molecule? What is the value in having it?

9-3. To help them study certain neuromuscular diseases, scientists have produced a strain of mutant mice. These mice have defects in their muscles and in the neurons that lead to the muscles.
A. The neurons that innervate the skeletal muscles have wider nodes of Ranvier than in normal mice. How will this affect the function of the neuron?
B. The muscle membrane is more permeable to Na^+ than in normal mice. What affect will there be on the resting potential of the muscle cell?

9-4. Consider what happens in skeletal muscle. Fill in the blanks with one or more of these terms:
myosin actin tropomyosin sarcoplasmic reticulum troponin a protein kinase calmodulin none of these some of these *plus* something else
A. Ca^{++} binds to _____.
B. The ATPase needed for contraction is associated with _____.
C. The crossbridges are formed between_____ _____.
D. An action potential triggers release of Ca^{++} from _____.
E. To form crossbridges, _____ must be phosphorylated.

9-5. Eserine inhibits acetylcholine esterase. Botulinum toxin inhibits acetylcholine release. Curare binds very strongly to nicotinic acetylcholine receptors (but does not activate them). Consider the effects these compounds should have on the neuromuscular junction.
A. Which of these drugs will cause paralysis? Which will cause spasms?
B. If you are told that a test tube contains one of these drugs, how could you determine which one of the three it is?
C. Is curare an agonist or antagonist of acetyl choline?
D. How could one of these drugs be used to help isolate acetylcholine receptors?

9-6. During pregnancy, action potentials do not propagate over long distances in the uterine smooth muscle (myometrium) and different areas of the myometrium contract independently. Shortly before delivery, the myometrial contractions become synchronous. What changes must occur in the membranes of muscle cells to explain this?

9-7. For each of the following traits, indicate whether cardiac muscle
a. shares this trait with skeletal muscle b. shares this trait with smooth muscle
c. shares this trait with both skeletal and smooth muscle d. does not show this trait
e. is the only one of the three muscle types to show this trait

(1)._____contains intercalated discs (2). _____is striated
(3). _____shows auto-rhythmicity (4). _____is innervated by motor neurons
(5). _____contains actin, myosin, troponin, and tropomyosin
(6). _____Ca^{++} activation of calmodulin is important in mediating contraction

9-8. Suppose you stimulate a skeletal muscle chemically at a synapse (end plate) by spritzing on acetylcholine.
A. What is the expected membrane potential change at the site of the synapse?
B. What is the expected change a centimeter away from the synapse?

9-9. Suppose you stimulate a skeletal muscle chemically at a synapse (end plate) by spritzing on acetylcholine, as in the previous question. However this time you also add a chemical, such as tetrodotoxin, that blocks the voltage-dependent Na^{+} channels in the membrane, but has no other effect.
A. What is the expected membrane potential change at the site of the synapse?
B. What is the expected change a centimeter away from the synapse?
C. If you stimulate the muscle electrically, will you get an action potential?

9-10. Myasthenia gravis is a disease characterized by extremely weak muscle contractility. In analyzing the cause of this weakness, the following results were obtained:

(1). When electrical stimulation was applied directly to the muscle membrane, the muscles contracted normally.

(2). When the nerve leading to the muscle was electrically stimulated, the muscle contracted less strongly than usual.

(3). When an inhibitor of acetyl cholinesterase was given, it restored the muscle to near-normal function.

A. Based on the above results, (1) to (3), which of the following might be defective?
(axonal transmission of action potentials) (neurotransmitter release)
(receptor in neuromuscular junction) (function of actin and myosin)
(function of sarcoplasmic reticulum). Pick at least <u>two</u> possibilities that could explain the results.
B. Explain how the results of the experiments above led to your conclusion.
C. Suggest an experiment to differentiate between the two possibilities you chose.

9-11. When calcium is absent from the sarcoplasm (= cytoplasm of muscle cell), the myosin head is in a _____(low/high) energy state, and has _____ bound to it.

9-12. For a skeletal muscle to remain rigidly contracted, it needs a continual supply of ATP. Yet, when a skeletal muscle runs out of ATP (in a corpse), it becomes rigid.
A. How can you explain this seeming paradox?
B. Explain how skeletal muscle may remain relaxed even if ATP levels are high.

9-13. Consider what happens in <u>smooth</u> muscle. Now answer the questions in 9-4 over again for smooth muscle.

9-14. (Circle all that are true.) Smooth muscle cells

a. contract when Ca^{++} binds troponin
b. are innervated by the autonomic nervous system
c. contain gap junctions
d. have thick and thin filaments in organized sarcomeres
e. are found in the walls of the coronary arteries
f. contain myosin kinase

9-15. Calcium channel blockers are drugs used to treat people with high blood pressure.

A. They mostly affect the smooth muscle in blood vessels. Why would blockage of calcium channels in these cells lead to a decrease in blood pressure?

B. These drugs also can affect the myocardial muscle of the heart, where they decrease the strength of myocardial contraction. Explain why they have this effect.

C. In some people, these drugs have a side effect of decreasing intestinal motility, but are not likely to have side effects of decreasing skeletal muscle contractions. Yet muscles in both places require calcium. Explain why side effects are more likely in intestine than in skeletal muscle.

9-16. Consider a normal skeletal muscle that is relaxed. Action potentials are fired by the motor neuron that innervates the muscle, and the muscle contracts.

A. The minimum number of AP's required to cause a twitch in the muscle should be (0) (1) (2) (3) (4) (>4) (depends on the neuron) (depends on the muscle).

B. When the muscle contracts, which of the following will shorten?
(space between the ends of the actin filaments and the Z line) (space between the ends of the myosin and the Z line) (length of the sarcomeres) (length of the actin filaments) (none of these).
Circle all that apply, and show how the items & spaces mentioned shorten (or not).

9-17. Here is a list of events that may occur in skeletal muscle. Refer to the list when answering this question.

(a) Split of ATP
(b) Myosin binds to actin
(c) Tropomyosin binds more tightly to actin
(d) Ca^{++} binds to its target protein
(e) Myosin separates from actin
(f) Tropomyosin binds more tightly to myosin

A. In skeletal muscle, the target protein for Ca^{++} referred to in (d) should be _____. If this were smooth muscle, the target protein should be (the same protein) (a different protein, namely _____).

B. Suppose the first AP reaches the relaxed skeletal muscle. Which of the events on the list would be the first to happen? (a) (b) (c) (d) (e) (f). Explain briefly what function is served by carrying out this step in skeletal muscle.

C. Put the events on the list in the right order, starting with the first thing that will happen when the first AP reaches the skeletal muscle. Use the letters given, and omit any steps listed that are not involved in the contraction cycle or will not occur as long as stimulation continues.

Order : _____

D. Suppose stimulation of the muscle ends.

(1) Which step(s) listed above must occur to end the contraction cycle and allow the muscle to relax? (a) (b) (c) (d) (e) (f).

(2) When the contraction cycle stops, which of the steps listed will occur first, and which will occur last? First will be _____ AND last will be _____.

Explain briefly where the cycle will stop, and explain the function of the last step that will occur.

Problem Set 9R – Review Questions on Muscle & Nerve Function

9R-1. When smooth muscle contracts, phosphorylation of myosin should_____, AND the binding of Ca^{++} to troponin should _____ .
Choices are 'increase', 'decrease', 'stay the same' and 'stay the same at zero'.

9R-2. Consider the voltage gated Ca^{++} channels in the plasma membrane of a myocardial (contractile) cell.

A. If the channels are open longer than usual, you expect the height of the AP of the cells to _____ AND the width of the AP to _____ .
(Choices are increase, decrease etc. as in question I)

B. When the Ca^{++} channels do close, the membrane potential should (stay the same) (become less -) (become less +) (become more +) (become more -) (go from - to +) (go from + to -) (can't predict).

C. Suppose the Ca^{++} channels in the myocardial cells stay open longer than usual, but the Ca^{++} channels in the pacemaker cells* are unaffected. Then the period between the start of one heart beat and the start of the next will (decrease) (increase) (either way) (neither -- it should stay the same)
(decrease or stay the same) (increase or stay the same) (can't predict -- any of these are possible).

*Note/hint: If the role of pacemaker cells has not been covered in class, look in the back – it is explained in the key without giving away the answer.

9R-3. This is a review of the set up of the nervous system. You are looking at a preganglionic neuron of the parasympathetic system. For each part below, circle as many choices as apply. Explain as indicated.

A-1). The neuron is (efferent) (offerent) (offensive) (afferent) (sensory) (ifferent) (none of these).
A-2 . The neuron could synapse on (skeletal muscle) (smooth muscle) (a gland) (another neuron) (cardiac muscle) (any of these) (none of these).
A-3 The cell body of this neuron should be (in a ganglion close to the spinal cord) (in the spinal cord) (in a ganglion close to the target organ) (any of these) (none of these).

Draw a picture that shows the set up. Be sure it is labeled to show the features mentioned above.

B. Consider the synapse made by the axon of the neuron described above on its target cell. For each part, circle all that apply and explain briefly how the structure(s) at the synapse are responsible for these properties.
B-1. The synapse should be (nicotinic) (muscarinic) (adrenergic) (cholinergic) (none of these).
B-2. The synapse on the target cell should be (excitatory) (inhibitory) (either way)
(both – both types of transmitters could be released at the synapse).
B-3. When transmitter is released at this synapse what effect should it have on the target cell?
(generate a second messenger) (open a channel) (close a channel) (open or close a channel)
(none of these).

9R-4. You have a skeletal muscle fiber that is not being stimulated. It contains the usual distribution of Ca^{++} for an unstimulated muscle cell.

A-1. Suppose the motor neuron controlling the muscle fiber fires several times, and the muscle responds. For the muscle to respond, Ca^{++} must bind to (calmodulin) (tropomyosin) (troponin) (actin) (myosin) (none of these). Circle all that apply.

A-2. Suppose the motor neuron that controls the muscle fiber fires only once. The muscle cell will (respond by relaxing) (respond by contracting) (not respond significantly)
(respond, but can't predict whether it will relax or contract).

B. Suppose a relaxed skeletal muscle fiber has the normal Ca^{++} distribution. While the muscle was relaxed, the cell ran out of free ATP. The motor neuron controlling the muscle fiber fires – enough times to make a normal muscle respond.

B-1. When the motor neuron fires, the muscle cell will _____ (For all parts of B, the choices are the same as in A-2.)

B-2. When stimulation stops, the muscle fiber will _____.

B-3. Suppose that after stimulation stops, you add an analog of ATP that cannot be hydrolyzed, but otherwise mimics ATP. The muscle cell will _____.

B-4. After adding the analog (and waiting a bit) you now stimulate the muscle again, the same amount as before. The muscle cell will _____.

9R-5. Some smooth muscle cells surrounding the blood vessels (vascular smooth muscle) can contract or relax in response to stimulation. The two major receptors involved that respond to NE (norepinephrine) and E (epinephrine) are alpha 1 receptors and beta 2 receptors.

NE (norepinephrine) binds to alpha 1 receptors and triggers the IP3 pathway.
E (epinephrine) binds to beta 2 receptors, triggers the cAMP pathway.
Phosphorylation of myosin light chain kinase (MLCK) by PKA inactivates the kinase.

A. Suppose some vascular smooth muscle cells have both alpha 1 and beta 2 receptors for NE & E. (No other receptors for E or NE.)

A-1. When you add E, you expect ***less*** phosphorylation of (MLCK) (myosin) (actin) (calmodulin) (troponin) (tropomyosin) (all of these). Circle all that apply.

A-2. E should cause (vasoconstriction) (vasodilation) (can't predict).

A-3. NE should cause (vasoconstriction) (vasodilation) (can't predict).

Note: On the original exam, we specified the effect that each signal molecule (E or NE) has -- whether it cases vasoconstriction or vasodilation. This time, you figure it out.

B. Suppose you have vascular smooth muscle cells that have both types of receptors. In these cells, a fixed amount of NE or E (amount = X) produces the appropriate response. Suppose you add equal amounts (=X) of both NE and E at once.

B-1. You would expect to find Ca^{++} binding to (calmodulin) (tropomyosin) (troponin) (actin) (myosin) (none of these).

B-2. You would expect to get (vasodilation) (vasoconstriction). Pick one, and explain how you decided.

9R-6. Consider the receptors for E and NE described above (or on the previous page).

A-1. Which receptors are metabotropic? (alpha 1) (beta 2) (both) (neither).

A-2. These receptors are (both adrenergic) (both cholinergic) (neither) (one is cholinergic and one is adrenergic) (can't tell).

A-3. The neurons that synapse on the smooth muscle cells should be (motor neurons) (inter-neurons) (post-ganglionic neurons) (pre-ganglionic neurons) (sensory neurons) (part of the adrenal medulla) (any of these) (can't tell from information given).

B. Suppose the smooth muscle cells have only alpha 1 and beta 2 receptors. (No other receptors for the standard neurotransmitters and hormones.) Now consider the neurons that synapse on the muscle cells.

B-1. Which type of receptor should these neurons stimulate? (primarily alpha) (primarily beta) (both types about equally well) (primarily either alpha or beta, but you can't predict which).

B-2. Consider the receptors on the neurons themselves. These should be (primarily alpha) (primarily beta) (both types of) (either type of) (neither type of) receptor.

9R-7. Each neuron releases one major transmitter. However, many neurons release several co-transmitters along with the major transmitter. Some neurons that synapse on vascular smooth muscle cells release both NE & ATP. Both ATP and NE are found in the same synaptic vesicles. The ATP receptor in the muscle is a ligand gated cation channel that is permeable to both Na^{+} and Ca^{++}.
(NE works as described above.)

A. In a normal organism, both NE and ATP are released together. Suppose you set up an experiment and add only one or the other to the muscle cells.

A-1. Which will produce a faster response? (ATP) (NE) (both should be about the same) (can't predict).

A-2. If the muscle cell plasma membrane contains voltage gated Ca^{++} channels, they can probably be opened by addition of (ATP alone) (NE alone) (neither) (either one) (both – you need ATP and NE).

B-1. ATP alone will probably cause (vasodilation) (vasoconstriction) (neither) (can't predict).

B-2. In a normal organism, when these neurons cause the vascular smooth muscles to contract, the Ca^{++} needed for contraction should come from (the ER) (outside the cell) (both) (neither).

9R-8. A. When a cell fires an AP, it usually returns to resting potential by opening channels for (Na^+) (K^+) (Ca^{++}) (Cl^-) (none of these) (can't predict -- depends on which type of cell you are talking about).

B. Compared to what happens in ordinary neurons, once the cell reaches maximum depolarization, the opening of the channels referred to in A is relatively delayed in (pre-ganglionic neurons) (post-ganglionic neurons) (either type of neuron)(skeletal muscle) (cardiac contractile muscle) (cardiac pacemaker cells) (either type of heart cell) (either type of striated muscle) (none of these – all open their channels at about the same time) (beats me).

C. In long QT syndrome, the cardiac contractile cells take longer than normal to repolarize after an AP – the AP lasts longer than normal. Long QT syndrome could be caused by a delay in which of the following? (opening of Na^+ channels) (opening of K^+ channels) (opening of Ca^{++} channels) (closing of Na^+ channels) (closing of K^+ channels) (closing of $Ca+^+$ channels) (any of these) (none of these) (several of these, namely _____).

D. In long QT syndrome, a stimulus that has no effect on normal people can cause a premature heart beat – one not triggered by the usual pacemaker -- and generate an irregular rhythm in the heart. In this case, a *longer* AP causes a *premature* heart beat. Consider the state of the cardiac muscle membrane. Which of the following is the best explanation for a premature heart beat in this syndrome?
(the refractory period lasts longer than the depolarized state)
(the depolarized state lasts longer than the refractory period)
(the depolarized state is about the same length as the refractory period) (beats me).
Explain briefly what the normal situation is, and how it is different in long QT.

9R-9. The heart normally gets input from both the sympathetic and the parasympathetic systems.
A. If you block all sympathetic input into a normal heart, you would expect the heart beat to (stop) (speed up) (slow down) (speed up or slow down) (stay the same).

B. If you want the heart to slow down, you could use (an adrenergic agonist) (a cholinergic agonist) (an adrenergic antagonist) (a cholinergic antagonist) (any of these) (none of these).

C. The parasympathetic input to the heart should cause release of a neurotransmitter (NT).
C-1. The NT should bind to a receptor that is (metabotropic) (ionotropic) (either way) (both) (neither).

C-2. The NT of the PS system has multiple effects on the heart. In order to affect the heart rate of a normal person, the NT should bind to receptors on
(contractile cells in the atria) (contractile cells in the ventricles) (cells in the bundle of His) (Purkinje fibers) (pacemaker cells in the atria) (pacemaker cells in the ventricles) (any cell with pacemaker activity) (any of these) (none of these).

PROBLEM SET #10 - Respiratory system

10-1. If the spinal cord were severed where it joins the brainstem, what would happen to respiration?

10-2. The Heimlich maneuver puts sudden pressure on abdomen. Why does this help a choking person?

10-3. During forceful exhalation used to blow up a balloon, you would contract your
a. diaphragm
b. intercostal muscles
c. abdominal muscles
d. smooth muscles around the alveoli

10-4. A doctor wants to listen to the sounds in a patient's respiratory system while he breathes. Since a relaxed, seated person may breathe only 12 times per minute, it could take as long as 15 seconds to listen to three inhalations… too much wasted time! So the doctor asks the patient to inhale at a faster than normal rate by asking for the next inhalation before it would normally occur: "Take a deep breath...Deep breath.... Another deep breath..." After taking several deep breaths very rapidly in succession, the doctor stops to record his findings, and the patient notices that there is a long pause before he next feels the need to inhale. Why?

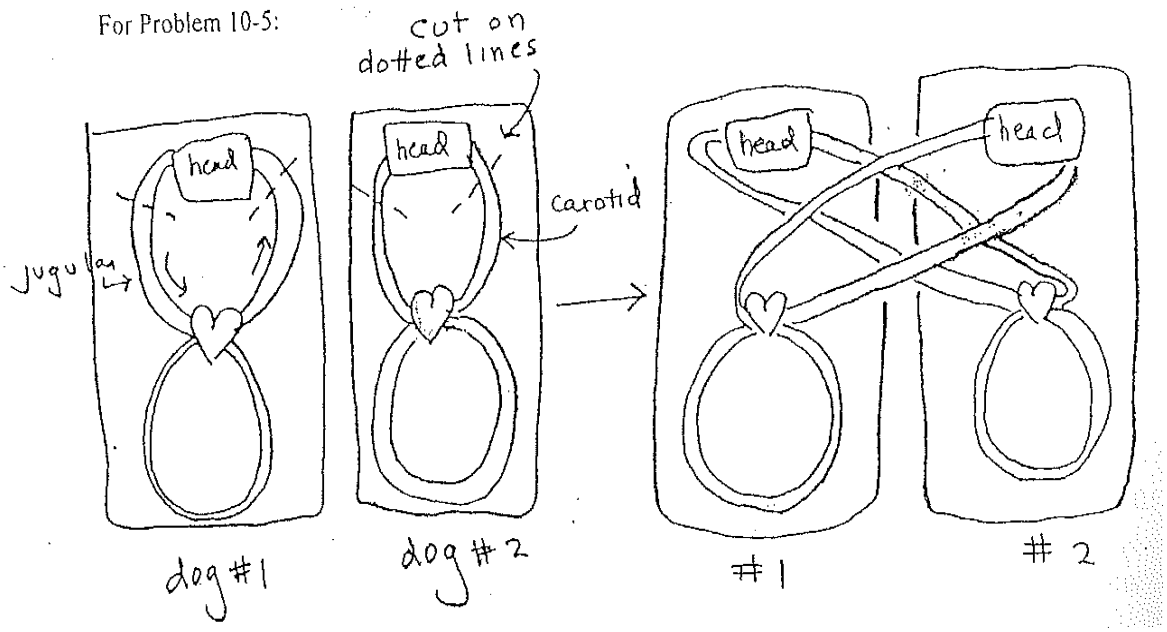
10-5. In the 1890's, Leon Fredericq elucidated the factors that control respiratory rate. He placed two anesthetized dogs back-to-back and surgically cut and reattached their carotid arteries and jugular veins as diagrammed below. The result was that blood went from dog #1 to the head of dog #2 and back to the heart of dog #1, while blood from dog #2 went to the head of dog #1 before returning to dog #2's heart. (See picture on top of next page.) He then measured the breathing rate in each dog. (Note: The anesthesia did not affect breathing and is not relevant to this question.) Fredericq then performed the following experiments:

A. He partially occluded the trachea of dog #1 and measured the rate of breathing in both dogs. Based on what *you* know about control of respiration, you could predict that the breathing rate he measured was _____(increased/decreased/unchanged) in dog #2 and _____ in dog #1.

B. He then rapidly and repeatedly blew air into the lungs of dog #1, hyperventilating it. This produced an increase in arterial oxygen and a decrease in arterial CO_2 in dog #1. Based on what *you* know, you could predict that the breathing rate of dog #2 was_____ (increased/decreased/unchanged)

C. The above experiments left him with at least <u>two</u> possible interpretations for the factor(s) that control respiratory rate. What were they?

D. He then performed <u>one</u> experiment on these animals that allowed him to differentiate between these possible interpretations. He _____and measured the breathing rate in _____. This breathing rate was _____. He concluded that _____.



10-6. An individual has thickened alveolar membranes caused by disease. How will this affect the rate of diffusion across the alveoli?

10-7. A person is breathing 100% oxygen. How much will the oxygen content of the arterial blood increase compared to when the person is breathing room air?

10-8. A person trapped in an elevator begins to deplete the supply of oxygen in the air, but it takes a long time before he is affected by this. Why?

10-9. A person is brought to the hospital with CO poisoning.
   A. Arterial pO_2 is normal. Why?
   B. The total O_2 in blood is low and person is oxygen starved. Why doesn't this low O_2 cause breathing rate to increase?

10-10. In a person with iron deficiency anemia, the amount of hemoglobin in the blood is lowered, because iron is required for hemoglobin synthesis. How will low hemoglobin levels affect the total amount of oxygen carried? How will this affect the pO_2? How will it affect breathing rate?

10-11. Why is it important that airplane interiors be pressurized such that the air pressure is 760 mm Hg? If there is a drop in air pressure, oxygen masks are used. What is the physiological value of breathing pure oxygen?

10-12. List 3 proteins found in mature red blood cells, and the function of each.

10-13. During fetal development, the pO_2 in the placenta is about the same as that in the mother's blood. To ensure movement of oxygen from the mother's to the fetus' blood, fetal hemoglobin has 2 alpha and 2 gamma subunits instead of the 2 alpha and 2 beta subunits seen in adults. The gamma subunit binds oxygen much more strongly, and so oxygen moves into the fetal blood in the placenta, despite the fact that the pO_2's are similar. Compared to the normal oxygen-hemoglobin dissociation curve, the curve for fetal hemoglobin would be shifted (to the right) (to the left).

10-14. The hemoglobin of an Andean llama becomes saturated with oxygen at a lower partial pressure than that of a human. What is the mechanism by which this occurs, and what is the advantage of it? Suppose we put equal numbers of moles of llama and human hemoglobin in separate beakers. Which beaker will contain more oxygen at sea level? Which will contain more oxygen in the Andes at 14000 feet?

10-15. Cardiopulmonary resuscitation (CPR) is a technique to revive an individual whose heartbeat and breathing have stopped. It includes exhaling into the mouth of the patient. If the air we exhale contains carbon dioxide, why isn't this dangerous to the patient?

10-16. An individual has iron-deficiency anemia (as in problem 10-10). Suppose hemoglobin levels in this person are half those of normal people. Some blood is taken from the anemic person and tested at different pO_2's. Draw the oxygen-hemoglobin dissociation curve you would expect to see, and compare it to that of a normal individual. Explain your answer.

10-17. For each pair of statements, circle whichever is greater, x or y. If x = y, circle both.

A. x. carbonic anhydrase activity in blood at the tissues
y. carbonic anhydrase activity in blood at the lungs

B. x. pCO_2 in blood leaving the tissue
y. pCO_2 in the alveolus

C. x. change in hemoglobin saturation that occurs when pO_2 drops from 100 to 90 mm Hg
y. change in hemoglobin saturation that occurs when pO_2 drops from 40 to 30 mm Hg

D. x. diameter of bronchioles during parasympathetic stimulation
y. diameter of bronchioles during sympathetic stimulation

E. x. calcium in the cytoplasm of the diaphragm during exhalation
y. calcium in the cytoplasm of the diaphragm during inhalation

F. x. oxygen dissociating from hemoglobin when temperature is high
y. oxygen dissociating from hemoglobin when temperature is low

G. x. oxygen binding to globin
y. oxygen binding to heme

10-18. Lowering the pH changes the binding of O_2 to hemoglobin.

A. Lowering the pH causes the oxygen-hemoglobin dissociation curve to shift to the (right) (left) (either way).

B. This would increase the tendency of hemoglobin to (pick up oxygen in the lungs) (release oxygen in the tissues) (pick up CO_2 in the tissues) (release CO_2 in the lungs) (some combination of these) (can't predict). Explain.

C. Mammals are constantly losing heat from their surface, and needs to produce more heat by muscle metabolism. Compared to humans, small mammals like mice have relatively little muscle mass compared to their surface area, so they need to produce heat a much greater rate. This higher metabolic rate of small mammals requires a more efficient delivery of oxygen to the tissues. When blood from these small mammals is tested, it is found to differ from human blood in that the hemoglobin has less affinity for oxygen and the response to lowered pH is even greater than described for humans, in A. and B. above. This benefits the mouse because it improves (saturation of their hemoglobin in the lungs) (release of oxygen in the tissues) (both) (neither).

Problem Set #11 Heart Function & Circulatory System

11-1. Fill in with the appropriate ion:

a. Inactivation of ___ channels could bring about the slow drift of membrane potential to threshold in pacemaker cells.

b. Sympathetic stimulation decreases permeability of the SA node to ___.

c. Slow inward diffusion of ___ is largely responsible for the plateau phase of the cardiac action potential.

11-2. What will happen to a cardiac pacemaker cell if its permeability to Na^+ is increased?

11-3. The sinoatrial node is the heart's normal pacemaker. Which of the following explains why?
a. The SA node has the fastest natural rate of autorhythmicity
b. The SA node has both sympathetic and parasympathetic innervation
c. The SA node lies in the right atrium, where blood flow begins
d. K+ channels in the SA node are activated more rapidly than elsewhere in the heart

*11-4. If you drink too much coffee or experience strong emotion, your heart may "skip a beat". Instead of the SA node, a different group of pacemaker cells depolarize and initiate an action potential a little early -- just after the previous contraction, but before the subsequent one should have occurred. A premature contraction occurs, but the subsequent contraction is delayed, even though the SA node continues as usual. Why is the subsequent contraction delayed?

11-5. The least oxygenated blood in the body is carried by the
a. left ventricle b. pulmonary artery c. pulmonary vein d. aorta

11-6. During ventricular diastole, the atrial pressure is _____
a. greater than b. less than c. equal to
the ventricular pressure, and so the AV valve is _____
a. open b. closed

11-7. Which of the following statements about interstitial fluid is <u>false?</u>
a. It contains the same glucose and salt concentration as plasma.
b. It contains a lower protein concentration than plasma.
c. Its osmotic pressure is greater than that of plasma.
d. Its hydrostatic pressure is less than that of plasma.

11-8. After exposure to second-hand cigarette smoke, rabbits had platelets that released more ADP than usual. The rabbits were more likely to
a. form blood clots b. bleed from capillaries c. secrete erythropoietin d. use myokinase e. eat off of little plates

11-9. Bradykinin acts as a local factor that causes vasodilation of arterioles. If, however, the endothelium of the arteriole is removed, bradykinin is no longer effective in stimulating vasodilation. What would you expect is the mechanism by which bradykinin stimulates vasodilation?

11-10. A sudden increase in pressure in the carotid sinus leads to _____ sympathetic nerve activity.
a. increased b. decreased

11-11. Due to liver damage, an individual makes less albumin and fibrinogen. This will decrease the movement of fluid from

a. capillaries to interstitial fluid b. interstitial fluid to capillaries
c. interstitial fluid to lymphatic vessels d. lymphatic vessels to blood
e. glomerular capillaries to Bowman's capsule

11-12. Explain why edema occurs in the following situations:

a. liver disease

b. an allergic response in which capillary pores enlarge

c. removal of lymph nodes in woman with breast cancer (edema develops in arm)

11-13. Circulatory shock occurs when insufficient blood reaches tissues, either because there has been a loss of blood, or because the size of the blood vessels has increased, or because the heart is diseased and unable to pump adequately. People have been told that accident victims should be kept warm. Unfortunately, this sometimes leads them to pile up too many blankets on an accident victim. For a person in shock, the increased heat can actually make the shock worse. Why?

11-14. EDTA, a chelating agent that binds Ca^{++}, is added to blood in a test tube to prevent it from clotting.
A. Why does EDTA prevent blood clotting?
B. What would be the problem in using EDTA to treat a person whose blood is prone to clotting?

11-15. Anemia refers to a decrease in the ability of blood to carry oxygen. Explain why anemia would develop in each of the following situations:

a. deficiency of iron in the diet

b. destruction of bone marrow as occurred in victims of Chernobyl explosion

c. diseased kidney

11-16. A person's heart starts to beat very fast. A physician presses on the neck, where the carotid artery is. Describe the pathway by which this leads to a slowing of the heart rate.

*11-17. You are studying fluid pressures during edema, in which excess fluid accumulates in the tissue outside blood vessels. The pressures measured during the normal (non-edematous) state are indicated in the table below (line a). (NOTE: In class, we discussed hydrostatic pressures measured at the beginning and end of the capillary; the numbers given here refer to the average hydrostatic pressure measured all along the length of the capillary.)

A. Edema may develop in Vitamin C deficiency, which causes increased capillary permeability. Which of the measurements in the table below (b, c, d, e) indicates the most likely pressures measured in this type of edema? Explain your answer.

B. Edema may develop when excess aldosterone secretion leads to salt and water retention, resulting in high blood pressure. Which of the measurements in the table below (b, c, d, e) indicates the most likely pressures measured in this type of edema? Explain your answer.

C. Edema may develop when damage to the glomerular capillaries allows proteins to enter glomerular filtrate. Which of the measurements in the table below (b, c, d, e) indicates the most likely pressures measured in this type of edema? Explain your answer.

	Capillary		Interstitial Fluid	
	Hydrostatic P	Osmotic P	Hydrostatic P	Osmotic P
a.	27	25	1	1
b.	27	6	6	1
c.	27	14	4	7
d.	41	25	8	1
e.	17	25	5	1

See also Problem Set 9R, problems 2, 8 & 9.

Problem Set #12 -- Urinary System

For problems 12-1 to 12-5, chose one or more answers.

12-1. Most of the Na^+ that is reabsorbed from the nephron enters the

a. afferent arterioles
b. efferent arterioles
c. peritubular capillaries
d. glomerular capillaries
e. collecting tubules

12-2. The ascending limb of the loop of Henle

a. actively transports NaCl into the surrounding interstitial fluid
b. is impermeable to water
c. is found in the renal cortex
d. drains into the proximal tubule

12-3. Substances that appear in the urine have been

a. filtered and reabsorbed
b. filtered and/or secreted but not reabsorbed
c. reabsorbed but not secreted
d. secreted and reabsorbed but not filtered

12-4. Blood pressure in the glomerular capillaries is higher than blood pressure in other capillaries because

a. the afferent arteriole has a larger diameter than the efferent arteriole.
b. the efferent arteriole has a larger diameter than the afferent arteriole.
c. of local factors.
d. the glomerular capillaries have larger pores.
e. the kidney is so close to the heart.

12-5. Of the following, protein concentration will usually be highest in

a. plasma
b. interstitial fluid
c. lymph
d. filtrate in Bowman's capsule

12-6. Some birds have exocrine glands that secrete salt. They are able to secrete fluid that is about 5% salt, five times as salty as normal body fluids. This is necessary because they drink salty sea water. Why can't they excrete the salt in their urine?

12-7. Eating a bag of salted pretzels causes the atria of the heart to release a hormone known as atrial natriuretic peptide (ANP). Its effect is to promote NaCl excretion by the kidney. ANP has at least three target tissues:

a. renin secreting cells of the kidney
b. afferent arterioles of the kidney
c. adrenal cortex.

Describe the effect that ANP should have at each of these targets, and how this would lead to an increase in NaCl excretion.

12-8. Aldosterone and ADH both affect the amount of water loss in the urine. Compare and contrast their modes of action. Consider the speed of response, mode of action of the hormone, & location of cells affected.

12-9. ADH can be over produced in certain medical conditions. If ADH is made in excess, what do you expect to happen?

A. The volume of urine produced should (increase) (decrease) (stay the same) (can't predict).

B. The osmolarity of urine should (increase) (decrease) (stay the same) (can't predict).

C. The osmolarity of blood should (increase) (decrease) (stay the same) (can't predict).

D. The blood pressure should (increase) (decrease) (stay the same) (can't predict).

Explain briefly how ADH produces these effects.

12-10. If you secrete aldosterone, what should happen to the movement of water in the kidney tubule?

A. More water should move (in different directions in different parts of the tubule) (into the tubule) (out of the tubule) (into none of these – no changes in water movement should occur).

B. How should water be transported across the membrane(s) of the cells surrounding the tubule? (actively) (passively) (both) (neither).

C. Consider the cells surrounding the tubule. Water should cross (the basolateral membrane) (the luminal membrane) (both membranes) (neither membrane) of the cells.

D. In response to aldosterone, you expect channels for water passage to be: (synthesized) (inserted into the luminal membrane) (inserted into the basolateral membrane) (unchanged).

12-11. Suppose you take a drug that inhibits Na^+ transport in the kidney. What will this probably do to the composition of your urine in terms of its content of glucose, Na^+ content, and water content?

12-12. Many diuretics (compounds that increase water loss in the urine) inhibit Na^+ transport. (Why would an inhibitor of Na^+ transport act as a diuretic? See previous question.) For each question, circle all that apply and explain in a sentence or two.

A. A diuretic that affects Na^+ transport must inhibit (secretion) (filtration) (reabsorption) of Na^+.

B. Different compounds affect different parts of the kidney tubule. Consider the following compounds. Each compound binds to proteins in the ***luminal*** membrane of the cells surrounding the specified region of the tubule.

- Compound PT binds to proteins in the proximal tubule
- Compound DH binds to proteins in the descending loop of Henley
- Compound AH binds to proteins in the ascending loop of Henley
- Compound DT binds to proteins in the distal tubule/collecting ducts.

B-1. Which compound is the least likely to act as a diuretic by inhibiting Na^+ transport? (PT) (DH) (AH) (DT) (none of these).

B-2. Which compound(s) are likely to affect the amount of amino acids in the urine by inhibiting Na^+ transport? (PT) (DH) (AH) (DT) (none of these).

B-3. Which compound(s) are likely to mimic aldosterone excess? (PT) (DH) (AH) (DT) (none of these).

B-4. Which compound(s) are likely to directly inhibit the Na^+/K^+ pump? (PT) (DH) (AH) (DT) (none of these).

12-13. If your hypothalamus or pituitary is damaged, you may make very large volumes of hypo-osmotic urine. What is the probable cause, and what can you do about it?

12-14. In Addison's disease the adrenal cortex does not produce steroids. This usually results in abnormal blood pressure (among other problems).

A. The blood pressure is likely to be (high) (low) (either way).

B. The primary effect of steroid absence is probably that the patient is (retaining water) (losing water) (retaining salt) (losing salt) (retaining both) (losing both).

C. The most reasonable thing to do (for the pressure problem) is to give the patient (renin) (aldosterone) (cortisol) (adrenaline) (estrogen) (angiotensin) (progesterone) (testosterone) (vasopressin).

12-15. The amino acid proline is injected into an individual with a certain disease. All of the proline is recovered from the urine. The same individual is given proline to drink. None of the proline is found in the urine. The inheritance of the disease indicates that it is caused by a single mutation. This individual probably has a defect in a protein found in

a. the digestive system
b. the urinary system
c. both the digestive and urinary systems

Describe the particular protein that would be defective and where it would be found. Be as specific as possible about the location.

*12-16. Coleridge tells a tale of an old sailor who was "Alone, alone, -- all, all alone; Alone on a wide, wide sea." His major problem, however, was not loneliness, but dehydration. He had "Water, water, everywhere, Nor any drop to drink." Why would drinking seawater not quench his thirst? (Hint: The salt in seawater gives it an osmolarity of about 2400 milliosmoles.)

*12-17. Two genes have been cloned that code for 2 related proteins, aquaporin 1 & 2. If you put either cloned gene (or cDNA) into a Xenopus oocyte, the oocyte makes the corresponding protein and the oocyte becomes highly permeable to water. Assume there are no other aquaporins in the ***luminal*** membranes of kidney cells.

#1 is expressed constitutively in the luminal membranes of the cells lining the proximal tubule and descending limb of Henle.

#2 is defective in a person with inherited diabetes insipidus (= person who produces very large volumes of dilute urine).

A. If #1 were defective, the filtrate at the bottom of the loop of Henle would be:
(normal) (higher in amount of salt than usual) (lower in salt) (more concentrated than usual) (less concentrated) (higher in protein than usual) (lower in glucose than usual) (higher in glucose).
Circle all likely possiblities and explain.

B. In a normal person, which protein(s) should be present in the cells lining the ascending loop of Henle? (#1) (#2) (both) (neither).

C. In a normal person, which protein(s) should be present in the cells of the collecting ducts? (#1) (#2) (both) (neither).

D. In a normal person, which gene(s) should be present in the cells of the collecting ducts? The gene(s) for aquaporins (#1) (#2) (both) (neither).

E. Suppose you can use the cDNA's to measure expression of the corresponding genes. (You can do this in an experimental animal, but not in a person.) Suppose a normal individual starts to secrete ADH. Then you would expect the level of hybridizing mRNA from the entire kidney to increase if you use the cDNA from gene (#1) (#2) (both) (neither).

F. To help the person with inherited diabetes insipidus described here, it would probably help to provide (ADH) (aldosterone) (both) (neither).

*12-18. Edema (excess fluid retained in the tissues) can be treated by using a diuretic, which increases the rate at which fluid leaves the body in urine. Some chemicals can cause water loss from the body and others cause water and salt loss from the body. If a chemical that just promotes water loss is given, it is not very effective in increasing water loss. What hormonal change, resulting from the loss of water alone, will negate the usefulness of this chemical as a diuretic, and why would each of the following treatments act as a diuretic?

a. large amounts of glucose injected into the blood

b. spironolactone, which binds to and inhibits aldosterone receptors

12-19. The proximal and distal tubules differ in their structure, function, and the fluid that passes through them. For each of the parameters listed below, indicate whether it is greater in the proximal tubule or distal tubule, and explain why.

	Where is it greater?	Explain *briefly* why it is greater there.
Microvilli		
Number of mitochondria		
Intracellular hormone receptors		
Osmolarity of filtrate		
Glucose concentration in cytoplasm		

12-20. President Kennedy's medical records were recently released, and showed that his physicians sometimes prescribed large doses of salt tablets. He needed to ingest more salt than most people, because he had a certain hormonal deficiency. He was not producing the hormone _____which is normally synthesized in the _____.

*12-21. The Na^+/K^+ pump in the proximal tubules of the kidney can be inhibited due to toxins, vitamin D deficiency or congenital abnormalities. The result is called Fanconi's syndrome. The primary symptoms include glucose and amino acids in the urine and metabolic acidosis (low pH in blood).

A-1. Normally glucose is (filtered) (reabsorbed) (secreted) (none of these), AND
A-2. Amino acids are not usually found in the urine because of active transport of amino acids (across apical/luminal surface of epithelial cells) (across basolateral surface of epithelial cells) (into capillaries) (out of capillaries) (none of these). For each part, circle one or more answers and explain.

B. From the results given, it looks as if under normal conditions the protons in the proximal tubules go (out of the lumen of the tubule) (into the lumen of tubule) (neither) (either way) AND protons are normally moved by (primary active transport) (secondary active transport) (neither) (either way).

12-22. A molecule of glucose is found in the lumen of the kidney tubule in a normal person.

A. To get to the tubule from the blood, the molecule was (filtered) (reabsorbed) (secreted) (actively transported across a membrane) (none of these). Circle all correct choices for all parts of this question (A-E).

B. Which of the following parts of the tubule is likely to contain the glucose molecule? (the distal tubule) (the descending loop of Henle) (the ascending loop of Henle) (the proximal tubule) (any of these) (none of these).

C. Suppose the molecule of glucose goes from the lumen to the general circulation, and is carried all the way to the heart. On its way from the lumen of the tubule to the heart, the molecule of glucose should pass through (a collecting duct) (the efferent arteriole) (the afferent arteriole) (a glomerular capillary) (a venule) (a peritubular capillary) (either type of capillary) (Bowman's capsule) (none of these).

D. To move from the tubule to the general circulation, the molecule of glucose needs to be transported by _____. Choices are: (a). simple diffusion (b). facilitated diffusion (c). secondary active transport (d). primary active transport (e). none of these.
In what order should these types of transport occur? Give the letters in order: _____.

*E. People with hyperargininemia (HAM) have a metabolic defect in degradation/production of the amino acid arginine (arg). People with HAM have high levels of arg in both the blood and urine. Their kidney proteins are normal.
E-1. In a normal person, the level of arg in the urine should be (negligible) (significant, but lower than the level in a person with HAM) (depends on what the person has been eating). Explain all your answers.
E-2. In a person with HAM, there should be *an increase* in the rate of arg (filtration) (reabsorption) (secretion) (all of these) (none of these – none increase).
E-3. In a person with HAM, there should be *a decrease* in the % of total arg in the blood or tubule that is (filtered) (reabsorbed) (secreted) (all of these) (none of these – none decrease).
E-4. In a person with HAM, the amount of arg found in the lumen of the kidney tubule should be higher than normal in (the proximal tubule) (the distal tubule) (the loop of Henle) (the collecting ducts) (all of these) (none of these).

12-23. BND (bad news disease) is an inborn error of metabolism. It is recessive; people with the BND genotype (homozygotes) have inherited two defective copies of the BND4 gene. Many people with the BND genotype have a normal phenotype (no symptoms), but some develop disease symptoms, including problems with the brain such as psychiatric problems and ataxia (lack of balance).

The BND4 gene is not expressed in the brain, but it is highly expressed in the kidney and in the intestine. It codes for a Na^+/amino acid co-transporter for neutral amino acids. It does not transport charged amino acids.

Normal people, and people with BND, also have a H^+/dipeptide co-transporter (HDCT) in the intestine and they have intracellular proteases that can hydrolyze intracellular dipeptides. Because of the HDCTs, most people with the disease genotype have normal levels of amino acids in their plasma, whether they have symptoms or not.

A. For each part of A, fill in the blank with all correct choices from the following list. Choices are: (the afferent arteriole) (the peritubular capillaries) (the glomerular capillaries) (the proximal tubule) (the distal tubule) (the ascending loop of Henle) (the descending loop of Henle) (the collecting ducts) (none of these) (all of these).

A-1. In the kidney, the BND4 protein should be made in the cells of _____.

A-2 An enhancer of the BND4 gene should be located in the cells of _____.

B. Consider a person with the BND genotype.

B-1. In the USA, people with a BND genotype usually do not develop symptoms. Why not ? Probably because they eat a diet that is (low in protein) (high in protein) (high in carbohydrates) (low in carbohydrates) (high in salt) (low in salt) (any of these – diet should have no effect).

B-2. The level of neutral AAs in their urine should be (higher than normal) (lower than normal) (normal, which is zero) (normal, which is nonzero) (can't predict – depends on phenotype).

B-3. The level of the AA aspartic acid in their urine should be (higher than normal) (lower than normal) (normal, which is zero) (normal, which is nonzero) (can't predict).

C. In a person with the BND genotype, the concentration of neutral AAs in the filtrate entering the collecting ducts of the kidney should be (about the same as in their plasma) (lower than in their plasma) (higher than in their plasma) (can't predict – depends on the person's phenotype).

D. If a person with the BND genotype ***stops*** secreting ADH, then what will happen?

(i) In the urine, the concentration of neutral AAs will (increase) (decrease) (stay the same) (can't predict).

(ii) In the urine, the amount of neutral AAs will (increase) (decrease) (stay the same) (can't predict).

*E-1. Suppose you could take neurons from the brain of a person with BND symptoms, and measure neutral AA uptake into the cells. The maximal rate of uptake, measured *in vitro,* should be (higher than normal) (lower than normal) (normal, which is zero) (normal, which is nonzero) (can't predict).

*E-2. When people develop brain symptoms in BND, the most likely explanation is (inability to transport AAs properly in brain) (low levels of neutral AA in the plasma) (tubular secretion of toxic byproducts of AAs in the kidney) (production of toxic byproducts from neutral AAs remaining in the intestine) (toxic compounds from either tubular secretion or intestinal metabolism) (any one of these – all are equally likely) (something else – none of these could explain the symptoms).

Problem Set 12R -- Review Questions on Physiology

Part I. Fill in the blank with increases, stays the same, decreases, or stays the same at zero.

12R-1. Suppose blood is carried from the left side of the heart to the right side, using the usual blood vessels.
The total amount of oxygen in the blood should _____, AND the total amount of hemoglobin should _____.

12R-2. Supposed you have a solution of hemoglobin that is 50% saturated with oxygen. If the solution gets more acid (but the pO2 remains the same), you expect the % saturation of hemoglobin to _____ , AND this is similar to what normally happens in the (tissues) (lungs) (both) (neither).

12R-3. Carbonic anhydrase is needed in RBC at (the lungs) (the tissues) (both) (neither), AND the enzyme catalyzes formation of (CO_2) (HCO_3-) (H_2CO_3) (CO_2 or H_2CO_3) (CO_2 or HCO_3-) (H_2CO_3 or HCO_3-) (none of these) (all of these).

12R-4. If renin is secreted, then
Then the % of the NaCl in the filtrate that is removed in the distal tubule should_____, AND

The % that is removed in the proximal tubule should _____ , AND

The amount of water retained in the urine when it exits the last part of the tubule should _____ , AND

The hormone(s) that are/is primarily responsible for any differences in salt removal in these parts of the tubule are/is (aldosterone) (ADH) (both) (neither).

12R-5. When RBC's arrive at the tissues, the net HCO_3^- in the blood (plasma + RBC) should _____ , AND the net Cl^- should _____.

12R-6. Suppose the filtrate in the kidney tubule moves from the middle of the descending loop of Henle to the middle of the ascending loop. Consider the movement of the following substances across the luminal (apical) surface of the epithelial cells.
The movement of NaCl should _____,
AND the movement of glucose should _____ ,
AND the movement of water should _____.

12R-7. Suppose a mutant hemoglobin has an affinity for oxygen that is higher than normal. In the tissues, the % saturation (relative to normal) should _____, AND in the lungs, the % saturation (relative to normal under same conditions) should _____.

12R-8. If the CO_2 in the blood rises, the rate of breathing should _____, AND the slope of the pacemaker potential in the brain stem respiratory center (medulla) should _____.

For the remaining questions, Fill in the blank, circle the right answer, etc. and explain.

12R-9. A. Consider blood that leaves the left ventricle.

A. The next part of the heart that it will enter will be the (left atrium) (right atrium) (right ventricle) (beats me). No explanation, for A & B, but be sure all your answers for A-C are consistent.

B. Suppose the flow from the left ventricle goes on its normal path. Once it leaves the heart, it loops through the usual blood vessels and returns to the heart.

B-1. On "the loop" an individual red blood cell could pass through (the lungs) (the liver) (both) (one of the other but not both) (either one or both).

B-2. In that RBC going through "the loop", the % saturation of hemoglobin with oxygen should (increase) (decrease) (stay the same) (stay the same at zero).

C. When the blood passes through "the loop" what should happen to the following?

C-1. The amount of HCO_3^- in the plasma should (increase) (decrease) (stay the same) (stay the same at zero)

C-2. The Cl^- level in the plasma should _____. Explain your answers to C briefly.

12R-10. The hemoglobin in a RBC should be maximally oxygenated when it enters (the left ventricle) (the right ventricle) (the left atrium) (the right atrium) (either of the ventricles) (either of the atria) (either part of the right half) (either part of the left half) (none of these – the level of oxygenation is the same in all parts of the heart).

12R-11. You measure the amount of CO_2 that can be added to each of the following:

(a) a solution of hemoglobin dissolved in buffer (same ion concentrations as in plasma)

(b) a solution of buffer (no Hb added)

(c) a suspension of RBC (red blood cells) in buffer -- same concentration of hemoglobin over all as in (a). Your results are as follows:

	Sample		
	(a) Hb	(b) Buffer	(c) RBC
Maximum CO_2 that can be added	2X	1X	17X

If you add the amount of CO_2 indicated, it goes into the sample. If you add more, it bubbles off.

A-1. If you examine the actual amount of free CO_2 present (dissolved) in the three samples it should be (about the same proportions as the numbers in the chart) (higher in (c) relative to the other two) (lower in (b) relative to the other two) (about the same in all three) (none of these) AND

A-2. The amount of added CO_2 in (c) is higher than in (a) because of (hemoglobin) (enzymes of glycolysis in RBC) (carbonic anhydrase) (DPG) (band 3 -- anion exchanger) (some other component of the RBC) (beats me). Circle one or more answers and explain.

B. Suppose you repeat this experiment with oxygen, and see how much oxygen you can add to each sample. (No CO_2 added.) You should get results that are (similar -- about the same proportions as those shown in the chart) (different -- (b) should hold less than the other two) (different -- all three samples should hold about the same amount of oxygen) (different -- in some other way) (beats me).

12R-12. Congestive heart failure (failure to pump properly) can cause swelling of the ankles, legs, feet and sometimes abdomen (= tissue edema due to liquid leaking out of the capillaries into the tissues) **or** difficulties with breathing (= pulmonary edema due to fluid leaking out of the capillaries in the lungs).

A. Survivors of heart attacks often have damaged heart muscle that leads to congestive heart failure. Damage to the right side of the heart often causes swelling; damage to the left side often causes breathing difficulties. Explain these results.

B. Individuals with congestive heart failure often have low blood pressure (because of insufficient pumping), even though the volume of the blood is normal (or high). The normal hormonal response(s) to low blood pressure should make the edema (better) (worse) (have no effect) (can't predict).

C. Conversion of inactive precursors to angiotensin II (active) requires an enzyme called ACE (angiotensin converting enzyme) in addition to renin. Normally it is the renin, not the ACE, which is rate limiting. ACE inhibitors are often given in cases of congestive heart failure. The primary hormonal effect of an ACE inhibitor should be to (stimulate release of aldosterone) (inhibit release of aldosterone) (effect release of ADH but not aldosterone) (have no effect on release of either hormone), AND another effect of an ACE inhibitor would be to [directly or indirectly] affect (skeletal muscle) (cardiac muscle) (smooth muscle), AND if the heart is not damaged, ACE inhibitors should be helpful for (high blood pressure) (low blood pressure) (either one) (neither one).

12R-13. Suppose you have a diuretic. that works by inhibiting Na^+ transporters in the luminal membrane of the epithelial cells lining the kidney tubule. This diuretic is secreted by the ***very last*** segment of the proximal tubule. (EC = epithelial cell; IF = interstitial fluid)

A-1. The diuretic should *directly* inhibit transport of Na^+ from (tubule to EC) (EC to tubule) (IF to EC) (EC to IF) (capillary to IF) (IF to capillary) (can't tell from information given).

A-2. The ***net*** effect (*direct or indirect*) of using the diuretic would be to decrease Na^+ transport from (tubule to EC) (tubule to IF) (tubule to capillary) (capillary to tubule) (capillary to IF) (capillary to EC) (can't tell from information given). Explain briefly.

B. What effect should this diuretic have on the overall handling of glucose and Na^+?

B-1. This diuretic should significantly inhibit filtration of (glucose) (Na^+) (both) (neither) (can't predict).

B-2. This diuretic should significantly inhibit reabsorption of (glucose) (Na^+) (both) (neither) (can't predict).

B-3. This diuretic should significantly inhibit secretion of (glucose) (Na^+) (both) (neither) (can't predict). Explain briefly.

C-1. This diuretic is most likely to be effective in increasing water loss if it works on the (DL = descending loop of Henley) (AL = ascending loop of Henley) (distal tubule) (DL or AL) (AL or distal tubule) (DL or distal tubule) (any of these – it should be equally effective wherever it works).

C-2. If this diuretic works on the appropriate segment, it is most likely to mimic the effects of (a deficiency of aldosterone) (an excess of aldosterone) (neither) (can't predict – depends on mode of action of diuretic). Explain briefly.

12R-14. Ca^{++} is reabsorbed in the distal tubules of the kidney. Reabsorption in the DT is regulated by PTH (parathyroid hormone), and by vitamin D. (PTH is a peptide; Vit. D is lipid soluble.)
Ca^{++} enters the cells surrounding the tubule by diffusion and exits the cells by primary active transport.

A. In the luminal membrane of the DT cells, you would expect to find: (a Ca^{++} pump) (Ca^{++} channels) (a Ca^{++}/Na^+ exchanger) (a receptor for PTH) (a receptor for vitamin D) (none of these).

B. In the basolateral membrane of the DT cells you would expect to find: (a Ca^{++} pump) (Ca^{++} channels) (a Ca^{++}/Na^+ exchanger) (a receptor for PTH) (a receptor for vitamin D) (none of these).

For each part, circle all that apply. Then explain your choices briefly. A picture might help.

12R-15. PTH stimulates release of both Ca^{++} and phosphate from bone and it affects their reabsorption in the DT of the kidney. PTH stimulates reabsorption of Ca^{++} in the DT. PTH acts to control phosphate reabsorption by triggering removal of a Na^{+}/Phosphate co-transporter from the luminal surface of the DT.

A-1. ***Insertion*** of the co-transporter -- in the absence of PTH -- should occur by (exocytosis) (endocytosis) (a process that does not involve vesicles).

A-2. Name one other transport protein whose activity is controlled in a similar way:
The transporter _____ is controlled by (removal) (insertion) in the plasma membrane in response to the hormone:_____.
For A-2, fill in the blanks and circle either (removal) or (insertion). On the exam, no explanation was required for A, but you should be able to explain all your answers!

B. Suppose all the molecules of the Na^{+}/phosphate co-transporter are inserted in the membrane. (No PTH.) Under these conditions, when the co-transporter is maximally active, it can be saturated at the high end of the normal physiological phosphate concentration.

B-1.This means that when phosphate is in the high normal range in the plasma, under optimal conditions for transport, you expect to find phosphate is (reabsorbed) (filtered) (secreted) (in the urine) (none of these) (all of these).

B-2. Suppose conditions for transport are maximal, as described above in part B-1, and PTH is released from the parathyroid gland. Then you would expect to find more phosphate is (reabsorbed) (filtered) (secreted) (in the urine) (none of these – the added PTH would have no effect) (all of these). Circle all that apply for B-1 & B-2 and explain both parts briefly.

12R-16. It is possible to put a clip on one of the renal arteries so that blood flow to one kidney is reduced. This causes secretion of renin, which leads to a rise in arterial blood pressure within minutes. This is followed by a long term increase in blood volume (and blood pressure), even though the levels of renin decline to normal.

A-1. The very rapid rise in blood pressure is probably due to the direct action of (aldosterone) (angiotensin II)* (ADH acting on the kidney).

A-2. The rapid effect is probably due to (vasoconstriction) (water loss) (salt loss) (water gain) (salt gain).
Circle all that apply and explain your answers to A briefly.

B. The increase in blood volume is due primarily to salt retention.

B-1. You would expect salt retention in (the clamped kidney) (the other kidney) (both kidneys).

B-2. The original secretion of renin should be in (the clamped kidney) (the other kidney) (both kidneys).

B-3. The secretion of renin in this experiment is triggered by baroreceptors (in the kidney) (in the systemic arteries) (both) (either one). Explain your answers to B briefly.

* If the actions of this hormone have not been covered in class, look it up!

Problem Set #13 -- Questions on Immunology

13-1. Suppose an antibody is drawn in the usual way as a branched or Y shaped structure. It is drawn with the Y on its side, so the open part of the Y faces to the left. In other words, the "variable" branched end of the molecule is on the left and its "constant" or straight end is on the right. (Draw the picture before you go any further.) Now suppose the molecule is split in the middle (to the right of the branch point) into a "right" and "left" part.

A. Will both parts bind antigen?

B. If you have two antibody molecules on the surface of the same B cell, will the right parts be the same? The left parts?

C. If a single B cell can simultaneously (or sequentially) make both bound and secreted antibody of the same class, then the two antibodies must have the same (right part) (left part) (both) (neither).

D. You can split a molecule of antibody into three pieces. The procedure cuts the molecule right at the branch point of the "Y", so that it separates each branch from each other and both branches are split off from the straight or "constant" part. (This can actually be done because the "hinge region" in the middle of the molecule is more sensitive to proteases than the rest of the molecule.) The straight part is known as the F_c fragment and each of the two branches is called an F_{ab} fragment. If you add whole, regular, antibody to bacterial cells (+ complement), the cells will lyse. If you add any of the fragments described here (+ complement) to bacterial cells, the cells will not lyse, whether you add F_{ab}'s, F_c's or a mixture. F_{ab} fragments do bind bacteria, and F_c fragments do bind complement, but the bacteria do not lyse. Explain these results.

13-2. If RBC of type A and antibody to type A (anti A) are mixed, the cells aggregate into clumps visible to the naked eye. Ditto if you mix RBC of type B and antibody to type B. If RBC of type B are mixed with antibody to type A (or vice versa), the cells do not clump. This is the basis of the classic test for blood type.

A. Explain how this procedure works -- why does anti A cause type A, but not type B, cells to clump?

B. How do you use this procedure to tell all 4 blood types, AB, A, B, and O, apart?

C. Would F_{ab} fragments (see Q 1D) or F_c fragments combine with RBC? Would either of them cause RBC to clump (assuming the right antigen/antibody combination)?

13-3. A real antibody is made up of several peptide chains. There are 2 different kinds of chains, called H and L, and one molecule of antibody (of the gamma G type) contains 2 of each. There are two identical H chains (H = heavy or longer chains) and two identical L chains (L = light, or shorter chains). The chains are held together by disulfide bonds. (For a picture, see texts, class handout, or search Google Images for 'antibody structure'.) Membrane bound antibody (on the B cell surface) is anchored in the membrane by the carboxyl end of the H chains. Which of the following should definitely have signal sequences?
(newly made L chains) (H chains of secreted antibodies) (L chains of membrane bound antibodies)
(H chains of membrane bound antibodies) (L chains of secreted antibodies) (newly made H chains)
(none of these).

13-4. A cell is thought to be an immediate precursor of B cells. It looks like an ordinary cell, and its DNA is the same as that of the zygote, but if you put it in culture (in a bottle) under the right conditions, the cell will differentiate further and produce membrane bound antibody.

A. In culture, this cell (or a single descendent) should produce antibody to a maximum of (1) (2) (3) (4) (>4) different epitopes (antigenic regions), AND

B. The epitope(s) the antibody will respond to (are already determined before you put the cell in a bottle) (will depend on what antigen you put in the bottle) (will be determined at random after you put the cell in a bottle).

13-5. Helper T cells can be activated by macrophages (which engulf everything indiscriminately) or B cells (which only engulf antigens that stick to their receptors).

A. What properties do macrophages and B cells have in common that allows them both to activate T cells?

B. It has been suggested that macrophages probably do most of the T cell activating in the primary response but B cells do most of it in the secondary response. Why does this make sense?

13-6. Compare and contrast the properties of the 3 major types of lymphocytes (helper T's, cytotoxic T's and B's). Consider significant surface proteins, cells and/or proteins recognized, physiological function, and anything else that's relevant. (If it was covered in class, or you are interested, you can include the kinds of cytokines made and/or needed for activation.)

*13-7. It has been reported recently that the X-linked form of severe combined immunodeficiency disease (SCID) is caused by a lack of IL-2 receptors. (Note: all activated T cells in normal individuals have IL-2 receptors & require IL-2 stimulation.)

A. Why should people with this disease (like the "bubble boy" who had to live in a bubble to avoid infection) have few if any functional B cells **or** T cells?

B. Why did the bubble boy have to avoid both viral and bacterial infections?

*13-8. Suppose a person recovers normally from viral infections, but then gets the same viral infections over again, unlike the rest of us.

A. What is probably wrong -- B cells, helper T cells and/or cytotoxic T cells?

B. Should this person be at a higher risk of cancer than the rest of us?

C. Should this person be able to lyse bacterial cells as well as the rest of us?

D. Should this person tolerate a graft (from an unrelated person) better than you or me?

13-9. You have B cells, T cells and macrophages -- all cells are from the same organism and all are naive (never exposed to antigen). You put a single B cell in a culture bottle, and you want the B cell to **secrete** soluble antibody.

A. You need to add (helper T cells) (cytotoxic T cells) (macrophages) (both types of T cells) (helper T's and macrophages) (all of these) AND

B. Addition of antigen should (be helpful but not required) (be necessary) (have no effect) (be inhibitory) AND

C. Suppose you can selectively remove MHC molecules from the surface of the B cell. The B cell should not make secreted antibody if you remove (class I MHC) (class II MHC) (both types of MHC) (either type of MHC) (neither type of MHC is needed).

13-10. You have the following cells from a single individual: a naïve B cell, a naïve cytotoxic T cell, and a skin cell. All cells are fully differentiated, but never exposed to antigen. *For each part of this question, choose **one or more** answers, and explain briefly*.

A. Consider the T cell and the skin cell.

A-1. On the surface of the T cell you should find (antibody) (CD4) (CD8) (MHC I) (all of these) (none of these).

A-2. On the surface of the skin cell you should find _____. (Same choices for all parts of A.)

A-3. The DNA of the two cells should be different in the loci for (sections that code for) _____.

B. Consider the T cell and the B cell. The two cells will probably have ***the same*** DNA in the loci for (TCR) (Immunoglobulin heavy chain) (MHC II) (none of these) (all of these).

Problem 13-10, cont.

C. Suppose you get a second naïve B cell from the same individual. You compare the two B cells. What should be ***different***?

(i) DNA encoding the constant region of the H chain.
(ii) DNA encoding the variable region of the H chain.
(iii) The class(es) of antibody found on the cell surface.
(iv) The majority of epitopes bound to MHC-I.
(v) Helper T cells that will potentially be able to activate the B cell.
(vi) None of these.

D. Consider the original naïve B cell; suppose it has both IgM and IgD on its surface. Which of the following are correct?

(i) The IgM and Ig D were made using the same DNA.
(ii) The IgM and Ig D were made from the same mRNA.
(iii) The light chains of IgM and Ig D have the same amino acid sequence.
(iv) The heavy chains of IgM and Ig D have the same amino acid sequence.
(v) none of these.

E. Suppose the original naïve B cell binds antigen, and you can check out the cell both before and after it meets the antigen. Suppose you check out the cell before it meets antigen, and you check out the cell &/or its descendants (all making IgM), afterwards. Which of the following should change after the cell binds antigen?

(i) The Ag binding site of the IgM.
(ii) The mRNA used to make the IgM.
(iii) The DNA coding for the IgM.
(iv) The epitopes bound to MHC-I.
(v) The epitopes bound to MHC-II.
(vi) none of these.

13-11. Immunology Alphabet Soup. For each question, circle all answers that apply. Be sure you can explain why the answers you circled are correct and the others are not.

A. A naive or virgin B cell (not exposed to antigen) makes no (antibody) (TCR) (RNA) (MHCII) (MHCI) (CD4).

B. An APC involved in the humoral response must have on its surface (CD4) (MHC1) (MHCII) (CAVA) (TCR) (Ig).

C. An activated T_H cell should produce or contain (antibody) (TCR) (CD8) (EGF) (TGIF) **(IL-1) **(IL-2) AND receptors for (IL-1) (IL-2) (MHC I) (MHC II) (RSVP).

D. You are infected by a particular bacterium for the first time. To get an optimal specific immune response to the bacterial infection, which kind(s) of cell(s) do you need? (T_S) (T_C) (T_H) (TF) (TSH) (APC) (TLC) (A) (B) (C) (D) (F) (INC) (none of these), AND, during this response, you would expect to find antigen in the endosomes of _____ (same choices).

**Note: In case the role(s) of specific IL's were not discussed in class, here is the necessary info: Activated T_H cells make their own IL-2 (acts as autocrine); to be activated they need to get IL-1 (paracrine) from APC's.

*13-12. Infection by some viruses alters expression of MHC genes in the infected cells.

A. Which type(s) of MHC would you expect to be affected?

B. Would you expect MHC production to be increased or decreased?

13-13. Suppose an infected cell makes 2 different viral proteins that are not made in uninfected cells.

A-1. The number of different T cell binding sites on the infected cell surface should be (1) (2) (≥ 2) (>2) (can't predict), and

A-2. Each T cell that binds is likely to bind effectively to (1) (2) (>2) (can't predict – could be ≤2 or ≥ 2) of the different sites on the infected cell surface. Explain both parts of A.

B. Each activated T cell that binds to an infected cell should have on its surface (MHCII) (MHCI) (antibody) (T cell receptors) (IL2 receptors) (CD4) (CD8) (none of these). Circle one or more correct answers and explain.

*13-14. You are studying mutant mice which respond to antigens normally, except that the only type of antibody which is secreted is of the type called IgM (immunoglobulin of class M). Their response to a second exposure to antigen is faster and larger, but the secreted antibody is the same kind (IgM) in the primary & secondary response. These mice have a single genetic defect (only one gene and protein is defective).

A. Consider the function of the normal version of the gene which is defective in the mutant mice. The normal version of the gene probably codes for a product that stimulates (proliferation of B cells) (proliferation of T cells) (class switching) (switching from membrane bound to secreted immunoglobulins) (antigen presentation). Explain why you think the gene is or is not involved in each of these processes.

B. To find out what is wrong with the mutant mice, you irradiate them (which destroys their own B and T cells) and treat them by injecting either proteins made by normal lymphocytes or injecting lymphocytes from either normal or mutant mice. You then measure their immune response and get the following results:

Treatment of irradiated mice	Immune response
None	Defective
Proteins made by B or T cells	Defective
B cells of normal + T cells of mutant	Defective
B cells of normal + T cells of mutant + Secreted or soluble proteins made by normal B or T cells	Defective
B cells of mutant + T cells of normal	Normal
B cells of mutant + T cells of normal + Secreted or soluble proteins made by normal B or T cells	Normal

B-1. Given all the information so far, the mutant mice probably have a defective protein made by their (B cells) (helper T cells) (cytotoxic T cells) (either B or helper T's, but can't tell which) (either type of T's, but can't tell which) (none of these) AND

B-2. It is likely that the protein is (a cytokine = a paracrine) (a surface protein that acts as a receptor) (a surface protein that acts as a ligand) (either a cytokine or its receptor) (a surface protein that acts either as a receptor or a ligand) (any of these) (beats me). Explain your answers to B-1 & B-2.

C. Suppose a normal mouse is infected with a virus that attacks liver cells.

C-1. Infected cells should carry viral antigens in association with (MHC I) (MHC II) (both) (neither) and the infected cells will probably be killed by contact with (complement) (helper T's) (cytotoxic T's) (either complement or cytotoxic T's) (either type of T cells) (none of these -- something else destroys infected cells) (none of these -- the mice should not be able to destroy infected liver cells), AND

C-2. In a mutant mouse the results will probably be (the same) (different) (can't predict). Explain your answers to C-1 and C-2.

*13-15. Consider cells that express both MHC I and MHC II (both are on the same cells). You incubate these cells with influenza virus (flu). You use two types of flu – normal, infectious virus and UV inactivated virus. (UV inactivated virus can't replicate, but has the normal structure and antigenic properties.) During some infections you add emetine (an inhibitor of protein synthesis) or chloroquine (an inhibitor of the acidification of endosomes & lysosomes). After each infection you check to see which type(s) of MHC display the virus epitopes. A table of the results is below. Researchers actually did experiments (1) to (4) to figure out how epitopes reach the cell surface. They didn't actually do experiment (5) -- you are asked to predict what would happen if they had done it.

For each part circle all answers that apply and explain briefly if asked.

A. What kind of cells could have been used here? The cells exposed to flu could have been (liver) (brain) (lymphocytes) (kidney) (RBC) (macrophages) (any of these).

B. There are rare cytotoxic T cells that bind to and kill only cells with antigen bound to MHC II. (These rare cells do not act as helper cells.) Both ordinary cytotoxic T cells and some of the rare cytotoxic T cells were used in this experiment.

B-1. You would expect the rare cytotoxic T cells to produce (CD4) (CD8) (perforin) (complement) (TCR) (MHC I).

B-2. You could use either the rare cytotoxic T cells or the common ones to lyse the cells in experiment(s) (1) (2) (3) (4).

B-3. Only the rare cytotoxic T cells could lyse the cells in experiment(s) (1) (2) (3) (4).

Explain briefly why the two types of cytotoxic T cells behave differently.

C. Fill in the blanks in the table for experiment (5), and explain briefly.

D. Suppose all the cytotoxic T cells in this experiment, both the rare ones and the common ones, come from one animal. The animal is highly inbred (homozygous). You take a sample of the T cells, isolate their DNA, and examine the DNA. You look at the region of the DNA that codes for CD4 (region 1), or at the region of the DNA that codes for the variable part of the TCR (region 2).

D-1. You compare region 1 DNA from rare and common cytotoxic T's. The base sequences in the two DNA samples should be (the same) (entirely different) (overlapping).
Overlapping means some sequences should be the same and some should be different. Explain briefly.

D-2. Suppose you take a large sample of (common) cytotoxic T's from an animal that has been immunized to flu. You isolate region 2 DNA from the cells. The DNA you get should be
(a mixture of sequences – some, but not all, molecules in the mix should be different)
(a pure sample – all the DNA sequences should be the same)
(a mixture – no two DNA molecules in the sample should be the same)
(a mixture – but I'm not sure exactly what type). Explain briefly.

Results of Experiments

Virus used	Flu epitopes with MHC I?	Flu epitopes with MHC II?
(1) Infectious virus	yes	yes
(2) UV inactivated virus	no	yes
(3) Infectious virus + emetine	no	yes
(4) Infectious virus + chloroquine	yes	no
(5) UV inactivated virus + chloroquine	?	?

*13-16. Type I diabetes is caused by destruction of the beta cells of the pancreas, the cells that produce insulin. In most cases, the beta cells are destroyed by an immune response. Both genetic and environmental factors are involved in triggering the immune response.

A. The immune system usually does NOT destroy the beta cells. If the cells are destroyed, it is a failure of (memory) (specificity) (tolerance) (any one of these) (none of these).

B. NOD mice develop a disease very much like type I diabetes. (NOD mouse = non-obese diabetic mouse). If you transfer plasma from a NOD mouse to a normal mouse, the normal is not affected. If you transfer lymphocytes (= WBC = white blood cells) from a NOD mouse, the normal mouse develops diabetes. The first people to do this found they had to transfer two types of WBC to transfer diabetes. After some experimentation, they found that under the right conditions, they only had to transfer one type of cell to cause the disease in the recipient.

B-1. Consider the one type of WBC that can cause diabetes by itself. Which type is it likely to be? (B cells) (helper T's) (cytotoxic T's) (either type of T cells) (any of these) (none of these)

B-2. What is the second type of WBC that was needed in the earlier experiments? It is probably (B cells) (helper T's) (cytotoxic T's) (either type of T cells) (any of these) (none of these).

B-3. The first type of WBC should make (CD4) (CD8) (TCR) (MHC I) (MHC II) (antibody) (none of these).

C. You have two identical twin patients. One develops diabetes type I, and the other doesn't. If you transplant a piece of pancreas from the normal twin to the twin with diabetes, what do you expect to happen? (the donor will develop diabetes) (the recipient will be cured)
(the recipient may get better temporarily, but will not be cured)
(the recipient may or may not be cured) (I'm clueless). Explain your prediction briefly.

Problem Set #14 – Development (Heicklen Questions)

14-1. Look at the following chart.

A. Put in a '+' or '-' indicating that K4 or K27 is methylated (+) or not (-).

B. Explain how you knew where to put the pluses and minuses.

	Methyl	ES_{cells}	Lens*	Heart	Blood	Mesoderm
Pax6 Lens*	K4					
	K27					
Nkx2.5 Heart	K4					
	K27					
GATA1 Blood	K4					
	K27					
Oct4 ES cells	K4					
	K27					
Bry Mesoderm	K4					
	K27					

*Note: Lens develops from ectoderm.
The tissue each MRG regulates is listed below the MRG.

14-2. An article in the *N.Y. Times* says "A complex known as polycomb… tags spools at a site called K27. This is a signal for another set of proteins to make the spools wrap DNA tight and keep it inaccessible. Another complex tags spools at their K4 site, which has the opposite effect…."

A. A-1. What does putting 'a tag on a spool' mean in this article? It refers to modifying: (protein) (RNA) (DNA) (splicing factors) (transcription factors) (histones) (hormone receptors) (nucleosomes) (regulatory regions) (any of these) (none of these).

A-2. What is the K4 site? It refers to the 4th (base) (amino acid) (codon) (transmembrane segment) (peptide chain) (domain) (protein) (intron) (exon) (variable region).

B. The gene Sry is a master regulator on the Y chromosome for testis.

B-1. In ES cells, which site in the region of the Sry gene would be tagged? (K27) (K4) (either one) (neither) (both).

B-2. Expression of Sry should turn on the gene regulatory network for which of the following cell types? (Sertoli) (thecal) (granulosa) (myocytes) (none of these).

Note: Sertoli cells are in testis; Thecal and granulosa cells are in ovaries; Myocytes are muscle cells of heart

Problem14- 2, cont.
For C-E below, choose an answer for each part, and explain briefly how you predicted the state of the chromatin. Explain case C first, and then why the others are, or are not, the same.

C. Consider the myocytes of a male.

C-1. In these cells, which sites in the region of the Sry gene would be tagged? (K27) (K4) (either one) (neither) (both).

C-2. In these cells, what would be the best description of the chromatin structure in the region of the Sry gene during interphase? (relatively loose euchromatin) (relatively tight euchromatin) (heterochromatin) (either state of euchromatin) (any of these) (none of these).

D. Consider developing Sertoli cells.

D-1. In these cells, which sites in the region of the Sry gene would be tagged? (K27) (K4) (either one) (neither) (both).

D-2. In these cells, what would be the best description of the chromatin structure in the region of the Sry gene during interphase? (relatively loose euchromatin) (relatively tight euchromatin) (heterochromatin) (either state of euchromatin) (any of these) (none of these).

E. Consider developing thecal cells.

E-1. In these cells, which sites in the region of the Sry gene would be tagged? (K27) (K4) (either one) (neither) (both).

E-2. In these cells, what would be the best description of the chromatin structure in the region of the Sry gene during interphase? (relatively loose euchromatin) (relatively tight euchromatin) (heterochromatin) (either state of euchromatin) (any of these) (none of these).

14-3. You have 2 DNA samples from two individuals. How can you distinguish between identical twins & two cloned animals derived from SCNT (such as the sheep Dolly and her sisters)?

A1. If the two individuals (whose DNA you are testing) are actually identical twins, & not derived from somatic nuclear transfer (SCNT), which of the following would be the same as the DNA of the biological mother and so <u>distinguish</u> between the two cases?
(all of the nuclear DNA) (mitochondrial DNA) (all of the DNA) (none of these).

A2. If the two individuals (whose DNA you are testing) are actually identical twins, & not derived from somatic nuclear transfer (SCNT), which of the following would <u>not</u> be the same as the DNA of the biological mother and <u>distinguish</u> between the two?
(all of the nuclear DNA) (mitochondrial DNA) (all of the DNA) (none of these).

B. If the samples are actually derived from somatic nuclear transfer experiments, which of the following should be the same as the DNA of the egg donor (=nuclear recipient)?
(all of the nuclear DNA) (mitochondrial DNA) (all of the DNA) (none of these)

C. If the samples are actually derived from somatic nuclear transfer experiments, which of the following should be the same as the DNA of the nuclear donor?
(all of the nuclear DNA) (mitochondrial DNA) (all of the DNA) (none of these)

14-4. Gene transcription is regulated to control the synthesis of specific proteins, and thus confer cells with a specific cell fate.

A. One method of transcriptional regulation during cell fate determination involves transcription factors. These TF's (are receptors) (act in the cytosol) (act in the nucleus) (act in the endoplasmic reticulum) (are located in the endosomes) (are lipids) (are proteins that bind directly to the DNA) (are none of the above)

B. A second method of transcriptional regulation during cell fate determination is methylation of the regulatory regions of genes. What is being methylated in this method? (proteins) (DNA) (receptors) (kinases) (none of the above)

C. A third method of transcriptional regulation during cell fate determination is histone methylation. What is being methylated in this method? (proteins) (DNA) (receptors) (kinases) (none of the above).

14-5. The mammary gland develops a tree-shaped network of tubes consisting of an outer layer of myoepithelium, and an inner layer of luminal epithelium (LE), lining the tubes. GATA-3 is a master regulatory gene (MRG) for the LE of the mammary gland. Expression of the GATA-3 protein induces progenitor cells to differentiate into the luminal epithelial cells of the mammary gland.

A. The primary effect of GATA-3 on gene expression in the LE is at what level? (transcriptional) (post-transcriptional) (translational) (post-translational) (none of the above).

B. During the differentiation process from mammary gland progenitors to LE, the regulatory regions of the MRGs that are not expressed in LE should have (increased methylation) (decreased methylation) (increased phosphorylation) (decreased phosphorylation) (no modifications). Explain A & B briefly.

C. Consider the basal promoter of the GATA-3 locus in differentiated luminal epithelial cells. In these cells, the GATA-3 locus should be wrapped around nucleosomes containing histone 3 with (K4 methylation) (K27 methylation) (neither) (both).

D. Consider the Oct-4 locus in differentiated luminal epithelial cells. In these cells, the Oct-4 locus should be wrapped around nucleosomes containing histone 3 with (K4 methylation) (K27 methylation) (neither) (both). Explain C & D briefly.

E. Decreased GATA-3 expression is frequently observed in breast cancer. Which of the following explain(s) this observation? In normal cells GATA-3 (drives differentiation) (drives proliferation) (triggers cell migration) (triggers apoptosis) (does none of these) (does all of these).

F. The normal version of GATA-3 is best described as a (oncogene) (tumor suppressor) (both) (neither). Explain E and F briefly.

14-6. Females diagnosed with breast cancer frequently must have a breast (or both) removed.

A. Doctors would like to develop embryonic stem cells that could replace the removed mammary tissue. (So implants would be unnecessary.) Researchers would start by trying to drive embryonic stem cells in a dish to become luminal epithelial cells of the mammary gland. See intro to problem 14-5. How should researchers try to change the gene expression pattern of the embryonic stem cells? They should try to (decrease GATA-3 expression) (increase Oct-4 expression) (increase GATA-3 expression) (increase Pax6 expression). Explain your answer briefly.

B. Suppose you are working on this project, and you want to decrease the complications of immune rejection when using the mammary luminal epithelial cells derived from ES cells. Therefore, you perform somatic nuclear transfer to generate the ES cells. Where will you get the nucleus for somatic nuclear transfer? Note: Assume breast cancer is caused by a somatic mutation in the cells of the breast, and the mutation has not occurred in, or spread to, other regions. *(Q cont. on next page.)*

Problem14-6, cont.

B-1. You would get the best results if you use a nucleus from a cell of: (the woman) (her sister) (the woman or her sister) (an unrelated woman) (any of these), AND

B-2. Assuming you pick the right person, you would get the best results if you derive the nucleus from: (a differentiated cell from the mammary gland) (an unfertilized egg) (a differentiated cell that is not part of the mammary gland) (either type of differentiated cell) (a cell from the 8 cell stage of an embryo created by *in vitro* fertilization of an egg from that person).

14-7. In 2008, scientists figured out how to reprogram fully differentiated skin fibroblasts to increase their developmental potential. These cells are referred to as induced pluripotent stem cells or iPS cells. These iPS cells are considered equivalent in their potential to the inner cell mass of a developing embryo. The reprogramming of skin cells is accomplished by adding the DNA coding for four proteins so that these four proteins are expressed in the iPS cells.

A1. Fully differentiated skin fibroblast cells under normal conditions are best described as: (totipotent) (pluripotent) (multipotent) (bipotent) (unipotent).

A2. When the skin cells have been reprogrammed to become iPS, the resulting iPS cells are best described as: (totipotent) (pluripotent) (multipotent) (bipotent) (unipotent).

A3. Once iPS cells are created, they can be induced to become various tissues. If iPS cells are induced to become fully functional neurons, they are (totipotent) (pluripotent) (multipotent) (unipotent) (differentiated) (none of these).

B. The four genes that are added to the iPS cells code for transcription factors.

B1. (i) These transcription factors ***directly*** affect (secretion) (levels of protein synthesis) (levels of RNA synthesis) (levels of DNA synthesis) (ion diffusion) (oxygen uptake), AND

(ii) These transcription factors probably work in (the cytoplasm) (the nucleus) (neither) (both) (either one).

B2. (i). The DNA that codes for these transcription factors should be transcribed in (the nucleus) (the cytoplasm) (the nucleolus) (none of these) (can't predict), AND

ii). The RNA that codes for these transcription factors should be translated on ribosomes that are (in the nucleus) (free in the cytoplasm) (attached to the outside of the endoplasmic reticulum) (inside the ER) (in the Golgi) (none of these) (can't predict).

C. One of the transcription factors expressed in the iPS cells is the Oct4 protein.

C1. The Oct4 gene is the master regulatory gene of what cell type?
(blastocyst) (trophoblast) (inner cell mass) (heart) (embryonic stem cells) (mesoderm)

C2. Which of the following would you expect to express Oct4 in normal development?
(sertoli cells) (granulosa cells) (cells at the 4-cell stage) (embryonic stem cells) (mesoderm)

D. What pattern of methylation would you expect to observe on the histones?

D-1. In fully differentiated fibroblast skin cells, in the region of DNA containing the Oct4 gene, what pattern of histone methylation would you expect? (K4 methylation) (K27 methylation) (K4 & K27 methylation) (neither K4 or K27 is methylated) (can't tell)

D2. In iPS cells, in the region of DNA containing the Oct4 gene, what pattern of histone methylation would you expect? _____ Same choices as in D-1.

D3. In iPS cells, in the region of the DNA containing the Nkx2.5 gene region, what pattern of histone methylation would you expect? _____ Same choices as in D-1. (Nkx2.5 is the master regulatory gene for heart muscle.)

E. In an iPS cell, which DNA matches the DNA of the donor of the skin cell? (mitochondrial) (nuclear) (nuclear and mitochondrial) (most of the nuclear DNA) (most of the mitochondrial) (can't tell) (none of the DNA).

14-8. A. Suppose that an infant is born with testes, the organs that result from the male but not the female duct, normal testosterone levels, but external female genitals due to a lack of the enzyme necessary to convert testosterone into DHT. What will this infant's primordial germs cells most likely form? (sertoli cells) (granulosa cells) (sperm) (eggs) (DHT induced derivatives) (none of the above)

B. This individual would be a (hermaphrodite) (pseudohermaphrodite), AND probably (XX) (XY) (XXY) (none of the above).

14-9. A. The first clinical trials of human embryonic stem cells are being done to help individuals with vision loss. The embryonic stem cells are first differentiated into cells that help maintain the retina, pigmented retina epithelial (PRE) cells. The PRE cells are then injected into patients with various types of retinal deterioration. First, put the following choices in order, from highest to lowest potency. Then, which cell types/potency levels would be required for cell fate determination to occur during the development of ES cells into PRE cells? List all choices in order from most potent to least potent, and circle the ones required for proper fate determination. Then, explain your ranking & choices.
Choices: (totipotent) (eye precursor) (germ layer) (pluripotent) (unipotent) (8 cell) (gamete).

B. What does H3K4 stand for? (4^{th} protein of histone 3) (4^{th} regulatory region of gene for histone 3) (4^{th} Lysine of histone 3) (4^{th} amino acid of histone 3) (4^{th} nucleotide of histone 3) (4^{th} potassium of histone 3).

C. Which of the following sites are known to be methylated on the histones that wrap the DNA that codes for the master regulator of PRE in <u>ES cells</u>? (H3K4) (H3K27) (H3K9) (CG sequences) (none of the above)

D. Which of the following sites are known to be methylated on the histones that wrap the DNA that codes for the master regulator of PRE in <u>liver cells</u>? (H3K4) (H3K27) (H3K9) (CG sequences) (none of the above).

E. Which of the following sites are known to be methylated on the histones that wrap the DNA codes for the master regulator of PRE in <u>PRE cells</u>? (H3K4) (H3K27) (H3K9) (CG sequences) (none of the above).

14-10. The cell differentiation cascade during normal development progresses from one cell with the potential to become any cell type (high potency) to fully differentiated cells (low potency) forming all of the human cell types.

A. Which of the following progresses in the opposite direction, becoming more potent? (mesoderm to heart) (inner cell mass to ectoderm) (creation of zygote using SCNT) (differentiation of iPS cells into neurons) (separation of trophoblast and inner cell mass) (iPS cell creation) (none of these).

B. Which of the following changes increases potency and occur in the natural process of human development? (mesoderm to heart) (inner cell mass to ectoderm) (creation of zygote using SCNT) (iPS cell creation) (differentiation of iPS cells into neurons) (separation of trophoblast and inner cell mass) (none of these). Explain A & B.

14-11. A. The sex of an individual that competed in the Olympics as a woman was contested. If this person was found to possess Wolffian duct derivatives, what could you expect regarding the fetal development of this individual considering what is known regarding the process of sex determination? (ovaries are present) (testes are present) (male levels of testosterone) (female levels of testosterone) (male levels of 5 α–reductase) (male levels of AMF) (female levels of 5α –reductase) (female levels of AMF) (penis would be present) (clitoris would be present).

B. What could you determine regarding this individual's chromosomes and the expression of genes on those chromosomes? (XX individual) (XY individual) (Sry expressed) (testosterone receptor expressed) (AMF receptor expressed).

C. If this individual had both Mullerian and Wollfian ducts what could you conclude? (granulosa cells function) (granulosa cells aren't functioning) (Leydig cells function) (Leydig cells aren't functioning) (thecal cells are functioning) (thecal cells aren't functioning) (Sertoli cells are functioning) (Sertoli cells aren't functioning) (none of these).

14-12. A. Recently it was found that H3 K9 modifications work like a switch. Methylation of H3 K9 decreases transcription. How would this most likely affect the chromatin in the region wrapped around the nucleosome with H3 K9 methylation? (tightens chromatin) (loosens chromatin) (increases TF accessibility to DNA) (decreases TF accessibility to DNA).

B. In addition, H3 K9 Acetylation increases transcription. How would this most likely affect the chromatin in the region wrapped around the nucleosome with H3 K9 acetylation? (tightens chromatin) (loosens chromatin) (increases TF accessibility to DNA) (decreases TF accessibility to DNA).

14-13. A. The sex chromosomes directly determine the fate of which reproductive organ(s)? (Mullerian ducts) (Wollfian Ducts) (gonads) (external female genitals) (external male genitals) (none of these).

B. Which of these reproductive organs are bipotential? (external genitalia) (gonads) (Mullerian ducts) (Wollfian ducts) (primordial germ cells) (none of these).

C. In the androgen insensitivity case study the gonads of an XY individual (Terry) were removed at birth. Terry developed testes and external female genitals but did not maintain either a Mullerian or a Wollfian duct. How would the reproductive organs of the individual with the same genotype be different if the gonad was removed after 6 weeks of embryonic development, before the gonad has differentiated into testes? (sperm develops) (eggs develop) (loss of female external genitals) (Mullerian duct remains) (Wollfian duct remains) (male genitals develop) (no male patterned baldness) (none of these features change).

Problem Set 15 -- Cancer & Regulation of the Cell Cycle

Part I. All the questions in this part (15-1 to 15-7) refer to the following list of genes:

p53
rb (= rbl = retinoblastoma gene = gene for rb protein, which is an inhibitor of transcription by E2F)
sis = gene for growth factor
erb-B = gene for growth factor receptor
gene for G1/S cyclin
ras = gene for a (monomeric) G protein stimulated by growth factors (mitogens); activated ras protein triggers a cascade of kinases.

All these genes (except the gene for cyclin) were discovered because they were mutated or abnormally expressed in cancer cells.

15-1. Which of these genes (in their normal form) would be considered proto-oncogenes and which would be considered tumor suppressors?

15-2. Mutations in some of these are considered "dominant" in that one abnormal copy (per cell) is sufficient to cause (or contribute to) uncontrolled growth of that cell; others are considered "recessive" in that both copies in the cell must be deleted or inactivated to cause loss of growth control. Which genes should be dominant in this sense (when mutant) and which should be recessive?

15-3. How could rearrangement (translocation) of sis cause loss of growth control if the coding region of the gene is unaffected?

15-4. How could a mutation in the coding region of ras or erb-B cause loss of growth control?

15-5. Mutations in rb and p53 are inherited as dominant mutations, that is, heterozygotes who inherit one defective copy of either gene almost always get cancer. Given your answers to question 2, why should this be so?

15-6. What should be the phenotype of a cell carrying a mutation that inactivated one of these genes (Assume both copies of "recessive" genes are inactive)? Will cells be blocked in the cell cycle? Grow uncontrollably? Something else?

15-7. Many cancers have mutations in more than one of these (or similar) genes. How could this happen? How did a single clone of tumor cells acquire so many different and useful (to it) growth promoting mutations?

Part II -- Old Exam Questions

15-8. Circle all choices that apply, and explain briefly at the end.

A. If growth factors are missing, most mammalian cells arrest (just before they enter M) (in G1) (in G2) (in S) (in M) (in G1 just before they enter S).

B. For the cells to start growing again, the first thing to be activated is (CDK) (E2F) (TK linked receptor) (G protein linked receptor) (either type of receptor) (p53).

C. If the DNA of the cell is damaged, which of the following will be activated – or increase in amount? (CDK) (E2F) (p53) (MAP kinases) (none of these).

D. Which of the following will be degraded almost immediately after the cell resumes the cell cycle? (CDK) (rb) (cyclin) (E2F) (ras) (none of these).

Explain briefly how the choices you picked are related. (On the exam you didn't have to explain any of the other items or steps in the cycle.)

15-9. Suppose that protein Z is made in all dividing cells. Synthesis of Z mRNA increases at the beginning of S and decreases after S. Degradation of Z mRNA increases just after S. Therefore the level of Z mRNA in S is about 50X the level during the rest of the cell cycle. Consider the DNA that codes for Z mRNA (Z DNA). Assume Z DNA has no introns, just to keep it simple.

A-1. Regulation of protein Z synthesis is probably best described as occurring at the level of (gene amplification) (transcription) (RNA processing/transport) (translation). Circle as many as apply. Note: regulation of translation = regulation of initiation of translation and/or regulation of stability of mRNA.

A-2. Z DNA is most likely to code for (a cyclin) (a histone) (a nuclear pore protein) (a lamin) (the protein kinase subunit of MPF) (either subunit of MPF) (a cyclin or a histone) (any of these).

B-1. In dividing cells, Z DNA is probably euchromatic (in M) (in S) (in all of interphase) (in G1 and G2) (throughout the cell cycle), AND

B-2. Z DNA is probably most sensitive to DNase (in M) (in S) (in all of interphase) (in G1 and G2) (throughout the cell cycle), AND

B-3. The transcribed region of Z DNA should be in nucleosomes (in M) (in S) (in all of interphase) (in G1 and G2) (throughout the cell cycle).

C. You have a mutant that doesn't make the usual amount of protein Z during S. The rate of Z mRNA synthesis in S is normal, but the rate of Z protein synthesis is less than normal. You discover that your mutant makes a regulatory protein that has one amino acid change -- a tyrosine is switched to phenylalanine. The normal regulatory protein (with tyr) can be phosphorylated by the usual protein kinases, but the mutant protein (with phe) cannot. What is the job of the normal version of the regulatory protein described here? Given all the data so far, the most likely explanation is that:

C-1. The normal version of the regulatory protein binds to (Z protein) (Z DNA) (Z mRNA) (more than one of these) (Z DNA or mRNA) AND

C-2. The binding regulates (transcription) (RNA splicing) (translation) (more than one of these) (transcription or translation) AND

C-3. Phosphorylation of the regulatory protein normally increases (Z DNA transcription) (Z RNA splicing) (initiation of translation of Z mRNA) (stability of Z mRNA) (degradation of Z mRNA) (initiation of translation or stability of Z mRNA) (initiation or degradation of ZmRNA) (any of these).

15-10. It has been reported that mutations in the cyclin genes can cause (or contribute to) uncontrolled growth (i.e. cancer) and it is assumed that mutations in the cyclin dependent kinase (CDK) genes will do the same.

A. Which of the following is (are) likely to convert a normal CDK gene into an oncogene?

____ A rearrangement that puts the CDK gene next to a very active enhancer.
____ A base change that causes the CDK to become resistant to degradation.
____ A base change that causes permanent inactivation of the CDK.
____ A base change that causes permanent activation of the CDK.
____ A regulatory mutation that causes synthesis of the CDK throughout the cell cycle.
____ None of these.

B. A normal CDK gene should be considered a (proto-oncogene) (tumor suppressor gene) (either one -- depends on the type of mutation) (neither). Explain your reasoning.

*15-11. Smooth muscle cells lining the blood vessels usually do not divide. However, *in vitro*, they can be stimulated to divide by addition of various growth factors.

A. Suppose you add growth factors to normal, nondividing, smooth muscle cells. What should happen to each of the following? Fill in each blank with "Increase," "Decrease," or "Stay the same."

_____ Level of G1/S cyclin/cell.
_____ Level of G1/S CDK (kinase) protein/cell
_____ Assembly of spindle
_____ Incorporation of thymine (T) into macromolecules
_____ Binding of transcription factors to the Rb protein
_____ Synthesis of histones
_____ Phosphorylation of Rb protein

Consider the items/events on this list that will change. Put them in order and explain briefly.

Problem 15-11, cont.

B. Using recombinant DNA techniques, it is possible to insert a gene coding for a mutant version of the Rb protein into smooth muscle cells. The mutant protein cannot be phosphorylated. The hope is that cells carrying the mutant gene will not respond to growth factors produced in response to injury and so the cells will not form the thickenings that narrow the arteries and cause heart disease. In which of the following cases should the mutant Rb gene work to prevent growth?

____ if the normal Rb gene is knocked out
____ if the mutant Rb gene is expressed at very high levels
____ if both the normal Rb gene and the mutant one are both expressed at equivalent levels
____ in none of these cases -- this is a crackpot idea that will never work

15-12 . Growth factor receptors often activate MAP-Kinases. The order of proteins is
GF → tyrosine kinase → ras → Kinase 1 → Kinase 2 → MAPKinase → Transcription factors.
(Assume each protein activates the next.) Once the TF's are activated, the appropriate genes are transcribed, and the cell makes proteins (such as cyclins) needed to enter S or M. The gene for kinase 2 would be best described as a (oncogene) (proto-oncogene) (tumor suppressor gene) (blue gene) AND if a mutation affecting kinase 2 causes cancer, the mutation is most likely to cause (inactivation of kinase) (permanent activation of kinase) (overproduction of kinase) (production of abnormally low levels of kinase) (beats me).

15-13. You are working in a laboratory on an independent study project. You have some epithelial cells from a human growing in a bottle. These cells normally divide throughout life. Before spring break, you replaced the medium (fluid surrounding the cells) with fresh medium and forgot to include any growth factors. Then you went home for spring break.

A-1. Now that you have come back after vacation, you will probably find that the cells have (grown normally) (arrested at G1/S) (arrested at S/G2) (arrested at G2/M) (grown much more than usual) AND

A-2. When you add the missing growth factor to the medium, the cells will probably start to synthesize (p53) (growth factor receptor) (MPF) (cyclin) (cdk = cyclin dependent kinase) (none of these).

B. Ink proteins bind to and inhibit one of the cdk's in the cell cycle. (Ink = INhibitor of Kinase.) Many cancer cells have mutations in the INK gene(s). (For clarity, the protein name is given in lower case and the gene name in upper case.) Some cancer cells have normal INK genes but have mutation(s) in the corresponding CDK gene(s) that eliminate cdk-ink protein binding. (Other aspects of cdk function are normal.) Given this information, what would you expect?

B-1. Levels of ink activity in tumor cells with mutations in the INK gene should be (normal) (above normal) (below normal) (can't predict).

B-2. Levels of ink activity in tumor cells with mutations in the CDK gene should be (normal) (above normal) (below normal) (can't predict).

C. Suppose you add growth factor to your cells, as in part A, but the ink protein that binds the "start" cdk is activated. (The ink's are for real, but what really regulates the ink's is not known.) Then your cells should (grow normally) (fail to grow) (grow uncontrollably) (can't predict).

D. What is the best term for a normal INK gene? (tumor suppressor) (inactive tumor suppressor) (oncogene) (proto-oncogene) (oncogene or tumor suppressor) (beats me).

E. Suppose you have cells that have permanently active ink protein as in C. The cells also have permanently active E2F or permanently active ras. (Consult texts or handouts for roles of E2F and ras.)

E-1. If your cells have active E2F and active ink, then your cells should (grow normally) (fail to grow) (grow uncontrollably) (can't predict).

E-2. If your cells have active ras and active ink, then your cells should (grow normally) (fail to grow) (grow uncontrollably) (can't predict).

15-14. Cells from the neural crest form melanocytes (pigment producing cells) as well as parts of the nervous system. A protein called "kit" that controls migration of melanocytes was discovered twice. It was discovered the first time because it is carried by a virus that causes cancer in cats; without the kit (kitten?) gene, the virus no longer causes tumors. Kit was discovered the second time because animals (and people) with mutations in the kit gene have a lack of normal pigmentation. In these kit mutants, the melanocytes do not function properly. The kit protein is a receptor tyrosine kinase. The receptor must bind its ligand for the melanocytes to proliferate (grow and divide) and for them to find their way to their final positions.

A. The normal version of the kit gene is best described as
(a proto-oncogene) (an oncogene) (a tumor suppressor) (none of these) (any of these).

B. Cells infected by the cancer causing virus probably produce (unusually low amounts of functional kit protein) (unusually high amounts of normal kit protein) (an altered version of kit protein) (high amounts of normal protein or altered protein) (low amounts of normal protein or altered protein) (none of these could explain the results)
(any of these).

C. The mutant version of the kit gene that causes abnormal pigmentation probably produces _____. (Same choices as in part B.) Explain your answers to each part.

*15-15. CDC 25 (cell division cycle #25) is a yeast gene that has been extensively studied. If all copies of the gene in a cell are inactivated, the cell arrests the next time it comes to the G1/S boundary. Human cells have a similar gene (human CDC 25) whose function has not yet been extensively examined. Researchers have just started to investigate the human gene because they suspect that alterations in the human gene may play a role in some cancers.

A. It turns out that many human cancer cells have mutations that affect the structure or regulation of the CDC25 gene(s). From the information given so far, the normal (unaltered) human CDC 25 gene is best classified as a (tumor supressor gene) (oncogene) (proto-oncogene) (tumor supressor or oncogene) (oncogene or proto-oncogene) (tumor suppressor or proto-oncogene) (any of these) (none of these). Explain.

B. From the information given so far, plus your general knowledge of how the cell cycle is regulated, which of the following could be coded for by CDC 25?

(1) ___enzyme that degrades a cyclin
(2) ___enzyme that degrades a CDK
(3) ___transcription factor that is required to transcribe a cyclin gene
(4) ___transcription factor required to transcribe a CDK gene
(5) ___enzyme that inactivates CDK
(6)___enzyme that activates a CDK

Put a L (likely) or U(unlikely) in each blank, and explain. First explain the general principle of how cyclins and CDK's work, and then explain (in one or two sentences) why each case is or is not likely.

*15-16. *BRCA-1* is a tumor suppressor gene that causes (or contributes to) breast cancer.

A. One goup reported that the protein encoded by the gene is mislocated in cancer cells -- the protein is found in the nucleus in normal cells but in the cytoplasm in cancer cells. (This finding is not necessarily correct, but answer the question assuming that it is.) Given this result, and the fact that the gene is a tumor suppressor, which of the following are likely roles for the normal protein (BRCA-1) made by the *BRCA-1* gene*?

(1). Transcription factor required for synthesis of a growth factor.
(2). Initiation factor required for translation of growth factor mRNA.
(3). Splicing factor required for production of mRNA for growth factor receptor.
(4). Inhibitor of DNA synthesis; inhibitor is activated by p53.
(5). Inhibitor of degradation of cyclin mRNA.
(6). None of these are reasonable.

Circle all reasonable choice(s) and explain briefly why each choice is or is not reasonable.

*Note: The standard convention for human genes/proteins is to write the gene name in italics (*BRCA-1*) and the protein name without italics (BRCA-1). The convention for different organisms is different.

Problem 15-16, cont.

B. It has been reported more recently by a different group of scientists that some sections of the BRCA-1 protein are similar to that of several secreted proteins and that the protein contains no transmembrane sequences. If this result is taken at face value, and the result in A is ignored, then which of the following are likely roles for the normal protein made by the *BRCA-1* tumor suppressor gene?

(1). Growth factor (stimulatory) (2). Receptor for growth factor (stim. or inhib.)
(3). Inhibitory growth factor (4). (1) or (2)
(5). (2) or (3) (6). (1) or (3)

AND if *BRCA-1* codes for a factor (or a receptor for it), the factor is probably (endocrine) (exocrine) (autocrine) (endocrine or exocrine) (endocrine or autocrine) (exocrine or autocrine) (any of these). Remember that cancer cells develop in the presence of large numbers of normal cells.

C. A third report says that in normal cells, the protein encoded by *BRCA-1* is found in the Golgi as well as in other parts of the cell. (This group used a different method of localization than the group described in A.) This is consistent with the result in (A) (B) (both) (neither).

*15-17. You have some normal human cells. The cells grow well at either 25ºC or 37ºC *in vitro* if EGF is added to the medium. (EGF = epidermal growth factor; it is required for growth of normal cells under these conditions.) You have several different mutants that are the same as the normal cells except that each mutant has a different temperature sensitive mutation. A cell with a temperature sensitive mutation makes an altered protein that works at low temperature, say 25ºC, but does not work at all -- is inactive -- at high temperature, say 37ºC. If you grow the cells at 25ºC the altered protein works and the cells have a normal phenotype. However when you shift the cells to 37ºC, you inactivate the altered protein, and the cells have a mutant phenotype. In other words, shifting the temperature allows you to turn the protein "on" or "off." (This allows you to maintain lines of cells that have serious defects.) You have normal cells and cells with temperature sensitive mutations in the genes for rb, ras, or p53. In each mutant line, both copies of the gene are mutant.

A. You grow the cells at 25ºC and then switch them to 37ºC. What will happen to the cells at high temperature? Remember the mutant proteins are inactive at high temperature. See class handout or text for diagram of pathways involved. (Class handout was included on the exam.) Fill in the blanks with one of the following choices:

Grow + EGF = continue to grow only if EGF is added.
Stop = stop growing even if EGF is added.
Grow +/- EGF = continue to grow whether EGF is present or not.
Can't predict.

(i)The normal, non mutant cells will _____
(ii) The temp. sensitive ras mutant cells will _____
(iii)The temp. sensitive rb mutant cells will _____
(iv) If you have a double mutant with both temp. sensitive ras ***and*** temp. sensitive rb, the mutant cells will _____
(v) Which of the cell types will make G1/S cyclin after the shift to high temperature? (normal) (ras mutants) (rb mutants) (ras & rb double mutants). Pick one or more answers, and assume EGF is present if needed.

Draw a simple diagram that shows how EGF, ras, rb, & cyclin relate in normal cells, and why (or where) the mutations affect growth (either to block or over-ride normal signals). Note that some features of the cycle need not be explained until part B.

B. Suppose you grow the cells at 25ºC, irradiate the cells with UV light (which damages DNA), and put the cells in fresh medium at 37ºC. What will happen in the short run? (Same choices as in A)

(i) The normal, non mutant cells will _____
(ii) The temp. sens. rb mutant cells will _____
(iii) The temp. sens. p53 mutant cells will _____
(iv). Which of the cell types will have active start kinase after the shift to high temperature? (normal) (rb mutants) (p53 mutants). Pick one or more answers, and assume EGF is present if needed.

*15-18. You add GGF (great growth factor) to some cells in G1, and activate the MAPK pathway. (You are not expected to do this problem without a diagram of the pathways in front of you. Consult a text or your class handouts.)

A-1. The proportion of Ras bound to GDP should (decrease) (increase) (stay the same) (stay the same at zero).

A-2. Phosphorylation of tyrosine side chains on the GGF receptor is likely to (decrease) (increase) (stay the same) (stay the same at zero).

A-3. Cyclin synthesis should (decrease) (increase) (stay the same) (stay the same at zero).

A-4. Binding of E2F to DNA should (decrease) (increase) (stay the same) (stay the same at zero).

List all the items that change in the proper order. (You can just say A-1, A-2 etc.) Please do not put in any other steps.

B. Assume normal cells, without UV treatment, take about 1 hr to start synthesizing DNA after you add GGF. Suppose you irradiate cells with UV (which damages the DNA) and then add GGF. You wait one hr. after the UV treatment.

Compared to normal cells (GGF added, no UV),

B-1. The level of active E2F is likely to be (lower) (higher) (the same).

B-2. The level (total amount) of p53 is likely to be (lower) (higher) (the same).

B-3. The level (total amount) of cyclin/CDK complex is likely to be (lower) (higher) (the same).

Explain briefly how the UV treatment (+ GGF) affects the levels of active E2F, p53 & cyclin/CDK complex. Please stick to the items mentioned.

C. NF-1 codes for a protein involved in response to GGF. The protein made by NF-1 affects activation/inactivation of Ras. NF-1 is a tumor suppressor.

C-1. NF-1 is most likely to code for a protein that catalyzes (binding of ATP) (binding of GTP) (hydrolysis of ATP) (hydrolysis of GTP) (phosphorylation of ATP) (phosphorylation of GTP) (binding of ADP) (binding of GDP).

C-2. The effect of NF-1 protein on Ras is most likely to be (inactivation) (activation) (either one) (both).

C-3. NF-1 protein is involved in the response to GGF. When GGF is added, you expect NF-1 protein to be (inactivated) (activated) (either way).

Explain briefly what happens to NF-1, MAPK, & Ras when you add GGF.

D. Suppose you have some normal cells that have been growing in vitro. The cells have been deprived of all growth factors for a few days, and GGF is added to the medium.

D-1. After GGF is added, what will the cells do first?
(divide) (synthesize DNA) (synthesize mRNA) (synthesize new proteins) (any of these).

D-2. When the cells do make proteins, after GGF added, what will they make first?
(histones) (enzymes for DNA replication) (TF's) (cyclin) (CDK).

D-3. After GGF is added, which of the following will be phosphorylated?
(MAP Kinase) (ras) (raf) (E2F) (rb) (TF's like jun, fos, or myc) (p53) (GGF) (GGF receptor).

D-4. Of the items listed in D-3, the first protein to be phosphorylated is _____, AND the last to be phosphorylated is _____.

Problem Set 15R – Review Questions for the Final

Some of the material below may not be covered thoroughly in some years. The notes marked with an asterisk (*) provide some information needed to fill the gaps.

15R-1. Myasthenia gravis is an autoimmune disease caused by an immune response to the nicotinic acetyl choline receptor. The response triggers destruction of the receptors at the neuromuscular junction. (The immune response causes RME of the receptors.) What triggers the autoimmune response is not known. Whatever the cause, the result is muscle weakness – skeletal muscles do not respond properly to normal nerve stimulation.

A. One treatment for myasthenia gravis (MG) is plasmapheresis. In this technique, the blood is separated into a fraction containing the cells and a fraction containing the plasma. Either fraction can be discarded, and the other fraction returned to the patient. If the plasma is discarded, and the cells are returned to the patient (plus a plasma substitute), the symptoms of MG improve in days, although the effects only last 6 to 8 weeks. If the cells are discarded, and the plasma returned to the patient, there is no improvement.

A-1. These results indicate that the effector cells that cause the damage in MG are probably (B cells) (cytotoxic T cells) (both B cells & cytotoxic T cells) (either B cells or cytotoxic T cells).

A-2. These results indicated that the damage in MG is probably due to (perforin*) (soluble antibody) (cell surface antibody) (either type of antibody) (antibody or perforin) (any of these). Explain briefly.

*Note: perforin = protein secreted by cytotoxic T cells that helps destroy their targets.

B. If you test the cells and blood of a patient with MG, you will find that the patient contains T cells specific for the acetyl choline receptor (AChR).

*Note: Helper T cells are needed for most B cells to produce secreted antibody. The helper T cells are specific for an epitope of the antigen involved.

(1)The specific T cells are probably (CD4+) (CD8+) (a mix – some are CD4+ & some are CD8+) (both – the cells are both CD4+ & CD8+) (neither – T cells do not express CD4 or CD8).

(2) The specific T cells produce proteins that combine specifically with epitopes from the AChR. These proteins are (soluble antibodies) (cell surface antibodies) (MHCI) (MHCII) (CD4) (CD8) (cytokines) (perforin) (complement) (cell surface TCR) (soluble TCR).

Explain your answers to (1) & (2). Explain what these particular factors/proteins are for. (Don't explain all of immunology.)

15R-2. For this question, assume that individuals with myasthenia gravis (MG) produce soluble antibodies to the AChR even if that does not fit with your answers to question 1.

A. Suppose you isolate soluble antibodies (all IgG) from the plasma of a single patient with MG. From these, you isolate all the antibodies that combine with the ACh Receptor. All the AChR antibodies will probably (be the same) (combine with the same epitope) (be of the same class) (none of these).

B. Suppose you isolate 2 different soluble antibodies (both IgG) from two different patients with MG. Both antibodies are directed against the same epitope of the ACh Receptor. Both the antibodies will probably (have the same L chain) (have the same H chain) (be the same) (have the same variable region) (have the same constant region of the H chain) (none of these).

For each part, circle one or more answers; then explain the answers briefly.

15R-3. Read the first paragraph of 15R-1 for a description of myasthenia gravis. The immune response in people with myasthenia gravis (MG) affects the neuromuscular junctions in the somatic nervous system. It does not affect the autonomic nervous system (ANS). NT = neurotransmitter.

A. Consider the junctions between smooth muscle and the sympathetic branch of the ANS. The lack of effect of MG at these junctions is probably because (the transmitter is not acetyl choline) (there is no endplate structure) (the transmitter is ACh, but the receptor is different) (the mode of release of NT at the synapse is different) (any of these – they are all equally likely explanations) (none of these – the sympathetic branch doesn't innervate any smooth muscle).

B. Consider the junctions between smooth muscle and the parasympathetic branch of the ANS. This lack of effect of MG at these junctions is probably because (the transmitter is not acetyl choline)) (there is no endplate structure) (the transmitter is ACh, but the receptor is different) (the mode of release of NT at the synapse is different) (any of these – they are all equally likely explanations) (none of these – the parasympathetic branch doesn't innervate any smooth muscle).

C. Consider the synapses between the pre and post ganglionic cells in the ANS. The most likely explanation for the lack of an effect of MG here is (ACh is not involved in these synapses)
(ACh is involved, but the receptor for ACh is different) (both the receptor and the transmitter are different)
(either the receptor or the transmitter is different, but can't tell which) (the synapses are inside the CNS)
(the synapses are inside ganglia) (any of these – they are all equally likely explanations).
Explain your answers briefly. Please do NOT tell us everything you know about the nervous system. Stick to the issues at hand.

15R-4. A standard test for with myasthenia gravis (MG) is to give someone a dose of a fast acting (and short lived) ACh esterase inhibitor and see if their muscle tone improves dramatically. (For this problem, it doesn't matter how the ACh esterase works on the muscles to increase tone.) However, you have to watch out for changes in heart function.

A. The most likely consequence of a ACh esterase inhibitor on the heart is (too fast a heart beat)
(too slow a heart beat) (failure of heart to beat).

B. The primary effect on the heart would be on (contractile heart cells) (cells of AV node) (cells of SA node)
(cells of either node) (any of these).

C. In the sensitive cells of the heart, which of the following is most likely to be affected? (height of AP)
(width of AP) (pacemaker potential) (receptor or generator potential) (slope of rise in AP)
(slope of decline at end of AP).

D. In the sensitive cells of the heart, the item you chose is likely to (increase) (decrease) (can't predict).
Be sure all your answers are consistent, and explain briefly.

15R- 5. In normal people, the amount of ACh released in response to an action potential (AP) in the motor neuron is not always the same. There is a variation of about 10% in the amount of neurotransmitter (NT) released per AP. Suppose you record the EPP's & AP's at a single neuromuscular junction. (You measure the EPP at the synapse and the AP in the surrounding muscle membrane).

A-1. In a normal person, you expect the EPP to be (proportional to the amount of NT released)
(always the same size) (inversely proportional to the amount of NT released)
(variable, but to have no relationship to the amount of NT released).

A-2. Consider the value of the average EPP triggered by a single pre-synaptic AP. In a normal person, the average EPP should be: (significantly above threshold) (significantly below threshold) (close to threshold) (can't predict).

A-3. In a normal person, you expect to get: (a muscle AP of the same size from every EPP)
(muscle AP's of different sizes – AP should be proportional to the EPP)
(muscle AP's of different sizes but sizes not related to size of EPP)
(a muscle AP of standard size from some EPP's but no AP from others).

Explain your answers to A briefly.

B. In people with with myasthenia gravis (MG), release of transmitter (and the variation) is the same as normal. However the number of ACh receptors is way below normal (as a result of damage from the immune response). In normal people, one AP in the neuron generates one twitch in ***every*** muscle fiber connected to that neuron. (Each motor neuron forms synapses with multiple muscle fibers.) However in patients with MG, only ***some*** of the muscle fibers respond with a twitch.

Suppose you record the EPP's & AP's at a single neuromuscular junction as described above, but this time you do it in a person with MG. What do you expect to find?

B-1. You expect the ***average*** value of the EPP to be (the same as in normal)
(smaller – less depolarized – than in normal) (larger – more depolarized than in normal) (can't predict).

B-2. You expect to get: (a muscle AP of the same size from every EPP)
(muscle AP's of different sizes – AP should be proportional to the EPP)
(muscle AP's of different sizes but sizes not related to size of EPP)
(a muscle AP of standard size from some EPP's but no AP from others).

B-3. The value of the average EPP in a person with MG should be:
(significantly above threshold) (significantly below threshold) (close to threshold) (can't predict).

B-4. An inhibitor of ACh esterase often reduces the symptoms of MG – when it is present, more of the muscle fibers respond to an AP in the neuron. What effect should the esterase inhibitor have on the average value of the EPP? (increase it – increase the depolarization) (decrease it – decrease the depolarization)
(reverse the sign – hyperpolarize) (zip – no effect on EPP) (beats me).

Explain how your answers in B lead to the results given. Explain why the relationship between neuron AP's and twitches is different in people with MG than it is in normal people.

15R-6. For each of the following questions, circle or write in ALL correct choices, and provide one sentence of back up. (State the principle underlying your selections.) Choices and instructions are the same for all parts except last part of F.

A. Which of the following items should be made on ribosomes bound to the ER? (TCR) (secreted antibody) (antibody attached to plasma membrane) (CD4) (MHCI) (MHC II) (perforins) (cytokine such as IL-2) (complement) (all of these) (none of these).

B. Which item(s) listed should be able to act as paracrine signal(s)? _____

C. Which item(s) listed should be made in naïve B cells? _____ Assume the B cells are not making their own growth factors.

D. Which item(s) listed should be involved in juxtacrine signaling between lymphocytes and virus infected cell(s)?_____

E. Which item(s) listed should be found in the plasma of a normal adult (not suffering from any disease, allergy, etc.)? _____

F. You have a culture of B cells. You divide the cells in half, and fix each half as described below. "Fix the cells" means you treat the cells with chemicals that kill the cells but leave all the structures in place. For sample (1), you add antigen, wait one hour, and then fix the cells. For sample (2), you fix the cells first, then add the (same) antigen and wait one hour. Sample (1) can activate helper T cells. Sample (2) cannot. The same antigenic determinants can be detected on the surfaces of both samples.

a. In sample 1, antigen should be attached to _____.

b. In sample 2, antigen should be attached to _____.

c. These results indicate that fixing the cells probably disrupts (antibody/antigen binding) (RME) (TCR/Antigen binding) (MHC/CD binding) (secretion of antibody) (lysis of target cells). Circle the best answer and explain briefly why only sample (1) activates helper T's. This explanation can take more than one sentence.

15R-7. You have a fully differentiated B cell from a sheep. The B cell is naïve – it has never been exposed to antigen. Assume that the sheep immune system works the same way as the human. For this question, circle one *or more* correct answers.

A-1. The naïve B cell should make: (secreted immunoglobulin) (surface immunoglobulin) (either secreted or surface immunoglobulin) (both) (neither).

A-2. Suppose you can isolate a large collection of fully differentiated naïve B cells from a single sheep. All these cells will make the same (TCR) (immunoglobulin) (class(es) of immunoglobulin) (heavy chains) (light chains) (all of these) (none of these).

A-3. All these cells will make (CD4) (CD8) (MHC I) (MHC II) (all of these) (none of these).

B. You transfer the nucleus from a somatic cell of a sheep (call it Polly) into an enucleated sheep egg (from Lolly), and stimulate the egg to divide. Then you implant the egg into a female sheep (Colly). Suppose it works, and you get a live adult sheep (Holly). Assume that Polly, Lolly & Colly are not related -- each one has mitochondrial and nuclear DNA that is distinguishable from the other two.

B-1. The mitochondrial DNA of Holly should be the same as the mitochondrial DNA of (Polly) (Colly) (Lolly) (all of these) (none of these) (depends on which type of differentiated cell you look at).

B-2. You isolate a kidney cell from Holly, and look at the nuclear DNA. The DNA should be the same as the nuclear DNA from (a skin cell from Holly) (a naïve B cell from Holly) (a kidney cell from Polly) (a kidney cell from Lolly) (a kidney cell from Colly) (all of these) (none of these).

C. Suppose you compare the antibodies made by all the different sheep in response to a human protein, say protein H. Assume sheep do not make a protein that is homologous to protein H. You inject all the sheep with the protein H, and then isolate antibodies to protein H from the blood of all the sheep. All the antibodies you isolate are of class M.

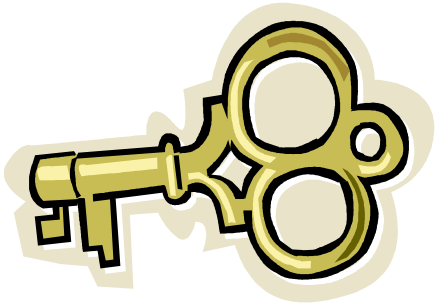
C-1. All the isolated antibodies to protein H would (be specific for the same antigen) (have the same heavy chain) (be specific for the same epitopes) (have the same variable region) (have the same light chain) (have the same amino acid sequence for at least half of the heavy chain) (have none of these things in common).

C-2. You would expect to find exactly the same antibodies to protein H in Holly and in: (Polly) (Lolly) (Colly) (all of these) (none of these). Explain the rationale for your answer to C-2 in one or two sentences.

Keys

To

Problem Sets



ANSWERS TO PROBLEM SET #1

1-1. 1000 nanometer = 1 micrometer. 0.2 micron = 200 nm, so the microtubules are below the resolution of the light microscope when the microscope is used in the ordinary way. When you use ordinary microscopy, the light you see is from the light source you used to illuminate your sample. The light you see has either bounced off, or gone through, the sample. The wave length of that incident light limits what you can resolve. When you use the fluorescence techniques, the light you see is not from the incident light -- what you see is the light emitted by the fluorescent material in the sample. (The sample was excited by the incident light, but the light that the sample gives off, which is what you see, is a different wave length. The incident light is filtered out.) You are looking at a point source in the sample that emits light, not the results of shining a light on the sample and seeing what is reflected, transmitted, etc. To put this more colloquially: there are things that are too small to see if you shine a flashlight on them in the dark. But if the items are lit up from inside, you can see them easily.

1-2. A.

(1) Table shows percent of phospholipids remaining after enzymatic digestion, so the amount accessible to enzyme degradation (in intact cells) would be 100% minus these numbers: lipid A -- 76%, lipid B -- 20%, & lipid C -- 0%.

(2) When the RBCs are lysed, the cell membrane is broken open, and the enzyme has access to the phospholipids inside as well as outside, so nothing is left.

B. When the membranes are disrupted after lysis, the membrane is broken into fragments. There is a 50% chance that each fragment will reseal in its original form, and a 50% chance it will reseal "inside out" -- with the inner face now on the outside. Once the fragment reseals, the enzyme has access to whatever layer of the membrane is now on the outside of the resealed fragment. Half the time the outer layer of the resealed fragment will be the ***outer*** layer of the original membrane, and the other half the time, time the outer layer of the resealed fragment will be the ***inner*** layer of the original membrane. Therefore the enzyme will, on average, degrade half of each type of phospholipid.

C. The normal distribution of these molecules is lipid A: 76% out, 24% in; lipid B: 20% out, 80% in; lipid C: 0% out, 100% in.

D. RBC's were good choice because they have no internal membranes and little cytoskeleton, so it's easy to disrupt and reseal them.

1-3. Membrane is fluid, but some proteins are anchored in place by connections to the cytoskeleton (on the inside of the membrane) or to the extracellular matrix (on the outside). Also, the structures that join cells (primarily tight junctions) often occur in strips or belts that form barriers in the membrane, and these structures limit ability of proteins to move from one side of the barrier to the other.

1-4. The hydrophobic nonpolar lipids are "hidden" from both the water in the cytoplasm and from the water in the blood by being sandwiched between two layers of amphipathic lipids. A Martian sandwich?

1-5. A. A weak acid dissociates slightly: $HA \leftrightarrow H^+ + A^-$. The HA, un-ionized form, can diffuse through the nonpolar bilayer, but the ions formed from it ($H^+ + A^-$) cannot. So the weak acid will be trapped if it ionizes (which occurs in a basic environment only).

i) Greater in 2. If HA is put in chamber 1, with a low pH, the excess H^+ present will drive the equation (above) to the left; none of the acid will dissociate, and so the un-ionized HA will diffuse across the bilayer and be trapped in chamber 2 (where it will dissociate.)

ii) Greater in 1. If chamber 1 has a high pH (low $[H^+]$ or high $[OH^-]$), the reaction will go to the right. If pH is high enough, acid will dissociate completely (as all H^+ produced will combine with free OH-). All ions formed will be trapped in chamber 1.

iii) Same in both. HA will remain un-ionized at low pH, and will diffuse back and forth freely between the two chambers.

iv) Greater in 1. At very high pH, acid will be completely ionized and ions will stay in the chamber where HA was put.

Answer to 1-5, cont.

B. A weak base dissociates slightly according to the equation: $BOH \leftrightarrow B^+ + OH^-$. At low pH, reaction goes to the right (as H+ combine with OH- and pull reaction) and vice versa at high pH (where high OH- drives reaction to left). In this case the weak base will be trapped in an acid environment. So answers are: i) Greater in 1; ii) Greater in 2; iii) Greater in 1; iv) Same in both.

C. In the lysosomes. This is the same case as B, part ii. The lysosomes are more acidic than the rest of the cell, so the weak base strychnine will be trapped in the lysosomes. The un-ionized base may diffuse in and out of all the organelles, but it will ionized and be trapped primarily in the lysosomes.

1-6. Assume the inner and outer nuclear membranes are so close together that their surface areas are about equal. In that case, the ratio of monolayer area/nuclear surface should be about 4, since there are 2 bilayers surrounding the nucleus. Exact number depends on the number and/or structure of the nuclear pores. (Pore proteins may displace a significant amount of lipid since the pores can take up as much as 25% of the nuclear surface; this would increase the surface area of the nucleus and/or decrease the amount of lipid present and thus decrease the value of M/N. Alternatively, pore proteins may simply push the membrane lipids into a different conformation, so that they form a rim around the edges of the pore instead of filling the pore; this would have no effect on the M/N ratio.)

1-7. Microtubules (MT) are hollow; microfilaments (MF) and intermediate filaments (IF) are solid. MT and MF are polymers of globular subunits; MT are made of tubulin and MF are made of actin. IF are made of fibrous subunits.

1-8. A. The component of the cytoskeleton with the largest diameter is **microtubules** and the one(s) NOT involved in movement are **intermediate filaments**.

B. If you add fluorescent antibody against actin to normal cells in interphase, you will "light up" **the microfilaments** AND you will see fluorescence in the **cytoplasm**.

C. If you add the same antibody (as in B) to a red blood cell, you will see **fluorescent staining over a more restricted area**.

In normal cells, the cytoskeleton, containing MF made of actin, extends through the entire cytoplasm. In RBC, most of the internal organelles and cytoskeletal components are lost. All that remains is the actin/spectrin/ankyrin web supporting the plasma membrane. So you would see fluorescence in a thin rim around the edge of the cell, under the plasma membrane (as vs all through the cytoplasm).

1-9. A. MT. Drug results rule out microfilaments, which are made of actin, and implicate MT which are made of tubulin. (IF are assumed not to be involved as they usually are required for structural support, not movement; drug results don't rule them in or out.)

B. Different. MT are known to act as "railroad tracks" for transport of molecules and vesicles; the MT stay put and the moving material is transported by motor molecules which use the energy of ATP hydrolysis to move along the MT. The motor molecules that move along MT are dynein and kinesin; they move in opposite directions. Since the organelle membranes are pulled in opposite directions, and the motor molecules move in opposite directions, reformation of the organelles should use different motor molecules.

C. Dynein. MT are usually arrayed in a pattern so that they spread out from a single area, the centrosome or MTOC, near the center of the cell. The MT are all arranged with their minus ends near the centrosome. Usually dynein moves down the MT from the plus to the minus end of the MT, while kinesin moves in the other direction. If the Golgi components are pulled in, toward the center of the cell, the most likely candidate is dynein. The Golgi is thought to be pulled in toward the centrosome by dynein and the ER to be stretched out along the MT by kinesin.

1-10. Microfilaments. MF are made of actin; MT of tubulin. (IF of neither.) Given drug results -- inhibition by actin-binding drugs, but not by tubulin-binding drugs -- process is dependent on MF, not MT. Drug results don't rule out IF involvement (in addition to MF) but IF aren't usually involved in movement, so unlikely they're involved here. Conclusion: MF are definitely involved; IF could be, but it's not likely.

1-11. A. Actin, cytoplasmic division, & MF. The cleavage furrow that divides the cytoplasm in half at telophase contains microfilaments made of actin (and myosin). If the furrow fails to form or contract the cell properly, the cells will never divide even though the chromosomes have separated correctly and the nuclei have divided properly. The cells will become multinucleate if the nuclei divide but the cell doesn't. This will occur if drug A binds to actin, and inhibits the action of MF.

B. Tubulin, chromosome separation, & MT. The chromosomes are lined up and the chromatids pulled apart by MT (spindle fibers) made of tubulin. If the MT's fail to form or slide properly, the sister chromatids will not be pulled apart and the nuclei will have a double number (4N) of chromosomes. If this process repeats, the nuclei will become 8N (4X the usual number) and so on. If sister chromatids separate, but fail to move apart (to opposite poles), and the cell (cytoplasm) divides, the daughter cells will be normal size. However one daughter cell will have no nucleus or a nucleus without chromosomes, and it will not survive; the other cell will be polyploid. This will occur if drug B binds to tubulin and inhibits the action of MT.

Note (FYI): MT are involved in some transport of vesicles within cells, but not in budding from ER or endocytosis.

1-12. A. Gap junctions. B. None of these. C. Adhesive junctions (desmosomes & adherens junctions; these are called spot desmosomes and belt desmosomes, respectively, in older books). D. Gap junctions (& Plasmodesmata in plants). E. Tight junctions.

1-13. A. *Increase, stay the same*. If gap junctions increase, flow of ions (under optimal conditions) should increase (when the channels are open), AND there should be no effect on water passing through the extracellular space between the two cells. Tight junctions, not gap junctions, limit water passage in between cells.

B. *Stay the same, increase*. There are proteins connecting the two cells at a desmosome, but the IF's associated with desmosomes are *inside* the cells. (The connecting proteins are not MF's or IF's.)

1-14. A. P & E represent 2 different sides on the inside of a bilayer. In some pictures, you see the E face of one bilayer and the P face of another; often you see the P & E faces of the same bilayer (in different pictures). The P and E faces look different because there are more molecules of connexin (& other transmembrane proteins) associated with the P side than the E side. (See B below.)

B. In gap junctions, all of these are connected to each other. Individual connexin molecules in the same cell are connected to each other to make a single connexon, which is inserted in the cell membrane. Two protruding connexons from adjacent cells are connected to form a channel. (Since the two connexons stick out, the membranes are separated by a gap -- that's how gap junctions get their name. There is no gap between the connexons. See pictures in texts.) The connexons must be attached to the cytoskeleton because they preferentially go with the P side (cytoplasmic side) of the bilayer when the cell membranes are broken by freeze fracture.

C. The bumps correspond to the extracellular domains of transmembrane proteins. When the lipid bilayer was fractured, the E side of the bilayer was separated from the P side, but the proteins remained with the P side because they were attached to the cytoskeleton. When you look down at the P face, the cytoplasmic domains of the transmembrane proteins are not visible in the freeze fracture picture -- they are underneath the P face. The extracellular domains that were pulled out of the E face are visible as bumps on the surface of the P face.

1-15. A. You want to look at the inside surface of the plasma membrane, which is inaccessible in right-side out vesicles and exposed in inside-out vesicles.

B. Carbohydrates are found only on the protein domains that are on the outside of the plasma membrane. When you make inside-out vesicles, the protein regions carrying the carbohydrates are buried inside the vesicle. So right side-out vesicles which have their carbohydrates exposed should bind to the column but inside-out vesicles should not.

1-16. A. Intrinsic, transmembrane protein.

B. Protein must be a transmembrane protein from freeze fracture results. Any transmembrane (TM) protein is by definition intrinsic. Remaining results indicate this is a "single pass" TM protein that goes through the bilayer once, with amino end on outside and carboxyl end in cytoplasm. Amino acids 1-50* = part of extracellular domain, since this section has the attached carbohydrate. This indicates the amino end is on the outside. 73-95 = TM domain, since it is only hydrophobic part. Part after amino acid 95 has no carbohydrates and is hydrophilic, so it = intracellular domain. Amino acids 51-72 must be part of the extracellular domain, as they are the "far side" of the TM segment (they are in between the TM domain and amino acids 1-50). Since amino acids 51-72 have no carbohydrates, they probably do not stick out as much as 1-50. Either 1-72 are folded up in a single globular (extracellular) domain with 51-72 on the inside of the glob, or 51-72 constitute a stalk-like structure with 1-50 forming a cap at the end. *Remember: You always count the amino acids in the protein from the amino end.

1-17. A. Amino end (of mature protein) AND domain B or domains B + C; B. Intrinsic; C. 1; D. Domain B. Mature protein P has only one hydrophobic region (C), so the mature protein should go across the membrane once. Since it is an integral part of the lipid bilayer, it is considered an intrinsic protein.

When you do freeze fracture, the bilayer splits into two leaflets, but the proteins that extend across the bilayer do not break -- they pop out of one side or the other and stay with the opposite side. So when you see a bump on the P face, which corresponds to the inside of the leaflet that was closest to the cytoplasm, you are looking at the part of a protein that used to be on the outside of the other leaflet (and therefore outside the cell) in the intact membrane. This represents the amino end of the mature protein (the B domain = extracellular domain). The "bump" may consist of both the C and B domains, but the B section should be on top. The "bump" is not just the transmembrane or C domain. (If you could make a clean slice through the middle of the bilayer you would see just the C domain, but you are looking at a ragged fracture through the bilayer instead.) Only the extracellular domain(s) should have carbohydrate attached.

1-18. 1-A. Intrinsic membrane protein.

1-B. Cadherin. RBC anion exchanger is a transmembrane protein, but it is a multipass protein, while cadherins are single pass. All the others are either intracellular or in the ECM.

1-C.(1). Movement of vesicles using dynein requires tubulin; tubulin makes up microtubules and dynein is the motor molecule that helps move vesicles etc. along the microtubules.

(2). Lamin has an NLS. NLS = nuclear localization signal; required of all proteins that enter the nucleus. Lamin is a type of IF found under the nuclear envelope. (None of the other proteins are nuclear.)

(3). Catalase. Peroxisomes and lysosomes both have single membranes, but only enzymes of peroxisomes (such as catalase) are made on free ribosomes. Acid hydrolases are in an organelle with a single membrane (a lysosome) but they are made on membrane bound ribosomes.

(4). Fibronectin or collagen are both part of the ECM. ECM = extra cellular matrix; only these two proteins are entirely extracellular. Collagen is the major structural protein of the ECM; fibronectin is an adhesive protein that helps hold it all together.

(5). Spectrin defects cause RBC fragility. Spectrin is part of the protein web that lies under the plasma membrane and helps strengthen the membrane. (Other members of the spectrin family are found under the plasma membrane in other cells. But spectrin per se is unique to RBC.) Actin helps connect the web, but is not unique to it -- actin is involved in multiple functions in virtually all cell types. Therefore defects in actin would cause many other additional problems. (I suspect most if not all actin defects are lethal.)

Answer to 1-18, cont.

(6). Cadherin defects cause blistering of the skin. Cadherins are part of the adhesive junctions (adherens junctions and desmosomes) that join the cells in an epithelial layer.

1-19. A. Hemidesmosome. (Only structure listed that contains integrins. The transmembrane protein in a gap junction is connexin; in all the other structures it is a cadherin.)

B. (i) IF. Integrins of hemidesmosomes are connected (through intermediary proteins) inside the cell to IF.
(ii) ECM -- Integrins are connected to a protein in the ECM.

C. Basal -- Hemidesmosomes are located on the basal part of the BL membrane;
All the other structures listed are located on the lateral side of the BL membrane, and connect to a transmembrane protein from the next cell. Inside the cell, transmembrane proteins of adherens junctions (cadherins) are connected to MF; transmembrane proteins of desmosomes (another type of cadherins) to IF. Transmembrane proteins of gap junctions (connexins) must be connected to the cytoskeleton (see answer to 1-14 B) but we did not go into the details.

Note: The problem refers to "an integrin in the plasma membrane of an epithelial cell. The cell is part of an epithelium surrounding the lumen." This means that '*the layer of epithelial cells'* surrounds the lumen, not that '*the part of the membrane containing the integrin'* surrounds the lumen. It does not make sense for the junction containing the integrin to be on the side of the cell (the apical side) surrounding the lumen -- the structures listed connect epithelial cells to each other and to the underlying layers, not to extracellular material in the lumen.

1-20. A. **Protein A has 1 extracellular domain and protein B has two.** A domain is defined as a distinct region of a protein. The two extracellular domains of protein B could be oriented in 3 dimensions so that they are closely connected, and/or form one functional unit, but they are usually considered two separate domains because they are two distinct regions of the protein. By the same reasoning, each individual transmembrane section of the protein is considered a domain in its own right -- the problem speaks of 4 transmembrane domains, not a single transmembrane domain.

B. (1) & (2). Both ends of the multipass protein should be on the cytoplasmic (P) side of the membrane; the amino end of the single pass protein should be on the extracellular (E) side of the membrane.
(3). The freeze fracture plane should go down the middle of the lipid bilayer, but not through the transmembrane proteins. The plane of fracture will go around, not through, the transmembrane proteins. (Think of almonds in a chocolate bar. When you break the chocolate, the almonds go with one side or the other.)
Note: The plane of fracture usually splits through one bilayer, so you see either the P or E face in a picture. Sometime, the plane of freeze fracture splits part of the membrane of one cell and part of the membrane of a neighboring cell. In that case, you see the P face of one membrane and the E face of the other in the same picture. (There are examples of this in the texts, for example Fig 17-12 (c) of Becker, 8th ed. or 15-10 (c) of 9th ed.)

C. **The multipass protein (B) could have oligos on 2 dif. domains.** Oligosaccharides are added enzymatically in the ER and Golgi. Therefore oligosaccharides can only be added to domains that are exposed to the enzymes inside the lumens of the organelles = the extracellular domains (see answer to A). Note that oligosaccharides are not added to the ends of proteins -- they are added to the side chains of the amino acids asparagine, threonine, and serine. The multipass protein has two extracellular domains that can be glycosylated -- the single pass protein has only one.

1-21 A. Passage of ions from **cell to cell will stay the same**; passage **across the epithelial cell layer will increase**. The passage of ions from cell to cell (in the plane of the epithelium) is through gap junctions, not through tight junctions. Changes in tight junctions will have no effect. The passage **across** the epithelial cell layer (from the apical side to the BL side or vice versa) normally occurs by passage through the cells themselves. Passage between the cells is blocked because the tight junctions form an impenetrable barrier between the cells. If the tight junctions are defective, they will not serve as an effective barrier and ions will be able to pass across the epithelial layer by passing through the spaces around/between the cells. (Answer to B on next page.)

Answer to 1-21, cont.

B. **Protein A belongs at the border between the BL and apical surfaces.** Tight junctions are what prevent mixing between the two areas of the cell membrane of a polarized cells -- the tight junctions prevent lateral diffusion of proteins and lipids in the plane of the membrane. So wherever the tight junctions are defines the border between the two regions of the cell. It was not sufficient to say that tight junctions have to be on the sides, not the ends, in order to join the cells of the epithelium, since (a) that issue was already covered in part A, and (b) that does not explain why these particular junctions are found at the border between the two areas.

1-22. A. ENaC binds to spectrin A, and is associated with an actin network.
Explanations: A-1. Antibodies combine with antigens, and an antibody-antigen complex precipitates because the antibody crosslinks multiple particles of the antigen into a large aggregate. Each antibody is specific for a particular antigen (or piece of it). When you add antigen, you therefore expect a precipitate containing only the antigen that matches up with the antibody. However, if a second antigen is linked to the first, the precipitate will contain both antigens, even though the antibody could only combine with the first antigen. In other words, when antibody to protein X causes co-precipitation of protein Y ("brings down Y"), it is assumed that protein Y was stuck to protein X (by noncovalent interactions). So if antibody to ENaC causes precipitation of spectrin A, it is because the ENaC and spectrin A are stuck together, not because the antibody binds both proteins.

A-2. If ENaC is linked to spectrin A *in vivo*, then ENaC must be linked to the part of the cytoskeleton that contains spectrin A. Spectrin is part of the web supporting the plasma membrane, which contains short microfilaments (MF) made of actin, but no tublin or microtubules (MT). This is an epithelial cell, not a red blood cell, so it contains both MF and MT. However the ENaC must be linked to the MF, not the MT.

B. (1) Spectrin A should be in the ppt. if you use antibodies to fusion protein 2 (last 50 AA of ENaC fused to GFP).
(2) ENaC is probably anchored to spectrin A by its carboxyl end.
(3) Spectrin A is probably on the apical side of the plasma membrane.
Explanations: If you have only the last 50 AA of ENaC present, the protein still binds to spectrin A. Therefore, it's the carboxyl end that binds to spectrin. (But see note.) Since the labeled ENaC is only detected in the apical half, spectrin A must also be in the apical half. We know spectrin is part of the web supporting the membrane, so putting it all together, the spectrin A must be under the apical membrane. (Other supporting proteins, such as other types of spectrin, must be associated with the basolateral membrane.) The basic idea that had to be explained: ENaC sticks to spectrin A, and when you block or remove one end of ENaC, it fails to stick.

Note: The fusion proteins described here do not include all of ENaC. Fusion prot. 1 includes ***only*** the first 50 AA of ENaC, and fusion prot. 2 includes ***only*** the last 50 AA of ENaC. In both cases, GFP is probably on the carboxyl end, as that's the way it's usually done. However GFP doesn't usually interfere with function, no matter what end it is on. The GFP forms a separate domain that folds separately. It tags along with the rest of the protein, but usually allows the protein to fold, function, and bind to antibodies in its usual way.

C-1. The two unlabeled antibodies were made in different species. We know this because the secondary antibodies are called 'anti-rabbit' (for ENaC) or 'anti-mouse' (for spectrin A), indicating that the secondary antibodies were directed against rabbit and mouse constant region respectively. In other words, the antibody to ENaC was made in a rabbit, and the antibody to spectrin A was made in a mouse. Since the two unlabeled antibodies have different constant ends, different labeled antibodies are needed to stick to each one.

C-2. No labeled bands with unlabeled antibody to ENaC; one labeled band with unlabeled antibody to spectrin A.
Explanation: The ppt. contains both ENaC and spectrin A. Therefore both are present on the gel (in the blot), and whatever unlabeled (primary) antibody you add will stick to its respective protein. But you still won't be able to see any bands, because the first antibody isn't labeled. So you add labeled (secondary) antibody to stick to the first one, and form a labeled band. The labeled secondary antibody used here is always goat anti-mouse, which only binds to primary antibody from mouse, not to primary antibody from rabbit. If the first unlabeled antibody is directed against ENaC, then it was made in a rabbit, and the labeled antibody (anti-mouse) will have nothing to stick to, so no bands will be seen. If the first unlabeled antibody is directed against spectrin, then it was made in a mouse, and the labeled antibody (anti-mouse) ***will*** stick to it, and you will see one labeled band.

1-23. A. Emerin is a single pass protein; if it's in the plasma membrane, it would probably have its amino end in the cytoplasm. If it's glycosylated, the sugars would be added near the carboxyl end, and the largest section in the cytoplasm would contain about 210 amino acids.

Complete Explanation: This protein has only one hydrophobic section, which probably makes up a single transmembrane domain. That means that emerin is a single pass protein with 11 amino acids on one side of the membrane, a transmembrane section of 20-30 amino acids (approx.) and the rest of the protein (about 210 amino acids) on the other side of the membrane. Glycosylation happens only to amino acids in domains that are exposed to the lumen of the ER or Golgi; these are the domains that end up on the extracellular side of a plasma membrane protein. Phosphorylation happens inside the cell, in the cytoplasm (not just inside the ER or Golgi), where the kinases and phosphatases are. Since there is one possible glycosylation site and many possible phosphorylation sites, it makes the most sense if the short 11 amino acid tail is on the extracellular side and the large AA domain is intracellular.

The fact that the protein is found in the membrane fraction has nothing to do with the freeze fracture procedure. (See note below at *.)

B. Emerin is in the inner nuclear membrane (for real), bound to an intermediate filament (IF). Lamin A is a type of IF found only in the nucleus. If either lamin or emerin fails to bind to the other, the result is EDMD. One domain of emerin is inside the nucleus, bound to lamin A, while the other domain is in the peri-nuclear space -- the area between the two nuclear membranes that is continuous with the lumen of the ER. If either lamin or emerin is defective, emerin is not properly localized and doesn't function properly. (Its function is unknown.) The most likely model is that the large domain of emerin (with the multiple phosphorylation sites) is in the nucleus, and the small domain of emerin (with the glycosylation site) is in the peri-nuclear space.

C. You won't find particles on either face; if emerin is inserted as expected with its large domain inside the nucleus. If it is inserted in the reverse orientation, you ***will*** see particles on the P face.
Explanation: When the membrane is broken open by the freeze fracture technique, the protein will go with the P half of the bilayer (= the side nearest the nucleoplasm or inside of nucleus), because the protein is anchored by lamin inside the nucleus. The part of the protein that was in the nucleus will not be visible -- it will be behind the P face, and the part that was on the E side of the membrane (in the perinuclear space) will stick out of the P face. If the protein is inserted as in B, the large domain will be attached to lamin, on the far side of the P face, and the 11 amino acid domain will stick out. It is too short to form a visible particle. So you won't see any particles. However, if the protein was inserted in the reverse orientation, the part sticking up will be the large domain, and that will form particles.

*Note: Many students said the particles would be on the P face because the large domain of the protein is on the P half of the bilayer. The large domain is on the P half, but what you see in freeze fracture is the ***inside*** of the bilayer. You don't see the whole P side -- you see the P ***face***, which is the surface on the inside of the P half of the bilayer. The large domain (or whatever was in the nucleoplasm) was on the ***outside*** of the bilayer. After freeze fracture, the domain that was in the nucleoplasm, sticking out of the P side of the bilayer, is underneath the P face, not on top of it. What sits on the P face and forms particles, if any, is what was originally on the outside of the OTHER (E) half of the bilayer.

KEY TO PROB. SET #2

2-1. Experiment 1 shows that the mechanism is passive transport, not active transport, because the final concentration inside the cell = $[C2406]_{in}$ is equal to the external concentration = $[C2406]_{out}$ = about 10mM. Uptake does not affect $[C2406]_{out}$ significantly because the volume of solution on the outside of the cells is so large compared to the volume inside the cells.

Experiment 2 shows that the rate of uptake reaches a plateau at high C2406 concentrations, suggesting that a saturateable carrier is involved. Putting the results of both experiments together, the most likely mechanism is carrier mediated transport, meaning facilitated diffusion using a permease or transport protein, not passage through a channel.

2-2. A. F & G are not ions, so ion channels are ruled out. Any of the remaining mechanisms which require carriers are equally likely; mutants imply the need for a specific protein = carrier. So anything BUT simple diffusion or ion channels is possible. (If it were an option, endocytosis without a receptor would be ruled out too.)

B. Secondary active transport is best bet here. Uptake depends on ion gradient. As ion flows down its own gradient, the energy released drives F into the cell. Na/K pump needed to set up ion (Na^+ or K^+) gradient in first place.

C. Primary active transport. Once cell runs out of ATP, transport stops, implicating primary active transport. Cell has some ATP to start, but can't make more. (Note: Receptor mediated endocytosis is not listed here; if it were an option, it could be possible too. Both primary active transport and RME use a carrier and require a direct source of energy. In RME, ATP is needed to uncoat the vesicles and recycle the receptors.)

2-3. A. See p. 22. Please do not attempt to do the rest of this problem until you are sure of the answer to part A.

B. $[Na^+]$ inside < $[Na^+]$ outside. $[Na^+]$ must be higher outside to drive AA transport. (See C for mechanism.) The ionophore allows Na^+ to flow in (or out), so it immediately eliminates any differential in concentration between the two sides of the membrane. Blocking the pump doesn't affect the Na^+ distribution directly, but it prevents pumping of Na^+ (used for AA transport) out of the cells. When pumping stops, the Na^+ gradient is not affected directly, but the gradient cannot be replenished, so AA transport continues until the Na^+ gradient "runs down."

C. Na^+ & amino acids. The carrier or transport protein binds Na^+ and amino acids on the outside; Na^+ binds because of the high concentration, and this binding promotes AA binding. Then the carrier changes shape and faces inside. Since the conc. of Na^+ is low on the inside, the Na^+ falls off and this causes the amino acids to fall off. The carrier then faces back outside and the cycle starts again. (See text or handout on Na^+ -- glucose co-transport; mechanism is the same.)
Note: the carrier does not bind ATP (or K^+) on either side. The Na^+/K^+ pump binds ATP on the inside, & K^+ on the outside, but the AA carrier doesn't bind either one.

D. Rate of AA transport is proportional to AA concentration only at low [AA]. Transport of amino acids requires a carrier protein. (Transport also required Na^+, but you have plenty of it.) At low [AA] there is plenty of carrier (& Na^+), and rate of transport is limited by AA supply. At high [AA], amount of carrier is limiting and rate of AA transport reaches a maximum when all molecules of carrier are saturated with AA. You can consider transport a standard chemical reaction (AA_{out} --> AA_{in}) catalyzed by an enzyme = carrier protein. The reaction then has a standard V vs [S] curve that is proportional to [S] (AA_{out}) for low values of [S] and plateaus (approaches V_{max}) for high values of [S].

2-4. A. See p. 22. Please do not attempt to do the rest of this problem until you are sure of the answer to part A.

B. Protein P must be a transmembrane protein. A carrier protein must extend through the membrane to facilitate transport.

C. At the point on graph #3 indicated by the arrow, $[ser]_{in} = [ser]_{out}$. At the point indicated by the arrow, the system has reached equilibrium and the flow in equals the flow out. (Flow does NOT stop, but the net transport across the membrane is zero, because the total transport in = total transport out.) Since there is no external source of energy other than the serine gradient itself, you can conclude that equilibrium will be reached when the concentration inside = concentration outside. At that point the driving force inwards = force outwards.

Graph #3 does NOT indicate that the two concentrations are equal at equilibrium. You have to conclude that from other information. Graph #3 only tells you that equilibrium has been reached, not what the equilibrium concentrations are.

Also note that graph #3 does not reach a plateau because the carriers are saturated -- this is because graph #3 shows uptake as a function of time, not as a function of concentration. (Graph #2 does reach a plateau because of saturated carriers.)

D. A carrier or permease must bind tightly to the amino acid that it transports. There must be a correct fit in terms of shape and chemical structure between the amino acid and peptide P just as there must be a good match between any enzyme and its substrate. Since peptide P promotes the transport of some amino acids and not others, it must bind to the amino acid side chains. Serine and threonine have similar side chains ending in OH. Both side chains must fit into the binding pocket of the peptide P and so both amino acids are transported readily giving the kinetics shown in graph #3. The other amino acids have side chains that are quite different -- either charged or nonpolar (and, in the case of lys, much bigger). These side chains must not fit well into the binding site of peptide P, so the amino acids do not bind to peptide P, and their uptake is not promoted by peptide P. To get full credit for your answer, you had to explain the general principle and give specific details based on the structures of the amino acids involved.

E. Transport through gap junctions will have the same shape as graph #1 (linear) and transport through tight junctions will not look like either graph.

Gap junctions contain connexons that form channels from cell to cell -- small molecules like serine should pass freely through, diffusing down their gradients. The rate of transport should not saturate with [serine] as channel proteins do not interact significantly with the substances that pass through them. (You can call passage through channels "facilitated diffusion" but that refers to the need for a protein to make up the channel, not for a need for the protein to interact with the material it transports.)

Ordinary tight junctions do not contain any structure or protein to promote transport. They consist of tight seals between the extracellular domains of transmembrane proteins in one bilayer and the transmembrane proteins from another. Liquid and dissolved solutes should not pass across tight junctions from cell to cell. Even if serine can diffuse somewhat across a simple lipid bilayer (as in graph #1) it will not diffuse across the fused membrane structures (containing proteins, not just lipids) that form at a tight junction.

2-5. A. On the inside, the following should occur: Na^+ conc. should rise; K^+ conc. should fall, and ATP conc. should stay the same. What you are looking at here is the functioning of the ATP driven Na^+/K^+ pump. The pump is an asymmetric protein complex located in the plasma membrane that pumps in only one direction (per ion). When you turn the membranes inside out, the pump should still work to pump Na^+ in one direction and K^+ in the other, but since the pump faces the "other way" it should pump the ions in the opposite direction (from usual). So Na^+ should be pumped in, and K^+ out. The pump will function by hydrolyzing ATP, as usual, but ATP will be split on the outside, not the inside, as the side of the protein that binds Na^+ and splits ATP is facing out.

The class handout shows a kinase and a phosphatase acting at different stages of the pump cycle. These are not separate enzymes but integral parts of the pump itself.

B. Cl^- must leave and bicarbonate concentration must remain higher outside. The membranes can be sealed either way since the transport system involved here has no innate direction. In this case, you are looking at the anion exchanger of the red blood cell plasma membrane (band 3). In some older texts, this protein is referred to as the "anion channel," but its properties indicate it is an exchanger or antiporter, not a simple channel. Ion movements through this transporter are dependent on the gradients of bicarbonate ion (and Cl^-), not on ATP, so transport can occur in either direction (depending on the concentrations of the two anions). This can be considered to be a case of facilitated diffusion, or a case of secondary active transport. (One ion is driven up *its* concentration gradient by the movement of the other ion down *its* concentration gradient. In a normal cell, the bicarb gradient drives the reaction, but in a test tube either ion can push the other.)

The transporter protein involved here (band 3 protein) is an exchanger; if one of the anions flows down its gradient in or out, the other anion must flow in the opposite direction. One ion cannot move without the other. In the set up described here, you start with equal [Cl^-] on both sides and add extra bicarbonate on the outside. So bicarb will flow in, down its gradient, and Cl^- will flow out. (The exchange is a consequence of the structure of the transporter protein; some carrier proteins transport one ion at a time, and some transport 2 or more simultaneously.) If the bicarb concentration on both sides becomes equal, no more bicarb will flow in (net).

2-6. A. Simple diffusion, pinocytosis, and ion channels are ruled out.

The curve shown indicates that uptake is saturated (reaches a maximum rate) at high concentrations of X. This means that there must be a carrier or transport protein that binds to X, moves it inside the cell, and then releases X. At very high concentrations of X, all the binding sites on the transport protein molecules are occupied, the protein is working as fast as it can, and a maximum level of transport is reached. (In other words, there is a permease with a V_{max}.) RME, active transport, and facilitated diffusion (carrier mediated) all involve carrier proteins, but the other processes do not.

Ion channels contain proteins that confer specificity on the channels, but the proteins do not usually interact with (or bind to) the ions that pass through, and so the channels do not saturate.
(Channels can be opened or closed, but when the channels are open, the ions pass through them essentially by passive diffusion. The rate of movement of the individual ions through the channels is so fast that the rate never becomes a limiting factor except at extraordinarily high (non-physiological) ion concentrations. It is the concentration of ions, not the rate of passage of individual ions through the channel, that is virtually always limiting.)

B. Receptor mediated endocytosis.

The mouse antibody binds to the cell surface proteins (receptors) that normally bind to X, and then the fluorescent goat antibody binds to the mouse antibody. So the fluorescence you see indicates where the receptors are. (There is no X in this experiment -- you are using antibodies, not X, to identify the location of the receptors.) The simplest interpretation of the results is that the receptors cluster in coated pits and are then internalized by endocytosis. The coated vesicles formed by endocytosis contain the receptors and their attached antibodies, removing the fluorescence from the cell surface. (Some receptors go to coated pits, whether they have bound their ligand or not. If these receptors normally go to the coated pits only after they have bound their ligand, then the antibody mimics X and triggers movement of receptor to coated pit.)

Answer to 2-6, cont.

FYI for the experts only: If the goat antibody could cross link the mouse antibodies (if both were multivalent = had multiple binding sites for the other), patching or capping could occur (that is, all the fluorescence would end up in one patch called a "cap" on the surface). This could explain some, but not all, of the observed results. In this case, the two antibodies could cross link the receptors to form clusters (patches) and then caps. However, this process alone, in the absence of RME, coated pits, etc., would eventually produce a fluorescent cap on the cell, not the disappearance of the fluorescence. On the other hand, RME, without patching, can explain all the results. Note: patching and cap formation is mentioned here just in case you already know about it. It is the basis of a famous experiment that was thought to demonstrate the fluidity of membranes; it is now known that patching (and capping) is an active, energy driven process that occurs only in certain kinds of cells (those that are polarized and capable of locomotion).

C. Sparse or clusters – depends on the nature of the defect in clathrin function.

Clathrin makes up the "coat" of both coated pits and coated vesicles. It is thought to help concentrate the receptors in the coated pits and to promote formation of endocytic vesicles. If the clathrin doesn't work properly, coated pits may not form, or they may form and fail to trap receptors; in either of these cases the fluorescence will remain scattered. Alternatively, the clathrin may form pits and trap receptors but not invaginate; in this case the fluorescence will cluster, but remain on the cell surface. So no internalization is expected to occur, and the only question is whether the fluorescence on the surface will cluster. The answer will depend on what is wrong with the clathrin.

2-7. Receptor mediated endocytosis (RME) is most likely, and receptor protein is probably abnormal. (If E binds to a carrier protein, the way iron binds to transferrin, then the carrier protein could be abnormal, instead of the receptor.) RME is the only specific mechanism that brings E into the cell in a vesicle that must pass through the endosomal compartment (to separate receptor and ligand) before ligand is free in cytoplasm. If E came in through facilitated diffusion or active transport, it would be free in the cytoplasm first and then enter an endosome or lysosome if necessary.

2-8. A. See page 22. Please do not attempt to do the rest of this problem until you are sure of the answer to part A.

B. *In < 5 min*. Ligand binds to receptor and complex ends up in coated pit. Pits invaginate to form coated vesicles. So label should appear in coated pits before it appears in coated vesicles.

C. *Endosomes & coated vesicles; recycled back to the plasma membrane*.

Ligand binds to receptors --> coated vesicles --> endosomes, where the ligand and receptors are separated and sorted ---> 2 separate vesicles. Vesicle with ligand goes to lysosomes and is degraded; vesicle with receptor goes back to membrane and fuses with it.

D. *By autoradiography*.

Protein/lipid ratio determines density & equil. centrifugation separates on basis of density. So if P/L ratio is same, equil. cent. won't work.

If size and density same, diff. centrif. won't work either.

Autoradiography should work -- you would put a photographic emulsion over a sample of your cells, leave the coated sample in the dark to expose the emulsion, develop it, and count grains over each organelle. The more grains, the more radioactivity in each organelle. (If all organelles are the same size, you would also have to do something to identify which organelle is which -- probably an *in situ* enzyme assay for marker enzymes.)

2-9. A. *Stay the same at zero; increase*. Apical surface faces the lumen; basolateral surface faces **away** from the lumen. Glucose is transported across the apical surface by co-transport with Na^+ (by secondary active transport); glucose is transported across the BL surface by facilitated diffusion (not a case of active transport). In other words, there is no active transport of glucose across the BL surface. The only active transport is across the apical surface.
If you consume a lot of glucose, **active** transport of glucose across the basolateral surfaces of the epithelial cells should stay the same – there isn't any. **Transport** across the BL membrane will increase, but it will be an increase in non-active (passive) transport by facilitated diffusion.
If you consume a lot of glucose, active transport of glucose across the apical surfaces (by co-transport with Na^+) should increase -- unless the carrier is already at maximum capacity (which is unlikely).

B. You should get a graph like #2 for transport into muscle cells, into epithelial cells & out of epithelial cells. This type of curve (hyperbolic) indicates the presence of a transport protein that saturates with increasing concentration of substance moved. Such a curve would be observed for either facilitated diffusion (used to get glucose into muscle cells and out of epithelial cells) or with active transport (such as the Na^+/glucose co-transport used to get glucose into epithelial cells). You can't tell active transport and facilitated diffusion apart using graphs of influx vs. concentration. Transport in and out of capillaries is by simple diffusion; the graphs would look like #1 (linear).

2-10. A. Transport using a Na^+ gradient must be *antiport.* Na^+ must flow down its gradient in order to provide the energy to move protons. In a normal cell, the Na^+ concentration outside is much higher than the Na^+ concentration inside the cell. So if Na^+ flows down its gradient, the Na^+ must flow into the cell, not out. Since Na^+ flows in and H^+ flows out, this must be antiport.

B. Transport using hydrolysis of ATP as a source of energy is *primary active transport* (by definition). ATP is split to move both K^+ and protons against their respective gradients. ATP hydrolysis is moving both ions (as in the Na^+/K^+ pump). You might think that the ATP moves the K^+, and then the K^+ flows down its gradient, pushing the protons out. That won't work since K^+ is moving in, against its concentration gradient. (The voltage gradient across the membrane is negative, balancing some of the push of the K^+ concentration gradient, but moving K^+ in still requires energy.)

C. In the short run, *primary active transport* will be inhibited. In the long run, *both* will be inhibited. Explanation: Primary active transport is directly dependent on the energy derived from splitting ATP. When the ATP runs out, transport should stop immediately. H^+ transport that is dependent on the energy of Na^+ running down its gradient (secondary active transport) is not directly dependent on ATP. Proton transport will not stop immediately if the ATP runs out -- transport will continue as long as there is a gradient of Na^+ to provide the energy to push the protons. Normally the Na^+ gradient is maintained by the Na^+/K^+ pump, using ATP. If the ATP runs out, there will be no way to replenish the gradient, and it will eventually run down.

2-11. A. Receptor will be found in: **Coated vesicles,** then in **uncoated endocytic vesicles,** and then in **endosomes** (lower pH).

B. Ligand will be found first in **coated vesicles** and last in **lysosomes**.
Explanation: The receptor binds its ligand, the combination goes to a coated pit, and a coated endocytic vesicle forms by invagination that contains both receptor and its bound ligand. The vesicle is then uncoated and goes to endosomes (or becomes acidified and turns into an endosome). The ligand ends up in lysosomes, where it is degraded, so it goes from endosomes to lysosomes. We can't be sure where the receptor goes -- whether it's separated from its ligand in the endosomes and recycled back to the plasma membrane or whether it's sent with its ligand to the lysosomes.

C. Receptor R-1 that has bound ligand ends up in **lysosomes**; it separates from its ligand in **none of these**. In this case, both receptor and ligand stay together throughout their passage through the cell and both end up in lysosomes, where they are degraded. (The receptor and ligand do not separate in endosomes.) In the absence of ligand, receptor either stays on the surface or makes a "round trip" without being degraded, so it has a long half life. When ligand is present, receptor binds to it and both are targeted to lysosomes for destruction. So the receptor doesn't last as long -- it has a shorter half life.

D. Receptor R-2 is more likely to enter coated pits **if it is bound to ligand**. The receptor is **recycled** because the ligand and its receptor separate in the **endosomes**. In the absence of ligand, receptor remains on the cell surface. In the presence of ligand, the receptor-ligand complex enters coated pits, goes to endosomes and the receptor is recycled as explained above. When ligand is absent, all of the receptor is on the surface. When ligand is present, the total amount of receptor is the same, but some of it is passing through the cell during the endocytosis/exocytosis "round trip." So the amount of receptor on the surface is reduced, even though the total amount of receptor (and its half life) remain the same.

2-12. A. Answers: Na^+ must pass through the cytoplasm of the epithelial cells, using simple diffusion without a protein, enter the cells using channel mediated facilitated diffusion & exit the cells using primary (direct) active transport. The rate of Na^+ absorption will be proportional to $[Na^+]$ at low concentrations.

Explanations:

A-1. The tight junctions prevent the Na^+ from diffusing around the cells; the other junctions connect the epithelial cells to each other, but don't matter here. The EMS is inside the cells, but Na^+ doesn't necessarily enter it. (You didn't need to explain this.)

A-2: Na^+ uses a channel to cross the apical membrane -- this is an example of channel mediated facilitated diffusion. Na^+ then diffuses across the cell to the basolateral membrane by simple diffusion. The Na^+ is finally pumped out of the cells, across the basolateral membrane, by the Na^+/K^+ pump. (All, or virtually all, epithelial cells, have a Na^+/K^+ pump on their BL side.) The Na^+/K^+ pump uses ATP and is therefore an example of primary active transport. So you need both types of transport to get the Na^+ across the epithelial cells, all the way from the lumen to the interstitial fluid. The pump is not needed directly to move the Na^+ into the cells -- it is needed only indirectly to keep the Na^+ low and pull the Na^+ in down its gradient, across the *apical* membrane. However the pump is needed directly to allow the Na^+ to get out of the cells across the *basolateral* membrane. Note there is no glucose/Na^+ co-transport here. (All the glucose is absorbed earlier, before the liquid in the lumen gets to the colon. See part C.)

Explanations to 2-12 continued on next page.

Answer to 2-12, cont.

A-3. Absorption depends on two processes – diffusion of Na^+ into the cells through a channel, followed by pumping of Na^+ out of the cells into the interstitial fluid (IF). Channels don't saturate at physiological concentrations of ions, so transport across the apical membrane should be proportional to the [Na^+] in the colon at all levels of concentration (assuming the concentration inside the cells stays at the same low value). However, the pump does saturate -- if there is enough Na^+ and K^+ to transport, the pump reaches a point where it is pumping as fast as it can and adding more Na^+ won't make any difference in the rate. This will directly slow down transport of Na^+ from the cells to the IF and indirectly slow down passage from the colon into the cells. If the pump is saturated, the concentration of Na^+ inside the cells will increase, decreasing the gradient that pushes Na^+ across the apical membrane. So transport into the interstitial fluid will be limited by the capacity of the pump at high Na^+ concentrations.

B. After you add the analog, the rate of Na^+ leaving the lumen will slow down after a while, and the rate of Na^+ entering the interstitial fluid will slow down immediately. The Na^+/K^+ pump will stop immediately, since it needs to hydrolyze ATP in order to run. Therefore transport across the BL membrane will stop immediately. Transport across the apical membrane will not be immediately affected, since transport through the channel does not require ATP. However, transport through the channel does require a gradient of Na^+ that is lower in the cells than in the lumen. The chemical gradient that drives Na^+ in is set up and maintained by the Na^+/K^+ pump. (If the pump is blocked, the gradient will run down as Na^+ comes in from the lumen. (The voltage across the membrane may also change as positive Na^+ ions accumulate in the cytoplasm.) As the electrochemical gradient across the apical membrane runs down, the 'push' on Na^+ will decrease, and the rate of transport into the cell will decrease.

C-1. The GLUT protein will transport glucose into the cells.
The epithelial cells of the colon do not have any other glucose transporters (other than the GLUT), so they are not bringing in glucose from the lumen. (No glucose/Na^+ co-transport here.) Therefore the cells must be nourished by glucose from the blood just like almost every other cell of the body. They must take up glucose from the interstitial fluid, not the other way around.

C-2. Glucose transport will either stay the same or decrease. Your choice of answer will depend on your reasoning.
Adding the analog will have no direct effect on glucose transport. GLUT proteins are carriers that can carry glucose across a membrane in either direction, but must always transport glucose down its gradient. (This is carrier mediated facilitated diffusion). The transport is driven by the gradient of glucose concentration and is not driven by the supply of ATP. You don't need ATP directly for transport or indirectly to maintain a Na^+ gradient for co-transport. Therefore, ignoring any side effects, the answer is 'stay the same.' However, if you block ATP hydrolysis, you will block phosphorylation of glucose, which will allow the concentration of free glucose in the cell to build up. This should slow transport of glucose by reducing the gradient of glucose responsible for driving transport. So if you consider this aspect of transport, the answer is 'decrease.'

D-1. On a high sodium diet, you should find some ENaC in vesicle membranes. Under these conditions, cells don't need a lot of ENaC in their plasma membranes, so the ENaC is removed from the plasma membrane by invagination of the plasma membrane (endocytosis). The vesicles that are formed will hold the ENaC in their membranes until it is needed back in the plasma membrane. ENaC is not a soluble protein; it is inserted into a membrane as it is made, and always remains in one membrane or another, never in the lumen of a vesicle or in the cytoplasm. ENaC can be passed from one membrane to another by vesicle fusion.

D-2. After a switch to a low sodium diet, you expect to see exocytosis – that is, vesicles containing ENaC will fuse with the plasma membrane. This will insert ENaC molecules (back) into the plasma membrane.

Key to Problem Set 2R

2R-1. ***A-1. Secondary active transport; A-2. Carrier protein.*** Amino acids are too big and polar to diffuse across a bilayer without a protein. So a protein of some sort is necessary. Since the AA are pumped into the epithelial cells from the lumen, they will reach a high concentration in the epithelial cells, and can exit the BL membrane by facilitated diffusion -- going down their gradient.). Energy is necessary to pump the AA into the epithelial cells, but no additional energy is necessary to move the AA out of the epithelial cells. Amino acids are transported by one or more carrier proteins, in the same way that glucose is transported by GLUT proteins. Usually only water and small ions, such as Na^+, Ca^{++} and Cl^- go through channels.

B-1. ***RBC won't be able to make pepT from the added gene.*** Mature RBC have no membrane bound organelles. They don't have a nucleus or DNA and so they don't have the enzymes for transcription. (They also don't have ribosomes, but even if they did, they couldn't make mRNA to translate.) Note: RBC do have contents other than hemoglobin, a membrane, and an ankyrin spectrin web. They have additional enzymes for glycolysis and carbonic anhydrase (for converting CO_2 to bicarb and vice versa).

B-2. ***(1). Because there is no pepT in normal oocytes.*** The gene for pepT may be present (it depends what organism you got the oocytes from). The critical issue is that there is no pepT *protein* in normal oocytes. You have to add a genetically engineered form of the pepT gene that is specially designed to be expressed (turned on) in oocytes. (The added gene must have a special promotor and/or regulatory sequence that is recognized by the transcription enzymes of the oocyte.)

(2). Neither. (No proton gradient to provide energy for transport.)

(3). Net transport of peptides out of the oocytes. Transporters like pepT or SGLT can move the material they transport in either direction -- in or out. The direction of transport depends on the gradient of the ion driving transport. The ion (H^+ or Na^+) runs down its gradient, driving symport of peptides or glucose respectively. You can explain this in terms of energy -- the direction of the ion gradient determines which way the ion flows, and that determines which direction you run the cycle (which way you "turn the revolving door" or run the cycle on the handout) and therefore which way you transport the peptides or glucose. Or you can explain this in terms of how the binding of the ion (which occurs on whichever side has the highest concentration of ion) changes the affinity of the co-transporter for the peptides or glucose.

2R-2. ***A. SGLT protein*** (Both proteins are co-transporters); ***B. in the cytoplasm; C. yes***; (Glycosylation must occur on the lumen/extracellular side of the protein); ***D. split.*** (The chymotrypsin site is exposed on the outside of the cells.) Picture should show both ends of protein in the cytoplasm, and the correct sites for glycosylation and chymotrypsin splitting on the outside

2R-3. Optional choices and explanations are in parentheses.

A. Single bilayer? ***Lysosomes & Peroxisomes.***

B. In cytoplasm, but not part of EMS? ***Peroxisomes, MF, and mitochondria.*** (Nuclei are not usually considered to be "in the cytoplasm," but that was okay. Nucleoli are within the nucleus, so that was *not* okay. Desmosomes and FA are optional -- they can be considered part of the membrane and/or ECM or part of the cytoplasm.)

C. Functional ribosomes? ***Mitochondria***. (There are ribosomal subunits in the nucleolus, but not complete ribosomes.)

D. Connected to/contain IF? ***Desmosomes & Nuclei*** (Nuclei contain lamins. You could add MF, since all components of cytoskeleton are interconnected.)

E. Connected to/contain actin? ***MF, FA.***

Picture should show nucleolus within nucleus, mito, peroxo, and lysosomes in the cytoplasm, and FA and desmosomes on different parts of the membrane (as they are connected to different things -- the ECM or another cell, respectively)

2R-4. ***A-1. Apical part of the membrane; A-2. Decrease.*** (but see note below.) Tight junctions prevent the proteins and lipids on the two sides of the epithelial cells from mixing -- some proteins and lipids stay on the apical surface facing the lumen and some stay on the basolateral side, facing the body. PepT normally faces the lumen. When tight junctions are disrupted, the materials from the two sides of the cell surface can mix, and the fluorescent pepT that was located on the apical side should be spread out over the entire cell surface.

Note: "Stay the same" was accepted if you explained clearly that pepT was probably anchored to the cytoskeleton, and therefore did not move when tight junctions were disrupted. I am not sure of the actual experimental result for this protein, although I think it would spread around somewhat even if anchored. (Tight junctions also prevent passage of materials across the membrane in the spaces between the cells, but that is irrelevant here.)

B. Exocytosis. The only way to get both more fluorescence and more transport is by insertion of more fusion protein into the apical side of the membrane in response to hormone. There is no change in the total amount of fusion protein in the cells but the protein is moved from vesicles in the cytoplasm to the apical surface. When the fusion protein is inserted into the apical membrane, the GFP part of the fusion protein increases the fluorescence on that side of the cell and the pepT part increases peptide transport. How does fusion protein get added to the plasma membrane on the apical side? When fusion protein is made it is inserted into the membrane of the ER. From there it goes to the membrane of the Golgi, and then into the membrane of secretory vesicles (or the equivalent). Before hormone binding, the fusion protein is in the membrane of the vesicles, which are inside the cell. Hormone binding triggers exocytosis -- it causes fusion of the secretory vesicles with the plasma membrane. When the vesicles fuse with the plasma membrane, the fusion protein becomes part of the plasma membrane. This is analogous to the way insulin promotes insertion of GLUT4 into the membrane. The hormone involved here is also insulin.

Many people thought this was a case of RME -- hormone receptors were trapping both hormone and fusion protein. (Or hormone receptors = fusion protein.) This would increase the concentration of the fluorescence in coated pits (temporarily). However this wouldn't increase transport of peptides -- it would decrease transport, as pepT would be removed from the surface. In addition, this isn't feasible because hormone binding to receptors would occur on the BL side of the membrane, away from the site of pepT. (Hormones travel through the blood, so they would reach the BL side, not the lumen side.)

C. C-1. both; C-2. neither; C-3. lysosomes. Hormone will bind to receptors, and both will be internalized by RME into coated vesicles. The vesicles will be uncoated and fuse with (or mature into) endosomes. So both will be in endosomes. The fusion protein is not internalized by RME so no fluorescence will be found in uncoated (or coated) vesicles. (The hormone triggers exocytosis, not endocytosis, of vesicles containing fluorescence.) Only hormone is degraded, so lysosomes should contain hormone but not receptors. The receptor and hormone must separate in endosomes so the hormone can be directed to lysosomes for degradation and the receptors can be recycled back to the plasma membrane. (Exocytotic vesicles could be carrying receptors, but not hormone, back to the surface. These are not what is usually meant by "uncoated vesicles".)

2R-5. ***A. Raise cell pH.*** (H^+ leave, so $[H^+]$ goes down and pH goes up.) ***B. Stop after a while.*** Peptide transport is dependent on the proton gradient. If you inhibit the exchanger, you don't abolish the gradient. However as proton & peptide transport occurs, the gradient will run down and will not be replenished. Peptide transport will continue until the gradient runs down. Peptide transport is not directly dependent on the exchanger itself -- it is dependent on the proton gradient set up and (normally) maintained by the exchanger.

2R-6. ***A-1. Transport of glucose will stop immediately; A-2. Transport of peptides will stop after a while.*** Glucose transport is directly dependent on the sodium gradient. The ionophore will abolish the gradient almost instantaneously, so transport will stop right away. Peptide transport is indirectly dependent on the sodium gradient. In the absence of the gradient, peptide/proton co-transport will continue (as when the Na^+/H^+ exchanger is inhibited) until the proton gradient runs down. Sodium is needed to replenish the proton gradient, but it isn't needed directly for peptide transport.

B. Inhibition, but it would be slower than adding a Na^+ ionophore. When you add an ionophore, you destroy the Na^+ gradient, you prevent any more Na^+/H^+ exchange, and the proton gradient that runs pepT starts to run down right away. When you inhibit the pump, you don't destroy the Na^+ gradient -- you just let it run down and don't replenish it. Na^+/H^+ exchange doesn't stop until the Na^+ gradient runs down. Unlike with the ionophore, the proton gradient is replenished for a while, so the proton gradient lasts longer, and peptide transport goes on longer too.

Another way to look at it is that the process of peptide transport can be broken down into 3 steps:
(1) The pump generates a Na^+ gradient
(2) the Na^+/H^+ exchanger uses the Na^+ gradient to set up a H^+ gradient
(3) pepT uses the H^+ gradient to run peptide transport.

Adding an ionophore blocks (2) -- no exchange without a Na^+ gradient. PepT catalyzes (3) until the the H^+ gradient runs down.
Blocking the pump blocks (1) -- but it leaves the existing Na^+ gradient intact, so (2) can run for a while, and there is no immediate effect on (3). When the Na^+ gradient runs down, (2) stops and then pepT catalyzes (3) until the the H^+ gradient runs down.

2R-7. ***A. Transmembrane proteins; B. Inside the cell in the cytoplasm.*** (You are looking at the E face head on. The proteins that you see were transmembrane proteins with cytoplasmic and extracellular domains. The extracellular domain is behind the E face and you can't see it. The cytoplasmic domain was pulled out of the cytoplasm and is sitting on top of the E face (and on top of the intracellular domain).

C-1. 6 nm2. A single molecule of connexin is 6 nm^2 in cross section because it has 4 transmembrane helices at 1.5 nm^2 each. 6 molecules of connexin make up one connexon.
C-2. Gap junctions. In gap junctions you expect to see connexons, which are made of connexin. Although a single molecule of connexin should be only 6 nm^2 in cross section, you see particles that are 36 nm^2 in oocytes. This indicates that connexin associates into hexamers. Therefore when you look at gap junctions, you expect to see hexamers, not monomers, of connexin. (The hole in the middle of the connexon is relatively small compared to the diameter of the whole thing. See diagrams in text or in Google images.)

2R-8. A. H/K pump does antiport. (K^+ in, H^+ out of cell; analogous to Na/K pump.)
B. Into the lumen. That's what keeps the stomach acid.
C. Energy comes from hydrolysis of ATP.
D. H/K pump is on apical (lumen) side; Na/K pump is on basolateral (opposite) side.
Explanation: You need to show tight junctions, K^+ coming in from lumen, H/K pump and Na/K pump on correct sides, and correct drugs on extracellular part of each pump. (No picture is provided on purpose – we urge you to compare your picture with the pictures of other students.)

2R-9. A. Ouabain binds on side facing the interstitial fluid (basolateral side).
B. Omeprazole binds on the lumen (apical) side.
C. Omeprazole (Prilosec). You want to inhibit the gastric H/K pump from pumping protons (acid) into the stomach.

2R-10. A. None of these -- the H/K pump doesn't bind the drug.

B. *Of the choices given*, only loop between TM3 & TM4 binds the drug.

C. The amino end of the protein should be in the cytoplasm.

D. The carboxyl end should be on the same side as the amino end.

E. The two pumps should have their carboxyl ends on the same side, since they are homologous.

Picture should show a transmembrane protein with both ends in the cytoplasm, and 10 TM segments. Drug binds to both the extracellular loop between TM3 & TM4, and the extracellular loop between TM5 & TM6. (Remember protein is folded in 3D, so both loops can be close together.)

You know which parts of the protein bind the drug from the figure. A protein that has only the two loops between TM3 & TM 4 and TM 5 & TM 6 from the Na/K pump is inhibited by the drug. A protein with only one of these loops is not inhibited by the drug. Therefore these 2 sections (domains) must **both** be needed to bind the drug. Since these binding sections must be on the outside of the protein, that indicates how the protein is inserted in the membrane.

F. Of the sections listed, only the region between TM1 & TM2 is on the E side, facing the inside of the lumen (when in the EMS) or facing the outside of the cell (in the plasma membrane). Only sections on the E side can be glycosylated.

2R-11. A-1. Cl^- enters lumen by passive transport (down its gradient, through a channel).

A-2. Cl^- goes into the cell from the interstitial fluid and bicarb exits -- both pass through the anion exchanger on the basolateral side.

A-3. Carbonic anhydrase is needed, and the reaction goes toward formation of bicarb (& protons -- see B-1 & B-2).

CO_2 enters the parietal cells (by simple diffusion, see C). Carbonic anhydrase catalyzes conversion of CO_2 to bicarb. Bicarb is exchanged for Cl^- on the basolateral side, using the anion exchanger. Cl^- level in cell rises, creating a gradient -- higher on the inside of the cell, lower in the lumen. Cl^- then flows down its gradient into the lumen through the channel on the apical side.

B-1. Neutrality is maintained by adding (generating?) protons using the carbonic anhydrase reaction. (If you chose 'none of these' meaning that no protons were added to the cell in the form of protons, it is okay if you explained that protons were being generated by the CA reaction. Similarly if you chose 'removing protons' meaning that the H^+ were constantly being generated and removed, that is okay if clearly explained.)

B-2. Generation of protons requires adding (using up) CO_2. ('Addition' was intended answer, as CO_2 is constantly *added* to the parietal cells from the blood. If you chose 'removal' meaning constant *consumption* by the carbonic anhydrase reaction, that is okay if clearly explained.)

When the parietal cells are active they are constantly losing protons -- the protons are secreted into the lumen. To replace the lost protons, you need to generate more, using the carbonic anhydrase reaction. This requires a constant input of CO_2.

C. Simple diffusion. Gases diffuse through membranes.

D-1. K^+ moves into the lumen.; D-2. K^+ moves passively (through the channel on the apical side).

D-3. K^+ will be higher on the inside.

The pumps create a high $[K^+]$ inside. (Since ATP is being split to move K^+, the level of K^+ inside will exceed the level outside. The exact level inside will depend on the amount of ATP split, properties of the pumps, etc.) Once the pumps create a high $[K^+]$ inside, the K^+ leaks out the channel into the lumen, driven by its concentration gradient. (The pumping in and leaking out actually happen simultaneously, but it's easier to see what's happening if you think of them as happening sequentially.) As the positively charged K^+ leaves the cell, going down its concentration gradient, the cell becomes negative on the inside (relative to the outside). The negative charge inside the cell (or potential difference across the membrane) attracts positive K^+ ions back into the cell. Eventually enough K^+ leaks out so that the push out (due to concentration) is balanced by the push/pull in (due to the potential difference). As with the usual K^+ leak channels, this balance is reached when the $[K^+]$ inside greatly exceeds the $[K^+]$ outside. In other words, not very much K^+ has to leak out to achieve this balance, and the level of K^+ inside the cells remains high. (If you have a reservoir, filled by a pump, and the reservoir has a small, slow leak, what happens? As long as the pump runs, the water level in the reservoir can remain high.) The cell reaches a steady state when the rate of K^+ being continually pumped in (to maintain the high internal level) = the slow rate of K^+ leaking out. If the level of K^+ inside falls, because of the leakage through the channels, the pump will bring in more K^+, to replace what leaked out. But then the 'extra' K^+ leaks out until balance is reached again. In other words, the pumps initially build up a high supply of K^+ and then replace the K^+ that leaks out.

2R-12. Answers:

A-1. Inhibition of Nedd4 could be used for up-regulation of Na^+ transport;

A-2. Endocytosis is triggered by covalent modification (adding Q);

A-3. If Nedd4 activity increases, ENaC in the cell will decrease (because it's degraded in lysosomes);

A-4. New ENaC must be constantly synthesized.

Explanations:
Addition of Q triggers endocytosis (and/or targeting of ENaC in endosomes to lysosomes) -- ENaC with Q added is not recycled. Therefore new ENaC must be made and added to the plasma membrane by exocytosis to balance the ENaC removed by endocytosis and degraded in lysosomes.

How does Nedd4 fit in? If Nedd4 is inhibited, there will be less Q added, and so less endocytosis & degradation of ENaC. Therefore there will be more channels on the surface and more Na^+ transport. Increasing the activity of Nedd4 will have the opposite effect (more Q addition, more degradation). In this context, up regulation = increasing the rate of transport or number of channels, not increasing the addition of Q.

B. Uncoated endocytic vesicles would contain all the receptors; vesicles from endosomes containing ENaC would contain only the EGF receptor.

Explanations:

B-1. Many different receptors can be captured in one coated pit and end up in one endocytotic vesicle. Sorting of contents doesn't occur until the vesicle fuses with (or becomes) an endosome.

B-2. In the endosome, in the acidic environment, many ligands and receptors are separated, and the various components accumulate in different parts of the endosome. Substances to be degraded accumulate in one part of the endosome, and are enclosed in vesicles that bud off and eventually fuse with lysosomes. Substances to be recycled to the cell surface, accumulate in a different part of the endosome, and are enclosed in vesicles that bud off and fuse with the plasma membrane. Everything that is going to be degraded should end up in vesicles headed for lysosomes; everything to be recycled should end up in vesicles headed for the cell surface. Since ENaC and EGF receptors are both degraded, they should be in one type of vesicle going to lysosomes; the other receptors (LDL and transferrin receptors) should be in a different type of vesicle headed for the plasma membrane. (If you thought that ENaC was recycled, not degraded, then the vesicles with ENaC should be headed for the cell surface and should contain the transferrin and LDL receptors.)

Answer to Problem 2R-12, Part C, is on the other side of this page.

Answer to Problem 2R-12, cont.

C. People with Liddle's syndrome have too many channels, because endocytosis (or degradation) is too slow, and the last AA section has a site for enzymatic modification (addition of Q). Changing the activity of Nedd4 (either up or down) should have no effect if the site for enzymatic addition of Q (by Nedd4) is removed.

Explanations: C-1. If there is too much transport, either there are too many channels, or the channels are open too often, or the channels are located in the plasma membrane when they should be sequestered somewhere else. (Some of the channels could belong in vesicles until needed, as for GLUT4 or pepT.) In this problem, the second alternative is ruled out (since it says each individual channel transports Na^+ normally) and the mislocation solution is not listed as one of the choices. So the only possibility is too many channels. (We have simplified the situation -- in Liddle's syndrome, it looks as if there are too many channels AND each individual channel is open too often as well.)

Note: Even though individuals' channels don't saturate, having more channels increases the rate of Na^+ transport. If you open more windows, more air comes in. Or to use the door analogy, when there are more doors there are more opportunities for Na^+ to come in -- each ion is more likely to be close to a door (or channel) and more likely to enter the cells. Even though there is no line (no waiting) at any individual channel, the more channels there are, the more likely each Na^+ ion is to bump into a channel and enter the cell.

C-2 & C-3. Why too many channels? The simplest explanation is that the channels are made normally, but not degraded properly, because of the missing amino acids. The site for addition of Q is missing, so Q cannot be added, and the signal for endocytosis (&/or degradation) is missing. When Q is not added, either the channels are not endocytosed, or they are endocytosed but recycled, instead of degraded. (Which case is correct is not settled, and either explanation was fine.)

C-4. If the site for addition of Q is missing, it doesn't matter if Nedd4 is active or inactive (inhibited) -- the result is the same. You can't add Q if there is no place to add it to. If you thought ENaC had a receptor, then the missing amino acids must include the part that binds to the receptor. In that case, Nedd4 modifies some other part of the channel, and this normally promotes or prevents binding of ENaC to its receptor. If the site for binding to receptor is missing, failure to add Q (or adding too much Q) won't make any difference in this case either.

Note: The term 'enzyme activity' always refers to the rate at which the enzyme catalyzes its reaction. So a change in enzyme activity means a change in the rate of the enzyme catalyzed reaction. It never means a change in the substrate specificity of the enzyme -- it doesn't mean a change in what the enzyme adds or where it adds it. If you could change those properties of the enzyme, not just its activity, then a change in the enzyme might affect the symptoms.

KEY TO PROBLEM SET #3

3-1. A. The protein is a transmembrane protein, so it should be made on ribosomes attached to the ER. A protein must be made on ribosomes attached to the ER for it to be inserted into the plasma membrane as described below.

B. You would expect to find some of this protein in or attached to the Golgi membrane. The newly made protein would be inserted in the ER membrane (it is an integral protein of the membrane) → membrane of a transport vesicle → Golgi membrane → membrane of a vesicle → plasma membrane.

C. Protein should have 1 signal sequence (before it enters the ER), and 2 additional start transfer sequences. The number of additional stop transfer sequences, and where the COOH end is, depend on whether the signal peptide is removed or not. If SP is removed (which is the usual case), there should be 3 additional stop transfer sequences, and the COOH end should be on the cytoplasmic side. If the SP is <u>not</u> removed, there should be 2 stop transfer sequences (in addition to the SP) and the COOH should be on the extracellular side. In either case, the finished protein will have 5 hydrophobic domains.

Note: 'Start transfer' sequences and the SP itself start passage of the protein through the pore complex, and then lodge in the hydrophobic layer of the membrane. Therefore they do double duty as both 'start transfer' and 'stop transfer' signals. Since they are critical to staring translocation, they are usually counted as 'starts' not as 'stops.'

3-2. A. Simplest Solution:

A-1. The carboxyl end is in the cytoplasm in both cases.

A-2. Signal peptidase cuts the SP off for the 5 transmembrane (TM) model only.

A-3. The SP is internal, not at the amino end, and remains in the mature peptide, in the 6 TM model.

A-4. The first TM sequence is a stop in the 5 TM model. (It's the SP in the 6 TM model.)

Explanation:

The simplest solution is that the difference depends on the location of the SP -- whether it is on the amino end or not. Other solutions are possible (see below) and are acceptable if the answers and explanations all fit together.

If the SP is on the amino end, it is the first hydrophobic section, and it begins transport of the peptide into the ER. The second hydrophobic section acts as a stop transfer sequence to prevent further transfer of the peptide through the translocon. After the SP enters the ER lumen, it is cut off by signal peptidase, and the stop transfer sequence (2nd hydrophobic sequence) becomes the first TM domain in the mature protein. (The next hydrophobic section restarts the transfer, the next stops it, and so on.) When the process is finished, 5 TM domains remain, and the amino end is in the lumen and the COOH end is in the cytoplasm.

If the SP is internal, the amino end remains in the cytoplasm, and the first hydrophobic section (the SP) starts transport of the peptide into the ER. The first hydrophobic section is not removed, but remains lodged in the membrane, and constitutes the first of 6 TM domains. The second hydrophobic sequence is a stop transfer, the next is a restart, and so on. When the process is finished, 6 TM domains remain, and both ends of the protein are in the cytoplasm.

Alternative explanations of the 5 TM model:

(1) One of the internal hydrophobic sections is not transmembrane. One of the middle hydrophobic sections could be buried in the membrane but not pass through it, as in problem 3-3B. This will work if the amino end is in the cytoplasm, and the signal peptide is not removed. (There is an internal SP.) Such a protein has 6 hydrophobic domains, but only 5 transmembrane domains. This solution won't work if the amino end is in the lumen. In that case, the SP on that end must have been removed, and the remaining protein must have a total of 5, not 6, hydrophobic domains. (So it would have 4 TM domains if one hydrophobic domain were buried in the membrane.)

(2). Alternative Splicing/Processing. If all the exons are used, the gene could code for a protein with 6 TM regions, and all of them could be retained in the mature protein. In this case, both ends of the protein would be in the cytoplasm. (The protein would have to have an internal SP that is not removed.) Alternative splicing of the primary transcript could remove the exon(s) coding for one of the hydrophobic sections. The resulting protein would still have an internal SP and its amino end in the cytoplasm, but it would have 5 TM domains, and its COOH end would be in the lumen. (Addition of poly A before the section coding for the last hydrophobic section would have the same effect.) If the protein has an SP on the amino end, which is removed, alternative splicing won't explain these results.

Answer to Problem 3-2, cont.

B. Insig must have its amino end in the cytoplasm. The correct answer to this question depends on your model for A. The answer is 6 TM (if the 5TM model is missing a SP) or either way (for the alternative explanations given above).

Explanation: The mutations indicate that the amino end is responsible for the inhibitory effects of Insig. The proteins that are inhibited are in the cytoplasm, so the amino end of Insig must be in the cytoplasm. Therefore it could be any model that has the amino end in the cytoplasm.

Note: Insig is a real protein (there are actually 2, Insig-1 and Insig-2). Insig is involved in regulating transport of other proteins to the Golgi. Its function doesn't matter here. It got is name because the gene that codes for it is induced (turned on) by insulin. Insig = ***Ins***ulin ***I***nduced ***G***ene.

3-3. A. 4 transmembrane domains (= sections that go all the way across the membrane); 3 extracellular domains (= sections on outside of cell); carboxyl and amino ends should be outside. Hydrophobic regions (1, 2, 3, & 4) should be buried in the lipid bilayer as transmembrane domains; hydrophilic regions should be on outside of cell or in cytoplasm (= outside of bilayer). Sections A, C and E should be on outside of cell; B and D on inside. Protein should insert in membrane as shown in answer to problem 3-1, case B, BUT signal peptide should be removed, leaving both amino and COOH ends on the outside.

When it is made, the protein should insert into the ER membrane one hydrophobic region at a time, starting from the amino end. The first hydrophobic region to insert (the SP) should be removed, leaving the amino end of the peptide on the inside of the ER (corresponding to the outside of the cell). If the SP were in the middle of the peptide, or on the cytoplasmic side of the ER, it could not be removed.

The next hydrophobic region (#1) should act as a "stop transfer" sequence and anchor the peptide in the bilayer. The next hydrophobic region (#2) should act as a "start transfer" sequence to re-insert the peptide in the bilayer, #3 should act as a "stop transfer" and #4 as a "start transfer" sequence. The hydrophilic region after #4 should be pulled or pushed all the way through the bilayer, leaving the COOH end on the inside of the lumen (= outside of the cell).

B. 3 transmembrane domains, 2 extracellular domains, the COOH end on the inside and the amino end on the outside. Sections A and D must be outside to bind glutamate. The COOH must be on the inside, since reagents do not react with it in intact cells. The two ends of section #2 must both be on the cytoplasmic side of the membrane since they are both degraded from that side and not from the other. (If #2 went through the membrane, then one end would be degraded from one side and the other end would be degraded from the other side.) So section #2 is not a transmembrane domain -- most of it is buried in the membrane, but doesn't go across the membrane -- it enters and exits from the same (cytoplasmic) side. This leaves 1, 3 & 4 as transmembrane domains; B, C, & E as cytoplasmic domains, and A and D as extracellular domains.

3-4. A. Domain A is clearly the signal peptide, and it is removed after the protein is inserted into the ER membrane. If Protein P is inserted into the ER membrane in the usual way, domain A (before it is removed) will be inserted in the membrane with its carboxyl end near the lumen side, B will form a loop on the lumen side, C will be inserted in the membrane with its amino end near the lumen side and D will stick out on the cytoplasmic side. In other words, A will act as a "start transfer" sequence and C will act as a "stop transfer" sequence. Once the signal peptide, is removed, you will have a single pass protein with B (with the free amino end) on the lumen side. (See class handout.)

B-1. In the cytoplasm or membrane. B-2. secreted; B-3. in the cytoplasm.

The region coding for section A is the signal sequence. If it is missing, what will happen depends on whether the protein can still bind the SRP or not. If the protein doesn't bind the SRP, it cannot be co-translationally imported into the ER and will remain in the cytoplasm.

It is likely that any hydrophobic sequence, such as C, can bind to the SRP, and act as a signal sequence. In that case, the protein (w/o A) will end up inserted into the ER, using C as both a start and stop transfer sequence (and signal peptide). In this case, the protein will be inserted in the opposite orientation to the usual, so you will have a single pass protein with B (with the free amino end) in the cytoplasm.

If you need a special sequence (to bind the signal recognition particle) not just any old hydrophobic sequence, then region C should not be able to act as a signal sequence and substitute for the missing region A. In that case, the protein will remain in the cytoplasm.

If the region coding for section C (only) is missing, the protein should be transferred all the way through the ER membrane into the lumen; it shouldn't stay in the membrane since there will be no hydrophobic region to anchor it (once region A is enzymatically removed inside the lumen.) From the lumen of the ER, it should follow the default pathway through the Golgi, transport vesicles, etc. to the plasma membrane. When the transport vesicle containing the protein fuses with the plasma membrane, its contents, including protein P, should be released to the extracellular space. (If region A were not removed after transport, the protein would end up in the plasma membrane.)

If the regions coding for sections A and C are both missing, the protein will never enter the ER, so the protein will remain in the cytoplasm.

C-1. inside; C-2. B; C-3. outside.

The inside of the ER corresponds to the outside of the cell. The enzymes that add carbohydrates are inside the ER and Golgi, and only the parts of proteins that are inside these organelles can be glycosylated. The parts that are inside the lumen of the ER or Golgi correspond to the parts that are inside transport vesicles and will end up on the outside of the cell. Therefore, carbohydrates should be added only to the parts of protein P (and all membrane proteins) that are outside the cell. Since protein P ends up with its amino end (part B) on the outside of the plasma membrane, part B should be on the inside of the vesicle and have carbohydrate; the carboxyl end (part D) should be outside the vesicle.

D-1. Golgi; D-2. ER; D-3. T. Grains represent radioactive precursors that have been incorporated into (added to) proteins. Radioactive precursors to sugar S are incorporated only in the Golgi, not in the ER, so S must be added in the Golgi. Precursors to T are incorporated right away in the ER, but do not show up in the Golgi until later, and the amount of radioactive T found in the Golgi is the same as the amount found in the ER.

The simplest explanation is that T is added in the ER, and then the protein carrying the radioactive T moves down the assembly line to the Golgi, where S is added. (No T is added in the Golgi; all radioactive T found in the Golgi was originally added in the ER.)

3-5. A. 4 membranes -- 2 around the mitochondrion and 2 around the nucleus. (There is none around the nucleolus.) The double nuclear membrane is sometimes called the nuclear envelope and considered a single structure composed of flattened sacs instead of a double structure composed of two bilayers of membrane.

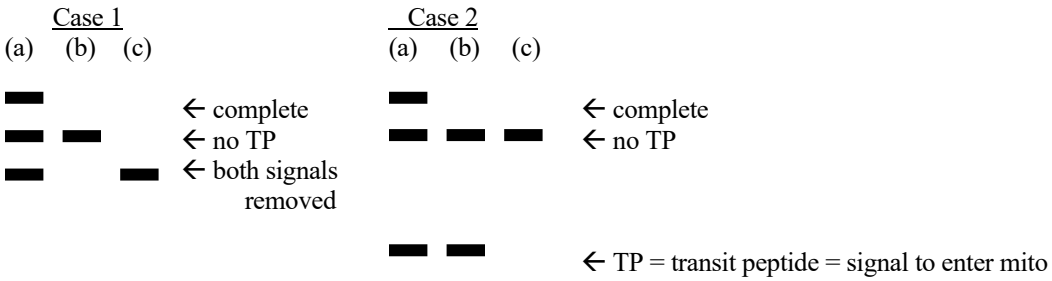
B. The answer depends on what you call "passing through a membrane." There are probably only 2 "crossing events" here, one to get across the 2 mitochondrial membranes at a contact point and one to pass through a nuclear pore (which may or may not be counted as passing though a membrane -- you're really passing through a gate or channel in the membrane).

3-6. A. Two localization signals or sequences (LS's) are required, one to enter the mitochondrion (a transit peptide or TP) and one to insert into the inner membrane.

B. One or none. The TP is used to enter the mito., and is removed after the protein enters the matrix. The second LS is probably removed too, but can be retained and used to anchor the protein in the inner membrane. (A separate stop transfer sequence is more likely to anchor the protein in the inner membrane.)

C-1. The three bands probably correspond to complete peptide (+ both LS's), processed peptide without the TP, and processed peptide without any LS's. (In this case -- case 1 -- all three bands would be of high molecular weight.) Alternatively, if second LS is not removed, the three bands could correspond to complete peptide, peptide minus the TP, and the TP itself. (In this case -- case 2 -- two of the bands would be of high molecular weight and one would be much smaller. This scenario is less likely since transit peptide is probably degraded rapidly after its removal.)

C-2. Gels should look like this:



Note: In case 1, a few peptides that are still "in transit" through the inner membrane might still have their second signal, so you might find a few medium length peptides (in case 1) in the inner membrane. The signal to enter mito. is removed before transit into the matrix is complete, so no complete length peptides are expected in the matrix in either case.

3-7. A. Gene must be nuclear; no DNA in peroxisomes.

B. One signal -- to enter peroxisomes (to cross the single membrane).

C. One -- signal/transit peptide is not removed on entering peroxisomes.

D. Only one -- protein is not shortened by removal of signal/transit peptides.

3-8. A. Nascent peptide chains (nascent = still attached to ribosomes) that are destined for mitochondria have TP's on their amino end. When you block protein elongation, the nascent chains attach to mitochondria because the amino ends of the nascent peptides bind to receptors on the outside of the mitochondrion. Normally this wouldn't happen because protein synthesis would be too fast -- the peptides would be finished and released from ribosomes before they would get a chance to meet a mitochondrion. When unfinished chains destined for mitochondria lie around in the cytoplasm, they eventually bind to a mitochondrion and the nascent chains enter as far as they can, trapping the ribosomes on the outside of the mitochondrion.

B. The peptide sequences for binding to and entering peroxisomes are on the carboxyl ends of the peptides. If the peptides don't get finished, there is no signal; there is nothing to bind to the receptors on the outside of peroxisomes.

3-9. A. This is an example of complementation. Hurler's and Hunter's patients lack different enzymes in the same breakdown pathway, so each is blocked at a different step. (One is aaB_, and has no enzyme A and the other is A_bb and has no enzyme B.) The hybrid cells make both enzymes and can carry out the whole breakdown pathway.

B. The scavenger pathway takes up the extracellular enzymes and targets them to lysosomes. Receptors on the cell surface bind the extracellular enzymes by their mannose-6-phosphate, and the cell internalizes the enzymes by receptor mediated endocytosis --> coated vesicles --> endosomes --> lysosomes.

C. No. First of all, enzymes targeted to the cytoplasm, mito., etc. do not enter the ER, so they can not accidentally take the default pathway and end up outside a cell. So they aren't likely to be in the medium. Even if missing enzymes from other compartments were present in the medium, the extracellular enzymes would not bind to receptors and be efficiently taken up; even if they were taken up by pinocytosis (without receptors) they would be destroyed in lysosomes.

D. Treatment with proteases will destroy the peptide chain; treatment with periodate or phosphatase will destroy the mannose-6-phosphate that binds to the receptors. Nuclease treatment should have no effect on enzyme uptake or function.

3-10. A. Proteins from one Golgi stack (stack = a pile of disk-shaped sacs) have been modified by enzymes from another, so vesicles must have ferried protein and/or enzymes from one stack to another. If you assume that vesicles move only in the forward direction, these results support the stationary (= vesicle shuttle) model, and the vesicles must carry the protein substrates (not the enzymes) forward. According to the progression model, proteins (& enzymes) should progress though a single stack and not be able to switch stacks, so no viral protein should be modified.

The sequence of events (*in vitro*) should be as follows: Vesicles must bud off from the cis or medial Golgi from the infected cells, carrying unmodified proteins from one stack, and fuse with the medial or trans Golgi from the uninfected cells. The proteins are then modified in the trans Golgi from the other stack, which contain normal modifying enzymes.

B. (1). If vesicles move in the retrograde direction (*in vivo)*, the vesicles must carry the "used" modifying enzymes back from one saccule to the previous one, so they can be reused. (In the previous case, the vesicles were assumed to carry the substrate proteins, not the enzymes that modify them.)

(2). In the Rothman experiment, proteins from one stack were modified by enzymes from another. Either the substrates (proteins) were ferried from one stack to another OR the modification enzymes were transferred. (In the original interpretation of the experiment, as in part A, the possibility of ferrying enzymes by retrograde transport was not considered.) If vesicle transport is retrograde, it is probably the enzymes that were transferred from one stack to another, not the substrate proteins. According to this model, the sequence of events *in vitro* is as follows: vesicles carrying modifying enzymes must bud off from the medial or trans Golgi from the normal cells and fuse with the cis or medial Golgi from the infected cells. The unmodified proteins from the infected cells can then be modified by the enzymes delivered by the vesicles.

3-11. A. Typical Marker enzymes:

Peroxisomes -- catalase, urate oxidase; lysosomes -- acid phosphatase (& other acid hydrolases)

ER -- glucose-6-phosphatase, cytochrome c reductase or cytochrome b5 reductase are usually used. Signal peptidase (for removing SP) should work too. Other typical enzymes of the ER are involved in lipid biosynthesis and first steps of N-glycosylation (although these are not usually used as markers.)

Golgi -- nucleoside diphosphatase, thiamine pyrophosphatase (function unknown); other characteristic enzymes are glycosyl tranferases and glucan synthetases.

Mitochondria -- cytochrome oxidase (and other carriers/enzymes of electron transport, Krebs cycle, and ox. phos.)

Note: the point here is to think about what enzymes are localized in the various organelles and/or to become familiar with the names of some the standard enzymes used in real experiments. Some of the enzymes actually used as marker enzymes are not ones you are likely to think of because their function is obscure (but they turn out to be easy to assay and unique to one organelle). You are not expected to memorize all of these marker enzymes – you are only supposed to remember the obvious ones.

B. To look at enzymes *in situ* you want reactions that will give precipitable products that are colored (for the light microscope) or electron dense (for the EM). This way you can pinpoint the positions of the respective enzymes. For isolation and purification, you usually use reactions that give soluble products (preferably colored), because you want to measure the amount of enzyme activity in each fraction or test tube, and soluble products are usually easier to quantify. (It's usually easier to measure the amount of color in a solution than in an insoluble precipitate.)

3-12. A. See Page 42. Please do not attempt to do the rest of this problem until you are comfortable with the answer to part A.

B. Catalase is the only enzyme listed that is found in (& characteristic of) peroxisomes.

C. Outside the cell. The 5' end of the mRNA codes for the amino end of an extracellular protein, so the 5' end of the mRNA should code for a signal peptide. The 3' end of the mRNA codes for the COOH end of an enzyme found in peroxisomes, so the 3' end should encode a peroxisomal localization signal. Therefore the recombinant protein should have a signal peptide (to enter the ER) on its amino end and a peroxisomal localization signal on its carboxyl end. Since translation starts at the 5' end, the growing recombinant protein chain will be inserted into the ER as it is made. (Once it enters, the SP will be removed since collagen is in the extracellular matrix, not attached to the cell membrane.) Once the recombinant peptide is inside the ER, its peroxisome localization signal can not be used. The signal will be present, since it is on the COOH end of the peptide, but the proteins that recognize it are in the cytoplasm and can not enter the ER to read the localization signal. There are no other localization signals, so the recombinant protein will follow the default pathway to the outside of the cell (ER --> Golgi --> transport vesicle --> exocytosis).

3-13. A. Lumen side. Lumen side = extracytoplasmic or extracellular side = E side = half of bilayer closest to lumen (for structures inside the cytoplasm) and closest to the outside of the cell (for structures in the plasma membrane). The E 'face' = surface on inside of E half of bilayer.)

B. Cytoplasmic side, because all lipid precursors are in the cytoplasm and lipid synthetic enzymes are on the cyto. side of ER membrane. So cyto side is where all new lipids are made.

C. Lumen side. See answer to A. Carbohydrates are found on extracellular surface of plasma membrane, not on cyto side. What's on lumen side is destined for extracellular space (or inside of organelle.)

D. Cytoplasmic side. SRP = signal recognition particle, binds to signal sequences on nascent (still growing, incomplete) peptides destined for ER.

E. Cytoplasmic side is more likely (following the model discussed in class and shown on handout). Peptides enter by a looping process, and original amino end is at one end of loop, near entry point from cytoplasm -- the amino end is not usually pulled through into lumen. It is side of the loop toward the carboxyl end that is the "loose end" and it probably is pulled/pushed through the membrane as it is made. (For post-translational transport, the "loose" end of the peptide, the end that's pulled through, may be the amino end, not the carboxyl end. In this case, the amino end would be all the way through the ER membrane, on the lumen side.)

F. Lumen side. LDL receptor will end up on outside of cell (same reasoning as in C).

G. Cytoplasmic side; see B.

H. Made by ribosomes on cyto side, and found on both sides. Finished protein is an intrinsic (integral) transmembrane protein that sticks out both sides. Protein is inserted into ER membrane as it is made.

3-14. A. <u>AP & EX.</u> AP (= Acid phosphatase) is a lysosomal enzyme; it must pass through (and bud off from) the Golgi to reach the lysosomes. EX (= Extracellular protein) must also pass through and bud off from the Golgi to reach the extracellular space. The transport vesicles that bud off from the Golgi must fuse with the plasma membrane to deposit their contents outside the cell.

B. <u>Cat, Hexo. H1 & K</u>. Cat (= catalase) is a peroxisomal enzyme; it should be made on cytoplasmic ribosomes and then transported post-translationally into peroxisomes.

H1 (= histone H1) nuclear protein; it should be made on cytoplasmic ribosomes and then transported through nuclear pores into nuclei.

Hexo. (= hexokinase) is a cytoplasmic enzyme; it should be made on cytoplasmic ribosomes and released directly into the cytoplasm.

K = an enzyme of the Krebs cycle = an enzyme located in the mitochondrial matrix. It should be made on cytoplasmic ribosomes and transported post-translationally into the mitochondria.

Answer to 3-14, cont.

None of these proteins enter the endomembrane system.

C. Hexo. Enzymes that go straight to the cytoplasm don't need any signal at all, since they don't need to attach to ER and don't need to be sent any place special after they're made.

All the other proteins need some sort of signal to direct them to their proper location, although in some cases the localization signal may be absent in the mature protein, because the signal is removed once the protein reaches its destination. For example, mature EX and mature K have no localization signals, but newly made EX & K should include them. Newly made EX needs a signal peptide to get into the ER, and newly made K needs a TP to get into the mitochondrion.

D. AP, Cat, M. AP --> lysosomes, Cat --> peroxisomes, M --> Golgi.

3-15. A. Clathrin -- Found on cytoplasmic side of plasma membrane (also found on outside of coated vesicles and trans Golgi); function is to gather receptors (in coated pits) and/or help membrane invaginate to form (coated) vesicles from plasma membrane or trans Golgi.

Docking Protein -- Found on cytoplasmic side of rough ER membrane. (It is actually an intrinsic membrane protein, but part that binds SRP sticks out on cytoplasmic side.) Function is to act as SRP receptor (signal recognition particle receptor or SRPR); binds SRP so signal peptide can enter ER and start transfer of nascent peptide to lumen of ER. Once peptide enters ER, peptide and ribosome will remain attached to ER, and SRP can be released and reused.

EGF receptor -- Part (domain) that binds EGF is found on the outside of the cell on extracellular side of plasma membrane. (Entire protein - integral transmembrane protein.) Function is to bind EGF in medium or extracellular fluid. EGF can be released into medium from other cells or from same cell that has receptor.

EGF -- See above. If it is acting on a cell, it should be found on the extracellular side of plasma membrane (bound to receptor). If it is being made inside a cell, it should be found on the lumen side of ER, Golgi, or vesicle prior to its release.

Lamin -- Found on inner (nucleoplasmic) side of the inner of the two nuclear membranes (or nuclear envelope). Provides support to nuclear membrane.

Signal Peptidase -- Usually refers to enzyme found on lumen (or E side) of rough ER that removes signal peptide during co-translational transport into ER. (Can also refer to similar enzymes in lumen of mitochondria or chloroplasts that remove signal or transit peptides post-translationally.)

B. E = EGF receptor, S = Signal peptidase of ER, EGF, and Docking Protein.

E -- Ends up on outside of cell, so should be made on ER and processed through endomembrane system. Remains attached to membrane on inside of ER, vesicle, etc. until vesicle fuses with plasma membrane.

S -- Is inside ER (not clear if on inner face of ER membrane or free in lumen) so needs to be made on ER and enter lumen. May or may not remain attached to ER membrane.

EGF -- Exits the cell, so should be made on ER and processed through endomembrane system. Remains in lumen of ER, vesicle etc. (not attached to membrane) until vesicle fuses with plasma membrane, releasing EGF to outside.

D -- Is integral membrane of ER, so must enter ER and remain inserted in membrane. May be retained in ER or passed on to Golgi and sent back to ER.

C. EGF, C = clathrin, L = Lamin.

EGF -- Since it's secreted, and not attached to a membrane, its signal peptide must be removed.

C -- Since its not made in ER in the first place, it never had a signal peptide. (It must have some sort of signal that sends it to the inner face of the plasma membrane, but that's not what is meant by a signal peptide.)

L -- Made on cytoplasmic ribosomes (like C) so never had a signal peptide. Does have a nuclear localization signal that is not removed.

Note: it is not clear whether SP of signal peptidase is removed or not (see part B above) so it was okay if you said it was removed or wasn't.

3-16. A. **The amino end of the single pass protein (A) should be in the lumen of the vesicle**. When a vesicle buds off the ER or Golgi, what was inside the lumen of the organelle ends up inside the lumen of the vesicle; when the vesicle fuses with the plasma membrane, what was inside the lumen ends up outside the cell.

B. **Both proteins should be made on the ER.** All the transmembrane proteins of the plasma membrane (but not all the transmembrane proteins of the cell) are made on ribosomes attached to the ER. These proteins enter the ER as they are made and then go to the Golgi, and from there (by vesicle) to the plasma membrane.

C. **Signal peptidase recognizes a sequence in the single pass protein (A) only.** If the signal peptide (SP) **were** removed from the multipass protein, it would have only 3 transmembrane segments. If the SP were **not** removed from the single pass protein, it would have 2 transmembrane segments -- assuming in both cases that the proteins are inserted co-translationally, starting with the SP.

The single pass protein must have had a signal peptide on its amino end, and a stop transfer sequence in the middle of the chain. The SP was inserted into the translocon (pore) in the ER, and then the growing chain passed through the pore in the ER as the chain grew, until the stop sequence entered into the pore and "got stuck" in the membrane bilayer. The remainder of the protein, including the COOH end, remained in the cytoplasm as it was made. The SP was cleaved off by signal peptidase in the lumen, leaving a single pass protein with its amino end in the lumen of the ER. So there must have been a sequence recognized by signal peptidase near the end of the SP.

The multipass protein has 4 transmembrane domains, and the last intracellular domain (the COOH end) is in the cytoplasm. Working backwards, starting from the COOH end and going back and forth across the membrane 4 times, the amino end must be in the cytoplasm or at the end of the first transmembrane domain. The SP could be at the very amino end or further down the chain -- in either case, the SP constitutes the first transmembrane sequence, and the place where movement of the chain into the pore starts. The rest of the chain is inserted as it grows; the 3 remaining transmembrane domains act as stop-start-stop sequences to lock the protein into the membrane. If the SP were removed, the protein would lose one of its transmembrane sequences. Therefore there must not be a sequence in the peptide recognized by signal peptidase. (It's not that the enzyme ignores a sequence -- the correct sequence of amino acids is not found in the protein.)

3-17. A. Free in the cytoplasm; no. IP1 acts as glue to hold clathrin to the inside (cytoplasmic side) of the plasma membrane. IP1 should be found either in the cytoplasm (when unphosphorylated) or stuck to clathrin and/or the inside of the plasma membrane (when phosphorylated). IP1 should not be an integral part of the plasma membrane but it should be found coating it and/or vesicles formed from it. IP1 must lack a signal peptide and be made on free ribosomes in the cytoplasm so it can be released into the cytoplasm. Then it can attach to clathrin (which is in the cytoplasm) and the plasma membrane. If IP1 had a signal peptide, it would enter the ER and be exported out of the cell (if SP removed) or become an integral part of the plasma membrane (if SP not removed).

B. IP1 should be found on the outside of coated vesicles and coated pits. (It was okay if you said it was "in" vesicles or if you said it was "on" but not "in" the vesicles.)

Uncoating should require a phosphatase to return IP1 to its unphosphorylated "unsticky" state. The likely series of events here is as follows:

(1) IP1 and clathrin are present in the cytoplasm. IP1 is phosphorylated --> IP1-P.
(2) IP1-P binds to a receptor in the plasma membrane and clathrin sticks to the IP1-P (or IP1-P binds to clathrin first, and the combo sticks to the membrane).
(3) The clathrin--IP1-P--receptor complex clusters in coated pits (and promotes curving of the membrane). When a vesicle buds off, the complex coats the vesicle.
(4) Shortly after the vesicle forms, a phosphatase converts IP1-P ---> IP1 (+ free phosphate). This causes clathrin & IP1 to disassociate from the membrane of the vesicle.
(5) End result: Vesicle is uncoated, and clathrin + IP1 are released into the cytoplasm ready to start again.

The data in this problem imply the need for a phosphatase for uncoating. Information provided in lecture and in some texts indicates that RME requires an input of energy, and the uncoating step may require hydrolysis of ATP. (Alternatively, some other step(s) in the process of RME may be the one(s) that requires ATP.) Even if ATP is split during uncoating, that doesn't mean a protein kinase is necessarily involved. There are many enzymes that split ATP that are not protein kinases because the phosphate is not used to phosphorylate a protein. If the scheme works according the steps listed above, a kinase (and ATP) are needed for coating in step (1), not for uncoating in step (4).

3-18. A. Ceramidase should end up secreted, and the SAPs should end up in lysosomes.
Explanation (not required): Ceramidase can't reach lysosomes unless it can bind to the M6P receptor. If ceramidase has no M6P (as in ICD) or the receptor that binds M6P isn't available, the result will be the same -- ceramidase will follow the default pathway and be secreted. SAPs reach lysosomes in ICD so they don't have to bind to the M6P receptor to reach lysosomes.

B. Normal people ***and*** people with ICD should take up ceramidase added from the outside.
Normal ceramidase should have M6P on it. Normal people, and people with ICD should have normal receptors. If there are receptors in the cell, some should recycle (accidentally?) from the endosomes to the plasma membrane instead of to the Golgi. So both normal people and those with ICD should be able to use the salvage/scavenger pathway to take in the added ceramidase using RME. Cells that have no M6P receptors will have no receptor for ceramidase, and will not be able to bind ceramidase for RME.
Note that the ceramidase here is normal -- it is not from a person with ICD. If the ceramidase has no M6P, even the cells with normal receptors won't be able to bind the M6P, so none of the cells listed will take it in the ceramidase by RME. (4/6 if you assumed normal ceramidase has no M6P, and explained the consequences properly.)
In a person with ICD, the receptors probably recycle from Golgi to endosomes and back, even though they have no ligand. (Just as many receptors on the plasma membrane recycle from the plasma membrane to endosomes and back, whether they have bound ligand or not.) Therefore some M6P receptors should be diverted to the plasma membrane. However, it is conceivable that 'empty' receptors do not recycle, and remain in the Golgi. In that case, the people with ICD won't have any receptors on their plasma membrane, and won't take up ceramidase. (This is okay if explained clearly.)

C. Sortillin should be concentrated in the trans part of the Golgi. Some sortillin should be found in two types of vesicles -- those going away from the Golgi toward the endosomes, AND those recycling back from the endosomes to the Golgi.
Explanation: Sortillin should help SAPS reach the lysosomes in much the same way that the M6P receptor helps hydrolases. In other words, sortillin is the SAP receptor. Both receptors should bind their respective cargo (hydrolases or SAPs) in the trans part of the Golgi. Then vesicles should bud off carrying both types of receptors (in the membrane) and both types of cargo proteins inside the vesicles. The vesicles should go to endosomes, where each receptor and its cargo should separate. Then vesicles carrying only cargo (SAPs and hydrolases) should go to (or mature into) lysosomes, and vesicles carrying only the receptors should recycle back to the Golgi.

D. The chimeric protein with the luminal & transmembrane domains of the M6P receptor and the tail (cytoplasmic domain) of sortillin should transport ceramidase (but not SAPs) to the lysosomes. SAPs & hydrolases bind to their respective receptors. They must bind to the part of the receptor that is inside the lumen of the Golgi. They can't bind to the cytoplasmic tail -- they must bind to the luminal domain. So which protein is picked up as 'cargo' will depend on which luminal domain the receptor protein has, not on the tail part. In this case, the luminal domain can bind to hydrolases (with M6P) but not to SAPs. The tail of sortillin must be responsible for transport to endosomes and/or lysosomes -- if you remove it, the normal sortillin cargo doesn't reach lysosomes. So the tail of sortillin should be just as good as the tail of the M6P receptor in sending proteins to lysosomes. (This experiment was actually done. See EMBO J. 2001 May 1; 20(9): 2180–2190.

E. The chimeric protein with the amino end of a mito. matrix protein and the carboxyl end of a SAP will end up in mitochondria. The protein has no signal peptide, so it will be synthesized entirely in the cytoplasm and will not enter the ER. It will never encounter the luminal domain of sortillin, so it will never go to lysosomes. (It's domain C will have no effect. It can't be 'read.') When the protein is finished, it will be transported (post-translationally, not co-translationally) into the mitochondrial matrix as usual, using the transit peptide on its amino end.

3-19. A-1. Both enzymes should be made in the cytoplasm on free ribosomes. (All peroxisomal enzymes are made on free ribosomes.)

A-2. Luciferase should co-localize with catalase. (The only peroxisomal enzyme on the list.)

A-3. Peroxisomes are surrounded by a single membrane (one bilayer).

B. The peroxisomal localization signal, the PLS, is on the carboxyl end of luciferase, and if an SP is added to the amino end, the protein should end up in the plasma membrane or outside the cell. (The luciferase will pass through the ER and Golgi, but it is not going to accumulate there unless it has the appropriate signal.)

Explanation: B-1. The data show that if you delete the amino end of luciferase it ends up in the right place, but if you delete the carboxyl end it never enters peroxisomes. This is how you know the PLS is on the carboxyl end. (Note: most peroxisomal enzymes have their PLS on the carboxyl end, but a few, including thiolase, have it on the amino end. See below.)

B-2. Once you put a signal peptide (which binds the SRP) on the amino end of luciferase, the ribosomes making the protein will bind to the ER, and protein will enter the endomembrane system as it is made. The protein may end up entirely inside the lumen or embedded in the ER membrane -- it will depend on whether the protein has any hydrophobic sequences that can act as transmembrane sequences, and whether the SP is removed. (Presumably they designed the recombinant protein to have a site recognized by signal peptidase, so the SP will be removed.) Once the luciferase enters the ER (lumen or membrane) it will be transported to the Golgi by a transport vesicle and from the Golgi to the cell surface by a default vesicle. If the luciferase is inside the lumen of the EMS it will end up outside the cell; if the luciferase is transmembrane, it will end up on the cell surface as part of the plasma membrane.

C-1 & C-2. Thiolase and Luciferase have different localization signals, and a receptor for the luciferase PLS is defective in type B cells.

Explanation for C: From the results, you can see that thiolase can enter peroxisomes with genetic defects of type B and D, & luciferase can enter peroxisomes with defects of type C & D. The enzymes you are adding are normal, so there can't be anything wrong with them -- they must have normal PLS's. If an enzyme doesn't enter the peroxisomes, it must be a defect in some protein involved in transport that is the problem. (It can't be a problem with the enzymes because they are normal.) Since type B cells can take up thiolase but not luciferase (and vice versa in type C cells) the two enzymes must use different pathways to enter peroxisomes. Therefore they must have different PLS's (and different receptors for those PLS's). The two pathways overlap, and use some of the same proteins for certain steps, but each pathway must have unique steps so that a defect in an uptake protein can wipe out uptake of one enzyme but not the other, as in type B and C cells. (One of the common proteins used by both pathways must be defective in A, and one of the other peroxisomal enzymes, not the import system, is probably defective in D.)

In type B cells, the pathway for uptake of thiolase into peroxisomes is okay, but the pathway for uptake of luciferase is messed up. This must mean there is a defect in some protein needed to take up luciferase, but not thiolase (and vice versa, in cell type C). There must be something wrong with the recognition of the luciferase PLS, but the recognition of the thiolase PLS is normal. (Remember, both PLS's are normal.) Given the choices listed, the defect must be in the receptor for the luciferase PLS.

Most peroxisomal enzymes use the same uptake pathway as luciferase, but a few use the thiolase pathway. A lot of this has been figured out by looking at peroxisomes from people with different diseases. If you are curious about the details see Motley et al, Differential Protein Import Deficiences in Human Peroxisome Assembly Disorders, J. Cell Bio, **125**, 755 -767 (1994).

3-20. yes, at a different time. The same ribosomes are used to synthesize cytoplasmic and ER-related proteins. If the protein contains an SRP recognition signal (i.e., a signal peptide), the ribosome will move to the ER and continue translation. Once the ribosome finishes synthesizing the ER protein, it will synthesize the next protein according to the signals in the amino acid sequence of the new protein.

3-21. A. Enzymes in lysosomes from a person with PX disease should be able to degrade the substrate(s) of **enzyme Z**. Why not substrate of enzyme X? Enzyme X won't work, so you won't be able to degrade its substrate. Why substrate of enzyme Z? Since the mutation is in the gene for a single lysosomal enzyme, and not in a processing or transport factor, all the other enzymes, including enzyme Z, should be okay.

B. In a normal person, which enzyme(s) should be enzymatically active, but only at low pH? **both enzymes**. Why both? All lysosomal enzymes have acid pH optima (or lysosomes are acidic).

***3-22. A.** In a normal person, which enzyme(s) should separate from M6P receptors in endosomes? **enzyme P**. Most acid hydrolases reach lysosomes by the M6P pathway, and this is the pathway disrupted in ICD. In the M6P pathway, the lysosomal enzymes bind to M6P receptors in the Golgi, and separate from the M6P receptors in endosomes. However, enzyme W must not use the M6P pathway, since enzyme W still reaches lysosomes in ICD.

B. Which of the following is most likely to help people without enzyme W? **treated to add or uncover M6P**. Enzyme W added from the outside must be picked up by the scavenger pathway (= RME by M6P receptors on the PM). Normal enzyme W does not use the M6P pathway, so normal enzyme W probably has no M6P on it, and the M6P must be added (or the pre-existing oligosaccharide must be modified to reveal it) for the enzyme to be taken up by the scavenger pathway.

This problem is based on the treatment of Gaucher's disease, in which the enzyme glucocerebrosidase is missing. http://www.gaucherdisease.org/gaucher-disease-treatments.php Glucocerebrosidase binds to a different receptor, not the M6P receptor, but is targeted to lysosomes by a similar salvage process (RME). The 'different receptor' recognizes a different sugar, not M6P, but proper glycosylation of glucocerebrosidase, which can only be achieved in animal cells, is required for the enzyme to bind to its receptor and to be an effective treatment.

Key To Problem Set 3R

3R-1. A-1. The transmembrane sequence that anchors AP in the lysosomal membrane is probably (a separate stop transfer sequence), AND

A-2. The enzyme that releases AP from the lysosomal membrane is probably found (on the lumen side of the lysosomal membrane) AND

A-3. The enzyme probably cuts (on the amino side of a stop transfer sequence) .

Explanation: The protein enters the ER using its SP on the amino end. If the SP were the only transmembrane segment, the entire protein would be inside the lumen (except for the SP) and the protein would not be a transmembrane protein with a cytoplasmic domain. So the protein must end up anchored in the membrane by a separate stop transfer sequence that is followed by a cytoplasmic section. Then the SP can be removed, as usual, leaving the protein with its amino terminal end inside the lumen, its transmembrane segment in the middle anchoring it in the membrane, and its carboxyl end in the cytoplasm. When the protein reaches the lysosome, an enzyme inside the lumen cuts next to the lumen side (which must be the amino side) of the transmembrane segment, releasing the AP into the lumen of the organelle. If enzyme cut on the cytoplasmic side of the transmembrane segment, the AP left would still be anchored in the membrane, not loose in the lumen.

If SP were left on in ER, and protein had a separate transmembrane segment too, one cut wouldn't release the AP → lumen of the lysosome. The protein would be anchored at two spots. The enzyme in the lysosome would have to recognize and cut twice, once next to the stop transfer sequence and once next to the SP. (And signal peptidase would have to NOT recognize either sequence.) This is possible, but seems less likely.

B-1. The sequence that targets AP to the lysosome should be (in cytoplasmic domain of AP), AND

B-2. It should be (some other sequence), AND

B-3. If the transmembrane segment of AP were deleted, then AP should end up in (extracellular space outside the plasma membrane). Explanation: The targeting sequence must be in the cytoplasmic domain, because a mutation there causes AP to end up in the wrong place. The cytoplasmic protein tail must bind to some protein in the cytoplasm or on the cytoplasmic side of the Golgi membrane that "collects" and targets membrane proteins to the lysosomes.

The targeting sequence can't be the usual one for adding M6P because in I cell disease (when M6P is not added) the protein still ends up in the proper place. The SP targets the protein to the ER, not the lysosome. The term "transit peptide" is usually reserved for sequences that target proteins to mitochondria and chloroplasts and the term "NLS" refers to sequences that target proteins to the nucleus.

If the transmembrane sequence is missing, the AP will enter the lumen of the ER, and there will be nothing sticking into the cytoplasm. The targeting sequence will be present, but it will be inside the ER instead of in the cytoplasm. So the sequence will not be able to bind to the cytoplasmic protein(s) that are its normal target, and the protein will follow the default pathway to the outside of the cell. (Since the SP is removed, the protein will end up outside the cell, not in the plasma membrane. If the SP is not normally removed, until the protein reaches the lysosome, then the protein would end up in the plasma membrane, anchored by the SP.)

C-1 & C-2. Probably in the Golgi (but see below); endosomes. M6P receptors in the Golgi normally bind to M6P that is attached to acid hydrolases destined for lysosomes. In the endosomes or sorting vesicles, the receptors and hydrolases separate (because of the acid pH of the endosomes) and the receptors are recycled back to the Golgi while the hydrolases are delivered to lysosomes. (Only a small fraction of the hydrolases and/or receptors are accidentally delivered to the cell surface.) If drug C interferes with the separation of hydrolases and receptors, it probably interferes with the acidification of the endosomes. (Lysosomes are acid too, but separation occurs before the hydrolases reach lysosomes.) If the hydrolases remain stuck to their receptors, the hydrolases will probably be dragged back to the Golgi with their receptors. So the hydrolases will never reach their intended target organelle, the lysosomes -- they will be carried back to the Golgi. If all the existing receptors become stuck to hydrolases, any new hydrolases will enter default vesicles and the new hydrolases will be secreted outside the cell. However receptor-hydrolase complexes already formed should not be diverted to the default pathway.

The pathway being followed here is not RME. It is synthesis of lysosomal enzymes. (Two different kinds of materials end up in endosomes. Some extracellular materials taken in by RME, end up in endosomes, and some materials made inside the cell and released from the Golgi also end up in endosomes. Both types of materials are normally separated from their receptors inside the endosomes.) In a normal cell, a few hydrolase receptors may reach the cell surface by the salvage pathway, but most of the hydrolase receptors are cycling between the Golgi and the endosomes; they are not cycling between the plasma membrane and the endosomes by endo/exo-cytosis. (Answer cont. on next p.)

Answer to 3R-1, cont.

Why should you expect the receptors to drag the hydrolases back to the Golgi? Why don't you expect the hydrolases to drag their receptors to the lysosomes? The receptors are transmembrane proteins and so are at least partially on the outside of the vesicles. The hydrolases are inside the vesicles. It is the receptors that normally target the vesicles carrying them to the Golgi. This makes sense, since the receptors, being transmembrane proteins, should have cytoplasmic tails that stick out of the vesicles. The cytoplasmic tails could stick to a protein on the surface of the Golgi and that could determine the final destination of the vesicles and receptors. Since the hydrolases destined for the lysosome should be completely inside the vesicles, they should not affect where the vesicle goes or what it sticks to.

It is possible that the tails of "full" receptors bound to hydrolases are folded slightly differently (have a different conformation) than the tails of "empty" receptors that have released their hydrolases. In that case, you can argue that the conformation of the tail determines where the receptor will go. Empty receptors may normally go back to the Golgi, but full receptors may be targeted to the lysosomes.

C-3. No. Acid phosphatase reaches lysosomes by a different mechanism than most hydrolases -- it does not use M6P receptors. You know this, as explained above, because people with I disease have AP in the right place.

3R-2. A. Both batches should have receptors; only batch B, made from plasma membrane (should bind fluorescent protein X).
Explanation: Vesicles made from plasma membrane (batch B) = fragments of plasma membrane resealed into spheres. These vesicles should have receptors on their outside surface, just like the plasma membrane, and bind fluorescent protein X. If you took this question to mean plasma membrane vesicles were the ones naturally formed from the plasma membrane during RME, then those vesicles should not bind tagged protein X, because the receptors would be on the inside, not the outside, of the vesicles. Partial credit was given for this answer & explanation.

Vesicles made from ER (batch A) should have newly made receptors (not yet shipped to the plasma membrane) but these receptors should be on the inside of the vesicle, so they should not bind protein X. Proteins do not pass membranes unless there is RME or some other special process involved, so fluorescent protein X could not get inside the sealed vesicles and bind to receptors on the inside.

B. You supply some cells with radioactive amino acids, etc.

B-1. You are likely to find radioactivity first in (exocytotic vesicles -- vesicles going to the plasma membrane on their way out) AND you are likely to find fluorescence first in coated endocytic vesicles.

B-2. You can conclude that in the case of protein X, (receptor) is recycled to the cell surface.
Explanation: Radioactive amino acids are used to make new proteins. The newest protein X receptors will be in exocytotic vesicles carrying the new receptors to the plasma membrane. Protein X receptors on the cell surface can bind fluorescent protein X and enter the cell by RME --> coated endocytic vesicles --> endosomes --> lysosomes (for degredation) or exocytotic vesicles (for recycling to cell surface). So the first fluorescence to enter the cell will be in coated vesicles. The labeling results indicate protein X (fluorescent) goes to lysosomes and the receptors (radioactive) go to exoctyotic vesicles.

3R-3. A-1. Extracellular domains will be B, & D; cytoplasmic domains will be A, C & E. Extracellular domains in the plasma membrane correspond to intra-lumenal domains in the vesicle.

A-2. No carbohydrates should be on A or 1. Domains A and 1 are cytoplasmic and transmembrane respectively; glycosylation enzymes are all in the lumen of the ER &/or Golgi and act only on domains in the lumen.

A-3. In the cytoplasm. The cytoplasmic domains remain in the cytoplasm no matter what organelle membrane the protein is in. Every time a vesicle buds off one organelle and fuses with another, the topology remains the same. Cytoplasmic domains remain outside the organelle and intra-lumenal domains remain inside.

B. The SRP should bind to domain 1. The SRP = the signal recognition particle. The SRP binds to the signal peptide, which is the first hydrophobic stretch in the peptide chain and the one that is inserted into the membrane channel to start passage of the growing chain into the lumen of the ER. Since the amino end of the peptide is in the cytoplasm, the SP cannot be on the amino end. The SP must correspond to the first transmembrane domain.

C. The bumps correspond to domain B, which is the extracellular domain. See answer to 1-14 part C for more details on connexin; answer to 1-17 for more on freeze fracture.

3R-4. A. H/K protein is probably transported to endosomes (en route to its final destination -- see C). The only other possible location, of those listed, is lysosomes. But the protein is not degraded, as no protein synthesis is required to activate the cells. So the protein can't be transported to lysosomes.

B. Clathrin. Plasma membrane is invaginating, forming a vesicle, and that requires a coat protein, clathrin in this case.

C. H/K pump is a transmembrane protein, so it must always be in a membrane -- it can't be inside the lumen of a vesicle or in the cytoplasm. Therefore it must end up in the membrane of a vesicle as a result of endocytosis (followed by additional cycles of vesicle formation and/or fusion.)

D. Process starts with clathrin.

Explanation: What is going on here is a cycle of endocytosis & exocytosis. Endocytosis removes and inactivates the pump (& the cells). Then exocytosis in response to a signal activates the cells by reinserting the pump back into the plasma membrane. There are medical implications of all this -- the drugs don't work unless the pumps are in the membrane. The process is like RME, but there is no receptor -- the protein being endocytosed is already a transmembrane protein. The pump protein moves laterally into a coated pit, and then a coated vesicle pinches off. The vesicle is uncoated, and the vesicle becomes or fuses with an endosome. When all the contents of the endosome are sorted out, the pump protein molecules end up in the membranes of vesicles. The pump remains in the vesicles as long as the cells are inactive. (In parietal cells the 'vesicles' are actually a group of tubes and vesicles under invaginations in the plasma membrane that are called "tubulovesicles.") In response to the correct signal, the tubulovesicles fuse with the plasma membrane, restoring the pump. and activating the cells. (The regulation of GLUT 4 by insulin works in a similar manner.)

3R-5. A. See Page 42. Please do not attempt to do the rest of this problem until you are comfortable with the answer to part A.

B. (i) The SRP is most likely to be found in some part of the ER (inserted in membrane of rough ER); (ii) The receptor domain of the SRP should be in the cytoplasm. Picture should show presidin A as a transmembrane protein in the ER membrane with its domain B on the cytoplasmic side of the membrane.

Just for the record:
For M6P: (i) The M6P receptor is most likely to be found in some part of the Golgi (inserted in membrane of trans Golgi sac) – but see answer on page 30 for details. (ii) The receptor domain would be inside the Golgi lumen.
For LDL: (i) The LDL receptor would be inserted in the plasma membrane. (ii) The receptor domain would protrude out of the cell.

3R-6. A & B. Presidin with multiple ubiquitins attached will end up being *degraded* in a *proteasome*. (Some membrane proteins with only one molecule of ubiquitin attached are targeted to lysosomes. This is what happens to ENaC – the 'Q' added by Nedd4 in problem 2R-12 is ubiquitin.)

3R-7. A. (i) Newly made molecules containing the complete amino acid sequence will first be detected *inserted in the membrane of the ER*; (ii) The protein will go in vesicles from the ER to the *cis* Golgi and from the *trans* Golgi to the membrane. Therefore, the last vesicles to get newly made molecules will be *vesicles that bud from the trans Golgi;* (iii) Domain X will be *inside the lumen of the vesicle*. For full credit you needed a picture, properly labeled, and some indication of how domain X gets from the inside of the vesicle to the outside of the plasma membrane.

B.(i) Neither; (ii) SP included in the 425 AA & nowhere; SP'ase doesn't cut presiding B. (iii) Entirely in the cytoplasm. Explanation should show how protein enters the ER membrane using an internal SP that is also a stop transfer sequence -- this leaves the part of the peptide before the SP (on the amino side of the SP) in the cytoplasm. The resulting protein should be anchored in the ER membrane by amino acids 201-226 = internal SP that is not removed. Recombinant protein is missing the SP, so it can't bind to SRP and ribosome making it can't attach to the ER. Ribosome will remain free in the cytoplasm, and release completed protein in the cytoplasm.

3R-8

A-1. Ribosomes making enzyme X should be attached to the ER.
A-2. Newest enzyme X should be in membrane of cis Golgi.
A-3. The tail (on the amino end) should always be in the cytoplasm.

The enzyme is still called "enzyme X" whether it is completely modified or in its final position or not. Therefore the 'newest' enzyme molecules should be the ones whose amino acid chains were made the most recently -- these are in the cis Golgi, on their way to the trans side. This is the way the term 'newest enzyme X' is usually used. (If you take 'newest' molecules of enzyme X to mean the newest completely modified ones that have already reached their final destination, then the newest enzyme molecules are in the trans Golgi.)

B-1. Best answer is stationary cisternal model; vesicle is moving toward the trans side, and enzyme will never enter a vesicle again. In the stationary model, new protein is transported from sac to sac in a cis to trans direction using the vesicles. You are looking at a vesicle carrying new enzyme X from a medial sac to a trans sac, and the enzyme X is on the way to its final destination. This answer assumes "newly made enzyme" means enzyme en route to its final destination, as explained above.

Many students took "newly made enzyme" to mean completely modified, finished enzyme X that is working in the trans Golgi to modify other proteins. The answers that fit this scenario are: cisternal progression model; the vesicle is moving towards the cis side, and enzyme is in a retrograde vesicle. In this scenario, the "new" enzyme X must have previously reached its final destination (and be indistinguishable from "old" enzyme X); the vesicle you are looking at is moving back (retrograde) toward the cis side, carrying the "old" enzyme X. According to this model, each maturing trans Golgi sac is moving up, cis to trans, carrying newly made protein, while the "old" enzyme X (and other "old" modifying enzymes) are being recovered from an "old" sac and are being moved back to join a "new" sac as it moves up from medial to trans. (The vesicles do not carry enzyme X all the way back to the cis side -- they move the enzyme back from one sac to the next sac in line.)

C-1. Mis-localized protein should be degraded in proteasomes.
C-2. Protein you inhibited is probably part of the SRP. SRP is required to bind to SP and ferry ribosome making new protein to the ER. If ribosome reaches the ER, the new protein chain can pass through the translocon into the ER lumen. (Enzyme X will remain lodged in the ER membrane; proteins without an internal hydrophobic region will pass all the way into the lumen.) If the SRP is not binding as effectively as usual, it will bind to and carry some ribosomes to the ER, but the remaining ribosomes will remain unattached to the ER, and release their proteins into the cytoplasm.
Inhibiting the signal peptidase should not affect transport through the translocon -- the peptidase acts on the inside of the lumen. However note that the signal peptidase has no role in location of this particular protein anyway -- enzyme X has an internal signal peptide that is not split by the peptidase, so inhibiting the peptidase would have no effect on localization of enzyme X. Inhibiting the coat protein would inhibit transport from one part of the endomembrane system to another, but would not affect distribution between endomembrane system and cytoplasm. Inhibiting the IF would reduce the amount of enzyme X, and problem states that you have the normal amount of enzyme X.

D. Location of acid hydrolase (but not catalase) should be affected. Acid hydrolase is found in lysosomes; catalase is found in peroxisomes. Proteins need to use the SRP (to enter the endomembrane system) to reach the lysosomes, but the SRP is not needed to reach peroxisomes.
Details: When inhibitor is added, and the SRP is not working properly, some of the ribosomes making acid hydrolase molecules will become attached to the ER, but some will remain unattached in the cytoplasm. The acid hydrolase molecules made by the attached ribosomes will enter the ER and reach the lysosomes, but the acid hydrolase molecules made on unattached ribosomes will remain in the cytoplasm. Therefore only some of the acid hydrolase molecules will reach their proper destination. However all the catalase molecules will end up in peroxisomes as usual. Proteins destined for peroxisomes are made on free ribosomes -- the proteins do not interact with the SRP and do not enter the endomembrane system. Therefore inhibition of the SRP will have no effect on location of catalase.

3R-9. A-1. M-N-Q-serine.; A-2. Only one sugar. Explanation:

Normal situation: the proteins to be modified pass through the Golgi regions in order, cis to trans. In the cis Golgi, enzyme Z adds sugar Q to the side chain of serine. In the medial Golgi, enzyme Y adds sugar N to the sugar Q. In the trans Golgi, enzyme X adds sugar M to the sugar N. The normal chain grows as follows:

	enzyme Z		enzyme Y		enzyme X	
	cis Golgi		medial Golgi		trans Golgi	
	Q added		N added		M added	
serine	→	Q-serine	→	N-Q-serine	→	M-N-Q-serine.
The serine is part of a protein chain, and the sugars are added to the OH of the side chain of serine.						

Hybrid situation: In the hybrid, no sugars will be added in the cis or medial Golgi. When the protein gets to the trans Golgi, sugar Q will be added. Why is this? In the cis Golgi, there is no enzyme to add sugar Q to serine. When the protein gets to the medial Golgi, enzyme Y is present, but it can't add sugar N directly to serine. (It will only add to the Q of Q-serine.) Therefore no sugars are added in the medial Golgi. When the protein gets to the trans Golgi, the hybrid enzyme Z (with the luminal/catalytic domain of enzyme Z) will add sugar Q to the side chains of serine. (Enzyme X won't do anything as there is no N-Q-serine for it to add sugar M to.) The chain in the hybrid grows as follows:

	no enzyme Z		enzyme Y		hybrid enzyme & enzyme X	
	cis Golgi		medial Golgi		trans Golgi	
	nothing added		nothing added		Q added	
serine	→	serine	→	serine	→	Q-serine.

There are two things to realize here.

(1) The protein to be modified moves through the Golgi in steps, and modifications occur in order -- if a reaction is skipped in an early step, the protein and modifying enzymes can't go back and make it up.

(2) The enzymes are specific, so each enzyme will only catalyze a particular reaction, using the proper substrates. Each enzyme will only add a particular sugar to a particular substance -- it can't catalyze addition of a different sugar, or catalyze addition of the normal sugar to a different substance. For example, enzyme Z can only add sugar Q to serine; it can not catalyze addition of other sugars (M or N) to serine or addition of sugar Q to a different amino acid or to a sugar.

B. The hybrid protein will end up in the medial Golgi. From the data given in the chart, it is clear that the transmembrane domain of the protein determines the final localization of the protein. (The luminal domain determines its catalytic activity, but not where it will be localized.)

C. The hybrid protein will end up outside the cell. The newly made hybrid protein will have the signal sequence of glycophorin. This will cause the ribosomes making the protein to attach to the ER, and start the newly made protein going through the translocon from the amino end. The signal sequence of glycophorin must be removed to produce a single pass protein with an extracellular amino end. So the signal sequence on the amino end of the hybrid protein will be removed. The hybrid protein will have no transmembrane section in the middle to anchor it in the membrane, so the entire protein will end up in the lumen of the ER. Proteins in the lumen with no additional localization signals go to the constitutive (default) pathway. The proteins are passed to vesicles that bud off the ER, and the vesicles fuse with the plasma membrane, releasing their contents to the outside of the cell.

Some students did not realize that the newly made hybrid protein would contain the signal sequence of glycophorin. Part credit was given for explaining that the protein will end up in the cytoplasm if it has no signal sequence.

Note: You can't assume that the protein will end up where glycophorin normally does because it has the localization signal of glycophorin. You have to consider all the parts of the protein that determine final location. (If the protein had a transmembrane section it would NOT end up outside the cell.) To figure this out correctly, and to get full credit, you had to consider the mechanism by which proteins reach their target -- which piece of the protein does (or does not) cause the ribosome to attach to the ER, which section causes the growing protein to enter the ER, which part causes the protein to lodge in the membrane or stay in the Golgi, etc.

KEY TO PROBLEM SET #4

4-1. **A**. The mini-chromosome is shorter than the naked DNA because the DNA in the chromosome is wound around histones → nucleosomes.

B. SV40 has 20-25 nucleosomes accord. to the Lodish & Darnell text. From calculations, you would expect 26. There are 200 base pairs (or .2 kb) per nucleosome, so 5.2 Kb/.2 kb = 26.

C. No. DNA in nucleosomes → ladder = sequence of bands = pieces of 200, 400, 600 ... base pairs if digestion is short enough, and pieces of about 140 (forming a single band on the gel) if digestion is longer. Digestion of naked DNA → smear = mixture of pieces of all different lengths, since DNA is cut at random, not just between nucleosomes.

4-2. **A.** Nucleosome formation.

The DNA in chromatin is in nucleosomes. A nucleosome consists of about 145 BP of DNA wrapped around histones (the core) and a linker region of about 55 BP. Psoralen can not crosslink the DNA inside the nucleosome core or in most of the linker region because the DNA is protected by the histones; it can only crosslink DNA in chromatin approximately once per 200 BP, presumably in a relatively exposed part of the linker region. Psoralen can crosslink isolated DNA at any spot along the chain.

B. A regular pattern as above.

Nucleosomes are usually found throughout transcribed regions, including right ahead of and right behind RNA polymerase. The nucleosomes are probably a little looser than in untranscribed regions, and the single nucleosome right where the RNA polymerase is may be partially unfolded or absent (temporarily), but there is no wholesale loss of nucleosomes. (Some regulatory regions may lose nucleosomes; see below.)

C. Binding of transcription factors & very high.

Tissue specific transcription factors bind to regulatory regions (enhancers) that are usually, but not always, upstream of the genes they regulate. The binding of the transcription factors displaces nucleosomes, exposing the DNA to DNase and/or psoralen. So DNA in the enhancer region of an actively transcribed gene should be very sensitive to DNase and should give a random cross-linking pattern with psoralen.

4-3. A. This structure represents a section of the chromatin containing 4 nucleosomes. In this structure you should have ***1 double stranded DNA molecule, 800 base pairs long, and 8 molecules of H2A.***

Explanation: The structure contains one continuous DNA molecule, and 4 core nucleosomes. (See lecture 9 for description and texts for pictures.) Each core contains 8 histone molecules -- 2 each of H2A, H2B, H3 and H4. (Therefore the structure, with 4 cores, contains 4X 2 molecules of H2A.) The DNA is wrapped twice around each histone core and includes linkers -- sections that stretch between the parts wrapped around the cores. In undigested chromatin, there is a linker of 50 bp between each core and about 150 bp wrapped around each core, for a repeating distance of 200 bp. When chromatin is exposed to micrococcal nuclease, a particular point in the linker DNA between the core nucleosomes is the most sensitive spot -- the DNA is digested preferentially at this point. Conditions for nuclease digestion are chosen so that some, but not all, linkers are cut. As a result, the chromatin is broken up into sections containing one or more nucleosomes. Each nucleosome is associated with 200 bp -- the DNA starts at the weak point in a linker and extends for a multiple of 200 bp until another weak point.

B. ***Mitotic chromatin should be much shorter and contain 4 molecules of H1***

Explanation: The section of interphase chromatin under consideration here is relatively extended, because you see the "necklace" structure described above. Mitotic chromatin is tightly folded. The chain of nucleosomes is folded back on itself and then the folded chromatin is folded back on itself again

several times to produce a much shorter and thicker structure. Histone H1 is required for higher orders of folding, and there is one molecule of H1 per nucleosome.

C. The chromatin could be from ***any region of the gene that is transcribed (to make mRNA).*** Explanation: The section under consideration must contain nucleosomes during interphase. Cis acting regulatory regions of active genes generally do not have nucleosomes -- the DNA is bound to proteins, but the proteins are transcription factors or co-activators instead of histones. This rules out the promotor, enhancer, and any other regulatory elements. Transcribed regions do contain nucleosomes -- the polymerase is able to loosen them or work around them. It may temporarily displace one nucleosome at a time but it doesn't remove them in a wholesale fashion. Both exons and introns are transcribed, so the region could be from either one.

4-4. A-1. Max. # of nucleosomes = length of DNA/200 = 4200/200 = 21.

A-2. Region A = part that can be transcribed; it is transcribed in nerve cells but not muscle cells. Nerve cells make mRNA for protein H; muscle cells do not.

A-3. The number of nucleosomes will be higher in muscle cells since they aren't making protein H, i.e. will be loose euchromatin & 30nM fibers. Protein H in nerve cells will be looser (transcribed region) & loosest euchromatin (basal promoter & enhancers).

B-1. DNA in region B in nerve cells is more likely to be degraded. Chromatin in active promoters is very loose (and therefore accessible to degradation) because nucleosomes have been removed and replaced by TF's.

Picture should go: 5' ..... region B....Start of transcription ..... region A.....3'.

Transcription goes from left to right.

4-5. A. Ribosome could be in a **mitochondrion** or the **cytoplasm**.

B. Finished protein P must pass through a **nuclear pore** and enter the nucleolus (no membrane) for assembly with rRNA; the ribosome must be attached to **none of these**. Ribosomes making proteins destined for the nucleus are not attached to any organelle -- they are free in the cytoplasm. Ribosomes making proteins destined for the endomembrane system are attached to the ER, but the nucleus is not part of the EMS. Ribosomal proteins must enter the nucleus, because the site of ribosomal subunit assembly (from ribosomal proteins and rRNA) is in the nucleolus. Note that the problem says the ribosome is making a protein that will be part of a *cytoplasmic* ribosome. It isn't making a protein destined for mitochondria.

C. 26,000 nucleosomes with 26,000 molecules of H1 and 52,000 molecules of H2A.
Calculation:

Total BP of r DNA = 13,000 BP per gene X 200 copies of gene per haploid genome X 2 copies of genome/diploid.

Total # nucleosomes = total BP of DNA/200 BP per nucleosome
= (13,000 X 200 X 2)/200 = 26,000.

One molecule of H1 & 2 molecules of H2A per nucleosome.

4-6. **A-1.** Protein synthesis inside the nucleus should *stay at zero* AND the rate of ribosomal proteins (that are part of subunits) moving **out of** the nucleus should *increase* (as subunits move out). However the rate of individual ribosomal proteins moving out the nucleus should *stay at zero* – ribosomal proteins do not move out of the nucleus as separate molecules.

A-2. Ribosomal proteins moving **in to** the nucleus should *increase (as proteins move in to meet the rRNA)* AND rRNA crossing the nucleolar membrane should *stay at zero (there is no membrane around the nucleolus).*

B-.1 During G2 to M, depolymerization of lamins should *increase* AND during G1 to S depolymerization should *stay the same at zero (or sts).*

B-2. Lamins are **(IF)** AND have a **(nuclear localization signal)**.

4-7. No. Bacteria don't have histones.

4-8. **A-1. Sample Y has 4 bands** (4 types of histones per octamer -- no H1); **sample X has 5 bands** (4 octamer histones + H1). H1 is not needed for the "beads on a string" conformation, but H1 is needed to form the 30 nm fiber. So sample X must have all 5 histone types. (Each band on the gel = one type of histone = H1 or H2A etc.)

A-2. About the same in 4 bands and lighter in one. There are 2 molecules of each H2A, H2B, H3 & H4 per nucleosome but only one H1.

A-3. 80. 1 nucleosome (without H1) contains 2 molecules each of 4 histones or 8 molecules total. There is one nucleosome per 200 BP of DNA. So total number of molecules of histone = 2000/200 X 8.

B. Actively transcribed region should look like sample **Y --** chromatin should be in the "beads on a string" state**.** DNA will still have nucleosomes, but nucleosomes will not be tightly packed -- chromatin must be relatively unfolded to be transcribed. So in the EM, chromatin will look like sample Y (whether H1 is present or not). Treatment used for original sample X does not loosen up chromatin beyond the 30nm fiber stage, or remove H1 -- the chromatin in this experiment looks like sample Y because the chromatin was already loosened up to the "beads on a string" stage inside the cell. (Note question does not ask how many bands are on the gel, but what the chromatin looks like in the EM. The chromatin could still have H1 associated with it and look like sample Y.)

4-9. A. See p. 42. Please do not attempt to do the rest of this problem until you are sure of the answer to part A.

B. DNA and primary transcripts should be the same (assuming polyA is added at the same spot); differences should be in mRNA. See bottom of page for an example of how the transcript could be alternatively spliced in the two cell types.

***4-10**. **A. The same.** The primary transcript is the same in both cell types; you are looking at the new nuclear RNA = primary transcript before splicing.

B. Neither -- both equally sensitive. Here you are looking at chromatin, not RNA, and you are comparing two regions of the gene in the same cell type (you are not comparing different cell types). Both regions of the gene are transcribed, so the chromatin in both regions should be in the same state. The whole gene should be in nucleosomes, and the nucleosomes in both parts should be super coiled in the same way. Since sensitivity to DNase depends on the state of the chromatin, and the chromatin in both regions is in the same state, both regions should be equally sensitive.

C-1. Cytoplasmic RNA hybridizing to **probe B should be the same** in both cell types, and C-2. Cyto. RNA hybridizing to **probe C should be higher in the cell type making form #1.** This time you are looking at cytoplasmic mRNA = primary transcript after splicing and transport to cytoplasm. Since splicing in the two cell types is different, the two RNA's should be different. Probe B hybridizes to a region that both mRNA's have in common; probe C hybridizes to a region found only in mRNA for form #1.

4-11. **A. Euchromatin, Loosest DNA, Basal promoter, Enhancers, The gene is actively being transcribed**
DNase hypersensitive sites (HSSs) indicate that the region(s) are very open - loosest euchromatin - and exposed to DNase as a result of nucleosomes being displaced (or very loosely bound to DNA) by transcription factors in regulatory regions such as basal promoters and enhancers. The gene associated with these HSSs would be actively transcribed.

B. Loosest - The regions where regulatory TFs are bound is loosest euchromatin, as is true of regulatory regions for actively transcribed genes.

4-12. A. Lens enhancer on the Pax6 gene - the most direct way to decrease the level of transcription of Pax6 only in the lens is to mutate the Pax6 lens enhancer because it decreases only Pax6 and only in the lens. The combination of TFs expressed specifically in the lens will not be able to bind and activate significant levels of Pax6 transcription.

B. Basal promoter of the Pax6 gene - The most direct way to decrease the level of transcription of Pax6 in all cell types where Pax6 is normally expressed is to mutate the Pax6 basal promoter because this will decrease Pax6 transcription everywhere. Pax6 expressed will be decreased because there won't be anywhere for the basal TFs and RNAPII to bind.

4-13. A. Just 5' of exon 1 & Intron 1: The basal promoter – just 5' of exon 1 – that promotes NFAT transcription in the heart and the heart enhancer will both be HSSs in the heart where they increase NFAT expression.

B. Intron 1 & Intron 10: The basal promoter –contained within intron 1 – that promotes NFAT transcription in the immune system and the immune system enhancer will both be HSSs in the immune system when they are actively promoting NFAT transcription.
Note that different binding sites in intron 1 can bind the regulatory TFs that enhance NFAT expression in the heart, while other binding sites in intron 1 bind to basal TFs to promote transcription of NFAT in the immune system.

C. None: NFAT is not expressed in the lens so the regulatory regions of the NFAT gene would be loose euchromatin, this region would not be in a chromatin configuration that would confer hypersensitivity to DNase (HSSs).

D. Just 5' of exon 1 & Intron 1: Just 5' of exon 1 and Intron 1 would loop around & interact with the basal promoter and basal TFs/RNA polymerase II via mediator to activate transcription in the heart.

E. TBP, Basal TFs, Regulatory TFs, Mediator & TFIID - all of these proteins would be present in the protein complex that drives NFAT transcription in the heart.

F. Heart: Deletion of the 5' basal promoter (P1) would result in loss of expression specifically in the heart. NFAT expression in the immune system would not change because this expression is driven by a different basal promoter and enhancer.

G. Immune System: Deletion of intron 10 would result in loss of expression in the immune system. Low levels of expression might still be seen in the immune system because the basal promoter that drives expression in the immune system is still present but these are usually low, non-physiological levels.

KEY TO PROBLEM SET #4A

4A-1. A. 4 lariats (one per intron)

B. The primary transcript, a lariat and the nucleus.

Details:

The primary transcript: the 1° transcript is a copy of the entire sequence present in the DNA – it contains all the exons and introns.

A lariat structure: since exon 3 is not recognized as an exon, it is considered by this liver cell to be simply a region within the intron between exons 2 and 4.

The nucleus: since the primary transcript is in the nucleus, and that is where the intron-lariats form and are degraded.

4A-2. A. #6. Note the question asks about the sites at the ends of the *introns*, not the sites at the ends of the exons. That's why the answer is #6, not #7. In kidney cells, exon 6 is excised along with introns 5 & 6 as one big intron. As a result, exon 5 is connected to exon 7. The splicing out of the big intron connects the 3' end of exon 5 (which is the donor site at the 5' end of ***intron*** 5) to the 5' end of exon 7 (which is the acceptor site at the 3' end of ***intron*** 6). Donor and acceptor sites for RNA splicing are defined by the ends of the introns, not by the ends of the exons. (For an explanation, it was sufficient to draw exons 5 to 7 with accompanying introns, and show where the splicing occurs.)

B. ***Answers:*** B-1. 160 AA; B-2. the same length; B-3. Smaller.

Explanations:

B-1. 30 nt corresponds to 10 codons, or 10 additional amino acids. Therefore, the protein in eye cells will be 10 AA longer than the protein found in kidney cells, which is 150 AA long.

B-2. The primary transcript is the same length in the two cell types; it's the way it's spliced that's different in kidney cells and eye cells. After splicing, 30 additional nt are included in the mRNA in eye cells.

B-3. In kidney cells, one large intron is spliced out as a lariat. The big intron or lariat includes introns 5 & 6 and exon 6. (See A.) In eye cells, the two introns, 5 & 6, are removed as separate (smaller) lariats. The individual lariats are smaller in eye cells, but the ***number*** of lariats is smaller in kidney cells.

Answers: C-1. both; C-2. eye cells; C-3. the mRNA (see note at **).

Explanations:

C-1. Probe 5 hybridizes to exon 5 in the mRNA. This exon is found in mRNA from both cell types.

C-2. Probe 6 hybridizes to exon 6 in the mRNA. This exon is only found in mRNA from eye cells. This exon is transcribed in both cell types, but spliced out of the primary transcript in kidney cells. (See above.)

C-3. The mRNA is separated by size on a gel first. Then the probe is added to visualize the location of the mRNA. The size of the probe does not affect the position of the band – the probe sticks and forms a visible band wherever the mRNA happens to be, and that depends on the size of the mRNA.

**Note: Some students chose 'neither' because they felt that a RNA this size was too long to code for a protein that contains only 160 amino acids. Therefore, they reasoned that the RNA in the gel or blot must be the primary transcript, not the mRNA. Nine hundred nucleotides may seem on the long side, but eukaryotic mRNAs often contain very long UTRs, and the problem states that it is messenger RNA.

C-4. ***Answer:*** mRNA from Eye cells with either probe. (See diagram below.)

Explanation: There are 4 possible cases here -- A, B, C, & D – because there are two different mRNAs and two different probes. Here are the patterns of bands you will get on the blots:

Kidney mRNA		Eye Cell mRNA	
Probe 5	Probe 6	Probe 5	Probe 6
		xxxxx	xxxxx
xxxxx			
A	B	C	D

You are looking for two cases that give the same pattern of labeled bands. You can see from the results above that C and D give the same pattern, but none of the other pairs do. If you want to get the same pattern in different cases, you have to use the same mRNA both times. That's because the position of the band depends on the size (length) of the mRNA, not on the size of the probe.

The bands in A and C are in different places because the two mRNAs (from the two cell types) are of a different length. Whatever probe you use, if you compare blots of the two mRNAs with the same probe (A vs C or B vs D) you won't get the same pattern.

Since it is the size of the mRNA that determines the position of the band, you need to use two different probes with the same mRNA (A vs B or C vs D), not two different mRNAs with the same probe. If you use kidney cell mRNA (A vs B), you'll get one band with probe 5 and no bands with probe 6. If you use eye cell mRNA (C vs D), you'll get one band with either probe, and both bands will be in the same spot (since the position of the band depends on the length of the mRNA, which is identical in both cases). The two different probes will bind to different parts of the mRNA, but the position of the band will not change.

In this problem, many students compared the wrong blots – they compared the bands you get when using probe 5 with the two different mRNAs (A vs C) instead of comparing the two different mRNAs with the same probe (A vs B and C vs D).

Additional Note: Many students wrote statements such as 'only eye cells have exon 6.' All cells have exon 6 in their DNA, and in their primary transcript, but kidney cells do not have exon 6 in their mRNA. I think the students meant 'only eye cell mRNA has exon 6' that is, mRNA from eye cells contains exon 6, but mRNA from kidney cells does not. This may seem trivial, but it is important to keep the DNA, primary transcript, and mRNA straight.

4A-3. A. 1 promoter, 1 stop codon, & one 5' splice site; 2 exons. First exon is about 1.8 kb long. (Exon goes from start of transcription, not translation, to first intron.)

Order: Promoter (at start of/before transcription) -- exon (#1) -- 5' splice site (at end of first exon/start first intron) -- stop codon (for translation, not transcription).

B. Eukaryotic cell. Prokaryotes don't have introns or enzymes to remove them.

C. 3.5 KB. Transcription starts at around 2.5 and goes to about 7.0. So primary transcript is about 4.5 kb. Intron (1 kb) is removed → mRNA of about 3.5 kb. This answer assumes intron ends at arrows. If intron goes all the way to the XX marks, then intron is longer and mRNA is shorter.

D-1. 1 band, 3.5 kb long (3.0 kb if intron is longer). The only RNA you will find in the cytoplasm is mRNA, which is identical to the probe. No restriction enzymes are used here -- the RNA is small enough so you don't need them, and restriction enzymes don't cut up RNA anyway. (This is a Northern blot.)

D-2. More, 4.5 kb long. Nucleus will contain unspliced primary transcript of 4.5 kb plus mRNA (on route to the cytoplasm). If primary transcript had more than one intron, you might also find partially spliced transcripts.

4A-4. A-1. The region labeled 'exon 2' should be spliced out in ***(heart)***.

A-2. The region labeled 'exon 1' should be spliced out of the primary transcript in ***(neither).***

Explanation for A-2 (only part required): Exon1 is not present in the mRNA from the immune system, but it isn't spliced out. It is just not transcribed at all. In the immune system, the promoter used is downstream of exon 1, so transcription starts after exon 1. In other words, exon 1 is skipped (in the immune system), not spliced out. (Exon 1 is present in mRNA from heart, but since this was fairly obvious it did not need to be explained.)

Additional info (not required): The dashed lines do not represent sequences in the mRNA – they are sequences that are removed from the primary transcript to make the mRNA. In heart, transcription begins with exon 1, so exons 1 & 2 are transcribed, but exon 2 is spliced out of the transcript. In the immune system, exon 1 is not transcribed, so the mRNA begins with exon 2.

B-1. These two mRNAs should differ in sequence ***(at the 5' end).***

B-2. Which mRNA should be longer? ***(neither – both should be about the same length).***

Explanation for B-2: The primary transcript in heart is longer, but after splicing, the 2 mRNAs will be about the same length. Why? After splicing, exon 1 (in A) or exon 2 (in B) will be connected to exon 3. (The rest of the mRNA is exactly the same in both cases.) Since exon 1 and exon 2 are approximately the same size, both mRNAs should be about the same size.
Details: The transcript in heart (that will be spliced to give mRNA A) will be longer than the transcript in the immune system, as transcription in heart starts at a promoter that is further upstream. However, in heart, introns 1 & 2 and exon 2 are spliced out of the transcript, while in the immune system, exon 1 and intron 1 are skipped, and intron 2 is spliced out of the transcript. Therefore, the final mRNAs will start with either exon 1 or exon 2, which are the same length, followed (in both cases) by exons 3 to 9.

C-1. The 5' donor site used for alternative splicing must be at one end of intron ***(6)***.

C-2. During alternative splicing, what should be joined? ***(3' end of exon 6 to 5' end of exon 8).***
Your picture should include – clearly labeled -- (1) introns & exons (2) the two splice sites (3) the orientation of the mRNA, from 5' to 3'. The so-called 5' donor site is at the 5' end of the intron to be excised, which means the site is at the 3' end of the preceding exon -- which is to be joined to the 5' end of the exon following the excised section.

C-3. The most reasonable estimate for the length of exon 7 is ***(306 BP).***

The smallest exon is 100 BP. So exon 7 must be approximately 300 BP. However, exon 7 must be a multiple of 3 BP in length. Why a multiple of 3? Because mRNA is read in groups of 3, and a deletion that is not a multiple of 3 BP in length will cause a frame shift. Of the numbers given, 306 is the closest number to 300 that is also a multiple of 3.

Note that not every individual exon must be a multiple of 3 BP long. The mRNA is read (translated) continuously from exon to exon, so a single codon can overlap two exons. However, if a section is deleted and causes no frameshift, then the deleted section must be a multiple of 3 BP long.

4A-5. A. TOR action is most likely to increase binding of ***(eXT to the 5' end of mRNA).***
Why the 5' end? Because eXT binds to the cap, which is on the 5' end of mRNA.
Note: A did not need to be explained. You had to explain B and C– how TOR, eXBP and eXT affect each other and protein synthesis.

B. You would expect unphosphorylated eXBP to ***(inhibit protein synthesis).***
TOR is a kinase, so activation of TOR should lead to phosphorylation of eXBP. We know that TOR action stimulates protein synthesis, so the phosphorylated form of eXBP should NOT block protein synthesis. Conversely, the unphosphorylated form of eXBP should block eXT action and inhibit protein synthesis.

C. Phosphorylation is most likely to directly ***(inactivate eXBP)***.

eXBP is a substrate of TOR, but eXT is not. So phosphorylation must directly affect eXBP, not eXT. As explained above phosphorylation must prevent eXBP from binding to eXT, thereby allowing eXT to bind to the cap on the mRNA, so protein synthesis can proceed.

4A-6. A. When the transcript from people with a normal LDLR gene is spliced, how many lariats? ***(17).***
B. In people with this deletion, how many lariats (looped out sections) should be formed? ***(14).***
C. The sequence of 55 AA not found in normal people could be encoded in ***(intron 15).***

The 4 things required in your picture:
(1). An accurate depiction of the deletion showing that the deletion cuts out everything from the middle of intron 15 to the middle of exon 18.
Additional Info: In the normal case, there are 17 introns, and each one will be removed individually to form a lariat (assuming no alternate splicing). In the deletion, there is no 3' (acceptor) end to allow splicing out of intron 15, and introns 16 and 17 are deleted. (There is a 5' donor site in intron 15, but no 3'acceptor site to connect it to.)

(2). You had to show that the mRNA does not extend beyond exon 18.
Additional info: The normal stop codon is deleted, but the mRNA will still end at the end of exon 18. (That's where poly A was added to end the transcript.) Therefore, it should be clear that translation cannot extend beyond the end of exon 18 until it hits a stop codon – there is no mRNA after exon 18 to translate. The 3' UTR is NOT encoded after exon 18. The 3' UTR is encoded in exon 18 in the part after the normal stop codon. That part of exon 18 could encode part of the 'extra' 55 AA and include a stop codon.

(3). You had to show where the 'extra' 55 AA come from. Some of the AA must come from translation that continues into intron 15 (from the end of exon 15). The simplest solution is that there is a stop codon in intron 15, after 55 codons in the same reading frame as exon 15. So all 55 AA are encoded in intron 15. Alternatively, translation may continue from exon 15 all the way through the remaining part of intron 15, and continue on into the remaining part of exon 18, until it hits a stop codon in exon 18.

(4). You needed to include some detail in your diagram that shows how splicing works. You could label the 5' and 3' ends of the introns, or show how lariats form, or show why you can't splice out intron 15, etc.

4A-7. A. The normal version of the gene for protein Q should encode ***(an amino terminal SP)*** ***(a site recognized by SPase).***
To get a single pass protein with its amino end in the lumen of the ER or a vesicle, the protein must have a SP on the amino end that is removed after the protein is inserted into the ER membrane. The protein must have a single TM section to anchor it in the membrane. (The TM section does not act as an internal SP or transit peptide.) If an internal SP were used, the protein would end up with its carboxyl end in the lumen.

B. The region that binds the paracrine is more likely to be ***(near the amino end).***
A paracrine must bind to the extracellular domain of a receptor. In this case, the amino end will be extracellular, and the carboxyl end will be in the cytoplasm. When a vesicle with a TM protein fuses with the plasma membrane, the part of the protein that was in the lumen becomes extracellular, and the part that was in the cytoplasm remains in the cytoplasm.

C. The TM segment is most likely to be encoded in ***(exon 7 or 8)***
If the protein is secreted, it must lack a TM section to anchor it in the membrane. (It will insert in the ER membrane as usual, but the SP will be removed, and the rest will go all the way through to the lumen.) Therefore, the normal TM section must be encoded in the mRNA after intron 6. The only choices on the list that are after intron 6 are exons 7& 8.

D. You expect the drug-treated batch to grow ***(slower than normal).***
When the receptor is secreted, the section that binds the paracrine (the extracellular part, on the amino end) is released from the cells, so there is no longer any connection between the receptor and the cell. Since the secreted receptor is no longer connected to the cell, the receptor cannot transmit a signal. The secreted protein can bind the paracrine, but binding won't have any effect on the target cells.
Some students thought that both cultures would grow at the same rate, because the drug treated batch would make a little normal receptor, and that would be enough for normal growth. They reasoned that the signal would be amplified so much that even a small amount of GF binding to normal receptor would be sufficient. Equivalent growth rates are very unlikely, as both the normal amount of signal bound to the cell surface (in the normal) and any greatly reduced amount bound (in the splice variant) would both be amplified by the same factor. So the actual amount of target protein made or activated would be much larger in the normal. In other words, the amount of productive GF binding is probably what limits growth.

E. The R type cells should grow ***(faster with G#2).***
If the mixed culture contains #1 G cells, the G cells will secrete receptor for the paracrine GF. The soluble secreted receptor will bind the GF, reducing the amount of GF available to bind to the receptors on the R cells. In other words, the secreted receptor acts as a decoy to trap GF. This will decrease the GF available to bind to the target cells, and the R cells will grow slower. If the mixed culture contains #2 G cells, there is no secreted receptor to soak up GF, so the R cells will get more signal.
In 8D, the amount of receptor on the surface of the R cells is limiting growth. In 8E, the amount of GF available to bind to surface receptor on R cells is limiting growth. In both cases, growth slows if signaling from the GF-receptor pair is reduced.

Note that this question asks about the growth of the R type cells in a mixed culture (mixed with #1 or #2 type G cells). It does not ask about the relative growth of the 2 types of G cells.

4A-8. Problem 4A-8 is based on the structure of the gene for PTHrP.

A-1. How many different translatable mRNAs can contain exon 4? __***1***____

A-2. The polyA addition site is probably in exon ***(4)*** at ***(the 3' end).***

Explanation: You have to eliminate the intron between exons 4 and 5 and keep exon 4. You can't splice out the intron after exon 4, so you have to end transcription (& add polyA) at the right (3') end of exon 4.

Additional info: The diagram represents the sense strand of the DNA, written left to right, 5' to 3'. Transcription has to start at the 5' (left) end of exon 1, and go left to right, as indicated by the diagram. You can't splice out exon 2, as it contains the start codon for translation. (In any case the problem says you can only use the splice sites and splices shown, not others.) So the only options are whether to connect the end of exon 3A to the start of exon 4 or exon 5. Only one of these choices will contain exon 4.

B. Assume that the splices and splice sites shown are the only ones possible.

B-1. How many different translatable mRNAs can be made from the IASH gene? ____***3****___ (See note at ** below.)

B-2 & B-3. There must be no acceptor splice junction at the start of exon ***(3B)*** and no donor junction at the end of exon ***(3B & 4).***

B-4. (i) The minimum #of splice sites is ___***2***____, and the (ii) number of exons that will be skipped will be ***(0).***

Explanation: The basic issue is that none of the translatable mRNAs can contain any introns. Using the splice sites and splices shown, that limits the choice of possible mRNAs to 3. (But see note below.) No additional explanation was required, but is provided below in 'additional info.'

** Note: The most common mistake was to overlook the possibility of an mRNA with exon 3B instead of exon 4 or 5. In that case, only 2 different mRNAs are possible. If you put '2' as the answer to B-1 for this reason, but then answered B-4 with the corresponding answers ('3 splices' and '1 or 2 exons skipped'), you were given full credit for B-4. I think some students overlooked the distinction between 'splice sites' and actual 'splices'. If you tried alternative uses of the sites shown, there are more possible translatable mRNAs. However, since transcription has to start before intron 1, and the mRNA must contain no introns, it is impossible to eliminate exon 1, even though it is not translated. It is also impossible to skip exon 2, since that is where translation starts, and you can't include both exons 4 and 5 in the same mRNA, since the intron between them cannot be spliced out. These factors greatly reduce the number of possible mRNAs that can be made.

Additional Info:

B-1. All the transcripts must contain exons 1, 2 & 3A. There are then 3 choices for the rest – either 3B, 4, or 5.

B-2. & B-3. The answers to B-2 & B-3 did not need to be explained, but here is a full explanation for the curious:

If you can't attach any 3' end to the start (5' end) of a specific exon, then there is no acceptor splice junction at the start of that exon. This is the case with exon 3B. If you can't attach the 3' end of an exon to anything, then there is no donor splice junction at the end of that exon. This is the case with exons 3B and 4.

B-4. If transcription stops at the end of 3B, no exons will be skipped, that is, no exons will be transcribed, but spliced out of the transcript. (Exons 4 & 5 will not be used, but that's because they won't be transcribed at all.)

If 3B is spliced out of the transcript, then 3A will be connected to exon 4 (splicing out one exon, 3B) or 3A will be connected to exon 5 (splicing out two exons, 3B and 4.)

C-1. All proteins made from the IASH gene will probably have the same ***(amino end).***

C-2. The number of AA in the mature protein made from that mRNA should be ***(<N).***

Explanation:

C-1. This part did not need to be explained directly – see 'additional info' for a direct explanation. What needed to be explained or demonstrated (2 pts) was the distinction between transcription (which starts in exon 1) and translation
(which starts in exon 2).

C-2. Proteins are often modified after synthesis by removal of amino acids, such as the met or the SP on the amino end, or the extra part that keeps the protein inactive until the extra is removed. The protein described in this problem is a hormone, so it must be secreted, and its SP must be removed. That's why the correct answer is <N not ≤N. Not all proteins are shortened after synthesis, but this one must be. This did not have to be explained to get full credit for the explanation.

Additional info for 7-C:

C-1. All the transcripts must contain exons 1, 2 & 3A, so the mRNAs will have the same 5' end, and translation starts at the same point in exon 2 for all of them, so the proteins will have the same amino end. Transcription (& the mRNA) must start with exon 1, even though translation doesn't start until exon 2.

C-2. Sections of the mRNA that are not translated don't count as 'coding.' So the untranslated bases/codons in the mRNA (the ones before the start codon and after the stop codon) are not counted as 'codons.' Therefore, the number of bases/3 is always larger than the number of codons that actually code for AA. Neither introns nor UTRs are translatable or coding. The issue here is not about total bases/codons in the mRNA vs bases/codons translated (& AA put into protein). The question is about the # of AA put into the protein (using translated codons) vs the # of AA found in the mature protein after post-translational processing.

4A-9. A. Binding of epinephrine to β-AR leads to an increase in AUF1 transcription. Which of the following are plausible explanations for this observation? ***(PKA phosphorylation activates a transcription factor that upregulates AUF1 transcription)***
(cAMP binds to and activates a transcription factor that upregulates AUF1 transcription)
Epinephrine is water soluble, so doesn't have intracellular receptors. Gαs is membrane bound, so doesn't go to nucleus to regulate transcription. It is feasible that cAMP can bind and regulate a transcription factor, similar to how cAMP binds and regulates the regulatory subunit of PKA. It is also feasible that PKA phosphorylates a transcription factor, altering its activity. [In reality, a transcription factor is phosphorylated by PKA.]

B. Propose how AUF1 acts on β-AR mRNA. AUF1 is most likely to ***(cause mRNA degradation).***
Epinephrine treatment leads to a decreased half-life of β-AR mRNA, suggesting that it is degraded more. The fact that AUF1 protein levels are upregulated by epinephrine treatment is consistent with AUF1 causing the degradation. The binding site for AUF1 is in the 3' end of the β-AR mRNA, also consistent with degradation instead of translation regulation. [Note: since inhibition of translation was not ruled out, credit was given for this answer, too.]

Key to Problem Set 4R

4R-1. A. Decrease; STS. The average number of nucleosomes per enhancer decreases because the histones are displaced by transcription factors when the DNA is transcribed. (The number of nucleosomes in the transcribed section of the gene should STS.) Enhancers are regulatory sequences in the DNA; the DNA stays the same, so the number of enhancers doesn't change, but the binding of regulatory proteins to the enhancers changes.

B. Larger than. There are 4 basic cell or tissue types (nervous, muscle, epithelial, & connective) and 3 nuclear RNA polymerases I, II & III).

C. Smaller than. There are 5 types of histone (H1, H2A, H2B, H3 and H4). Each nucleosome has 8 or 9 molecules of histone. Each nucleosome is an octamer -- 2 molecules each of H2A-H4 and (usually) one molecule of H1.

D. Estrogen receptors and PKA. These two proteins are not secreted or inserted in a membrane; they should not enter the RER. PKA is cytoplasmic; steroid receptors are either nuclear or cytoplasmic.

E. Insulin. This is the only protein that is secreted. It must have an SP to enter the ER and its SP must be removed to allow the protein to go all the way through the ER membrane and be released on the other side. PKA and estrogen receptors have no SP to begin with. Insulin receptors and glut 4 are transmembrane proteins that need an SP to insert into the membrane; the SP is probably removed but it need not be.

4R-2. A. Binding site for STAT is farthest upstream. (The start of transcription = 0. Bases are counted up in both directions, + in direction of transcription (towards the 3' end of the sense strand, starting from 0) and - in the other direction (towards 5' end of the sense strand, starting from 0). Bases on 5' side of start (before the start) are upstream; bases on 3' side (after the start) are downstream.)

B. The binding site for STAT is an enhancer, and a cis acting element.

C. The indicated region must include the start of transcription and the basal promotor.

4R-3. A-1. muscle; A-2. by insertion of pre-existing transporters into the membrane.

A-1. You expect to find glut 4 mRNA only in cells that make the protein (GLUT 4). Muscle makes glucose transporters that respond to insulin; the other tissues mentioned do not. So only muscle (of the organs/tissues listed) should make GLUT 4 protein and contain glut 4 mRNA.

A-2. Insulin triggers insertion of the transporters (for carrier-mediated facilitated diffusion) into the plasma membrane by causing fusion of vesicles containing the transporters (inserted into the membrane of the vesicle) with the plasma membrane (exocytosis).

B. All the other cell types. The DNA in virtually all cell types is the same; it's the expression (transcription) that's different. So it doesn't matter where the DNA comes from -- the number of pieces it will be cut into (& the number that will hybridize to the probe) will be the same. However the mRNA made from the DNA will be different. Note this question asks about the sequence of the DNA, not the state of the chromatin.

C-1. pancreas; C-2. multiple distinct smaller bands. Insulin is not made in brain, so the chromatin containing the insulin gene should be relatively tight and resistant to DNase degradation in brain. The chromatin should be in the same state in all the other organs/tissues except pancreas. In the pancreas, the only organ where the gene is transcribed, the region of the chromatin containing the insulin gene should be looser and more susceptible to degradation by DNase. So when you pretreat the chromatin with DNase and then cut up the DNA, the DNA from pancreas should be more degraded than the DNA from the other sources. The transcribed region will form smaller bands at 200bp increments, i.e. a “ladder” that hybridize to the insulin gene probe. (Note you are looking for undegraded pieces that hybridize to cDNA for insulin, not to cDNA for glut 4.) The insulin enhancers in pancreas will be even more degraded and not match the insulin cDNA probe (corresponds to RNA).

For the explanation, the general principles to state for full credit are as follows: (1) transcribed chromatin is looser and therefore more susceptible to DNase and (2) the insulin gene is transcribed only in pancreas.

D-1. exocytosis; D-2. lower. Insulin is a secreted protein; it is packaged into secretory or storage vesicles that release the insulin by exocytosis (= vesicles fuse with plasma membrane) only in response to the appropriate signals (such as high blood glucose or neuronal stimuli).

Exocytosis leading to release of insulin occurs in response to high blood glucose levels after eating.

Since mRNA levels in the cells are the same in fasting and fed animals, the regulation of insulin release and/or production must be at the post transcriptional stage (& not at degradation of mRNA).

4R-4. A-1. SRE affects transcription, not translation. (It can't affect translation directly because it is in a region that is not transcribed -- it is located before the start of transcription. The mRNA won't contain the SRE.)

A-2. Without SREBP, cellular cholesterol levels should be low.

SREBP acts as a (regulatory) transcription factor. It binds to the SRE (a proximal regulatory site, or part of the promotor, depending on your terminology) and stimulates transcription of genes for uptake of cholesterol (LDL receptor) and synthesis of cholesterol (enzyme H).

SREBP is an activator that is required for transcription of the genes for enzyme H and the LDL receptor. If there is no SREBP, transcription will be low, and the amount of enzymes/receptors made will be low. Therefore uptake and synthesis of cholesterol will be lower than normal.

B. An NLS is found in both SREBP and nSREBP.

nSREBP must have a NLS to reach the nucleus. Since nSREBP is part of SREBP, the SREBP must include the sequence of amino acids that make up the NLS.

How does this work? The NLS must be in the nSREBP part of the protein, but the NLS is ineffective as long as the nSREBP is attached to the rest of the protein. The NLS in the complete protein can't be used, either because it is masked, or because the TM domains anchor the protein in the ER membrane. The protein is tied down and can't move. As soon as the nSREBP is detached from the TM domains, the NLS in the nSREBP can be read, and the detached part of the protein moves to the nucleus, leaving the remaining part of the protein (containing the TM domains) embedded in the ER membrane.

C. SREBP is made on ribosomes attached to the ER, and is inserted into the membrane so its COOH end is in the cytoplasm. A vesicle buds of the ER membrane and goes to the Golgi, so the SREBP travels to the Golgi in the membrane of a vesicle, with its COOH end in the cytoplasm.

D. SREBP (not nSREBP) should contain a sterol binding domain; if SREBP has no TM domains, it should be made on free ribosomes and remain in the cytoplasm, and the cellular cholesterol levels should be higher than normal. (If the mutant SREBP entirely enters the ER lumen, the cellular cholesterol levels will be lower than normal. See * on next page.)

Detailed explanation for D:

D-1. Cholesterol controls release of nSREBP. It doesn't affect the activity of nSREBP once it is released. The cholesterol binding domain is in the membrane, not in the TF. It works like this: Cholesterol in the membrane binds to the transmembrane domains of SREBP, inhibiting vesicle formation and transport of SREBP to the Golgi. When cholesterol levels in the membrane fall, vesicles form from the ER and carry the SREBP to the Golgi. There a protease cuts the peptide chain and releases the nSREBP part into the cytoplasm. nSREBP itself doesn't bind cholesterol. Cholesterol controls the release of nSREBP, not the activity of the TF itself. This is different from the way most steroids control transcription. Having the 'cholesterol sensor' in the membrane instead of in the TF makes sense because cholesterol is found entirely in membranes.

The protease in the Golgi is bound to the Golgi membrane, but it doesn't have to be entirely inside the lumen of the Golgi. That's how it is able to cut the SREBP peptide near the cytoplasmic side of the ER membrane, releasing the nSREBP into the cytoplasm.

Note: The regulation of SREBP has been considerably simplified for this problem. This whole process involves two proteases, and (at least) two additional proteins, SCAP and Insig. For the general scheme and the role of SCAP see Horton et al, J Clin Invest, May 2002, Volume 109, Number 9, 1125-1131. For a diagram showing how nSREBP is released, see fig. 1. If you don't know how to find an article online, ask Dr. M. If you want to know how Insig fits in, see the 5th edition of Lodish et al, or Goldstein et al, Cell **124**:35-46 (2006). Alternatively, go to the pubmed bookshelf and type 'srebp insig' in the search box.

D-2. The SREBP without any TM domains should be made on free ribosomes and stay in the cytoplasm, not go inside the lumen. SREBP normally has an internal SP -- we know this because both ends are in the cytoplasm. Without the SP it will not enter the ER. See * on next page if you thought the SREBP would enter the ER lumen.

D-3. The mutant SREBP won't just lie around in the cytoplasm, because it has an NLS to target it to the nucleus. It doesn't have to go to the Golgi to get cleaved -- it already has lost its anchor. (Also, if it's in the cytoplasm, it won't get close enough to the Golgi for the Golgi proteases to act on it.) Since it has an NLS and no TM domains to anchor it in the EMS, it should go directly to the nucleus and stimulate transcription of genes for LDL receptor and enzyme H. The result will be overproduction of cholesterol.

Answer to 4R-4, cont.

*Explanation of D-2, cont. -- if you thought the SREBP would enter the ER lumen.

If you don't realize that normal SREBP must have an internal signal peptide, you will probably conclude that the mutant SREBP will enter the lumen of the ER. If the SP is on the amino end, it allows normal SREBP to enter the ER, and then the SP is removed, leaving the two remaining hydrophobic (TM) sections to anchor the protein in the membrane. If the two hydrophobic (normally TM) domains are removed, the mutant SREBP will go all the way into the ER lumen and remain entirely inside. In that case, the cellular cholesterol levels will be unusually low. Why? If the SREBP enters the ER, it can never get to the nucleus and stimulate transcription of the genes for synthesis and uptake of cholesterol. (Protein can't exit the Golgi to the cytoplasm.) If the enzymes for synthesis and uptake aren't made, the intracellular cholesterol levels should be low. (Where will the SREBP go in this case? If the SREBP enters the lumen of the ER, it would probably be secreted by the default pathway, even if it has a NLS.) When this was an exam problem, full credit was given for this explanation, but not for the answer.

4R-5. **A.** Basal promotor is in **none of the above.** (The basal promoter is mostly in the region 5' to the first exon – but overlaps a little of the first exon). Control elements can be **either way,** i.e. anywhere relative to the gene.

B. Exon 2 codes for a **transit peptide**; Exon 3 codes for a **localization signal to enter mito. inner membrane**; Amino acids not in mature enzyme are encoded in **exons 2 & 3**; Enzyme M should end up in **cytoplasm** (if exons 2 & 3 deleted) and in matrix of mitochondria if exon 3 is deleted.

Deletions (expts 2 & 3) indicate you need exon 2 to enter mitochondria and exon 3 to go from matrix to transmembrane space. (Exon 1 should encode all or part of the 5' untranslated region, the section before translation starts.) This indicates that enzyme M is made on cytoplasmic ribosomes, enters mitochondria at a contact point (using transit peptide encoded in exon 2 as localization & entry signal), and then passes from matrix across inner membrane to intramembrane space (using SP encoded in exon 3). Once enzyme M is in the matrix, TP is removed to expose SP. Then SP allows protein to enter the mito. inner membrane in the same way that proteins destined for the endomembrane system enter the ER membrane (but post-translationally as vs. co-translationally). SP is removed after protein passes across the inner membrane (by equivalent of signal peptidase) -- this detaches protein from membrane. If SP and TP are not present (as in expt. 6) there will be no localization signals on the protein, so enzyme M will remain in the cytoplasm where it is made.

C. Low enzyme levels are probably due to **changes in rate of transcription** due to removal of a **cis acting control element**.

The intron between exons 9 & 10 probably contains an enhancer; when it is removed, transcription can not be activated (enhanced) by the appropriate transcription factors and levels of transcription decline. A cis acting element such as an enhancer only effects transcription on the DNA molecule on which it resides.

How to rule out effects at translation or after? The mRNA made in this experiment is the same as the normal mRNA -- the intron would be spliced out anyway (assuming no alternative splicing). So there should be no differences in translation or protein made. It is possible that you have removed an alternative polyA addition site and altered the 3' untranslated part of the mRNA (without changing the translated part of the message). This could increase degradation of the mRNA and decrease protein levels without changing the structure or localization of the protein.

D. Differences here are probably in **splicing**; Newly made enzyme M should have **no localization signals on its amino end and 1 on its carboxyl end** (that's where the signal to enter peroxisomes usually is).

You need different localization signals to enter mitochondria or peroxisomes (on different ends of the protein) and none to remain in the cytoplasm. So you must make proteins with different carboxyl and/or amino ends to achieve these different localizations. Since there is only one gene for the protein, the same gene must be used to make more than one different mRNA -- one mRNA coding for each differentially located version of enzyme M. This can be done by alternative splicing. If the mRNA is lacking exon 2 and the section with the peroxisomal localization signal (somewhere near the 3' end), the protein will lack signals to enter organelles and will remain in the cytoplasm. If the mRNA has the sequence from exon 2, but does not have the sequence for the peroxisomal LS, the peptide will enter the mito. If the mRNA lacks exon 2 but includes the exon with the perox. LS, the peptide will go to peroxisomes. (It is not clear what will happen to the protein if the mRNA codes for both a TP and a PLS. It depends on how the peptide interacts with the various other proteins that ferry it to the organelles.)

Answer to 4R-5D and E continued on the next page

These differences in mRNA can be achieved by alternative splicing of the primary transcript. For example, you can add exons to the 3' end of the mRNA by splicing out early poly A addition sites. As a result, additional exons can be transcribed into the message, adding a perox. LS to the carboxyl end of the protein. Alternatively you could splice out exons near the 3' end (such as #9) to remove a peroxisomal localization signal (to get the cytoplasmic or mito. form).

Note: you can't use splicing to make changes in the extreme 3' or 5' end of the mRNA because you can't splice out the first or last exon. (Draw it out and see!) That is why this problem is set up so translation starts in the second exon. It is possible to get proteins with different amino ends by having transcription start in different places (in different cell types) instead of, or in addition to, alternative splicing.

E. If you remove exon 2, the protein will have a localization signal to enter the inner membrane (mito LS), but no transit peptide. The mito. LS is normally recognized by the mitochondrial equivalent of the SRP, and that allows the peptide to enter the inner membrane of the mitochondrion. The fate of the protein without exon 2 depends on how well the cytoplasmic SRP recognizes the mito. SP. If the mito. SP is not recognized at all by the cytoplasmic SRP, all the protein should stay in the cytoplasm. On the other hand, if the mito. SP is recognized as well as a standard cytoplasmic SP, all the protein should enter the ER. The results given indicate that neither of these is the case, since all the protein molecules don't end up in the same place. This suggests that some, but not all, molecules enter the ER (and travel on to the plasma membrane). The most reasonable explanation is that the regular (cytoplasmic) SRP recognizes (binds to) the mito LS somewhat, but not very well. Therefore only a fraction of the chains bind to the SRP, are ferried to the ER and enter the ER. The remaining growing chains do not bind to the SRP and stay in the cytoplasm.

4R-6. A. **Transcription of gene Z**. The mRNA is the same size in both types of T cells, making alternative splicing a very unlikely explanation for the difference. Although the product of transcription is the same in both cases, that doesn't mean that there is no regulation of transcription -- there can still be a change in the amount (or rate) of transcription.

The amount of cytoplasmic RNA hybridizing to the probes (mRNA) and the amount of enzyme activity are both higher in mature T cells. These results indicate that maturation causes an increase in the amount of cytoplasmic mRNA (either because of an increase in transcription or a decrease in mRNA degradation). Differences in the level of mRNA are sufficient to explain the differences in enzyme Z activity -- the more mRNA there is, the more synthesis of enzyme Z occurs, and the higher the amount of enzyme Z. There is no need to invoke regulation at translation or by modification after translation -- if the amount of mRNA is limiting, having more mRNA will lead to more enzyme Z activity.

Note: Many students confused (or misused) the terms "translation" and "transcription." In molecular biology, these are technical terms with different meanings, and cannot be interchanged. See table below. If you want to be understood (and to receive full credit on exams) you have to use the terms correctly. Ask Dr. M or your TA if you need clarification on this.

B. **Regulatory TFs.** Which would differ between immature and mature T cells

C-1. **In between nucleosome cores.** – linker DNA will be randomly cut

C-2. Region in question is a cis acting regulatory one that is not transcribed in either cell type. However the region is active (binding TF's) in mature cells but not immature ones. "No distinct bands" means that sample was degraded.

(1). Sample of chromatin from immature cells has more histone H2B. (DNA in sample from immature cells is in nucleosomes; DNA in sample from mature cells was degraded and has less H2B because it's a regulatory region. It has TF's bound to the DNA instead of histones.)

(2). Any of these -- a regulatory region can be near the start (proximal on the 5' side, near the basal promotor) or far away (distal) on either side of the start of transcription.

D. An enhancer. The region cannot be a silencer (cis acting) as explained below.

Note on terminology: A transcribed region that produces a TF is not called a silencer or enhancer. The cis acting region that binds the TF is what is called a silencer (if binding inhibits transcription) or an enhancer (if binding activates transcription).

ANSWERS TO PROBLEM SET 5

5-1. d. is negative feedback -- more A gets you more B, but that gets you less A, correcting the over supply of B, and so on. In this case, neither A nor B gets too high (as in e) or goes to zero (as in c).
e. is positive feedback -- more A gets you more B, which gets you more A, etc., and the supply of A and B spirals out of control.
a. & b. are not feedback -- there is no loop. The end product or process doesn't affect the first.
c. is a general shut down – less A gets you less B, which gets you less A, which cuts down on B, etc., until there is no A or B.

5-2. True/False
A. False. Blood is a type of *connective* tissue.
B. False. In an organism that maintains homeostasis, blood glucose levels stays *within narrow limits.* Homeostasis does not imply an unchanging situation, but rather one in which changes are kept within narrow limits.
C. False. On a hot day, blood vessels in the skin *dilate* to maintain body temperature. Vasodilation allows heat loss from the surface of the body.

5-3. It is critical that internal temperature remain fairly constant, so central receptors are critical to provide information about core temperature. The peripheral receptors send signals that allow the hypothalamus to make adjustments <u>before</u> changes in temperature occur centrally.

5-4. The hypothalamus is able to sense a decrease in temperature as small as 0.01 degrees C. When the temperature drops just a few hundredths of a degree, the hypothalamus stimulates muscle contraction and vasoconstriction, which raise the temperature back up to 100 deg F. So the temperature in the hypothalamus will vary between about 99.98 and 100.02. Peripheral temperature is another matter; skin temperature can vary from 68 to 104 degrees F.

5-5. As water evaporates from the skin surface, the heat required to transform water from a liquid to a gas is transferred from the skin to the water, so the skin cools off in the process. Humans also spread fluid on the body surface, but this occurs by activating sweat glands. Rats lack sweat glands, so use this behavioral mechanism to cool off.

5-6. a. epithelial
b. In general, the body does not actively transport water. Rather, water moves passively by osmosis into more concentrated areas. So in order to make sweat, the epithelial cells actively transport Na^+ into the gland lumen, and water osmoses after it. To maintain homeostatic levels of Na^+, the duct cells then reabsorb this Na^+.

5-7. Alcohol causes dilation of peripheral blood vessels. Initially, this brings warm blood to the peripheral thermoreceptors, which send a message to the hypothalamus that it is warm, the brain gets the message, and the fan notices he feels warm. But it's really <u>not</u> warm outside, and there is a large temperature gradient between the body and the air. With continued dilation of the blood vessels, heat is lost to the cold environment, and he ends up feeling chilly.

5-8. A. The same as. Both Ann and Mary have the same internal temperature (because of homeostatic negative feedback mechanisms), but Ann generates the necessary heat by basal metabolism alone and Mary by basal metabolism plus shivering. The hypothalamus is the main internal temperature sensor, but it itself only changes slightly in temperature and only temporarily -- it responds to these changes by sending messages that restore the normal state.
Answers to 5-8 cont. on next page.

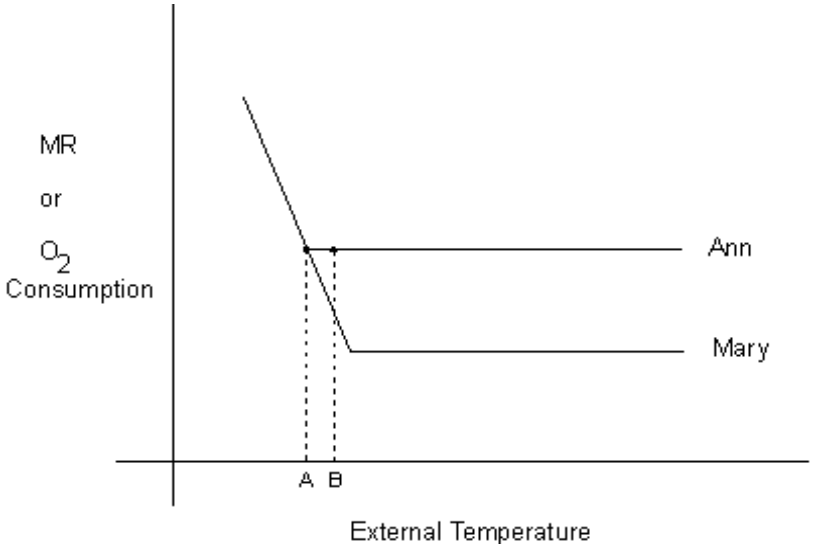
Answer to 5-8, cont.

B. Mary has a lower basal metabolic rate, so she needs to shiver to generate some extra heat and keep warm in a cold room. Ann has a higher basal metabolic rate, so she generates enough heat to maintain her metabolism *and* to warm the body, so she has less need for shivering to generate heat. Of course, if the room got even colder, Ann would start shivering, too.

In other words, Mary's critical point for shivering (in response to external cold temperature) is higher than Ann's. Therefore, as the room cools off, Mary will start to shiver first, at a higher temperature, and Ann will only shiver if it gets even colder. Shivering will kick in first for Mary as the room cools off. The temperature that will activate the shivering mechanism is lower for Ann, and it will not be reached until the room cools down even more.

C. Same or higher. There are two possibilities for relative oxygen consumption. Ann is still in her thermoneutral zone (where enough heat is generated by metabolism alone to keep warm); Mary has started shivering, so she is generating some heat by metabolism and some by shivering. If both are generating the same amount of heat (as at temperature A in diagram below), oxygen consumption should be about the same. However, Ann is not yet shivering, so she could still be generating excess heat from metabolism (even in the cold room) and so she could be using more oxygen (as at temperature B).

To interpret the curves, remember that temperature is higher to the right. The room is cooling off, so you follow the curves to the left. At the bend in each curve, the person starts to shiver and to use up more oxygen. The room has to get down to temperature A before Ann starts to shiver; Mary starts to shiver before the room even reaches temperature B. Below temperature A, they both shiver.



5-9. The difference is in salt recovery, not salt secretion. The salt must be reabsorbed by a carrier protein which can be saturated. Guinevere and Arthur both secreted the same amount of salt into the gland to produce sweat, but Arthur did it over a shorter interval than Guinevere. They both needed to reabsorb the same amount of salt out of the gland. Arthur tried to reabsorb this salt in a short time interval, saturated his carriers, and some of the salt left the duct with the sweat.

5-10. A-1& A-2. In the short run, core body temperature should **decrease, &** the face should feel **hotter.**
A-3. After the response, core body temperature should **rise**.
A-4. This similar to what happens when someone **finishes with a fever.**

Explanation of A:

A-1. Person responds by trying to cool off either through behavior (removing clothing, etc.) or by physiological mechanisms (sweating, flushing, etc.)

A-2. Face feels hotter because of vasodilation -- smooth muscles around the arterioles relax, and blood flow to face increases. The heat radiation from the blood near the surface makes the face feel hot.

A-3. Person has reduced core temperature below normal (due to erroneous signals), and now needs to restore the temperature to normal.

A-4. When fever breaks, person feels hot and sweats and vasodilates to cool off. The same thing happens to a person with a hot flash -- she suddenly feels hot, so her physiological response is the same -- sweating and vasodilation. (Her behavioral response may also be the same -- she may throw off the blankets, take off a sweater, etc.) This is the part you had to explain.

In the case of a fever & a hot flash, the physiological response is the same, but the mechanism behind the 'feeling hot' is different. In the case of a fever, the change from feeling normal to feeling hot is due to a shift in the set point. People used to think the set point changed to cause a hot flash, but now they think otherwise – they think the width of the thermo-neutral zone is what is changed. See below.

B-1. The effectors causing the flush are **smooth muscle**.
B-2. The regulated variable here is **core body temperature.**
B-3. The set point should be **the same** for both types of women.

Explanation of B:

B-1. The smooth muscle relaxation is what is primarily responsible for the redness of the face. Sweat glands help you cool off overall, but don't make your face red.

B-2. The negative feedback system here senses and regulates the core body temperature. The extent of vasoconstriction and redness of face are 'controlled' -- that is, adjusted to keep the core body temperature in line. The (face) surface temperature may be sensed, and that information may be used by the IC, but it is the core body temperature that is maintained within narrow limits, not the surface temperature (which can vary a lot).

B-3. The info is given that both types of women have the same **average** body temperature.

C-1. The maximum core body temperature should be about **37.2^{o}C**.
C-2. The thermo-neutral zone should be wider in **women without hot flashes.**
C-3. Yominbine should **lower** the critical pt for sweating (& raise the critical pt for shivering).
C-4. **The one who <u>does</u> have hot flashes** will start shivering first.

Explanation of Part C is on next page.

Explanation of Answer to 5-10, Part C.

Explanation of C:

C-1. As defined here, the thermo-neutral zone is the distance between the critical point for sweating and the critical point for shivering. In other words, it is $37^{o}C \pm 0.2^{o}C$. The thermo-neutral zone is not $37^{o}C \pm 0.4^{o}C$.

C-2 to C-4. The data indicate that women who have hot flashes have the same set point as those who don't, but the women with hot flashes have a narrower range of tolerance for temperature changes. They are more sensitive to changes in temperature, and both sweat and shiver more easily. (Therefore the critical point for sweating is lower, and the critical point for shivering is higher.) A small change in temperature that would not trigger a corrective action in a person without hot flashes will trigger corrective action in a person with them. This has been shown by putting people in a room and heating or cooling it and seeing who sweats or shivers and who doesn't with small temperature changes.

For an article on the current understanding of hot flashes, see
http://www.medscape.com/viewarticle/510409_3

ANSWERS TO PROBLEM SET 6 - Signal Transduction & Hormone Action

6-1. A. Domain B. The extracellular domain (B) should bind ligand.

B. No. G-linked receptors are generally multiple pass proteins that go through the membrane seven times. This receptor is more likely to *be* a tyrosine kinase or be linked to a tyrosine kinase, since those receptors are "single pass" proteins.

6-2. A. Hydrolysis of GTP. When triggered by the appropriate receptor, a G protein binds GTP, which displaces a GDP. The GTP is then quickly hydrolyzed to GDP by the G protein itself, which is a GTPase.

B. Either inhibits or activates. The form bound to GTP is considered the "active" form, but when activated, it might stimulate or inhibit its target protein, depending on whether it's a G_s or G_i , that is, a stimulatory or inhibitory G protein.

C. Neither. The active forms of the G proteins usually act by altering the activities of <u>other</u> (target) proteins, which may be kinases and phosphatases. It is these target proteins, not the G protein itself, that may phosphorylate and dephosphorylate other target proteins.

6-3. These are the only amino acids with free hydroxyls in their side chains; an OH is needed to form an ester bond with a phosphate.

6-4. A. down-regulation of receptors for thyroid hormone in the skin, so these cells are no longer sensitive to thyroid hormone.

B. Inhibit secretion of TSH, which is produced by the anterior pituitary gland. This would inhibit TH secretion, providing another way to prevent TH from stimulating the gland to develop.

6-5. Both act in the IP_3/DAG pathway. PLC generates IP_3 and DAG from PIP_2; PKC is activated by DAG and Ca^{++}. Ca^{++} is released from the ER by IP3.

6-6. A. If protein kinase A (PKA) is always "on," then glycogen breakdown will always be stimulated and glycogen synthesis will be inhibited because of the activation/inactivation of the corresponding enzymes (see handout). This is equivalent to having a constant high level of cAMP or continual hormone stimulation. (Normally, if PKA is active, cAMP is high and the phosphatase is inactive. In this case it is possible that both PKA and phosphatase are active. We are assuming that the effects of the kinases will overwhelm any possible effects of the phosphatase.)

B. If phosphorylase kinase is always "on," glycogen phosphorylase will be active, breaking down glycogen. But unlike the case in A, glycogen synthesis will not be inhibited by phosphorylation of glycogen synthetase. Phosphorylase kinase is part of the path leading to glycogen breakdown but it is not part of the path leading to glycogen synthesis. If cAMP is low, and glycogen synthetase is active, glycogen synthesis may be occurring at the same time as glycogen breakdown. In the normal pathway, PKA is a branching point, which ensures that one pathway is always stimulated while the other is inhibited, so that either glycogen synthesis or glycogen breakdown occurs. In the situation here, the system has a short circuit, and both processes can occur at the same time.

6-7. Different authorities come up with different answers for this one. The usual range is from millions to billions (i.e. a very large number). For an estimate, see Sadava p. 143 (9^{th} ed.) or p. 346 in 8^{th} ed. or Becker fig. 14-3 (6^{th} or 7^{th} ed).

6-8. Drug is acting as an ***agonist*** (mimic) of the hormone.

6-9. A. Phosphodiesterase breaks cAMP down into AMP, inactivating it. If no phosphodiesterase is present, cAMP will accumulate, prolonging the activity of the kinases and phosphorylases that lead to hydrolysis of glycogen to glucose. Liver glycogen will decrease.

B. Protein kinase is necessary for activation of glycogen phosphorylase and inactivation of glycogen synthetase. Without protein kinase, glycogen synthetase is active, so glycogen will increase.

C. The agonist combines with epinephrine receptors and mimics its effects, so it will activate G protein, leading to increased adenyl cyclase, cAMP, protein kinase, phosphorylases, and will lead to decreased glycogen, just as could occur with epinephrine itself.

6-10. **A**. IP_3; **B**. ER. Ca^{++} ionophores mimic IP_3, which triggers release of Ca^{++} from the ER.

6-11. It is most likely that the drug is affecting something downstream of (acting after) cAMP. cAMP activation activates protein kinase A (also called PKA or cAMP-dependent kinase). We usually assume there is only one PKA. (There are actually 2, so the drug could be affecting only one of them.) If there is only one kinase, the difference must be further downstream, after the PKA. This is quite possible, as PKA phosphorylates many different proteins, and these in turn activate or inhibit other proteins, and so on. In other words, there are a large number of enzymes and target proteins that occur downstream of cAMP, and it's likely that the drug was specific for one of these. (If we consider that there are multiple protein kinases, the drug could affect them differently.)

Most cells have only one type of receptor for any particular hormone, but some cells have more than one receptor for any given hormone. If there are multiple receptors, the drug might be acting on one particular receptor, or its associated G protein, which could result in activation of only one protein target. (One target protein could increase the level of cAMP and one could decrease it.)

6-12. A. A is a peptide, B is a steroid.

B. Peptide hormones bind to membrane receptors, which are proteins. Hormone A shows most binding to intact cells and membrane fragments which would include the receptors. It doesn't bind to trypsin-treated cells where the receptors were destroyed.

Steroid hormones bind to receptors that are in (or go to) the nucleus. The steroids don't bind membrane fragments, and do bind intact cells, since trypsin couldn't enter the cell and get to the steroid receptors. The large amount of binding in cytosol may reflect binding to receptors in cytosol near nucleus, or to newly synthesized receptors that haven't yet been brought to nucleus.)

C. Peptide hormone has hydrophilic regions, can circulate free in blood. Steroids are lipids and hydrophobic (lipophilic). To prevent them from forming fat globules in the watery blood, they are carried in association with the hydrophilic proteins.

6-13. Epinephrine induces a more rapid response - it simply activates certain proteins that already exist in the cell. Making new proteins from scratch takes longer, which is what the steroid hormones, like cortisol, do.

6-14. No. Testosterone is a steroid hormone, and lipid-soluble. It would have been in the greasy gunk he threw out. It seems unlikely that the water-soluble substances would have had this effect, though thinking it would may have given Brown-Sequard all the encouragement he needed. Note: While testosterone can improve sex behavior in those with testosterone deficiency, it has no effect on sex behavior of those who already have normal testosterone levels.

6-15. Let's say each receptor is a sphere imbedded in the membrane so that the bilayer of the membrane bisects the sphere. Then the receptor takes up an area of the membrane = cross section of the sphere (NOT the area of the sphere).

area receptors take up = πr^2 not $4 \pi r^2$

area of receptors/area of cell = $\pi r^2 \times 10^5 / 4 \pi R^2$.

r = radius of receptor = 10 A = 10×10^{-10} m.

R = radius of cell = 50 microns = 50×10^{-6} m.

Area of receptors/area of cell = $(10 \times 10^{-10})^2 \times 10^5 / 4 \times (50 \times 10^{-6})^2 = 10^{-5}$ = .001%.

0.001% of the membrane is covered with this particular receptor; in other words, 99.999% of the membrane is free of this particular receptor. Thus although there may be many receptors per cell, they constitute only a tiny fraction of the total and may be hard for scientists to isolate. Also, the cell surface is by no means saturated with receptors -- they are relatively far apart and the probability a ligand will meet its receptor can be regulated by changing the number of receptors.

6-16. A. a cell surface receptor; since the effects of F on Enzyme A are prevented by an adenylyl cyclase inhibitor, and since adenylyl cyclase is generally activated by G-protein coupled receptors, F must be acting on a membrane receptor.

B. cell surface receptor, protein kinase in cytoplasm, G protein.
F should be a ligand for a surface receptor; binding of F should activate a G protein which should activate a protein kinase. The series of events should go like this:
F binds to receptor on outside of cell → activates G protein (GDP released; GTP bound) → activates adenylyl cyclase → generates cAMP → activates protein kinase A → phosphorylates some enzyme Q, changing its charge from neutral to negative.

Note: The receptor is always separate from the G protein (if there is a G protein involved). The receptor can function without a G protein in some signaling systems if the receptor is part of a channel or if the receptor has an intracellular domain that has tyrosine kinase or other catalytic activity (or is linked to a protein that does). So a receptor can function without a G protein but a G protein can't function without a receptor. The surface receptor here is probably the G protein-linked type because adenylyl cyclase is involved.

6-17. A. Protein kinase C. Ca^{++} probably activates some enzyme that modifies gelsolin. Protein kinase C is dependent on Ca^{++}, as well as DAG, so the simplest explanation is that Ca^{++} activates PK C which phosphorylates gelsolin, and thereby and activates it. There are other possibilities -- the Ca^{++} could act through a series of steps to activate some other protein kinase -- but the answer given here is the simplest one.

B. ER & IP_3. If intracellular Ca^{++} remains constant, the Ca^{++} must be released from some internal compartment, and the ER (really some specialized part of it) is the Ca^{++} storage compartment. (The sarcoplasmic reticulum is a modified ER found only in muscle.) The Ca^{++} storage compartment has a receptor for IP_3, and binding of IP_3 opens Ca^{++} channels. The whole sequence is as follows:
Hormone binds to extracellular receptor → activates G protein → activates phospholipase C →breaks down PIP2 to release IP_3 → binds to receptor on ER membrane → opens Ca^{++} channels in ER membrane → Ca^{++} flows down gradient into cytoplasm.

6-18. A. See p. 70. Please do not attempt to do the rest of this problem until you are sure of the answers to part A.

B-1. Membranes. CDE activation requires phospholipase C, which is membrane-bound.

B-2. IP3 and Ca^{++}. You can activate the cascade if you add something that comes after (downstream from) phospholipase C action, that is, if you eliminate the need for phospholipase C. You can do that by adding the product of phospholipase C action, namely IP3, or the substance released by the ER in response to IP3, namely, Ca^{++}.

C. Ca^{++}.
The IP3 receptors are on the surface of the ER. This time you have to add something downstream of the point where IP3 acts, and that leaves only Ca^{++}. Normally, the ER releases Ca^{++} in response to IP3; if you add Ca^{++} you don't need IP3 or its receptors.

6-19. A-1. Free in the cytoplasm; A-2. Unmasks a localization signal.

The receptor is in the cytoplasm (& later goes to the nucleus); proteins destined for the cytoplasm or nucleus are made on free ribosomes in the cytoplasm. If the receptor were made on ribosomes attached to the ER or outer membrane of the nucleus, it would end up in the endomembrane system or cell surface, not in the cytoplasm.

No signal is needed to remain in the cytoplasm, but a nuclear localization signal (NLS) is needed to enable a protein to bind to the nuclear pore complex and enter the nucleus. The NLS must be masked until hormone binds; otherwise the receptor would enter the nucleus without hormone. Since only the receptor-hormone complex, but not the receptor alone, enters the nucleus, binding of hormone must cause a change in the shape of the receptor so that the NLS is exposed and the hormone-receptor complex can enter the nucleus.

B. 1 H2B/100 bp, # H2B stay the same and sensitivity to DNase should increase.

There is one histone octamer with 2 molecules of H2B per 200 base pairs of DNA = one nucleosome per 200 bp of DNA. So 2 H2B/200 bp = 1 H2B per 100.

The transcribed region of chromatin should retain all or most of its nucleosomes when the RNA polymerase passes through, so the number of H2B molecules should stay about the same. (The number of nucleosomes may decrease significantly in the non-transcribed regulatory regions as the nucleosomes are replaced by regulatory proteins, but this part of the question asks about the transcribed regions.)

When transcription occurs, the nucleosome fiber will probably become more loosely coiled, going from a tighter, more closed configuration (such as the 30 nm fiber) to a looser, more open configuration (like "beads on a string"). The number of nucleosomes doesn't change, but their state of packing does. The looser structure will be more sensitive to DNase as the DNA will be more accessible to nucleases.

C. #H2B decrease, and sensitivity to DNase increase.

In the regulatory, nontranscribed, regions (enhancers, promotors, and other cis acting elements) the histones are replaced by regulatory proteins (basal and regulatory transcription factors). DNA with regulatory proteins instead of histones is much more sensitive to DNase.

D-1. Z has a nuclear localization signal. Z (plus hormone) enters the nucleus, but X and/or Y without Z do not (result (1)), so Z must carry the localization signal.

D-2. Y binds to a cis acting regulatory sequence. Z (+ hormone) enters the nucleus, while Z-Y complex (+ hormone) enters the nucleus and binds to the DNA. Therefore Y must bind to DNA. The sites on the DNA where transcription factors bind are known as cis acting regulatory sequences; the special class of sites where hormone receptors (acting as regulatory transcription factors) bind are usually called hormone response elements (HRE's). The details of hormone action will be discussed in class later; only lipid soluble hormones act in this manner (binding to receptors that act as TF's).

D-3. X binds to transcription factors or other proteins. X-Y stimulates transcription in cell extracts (ground up cells with no intact nuclear membrane), which indicates that Y binds to the DNA and then X actually activates transcription. In intact cells, X-Y can't get to the DNA because it can't enter the nucleus. Once X-Y binds the DNA, Y anchors the complex to the DNA and X binds to other transcription factors and/or co-activators, positioning them (& RNA polymerase) in the right place to start transcription.

D-4. Terminology: Y is the DNA binding domain. X is the transcription activating domain. Z is the cortisol (hormone) binding domain (which also includes the NLS).

E. Many genes have the same cis-acting regulatory sequences associated with them. All the genes that share the same regulatory sequences can be turned on simultaneously by action of the same TFs. The genes can be located far from each other, but each has the same "switch" located in the proximal or distal regulatory region. When the "switch" is activated in response to addition of a hormone, the switch (cis-acting sequence) is known as a hormone response element or HRE. The HRE's for cortisol are called cortisol response elements; the HRE's for estrogen are called estrogen response elements, and so on. All the cortisol response elements bind the same TF = the activated cortisol receptor; all the estrogen response elements bind a different TF = the receptor activated by estrogen. When steroid hormones are added, the hormones bind to their respective receptors. The receptors are then activated, go to the nucleus, bind to their respective hormone response elements, and activate (or inhibit) transcription.

Note: Adding hormone, even at very low concentration, always means adding many molecules of hormone. Thus adding hormone always activates many copies of the receptor, and there is enough activated receptor to bind to multiple HRE's and turn on multiple genes.

6-20. A. cAMP & IP3. Ca^{++} activates calmodulin, so activity of subunit A should depend on Ca^{++} levels. Ca^{++} is released from the ER by the action of IP3, so the level of Ca^{++} (and level of activity of subunit A) should depend in turn on the level of IP3.

Binding of cAMP activates Protein Kinase A. PKA phosphorylates subunit B of PKK, and phosphorylation usually alters the activity of target protein. So activity of PKK should depend on levels of cAMP as well as on levels of Ca^{++}.

B-1. adenyl cyclase; B-2. Ca^{++}; B-3. subunit B; B-4. subunit A. Explanations:

For H1: H1 binds to a G-protein linked transmembrane receptor → activation of a G protein → activation of adenylyl cyclase → cAMP → binding to PKA → phosphorylation and activation of subunit B → activation of PKK.

Phosphodiesterase destroys the cAMP, converting it to AMP and preventing activation of PKA.
DAG and PLC are not involved here, so antagonists or inhibitors of those components have no effect.

For H2: H2 binds to a transmembrane receptor that activates the DAG/IP3 pathway, so the receptor must activate PLC. Both a TK receptor or a G protein coupled receptor can trigger the IP3 pathway. (Only a GPCR can activate the cAMP pathway).

A G protein coupled receptor can activate PKK through the DAG/IP3 pathway as follows:
The receptor-H2 complex → activation of a G protein → activation of phospholipase C → generation of DAG and IP3. The DAG is not involved in signaling here, but the IP3 → ER → release of Ca^{++} → binding to subunit A (calmodulin) → activation of PKK.

PLC inhibitors block generation of IP3, thereby blocking generation of Ca^{++} and activation of calmodulin. Antagonists of DAG have no effect as DAG (& PKC) are not involved here; phosphodiesterase has no effect as cAMP is not involved either.

A TK receptor could work like this: H2 could bind to a TK receptor → activation of the receptor → self phosphorylation of cytoplasmic domain → activation of phospholipase C (by binding of PLC to the receptor or to adapter proteins that bind to the receptor). Once PLC is activated, the rest of the pathway is the same.

C: C-1. Both; C-2. One or the other but not both; C-3. Hormone 2.

Explanation for C-1 & C-2. A G protein can activate either the cAMP pathway &/or the DAG/IP3 pathway. So both hormone receptors could be coupled to G proteins. However the two receptors must activate different G proteins, as the two hormones activate different enzymes -- PLC vs adenylyl cyclase. The same G-protein can activate both the IP3 & cAMP pathways (if it binds to & activates both PLC and AC). However in this case, one hormone produces only the IP3 response, and the other hormone produces only the cAMP response. So it must be two different G proteins -- one that activates PLC and one that activates AC.

Explanation for C-3: Hormone 2 activates the IP3/DAG pathway. Either a G protein coupled receptor (which is a multipass protein) ***or*** a TK linked receptor (which is a single pass protein) can activate this pathway. Hormone 1 activates the cAMP pathway; only a GPCR (multipass) can activate this pathway**.**

6-21. A-1. The G proteins should be different (since they bind to different target proteins).

A-2. α-1 receptors should change the IP3 concentration (since α-1 receptors activate PLC).

B-1. Activity of PKA (protein kinase A) should decrease when epinephrine is added.

B-2. Effect should be less if an inhibitor of PDE (phosphodiesterase) is added along with the epinephrine.
("Greater" was the correct answer if you said "increase" for B-1.)

Explanation of B: You start with some activated PKA = PKA bound to cAMP. Then epi binds to the α-2 receptors. The activated receptor activates a G protein. The G protein binds to and inhibits adenyl cyclase (AC). Therefore no more cAMP will be made. The old cAMP will fall off the PKA, and be degraded by PDE. As cAMP leaves the PKA, the PKA will become inactive. So PKA activity will decrease. If PDE is inhibited, the old cAMP will not be degraded as rapidly, so the PKA will remain active longer, and the decrease will be less (or occur more slowly).

6-22. A. See p. 70. Please do not attempt to do the rest of this problem until you are sure of the answer to part A.

B. From the information given in the problem itself, pertussis toxin should inhibit binding of GTP (required for activation) or hydrolysis of GTP (required for deactivation). If you don't look at the table, you can't tell which process is blocked, binding or hydrolysis. If you take the results of experiment 3 in table 2 into account, you see that pertussis blocks all the effects of epinephrine on cells with α2 receptors, so it must block the activation of Gi, and therefore it must block the binding, not hydrolysis, of GTP. (If pertussis toxin blocked hydrolysis of GTP, adding pertussis toxin would give you too much IP3 and Ca^{++}, not block Ca^{++} release.)

C-1. Both receptors probably activate PLC. (From the 1st experiment -- because both generate IP3 as well as cytosolic Ca^{++} in response to epi.).

C-2. The Ca^{++} enters the cytosol from the ER. (From the 2nd experiment -- if you block channels in the plasma membrane, and prevent Ca^{++} from entering from the outside, you still get a burst of Ca^{++}.)

C-3. The alpha subunit must activate PLC in cells with α1 receptors, but the β/γ subunits must activate PLC in cells with α2 receptors. This follows from the 4th experiment -- the β/γ binding proteins do not block Ca^{++} release in cells with α1 receptors, so the alpha subunit must be triggering the IP3 pathway. In cells with α2 receptors, the β/γ subunits must be the part of G_i that triggers the IP3 pathway, since release of Ca^{++} is blocked by the binding proteins. (In these cells, the alpha subunit inhibits AC and the PKA pathway.)

Here is the pathway overall for cells with α2 receptors: Epinephrine activates the α2 receptors, which activate Gi (by promoting GTP/GDP exchange). When G_i binds GTP it is activated, and it falls apart into the α subunit and the β/γ subunits. The α subunit inhibits AC, preventing synthesis of cAMP, thus preventing the activation of PKA. The β/γ subunits activate PLC. The activated PLC splits PIP2 in the membrane, to generate IP3. The IP3 diffuses through the cytoplasm and binds to Ca^{++} channels in the ER membrane. This triggers opening of the channels, and Ca^{++} flows down its gradient out of the ER into the cytosol.

Pertussis toxin blocks the disassociation of the subunits and thus blocks effects on both PLC and AC. The binding proteins only block the β/γ subunits, so they only block the effects on PLC.

Pertussis toxin has no effect on signaling with α1 receptors, because that involves a different G protein. Pertussis toxin is specific for Gi -- it doesn't bind to other G proteins. The binding proteins are not specific, and bind to all β/γ subunits. However, the binding proteins don't affect signaling by the α1 receptors because the β/γ subunits are not involved in the process -- only the α subunit of the G protein is involved in α1 signaling.

6-23. This question is based on an article in Science, 30 September, 2005. Vol. 309, p. 2232.

A. Ligand binding domain of IP3 receptor should be in the cytoplasm; Ligand binding domain of acetyl choline receptor should be extracellular.

The IP3 receptor is a transmembrane receptor in the ER membrane. It binds IP3 in the cytoplasm, so its ligand binding domain must be on the outside of the ER, not the inside.

B-1. PLC (phospholipase C) would be activated first. (ACh esterase could be activated at the same time; once receptor is triggered, ACh could be destroyed.)

B-2. The cytoplasm would contain IP3 and Ca^{++} but not DAG.

Explanation: PLC splits PIP2 (which is in the membrane) into IP3 and DAG. The DAG remains in the plasma membrane, but the IP3 is soluble and enters the cytoplasm. IP3 binds to receptors on the ER, and the binding opens Ca^{++} channels in the ER, releasing Ca^{++} from the lumen of the ER into the cytosol.

Note: PLC does not 'activate' PIP2. The enzyme PLC gets activated, and its substrate gets acted upon. In this case, the substrate PIP2 is hydrolyzed ('split') into IP3 & DAG. You have to be careful about the distinction between enzymes/proteins and substrates/small molecules.

B-3. ACh triggers exocytosis.

The normal scheme is ACh binds to its receptor, and the activated receptor activates a G protein, that in turn activates PLC. The PLC generates IP3 which triggers release of Ca^{++} as explained above. The Ca^{++} triggers fusion of secretory vesicles with the plasma membrane, causing secretion of digestive enzymes. If IP3 receptors are missing, there is no release of Ca^{++} and no exocytosis. You know the Ca^{++} must trigger exocytosis because the problem states that ACh triggers immediate secretion of digestive enzymes. If ACh stimulated any earlier step in secretion, it would take a while to show an effect.

Note: Release of Ca^{++} from the ER into the cytosol is not called secretion. Secretion is usually reserved for release of material to the outside of a cell. *(Answers to rest of problem 6-23 are on the next page.)*

Answer to 6-23, cont.

B-4. A Ca^{++} ionophore will trigger secretion of digestive enzymes. How does it work? A Ca^{++} ionophore makes channels in the plasma membrane, not the ER membrane. The net result of opening channels in either membrane (plasma membrane or ER) is the same -- Ca^{++} rushes into the cytosol, down its gradient. (The Ca^{++} level is kept low in the cytoplasm by constantly pumping Ca^{++} back into the ER or out of the cell. So the gradient is always "downhill" into the cytoplasm.) When you add an ionophore, Ca^{++} enters the cytosol from outside the cell, not from the ER. However, Ca^{++} in the cytoplasm has the same effect, no matter where it comes from, or what triggers its release. In all cases a pulse of cytosolic Ca^{++} triggers exoctyosis.

C. ACh causes different effects in the two cell types because of different targets of Ca^{++}. Both cells use the IP3 pathway to respond to ACh, as a lack of IP3 receptors blocks both responses. So both cell types must release Ca^{++} in response to ACh. The difference must be after Ca^{++}, not before -- different proteins must be activated or inhibited in the two cell types by Ca^{++} (directly or with calmodulin). In salivary cells, water channels are opened so water is released; in pancreatic cells exocytosis occurs so enzymes are released.

6-24. A. To activate the G protein, you need the GPAF and GTP.
B. When the G protein is (re)activated, you will release radioactive GDP.
Activation of a G protein requires GTP for replacement of a GDP with a GTP. Deactivation occurs by hydrolysis of the GTP to GDP. In part B, the phosphate has already been hydrolyzed off, and washed away, but the inactive enzyme still has a bound GDP. To reactivate, you have to exchange the GDP for a GTP, not phosphorylate the GDP to GTP.

6-25. You should get just as much binding if you omit GTP & epinephrine (epi).
(1) You need cAMP to activate PKA, so you can't omit it. (There is no adenyl cyclase to generate it.)
(2) You need ATP to phosphorylate protein Q -- PKA is a kinase that requires ATP as a substrate.
(3) You do not need GTP or epi, because (a) you already have cAMP, and (b) they are useless without a signal transduction system (GPCR, G protein & adenyl cyclase). In other words, you have the end product, cAMP, so you don't need to carry out the steps to produce cAMP in response to epi, and you don't have the proteins to do it anyway.

6-26. A. Intracellular receptors act as transcription factors; these factors must first stimulate transcription of a certain gene, the mRNA must travel to the cytoplasm, be translated into a protein, and there must be enough of this around for some obvious effect to occur within the cell. It takes minutes to days to note a biological response to a steroid hormone. Membrane-bound receptors don't require the production of new protein, but simply the activation of a protein that already exists: either the opening/closing of a channel, or the activation/inactivation of an enzyme, so this can occur much more rapidly. You got no credit for saying "Responses involving intracellular receptors are slower, because it takes time for the hormone to diffuse to the nucleus." While it does take more time to reach the nucleus, molecular diffusion at the level of a single cell is rapid enough that this increased time is negligible.

B. The question tells you that either DA or P can stimulate lordosis, and asks whether DA and P use the same signal transduction pathway. Here's how I expected you to reason through this (You didn't necessarily have to use the bits of information in this particular order to get to the answer. Basically, you got part credit for having each step in the pathway in the correct order and additional credit for mentioning that P and DA seem to be able to use the same pathway):

Diagram of pathway is given after the details below, and the numbers below refer to results listed in question.

Detailed Answer to 6-26, Part B:

1. If a phosphodiesterase inhibitor is injected into the brain, females show lordosis more than usual.
Since phosphodiesterase inhibition leads to increased cAMP, this suggests that cAMP is involved in DA and/or P stimulation of lordosis.

2. Rats with a mutation in the progesterone receptor don't show lordosis at all.
This suggests that both DA and P must make use of the same P receptor.

7. When a DA receptor agonist and a progesterone receptor antagonist are given together, lordosis does not occur.
This confirms that DA stimulates a receptor, and then somehow sends a message to the progesterone receptor.
DA-> R.....P receptor

3. DA has a strong affinity for a 7-pass protein found in the cell membrane.
You know that membrane receptors that stimulate G proteins are 7-pass proteins, so this suggests that DA activates such a receptor.
DA -> R -> Gprotein.....P receptor

4. DA receptor agonist leads to an increase in cAMP in the brain.
5. DA receptor agonist leads to activation of protein kinase A.
These suggest that the particular pathway activated by the G protein is the cAMP pathway we discussed in class:
DA -> R -> Gprotein -> cAMP -> PKA.....P receptor

9. When DA receptor agonist and antisense oligonucleotide to DARPP-32 are given together, lordosis does not occur.
This suggests that **DARPP-32 is required** for DA action, somewhere after the receptor activation.
DA -> R -> Gprotein -> cAMP -> PKA.....?DARPP-32.....P receptor

10. When the nuclear progesterone receptor is phosphorylated, it can bind to DNA, even if P is absent.
You'd expect that the progesterone receptor requires progesterone to act as transcription factor, but this result suggests that DA could activate the receptor simply by getting it phosphorylated.
DA -> R -> Gprotein -> cAMP -> PKA.....?DARPP-32.....Phosphorylated P receptor

8. When phosphorylated, the enzyme DARPP-32 inhibits the activity of protein phosphatase-1.
This suggests that DARPP-32 needs to phosphorylated to be active, so it suggests that one of the proteins phosphorylatd by PKA is going to be DARP-32.
DA -> R -> Gprotein -> cAMP -> PKA ->DARPP-32.....Phosphorylated P receptor

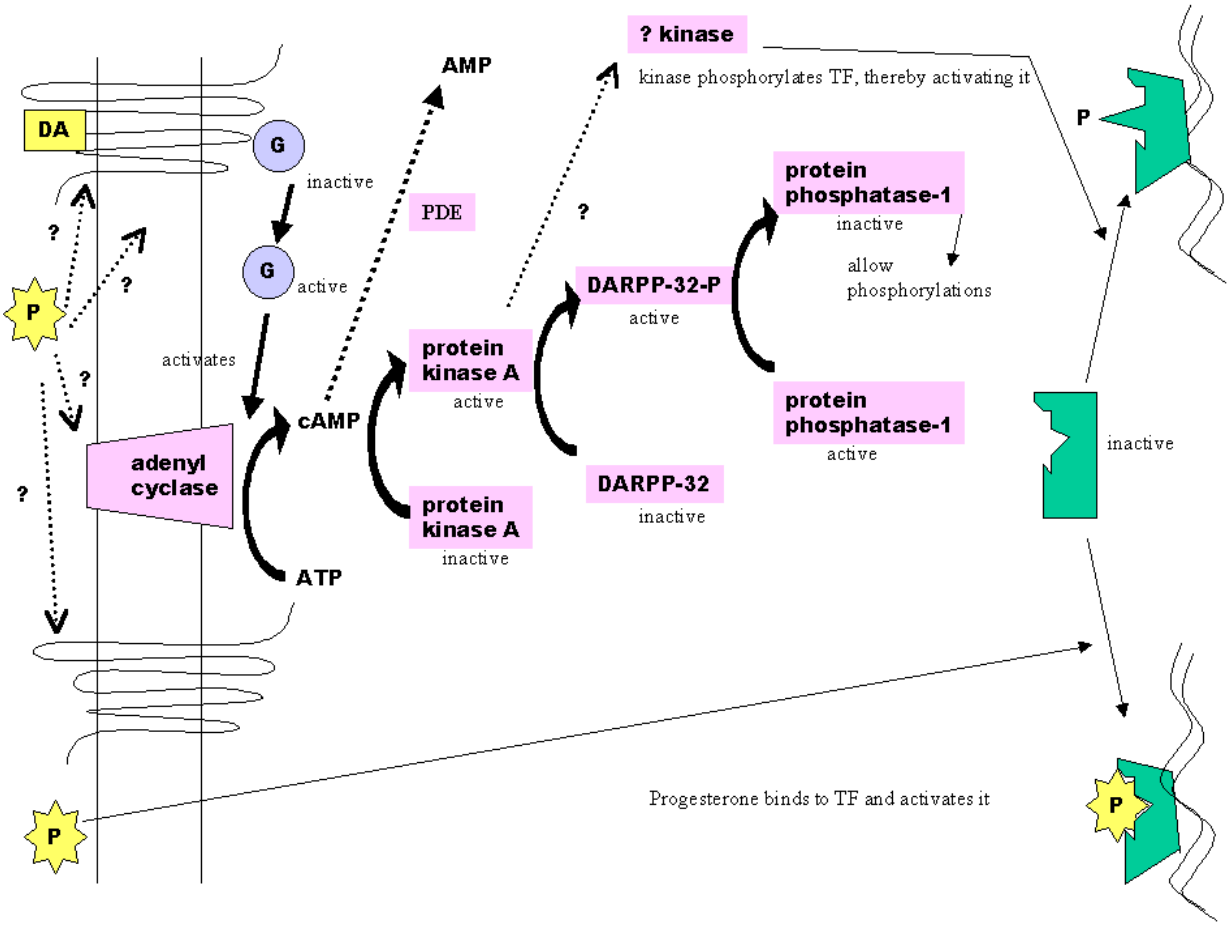
It also suggests that the activated DARPP-32 has its effect by inhibiting some of the phosphatases within the cell, or, to put it another way, allowing some kinases to do their phosphorylating work unopposed. Since we know that this pathway will end at a phosphorylated protein, the P receptor, we can surmise that DARPP-32 allows phosphorylation of the P receptor, either by PKA itself, or by some other kinases present in the cell.
DA -> R -> Gprotein -> cAMP -> PKA ->DARPP-32-> allows phosphorylation of P receptor

6. Progesterone treatment leads to an increase in cAMP in the brain, and an activation of protein kinase A.
This suggests that progesterone is able to use the same pathway as outlined above for Dopamine. (See note at end of answer.)

C. Phosphodiesterase catalyzes breakdown of cAMP to AMP, so an inhibitor of phosphodiesterase will keep the pathway turned on. cAMP activates the protein kinase A, which, in turn, phosphorylates DARPP-32. The phosphorylated DARPP-32 inhibits a protein phosphatase, so there will be more opportunity for protein kinases to do their thing. One of the things that will be phosphorylated by a kinase (could be PKA or some other kinase) is the progesterone receptor, and phosphorylated progesterone receptors can bind to DNA, even if P is absent. So transcription of this gene important in lordosis will continue without the usual inactivating factor (breakdown of cAMP), and there will be more than the usual amount of lordosis.

D. estrogen given 24 hours before progesterone. In the previous part, progesterone was the signal, and it bound to the progesterone receptor, stimulating transcription of some gene important in lordosis. In this question, estrogen is the signal, which binds to a gene at the Hormone (Estrogen) Response Element, initiating transcription. The gene being transcribed codes for the progesterone receptor. The resultant Progesterone Receptor mRNA must first be translated to produce the P receptor, which then must return to the nucleus to be activated by P or DA. All this takes time, so to maximize response to P, you should give estrogen first, so it has enough time to maximize synthesis of the receptor for P, and only then inject P. Estrogen may be said to have a permissive effect on progesterone action.

Diagram of pathway:



Note: This question is based on a research article reported in ***Science*** several years ago. Although textbooks generally maintain the classical view, that steroid hormones act on intracellular receptors, there is increasing evidence in the scientific literature to suggest that *all* steroid hormones probably have some effects on the membrane, or what are called "non-genomic effects". The basis for this is an exciting current area of research: Are there membrane receptors for the steroids? (There is some recent evidence that there are steroid receptors on the ER membrane &/or plasma membrane. See ***Science***, March 11, 2005 p. 1572 or http://www.sciencemag.org/cgi/content/full/sci;307/5715/1572) Do the steroids act as some kind of cofactor and modify other receptors, like the DA receptor in this case? Or are the steroid effects not mediated by receptors? One suggestion is that steroid hormones, being lipid soluble, can insinuate themselves into the membrane in the area of receptors such as this DA receptor, and thereby change some of the binding properties or signal transduction pathways associated with that receptor. Part of the excitement generated by this issue is that it suggests that some of the effects of a hormone might depend only on the intracellular effects, and some only on the membrane effects, and therefore drugs might be developed to mimic or inhibit one of these effects without the other.

ANSWERS TO PROBLEM SET #7

7-1. e. is incorrect. Can you think of examples of a-d?

7-2. a, b, c, d are incorrect; only e is correct. For b: hormones are secreted at a variable rate to turn rates of other processes up or down as needed to maintain homeostasis. For d: applies to water soluble hormones only.

7-3. Follicle cells: hormonal (TSH).
Hypothalamus: hormonal (eg, hormones produced by target organs, such as the thyroid and ovary); nervous (Neuronal messages come from other brain areas and synapse on the hypothalamus) and chemicals (such as glucose, Na^+ and other osmotically active substances).
Anterior pituitary: hormonal (from hypothalamus).
Posterior pituitary: neuronal (input is actually to the cell bodies of these neurons, in the hypothalamus).
Adrenal cortex: hormonal (ACTH).
Adrenal medulla: neuronal (from sympathetic division of autonomic nervous system) .

7-4. 1. e (exocrine glands use ducts. These are all endocrine glands.)
2. b,c (Ant. Pit originates from outpocketing of mouth; post pit & HT from brain.)
3. e (endocrine glands are generally of epithelial origin; neuroendocrines are neural)
4. d (thyroid hormone uses intracellular receptors like a steroid)
5. d (& c). TH is made from tyrosine. (One of the release inhibiting factors from the HT is dopamine, which is also derived from tyrosine.)
6. a (portal vein from HT to Ant. Pit.)

7-5. Table: i = increase; d = decrease

Condition	**TH**	**TRH**	**TSH**	**Goiter?**
Thyroid gland doesn't produce hormone	d	i	i	yes
Thyroid tumor: overactive in producing hormone	i	d	d	no
Anterior pituitary doesn't produce hormone	d	i	d	no
Anterior pituitary tumor: produces too much hormone	i	d	i	yes
Lack of dietary iodine	d	i	i	yes
Graves' disease	i	d	d	yes

7-6. In hypothyroidism due to pituitary damage, there will be low TSH and low TH, because anterior pituitary doesn't produce TSH which is necessary for thyroid to secrete TH. In hypothyroidism due to thyroid damage, the thyroid gland doesn't produce TH and so there is no negative feedback on the anterior pituitary gland, and TSH is produced at even higher levels. So in this case, there is high TSH and low TH.

7-7. a. and b. increase. (Anti-TSH-receptor antibodies act like the tropic hormone, and stimulate the thyroid gland to grow and secrete more thyroid hormone.)

c. decrease. (Due to negative feedback by TH)

d. not change. (TH combines with intracellular receptors, doesn't affect cAMP.)

e. decrease. (Due to increased metabolic rate)

f. not change. (The basal metabolic rate is higher, and more heat is produced, but the heat is dissipated by sweating, dilation of blood vessels, so that the body temperature remains normal. The hypothalamus senses small changes in temperature, and immediately sends messages to restore temp to normal, so the temperature of the hypothalamus itself changes very little, and that change is temporary.)

g. not change. (Thyroglobulin is not generally secreted into the blood.)

7-8. a. Thyroid hormone (thyroxine) will be low because of failure of thyroid to respond to stimulation; thyrotropin and its releasing factors (TSH & TRH) will be over produced because of failure of negative feedback.
b. Goiter will not occur since thyroid is not over stimulated -- it is under stimulated.
c. Treat with thyroxine. It won't affect thyroid function, but it will replace the missing thyroxine's effects on target organs. (It will also reduce TSH and TRH levels, but that will have no practical effect.)

7-9. c. The cells of the anterior pituitary gland that secrete TSH are going to respond to stimulation from the hypothalamic TRH (which is a peptide hormone, and so stimulates membrane receptors) and are also sensitive to negative feedback from thyroid hormone (which diffuses through the membrane and combines with intracellular receptors).

7-10. inhibit. Inhibition of I uptake can cause goiter. Goitrogens, or substances that generate goiter, are found in certain vegetables, such as turnips, kale, cabbage, brussel sprouts, cauliflower.

7-11. a. gonadotropins b. Since gonadal hormones are lower, there is less negative feedback on the anterior pituitary and/or hypothalamus, and so there will be increased levels of the gonadotropins in the blood.. There will also be increased levels of GnRH in the portal vessel, but this is diluted so much by the time it reaches the general circulation, that it will not be found in significant levels in the urine.

7-12. A. False (they diffuse through membrane); B. True; C. False (only peptide hormones made this way)

7-13. A. See p. 70. Please do not attempt to do the rest of this prob. until you are sure of the answer to part A.

B. GnRH will increase. (Prolactin should increase too; see below.) If no FSH or LH is released, there will be no negative feedback (inhibition) on the production/release of GnRH, and more GnRH will be produced.

PIH release will not be affected, because its production is not controlled by negative feedback from prolactin. (Mechanical stimulation of the nipple inhibits PIH release.) However, any PIH produced will not reach the AP.

Release of prolactin will probably increase, as explained in part A. Prolactin synthesis is largely under negative control of PIH -- prolactin release is inhibited until release/production of PIH is turned off. (No one is sure if there is a PL-RH at all; if there is, it doesn't seem to be a major player.) Therefore, if PIH can't reach the AP, prolactin release should increase.

C. No goiter. Because TRH will not reach the AP, TSH will not be released. Therefore thyroid will not be stimulated, and will not become enlarged. (TRH will be higher than normal, because of a lack of negative feedback, but that will have no consequences for thyroid function. If TRH cannot stimulate production of TSH, it doesn't matter how much TRH there is.

D. Individual will need TH pills. Individual will not be able to make thyroglobulin or TH, because TSH is needed to stimulate all steps of TH production. Whether iodine is available or not is immaterial.

7-14. Prolactin secretion must usually be under inhibitory control, probably by a hypothalamic inhibitory hormone, since it is produced by the anterior pituitary gland, which receives blood from the hypothalamus by way of a portal vessel. Removing the anterior pituitary from this portal vessel results in increased prolactin secretion, suggesting that secretion is usually inhibited by the hypothalamus. The prolactin-inhibiting hormone would be diluted in the general circulation by the time it reaches the kidney, and so have no effect on the anterior pituitary gland when it is transplanted there.

7-15. A suckling infant stimulates a neuron near the nipple, which conveys nerve impulses to oxytocin-containing cells in the HT/post. pit. These cells are stimulated to release oxytocin from their terminals in the posterior pituitary gland. There are receptors for oxytocin in the myoepithelial cells of the breast (where it stimulates milk let-down) and in the uterus (where it stimulates uterine muscle contraction). The contracting uterine muscles clamp down on the blood vessels, so less bleeding occurs. Suckling triggers release of both oxytocin and prolactin, but it is the oxytocin which promotes uterine involution after birth.

7-16. A. Never. Paracrine secretions are released out of the cell into the immediate vicinity, and affect nearby cells. Exocrine secretions are released through ducts, but not into the blood stream.

B. Never. Thyroxine, or thyroid hormone, is released into the blood. Monoiodotyrosine is a building block for thyroid hormone. If not used to make the hormone, it is recycled within the cell.

C. Sometimes. For temperature regulation, the hypothalamus is the integrator, but for regulation of calcium levels, the hypothalamus is not involved; the parathyroid gland is the integrator.

7-17. a, d (from HT and post pit. respectively).

7-18. Decrease. ADH causes water retention - more water channels are put into kidney cell membranes, so more water is reabsorbed and less leaves in urine. Decreased ADH -> less water reabsorbed -> more urine produced.

7-19. Normal pathway responsible for lactation: infant sucks on nipple → stimulates sensory neuron leading towards hypothalamus → signal to HT to decrease dopamine (=PIH) release (which will release brakes on ant. pit. production of PL) and HT will stimulate post. pit. to produce oxytocin. PL stimulates mammary gland to produce milk; oxytocin stimulates release of milk.

Pressure from the crutch is put on sensory neuron, which travels from breast, under arm towards spinal cord. This pressure caused action potentials to travel along this neuron, mimicking the situation that occurs when the infant sucks. NOTE: This was published as a single case study in a medical journal because it was so unusual; external pressure from crutches is usually not enough to mess around with underlying neuronal pathways.

7-20. A. For hydrophilic hormones, you could name any peptide hormone (insulin, glucagon, prolactin, TSH, ADH, TRH, etc.) or epinephrine.

B. Hydrophobic hormones include all the steroid hormones (testosterone, estrogen, aldosterone, cortisol, etc)

C. Thyroid hormone is the one hydrophobic hormone that can be stored. It's stored in the form of thyroglobulin, which is chopped up to release the actual thyroid hormone.

D. (b). Since the mineral supply of the human diet can change daily, iodine is not necessarily taken in every day. Dietary iodine is required for thyroid hormone synthesis. If we synthesized TH "on demand" as occurs for the other hydrophobic hormones (steroids) then we would be up a creek if we needed TH on a day when we hadn't eaten any iodine. Therefore, the thyroid gland makes thyroid hormone when iodine is present and can store as much as a 6 months supply. Since TH is lipid soluble, it would slip right out if stored in its active form. Instead, it is stored as part of thyroglobulin, and cleaved from there as needed.

7-21. A. *Q: Is the reason that subordinates have higher cortisol in their blood because they destroy the secreted cortisol more slowly than dominants do?*
Conclusion: Results show that when cortisol is injected into the animals, there's no difference in the amount remaining in the blood, so he could conclude that it is not metabolized differently by the two groups. Rather, the subordinates must really be secreting more cortisol.

B. *Q: Is the reason the subordinates secrete more cortisol because their adrenal glands are more sensitive to ACTH stimulation? (or: have more receptors for ACTH)* Conclusion: The greater secretion of cortisol by subordinates is not due to greater sensitivity of adrenal cortex to ACTH, but must be due to greater amounts of ACTH reaching the adrenal cortex.

C. *Q: Is the reason the subordinates secrete more ACTH because their pituitary glands are more sensitive to CRH than the dominants?* Conclusion: The pituitary gland of the subordinates is actually LESS sensitive to CRH, so this can't explain their higher cortisol levels.

D. *Q: Is the reason the subordinates secrete more ACTH because their hypothalamus is less sensitive to negative feedback of cortisol?* Conclusion: Probably! When given dexamethasone, dominants shut off cortisol production due to negative feedback of dex (which is mimicking cortisol), but subordinates don't respond.

Overall, then the higher cortisol found in subordinates seems to be due to less sensitivity to the negative feedback effects of cortisol on the hypothalamic level.

7-22. For hormones to have a physiological effect, they must combine with receptors in target cells, so perhaps the thin-bearded man had fewer androgen receptors in the hair follicle cells, so he grew less hair in response to the same hormonal stimulus. Receptors may differ in their affinity for a particular hormone, so even if the same number of receptors is present, they may be less efficient at binding androgen, so less hair growth occurs. The hormone that stimulates a receptor starts a signal transduction process, that leads to the final physiological effect, such as beard growth, so it could be some part of the signal transduction pathway that is different between the two men. Androgen concentrations were measured at a single point in time. Hormone secretion may fluctuate over the the day, so the 7:00 measurement may have been at the peak of a pulse for thin-beard, and the trough of a pulse for thick-beard, and the *average* androgen levels would then be higher in the thick-bearded man, supporting your original hypothesis, that beard growth reflects (average) androgen levels. The assay you did to measure androgens may have measured the total amount of hormone in blood, but most of that hormone would be bound to a transport protein. If there are different amounts of transport protein, they would have different amounts of free hormone that can bind to the target cell receptors. "Androgens" refers to a class of hormones, which includes testosterone, androstenedione (ASD), DHEA, and others. Perhaps only one of these hormones is able to stimulate beard growth, and the two men have similar levels of *total* androgens, but differ in the amount of the beard-stimulating androgen.

7-23 Insulin. Normally secreted when blood glucose is high (OR: after a meal).
Explanation: Glucose entry into cells is facilitated by either phosphorylating glucose as soon as it enters the cell, thereby maintaining a high concentration gradient that allows additional glucose to enter (liver does this) or by increasing the glucose transporter that are present in the membrane, so more of the glucose that passes by can get in (muscle does this).

7-24. Insulin stimulates glucose utilization and phosphorylation (in liver), and this "pulls" glucose into the cells. Glucose gets into liver cells by facilitated diffusion (the carrier is always there). The glucose is trapped inside the cells by conversion to G-6-P, and then the G-6-P is broken down to produce energy or converted to G-1-P and made into glycogen. The more utilization and phosphorylation of glucose, the lower the concentration of free glucose inside the cell is, the greater the gradient is relative to the blood, and the more glucose is transported into the cell. In the absence of insulin, phosphorylation of glucose is low, so free glucose builds up in the cell, and additional glucose cannot be transported in, against the gradient. In the presence of insulin, free glucose in the cell is minimal, the gradient is favorable, and transport into the cell occurs.

7-25. A. Insulin acts on many tissues; the main ones are muscle, liver, and adipose. Its effects are legion; here is a non-exhaustive list: In muscle, insulin increases glucose and amino acid uptake, increases protein and glycogen synthesis, and decreases glycogen and protein breakdown. In liver insulin increases glucose phosphorylation, glycolysis, and glycogen synthesis and decreases gluconeogenesis and glycogen breakdown. In adipose tissue, insulin increases glucose uptake and fat synthesis; it decreases fat breakdown.

B. Glucagon is the most obvious choice, but glucagon, epinephrine, vasopressin, cortisol and growth hormone are all antagonists of insulin -- that is, at least some of their effects are opposite to some of the effects of insulin. Glucagon acts primarily on the liver to increase gluconeogenesis and glycogen breakdown; it does not affect muscle. Epinephrine acts on liver, adipose and muscle as well.

7-26. Transport of glucose and amino acids into most cells is dependent on insulin. In the absence of insulin, the transporter proteins do not operate and the cells starve, glucose builds up to toxic levels in the blood, and disaster ensues. Release of glucose into the blood from the liver is stimulated by glucagon, but is not absolutely dependent on it. All the processes that are stimulated by glucagon can be stimulated by other hormones and/or can proceed (if not optimally) in the absence of glucagon. However glucose uptake in some tissues is absolutely dependent on insulin. (Exercise can mobilize some of the transport proteins in some tissues, but no other hormone can substitute for insulin.)

Key to Problem Set 7R

7R-1. A. The receptor for TRH should be ***a multipass membrane protein***. Explanation: TRH uses a G protein linked receptor, and all such receptors are multipass proteins. Note it is the ***receptor*** that is a multipass protein, NOT the G protein. G proteins are not integral membrane proteins.

B. When TRH binds to its receptor, you expect ***activation of PKC & PLC*** *and* ***an increase in cytosolic*** Ca^{++}**.** (Total intracellular Ca^{++} would stay the same, but Ca^{++} in the cytosol would increase.)
Explanation: TRH uses a receptor that activates a G protein; the G protein activates PLC. The PLC hydrolyses the PIP2 in the membrane and generates IP3 and DAG. The IP3 triggers release of Ca^{++} from the ER and the DAG (possibly with the help of Ca^{++}) activates PKC. Therefore the activity of PKC and PLC goes up for the cell as a whole, and the level of Ca^{++} goes up for the cytosol.

C. RME could be used to achieve down regulation of receptors for both ***TRH & TSH***.
Explanation: Down regulation means decreasing the number of receptors. RME can only be used to down regulate proteins on the cell surface. TH is a lipophilic hormone that uses an internal (cytoplasmic) receptor. This receptor can not be targeted for destruction using RME.
Details: Surface receptors can be down regulated in response to repeated stimulation as follows: In the absence of stimulation, receptors will not be degraded -- either they will not be internalized (they won't go to coated pits) or they will be internalized, but the receptors will not be targeted to lysosomes, and will return to the cell surface. In the presence of stimulation (= presence of ligand) the receptors will bind their ligands, and the ligand-receptor complexes will be internalized by RME. Then the complexes will be targeted to lysosomes for degradation. The more ligand (stimulation) is present, the more receptors will bind ligand and be degraded.

D. Of the choices given, the most likely additional hormone secreted in response to TRH is ***prolactin***.
Explanation: TRH is released by the hypothalamus and goes by the portal circulation directly to the anterior pituitary. In other words, the anterior pituitary is the normal target of TRH. Therefore TRH is most likely to act only on cells of the anterior pituitary; it is unlikely to affect cells in other glands. (If it reaches other glands, it will be in such low concentration that it is unlikely to have any effect.) The only hormone on the list that is made by the ant. pit. is prolactin.
There are several different cell types in the anterior pituitary, and each cell type produces different hormones. TRH binds to receptors on one type of cell in the anterior pituitary and triggers release of TSH. TRH could also bind to receptors on different cells of the ant. pit, triggering release of prolactin.

7R-2. A. Activation of Gs should lead to activation of ***PKA.***
Explanation: Gs activates adenyl cyclase, and AC catalyzes formation of cAMP from ATP. cAMP binds to PKA and activates the kinase. Note: PKA can phosphorylate many different proteins, but generally does not lead to increases in Ca^{++} or activation of other kinases listed. PUKE is a joke.

B. Activation of Gs normally involves ***binding of GTP.***
Explanation: Activation of G proteins requires GTP exchange -- the GTP binds to the protein, displacing bound GDP. You do not convert the bound GDP to GTP by phosphorylation -- you replace the GDP with GTP. The released GDP is phosphorylated to GTP and reused.

C. You would expect Gs to be involved in response to ***TSH.***
Explanation: The TSH receptor is linked to Gs -- the G protein that activates adenyl cyclase. The TRH receptor must be linked to a ***different*** G protein -- one that activates PLC. TH doesn't use a G protein linked receptor at all -- it uses an intracellular receptor that is a transcription factor.

D. In the pathway controlling levels of TH, you expect Gs to be involved in the ***thyroid***.
Explanation: The 3 hormones in the pathway leading to TH are TRH, TSH and TH. As explained above, only TSH uses Gs. TSH is released by the anterior pituitary but acts on the thyroid. So TSH receptors must be on the thyroid. TH has negative feedback effects on the ant. pit and the hypothalamus, but TH doesn't use a G protein linked receptor, as explained above.

7R-3. A. Reasonable candidates are ***(adreno)corticotropin aka ACTH*** or ***the gonadotropins (LH & FSH).***

B. Secretion of this tropic hormone should be ***decreased*** in response to high levels of ***glucocorticoids or sex steroids*** (respectively) and ***increased*** in response to high levels of the appropriate releasing hormone -- ***CRH or GnRH*** respectively (or stress instead of CRH). The circuit goes like this:

Hypothalamus → releasing hormone → ***Ant. Pit*** → tropic hormone → ***endocrine gland*** → steroid hormone

In addition, the steroid hormone produced at the end has a neg. feedback effect on the ant. pit. and hypothalamus. It inhibits production or release of releasing hormone from the HT, and production or release of tropic hormone from the AP (probably by downregulating receptors for the RH on the AP). The steroid hormone also affects its target organs appropriately.

7R-4. A. Hormone is ***insulin or glucagon.***

For insulin:

B. Secretion should ***decrease*** in response to ***low*** levels of glucose ***in the blood.***

C. Conditions that ***increase*** secretion normally occur during ***the absorptive state***.

D. This hormone stimulates insertion of glucose transporters into the membrane of ***muscle & adipose cells.***

For glucagon:

B. Secretion should ***decrease*** in response to ***high*** levels of glucose ***in the blood.***

C. Conditions that ***increase*** secretion normally occur during ***the post-absorptive state & stress.***

D. ***none of these.***

Explanation: For maintenance of homeostasis: Hormone (which ever one it is) acts to return the glucose levels in the blood to desired levels (to set point). Hormone acts by promoting uptake and use of glucose (insulin) or by promoting generation of glucose (& inhibition of its use), thus allowing release of glucose by liver (glucagon). Stress depresses insulin release and increases release of glucagon, thus maintaining higher blood glucose levels for use by the brain.

7R-5. A-1. Mutant version should be ***harder to inactivate.***

A-2. Mutants should have levels of ***cAMP; levels should be too high.***

Explanation: Gs will stay active for too long, because GTP will not be hydrolyzed normally to GDP. Therefore adenyl cyclase will be active for too long and too much cAMP will be made.

Note: In some smooth muscle cells, PKA -- activated by cAMP -- phosphorylates a Ca^{++} pump, and this causes a lowering of Ca^{++}. This result only occurs if the appropriate pump protein is present. You can't predict what will happen to Ca^{++} in the cells of the pituitary without knowing more about which proteins (pumps etc.) are present in pituitary cells.

B-1. ***Normal version of gene is a proto-oncogene; abnormal version is an oncogene*** Gs is needed for normal growth of some of the cells in the AP. (Different cells in the AP are stimulated by different RH's. The tumor is derived from cells that normally grow in response to RH's that use the cAMP pathway.) When Gs is over active (or remains active too long) as in this case, the cells grow even without stimulation from the HT, and form a tumor. The gene (allele) that codes for over active Gs is considered an oncogene, because cells carrying that form of the gene escape normal growth control. (They grow without external stimulation.) The normal form of the gene that codes for normal Gs is considered a proto-oncogene.

If Gs is too active, secretion of hormones will be too high as well as cell growth (see B-2).

B-2. You expect ***>100 units of GH secreted.***

Explanation: Each allele (version of the gene) will act independently to make the corresponding version of Gs. About half of the Gs will be overactive and half of the Gs will be normal. Each molecule of Gs will function independently. Therefore, overall, there will be too much active Gs, and it will activate too much adenyl cyclase and lead to the release of too much GH. In other words, the effect of the overactive Gs will over ride the effect of the normal Gs.

7R-6. A: TH below normal, Set point about the same in both; Contraction higher for Sam.
Explanation: A-1. Sam is probably cold because he has a lower metabolic rate, and generates less heat from basic metabolism than Joe does. TH controls the basal metabolic rate, so if Sam has a low metabolic rate, he probably has a low level of TH.
Note: Sam is cold, so his metabolic rate is too low. This is a result of chronic low TH, not a temporary dip in temperature that acts as a signal to raise the TH. (He won't have elevated TH as a response to the low BMR -- he has a low BMR because the TH level is too low.) In a normal person, if metabolism slows down too much temporarily, homeostatic mechanisms will kick in to raise TH secretion and therefore metabolism. But Sam's temperature control system is not working normally -- it has failed to keep up with the demand. So either he isn't making enough TH or the response to TH is abnormally low. The TH level is controlling the rate of heat production, not the other way around. (They should influence each other in a feedback loop, but the circuit isn't working properly.)

A-2. Homeostatic feedback mechanisms keep everyone at about the same internal body temperature (except when they have a fever). The set point is the same, but the mechanisms used to maintain it are different for Joe and Sam. Sam generates less heat, but conserves it better.

A-3. Constricting peripheral blood vessels reduces heat loss by radiation. At normal indoor temperatures, Joe doesn’t need to conserve heat to keep warm, but Sam does. Joe generates enough heat to keep him comfortable. Sam doesn't generate as much, so he reduces heat loss by constricting his perif. blood vessels, wearing a coat, etc. (These actions don’t generate more heat, but they conserve what he has.)

B-1. TSH. Explanation: TSH and TRH control TH production. All the other choices are irrelevant. You have to measure TSH, not TRH, because the concentration of TRH in the peripheral circulation is too low. TRH is released into the portal circulation that goes directly from the HT to the anterior pituitary. The only part of the circulation that has a significant level of TRH is the portal vessel.
If you answered "TSH or TRH" because the rest have nothing to do with TH production, you received credit for the explanation but not the answer.

B-2. TSH will be below normal, and TRH will be elevated. (Credit was give for explaining either case.) Anterior pituitary is not responding to TRH by making the usual amount of TSH. Therefore there is not enough TSH to stimulate the thyroid properly, and that's why TH is low. If TH is low, negative feedback to hypothalamus will be reduced and TRH secretion will be elevated. (There will be less negative feedback to ant. pit. too, but since ant. pit is already not responding to TRH, it doesn't matter.)

C. C-1. TSH in Sam's blood is probably elevated, and C-2. Release of free TH to the blood is below normal. ("Proper modification of thyroglobulin" was accepted instead of "release of free TH" if you took "modification" to include "cutting up the protein to release T4.")
Explanation: If TH is low, there is no negative feedback on the anterior pituitary (or hypothalamus), so TSH production will be higher than normal. Lithium inhibits endocytosis of modified thyroglobulin ***from*** the lumen ***back into*** the cells of the follicle. Therefore transport of the modified thyroglobulin to lysosomes is inhibited, and so is the hydrolysis of the protein. The protein must be cut up to release free TH to the blood.

7R-7. A-C. Epinephrine. D. Cortisol.

Explanations:
A. Epi is water soluble; it is made in advance and stored in vesicles. The hormone is released by exocytosis when the adrenal (medulla) receives the proper signal. Cortisol is a steroid and cannot be stored. Cortisol must be "made to order" when the adrenal (cortex) receives the proper signal. If both signals are received at the same time, epi. can be released immediately, but cortisol must be made first. (This question does not ask about the nature of the signals from the brain to the two parts of the adrenal -- that's part C. This question asks what happens after the signals both get there.)

B. Epi. binds to transmembrane receptors on the cell surface. These are GPCR's that trigger second messenger pathways. These pathways general affect enzymes that modify (activate or inhibit) pre-existing proteins. Cortisol binds to a receptor that is a transcription factor. Binding of cortisol triggers production of new proteins -- this requires transcription and translation and takes longer than modifying old proteins. (Note that this question is about the action of the two types of hormones; the previous question is about the production and/or release of the two types.)

C. Epi. is made by the adrenal medulla; cortisol is made by the adrenal cortex. Nerves connect to the adrenal medulla and stimulate release of epi. There are no nerves to the adrenal cortex -- the release of cortisol is controlled by production of ACTH from the ant. pit.

D. Cortisol production is controlled by the HT/ant. pit/tropic hormone system, which includes a negative feedback loop. Cortisol inhibits its own production by neg. feedback on the HT and ant. pit -- it inhibits production of CRH by the HT and the production of ACTH by the ant. pit. Epi. production is not part of a feedback loop -- its production is controlled by neuronal input.

7R-8. A. Hypoglycemia will cause secretion of glucagon. It is NOT the GH that causes glucagon release; the low blood sugar triggers GH and glucagon release independently.

B. Given that GH is secreted in response to stress & hypoglycemia, and that GH has one direct effect on skeletal muscle, the best answer is that GH acts to reduce glucose uptake (thus sparing the glucose for the brain and other tissues). This is what actually happens. GH does not affect glycogen breakdown directly. However, if no glucose is coming in, the cell will use up its available glucose (really glucose phosphate) and that will cause breakdown of glycogen to generate more glucose for energy metabolism. (Therefore 'breakdown of glycogen increases' is a good answer, if not the best one. GH does stimulate breakdown of glycogen, but not directly.)

C. Excess GH secretion will cause hyperglycemia. You have to come up with a reasonable explanation of how too much GH leads to high blood sugar.

(a) One possibility: GH inhibits glucose uptake in response to insulin, which is what causes hyperglycemia if there is too much GH. Muscle cannot release glucose, so GH can't stimulate an increase in glucose release from muscle. If it's going to increase the blood sugar, it has to do so by reducing uptake, not increasing release. (This is what really happens.)

(b) Another possibility: If GH stimulated glycogen breakdown, then that would help the muscle produce energy from its own stores when glucose is unavailable from the blood. But it wouldn't cause hyperglycemia directly, because muscle cannot covert glucose phosphate to glucose. However, muscle could breakdown glucose to lactate, the lactate could go to the liver, and the liver could use gluconeogenesis to generate glucose for release to the blood. (This isn't what happens, but it was accepted as a reasonable solution.)

Note: GH probably does not affect the liver directly, so it probably does not promote release of glucose into the bloodstream either directly or indirectly. Its main short-term effect is on uptake of glucose, not its release.

7R-9. A. Release of hormone requires cleavage of peptide bonds (to remove the signal peptide 'pre' part and then the extra 'pro' part that keeps it inactive) and then exocytosis is required for release of the hormone into the blood stream.

B-1. Only GH could use a TK receptor. TK receptors don't affect the cAMP pathway, so GH-RH and somatostatin must use GCPR's.

B-2. No two of these are likely to share a G protein. GH-RH and somatostatin have different effects. One raises the cAMP (by activating adenylyl cyclase) and the other lowers the cAMP. (It does it by inhibiting the adenylyl cyclase, but it could in theory do it by activating PDE). Therefore one receptor should activate a G protein that inhibits adenylyl cyclase (or the equivalent), and the other should activate a different G protein that activates adenylyl cyclase. (This is the actual case.)

7R-10. A. When the TK receptor binds IGF, the receptor should dimerize and catalyze hydrolysis of ATP (to phosphorylate cytoplasmic domains of the dimerized receptor).

B-1. Added IGF should inhibit GH synthesis and GH-RH release.

B-2. Added IGF should interfere with the paracrine effects of IGF.

Explanation: GH is a 'pseudo tropic' hormone, and its release is regulated by negative feedback in a way that is parallel to the way release of tropic hormones is controlled. High levels of IGF feed back to the HT (and AP) and inhibit release of GH-RH and GH. If IGF is injected and reaches high levels in the general circulation, it will lower the levels of GH. This will reduce the ability of the GH to stimulate its targets to produce IGF. It won't matter if the liver doesn't make IGF, as you've added plenty by injection. The endocrine production of IGF may be inhibited, but it won't affect growth (because you have replaced the circulating IGF). However there is nothing to replace the paracrine production of IGF by bone (in response to GH, not IGF). (There is no reason to posit feedback of IGF on synthesis by the liver, but even if there were such feedback, adding excess IGF wouldn't inhibit growth, because there would be plenty of IGF present to replace what the liver usually makes.)

In the short kids described here who don't respond to extra GH, there is some IGF already, so GH must stimulate IGF production. However, the IGF levels are unusually low, and adding extra GH doesn't seem to help. One possibility is that the GH receptors are defective; another is that signaling 'downstream' of the receptors is not working properly. It any case, it is likely that the GH receptors are already binding as much GH as they can, and that's why adding more GH doesn't help. But having less GH might make a difference in the paracrine response.

7R-11. A-1. Release of CRH should increase. (No feedback inhibition on hypothalamus to prevent release.)
A-2. Release of ACTH should decrease. (No ant. pit. to release it.)
A-3. Release of cortisol should decrease (No ACTH to stimulate its release from adrenal cortex.)
A-4. Release of CRH will change if you add dexamethasone.
Explanation: Dex. is an agonist (mimic) of cortisol. Therefore it will have the same effects as cortisol -- it will act as a feedback inhibitor on the HT/ant. pit. axis. It will bind to receptors in the hypothalamus and reduce release of CRH. If the ant. pit. were present, it would bind to receptors in the ant. pit. and reduce release of ACTH. However, in this case, it will have on effect on release of ACTH, as there is no ant. pit. present. (There was no release of ACTH to inhibit.)

B. Epi. release should be unaffected by removal of ant. pit. OR addition of ACTH.

C-1. (a). ACTH stimulates synthesis of cortisol, and (b) adrenal medulla has receptors for the cortisol (from the medulla).
C-2. Cortisol increases synthesis of new enzymes for making epi. .
Cortisol must be the active agent here, as metyrapone (which inhibits steroid synthesis) blocks the effect of ACTH. So it isn't the ACTH itself that stimulates epi. synthesis -- instead ACTH stimulates cortisol synthesis (in the adrenal cortex), and the cortisol stimulates epi. synthesis (in the adrenal medulla). Since cortisol is a steroid hormone, it probably exerts its effects by promoting synthesis of new enzymes, not activating pre-existing enzymes.

C-3. (a). Blood flows from cortex to medulla, and (b) the portal circulation contains high levels of cortisol.
The normal circulation pattern is general circulation → organ → general circulation. A portal circulation goes directly from one organ to another. This means that any secretions from the first organ are carried directly to the second organ. Therefore the secretions are present in relatively high concentration when they reach the second organ, but are greatly diluted when they reach the general circulation. This is how releasing hormones go from the HT to the ant. pit, and how cortisol must go from the adrenal cortex to the adrenal medulla. There is no portal vessel that connects the two parts of the adrenal, but the pattern of blood flow is equivalent to what you would have if there were a portal vessel. The general circulation feeds into capillaries in the cortex, and then the blood flows directly through the cortex, then through the medulla, and then out into the general circulation. Therefore the hormones of the adrenal cortex are quite concentrated in the portal circulation that flows through the adrenal medulla. Once the hormones reach the general circulation they are greatly diluted. If the adrenal cortex is making cortisol, then the levels in the portal circulation will be much higher than the levels in the general circulation. If the cortex is not making cortisol, and you add it to the blood, the level in the portal circulation will be the same as the level in the general circulation. This explains why adding normal levels of cortisol to an animal has no effect on the medulla -- the normal levels in the general circulation (& the levels in the portal circulation) are too low to produce an effect. You have to add extremely high levels to the blood, levels that approach the levels in the portal circulation (when the cortex is making cortisol), to get any noticeable effect.

Answers to problem set 8 – Nervous System

8-0. A. The volume of water will on left side will ***(increase).*** The osmolarity on the left side is 21 mOsM and on the right side is 12 mOsM. Water moves from low osmolarity to high osmolarity, so will move to the left side through this water permeable membrane.

B. At equilibrium, the glucose on the left side will ***(increase to be equal to the final concentration on the right side).*** Glucose is uncharged and will move from right to left down its concentration gradient. At equilibrium, the concentration of glucose will be equal on both sides, with 0 net movement.

C. Cl^- will initially ***(move from the left side to the right side).*** Since we start with neutrality on both sides, initially Cl^- moves down its concentration gradient from left to right.

D. The concentration of Cl- on the left side after equilibrium is reached will be ***(~10 mM).*** Very few ions need to move to reach the equilibrium potential for Cl^-, so the starting concentration doesn't change appreciably. [Remember, if the Na^+/K^+ pump is blocked, a neuron can fire 150,000 times before the concentrations of Na^+ and K^+ change enough to prevent firing.]

E. 7E. The membrane potential on the left side after equilibrium is reached is __***+58 mV***__. This is an equilibrium potential problem. Since I've asked for the potential on the left side, it is easiest to think of the left side as the inside of the cell. [Remember, the equilibrium potential is the membrane potential that exactly opposes a particular concentration gradient, in this case, 10 mM Cl^- on the left and 1 mM on the right.

$$E_{Cl} = (58/-1) * \log (1/10) = -58 * -1 = +58 \text{ mV}.$$

8-1. Some arguments that the endocrine and nervous systems are really part of a single "regulatory" system:

- Similar or identical signaling molecules are used in both the endocrine and the nervous systems. e.g., norepinephrine is a neurotransmitter, and epinephrine, a similar molecule, is a hormone.
- Both systems produce molecules that allow communication with other cells by combining with cell receptors.
- The two systems work together to control homeostasis, though the time courses may vary. e.g., neuronal stimulation of blood vessels and sweat glands are involved in short term response to decreased temperature, while epinephrine affects longer term response to temp change. Both systems also work together to control stress (sympathetic nervous system and endocrine secretions, especially adrenal medulla.)
- The developing nervous tissue gives rise to both the nervous system and to some parts of the endocrine system (e.g. hypothalamus, posterior pituitary, adrenal medulla)
- The hypothalamus has a central role in controlling many endocrine glands (by its control of the anterior pituitary gland) and is in turn controlled by neuronal input from the brain and peripheral sensory neurons.

8-2. Answers:

A. X
B. Y
C. X (though difference is so incredibly small that one might suggest that X = Y.) We are assuming the pump is not fast enough to correct the difference immediately.
D. X
E. X = Y; number of directions AP will travel is equal whether stimulated at axon hillock, in which case it will travel down axon, or whether stimulated at dendrite, in which case local potentials will be produced, which, when the axon hillock is reached, will stimulate an AP to travel down axon
F. X - Na^+ ions moved out by Na/K pump (3 Na^+ out for 2 K^+ in).

8-3. A. Increase - less K^+ would diffuse out of the cell down its concentration gradient
B. Potential becomes less negative, or more positive, since more K^+ remains inside.
C. Easier, since potential is closer to threshold
D. Decrease the number of K^+ leak channels, or block the ones that are there.

8-4. A. Na^+ inside cell would increase; K^+ inside would decrease (less electrical "pull" back in)
B. More positive (net Na^+ in will be greater > net K^+ out. K^+ is close to its equil. membrane potential but Na^+ is very far from its.)
C. Easier
D. Increase the number of Na^+ leak channels or ligand-gated channels for Na^+

8-5. Initially there was no action potential because the upswing of the action potential is caused by sodium influx, and there was no Na^+ available to enter the cell. We now know that the voltage-gated potassium channels are stimulated to open by increasing membrane potential to threshold, but that they do so after a small delay. When the membrane was depolarized in this experiment, the K^+ channels opened after this delay, K^+ left the cell, bringing a positive charge to the outside of the cell, which is what Hodgkin and Huxley measured.

8-6. c. When the action potentials moving towards each other meet in the middle of the axon, the patches of neuron on either side would be in a refractory period, and the action potential could not be propagated in either direction.

8-7. A. Increased permeability to Ca^{++}. Ca^{++} is higher outside the cell. If permeability to Ca^{++} increases, the ions will flow in and maintain the inside at a positive potential, relative to outside.
B. A decrease in K permeability, or a delay in opening of K channels. In neurons, the membrane repolarizes after peak of action potential due to opening of voltage-gated K^+ channels. If these channels remain closed, the membrane will stay depolarized.

8-8. A. Ligand gated ion channels at A & B.

Note: A & B refer to the membrane on the post-synaptic side only.

B. Voltage gated Na^+ and K^+ channels at D, E & F; Voltage gated Ca^{++} channels at G.
C. Graded local potentials at A, B, C, & D. (Generated at A & B, but spread passively to C & D.)
D. AP normally starts at D
E. IPSP at A
F. AP should go both ways toward D on left; toward F & G on right.
G. Synaptic vesicles at G
H. Gated ion channels without refractory period at A (K^+ and/or Cl^- channels), and at B (Monovalent cation channels, mostly for passage of Na^+).

Note: Ca^{++} channels at G are gated and don't have a refractory period but they don't generate potentials. Increase of $[Ca^{++}]$ may change the local potential, but it's the concentration of Ca^{++}, not the change in potential, that matters here and triggers release of transmitter.

I. Efferent; Excitatory. (Motor neuron refers to the somatic efferent neuron which innervates skeletal muscle; the neuromuscular junction is always excitatory.)

J. Either one; norepinephrine. (In the autonomic nervous system, the neurotransmitter at the second synapse is usually norepinephrine in the sympathetic branch and acetylcholine in the parasympathetic branch.)

8-9. A. X .(K^+ channels open more slowly.)
B. X (Na^+ has both electrical and chemical gradients pushing it in)
C. Y (Channels in terminal needed for release of neurotransmitter)
D. X=Y (Depolarization here is due to opening of Na^+ channels, not closing of K^+ channels.)
E. Y. (There is no graded potential on the presynaptic side – it's an all or nothing action potential.)
F. X (Neurotransmitter release is unidirectional – released from presynaptic only.)

8-10. A. Inhibitory. K^+ is higher inside cell than outside. If you increase permeability, more K^+ leaks out of the cell, so inside of membrane becomes more negative (or hyperpolarized) and closer to K's equilibrium potential. The membrane potential is therefore further from threshold, and it will take a larger stimulus to trigger an action potential.

B. Stimulatory. K^+ is high inside. If permeability to K decreases, less leaks out, so membrane becomes more positive on the inside than usual (i.e, depolarizes). It is closer to threshold, and it should take less of a stimulus to bring it to threshold and trigger an action potential.

C. Inhibitory. Cl^- is higher outside than inside. If permeability to Cl^- increases, Cl^- will flow in, so membrane becomes more negative than usual, or hyperpolarizes. Since membrane potential is now further from threshold, it is more difficult to reach threshold and will take more of a stimulus to trigger an action potential.

D. Stimulatory. Na^+ is higher outside cell than inside. If you increase permeability to both, Na^+ will enter and K^+ will leave. But the "push" on Na^+ is greater than on K^+, since there is both a concentration and electrical gradient directing Na^+ into the cell. (For K^+, chemical and electrical gradients are opposed.) So Na^+ influx exceeds K^+ efflux. The membrane will become more positive inside (i.e., depolarized), bringing it closer to threshold, so it will be easier to trigger an action potential.

8-11. Stimulate the presynaptic neuron and measure the time it takes for depolarization to occur in the postsynaptic neuron. Message conduction is slower in chemical synapses than in electrical synapses. OR: Apply a calcium-channel blocker to synapse. Opening of calcium channels is necessary for release of neurotransmitters, but not necessary for transmission across electrical synapses.

8-12. Both the sympathetic and parasympathetic nervous systems are divisions of the autonomic nervous system (see question #13). They control smooth and cardiac muscle, glands, and neurons in the gastrointestinal tract. Both have first cell body in the spinal chord and exit through the ventral root. Both have one neuron to neuron synapse. This synapse uses the neurotransmitter/receptor pair ACh /nicotinic.
SYMPATHETIC--1) Exits through thoracic and lumbar regions of the spinal chord, 2) Has short pre-ganglionic neuron and long post-ganglionic neuron, 3) Uses norepinephrine at neuron-effector synapse, 4) Stimulates "fight or flight" response, dampens vegetative/housekeeping functions.
PARASYMPATHETIC--1) Exits through cranial and sacral regions of the CNS, 2) Has a long pre-ganglionic neuron and a short post-ganglionic neuron, 3) Uses ACh /muscarinic receptor at neuron-effector synapse, 4) Stimulates vegetative, housekeeping functions, dampens "fight or flight" response.

8-13. Both the somatic and autonomic nervous systems are efferent and have their first (and only for the somatic) cell body in the spinal chord.
SOMATIC--1) Consists of a single neuron between central nervous system and effector organ, 2) Innervates skeletal muscle, 3) Can lead only to muscle excitation, 4) Neurotransmitter is ACh and the receptors are nicotinic.
AUTONOMIC--1) Has two neuron chain (connected by synapse) between central nervous system and effector organ, 2) Innervates smooth and cardiac muscle, glands, and neurons in the gastrointestinal tract, 3) Can lead to excitation or inhibition of effector cells, 4) Neurotransmitter/receptor is almost always ACh /nicotinic at first synapse and at second synapse is either ACh /muscarinic (PNS) or Norepinephrine/alpha or beta (SNS).

8-14. A. Y B. X C. X D. X E. Y** F. X

**** Note that this Q is not about transmission of APs in the membrane.**

8-15. A. #2. Sensory is afferent; goes toward CNS.
B. Both, since both produce action potentials.

C. ***Before continuing to part D on next page, please look up the answer to part C on p. 70 to be sure you are correct.***

D. #1. In cell #1, binding of acetyl choline on outside of cell leads directly to opening of Na^+ channels (ligand gated) and EPSP; this then triggers action potential. In cell #2, binding of odorant does not open ligand gated channels directly; it triggers a second messenger cascade which indirectly leads to opening of channels and action potential (see above). The cascade takes longer but allows for amplification of the signal (amount of odorant).

E-1. Both; E-2. Neither; E-3. EPSP; E-4. Receptor potential (=generator potential).
Both acetyl choline and odorant bind to receptors and cause ligand gated Na^+ channels to open (directly or indirectly) but neither should open enough channels to trigger an action potential, assuming that in part D you added just enough to trigger one AP. In both cases, you should generate a graded potential, proportional to the stimulus, that should be below threshold (since you used less stimulus than you used in part D).

The amplification caused by the cascade in cell #2 should not be sufficient to trigger an action potential. The amplification increases the number of ligand gated channels that can be opened by a single molecule of odorant, but it does not change the proportionality between the number of channels opened and the amount of odorant. If there is less odorant, there should be less cAMP, less open channels, and a smaller receptor potential. (Amplification changes the sensitivity, not the proportionality, of the system. If you used equal numbers of molecules of acetyl choline and odorant, you would get a larger graded potential from cell #2.)

F-1. Depends on cell that patch is from; F-2. Either way – depends on concentration gradient (*in vitro*).
The inside of the pipette corresponds to the outside of the cell; the medium corresponds to the inside of the cell.

For cell #1, the ligand gated channels are opened by binding of ligand (acetyl choline) to the outside of the cell, so you should put the ligand in the pipette. For cell #2, the ligand gated channels are opened by binding of cAMP on the inside of the cell, so you should put the ligand (cAMP, not the odorant) in the medium. Odorant binding on the outside of the patch won't open the channels unless you have all the necessary ingredients for the cascade in the patch and/or medium. (All the enzymes, G proteins etc. are probably in the membrane, but you need to add at least ATP & GTP.)

In an intact cell (under normal conditions), Na^+ will always flow in, because the Na^+/K^+ pump maintains a low intracellular Na^+ concentration. If the Na^+ concentrations in the pipette and medium resemble the usual concentrations in the extracellular fluid and cell respectively, then the Na^+ will go from pipette to medium. But in this *in vitro* experiment, you can manipulate the ion concentrations so that the Na^+ gradient goes either way, and when you open channels, the Na^+ will flow down the gradient (which ever way that happens to be).

8-16. A. Outline of the system: Stimulate receptor on outside of receptor cell → 2nd messenger inside receptor cell (G protein activated which then activates adenylate cyclase, etc.) → Ca^{++} channels open → causes release of ACh → opens post synaptic ligand gated Na^+ (or general cation) channels on neuron → Graded potential in postsynaptic membrane of neuron → opens voltage gated Na^+ channels → causes action potential in neuron. Note that these are Martians, and the situation is different from that in all terrestrial cases. In all real cases on earth, but not in the Martian case in this problem, there is a receptor potential in the receptor cell. See *.

B. 1, 3*, 4, 5, 7 & 8 stay the same; 2, 6 & 9 increase; 10 decreases (depolarizes).

C. 1. WORSE: Agonist mimics effects of ACh
2. BETTER: antagonist blocks the effects of ACh
3. WORSE: ACh -esterase removes signal, if inhibit or block this, the signal persists.
4. NO EFFECT: There are no presynaptic Na^+ gated channels because there is no action potential on this side.
5. BETTER: Blocking the Ca^{++} channels prevents transmitter (ACh) release.

* for 3: When stimulus increases, you get more Ca^{++} inside the cell, which causes release of more ACh and a graded potential in the neuron, and if that signal is large enough, opening of voltage-gated Na^+ channels in the neuron (when it fires an AP), not in the receptor cell. In some systems, Ca^{++} channels are voltage-gated, but in this Martian system, a change in potential in the receptor cell does not lead to transmission, so the Ca^{++} channels in this system are not voltage-gated.

8-17. Depolarization is due to Na^+ channels opening, so the membrane should depolarize normally. Once the Na^+ channels open, they won't close, so the membrane cannot repolarize properly. Since the Na^+ channels remain open, the membrane potential should remain high -- closer to the equilibrium potential for Na^+. (In other words, Na^+ rushes in until membrane gets so positive on the inside that the plus charge on membrane balances concentration gradient pushing in.) The opening of K^+ channels will cause some decrease in the membrane potential, but the membrane won't drop to normal resting potential because Na^+ channels are still open. (K^+ channels may not close, as their closing is voltage dependent.) A picture should include the following: labeling x axis (time, in msec); y axis (membrane potential, in mV); initial resting potential at around -70mV and depolarization of membrane to +30-40mV (usual rising part of spike) followed by failure to repolarize normally. (It is not entirely clear what the curve should look like after the depolarization hits a maximum; the membrane potential will probably decrease to an level in between the equil. values for Na^+ and K^+ and remain flat.)

8-18. a. K^+ channel - mechanically gated - on posterior end. Expect this because touching posterior end causes hyperpolarization, which would be caused by positive ions leaving or negative ions entering. Since the only two options are K^+ and Ca^{++}, it must be exit of positive ions that causes hyperpolarization. Concentration gradients are K^+ higher inside, Ca^{++} higher outside. If opening channels causes positive ion to exit, that ion must be K^+ which would exit the cell if moving down its concentration gradient.

b. Ca^{++} channel - mechanically gated - anterior end. Light touch here causes depolarization, so must be positive ions entering. Since Ca^{++} is higher outside the cell, only Ca^{++} entering could cause this change.

c. Ca^{++} channel - voltage gated - all over. With larger touch stimulus, an action potential develops across the cell, implying that voltage-gated channels are present. As in b. the depolarization could occur only if these were channels for the positive ions that is higher outside the cell, namely Ca^{++}.

Note: There may be more channels, and the actual situation may be somewhat different and/or more complex.

8-19. Answers *For GABA:*

A: increase, into the cell, more negative.

Explanation: GABA opens Cl^- channels, and increases the permeability of the membrane to chloride, so permeability goes up. The concentration of chloride is higher outside the cell, but there is an electrical gradient opposing the entrance of chloride. So which way will chloride go? We know that GABA is an inhibitory transmitter. Therefore opening channels by GABA should lead to hyperpolarization of the cell, shifting the membrane potential away from threshold, and making it harder to fire an AP. Since the response to GABA should be a shift toward a more negative potential, Cl^- must enter the cell to make it more negative inside. (We also know that $[Cl^-]$ is much higher outside the cell, so that it is likely to flow in, even with the opposing electrical gradient.) Note: in reality, RMP in many cells is close to the equilibrium potential for Cl^- (E_{Cl-}). In this case, there would be no net movement of Cl^-. However, if such a cell depolarized, say, in response to an excitatory signal, Cl^- would enter the cell.

B. In cell #1. The action potential coming down the axon opens Ca^{++} channels on the presynaptic side. Ca^{++} comes in from the outside, and the high Ca^{++} level triggers exocytosis of vesicles containing transmitter. The vesicles fuse with the plasma membrane and release neurotransmitter into the synapse.

C-1. At synapse: Potential will be larger (more negative); ligand gated channels open; IPSP. Explanation: The more AP's are fired in cell #1 on the presynaptic side, the more transmitter is released and the more channels are opened in cell #2 on the post synaptic side of the synapse. So you get a graded response at the synapse -- the more AP's, the more hyperpolarization (more IPSP's) at the synapse in cell #2.

C-2. At middle of axon: The same, namely zero, and neither. An AP is not a graded response -- it is an all or nothing event. You will get nothing in both cases because the synapse is inhibitory. Neither 1 nor many action potentials in cell #1 will trigger an action potential in cell #2. The IPSP's at the synapse will drive cell #2 away from threshold, not toward it. Even if the hyperpolarization from the synapse reaches the axon hillock without dying out it won't trigger an AP -- it will make an AP less likely, not more.

Answers For glutamate (continued on next page):

A. Stay the same, not at all, less negative.

Explanation: Glutamate is an excitatory transmitter. It does not open Cl^- channels -- it opens Na^+ channels*. So it will have no effect on Cl^- permeability or flow of Cl^- ions, but voltage difference will probably become less negative, due to increased permeability for Na^+. (See * below.)

B. Same answer as above.

C-1. At synapse: Potential change will be larger (toward the positive); ligand gated channels open; EPSP. This time you get more depolarization (EPSP) instead of more hyperpolarization (IPSP).

C-2. At middle of axon. Potential change should be larger (you'll probably generate an AP the second time, but not the first), and voltage gated channels will open. You will get nothing the first time since one AP in cell #1 is certainly not enough to generate an AP in cell #2. (One EPSP is not enough to reach threshold.) The second time you will probably get an AP -- it depends on the number of AP's you generated in cell #1 and the size of the threshold in cell #2. You have to open voltage gated channels to generate an AP. If you stimulate cell #1 even more, you'll get more AP's in cell #2, but all AP's will have the same size -- same change in potential for each AP.

* Excitatory transmitters probably open nonspecific cation channels that allow both Na^+ and K^+ to flow. However the push (in) on Na^+ is very high, and the push (out) on K^+ is relatively low, so the net effect is primarily on Na^+, resulting in a depolarization.

8-20. Picture should show a synapse with cell A on the pre-synaptic side and cell B on the post synaptic side, properly labeled. Note that synaptic endings are really very small, relative to the size of the dendrite, and each ending must be very close to a dendrite to form a synapse. The crude pictures drawn in class do not always reflect this clearly. (Many pictures had the pre- and post-synaptic cells in the right relative positions, but were not anatomically feasible. See texts for clear pictures.)

A-1. Transmitter T must be excitatory. (Otherwise, you wouldn't get an AP in cell B.)

A-2. Transmitter T normally acts at a synapse. It is released from the presynaptic side by exocytosis, and binds to receptors on the postsynaptic side, opening ligand gated channels. Receptors and their associated ligand gated channels are not found all over the cell surface at random -- they are clustered at synapses.

A-3. No (but see *). A signal cannot be transmitted across a synapse from the post to the presynaptic side. Transmission requires release of transmitter -- which can only happen on the presynaptic side, and binding of transmitter to receptors -- which are only located on the postsynaptic side. So if you locally stimulate a synapse on a dendrite of cell B, it can't release packets of transmitter and generate an AP in cell A. (This is unrelated to the issue of refractory periods and why AP's don't normally go backwards along the axon from synapse to cell body. If you did manage to stimulate cell A near the synapse, the AP would go backwards toward the cell body.)

*Since this experiment is in a test tube, if you stimulate cell B with enough excitatory transmitter, you might release enough transmitter into the medium to bind to synaptic receptors on the dendrites of cell A and trigger an AP in the axon of cell A. (As in the Loewi experiment with the frog hearts.) In this case, you don't transmit "backwards" across a synapse -- you stimulate cell A (with the excess transmitter intended for cell B), and then send a signal across the synapse from A to B in the "forward" direction (using the usual mechanism).

B. Recording at axon will look like (c) -- a typical action potential. Recording at B at the synapse will look like (b) -- a typical EPSP. Explanation:
We know that stimulating cell A generates an AP in cell B. This will only work if there is are multiple AP's generated in the axon of cell A, which in turn generate the AP in cell B. Therefore, we expect a standard AP in the axon of cell A.
AP's in cell A will only generate an AP in cell B if the synapse between the cells is excitatory. Therefore, we expect depolarization, not hyperpolarization at the synapse. We expect a graded response (depolarization in this case) but not an AP at the synapse -- there are no voltage gated channels on the dendrites and cell body, so you can't start an AP there.

C. If there is no Ca^{++}, that will affect the recording from cell B at the synapse, but it will not effect the recording along the axon of cell A. Explanation:

Transmission of the signal across the synapse requires exocytosis of vesicles containing the neurotransmitter on the presynaptic side. This exocytosis is dependent on Ca^{++}. When the AP reaches the synaptic terminal, voltage gated Ca^{++} channels are opened, and Ca^{++} normally flows into the cell. This influx of Ca^{++} is what triggers exocytosis of the vesicles containing transmitter. (And the transmitter is what generates the EPSP in cell B.) In the absence of Ca^{++} on the outside, Ca^{++} channels on the presynaptic side will open, but Ca^{++} will not flow in and exocytosis will not occur. So the postsynaptic side will receive no neurotransmitter, no channels will open, and no depolarization will occur (on the postsynaptic side). There will be no EPSP -- the recording trace will be flat.

The AP will not be affected because the AP is not dependent on Ca^{++} at all -- the AP is dependent only on flow of Na^+ and K^+ through voltage gated Na^+ and K^+ channels.

Answer to 8-20, cont.

D. Both recordings will be affected. (No points were awarded for answer -- all for explanation). Explanation is as follows:

The higher level of Na^+ will change the equilibrium potential for Na^+, so that when channels are opened, and Na^+ rushes toward its equil. pot., more Na^+ will come in -- there will be a larger chemical gradient, and a bigger "push."

Because of the larger Na^+ influx, the EPSP will be larger.

The AP will be different too. Under normal conditions the size of the AP and the ease of firing an AP are invariant, but that's because the ratio of Na^+ concentration in/out is invariant. However, here you changed the conc. of Na^+. Therefore:

(i) The AP will be higher because of the higher influx of Na^+.

(ii) You may get more AP's (from the same stimulus) because of the movement of the RMP closer to threshold (see below) and/or the larger EPSP's in response to the same amount of transmitter.

The RMP will not change significantly, as it is largely dependent on K^+, not Na^+, and it is close to the equil. pot. for K^+ all along. If there is any change, the RMP will be more depolarized and closer to threshold -- less K^+ may leak out if the added Na^+ makes the outside temporarily more + relative to the inside. (The change in charge due to added Na^+ is not the only effect of added Na^+. You also have to consider the effects on movement of K^+, which may offset the charge difference.) This issue is complex, and so no points were either awarded or subtracted for a discussion of what happens to the RMP.

Answers to Problem Set #9

9-1. A. x = length of actin filament in skeletal muscle. One tropomyosin covers every seven G-actins; hundreds of G actins are along one actin filament. See pictures in texts or on class handout.

B. x = y (length of sarcomere changes, not length of individual actin filaments

C. x = y (oxytocin stimulates contraction of uterine smooth muscle; smooth muscle actin doesn't have troponin bound to it in either condition)

D. y (in cold, arteriolar vasoconstriction occurs in skin; this requires smooth muscle contraction, which is dependent on calcium-calmodulin)

E. x ...ADP...

9-2. In the sarcoplasmic reticulum. It binds Ca^{++}, decreasing the concentration of free Ca^{++} in the SR. This helps lower the concentration gradient between the sarcoplasm and the inside of the SR, so that less energy need be expended in pumping Ca^{++} back into the SR.

9-3. A. slow down conduction of action potentials.
B. It will be closer to threshold.

9-4. In skeletal muscle:
(1). Troponin binds Ca^{++}.
(2). Myosin has the ATPase.
(3). Myosin & Actin form cross bridges between them.
(4). Sarcoplasmic Reticulum releases Ca^{++}.
(5). None of these.

9-5. A. Eserine will allow ACh to activate receptors for longer time, and so cause spasmodic contraction of muscles. Botulinum toxin (by preventing ACH release) and curare (by blocking ACh receptors) both prevent muscle contraction, and so paralysis results.
B. Put unknown drug on neuromuscular junction and stimulate nerve. If the muscle contracts, the drug was eserine, since the other two block functioning at the synapse. If the muscle doesn't contract, put some ACh in synapse. If the drug was Botulinum toxin, the muscle will contract. If it was curare, the receptors are blocked, and the muscle won't contract.
C. Curare is an antagonist; it binds to the receptor and blocks the effects of the normal ligand (ACh). If curare bound to and stimulated the receptor, it would be an agonist.
D. Use radioactively labeled curare, which will bind to the receptors.

9-6. Additional gap junctions form between adjacent cells, allowing more rapid conduction of action potential along cell membranes, and therefore synchronous contraction of cells.

9-7(1) e, (2) a, (3) b, (4) d, (5) a, (6) d

9-8. A. depolarization
B. If the amount of ACh is sufficient, the depolarization will spread to surrounding plasma membrane where voltage-gated channels are, and an action potential will develop all along the surface of the muscle.

9-9. A & B. You will still get depolarization at the synapse, since voltage-gated channels are not involved, but since voltage-gated Na^{+} channels are needed for the action potential, the depolarization will remain local, and no change in membrane potential will occur a cm away from the synapse.
C. No. A local potential would develop, but voltage-gated channels are still necessary for an action potential, so none will develop.

9-10. A. shows that the muscle itself is not defective, because it contracts with direct stimulation, so it must have functioning actin, myosin and sarcoplasmic reticulum.

B. shows that the nerve is able to stimulate the muscle, so action potentials must be transmitted down the axon. So the problem must be in the area of the synapse.

C. Inhibiting acetylcholine esterase as in C will prevent acetylcholine (ACh) destruction, leading to more acetylcholine in the synapse. Since this reverses the defect, the problem was either that insufficient ACh was produced (and ACh-esterase inhibitor reversed the deficit by allowing more ACh to remain in the synapse), or sufficient ACh was produced, but there were fewer, or defective, receptors (and A Ch-esterase inhibitor reversed the deficit by allowing the ACh to stick around for longer, and so stimulate the receptor enough times to eventually cause contraction to occur).

Experiment: Put ACh directly on muscle membrane. If get normal contraction, problem was insufficient ACh release. If get weak contraction, problem was receptor. (When actually done on muscles of MG patients, these experiments showed that ACh receptors are defective.)

9-11. high...ADP + P_i

9-12. A. In normal contracting muscle, cross bridges are not stable. They must constantly be broken and reformed, and this requires ATP. The actin-myosin complex must bind ATP to separate A and M, and hydrolysis of ATP follows binding. To maintain a contraction, complex must reform cross bridges and repeat cycle, using up ATP. In a corpse, when the ATP runs out, A and M can't separate, bridges are stable, and rigidity results.

B. Low Ca^{++} blocks the cross bridge cycle. Ca^{++} is needed to bind to troponin in order to twist tropomyosin out of the way and expose myosin binding sites on actin. In absence of Ca^{++}, tropomyosin blocks binding of myosin heads to actin, so no contraction can take place. (Low Ca^{++} blocks cross bridge cycle at different step from low ATP.)

9-13. In smooth muscle:

(1). Calmodulin binds Ca^{++}.

(2). Myosin has the ATPase. (Same as skeletal muscle.)

(3). M & A form cross bridges. (Same as skeletal muscle.)

(4). None of these. Action potential usually affects Ca^{++} channels in plasma membrane so Ca^{++} enters from outside the cell. (Smooth muscle has relatively little SR so relatively little Ca^{++} is usually released from SR. This is different from case in skeletal muscle; see above.)

(5). Myosin (and caldesmon, if present) must be phosphorylated to form cross bridges. (A protein kinase, MLCK, phosphorylates the myosin, but it is not activated by phosphorylation -- it is activated by Ca^{++} and calmodulin. The kinase is **in**activated, not activated, by phosphorylation. Caldesmon [if unphosphorylated] blocks actin in some types of smooth muscle.)

9-14. b, c, e, & f are true.

9-15. A. Smooth muscle contraction requires Ca^{++}, which enters cells from the ECF. If channels are blocked, less Ca^{++} enters the cell and the muscle contraction is attenuated. When the smooth muscles encircling the arteries are relaxed, the vessel dilates, decreasing blood pressure.

B. Heart muscle requires Ca^{++} for muscle contraction. Some of this Ca^{++} enters through membrane channels, so blocking them decreases contraction. Also, Ca^{++} is needed to sustain the depolarization, so if less Ca++ enters, the action potential has a shorter plateau and the contraction won't last as long.

C. Both intestinal muscle, which is smooth muscle, and skeletal muscle contract in response to high Ca^{++} levels. However in smooth muscle, most of the Ca^{++} comes from outside the cell. It enters the cell through channels in the plasma membrane and combines with calmodulin. Skeletal muscle, on the other hand, contracts when intracellular Ca^{++} is released from the sarcoplasmic reticulum and combines with troponin. Therefore, drugs which block Ca^{++} channels in the plasma membrane will have a greater effect on smooth muscle.

9-16. A. One. The transmitter secreted by a motor neuron in response to one AP generates a large excitatory post synaptic potential (EPSP) in the muscle membrane. In this case the EPSP is called an EPP or endplate potential. One EPP is sufficient to depolarize the muscle cell's membrane to threshold and generate an AP in the muscle, which causes a twitch (brief contraction). The EPSP generated at a neuron-neuron synapse is much smaller, and is insufficient to generate an AP in the post synaptic neuron.

B. The following will shorten: **(length of the sarcomeres) & (space between the ends of the myosin and the Z line)** For an explanation, you needed to draw or describe a muscle sarcomere = distance from one Z line to another. You had to make it clear where the actin and myosin are relative to each other), and where they are relative to the Z line as the muscle contracts (or relaxes).

9-17. A. The target protein for Ca^{++} should be **troponin** in skeletal muscle and **calmodulin in smooth muscle.**

B. **(d)** Tropomyosin (& troponin) are bound to actin, and the tropomyosin blocks the sites where actin and myosin bind. When Ca^{++} binds to its target, the target protein (troponin) changes conformation and pushes the tropomyosin on the actin out of the way, exposing binding sites for myosin. Myosin can now bind to actin and push the actin relative to the myosin.

C. **Order: (d) → (b) → (e) → (a).** Brief explanation: ATP binding is required for (e) to occur, but it is not listed. ATP hydrolysis (split of ATP) does not occur until later -- after step (e). See class handout for all steps.

Note: On an exam, you would probably not get full credit if you simply listed all the steps that occur during muscle contraction. To get full credit, you would have to be more selective. In other words, you can't just reproduce the class handout. We know it is tempting to put everything in, just in case, but (a) it takes too long and (b) you aren't given enough space. You might have to write out the whole cycle on scratch paper in order to figure out the answers, but in your explanation on the exam paper, you are better off sticking to the facts you need to answer the question. You are most likely to get full credit if you demonstrate the ability to pick out the important or relevant facts, and the ability to explain how these facts or principles lead to the answer.

Note that the explanations in this manual are usually much more thorough than those required on exams.

D. (1) **(c) & (e) must occur for relaxation to occur. (c)** is the critical step given all the choices on the list; **(e**) is the critical step if you stick to the choices picked in part C. First myosin will separate from actin (step e), and this must happen whether either contraction or relaxation is going to occur. If the muscle is going to relax, tropomyosin will bind to the actin (step c), preventing the myosin from binding again to myosin for another stroke. Therefore, of the steps listed in C, only **(e)** is critical to relaxation. (You don't need step a; see below.)

(2) Of the steps listed, **(e) will occur first, and (a) will occur last** when the muscle relaxes. ATP hydrolysis (step a) isn't needed to allow relaxation; it's needed to get the myosin ready for the next round of contraction.

More Extensive Explanation:

Why cycle stops: When stimulation stops, Ca^{++} is pumped back into the ER, and the level of Ca^{++} in the cytosol drops. Troponin loses its Ca^{++} and returns to its original conformation, allowing the tropomyosin to bind to actin and cover the myosin binding sites of the actin. Therefore myosin remains separated from actin.

Note: There are two places that the cycle can stop -- at the point where ATP binding is required or the point where Ca^{++} is required (to move the tropomyosin out of the way). In normal skeletal muscle, ATP isn't the limiting factor -- Ca^{++} is. (It's only in dead organisms that cytosolic Ca^{++} is abundant and ATP runs out.) So in normal cells, myosin and actin always separate whether the cycle is going to stop or not. The separation (& binding of ATP) doesn't end the cycle -- it just allows it to proceed to the next step. Whether the cycle continues or stops depends on the Ca^{++} concentration.

Where the cycle stops & function of last step: If Ca^{++} is low, myosin and actin can't form a cross bridge because tropomyosin is in the way. Once the myosin binds ATP it will separate from the actin, and the actin and myosin will stay apart. The ATP already bound to myosin will be split, energizing the myosin so it will be ready to bind to (& move) actin. The cycle can't go any farther until Ca^{++} rises and inactivates troponin. So the cycle stops with "cocked" myosin, not myosin bound to actin.

KEY TO PROBLEM SET 9R

9R-1. Phosphorylation of myosin should **increase** (phosphorylation is necessary for myosin-actin binding and contraction); troponin should **stay the same at zero** (it's in striated muscle, not in smooth muscle).

9R-2 A. & B. The **width of the action potential will increase**, as the extended period of depolarization found in heart muscle AP's is due to an influx of Ca^{++}. The **height will stay the same**, as the initial depolarization is determined by influx of Na^{+}. Once the Ca^{++} channels close, Ca^{++} will stop entering the cell, and voltage gated K^{+} channels will open, allowing K^{+} to exit. The depolarized cell (positive membrane potential) will then repolarize to its normal negative resting potential. So the potential will go from + to -.

C. Best answer is **increase or stay the same**, but increase was ok too. Pacemaker cells give the signal for myocardial cells to contract. Normally an AP from the pacemaker cell triggers an AP in the myocardial cell, and spacing between contractions (heart beats) depends on events in the pacemaker cell. But if Ca^{++} channels in the myocardial cells stay open, the myocardial cells will remain depolarized and will be in a refractory state where they cannot fire an AP. The myocardial cells will not respond to signals from the pacemaker cells as long as the Ca^{++} channels in the myocardial cells remain open. If the channels stay open long enough, the "next" signal from the pacemaker cells will be ignored, and the heart will "skip a beat." In this case, the myocardial cells will only contract in response to every other signal from the pacemaker cells, so the period from one contraction to the next will be longer. If the Ca^{++} channels stay open longer than normal, they may still close in time for the myocardial cells to repolarize before the next pacemaker AP. Then the next contraction will occur at the usual time, and the interval between heart beats will stay the same.

9R-3. A-1. The neuron is **(efferent)** .

A-2 . The neuron could synapse on **(another neuron)** .

A-3 The cell body of this neuron should be **(in the spinal cord).** *See text or handout 20C. The neuron is efferent because it carries info away from the CNS, toward an effector.*

B-1. The synapse should be **(nicotinic) & Cholinergic.**

B-2. The synapse on the target cell should be **(excitatory) .**

B-3. Transmitter should **(open a channel)**.

This neuron is the first in a two neuron relay, and the synapse discussed here is the synapse between this neuron and the next in the pathway, not between the second neuron and the effector. It is the first synapse outside the spinal cord, not the second. (See class handout or text.) The first synapse in the autonomic nervous system (parasympathetic or sympathetic) uses acetyl choline as a transmitter and the receptor is an ion channel (ionotropic or direct receptor). The receptor is cholinergic because it uses acetyl choline as a transmitter and nicotinic because it is stimulated by nicotine (a property of the ionotropic ACh receptor/channel). ACh always opens the channel to cations and Na^{+} moves into the cell, depolarizing it. So the synapse is always excitatory.

9R-4. A-1. Troponin; A-2. Contracting (one AP generates an EPP in the muscle; this is sufficient to cause the muscle to fire an AP & contract).

B-1. Contract; B-2. Not respond (stay contracted); B-3. Relax; B-4. Not respond (stay relaxed).

Explanation: (B-2 & B-3) You need to bind ATP to separate actin from myosin, and allow muscle to relax. If there is no ATP available, Actin and myosin will remain stuck together, as in B-2 (or in rigor mortis). Binding of ATP, but not the splitting, of ATP, is required for separation of actin and myosin, so the analog will allow relaxation, as in B-3.

(B-1 & B-4) You need to split the bound ATP in a relaxed muscle to allow the muscle to contract. When a muscle is relaxed normally, ATP binds as explained above. Then the bound ATP is split, 'cocking' the myosin (converting it to the high energy state). When the motor neuron fires, Ca^{++} is released from the SR , the myosin binds to the actin and moves, causing the actin to slide past the myosin, and causing the muscle to contract. This is what happens in B-1. The myosin in the relaxed muscle is in the high energy state, all ready to bind actin, so the muscle will contract (once) in response to Ca^{++}, even if no free ATP is available.

If the ATP analog used to relax the muscle cannot be split, as in B-4, then the muscle will be stuck in its low energy state. When the motor neuron fires, Ca^{++} will be released, but the myosin in the relaxed muscle will be in the low energy state, so it will not contract in response to Ca^{++}.

9R-5. A. Answers: A-1. Myosin; A-2. Vasodilation; A-3. Vasoconstriction.
Explanation:
For E: E triggers the cAMP pathway, so the activity of PKA increases. The PKA phosphorylates MLCK, and inactivates it. If the MLCK is inactive, it can no longer phosphorylate myosin. Since myosin in smooth muscle must be phosphorylated by MLCK before it can bind actin, the muscle cannot contract. If the muscle around blood vessels cannot contract, it must remain relaxed, and the blood vessels must remain dilated. Note that although phosphorylation of myosin decreases, phosphorylation of MLCK increases.
For NE: NE triggers the IP3 pathway, which leads to release of Ca^{++} from the ER. This will trigger contraction of the muscles around blood vessels, causing vasoconstriction.

B-1. Calmodulin; B-2. Vasodilation. -- the phosphorylated MLCK can no longer bind Ca^{++}- calmodulin.
Explanation: NE triggers the IP3 pathway, so it causes release of Ca^{++} from the ER. In smooth muscle, Ca^{++} binds to calmodulin, and it is the Ca^{++}/calmodulin complex that actually triggers contraction.
The Ca^{++}/calmodulin complex normally activates MLCK, which phosphorylates myosin, allowing contraction. E blocks activation of MLCK, as explained above. (Phosphorylated MLCK can no longer bind to Ca^{++}/ calmodulin.) If MLCK is phosphorylated, release of Ca^{++} from the ER will have no effect on contraction. The Ca^{++}/calmodulin complex will form, but it will not activate MLCK, myosin will not be phosphorylated, and no contraction will occur.

9R-6: A-1. Both; A-2. Both adrenergic; A-3. Post-ganglionic neurons. (See handouts)
B. Answers: B-1. Primarily alpha; B-2. Neither type.
Explanation:
(1). NE (or ACh) is the usual transmitter in the autonomic nervous system; E is primarily a hormone released by the adrenal medulla into the blood stream.
Some students reasoned that the answer was NE because this is clearly the sympathetic division of the ANS (since it uses adrenergic receptors), and you expect vasoconstriction, not vasodilation, from the sympathetic system. (This was not the intended answer, but was accepted.)
(2). The neurons described here are post-ganglionic neurons of the ANS. They synapse on smooth muscle and release NE. However the receptors on their dendrites/cell bodies are nicotinic cholinergic receptors. (In other words, the pre-ganglionic neurons that synapse on these neurons release acetyl choline, not NE or E.)

9R-7. Answers: A-1. ATP; A-2. ATP alone.
Explanation: A-1. NE uses a metabotropic (indirect) receptor that acts through a G protein to generate a 2nd messenger that in turn opens a channel in the ER. ATP uses an ionotropic (direct) receptor -- it binds to and opens a channel in the plasma membrane. Therefore ATP will have a faster effect.
A-2. To open voltage gated channels, you need to depolarize the cell -- to make the voltage across the plasma membrane less negative. ATP opens a channel that allows positive ions to flow into the cell; this will (temporarily) change the charge across the plasma membrane and depolarize the cell. NE generates a 2nd messenger that opens channels in the ER, temporarily increasing the Ca^{++} in the cytosol. This is unlikely to directly affect the voltage across the plasma membrane. (However, release of Ca^{++} from the ER sometimes leads to opening of Ca^{++} channels in the plasma membrane, and this in turn will depolarize the cell.)

B-1. Vasoconstriction; B-2. Both.
Explanation. B-1. ATP would cause a rise in Ca^{++}, which would cause contraction of the vascular smooth muscle, and therefore vasoconstriction. B-2. In smooth muscle, Ca^{++} usually comes from the outside, but some may come from the ER. In this case, both NE and ATP are released at a normal synapse. NE acts through the IP3 pathway, releasing Ca^{++} from the inside, while ATP opens channels in the plasma membrane, allowing Ca^{++} to enter from the outside.

9R-8

A. K^+

B. Cardiac contractile muscle.

C. Opening of K^+ channels, or closing of Na^+ channels or closing of Ca^{++} channels.

D. The depolarized state lasts longer than the refractory period.

Explanation of A-C: All cells repolarize by opening K^+ channels. During an AP, the cell is depolarized by an inrush of positive ions, Na^+ or Ca^{++}. Then K^+ channels open, and K^+ rushes out of the cell, down its gradient. The loss of positive ions restores the resting potential of the cell -- negative relative to outside. In cardiac contractile muscle, the opening of the K^+ channels is delayed. In addition, the AP is prolonged by the opening of Ca^{++} channels after the initial inrush of Na^+. Anything that increases the Na^+ or Ca^{++} moving into the cell, or decreases the K^+ leaving the cell, will prolong the period of depolarization.

Explanation of D: During the cardiac contractile muscle AP, the cell is refractory, and cannot respond to another AP (from a pacemaker cell or anywhere else). The muscle AP, and the refractory period, end at about the same time as a contraction. Therefore a second contraction cannot be triggered before the end of the first. This prevents the heart muscle from contracting before it has completely relaxed -- each pacemaker AP causes one contraction (if the previous contraction has ended, or none (if the muscle is still contracted). Multiple AP's from the pacemaker can't produce an additive effect, so the heart can't sum input and reach tetany. Each chamber of the heart must relax (& fill) before a second contraction (and pumping action) can occur.

Long QT syndrome has multiple causes, both genetic and environmental. The fine points are different, but in all these cases, the cell does not repolarize properly, and there is a risk of generating an irregular heart beat. In at least some cases, the cell does not repolarize completely by the time the refractory period ends. Therefore the cell is closer to threshold than normal, and a weak signal (from the normal pacemaker or elsewhere) can trigger a premature contraction. This is quite different from the case where the heart "skips a beat." In that case, a signal from the pacemaker is ignored because the muscle cell is still in its refractory period, and the muscle cell does not contract until the next AP from the pacemaker.

9R-9.

A. Slow down.

The sympathetic system releases norepinephrine (noradrenaline) which makes the heart beat faster; the PS system releases acetyl choline which makes the heart slow down. Under normal conditions, both systems release NT, and the balance between the two transmitters determines the heart rate. Blocking the sympathetic input, or increasing the PS input, will both have the same effect -- they will slow down the heart.

B. A cholinergic agonist or an adrenergic antagonist.

A cholinergic agonist has the same effect as acetyl choline. An adrenergic antagonist has the opposite effect from norepi. Mimicking acetyl choline (= input from the PS) or blocking norepi (= blocking input from the S) will have the same effect -- slowing the heart.

C-1. Metabotropic.

C-2. Pacemaker cells in the atria.

Explanations:

C-1. The input to the heart is from the post-ganglionic neurons of the autonomic system. They release NT's that bind to G protein coupled receptors that are not ion channels. The G proteins generally open ion channels indirectly, using 2nd messengers. The NT's don't open channels directly.

C-2. The SA node is the major pacemaker, and it is in the right atrium. If you want to adjust the heart beat, you adjust the frequency of AP's fired by the SA node. The other pacemaker cells are normally slower to fire than the SA node, and so it is the firing of the SA pacemaker that controls the contraction of all parts of the heart.

ANSWERS TO PROBLEM SET #10

10-1. It would stop. Respiration depends on neurons from the medulla oblongata descending the spinal cord to the diaphragm and intercostal muscles.

10-2. Increased pressure on abdomen forces diaphragm up, which decreases volume of lungs, increasing pressure, causing a sharp expulsion of air leading one to cough up the object choked on.

10-3. b, c

10-4. Hyperventilating, or exhaling CO_2 at a faster than usual rate will lower blood CO_2, which will decrease the amount of stimulation of central chemoreceptors, and breathing rate will decrease.

10-5.

A. Increased in #2 and decreased in #1. Explanation: Dog #1's blood has less O_2 more CO_2 so Dog #2's respiratory sends message to increase breathing. Dog #2's increased breathing causes increased O_2, decreased CO_2, which goes to #1's brain, so #1 decreases breathing.
B. Decreased.
C. The decreased breathing was due to
   (1) decreased CO_2 OR
   (2) due to increased O_2 OR
   (3) due to both decreased CO_2 and increased O_2
D. He gave dog #1 air high in oxygen to breathe and measured the breathing rate in dog #2. The breathing rate was unchanged, leading him to conclude that the decreased breathing measured above was due to the decrease in CO_2 not to the increase in O_2.

10-6. Gases must now diffuse over a longer distance, so diffusion is slower.

10-7. Very little (from about 200 ml O_2/L to about 215 ml O_2/L. The reason the increase is so small is that almost all the oxygen in blood is carried bound to hemoglobin and Hb is almost 100% saturated at the arterial oxygen pressure achieved by breathing room air. Breathing 100% oxygen does cause an increase in the amount of oxygen that dissolves in blood, but this is a small fraction of the total oxygen in the blood.

10-8. Can decrease arterial PO_2 greatly without affecting the total O_2 carried (see plateau part of O_2-Hb dissociation curve)

10-9. A. pO_2 is dependent on the dissolved O_2, which is not affected by CO.

B. Total O_2 refers to both dissolved O_2 and hemoglobin-bound O_2. The amount bound is abnormally low, and so insufficient O_2 reaches the tissues. But the amount dissolved, which determines pO_2, is normal. A low pO_2 will stimulate chemoreceptors, but since pO_2 is normal in this case, breathing doesn't increase.

10-10. Total amount of oxygen carried will decrease, because there is less hemoglobin to carry it. But pO_2, which is dependent on the dissolved O_2 will not change, and breathing rate, which is more dependent on pCO_2 will not change, unless the anemia is quite severe and insufficient oxygen reaches the tissues, leading to buildup of CO_2.

10-11. At 30,000 feet, air pressure is 226 mm Hg. If 21% of that is oxygen, the PO_2 would be 47 mm Hg in the air. When this air mixes with air in the alveoli, the pO_2 in alveoli is only 21 mmHg. From the oxygen-hemoglobin dissociation curve, you can see that at this pO_2, hemoglobin is only 20% saturated. To get enough oxygen, you have to either increase the pressure in the cabin, or increase the percentage of the air which is oxygen. In the latter situation, when you breathe 100% oxygen at 226 mm Hg, the pO_2 is 226 mm Hg in the air and 140 in the lungs, at which pressure hemoglobin is almost 100% saturated.

10-12. The major proteins discussed in class that are relevant to RBC structure and function are:

(1) Hemoglobin - carries O_2 to tissues, CO_2 from tissues, buffers H+ ions produced by dissociation of H2CO3
(2) Carbonic anhydrase - enzyme that catalyzes CO_2 + H20 <---> H2CO3
(3) Anion exchanger (band 3 protein) - exchanges HCO3- for Cl-
(4) Ankyrin or spectrin - cytoskeletal proteins

10-13. To the left. (less O_2 dissociates from Hb at the same pO_2.)

10-14. Llama Hb, like fetal Hb, has a higher oxygen affinity than human Hb; partial pressure of oxygen is lower in the Andes, so it takes a higher affinity to get the molecule completely loaded with oxygen.
Both would be saturated at sea level, but the llama HB would hold more oxygen at high altitude where oxygen partial pressure is lower.

10-15. The CO_2 is a small percentage of the air exhaled. A larger fraction of the air is oxygen, since the exhaled air contains the oxygen that was in the dead space in the respiratory tract.

10-16. The curve of oxygen bound vs. pO_2 would be the same shape as normal, and the midpoint would be at the same pO_2. However the curve would plateau at a lower level of oxygen bound, so the total amount of oxygen carried in the blood would be less in the anemic individual.

10-17. x, y or x = y

A. x = y carbonic anhydrase activity in blood at the lungs = carbonic anhydrase at tissues (the enzyme catalyzes the reaction in both directions)

B. x Blood leaving the tissue is high in CO_2, but when that CO_2 diffuses into the alveolus, it's diluted with incoming air, which is higher in O_2)

C. y This is the steep part of the curve change in hemoglobin saturation...from 40 to 30

D. y Sympathetic stimulation leads to bronchodilation

E. y The diaphragm, a skeletal muscle, contracts during inhalation, so Ca++ is released from SR into cytoplasm

F. x Heat shifts the curve to the right, meaning that oxygen dissociates more easily.

G. y O_2 binds to the iron in the heme group, not to globin

10-18. A. Lowering the pH shifts the binding curve to the right & increases the tendency to release oxygen in tissues.

B. Lowering the pH increases the [H+], which decreases the ability of hemoglobin to hold onto O_2, i.e., it takes more O_2 to reach the same percent saturation = shift curve to right. In tissues, H+ are released as byproduct of metabolism, so if keep overall [O_2] constant, and ability of hemoglobin to hold onto O_2 goes down, O_2 will be released.

C. Release of oxygen in the tissues. In general, hemoglobin is 98% saturated with O_2 in the lungs, so it wouldn't help much to increase hemoglobin saturation there. The step of the process that *was* subject to evolutionary modification was the release of oxygen when hemoglobin reaches the tissues. Two ways to get hemoglobin to give up more oxygen are a) to decrease hemoglobin's affinity for oxygen, and b) to increase the ability of H^+ to cause hemoglobin to give up oxygen. The tissues in these small mammals are producing a lot of H^+ from their rapid metabolism, and this acid helps ensure that the needed high level of oxygen reaches the tissue.

Answers to Problem Set #11

11-1. a. Inactivation of K^+ channels would do it. Na^+ would continue to leak in, but less K^+ would leak out. (This may not be the way it actually happens.) b. K^+ c. Ca^{++}

11-2. Pacemaker potential will be steeper, so threshold will be reached sooner, and rate of firing of action potentials will increase. Size & shape of Act. Pot. will stay same.

11-3. a.

11-4. The depolarization (due to the action potential) occurring in the myocardium during this premature contraction has a refractory period. The action potential produced by the SA node can't spread during this refractory period, and the heart skips a beat.

11-5. b

11-6. a, a

11-7. c

11-8. a

11-9. Bradykinin is affecting something in the endothelium which has an effect on the adjacent smooth muscle. The endothelium is known to produce nitric oxide, which causes smooth muscles relaxation and vasodilation. Bradykinin might stimulate the release of NO from endothelium.

11-10. b. The increase in blood pressure is sensed by the baroreceptor in the carotid sinus, which sends a message to the medulla oblongata to relax arteriolar smooth muscle, and to decrease rate of heart beat, both of which will lead to a decrease in blood pressure. These effects are dependent on increased parasympathetic activity, and decreased sympathetic activity.

11-11. b. Albumin and fibrinogen are secreted into the blood, where they increase the osmotic pressure of blood. If osmotic pressure in blood is lower, there's an increase in fluid moving out of capillaries, and a decrease in fluid moving into capillaries.

11-12. a. Liver makes blood proteins such as albumin, globulin, clotting proteins, that contribute to the osmotic pressure of the blood. A dysfunctional liver makes less protein, resulting in lower osmotic pressure in blood, and an increase in the net force pushing fluid out of the capillaries.

b. The hydrostatic pressure forces fluid out of capillaries though pores between cells. If these pores are larger, more fluid will flow out during the time the blood is in the capillaries, and fluid will accumulate in the tissues. (If it is the eyes or nose where the allergic response occurs, this excess fluid is obvious as watery eyes and runny noses, familiar to hay fever sufferers.)

c. Fluid which enters the tissue leaves either by bulk flow back into the capillary, or by movement through the lymphatic vessels. Removing lymph nodes block fluid flowing in lymphatic vessels, and the backed up fluid accumulates in the tissues.

11-13. An increase in temperature stimulates vasodilation, which will result in heat loss. The vasodilation will further intensify the decrease in blood pressure.

11-14. Ca^{++} is cofactor for clotting factors (proteases). EDTA binds Ca^{++} and takes it out of circulation, so other calcium-dependent processes will be affected, such as calcium uptake into heart muscle for action potential of pacemaker an myocardial cells, and calcium uptake into nerve terminals for neurotransmitter release.

11-15. a. Fe^{++} is needed for production of hemoglobin. With iron deficiency, less hemoglobin is made, and each red blood cell can carry less oxygen.

b. Stem cells in bone marrow are progenitors of red blood cells. With a decrease in stem cells, fewer red blood cells are produced and less oxygen can be carried by blood.

c. The kidney normally produces erythropoietin which stimulates stem cells to differentiate into red blood cells. A diseased kidney produces less erythropoietin and fewer red blood cells are made.

11-16. Pressure on neck exerts pressure on carotid sinus where baroreceptor is located. This increases firing rate of afferent neuron to cardiovascular center in medulla oblongata, which in turn, sends more parasympathetic stimulation to the SA node. Acetylcholine released there causes an increase in K^+ permeability of the pacemaker cells, so they depolarize less often and the heart rate decreases.

11-17. A. Increased capillary permeability means that more stuff can exit the capillaries. Small solutes, like glucose, normally exit the capillary down their concentration gradient; since blood flows through the capillaries so slowly, there's enough time for these substances to reach equilibrium and increasing the size of capillary pores wouldn't affect diffusion of these small molecules. However, the small pores normally prevent proteins from leaving the capillary. Increase in pore size allows these proteins to enter the interstitial space. They were the major factor contributing to osmotic pressure, so this movement will lower osmotic pressure in the capillary while raising osmotic pressure in the interstitial fluid. Only c. shows this change.

B. Hydrostatic pressure on blood derives from force exerted by heart. High blood pressure signifies high hydrostatic pressure. Only d. shows this change.

C. In this case there is movement of protein out of the capillaries, as in A., but here it is only happening in the glomerular capillaries. Stuff that exits the capillaries and enters the nephron has two choices: it will either be reabsorbed or it will exit the body in the urine. Protein does not normally enter the nephron, so there are no carrier proteins in the nephron to reabsorb it, and the protein here will be lost in urine. Thus, there is a decrease in osmotic pressure in the blood, with no increase in osmotic pressure in the interstitial tissue. Only b. shows this change.

NOTE: Some students used choice e. for one or another question, suggesting that the edema causes water loss from the capillary and so the hydrostatic pressure there decreases. However, as soon as the hydrostatic pressure decreases, water would be pulled back into the vessel, and there wouldn't be edema! In e., the forces pushing water out of the capillary (17 + 1) are less than the forces pulling water back in (25 + 5), so this case describes a situation in which water would be prevented from leaving the capillary.

Answers to Problem Set #12 – Urinary system

12-1. c **12-2**. a, b **12-3**. b **12-4**. a **12-5**. a

12-6. It would require diluting that salt in water, and the bird would lose too much water. Kidneys cannot produce urine which is concentrated enough.

12-7. a. Renin from the kidney stimulates conversion of angiotensinogen (from liver) to angiotensin, which stimulates the adrenal cortex to release aldosterone, which in turn promotes Na^+ reabsorption in the distal tubule. So if ANP promotes NaCl excretion, it must be decreasing renin production.

b. ANP dilates the afferent arteriole. This increases the push "in" relative to the rate of flow "out." This would increase blood pressure in the glomerular capillaries and increase filtration, so that more Na^+ enters the filtrate and can be excreted.

c. If ANP increases salt excretion, it must be decreasing secretion of aldosterone from the adrenal cortex, since this hormone decreases salt excretion.

12-8. ADH (vasopressin) is produced by the hypothalamus and secreted by the post. pit. in response to stretch sensed by receptors in arteries or osmoreceptors in hypothalamus. ADH acts on the collecting ducts of the kidney to mobilize water pores and increase water reabsorption. (Water flows out of the ducts because of the high salt gradient in the medulla.) ADH acts quickly through a second messenger system. (See also ans. to 12-9)

Aldosterone is produced by the adrenal cortex in response to angiotensin. Angiotensin is in turn produced by action of renin (from the kidney) on a circulating protein precursor to angiotensin. The sympathetic system (input from brain) or stretch receptors in the kidney can trigger production of renin. Aldosterone increases Na^+ reabsorption by the kidney, as well as by other tissues including the large intestine and sweat glands. In the kidney, aldosterone acts on the end of the tubule and/or the start of the collecting ducts. Na^+ reabsorption increases water reabsorption, since water follows the salt. Aldosterone is a steroid that activates transcription, so it is slower acting than ADH. (See also ans. to 12-10).

In summary, aldosterone acts primarily on Na^+ reabsorption near the end of the tubule and ADH acts primarily on water reabsorption in the collecting ducts, but both ultimately increase water retention since removal of salt leads to removal of water.

12-9. A. & B. Volume of urine decreases & osmolarity increases (because water is removed from the urine, but the salts stay the same).

C & D. Osmolarity of blood decreases, and BP increases (because the water retained in the body dilutes the blood and increases the volume in the circulatory system). ADH also acts as a vasoconstrictor, which would increase the BP further by causing constriction of the peripheral blood vessels and decreasing the capacitance of the circulatory system.

How ADH works: ADH increases the insertion of water channels (aquaporins) into the luminal membrane of the epithelial cells surrounding the collecting ducts. (There are constitutive water channels in the BL membranes of the cells.) The channels allow water to flow osmotically out of the collecting ducts into the medulla (because of the high salt gradient outside the tubule). The water that flows out of the tubules is retained in the body. Some stays in the IF, but most enters the capillaries.

12-10. A. (out of the tubule).

B. (passively) .

C. (both membranes)

D. (unchanged). Explanation: Aldosterone stimulates the movement of salt. The movement of water is entirely passive -- water follows the salt (Na^+). When salt is reabsorbed, it moves out of the tubule, across both cell membranes, and into the interstitial fluid. (It then moves into the capillaries.) As the osmolarity of the IF (& blood) increases, water flows out of the cell, which pulls water in from the tubule, and so on. It is ADH, not aldosterone, that affects insertion of water channels into the luminal membrane of cells surrounding the tubule. Aldosterone stimulates the synthesis of proteins needed to move Na^+, not water. (The two hormones also do not affect the same region of the tubule -- aldosterone affects primarily the distal tubule and ADH affects primarily the collecting ducts.) The part of the distal tubule affected by aldosterone may or may not be permeable to water. One possibility is that there are constitutive water channels in both luminal and BL membranes of the cells surrounding the distal tubule, and water simply follows the Na^+ locally. Alternatively, the distal tubule may be impermeable to water. In that case, the water that is absorbed as a consequence of Na^+ retention is reabsorbed in a different part of the tubule (as a consequence of overall higher Na^+ concentration in the IF).

12-11. If Na^+ transport is blocked, Na^+ gets into the tubule, but can't come out. Water (which follows Na^+ by osmosis) will stay in the tubule with the Na^+, instead of following the Na^+ out. Glucose is reabsorbed by co-transport with Na^+. If Na^+ transport cannot occur, glucose cannot be moved either. Glucose transport can be blocked directly, if the co-transporter is blocked. Glucose transport can be blocked indirectly if Na^+ movement out of tubule cells is prevented, say if the Na^+/K^+ pump is inhibited. Then there will be no Na^+ gradient across the lumenal membrane, and reabsorption of glucose (by co-transport) will stop. (Even if the co-transporter is not directly blocked.) Therefore the concentration of all three will be higher in the urine.

12-12. A. Reabsorption of Na^+. The diuretic must increase the sodium in the tubule (in the filtrate). Therefore it must inhibit recovery of sodium by the cells surrounding the tubule. Reabsorption and secretion in the kidney always refer to the process from the point of view of the cells surrounding the tubule -- not from the point of view of the tubule. So transport out of the tubule into the surrounding cells is known as 'reabsorption', and secretion means the reverse.

B-1. DH is the **least** likely to act as a diuretic. There is no sodium transport from the tubule (reabsorption) to inhibit in the descending loop of Henley. So what ever compound DH does, it can't bind to proteins and inhibit sodium reabsorption. Sodium transport from the tubule (reabsorption) occurs in all the other segments listed, so all the other compounds could be binding to (& inhibiting) proteins involved in sodium transport. There is an active sodium pump in the BL membrane of all these cells, which keeps intracellular sodium low. What's different about the DH is that it has no channels or transporters for sodium on the luminal surface. Therefore no sodium enters from the tubule. Any sodium that enters comes from cells on the BL side.

B-2. PT is most likely to affect the amount of amino acids in the urine. Most amino acids are reabsorbed in the PT using co-transport with sodium. So if you block sodium transport into the PT, amino acid reabsorption is likely to stop too.

B-3. None of these. Aldosterone stimulates sodium reabsorption in the distal tubule; DT works in the distal tubule, but it has the opposite effect.

B-4. None of these are likely to inhibit the pump. The pump is in the BL surface, not the luminal one. It says in the problem that the compounds bind to proteins in the luminal membrane. So none of the compounds can be inhibiting the pump. Also, if the compounds are specific for different parts of the tubule, they shouldn't affect the pump, which is present in all cells in all parts of the tubule.

12-13. No ADH. Many such cases of diabetes insipidus result from trauma to the posterior pituitary gland which occurs in car accidents. The damaged pituitary secretes less ADH; treated by giving ADH.

12-14. A. low.
B. losing salt is the primary effect. Water follows the salt, so ultimately, both are lost.
C. aldosterone

12-15. (c). Both systems have defects, and this makes sense if the protein that is defective in the disease is responsible for reabsorption in the kidney (from filtrate back into body) and absorption in the intestines (from digestive tract into body).
Details: Presence of injected proline in urine means the proline gets from the blood into the filtrate, but it's not recovered from the filtrate. Amino acids in the filtrate are usually (re)absorbed by 2^o active transport in the proximal tubule of the nephron, so if there is no reabsorption, the transporter is not working. Therefore all the injected proline is lost in the urine. When proline is taken by mouth, it must get into the digestive tract, but none of it goes into the urine, so it must not be getting into the filtrate. We know proline can get to the filtrate from the blood (from the injection experiment), so the proline must not reach the blood in the first place. The carrier protein that normally transports proline across the apical membrane of the epithelial tissues lining the digestive tract in the intestines must not be working. In other words, the same protein carrier acts as a transporter for proline in both these epithelial tissues - in the nephron and in the intestines. A single mutation can knock out the transport protein, prevent transport in both places, and cause the symptoms of the disease. (Where is the proline the person drank?)

12-16. After drinking salt water, he would excrete a volume of urine larger than the volume of water he drank, so he would become even more dehydrated. The increased [Na^+] in blood would cause water to osmose out cells, causing dehydration. The body would excrete salt (by decreasing aldosterone secretion) and increase water retention to dilute the salt (by secreting ADH). But when excreting salt maximally, the kidney can still produce a urine which is only 1200 mOsm. So if you drink, say, 1 L of 2400 mOsm salt solution, you can get rid of all that salt only by producing 2 L of 1200 mOsm urine. So as he drinks sea water, he becomes dehydrated.

12-17. A. Less concentrated. The descending limb of loop of Henle concentrates urine by removing water. If the protein forming the water pores or channels is missing, the water cannot diffuse out and urine will remain dilute.

From information given, aquaporins must make up pores that allow water to flow across luminal membranes; #1 must make up the unregulated pores of the descending limb; #2 must make up the pores of the collecting duct that normally respond to ADH. (There are constitutive aquaporins in the BL membrane of the cells, so any water that flows into the cells from the filtrate, will flow out of the cells on the BL side.)

B. Neither protein should be found in ascending limb of loop of Henle since no water diffuses out or is reabsorbed here.

C. #2 should be present in luminal membrane of cells of collecting ducts. Water normally flows out of the filtrate here, concentrating the urine, so pores are needed. If these pores are nonfunctional, urine will remain dilute = diabetes insipidus.

D. Both genes should be present in cells of collecting ducts (& in virtually all cells), but genes for both aquaporins will not be expressed in collecting ducts, so both proteins will not be present.

E. Neither. Both genes are expressed constitutively -- both proteins are present all the time (in the appropriate cell types). The activity or permeability of the pores in the collecting ducts (composed of aquaporin 2) is regulated by ADH. In the short run, ADH does not affect the synthesis or amount of aquaporin 2 -- it only regulates insertion of the protein into the cell membrane. (In the long run, it may also affect synthesis of the pore protein.)

F. Neither. In this person, aquaporin doesn’t respond to ADH. Therefore, adding more ADH won't help. (The usual cause of diabetes insipidus is a failure to make ADH. This isn't the problem here.) Adding aldosterone will increase Na^+ retention and water will follow the Na^+, reducing the urine volume, but the Na^+ that is retained may cause additional problems, and the urine volume will still be much too large (and it will be very dilute).

12-18. If only water leaves, there will be the same amount of solute in less water, which means an increased osmolarity. There are osmoreceptors in the hypothalamus that sense this increased osmolarity and stimulate the posterior pituitary gland to release ADH (vasopressin). ADH causes more water channels to be inserted in cell membranes of collecting duct cells, leading to an increase in water reabsorption, which is an anti-diuretic effect. (Loss of both water and salt would decrease the volume of blood, but not affect its osmolarity.)

a. Glucose in blood would enter the glomerular filtrate. Large amounts of glucose would saturate the carriers for glucose re-uptake, causing an increase in osmolarity of the filtrate. Less water would leave the filtrate and more water would be excreted in urine.

b. Aldosterone binds to nuclear receptors in the collecting duct and stimulates Na^+ reabsorption (by increasing the number of sodium channels in the membrane and increasing mechanisms of energy production for Na^+/K^+ pump). Water then passively follows the Na^+ by osmosis. If aldosterone's binding to receptors is inhibited, less water will be reabsorbed and more will leave in the urine.

12-19.

Feature	Where is it greater?	Explain *briefly* why it is greater there.
Microvilli	*proximal*	*Reabsorption of all glucose, amino acids, and most Na^+ and water takes place here, and microvilli increase surface area available for reabsorption.*
Number of mitochondria	*proximal*	*Most reabsorption takes place in proximal tubule, requires Na^+ concentration gradient which is based on energy-dependent Na^+/K^+ pump.*
Intracellular hormone receptors	*distal*	*Aldosterone, a steroid hormone, binds intracellular receptors in distal tubule (and collecting duct).*
Osmolarity of filtrate	*proximal*	*Osmolarity is 300 mOsm in proximal. Distal tubule receives fluid from ascending limb of loop of Henle, which is 100-200 mOsm.*
Glucose concentration in cytoplasm	*proximal*	*Glucose co-transported into proximal cells with Na^+, then diffuses down concentration gradient out of cell by facilitated diffusion. So glucose concentration within these cells must be higher than in usual cells.*

12-20. He was not producing the hormone _***aldosterone***_which is normally synthesized in the _***adrenal cortex***__.

12-21. A-1. & A-2. Glucose and amino acids are both **filtered** (passed through the glomerulus into the filtrate) **and then reabsorbed,** using co-transport with Na^+. Both substances are transported actively (up their gradients) into the epithelial cells lining the lumen; both substances then move passively (down their gradients) out of the cells and into the capillaries. The movement into the cells across the luminal membranes is a case of secondary active transport. The Na^+/K^+ pump keeps Na^+ levels low in the epithelial cells, so Na^+ can flow down its gradient from the lumen into the cells. When the Na^+ goes down its gradient, it drives glucose and amino acid transport into the cells across the luminal membrane. The amino acids and glucose then flow down their gradients out of the cells. So **active transport occurs across the luminal surface** and passive transport occurs across the basal surface and into the capillaries.

B. Under normal conditions, the **protons go into the lumen** by secondary active transport (anti-port or exchange with Na^+). When the pump is blocked, there is no Na^+ gradient so there is no driving force to move the protons out of the cells into the lumen. Consequently, the protons flow down their gradient into the extracellular fluid and into the capillaries, causing acidosis. Since the gradient of Na^+ produced by the pump is the driving force, this is **secondary active transport.**

12-22. A. filtered.
B. the proximal tubule.
C. a venule & a peritubular capillary.
D. all but primary active transport in the order: c-b-a.

Explanation: Filtration is the first step in formation of filtrate/urine -- cells and proteins are retained, and the liquid part of the blood (containing small molecules like glucose) is pushed from the capillaries into Bowman's capsule. The glucose carried by the filtrate enters the proximal tubule, where the glucose is reabsorbed. All the glucose should be reabsorbed, and none should be found in other parts of the tubule. How does reabsorption work? Glucose must move across both membranes of the cells surrounding the tubule. First glucose moves by secondary active transport across the luminal membrane, followed by facilitated diffusion across the BL membrane. The glucose then moves across the IF and into the peritubular capillaries by simple diffusion. (Primary active transport is needed to maintain the Na+ gradient needed for secondary active transport, but primary active transport is not needed directly to transport glucose.) The peritubular capillaries feed into a venule, and the venules into a vein. It is the capillaries, not the the arterioles or venules, that exchange material with the tissues. The efferent arteriole may parallel the proximal tubule, but it is not up picking up material to return to the general circulation. (Capillaries are thin walled and porous; arterioles and venules are not -- they are surrounded by smooth muscle.)

E-1. negligible
E-2. filtration & reabsorption.
E-3. reabsorbed.
E-4. all of these.

Explanations

E-1. Amino acids are filtered and then reabsorbed by the proximal tubule in the same manner as glucose (but using different transport proteins). Normally, the amino acids are completely reabsorbed and none ends up in the urine

E-2. In a person with HAM, the level of arg in the blood is higher, so more arg is filtered (carried into the tubule). Since the concentration of arg in the tubule is higher, the rate of reabsorption is higher. The transport proteins that carry out secondary active transport act like enzymes -- the rate of the reaction (transport) is proportional to the amount of substrate (arg) -- until the transporters are saturated. In a normal person, all the arg is reabsorbed, so the transporters are not normally saturated. In enzyme terminology, the transporters are working well below their K_m (in a normal person). In a person with HAM, there is much more substrate (arg), so the rate of the transport reaction increases (until the transporters are saturated).

E-3. In a person with HAM, the arg is so high that not all of it can be reabsorbed, so some of it remains in the tubule and ends up in the urine. The transporters are saturated -- the concentration of arg is so high that the transporters reach their maximal velocity and can't work any faster. They remove as much arg as possible, but they can't remove it all. The % of arg reabsorbed is 100% in a normal person, but it is less than 100% in a person with HAM.

E-4. Normally all the arg is reabsorbed from the proximal tubule, so none is left to reach the loop of Henle, prox. tubule, etc. In a person with HAM, the arg is higher in the blood, so more enters the proximal tubule. The level of arg is so high that not all of it is reabsorbed (see above), so some of the arg is left in the proximal tubule, and it travels all the way through the tubules & collecting ducts and on to the ureter.

Notes:

1. This condition (hyperargininemia) is real, but it is not called 'HAM.' I made that acronym up.
2. Secreted is not the same as ***ex***creted. Secreted (in the kidney) means actively transported from the blood to the filtrate; excreted means lost in the urine or feces.
3. Why isn't arg secreted in this case? It seems like a reasonable way to decrease the abnormally high blood level of arg. Unfortunately for people with this condition, there are no transport proteins for secretion of amino acids. Excess amino acids are normally converted to something else, or broken down to smaller molecules which can be eliminated from the body. Since there are no excess amino acids to be disposed of in normal people, no mechanism for secreting them has evolved.

12-23. Note: BND (bad news disease) is based on Hartnup disease.
A-1. In the kidney, the BND4 protein should be made in the cells of the **proximal tubule**. BND protein is an amino acid transporter. All transport of AA takes place in the proximal tubule.
A-2 An enhancer of the BND4 gene should be located in the cells **of all of these**. An enhancer is a cis-acting regulatory DNA sequence. The enhancers in every cell are the same.

B-1. In the USA, people with a BND genotype usually eat a diet that is **high in protein**. A person with the BND genotype must eat a high protein diet to maintain a normal level of neutral AAs in the plasma – there must be a high rate of neutral AA uptake from the intestine (using HDCTs) to balance the high rate of neutral AA loss in the kidney (due to the absence of BND protein).
B-2. The level of neutral AAs in their urine should be **higher than normal**. If BND protein is missing, any neutral AAs that are filtered into the kidney tubule cannot be recovered – they will be lost in the urine. The kidney does not have any other proteins that can substitute for the transport function of the missing BND protein. However, the situation is different in the intestine, which has multiple proteins (hydrolases, transporters, etc.) for absorbing nutrients from food. So even if BND protein is missing, neutral AA can enter the plasma, because dipeptides containing neutral AAs can be absorbed from the intestine using HDCTs.
B-3. The level of the AA aspartic acid in their urine should **be normal, which is zero**. Charged AAs are transported by a different protein, in both intestine and kidney, not by the BND protein. So the charged AA will be transported normally, even if BND protein is missing. Why is the normal AA level zero? If normal carriers are present, all the AAs will be removed from the urine and reabsorbed by the body. AA will only appear in the urine if the level of AAs in the plasma is very high, or there is something wrong with the kidney, such as the absence of BND protein.

C. H**igher than in their plasma**. When the filtrate enters the kidney tubule, the [neutral AA] is the same as in the plasma. If there is no BND protein, all the neutral AAs will remain in the filtrate. In the descending loop of Henle, water will be removed, increasing the [neutral AA]. There are changes in Na^+, and changes in osmolarity, but the question only asks about the concentration of neutral AA.

D. (i) In the urine, the <u>concentration</u> of neutral AAs will **decrease &** (ii) the <u>amount</u> of neutral AAs will **stay the same.** Secretion of ADH increases water reabsorption and decreases the volume of urine, but it does not affect the transport of neutral AA. (Therefore, if ADH secretion stops, the reabsorption of water will decrease, and the volume of urine will increase, diluting the concentration of neutral AAs.)

E-1. Uptake of neutral AA *in vitro* should be **normal, which is nonzero.** Uptake in the brain is necessary, as amino acids from the plasma are needed by all cells for growth and maintenance. (Neutral amino acids are especially important since trp is needed to make NAD, and cells cannot make trp themselves.) The blood-brain barrier does not prevent transport of essential molecules into the brain, but it does affect how transport occurs. For small molecules to reach brain cells, the molecules must be transported by carrier proteins out of the capillaries; they can't just diffuse out through the pores between the endothelial cells. Since the BND gene is not expressed in brain, meaning BND protein is not made in the brain, there must be a different carrier for transport of neutral AAs in the brain. The actual carrier for neutral amino acids should be normal in brain cells from a person with BND.

E-2. The most likely explanation is **production of toxic byproducts from neutral AAs remaining in the intestine.**
There are only two possibilities here – (a) that a lack of BND protein causes a buildup of toxic byproducts that reach the brain, or (b) that a lack of BND protein results in a shortage of neutral AA in the brain. Possibility (a) is thought to be correct, since BND protein is not made in the brain, and the plasma levels of neutral AAs are usually normal* in Hartnup disease. (However, the urine levels *are* elevated, as explained above.)
Since toxic byproducts probably cause the problem, as explained above, and since any toxic byproducts in the kidney would be excreted in the urine, the source of the toxic byproducts is probably the intestine.
More details: In the absence of BND protein in the GI tract, neutral amino acids will build up in the stomach or intestine. These can be converted to toxic byproducts. Some will be lost in the feces, but some could enter the plasma, and be carried to the brain. Toxic products might also accumulate in the kidney filtrate, but if they are secreted into the tubule, they would end up leaving the body in the urine; they wouldn't get into the plasma, so they couldn't reach the brain.

*Given the normal Western high protein diet. On a low protein diet, the levels of neutral AA in the plasma would be below normal, and then a lack of neutral AA in the brain could be the problem, and 'inability to transport AAs properly' would be a reasonable answer.

Key to Problem Set 12R -- Review Questions on Physiology

12R-1. Oxygen levels should **decrease** (as blood is pumped through the body) and the hemoglobin should **stay the same** (it's the amount bound to oxygen that changes).

12R-2. % saturation should **decrease**, as acid (low pH, high [H^+]) decreases affinity of hemoglobin for oxygen (shifts binding curve to right). As solution gets more acid, oxygen should fall off the hemoglobin; this is what happens to the RBC passing through capillaries **in the tissues** as metabolism produces acid (mostly from carbon dioxide → carbonic acid → H^+ and bicarbonate in the RBC).

12R-3. Carbonic anhydrase catalyzes the interconversion of **CO_2 and HCO_3^-**. (See note at end of 12R-13.) The same enzyme always catalyzes any given reaction in both directions. The concentrations of reactants and products, and overall changes in standard free energy, determine which direction the reaction will actually go in any particular situation. The reaction catalyzed by carbonic anhydrase is needed in **both lungs and tissues** (but goes in opposite directions in the two because of different concentrations of substrates/products in the two locations).

12R-4. **Aldosterone** will be secreted in response to renin; the aldosterone will promote NaCl uptake in the distal tubule but not affect uptake in the proximal tubule (which is constitutive and not controlled by hormones). Therefore the % NaCl removed will **increase in the distal tubule** and **stay the same** (not at zero) **in the proximal tubule**. If more salt is removed, water will follow and the **water retained in the urine will decrease**.

12R-5. The net bicarbonate will increase (as it is formed from the CO_2 generated by metabolism) but net Cl^- will stay the same (although its location will change). The amount of Cl^- in the RBC will increase and the amount in the plasma will decrease as Cl^- is exchanged for bicarbonate.

12R-6. Normally, water flows out of the descending loop (because of high salt in the ECF) and NaCl is pumped out of the ascending loop (which is impermeable to water). So if filtrate moves as described, **water movement will decrease and NaCl increase**. Glucose is primarily reabsorbed in the proximal tubule, so **glucose movement stays the same (at very low levels if at all)** whether filtrate is in ascending or descending part of loop.

12R-7. The % saturation will **stay the same in the lungs** (as there is more than enough oxygen in the lungs to saturate normal hemoglobin) while the % saturation **will increase in the tissues** (as the hemoglobin will hang on to its bound oxygen more efficiently -- binding curve shifts to left).

12R-8. High CO_2 triggers an **increase in breathing rate** (so more CO_2 can be exhaled) and the **slope should increase** (so threshold is reached sooner and muscles of inhalation contract sooner).

12R-9. A. Next is the **(right atrium).**

B-1. The best answer to this was **either one of the other but not both.** The answer **the liver** was accepted too. The "loop" described here is the systemic circulation which carries the blood throughout the body. A branch of the systemic circulation goes through the lungs, nourishing the lung tissue itself, and providing oxygen to the part that is not close to the alveoli. So the RBC could go through the part of the systemic circulation that supplies the liver or the part that supplies the lung. (The part supplying the lung and the part supplying the liver are in parallel, not in series, so the RBC cannot go through both.) The "loop" described here cannot carry the RBC through the pulmonary circulation that goes through the lungs and exchanges gases. Since most people who circled "the liver" clearly meant that the RBC could not go through the pulmonary circulation, that answer was accepted too.

B-2. The % saturation of hemoglobin with oxygen should **(decrease).**

C-1. The amount of HCO_3^- in the *plasma* should (**increase**).

C-2. The Cl^- level in the *plasma* should **decrease**.

The carbon dioxide from the tissues diffuses into the plasma (liquid part of the blood) and from there into the RBC. Inside the RBC it is converted to carbonic acid by carbonic anhydrase, and then disassociates spontaneously into bicarbonate ions and protons. (It is actually released from the carb. anhydrase as bicarb.) The bicarb. then leaves the RBC in exchange for chloride, using the band 3 anion exchanger. Overall, the carbon dioxide, which is relatively insoluble in water, is converted to bicarbonate, which is quite water soluble. Large amounts of the soluble bicarb can be carried in the plasma, and equivalent amounts of chloride end up inside the RBC (to maintain a constant anion concentration).

12R-10. Either part of the left half. The blood returns from the pulmonary circulation (where it is oxygenated) to the left side of the heart. It enters the atrium first, but it is still fully oxygenated when it enters the ventricle. The heart uses up a lot of oxygen, but that oxygen comes from capillaries that exchange materials with the heart tissue, not from the blood that is pumped by the heart. In other words, the heart gets oxygen the same way every other part of the body does.

12R-11. A-1: **CO_2 dissolved in solution should be the same** in all three cases. In (a) a small additional amount of CO_2 is bound to Hb. In (c) a large amount of additional CO_2 is present in the form of bicarbonate + H^+ (see below).

A-2. Both **carbonic anhydrase and the anion exchanger (band 3)** are required for blood to "soak up" the maximum amount of CO_2. (In addition, deoxy-hemoglobin binds a little CO_2.) Carbonic anhydrase catalyzes conversion of CO_2 to bicarbonate and H^+. (See note at end.) The anion exchanger is needed to trade bicarb in the RBC for Cl^- in the plasma. The whole process of "soaking up" CO_2 is as follows: CO_2 diffuses out of the tissues, first into the plasma and then into the RBC. Inside the RBC, carbonic anhydrase converts the CO_2 to carbonic acid, which then forms bicarb and protons. The reaction is driven to the right by the high level of CO_2 in the tissues and by the removal of bicarb from the cell in exchange for Cl^- from the plasma (catalyzed by the anion exchanger).

B. Sample (b) should hold less than the other two. The primary carrying capacity of the solution depends on the amount of hemoglobin present. (Oxygen is not very soluble in solution by itself.) The more hemoglobin (whether in the RBC or in solution) the more oxygen should be soaked up by the sample. Since (a) and (c) have the same amount of hemoglobin, they should pick up the same amount of oxygen.

Note: The carbonic anyhdrase reaction is usually written as if the enzyme produces carbonic acid, which spontaneously ionizes to bicarbonate and H^+. However the enzyme actually produces bicarbonate and H^+ directly.

12R-12. A. The left side of the heart pumps blood into the arteries (body); the right side pumps blood into the lungs. If either side of the heart is damaged, the blood is not pumped out properly, and blood accumulates BEFORE the point of damage. This build up (back up) of blood causes a local high blood pressure and blood seeps out of the blood vessels causing tissue or pulmonary edema. Damage to the right side causes blood to pool in the veins (since the blood is not pumped properly out of heart into lungs). This causes swelling in the tissues. Damage to the left side causes blood to pool in the lungs, and the liquid that leaks interferes with gas exchange, causing breathing problems.

B. **Worse**. The normal response is to retain more fluid, so blood pressure increases. But as BP goes up, more liquid leaks out of the capillaries into the tissues, causing more edema. The increased BP increases the load on the heart but does not help increase flow to the tissues/lungs because heart cannot pump properly.

C. ACE inhibitor will inhibit aldosterone release, affect smooth muscle, and help high blood pressure. ACE inhibitor will inhibit formation of angiotensin. That will block angiotensin's effect on smooth muscle (block vasoconstriction, promote vasorelaxation) and prevent angiotensin from stimulating aldosterone release (thus reducing salt and water retention). So there will be more (or continuing) fluid loss, reducing blood volume, and increased circulation. This reduces the load on the heart and/or reduces the BP.

12R-13. A. Diuretic inhibits transport from tubule to EC directly; it inhibits transport from tubule to capillaries overall

Explanation: Diuretics have the opposite effect from ADH (which is antidiuretic hormone). Diuretics increase the water lost from the body by increasing the volume of urine. (They do NOT increase the water removed from the urine and retained by the body.) In this case, the diuretic prevents removal of (some) Na^+ from the tubule, causing retention of Na^+ and water in the urine. Normally Na^+ moves into epithelial cells through their luminal membranes, and is pumped out the other side into the IF, using the Na^+/K^+ pump. The Na^+ then diffuses through the IF into the capillaries, which carry the Na^+ away. Water follows the Na^+, either at the same time (in the same location) or later -- if there are water permeable membranes, and high Na^+ on one side, water will flow osmotically across the membranes to the side with the Na^+. When the diuretic is present, less Na^+ leaves the tubule and enters the EC (whether Na^+ transport is active or passive). Therefore less Na^+ is available to exit the EC, less enters the IF and less reaches the capillaries. The less Na^+ is retained in the body, the less osmotic pull on water, and the less water is retained as well.

Answer to 12R-13, cont.

B. No effect on filtration or secretion; inhibition of reabsorption of Na^+ only.

Explanation: Filtration occurs at the beginning of the tubule, before the proximal tubule. So it will not be affected. Neither substance is secreted, that is, specifically transported across the EC's into the tubule, so the diuretic will have no effect on their secretion. Reabsorption of glucose occurs in the proximal tubule, using co-transport with Na^+ across the luminal membranes of the EC. The diuretic is not present in high concentration in most of the proximal tubule, so it should have no significant effect on glucose/Na^+ co- transport. By the time the filtrate reaches the part of the tubule containing high concentrations of the diuretic, virtually all of the glucose will already have been reabsorbed, that is, specifically transported across the EC's, out of the tubule. The diuretic must primarily inhibit Na^+ transport at some point after the end of the proximal tubule.

C. Most effect in AL; AL or distal tubule is next best choice. Diuretic mimics a deficiency of aldosterone.

Explanation: The diuretic works by affecting Na^+ transport; water passively follows the Na^+. (Water doesn't necessarily follow immediately, in the same section, but overall, water follows the salt. If body Na^+ increases, total water retained will increase, and vice versa.) The diuretic does not affect water transport directly. Therefore the diuretic must work on a section where there is Na^+ transport to inhibit. It will have no direct effect on portions of the tubule, like the DL, that do not transport Na^+. Therefore it must affect the DT or AL. Since only a little Na^+ (max) is removed in the DT (under the influence of aldosterone), the diuretic will have only a small effect if prevents Na^+ reabsorption in the DT. The diuretic will have more effect if it inhibits Na^+ transport in the AL, which reabsorbs a lot of Na^+.

Aldosterone affects Na^+ reabsorption, and so does the diuretic, but they work in opposite directions. The diuretic prevents reabsorption, and aldosterone promotes it. If aldosterone is absent, or you add the diuretic, you get low reabsorption.

12R-14 A. Ca^{++} channels. B. a Ca^{++} pump & a receptor for PTH.

Explanation: Ca^{++} must be reabsorbed from the tubule into the circulation. (The term "reabsorbed" always means recovered from the tubule, not released from the cells into the filtrate.) In other words, the net movement of Ca^{++} is out of the lumen into the capillaries. Ca^{++} must leave the lumen across the luminal/apical surface of the tubular epithelial cells (by diffusion) and then exit the cells on the basolateral side (using primary active transport). For this to work, there must be channels in the luminal surface of the cells and a pump (using ATP) in the BL surface. Channels are needed for (facilitated) diffusion -- the ion cannot diffuse across the membrane by itself. Primary active transport means a ATP dependent pump, not an exchanger. There is no secondary active transport, so an exchanger or co-transporter is not involved.

How this works: Ca^{++} is pumped out of the cells on the BL side, using the energy of ATP. The reabsorbed Ca^{++} then diffuses through the IF and into the capillaries. The ATP driven pump keeps the concentration of Ca^{++} inside the cells low, lower than the concentration in the filtrate. Therefore, Ca^{++} flows down its gradient out of the tubule into the cells, passing through the channels in the luminal membrane. The net effect is that Ca^{++} moves from the lumen, through the epithelial cells, into the IF and from there to the capillaries.

Vit. D is a lipid soluble hormone, so its receptor should be intracellular. PTH is a water-soluble hormone, so it needs a transmembrane receptor with an extracellular domain. Since PTH is distributed through the blood, it will bind to receptors on the BL side of the epithelial cells. Binding of PTH will trigger signal transduction pathways inside the cells (IP3, cAMP etc.) and these pathways can affect transport processes on either surface of the cell -- the state of channels on the luminal surface or the state of the pump on the BL surface. The hormone binds to the outside of the cells, but the second messengers exert their effects on the inside of the cell.

12R-15. A-1: exocytosis (vesicles with co-transporter in membrane fuse with plasma membrane).

A-2: Glut 4 or aquaporins are inserted in the membrane in response to insulin or ADH respectively. Na^+ channels and the Na^+/K^+ pump are inserted in the DT membrane in response to aldosterone, but this insertion is not reversible in the same way as the insertion of glut 4 or aquaporins.

B-1: filtered, reabsorbed & in the urine. (All but secreted.) B-2. Filtered & in the urine.

Notes on terminology:

(1) Secreted is not the same as excreted. Excreted by kidney = lost in urine; secreted in kidney = actively transported from circulation to the lumen. (What is secreted is later excreted. However, many of the things that are excreted in the urine were not secreted -- they were filtered and not reabsorbed.)

Answer to 12R-15, cont.
(2) Reabsorbed is not the same as retained. Reabsorbed in the kidney = recovered from the filtrate. Retained (in the body) = not lost in urine by any method -- either was not secreted or was reabsorbed. Things can be retained without being reabsorbed. (They were never in the filtrate in the first place.) This issue comes up if you answered the question backwards and thought phosphate was secreted into the lumen. (Then PTH increases retention, not reabsorption.)

Explanation: Phosphate is released from bone by PTH along with Ca^{++}. Bone is made of calcium phosphate salts, so you can't release one without releasing the other.

Phosphate is reabsorbed by the Na^+/phosphate co-transporter. In other words, the co-transporter moves phosphate out of the lumen into the circulation and not the other way around. How do you know this? Either Na^+ stays in the tubule, or it moves out of the tubule to the cells surrounding the tubule, and from there into the capillaries. The Na^+ goes from the tubule to the body, not the other way around. (Na^+ can be reabsorbed, but it can't be secreted.) The Na^+ moves across the luminal membrane into the tubule epithelial cells, and then out across the BL membrane into the IF. The process is driven by the Na^+/K^+ pump. The pump moves Na^+ out of the cell across the BL surface, and that pulls Na^+ into the cell -- the Na^+ flows down its gradient across the luminal surface. Sometime the Na^+ moves alone and sometimes it pushes other substances. If phosphate is moved by a Na^+/phosphate co-transporter, then phosphate must move with the sodium in the same direction. (An exchanger would move the phosphate in the opposite direction from the sodium.) Both phosphate and sodium must be reabsorbed from the lumen, not secreted into the lumen.

When the co-transporter is saturated, which occurs at high levels of phosphate, all the phosphate is filtered, some is reabsorbed, and some remains in the filtrate/urine. At lower levels of phosphate, all the filtered phosphate can be reabsorbed.

When PTH is present, more phosphate is released from bone but less phosphate is reabsorbed, so more is filtered, and more of it remains in the urine.

Note: If you thought phosphate moved into the lumen, not away from it, then PTH would increase the phosphate retained in the body. It would increase retention by reducing secretion, not by increasing reabsorption.

12R-16. A-1. The very rapid rise is caused by angiotensin II. A-2. The rapid effect is probably due to vasoconstriction.

Synthesis of renin leads to production of angiotensin II, & angio II causes vasoconstriction of systemic arteries, raising the arterial blood pressure. Of the choices given, this is the only one that will increase the arterial BP within minutes.

Vasoconstriction must occur throughout the peripheral circulation, not just at the kidney. Constriction of the efferent arteriole of the kidney would raise the BP at the glomerulus and increases the GFR but it wouldn't change the arterial BP. Angiotensin II causes synthesis and release of aldosterone (and also release of ADH). The effects of aldosterone and of ADH (on the kidney) are much slower -- they won't change the arterial BP in minutes.

ADH can have two different effects on the kidney -- it is a vasoconstrictor, and it increases insertion of water channels in the luminal membrane of the cells in the distal tubule. As explained above, vasoconstriction in the kidney won't increase the arterial BP. The insertion of water channels will raise the system BP, at least temporarily, but it isn't fast enough. Insertion of the channels is fast, but the resulting retention of water takes a while -- filtrate must pass through the tubule and water must be recovered from it. Not enough water can be recovered to have an effect in minutes on overall blood pressure.

What about aldosterone? Aldosterone can raise the BP by increasing retention of salt and water, but the process is slow -- it requires production of aldosterone in the adrenal cortex, and protein synthesis of transport proteins in the target tissues.

B-1. You would expect salt retention in both kidneys.

B-2. The original secretion of renin should be in the clamped kidney.

B-3. The secretion of renin *(in this experiment)* is triggered by baroreceptors in the kidney.

The clamped kidney has a lower local BP and lower GFR than normal, and these changes are detected by baroreceptors in that kidney. This triggers local responses in the affected kidney -- secretion of renin and constriction of the efferent arteriole or dilation of the afferent (there is disagreement about which happens here)*. Renin acts with other components to activate angiotensin and this in turn leads to production of aldosterone by the adrenal cortex. Aldosterone is secreted into the blood and reaches both kidneys, causing salt retention in both of them. The original effect is local, but once a hormone is produced, it can reach all its targets throughout the body. Renin can be secreted in response to signals from the nervous system, but they aren't involved here.

*The afferent only constricts in response to systemic input which overrides the local response in a case of a big drop in overall BP.

KEY TO PROBLEM SET 13 -- QUESTIONS ON IMMUNOLOGY

13-1. A. No. Only the "variable" (left) part will bind antigen = the "grabber" end with the two antigen binding sites. (See text books for how actual structure of antibody peptide chains corresponds to our usual diagram.)

B. All the antibodies will have the same left or variable part -- a single cell is only capable of making one type of variable region. If all the antibodies are of the same class, they will have the same constant part as well.

As the cell matures, the same variable part may be hooked up to different "constant" parts as a result of alternative splicing and/or additional rearrangement of the DNA. All the constant parts of membrane bound immunoglobulin are hydrophobic, and lock the antibody in the membrane, but constant regions of each class (IgA, IgM, etc.) are different. See texts if you are interested in the details of the differences between the different classes of immunoglobulin and class switching. Because of class switching, a cell can have antibodies of two different classes on its surface. The two types of antibody have the same antigen combining site but different constant regions. (Cell may not be able to make both types simultaneously; they may be made sequentially.)

C. Left or variable. Whether antibody is bound or secreted depends on the constant region of the antibody. Cell can make two different antibodies with same variable region (& class) but different COOH ends by alternative splicing of the same transcript.

D. Each F_{ab} fragment can bind antigen, and each F_c fragment can bind complement, but the complement isn't attached to the bacteria, so it doesn't puncture the bacteria and trigger lysis. Normally, when an antibody binds to antigen, the conformation of the antibody changes, altering the shape of the constant part. (A change of conformation is the common result when any receptor binds its ligand.) The change in conformation triggers the biological effects mediated by the constant part -- binding and activation of complement, binding to mast cells, etc. as appropriate. (See text for details if you are interested.) The F_c end of free antibody doesn't normally bind or activate complement -- the Ab has to bind to Ag first. In this particular set up (which is from a real, famous experiment) free F_c fragments probably bind complement because they have the same conformation as the constant part in an Ab-Ag complex. In other words, binding Ag or cutting off the V part allows the C part to change to the complement-binding shape.

13-2. A. Each antibody is bivalent (has 2 antigen binding sites) and each RBC has many copies of the A (or B) substance on its surface. Each molecule of the appropriate antibody cross-links two RBCs until all the RBCs are linked into one big clump. The "wrong" antibody can't bind to the RBC surface so it can't link the RBCs.

B. RBC's of type A are clumped by anti-A but not anti-B. Vice versa for type B. RBC's of type AB are clumped by both anti-A and anti-B; RBC's of type O are clumped by neither. So you need to test the reaction of RBC with both anti-A and anti-B.

C. F_{ab} fragments can bind to RBC's, but they can't crosslink and clump the RBC's because the F_{ab} fragments are monovalent -- they have only one antigen binding site per fragment. F_c fragments can't bind to RBC's at all.

13-3. Only newly made H and L chains should have signal sequences. Explanation: antibodies end up on the cell surface or secreted; therefore the peptide chains that make up antibodies must be made on the rough ER and enter the endomembrane system. The H and L chains must enter the ER separately and combine in the lumen to make antibody; all S-S bonds are formed in the ER. Therefore newly made H and L chains should have signal sequences on their amino ends. The antibodies pass through the ER, Golgi, etc. in the usual way for a protein that is designated for the outside of the cell. If the H chain has a hydrophobic COOH end, the Ab will end up anchored in the plasma membrane; if the H chain has a hydrophilic COOH end, the Ab will be secreted. Whether the protein ends up secreted or not depends on the COOH end, not the amino end (where the SP is). Since the signal sequences of most proteins are removed in the ER, and signal sequences can not be used here to anchor the peptides in the membrane, you would expect that completed antibodies, whether secreted or membrane bound, would not have signal sequences.

13-4. A. Antibody should be made to **one** epitope = antigenic region. Because of somatic mutation, which occurs after exposure to antigen (and was not discussed in class) different descendants of the B cell precursor may make slightly different antibodies, but these will all bind to the same epitope (although some may bind it more strongly than others).

B. The shape of the antibody will be determined at random (by rearrangement of the DNA) after you put the cell in a bottle. Antigen is not involved in the rearrangement or "choice" of the binding site for epitope. ("Choice" is in quotes because the epitope is not picked out in advance; the shape of the antibody is specified at random and that determines which epitope will stick to the antibody.)

13-5. A. Activation of helper T cells requires both juxtacrine and paracrine signals. In order to be activated, helper T cells must bind to (and therefore have receptors for) both MHC II + antigen (the juxtacrine) and IL-1 (the paracrine). Details regarding the paracrine part are usually not discussed in this class, and are only included here for completeness. Both macrophages and B cells can present antigen fragments in combination with MHC II (provide the juxtacrine signal) and both produce the necessary cytokine (IL-1 or the equivalent). Details: Both cell types can take in and process extracellular antigens so that pieces of the antigens (epitopes or antigenic determinants) can stick to the MHC and be displayed on the plasma membrane (in association with the MHC II). Thus both cell types can act as "antigen presenting cells." Both cell types also both produce IL-1, which is required for helper T cell activation.

B. B cells specific for an antigen should bind that antigen better than macrophages. However B cells that bind any particular antigen should be rare before antigen stimulation, so there are probably not enough B cells to stimulate helper Ts the first time around. The macrophages may not be specific, but there are more of them. After stimulation and clonal expansion, there should be more B cells (memory cells) that bind a particular antigen, and so the B cells should be more effective at trapping that antigen than macrophages.

Another possibility (which is only included for completeness): The naïve B's -- those unexposed to antigen -- may make relatively low levels of IL-1 and/or naïve Ts may need high IL-1. So the first time, macrophages may be needed to supply enough IL-1. Stimulated B cells may make more IL-1 or stimulated T cells may require less of it, so the second time, the B's may be able to supply enough IL-1.

13-6. Similarities: All three types are derived from the same stem cells. Their surface immuno-proteins are similar – both immunoglobulins (antibodies) and T cell receptors have variable and constant regions (and two types of peptide chains) and their synthesis requires DNA rearrangements. To emphasize these similarities, some authorities call immunoglobulins attached to the B cell surface “B cell receptors” instead of antibodies. All 3 types need cytokines to grow and differentiate, and all three make cytokines as well. Both types of Ts behave similarly, in that they recognize an antigen + MHC, and produce proteins (cytotoxic like perforins or cytokines) in response to activation.

Answer to 13-6, cont.

Differences: B cells have MHC II on their surface and most T cells do not. (All have MHC I.) B cells can secrete their immuno-proteins --> soluble antibodies; T cells do not secrete their immuno-proteins, so all T cell receptors remain on T cell surfaces. B cells (really the antibodies on B cells) can recognize free antigen; T cells (really the T cell receptors) can only recognize antigen bound to MHC. (T cells are "MHC restricted.") See table below.

Cell Type	Need**	Make	Bind To	Marker	Immuno-proteins
Helper T	IL-1	IL-2	MHC II + Ag*	CD4	T Cell Recept.
Cytotoxic T	IL-2	?	MHC I + Ag*	CD8	T Cell Recept.
B Cells	many IL's	IL-1	Antigen	other	IgA, D, G, etc.

Notes on table:

**Note 1*: the pathways for processing antigens are different for extracellular and intracellular antigens. Extracellular antigens (taken up by B cells & macrophages from the outside) are processed one way and end up associated with MHC II; intracellular antigens (such as proteins made in virus-infected cells), which are synthesized inside the cells, are processed another way and end up associated with MHC I. So the source of the "Ag" listed in the table differs, depending on which MHC type it is associated with.

** *Note 2*: The cytokines made and needed are included here for reference only. "Need" here refers to what is needed from other cells for activation and/or growth. All Ts need IL-2 to grow; Helper Ts make it themselves (after activation with IL-1) and cytotoxic Ts get it from helper Ts. What B cells need is complex; consult an advanced text if you are interested. (There are really two classes of helper Ts that make different IL's that have contrasting effects on cytotoxic Ts and Bs; the details are beyond the scope of this course.)

13-7. A. Without IL-2 receptors, T cells can't multiply and so there are few if any cytotoxic T cells and few if any helper T cells to stimulate the B cells. So the net result is that neither type of lymphocyte (B or T) can differentiate and function properly.

B. Without cytotoxic T cells, virus infected cells cannot be destroyed. Without soluble antibody (made by B cells with the aid of helper T cells) virus particles and bacteria cannot be attacked.

13-8. A. Looks like a problem with B cells. In a first infection, there is no humoral antibody at the start of infection, so the virus infects cells. Eventually, cytotoxic T cells are activated and destroy infected cells, and person usually recovers. In normal people, B cells are stimulated to divide and produce humoral antibody (and memory cells) that prevent the virus from re-infecting. Since this person recovers normally, cytotoxic Ts should be OK. Since helper T cells are required for function of cytotoxic Ts, and cytotoxic Ts are working, helper Ts must be okay. But person gets re-infected, which suggest the B cells are not making antibody to produce a secondary response so there is nothing to prevent additional rounds of infection.

B. No. It's not clear what part of the immune system (if any) knocks out cancer cells, but it isn't B cells. It's probably cells called natural killer cells which should be working normally. (Cytotoxic Ts are also working okay and they should recognize cancer cells as MHC class I + foreign stuff and knock them out. However people with cytotoxic T deficiencies don't get cancer at high rates, so cytotoxic Ts are probably not the major players here.)

C. No. Bacterial cells are lysed by a different mechanism than human cells infected with viruses. Bacterial lysis is caused by complement sticking to antibody on the surface of bacterial cells. If the B cells don't make normal antibody to stick to the bacterial cells, the complement won't act. (Cytotoxic Ts don't bind to bacteria -- no MHC.)

D. No, because cytotoxic Ts are okay. Grafts probably fail because the MHC class I's don't match, and foreign MHC-I can not be distinguished from self MHC-I + antigen by cytotoxic Ts.

13-9. A. & B. You need helper T cells and antigen is necessary too; C. Class II MHC. Explanations:

A & B. You need an activated helper T cell to activate the B cell; to activate the helper T cell you need an antigen presenting cell. The B cell itself could act as an APC or you can use a macrophage. In either case, the APC has to have processed antigen on its surface. (The macrophage might be a better APC than the B cell, as explained in the answer to 13-5B, so the macrophage might help, but it isn't absolutely necessary.)

C. The helper T recognizes class II MHC (+ processed antigen). No MHC II, no binding and no activation by T cell.

13-10. A. Cytotoxic T cell surface has CD8 & MHC I. Skin cell surface has MHC I. DNA should be the same in all the loci listed.

Explanation: All nucleated cells, including T and B cells, have MHC I on their surfaces. Cytotoxic T cells also have CD8 and TCR. The genes for the CD proteins and MHC's do not rearrange; only the genes for TCR and Ab do. The DNA of the skin cell would be different from that of the T cell in the locus coding for the TCR, but that is not included. The state of the chromatin will be different in the T cell and the skin cell, but the DNA itself will be the same (have the same base equence).

Note: TCR is not the same as antibody. Both TCRs and antibodies are very variable antigen binding proteins whose gene segments rearrange during development. However the structures of TCRs and antibodies are different, and the loci that encode them are different.

B. MHC II. The loci for the TCR and Ab (Immunoglobulin) both rearrange, but the locus coding for MHC II does not. The TCR locus will be rearranged in the T cell and the Ig Heavy chain locus will be rearranged in the B cell.

C. (ii) & (v) will be different.

Explanation: Each naive B cell has rearranged the DNA in its Ig locus separately. Therefore each individual B virtually always has a unique DNA sequence at its Ig loci, and codes for an antibody with a unique variable region.

The T helper cell needed for activation has to bind to an epitope displayed on the B cell surface (with MHC II); the epitope comes from a protein trapped by the surface antibody. Each B cell makes a different antibody, therefore each antibody should 'grab' a different protein (antigen), and each B cell should display a unique collection of epitopes with MHC II. The T helper that is needed will be different for each B cell, because the TCR of the T helper will have to match the epitopes displayed, which will be different for each B cell.

The majority of epitopes bound to MHC I will be the same -- these epitopes are derived from the proteins made by the cell. Since these are both normal B cells, most of their proteins should be the same. (Only the variable reasons of the H and L chains should be different.)

D. (i) and (iii) are correct. The DNA is rearranged before IgM is made; the DNA is transcribed to make IgM. The same DNA is then used to make IgD by alternative processing of the primary transcript to produce a different mRNA. (See handout.) Different sections of the DNA code for the constant part of the IgM and IgD chains, but the DNA in the cell is the same, whether it is used to make IgM or IgD.

The rest did not need to be explained, but is included fyi.

Alternative splicing and/or addition of polyA produces two different mRNA's that code for the two different types of antibody. A single mRNA can only code for IgM or IgD -- depends on whether mRNA contains the sequence encoding the mu constant region or the delta constant region of the heavy chain.

There is no class switching in light chains -- the same light chain is used for all 5 classes of antibody. It's the heavy chain (constant region) that switches. Once the 'Vee' region of a lambda or kappa light chain gene is rearranged, that's it. The same DNA is used to make the same primary transcript, the same mRNA, and the same light chains, whether the antibody is IgM, IgD, IgE, IgA or IgG.

Note: It is not correct that the entire L chain is variable, or part of the 'grabber' of the antibody. Part of the L chain is variable and part is constant, but the 'constant' part is really constant -- once the variable part of the DNA is rearranged, it is always next to the same constant region.

Answer to 13-10, cont.

E. Since the descendants make IgM after meeting antigen, the cell was activated to divide and produce effector cells, and the answers are (ii) & (v).

Explanation: The cell had to capture some Ag and display it to be activated. If its descendant cells make IgM after meeting antigen, they must be effector cells, not memory cells. Therefore there is no class switching or somatic mutation to consider. The Ag binding site of the IgM will stay the same, and the DNA coding for the antibody will also be the same. The following will change after binding antigen;

(i) Processing /display of antigen will change in the original B cell. When antigen binds to antibody, the complex will be internalized by RME, the antigen degraded to short peptides, and the peptides (epitopes) will be bound to MHC II. The MHC II + the new epitopes will be placed on the cell surface using exocytosis. Nothing will happen to the epitopes bound to MHC I, as these come from proteins made inside the cell, which are still the same.

(2). Once the original B cell meets a helper T and is activated, the B cell will divide, and its descendants will switch from making surface antibody (membrane bound IgM) to secreted antibody (soluble IgM). This switch occurs by alternative splicing/processing of the same RNA (see handout). Two different 'readings' of the DNA -- different splicing and/or different points of poly A addition to the transcript -- lead to two different mRNAs. Before meeting antigen, the mRNA codes for a protein with a hydrophobic tail; after meeting antigen the mRNA codes for a protein lacking the tail.

13-11. Immunology Alphabet Soup Answers:

A. TCR & CD4; these are found on the membranes of T cells, not on the surface of B cells. (Cell makes antibody but it is membrane attached, not secreted.)

B. MHC-I (which is on the surface of all nucleated cells) and MHC-II (which is needed for antigen presentation). MHC-II is needed to bind antigen fragments so they can recognized by T cells. The TCR on T cells can bind to an MHC-antigen combination but cannot bind to free antigen. Binding of T cells to antigen (+MHC) is required (but not sufficient) for T cell activation.

C. T_H should contain TCR and IL-2 AND receptors for IL-1, IL-2 and MHC II. Naive T_H cells contain TCR and receptors for IL-1. On activation, they produce IL-2 and IL-2 receptors. The T cell receptor is on the surface of T cells but is the receptor for antigen + MHC (MHC-II in this case).

D. T_H, APC and B cells are needed; APC and B will contain antigen in their endosomes. You need antigen presenting cells to activate T helper cells which in turn will activate B cells. In the primary response you need an APC (presumably other than a B cell) to engulf, partially digest and display antigen so the APC can activate a helper T. The activated helper T cell can then bind to a B cell (which has endocytosed and processed the same antigen) and activate the B cell to secrete antibody. The secreted antibody will bind to bacteria and trigger their destruction. B cells and APC's need to process antigen before they display the pieces (for recognition by a T cell), so they should have antigen in their endosomes.

13-12. A. MHC-1; B. Decreased. Explanation: MHC-1 is required for recognition of infected cells by cytotoxic Ts. If the virus can down-regulate expression of MHC-1, the infected cells are less likely to be recognized and lysed by cytotoxic Ts. Without MHC-1, the infected cell can not "display" viral epitopes and cannot act like a classic APC (which displays epitopes with MHC-II). Therefore an infected cell with no (or less) MHC-1 is more likely to survive, the virus is more likely to reproduce, and the cell is more likely to release progeny virus. Good news from the virus's point of view; bad new from the point of view of the infected cell/organism.

13-13. A. >2, and 1. Each protein contains many epitopes, and each T cell binds one epitope (associated with MHC I), not one complete protein. If an infected cell makes 2 special proteins it will contain many epitopes (antigenic determinants). Each epitope or peptide fragment will be bound to MHC I and will be displayed on the surface of the infected cell. Each T cell has only one type of T cell receptor and each receptor binds effectively to only one epitope + MHC I combo (or to a small number of closely related combos.)

B. MHCI, T cell receptors, & IL2 receptors, & CD8. The T cells that bind to infected cells are cytotoxic Ts. All Ts should have T cell receptors. (All nucleated cells have MHC I.) Cytotoxic Ts have CD 8 (as vs helper Ts which have CD 4). Activated Ts produce IL2 receptors. (Some T cells may have MHCII at some times, but in general, T cells do not have MHC II.)

13-14. A. Class switching is the only thing that is messed up here; all the other processes are occurring normally in the mutant, so they must be unaffected by the gene in question.

These B cells can switch from making membrane bound antibody to secreted antibody, and their second response is stronger than their first. This means that B and T cells are prob. proliferating normally, so there are more antibodies the second time around (B cells actually make the antibodies, but helper T cells are needed for B cells to respond properly) and switching from membrane bound to secreted antibody is also okay (since it works for IgM). Antigen presentation must be working or mice wouldn't respond to antigen at all. The obvious thing that is wrong is class switching -- by the secondary response the mice should be making a different class of antibody (IgA, IgE or IgG) not IgM.

FYI: Producing secondary antibodies requires rearrangements of the DNA encoding the heavy chains. (Light chain genes are not rearranged during class switching.) Sections of the DNA must be removed to put the DNA region encoding the variable part of the heavy chain next to a new DNA region encoding a new constant part of the heavy chain; this is what is failing to occur.

If the problem were with secretion, not switching, the mice would produce membrane bound antibody of a different class the second time, not secreted IgM. They wouldn't be able to make IgM anymore, because the DNA for the constant region of the IgM heavy chain would have been deleted during switching

B-1. Helper T cells; B-2. A surface protein that acts as a ligand. The data show that the T cells, and not just their secreted proteins, must be normal for the immune response to be normal. This means the T cells, not the B cells, must be defective in the mutant mice. (From the previous part we know B cell response is abnormal; this part indicates that the T cells have the primary problem, and therefore must fail to trigger the proper response in the B cells.) Helper Ts are the cells that signal and/or regulate B cell response, so it must be the helper Ts that are defective. So what is wrong with the interaction between the B cells & the helper T cells? The simplest solution is that a surface protein on the T cells, one that normally binds to B cells, is defective. No surface protein, no signal to B cells, and no class switching by B cells.

If a cytokine normally made by T cells were the missing or defective protein, then secreted proteins made by T cells would help the mutant mice, but they don't. If a T cell receptor were missing, it would have to be a receptor that triggers the T cell to signal the B cell. But the signal can't be a secreted protein, as explained above; it must be an integral part of the T cell itself. So if there's a receptor problem, the receptor must also act as a ligand.

Note: Some of the results described in this problem are made up, and may be unrealistic. The data presented here indicate that the T cell surface protein required for B cells to switch classes is not required for proliferation of B cells. It is likely that the same protein is needed for both switching and proliferation, since a common precursor cell gives rise to both memory cells (that switch) and effector cells (that proliferate and make antibody). If the T cell surface protein acts by stimulating this common precursor cell, you shouldn't get a larger secondary response or switching when the protein is defective.

Answer to 13-14, cont.

C-1. MHC I; C-2. Cytotoxic Ts; C-3. The same. Ordinary cells (like liver cells) have MHC-1 on their surfaces, not MHC-2. Cytotoxic Ts recognize infected cells because the infected cells carry (on their surfaces) MHC-1 bound to fragments of viral antigens. The cytotoxic Ts kill the infected cells (they induce apoptosis) using TNF or a pore-forming complex similar to, but distinct from, complement. Complement is primarily involved in lysis of bacterial cells that have bound antibody, not infected or abnormal host cells.

The mice have a problem with helper Ts, not cytotoxic Ts. The defect in the helper Ts seems quite specific -- it affects only class switching, not B or T cell growth, antibody secretion etc. (In other words, the helper Ts are defective in a single, specific function, not generally defunct.) So it seems likely that the helper Ts of the mutant mice will be able to activate cytotoxic Ts and the mutant mice will kill infected cells just like normal mice. (The secondary response would be different in the mutant mice.)

13-15. A. The cells exposed to flu could have been lymphocytes or macrophages. These cells have both MHC I (since all nucleated cells do) and II (since they are APC's).

B-1. You would expect the rare cytotoxic T cells to produce CD4, perforin, TCR, & MHC I.

B-2. You could use either the rare cytotoxic T cells or the common ones to lyse the cells in experiment (1) .

B-3. Only the rare cytotoxic T cells could lyse the cells in experiments (2) & (3).

Rare cytotoxic Ts kill cells just like normal cytoxic Ts do. They just kill a different target. All cytotoxic Ts must have a TCR to bind to antigen on a target cell, and they must make perforin to help kill the cells. They must have MHC I because all nucleated cells do.

The simplest explanation of the behavior of the two types of cytotoxic Ts is that the normal cytotoxic Ts have CD8 on their surface and the rare killer Ts have CD4 instead. T cells with CD8 stick to cells with MHC I (+ antigen) on their surface; cells with CD4 stick to cells with MHC II (+ antigen). The rare cytotoxic Ts act on the normal targets of cells with CD4 -- namely, cells with MHC II (+ antigen) on their surface. But instead of acting as helper cells, the rare cytotoxic Ts act as killers. In other words, the type of CD (4 vs 8) determines the target cell; the presence of other proteins, such as perforin, determines whether the cell will be a helper or a killer. Usually cells express either CD4 and the "helper proteins" or CD8 and the "killer proteins." The rare killer Ts have an unusual combination -- they express CD4 and the "killer proteins."

C. No and No.

Why not MHC I? Experiments 2 & 3 indicate that only viral proteins made inside the cell are displayed with MHC I. Or to put it the other way, proteins that are made outside the cell are displayed with MHC II, not MHC I. In experiment 5, the virus can't replicate (as in expt. 2). The only viral protein is the stuff that is carried into the cell in the virus particle itself. This material can not be displayed with MHC I.

Why not MHC II? If there were no inhibitors, the viral antigens (from the virus particle) could be displayed with MHC II. However chloroquine (which inhibits the proton pumps of the organelles) is present in expt. 5. We know from experiment 4 that acidification of lysosomes (and/or endosomes) is needed for proteins added from the outside to reach MHC II. Therefore in expt. 5, the viral antigens will not be displayed with MHC II.

Why doesn't chloroquine affect display with MHC I? Inhibiting the endocytic pathway with chloroquine has no effect on display of epitopes with MHC I because proteasomes, not lysosomes, are used to digest proteins for display with MHC I.

D-1. The two DNA samples should be the same. Region 1 DNA is the same in all the cells of the body -- it is not part of the section that rearranges in lymphocytes. One type of killer T cells is transcribing (expressing) the region 1 DNA to make CD4 and the other one is not. (The other type of killer T cells is transcribing the region with the CD8 gene instead.) However, the DNA coding for CD4 (or CD8) is the same in both.

Answer to 13-15 D, cont.

Note that CD4 and CD8 are not coded for by 2 different alleles of the same gene -- they are encoded by 2 different genes. (Each CD or surface protein is encoded by a different gene.)

The two genes that encode CD4 and CD8 may be members of the same gene/protein family, but this does not affect the argument. Members of the same gene family diverge and become different, but every cell has copies of every family member. Different genes (from the same family) may be used (transcribed) in different cells, but all cells have the same genes for ordinary proteins like CD4, CD8, etc.

D-2. The DNA you get should be a mixture of sequences – some, but not all, molecules in the mix should be different .

Why would the DNA be a mixture? Rearrangement of the DNA coding for the variable region of the TCR occurs during T cell development, so region 2 DNA of different (naive) T cells is usually different. However in this case you are probably looking at DNA from a mixture of different T cells, not DNA from a single clone. When you immunize to flu, you expose the organism to one or more flu proteins. There should be multiple (naive) T cells that have TCR's that respond to the same flu proteins (antigens) -- the different responding T cells should have different TCR's that recognize different epitopes of the proteins. Therefore, when you immunize to flu, you get clonal expansion of multiple T cell clones. When you take a large sample of T cells after immunization to flu, you will probably have a mixture of cells from more than one clone, so you will get a mixture of DNA's.

There are two additional but unlikely possibilities: Either all the T cells come from the same clone, or each T cell in the mixture comes from a different clone. In the first case, the DNA's will be the same, but in the second case, you will still get a mixture. (The reason for this -- allelic exclusion -- was not covered in class and is included FYI only.) Even if each T cell you isolate comes from a separate clone, some of the DNA from region 2 should still be the same. That's because of allelic exclusion -- only one copy of the TCR DNA is rearranged successfully. A diploid cell has two copies of each gene, but only one is rearranged and transcribed in each T or B cell, so that all copies of the TCR (or Ig) in that cell are the same. All the un-rearranged copies of region 2 should be the same. So if you take region 2 DNA from many T cells, some sequences should be the same (the un-rearranged ones) and some should be different (the rearranged sequences).

Note that it is not sufficient to explain that the TCR's of different T cells are different -- that doesn't explain why the DNA of different T cells is or isn't different. In most cases when cells make different proteins, the DNA is still the same. (As in part D-1.)

13-16. A. tolerance.
B-1. cytotoxic Ts.
B-2. helper Ts.
B-3. CD8, TCR, & MHC I
C. The recipient may get better temporarily, but will not be cured.

Explanation: The transplanted cells might make insulin for a while, but the immune system of the recipient would undoubtedly destroy the transplanted cells. The proteins of the two twins are the same (except for TCRs & Abs), and normally a transplant would be okay -- each twin would be tolerant to its own proteins, and therefore of the identical proteins of the other twin. But in this case, the recipient is already making cytotoxic T cells to its own beta cells, and the cytotoxic Ts will recognize the transplanted beta cells (which have the same surface proteins as the original beta cells) and destroy them too. The problem is with the immune system, not the beta cells, of the twin with diabetes. (Note that this is a theoretical question -- no one is suggesting that you do such a transplant. It would not be ethical, since you know it would fail.)

In normal people, the cytotoxic T cells that are capable of destroying beta cells are deleted or suppressed. One plan for treating diabetes is to try and boost the regulatory cells that (normally) suppress the cytotoxic Ts.

Note: Beta cells are not the same as B cells. Beta cells are specialized cells in the pancreas that secrete insulin. B cells are WBC that make antibodies.

Key to Problem Set #14

14-1. A.

Meso derivatives are still poised to go either way

	Methyl	ES	Lens	Heart	Blood	↓ Mesoderm
Pax6 Lens	K4	+	+	-	-	-
	K27	+	-	+	+	+
Nkx2.5 Heart	K4	+	-	+	-	+
	K27	+	+	-	+	+
GATA1 Blood	K4	+	-	-	+	+
	K27	+	+	+	-	+
Oct4 ES cells	K4	+	-	-	-	-
	K27	-	+	+	+	+
Bry Mesoderm	K4	+	-	-	-	+
	K27	+	+	+	+	-

Bry turns off in meso derivatives

14-1-B. TFs, except Oct4 which is on in embryonic stem cells (ES cells), are poised to go either way (K4 & K27 methylated) in ES cells and until they get to the stage where they are turned on. For example, Nkx2.5 has methylation of K4 & K27 at the ES cell and mesoderm stage but has K4 methylated but not K27 in heart turning Nkx2.5 gene transcription on. Nkx2.5 would have K27 methylated but not K4 in all of the cells where Nkx2.5 isn't expressed such as lens or blood.

14-2. A-1. Putting ‘a tag on a spool’ means modifying protein, histones, and nucleosomes. The spools here are nucleosomes, and the part that is modified is the protein, not the DNA. The proteins in the nucleosomes that are modified are histones.

A-2. K4 means the 4th amino acid, and the K indicates it is lysine. (K = lysine using the one letter symbols for the amino acids. We usually use the 3 letter symbols, such as lys.) K4 doesn't mean the 4th lysine -- it means the 4th amino acid, which happens to be lysine.

B. In ES cells, both sites would be tagged, and expression of Sry should turn on the gene regulatory network in Sertoli cells. The paper explains that in embryonic stem cells (ES cells), master regulator genes are in regions of chromatin that have both types of modifications on the histones -- the "turn on" and the "turn off" modifications. Therefore the cells are 'poised' and ready to go in multiple directions. The cells can give rise to multiple cell types, depending on which regions of chromatin have the "turn off" or "turn on" modifications removed. Sry should be active only in the cells of the developing male gonad, not in cells of the female gonad (thecal or granulosa cells) or somatic cells (myocytes).

C. In the myocytes, the K27 of the Sry gene would be tagged, and the chromatin including Sry would be relatively tight euchromatin. The Sry gene is not used in heart cells (at any stage) so it will be in chromatin that is tightly coiled and inactive. In interphase, inactive genes are generally in areas of tightly coiled euchromatin, not in heterochromatin.

D. In developing Sertoli cells, K4 would be tagged, and the Sry gene would be in relatively loose euchromatin. Only males have Sertoli cells, so this refers to a male. Sry is the master regulator gene that starts the sexual differentiation of the gonad in the male. If the Sry gene is inactive, an XY person develops into a female. At the appropriate stages of development, the Sry gene is transcribed in Sertoli cells, and the protein is used to turn on other genes leading to male gonadal development. At that stage, the chromatin containing the Sry gene must be relatively loose so the gene can be transcribed.

E. In thecal cells, neither site would be tagged, and 'none of these' is the best choice for the state of the chromatin containing Sry (since there isn't any). Only females have thecal cells, so this must refer to a female. There is no Sry gene in females, as the gene is on the Y chromosome.

14-3.

A. **Mitochondrial DNA; All nuclear DNA**

The important differences between identical twins and SCNT is:
1. Identical twins mitochondrial DNA matches biological mother’s mitochondrial DNA, whereas SCNT’s mitochondrial DNA does not match the biological mother’s (nuclear donor) mitochondrial DNA.
2. Identical twins nuclear DNA only matches half of the biological mother’s nuclear DNA. The other half of the nuclear DNA is derived from the father (sperm).

B. **Mitochondrial DNA** In SCNT the egg donor contributes the egg cytoplasm which contains mitochondria.

C. **Nuclear DNA** In SCNT all of the nuclear DNA matches the nuclear donor rather than being derived half from mom (egg) and half from dad (sperm).

14-4. A. ACT IN THE NUCLEUS & ARE PROTEINS THAT BIND DIRECTLY TO DNA: The TF's involved in developmental regulation act in the nucleus and bind DNA. (The TF's we discussed are not receptors.)

B. DNA; C. PROTEINS. Regulatory regions of genes are DNA; histones are proteins. Therefore you are methylating DNA and proteins respectively.

14-5. A. & B. TRANSCRIPTION & INCREASED DNA METHYLATION. Gata-3 protein is a TF that affects transcription; the MRGs that aren't expressed in LE would have increased methylation to keep them silent & maintain LE fate.

C & D. K4 METHYLATION; K27 METHYLATION: When genes are active, the histone 3 is usually methylated on K4 and not on K27. When genes are inactive, the situation is reversed. The GATA-3 gene is active during differentiation & maintenance of luminal epithelial cells, but the Oct-4 locus is not. (Oct-4 is 'on' in ES cells.). MRG normally remain transcriptionally active in the terminally differentiated cells they control.

E & F. DRIVES DIFFERENTIATION & TUMOR SUPPRESSOR: GATA-3 drives differentiation (at the expense of cell proliferation); when GATA-3 protein is present, cells stop dividing and remain in G-0. When the GATA-3 protein is absent, the cells grow instead of differentiating, so GATA-3 is considered a tumor suppressor. Triggers apoptosis is incorrect. If this was true then you would never get luminal epithelial cells because GATA-3 protein would kill them all.

14-6. A. INCREASE GATA-3 EXPRESSION: If you want differentiation to occur, you want to increase GATA-3 expression. (See above.)

B1 & B2. THE WOMAN & A DIFFERENTIATED CELL THAT IS NOT PART OF THE MAMMARY GLAND: You want a nucleus from the woman herself, and you want a nucleus from a differentiated cell that is NOT mammary gland. Explanation:

The mammary gland nucleus probably has genetic changes (somatic mutations) that affect regulation of cell growth. A nucleus from a different differentiated cell should be normal – no somatic mutations, and no extraneous DNA or proteins to cause an immune response.

An unfertilized egg isn't viable.

An IVF embryo made from the woman's egg cells (+ male sperm) would have genes from the sperm DNA that would provoke an immune response.

14-7. A1. **Unipotent**; fully differentiated skin fibroblast cells under normal conditions are expressing the proteins to enable them to have the structure and function of a fibroblast skin cell, they can only remain a fibroblast skin cell

A2. **Pluripotent**; reprogrammed skin fibroblast cells or iPS cells are pluripotent – can become any of the three germ layers that makes up the embryo but can not form the trophoblast or embryonic portion of the placenta

A3. **Unipotent & differentiated**; fully functional neurons are unipotent and differentiated.

B1. **Levels of RNA synthesis & the nucleus**; Transcription factors directly contact the DNA to increase or decrease the synthesis of RNA and the transcription factors do this in the nucleus where the DNA is located,

B2. **Nucleus & Free in the Cytoplasm**; The RNA for transcription factors, as for other genes, is synthesized in the nucleus; rRNA rather than transcription factors are synthesized at the nucleolus; The RNA for transcription is translated by the ribosome in the cytoplasm. The transcription factor that forms contains a nuclear localization signal that targets this protein to the nucleus where it functions to increase or decrease levels of RNA synthesis. Transcription factors are not synthesized in the ER – this would localize them in the ER membrane or cause them to be secreted depending on if they were embedded in the ER membrane or deposited into the ER lumen

C1. **Inner cell mass and embryonic stem cells**; these terms are equivalent, Oct4 is the master regulatory genes of the ICM and the embryonic stem cells – Oct4 confers pluripotency

C2. **Cells at the 4-cell stage & embryonic stem cells**; Oct4 and Cdx2 are both on from the zygote to the 8 cell stage in all cells, Oct4 turns off in the trophoblast cells and stays on in the inner cell mass at the 16 cell stage

D1. **K27 methylation**; Oct 4 is not actively transcribed in fully differentiated skin fibroblast cells - K27 methylation, without K4 methylation, tightens the DNA decreasing transcription

D2. **K4 methylation**; Oct 4 is on in iPS cells and therefore needs to be transcribed – K4 methylation, without K27 methylation, opens the DNA allowing RNA transcription

D3. **K4 & K27 methylation**; Nkx2.5, the master regulatory gene for heart muscle cells, isn't yet active in iPS cells but is poised to go either way since it may still be turned on in the differentiation cascade that the iPS cells have the potential to perform, K4 & K27 methylation keeps the DNA in the region of the DNA containing the Nkx2.5 gene poised to be able to be transcribed or not be transcribed depending on the cell type.
These modifications would occur for the endogenous Oct4 and Nkx2.5 genes and for the added Oct4 gene.

E. **Mitochondrial & most of the nuclear DNA**; The mitochondrial DNA will match the mitochondrial DNA of the DNA donor fibroblast skin cell, however, most of the nuclear DNA of the iPS cell will match (in base sequence) the DNA of the donor fibroblast skin cell. The only DNA sequences that will not match will be the DNA encoding the four proteins that were added to the skin cell. The state of the reprogrammed nuclear DNA will be different in the sense that reprogramming will change the modifications on the DNA and/or on the histones in the iPS cells. However the sequence of the bases in the DNA will be the same.

14-8. A. This infant's primordial germs cells will probably form **(sperm).**
B. This individual would be a (**pseudohermaphrodite**) & (**XY**).

Explanations (all 3 parts required for full credit – remaining parts on next page):
1. Since PGCs (primordial germ cells) in this case migrate into a testes, which contain Sertoli & Leydig cells, sperm results.

2. Pseudohermaphrodite, because the gonad contains only the somatic cells of the testes (Sertoli & Leydig cells), so not a hermaphrodite. The other sex organs, ducts and external genitals, do not all match the sex of the gonad. The ducts in this individual are those that occur in males when testosterone and AMF (secreted by Leydig & Sertoli cells respectively) are both present. Since DHT is not present, male genitalia do not form, and external female genitals result.
3. XY because the infant has testes; formation of which requires the Sry gene on the Y chromosome

14-9. A. Potency: Most_____**Pluripotent — Germ Layer — Eye Field — Unipotent**_____Least

Explanation: When ES cells (embryonic stem cells) develop into neurons they are first pluripotent, then they form the appropriate germ layer, then the eye field and then they become unipotent. as they develop into a specific cell type of the eye.

B. H3K4 = (**4th amino acid of histone 3**). The convention numbers every amino acids from the **N** terminus in increasing order, rather than numbering only the lysines.
C. Histone methylation of the master regulator of PRE in ES cells = (**H3K4) (H3K27**). MRGs in ES cells that haven't yet been activated are bivalently methylated.
D. Histone methylation of the master regulator of PRE in liver cells = (**H3K27. T**he MRG for part of the retina isn't transcribed in liver so the gene region for this MRG will be condensed and H3K27 methylated.
E. Histone methylation of the master regulator of PRE in PRE cells = (**H3K4).** The MRG for part of the retina is transcribed in the PRE so the gene region for this MRG will be open and H3K4 methylated.

14-10. A. The following become more potent: **(creation of zygote using SCNT) (iPS cell creation).**

B. **(none of these)** None of the items increase potency and occur in natural human development.

Explanation: iPS cells and zygotes created by SCNT are pluripotent and totipotent, respectively. The differentiated cell whose nucleus was used as a donor for SCNT was less potent, as were the cells transformed by the 4 genes used to make iPS cells. Natural human development does not include SCNT or creation of iPS cells – these require laboratory manipulations. All the other changes listed occur during human development, but they all involve increase in differentiation and decreases in potency.

14-11. A. You would expect: **(testes are present) (male levels of testosterone).**
B. You would find: **(XY individual) (Sry expressed) (testosterone receptor expressed)**.
C. If this individual had both ducts you could conclude: **(Leydig cells function) (Sertoli cells aren't functioning)**.

Explanation:
A. If tissues derived from Wolffian ducts are present, the fetus must have developed testes that produce male levels of testosterone to sustain the Wolffian ducts. (If the woman has no visible penis, she may have female levels of reductase.)
B. The person must be XY. The SRY gene must be expressed (to trigger testes formation and testosterone production) and the testosterone receptor must be expressed (for the Wolffian duct to respond to the testosterone).
C. If the person had both Mullerian Ducts and Wolffian Ducts, you can assume the person had high testosterone from working Leydig cells but not AMF, so Sertoli cells aren't functional.

14-12. A. Methylation of H3 K9 most likely **(tightens chromatin) (decreases TF accessibility to DNA)**
B. H3 K9 Acetylation most likely **(loosens chromatin) (increases TF accessibility to DNA)**
Explanation: Decreased transcription, caused by H3 K9 methylation, is associated with tighter chromatin and decreased TF accessibility to DNA.
Increased transcription, as a result of H3 K9 acetylation, is associated with looser chromatin and increased TF accessibility.

14-13. A. The sex chromosomes directly determine the fate of **(gonads).**
B. Bipotential organs include: **(external genitalia) (gonads) (primordial germ cells).**
C. If the gonad was removed at 6 weeks, **(Mullerian duct remains)**

Explanations:
A: The sex chromosomes only determines the fate of the somatic tissues of the gonads and then once this is determined the somatic tissue of the gonad determines the fate of the germ cells, the ducts and the external genitalia.
B: Unlike most of the organs in our body, the external genitalia and the gonads can become two different tissues. The Mullerian ducts and the Wollfian ducts can each only form specific organs.
C: This question asks what would be different from Terry in the case study if we removed the gonad before the gonad differentiated into testes. The testes secretes two molecules that are important for sex determination of the other sex organs, testosterone and anti-Mullerian factor (AMF). The only tissue that responds to AMF is the Mullerian duct. In the case study, where the testes were present until birth, the AMF secreted by the testes would destroy the Mullerian ducts. If the gonads were removed before they differentiated into testes, AMF would not be produced and the Mullerian ducts would remain. All of the organs that are normally affected by testosterone would respond in the same way, i.e. no response. If you don't have the receptor for testosterone (the androgen receptor) testosterone will not be detected, regardless of whether or not testosterone is produced.

KEY TO PROBLEM SET #15

15-1. Tumor suppressors = p53 & rb; rest are proto-oncogenes (= growth promoting genes).
A proto-oncogene is a gene with two properties:
(1) It makes a factor that promotes growth (as opposed to a gene that makes a factor that inhibits growth = tumor suppressor) *and*
(2) It can be mutated or over expressed (= converted into an oncogene) so that its product overstimulates growth.

15-2. Recessive (but see note) = p53 & rb = tumor suppressors; rest = oncogenes are dominant. To cause cancer or lose growth control, there must be no rb protein to block transcription by E2F. So usually both copies of the rb (or p53) gene must be inactive to cause a problem.
Note: Mutations that inactivate the p53 protein completely are recessive. However, some p53 mutations that alter the protein are so-called "dominant negatives" because the protein acts as a tetramer. The mutant protein interferes with tetramer formation by the normal protein, so heterozygotes can't make tetramers and have inactive p53.)

15-3. Rearrangement could put *sis* next to a strong promotor and/or enhancer so that large amounts of the *sis* product (a growth factor) are made. Then the cell would no longer be dependent on supplies of exogenous growth factor. (Most normal cells don't make their own growth factors.)

15-4. A mutation could alter the amino acid sequence of one of these signaling proteins so that it was permanently stuck in the "on" or stimulated state. Then the cell would proceed to enter S as if it were stimulated even when the stimulus was absent.

15-5. When both copies of rb or p53 are inactivated, the cell loses the ability to stop growing in response to damage and/or other appropriate signals. If one (inherited) copy is already shot, there is a reasonable chance that at least one cell in the body will sustain a mutation in the second copy. If both inherited copies are okay, a single cell must get two mutations to lose p53 or rb function. Since mutations are rare, this is a rare occurrence. So inheritance of cancer susceptibility is dominant, but at the molecular level, expression of p53 mutant phenotype is recessive.

15-6. No p53, no cell cycle arrest or apoptosis (programmed cell death) in response to DNA damage, so mutations tend to accumulate, and sooner or later one of them usually → lack of growth control. No rb product, no repression of genes controlled by E2F, and no stopping at G1/S border. (The step that requires CDK/cyclin is short circuited.) No *sis* function --> cell is dependent on added growth factor (which is probably normal for most cells); if that cell is supposed to make its own GF it won't enter S. No erb-B or ras product → no response to own or exogenous growth factor (stuck in G1).

15-7. Each mutation allows a clone of cells to grow (at expense of other cells of organism). The more cells there are in the clone and the more mutations like p53 (lack of repair, surveillance, etc.) the more likely it is that a second mutation will occur and/or be passed on. The clone with both mutations grows even better, spreads more, etc., and increases the chances of a third mutation and so on. In other words there are multiple rounds of mutation and selection and each new mutation that messes up growth control helps promotes the spread of cells carrying it. So cancers tend to become more aggressive and spread better as they grow and accumulate mutations.

15-8. A. If growth factors are missing, most mammalian cells arrest in ***G1 just before they enter S.***
B. For the cells to start growing again, the first thing to be activated is ***a TK linked receptor.***
C. If the DNA of the cell is damaged, ***p53*** will be stabilized and increase in amount.
D. ***Cyclin*** will be degraded.

Explanation: A cell in G1 will not enter S until a growth factor binds to the appropriate receptor. Growth factors generally use tyrosine kinase linked receptors -- receptors that are themselves tyrosine kinases or linked to TK's. Activation of the TK linked receptor triggers synthesis of cyclin (using ras, to activate the MAP kinase pathway which in turn activates TF's needed for synthesis of cyclin).

In healthy cells, p53 is unstable and does not accumulate. DNA damage triggers stabilization of p53. The p53 then builds up in amount and blocks the activation of the cyclin/CDK complex.

Once the cyclin/CDK is activated, and it phosphorylates its targets, the cyclin is degraded, leaving the CDK inactive until the next cycle. This, and the roles of ras, MAP kinases, E2F & rb did not need to be explained.

15-9. A-1. transcription & translation; A-2. histone**.**

The synthesis of mRNA is controlled here; that's what is meant by transcriptional control. The degradation of mRNA is controlled too; that affects the rate of translation. There are no introns, so regulation of splicing doesn't occur, and there is no indication of regulation of other steps in processing, transport or gene amplification (which is rare anyway).

Note: Most, but not all, eukaryotic genes have introns. (Histone genes generally do not have introns.) So you can't assume this organism is a prokaryote just because there are no introns in gene Z. (This organism must be a eukaryote anyway because many of the terms in the problem, such as histones, lamins, cell cycle, etc., don't apply to prokaryotes.)

New histones are made during S so the new DNA can be packaged into nucleosomes as soon as it's made. None of the other proteins listed are made only during S. Cyclin levels may trigger S, but cyclin is not made only during S; it probably isn't made in S at all. Nuclear pore proteins, lamins, and protein kinase subunit(s) of MPF are made and accumulate throughout interphase. (Protein kinase may be activated in or at start of S, but the protein isn't made then.)

B-1. all of interphase, B-2. in S, B-3. throughout the cell cycle.

Virtually all DNA is in nucleosomes whether cell is in M or interphase. During M, all the nucleosomes are tightly coiled into heterochromatin. During interphase, the nucleosomes can remain tightly coiled (DNA stays heterochromatic) or loosen up (DNA becomes euchromatic). If the DNA of interest can be transcribed at any time during interphase, the nucleosomes in that region should loosen up at the start of interphase and the DNA should remain euchromatic throughout interphase. When the DNA is actually transcribed, the nucleosomes should loosen up a bit more and the DNA should be most sensitive to DNase.

C-1. Z mRNA; C-2. translation; C-3. stability of Z mRNA.

The mutant described here makes an abnormal regulatory protein. Since the mutant has a problem with translation, but transcription is normal, the regulatory protein must affect translation. (There is no splicing here; see part A.) The simplest explanation is that the phosphorylated form of the (normal) regulatory protein binds to the mRNA and increases translation; the mutant protein either doesn't bind to the mRNA, or binds but has no effect (because it is not phosphorylated). So the only question is whether the regulatory protein affects initiation of translation or stability of the mRNA. You know from part A that the stability of Z mRNA is regulated, and there is no evidence for regulation of initiation. So it seems most likely that the the phosphorylated (normal) regulatory protein stabilizes Z mRNA during S; the unphosphorylated (mutant) regulatory protein does not.

Note: Many students thought that regulation of initiation (but not of degradation) should be all or nothing, but this is not the case -- regulation of most processes can produce a graded response. For example, in the hemin regulation of globin synthesis, intermediate levels of hemin should allow intermediate rates of synthesis of globin.

15-10. A. A base change that causes permanent activation of the CDK.

CDK's normally require cyclin for activation, and synthesis of cyclin normally requires the proper signals. If a CDK becomes cyclin independent, it will phosphorylate proteins at the wrong times and allow cells to proceed through the cell cycle (primarily at G-1 → S or G-2 → M) in the absence of the usual signals. This will lead to uncontrolled growth, i.e. cancer.

If the CDK becomes inactive, you won't get uncontrolled growth -- you'll get the opposite.

If the CDK gene is over expressed or the CDK becomes resistant to degradation there may be more CDK than normal. However it probably won't make any difference as cyclin is the limiting factor in CDK activity and so extra CDK (without cyclin) probably won't do anything. CDK is not normally inactivated by degradation; cyclin is.

B. CDK = a proto-oncogene.

15-11. A. Binding of TFs to Rb will decrease. Level of G1/S CDK will stay the same (or increase, see (2)), All the others will increase. The order of events, both those listed and the intervening ones, are as follows:

(1). Growth factors stimulate cells in G-1 (or G-0) using receptors. (Growth factors generally use tyrosine kinase receptors and trigger modification of TF's.)

(2). Cyclin synthesis occurs --> accumulation of cyclin. If cells were in G-0, not G-1, it may be necessary to synthesize CDK as well as cyclin. [CDK activity will increase, due to the increase in cyclin levels – see step 3. However the amount of CDK protein will not necessarily change.]

(3). Cyclin binds to and activates CDK.

(4). CDK phosphorylates Rb protein. (Note: Rb protein is called "rb" because a lack of it causes retinoblastoma, a childhood cancer.)

(5). Rb protein binding to transcription factor (E2F) decreases, releasing E2F.

(6). E2F promotes transcription of appropriate genes --> proteins --> cell enters S

(7). T incorporation (DNA synthesis) and histone synthesis increase at same time.

(8). Cell finishes S --> G-2 -->M.

(9). Spindle assembly and other events of M occur; cell completes M and goes back to G-1.

B. This should work in all of these cases (the first 3 listed). Adding mutant Rb protein should be effective even if normal Rb protein is present. Unphosphorylated Rb protein binds E2F so the E2F does not trigger transcription, and cell does not enter S, but remains in G-1 and does not divide. If only mutant Rb protein that cannot be phosphorylated is around, it will permanently bind Rb protein, and E2F will never be able to work.

If normal Rb protein is around, the mutant Rb protein should still prevent cell growth. It doesn't matter if normal Rb protein is around or not -- as long as there is mutant Rb protein present, free E2F will never be available. If the normal Rb protein is phosphorylated, it will release E2F but the free E2F will immediately be bound up again by the mutant unphosphorylatable Rb protein. So adding the mutant Rb gene should work even if the normal gene is still working.

This procedure has actually been tried in rats and seems to work. It is of great practical interest, because balloon angioplasty, which is often used to clear out obstructions (plaques) in the arteries, causes damage to the artery lining and triggers growth of the smooth muscle cells lining the artery. So the plaques that clog the artery grow back again. If balloon angioplasty can be combined with transfer of the mutant Rb gene, then the arteries will stay clear and future heart attacks will be prevented.

15-12. Gene for kinase 2 is a proto-oncogene; permanent activation of kinase is most likely to cause cancer. A proto-oncogene is a normal gene whose product is required for growth, as opposed to an oncogene (which is a mutated version of such a gene that over stimulates growth) or a tumor suppressor gene (which is a normal gene whose normal product is required to prevent uncontrolled growth). Kinase 2 protein (not just the kinase 2 gene) must be active to trigger a critical step in the cell cycle. Normally, activity of kinase 2 is controlled, and kinase 2 is only activated when GF binds to its receptor. If kinase 2 is always active, growth will no longer be properly controlled by the supply of GF, but will occur all the time. It is the activity of kinase 2, not its amount, that is critical here.

15-13. **A.** Arrested at G1/S; cyclin (& possible cdk -- depends on length of vacation).

Growth factors are normally needed to allow animal cells to go from G-1 to S; in the absence of growth factors or essential nutrients, animal cells generally stop in late G-1. (These are "cycling" cells that have temporarily lost a supply of growth factor, so they will probably be in G-1, not G-0. If they are starved for growth factors long enough, they will enter G-0.)

Growth factors are needed to trigger synthesis of cyclin, which is needed to activate cyclin-dependent kinases. The active cdk called the start kinase is necessary to allow synthesis of components needed for S phase. If cells are in G-1, they will have cdk (which is relatively stable, but inactive by itself) but not cyclin (which is degraded). If cells are in G-0, they will lack both cyclin and cdk and have to re-synthesize both.

MPF is the kinase (cdk) that controls the G-2/M transition which is where some cell types, but not adult mammalian cells, usually arrest.

p53 is made continuously (although it is normally degraded) -- adding growth factors doesn't affect its synthesis.

GF receptor is continually present -- it's the GF that's limiting.

Answer to 15-13, cont.

B-1. Ink activity should be below normal (if INK is mutant).; **B-2**. Ink activity should be normal if CDK is mutant. (See note.) Activated cdk is needed to phosphorylate proteins (to activate transcription factors) and allow cells to make the necessary proteins to enter S. If there is too little ink protein, there will be nothing to inhibit the cdk, and cells will grow when they shouldn't, because the cdk/cyclin will be active (when it shouldn't). If the cdk is altered so it can't bind ink, the results will be the same in spite of the presence of normal ink protein. In other words, too little ink protein (or too little binding to ink protein) leads to too much growth.

Note: "activity" of ink or cdk in this context means capacity or potential activity -- how much activity or reaction you can get if you supply an excess of partner, substrate, ligand etc. It doesn't necessarily mean how much activity the enzyme is exhibiting right now – the enzyme may not be working at all, if an earlier step is blocked and no substrate is available. "Low activity" means the enzyme is not catalyzing the reaction at a high rate because something other than low substrate is the limiting factor. (The enzyme is usually mutant or inhibited.)

C. Fail to grow

If the ink protein is activated (for whatever reason), it will bind to cdk and prevent phosphorylation of the cdk target proteins. The cell will be unable to transcribe genes and make proteins needed for S. So cells will not enter S and will not grow.

D. A tumor suppressor.

Ink protein is used to block the cell cycle (under appropriate conditions). When ink-cdk binding is prevented by mutations in either INK or CDK genes, the cell cycle cannot be blocked and cell will grow under conditions where it should not, and may form a tumor. Since ink is needed to stop the cycle, the gene that codes for ink protein (INK) is classified as a tumor suppressor gene (a "brake" gene), and lack of ink protein will cause uncontrolled growth.

E-1. Grow uncontrollably. **E-2**. Fail to grow.

It all depends on what comes last -- and what state it's in. If it is a stimulator of growth, and it is always active (stimulating), the cells grow uncontrollably. If it is an inhibitor, and it is always active (inhibiting), the cells fail to grow.

The order is ras → (many steps) → ink → (many steps) → E2F. In the normal pathway, GF stimulation triggers the ras pathway, which leads to synthesis of cyclin, and activation of cdk. Ink binds to cdk and blocks its activation, stopping the cycle. When the ink is inactivated, the cdk can bind cyclin and become activated, and the cycle can continue. The active cdk phosphorylates rb, which frees up E2F. E2F triggers synthesis of proteins needed to enter S. (See texts or class handouts.)

Details for E-1. If both E2F & ink are always active, the E2F will trigger entry into S even though ink is active. The ink blocks the cdk 'upstream' of E2F, but the E2F works anyway; it is not dependent on the cdk to activate it.

Details for E-2. If both ink and ras are always active, the ink will block the effects of ras and the cells will fail to grow. Even if ras is active, ink is 'downstream' and will remain active, blocking the cycle by binding to cdk. (This is assuming that other factors, not ras, control activity of ink.)

15-14. **Proto-oncogene, altered version** (always active as kinase); **low amounts or altered** (always inactive). The normal version of the gene, found in all normal mammals, controls maturation and migration of several cell types including melanocytes. The viral version (of the gene and consequently the protein) is altered so it is always active -- it does not have to bind its ligand to have kinase activity and trigger a signal transduction pathway. Since the protein is ligand independent, cells that have it grow uncontrollable and do not wait for the proper signal. In abnormally pigmented animals (& people) the kinase is relatively inactive or low in concentration, so that binding of the ligand does not give sufficient response. Therefore the organisms have spots without normal pigment.
Note: In order for melanocytes to migrate in a particular direction, the cells they crawl over must have the ligand on their surface and the receptor kinase must be able to bind the ligand. If ligand or receptor are defective, the melanocytes stay put.

15-15.

A. Proto-oncogene. If CDC 25 is knocked out, the cell fails to enter S. This means that the normal product of CDC 25 is needed for growth, i.e. to enter S. If CDC 25 is over-expressed (not knocked out) and makes too much product, the cell will grow uncontrollably and the over active gene will be called an oncogene. The normal, properly expressed form of such a growth-promoting gene is called a proto-oncogene. If CDC 25 were a tumor suppressor gene, then its product would keep growth in check, and knockout of the gene would result in uncontrolled growth, the opposite of what happens here. (When this was on an exam, you had to explain (1) the difference between a tumor suppressor gene and a proto-oncogene; and (2) the difference between an oncogene and a proto-oncogene.)

B. (1), (2), (4) & (5) are unlikely; (3) & (6) are likely. The general principle is that CDK must combine with cyclin to activate the kinase activity of the CDK and trigger entry into S (see handouts for details). The CDK is stable, and is continuously present, but the cyclin is made and degraded in a cyclic manner. (This difference in stability explains why (3) is likely but (4) is not; see details below.)

CDC 25 must code for something that promotes entry into S (since knockout of CDC 25 prevents entry into S). So the gene product must promote formation or activation of the CDK-kinase complex. This rules out choices (1), (2) & (5)

To get full credit for the explanation (when this was on an exam), you had to mention the points listed so far either in your general introduction or your list of cases. You didn't need all the details below, but they are included FYI. If CDC 25 coded for (1), (2) or (5) the normal gene product would block entry into S, not promote it. To put it the other way, if CDC 25 coded for any of these, knockout of CDC 25 would cause uncontrolled growth, not arrest.

(3) is likely because cyclin is unstable and must be made anew each time a cell needs to go from G1 to S. If a transcription factor for cyclin were knocked out, the cells would not be able to make any new cyclin and would arrest at the next entry into S (all old cyclin would be degraded).

(4) is unlikely because CDK is stable but knockout of CDC 25 causes cells to arrest at the next G1/S. If a transcription factor for CDK were inactivated, no more CDK would be made, but there would be enough CDK to continue for at least several cell cycles. Eventually, the amount of CDK per cell would fall below some critical value, and cell division would stop. But the cells would not stop at the very next entry into S. (That would only happen if no cyclin were made; see above.)

(6) Activation of CDK requires binding of cyclin, but other factors as well are needed to convert the complex to an active form. CDC 25 could code for one of these factors; this is actually thought to be its real function. Activation of CDK requires both addition of phosphates (by kinases) to certain amino acid side chains and removal of phosphates (by phosphatases) from other amino acid positions. CDC 25 is thought to code for a phosphatase involved in removing a critical phosphate from the CDK-cyclin complex.

15-16. A. (4). Since *BRCA-1* is a tumor suppressor gene, that means that it makes a product that normally blocks growth or entry into S (until the proper signal is received). When the gene is inactive, there is no block to growth and cell grows uncontrollably (cell enters S whether proper signals are received or not). So the normal product of *BRCA-1* must be something that blocks growth, not something that promotes it. (See note at end for update on "real" function of the gene.) The report that the *BRCA-1* gene product is present but mislocated in the cytoplasm in cancer cells indicates that the protein works in the nucleus in normal cells. So the normal product of the *BRCA-1* gene must be a protein that (a) blocks growth and (b) works in the nucleus. (You had to mention both of these to get full credit for explanation.) All choices but (4) describe proteins that promote growth, not block it. Choices (2) & (5) are also ruled out because they describe proteins that work in the cytoplasm, not the nucleus.

B. (3); autocrine (*if* the gene encodes a growth factor or receptor). Since the *BRCA-1* protein (usually written BRCA-1) must block growth, choice (1) is ruled out. Since the protein has no transmembrane sequences and is similar to secreted proteins, choice (2) is ruled out. That leaves choice (3), which is consistent with the role of BRCA-1 as a tumor suppressor gene. Since cells without BRCA-1 function develop into tumors even though normal cells are around, it means that normal cells cannot supply BRCA-1 protein to control growth of their neighbors. In other words, the *BRCA-1* product can only act in/on the cell that produces it. So if it is a growth factor, this makes it an autocrine factor by definition. (An endocrine hormone or growth factor is secreted into the blood and travels through the blood to affect the growth of other cells. That is not happening here.)

C. (B). A secreted protein should be made on ribosomes attached to the ER and should be processed in the Golgi on its way through the endomembrane system (ER → Golgi → vesicles → exocytosis). A nuclear protein should not be found in the Golgi -- it should be made on free ribosomes in the cytoplasm (not in the nucleus), and should never enter the endomembrane system. The completed protein should be released into the cytoplasm, not the ER, and transported through the cytoplasm to the nucleus. (To get full credit here, you had to explain both how this result was consistent with B and inconsistent with A.)

Note: this question is based on some early research results regarding the protein made by the BRCA-1 gene. One research group got the results in part A and a second group got the conflicting results in part B. More recent results indicate that the BRCA-1 protein is nuclear and involved in the repair of DNA.

15-17. A. (i) Normal cells will grow + EGF.
(ii) Temp. sensitive ras mutants will not grow.
(iii). Temp. sensistive rb mutants will grow w/ or w/o EGF.
(iv). ras/rb double mutants will grow w/ or w/o EGF, just like the rb mutants.
(v). normal and rb mutants will make cyclin.

First part of pathway -- EFG to cyclin: EGF binds to its receptor and triggers activation of the ras/MAP Kinase pathway. Net result of cascade is activation of transcription factors for synthesis of cyclin. Cyclin is needed to form active start kinase and proceed to S phase. What happens if ras is inactive? Kinases are never activated, transcription does not occur, and cells do not make cyclin or enter S; growth stops.

Second part of pathway -- Cyclin to rb/E2F: Cyclin combines with CDK to form start kinase, which is then activated and phosphorylates target proteins. One target protein is rb. Once rb is phosphorylated, it releases E2F, which triggers transcription of genes that make proteins to enter S. Normal unphosphorylated rb binds to E2F and prevents E2F from working. In normal cells, EGF binding leads to phosphorylation of rb and activation of E2F. What happens if rb is inactive? E2F will always be available to trigger entry to S -- activation of E2F will be independent of EGF and the ras/MAPK pathway.

Problem 15-17, cont.

B. (i) normal cells not grow.
(ii). rb mutants will grow w/ or w/o EGF as above.
(iii) p53 mutants will grow + EGF.
(iv) p53 mutants will have active start kinase.

Explanation: What p53 does and where it acts: p53 (if present) blocks activation of start kinase. In undamaged cells, p53 is unstable and does not accumulate. If DNA is damaged, p53 destruction is prevented, and p53 builds up, blocking activation of start kinase. (Without start kinase, rb cannot be phosphorylated, E2F cannot be activated, and growth stops.)

Mutants and activation of start kinase: The normal cells and the rb mutants have normal p53. It builds up and blocks activation of start kinase. That stops the cycle for normal cells, but the rb mutants grow anyway -- they have active E2F, regardless of what state start kinase is in. The p53 mutants are the only cells with active start kinase -- their p53 is inactive and cannot block activation. The p53 mutants act like normal cells whose DNA is undamaged -- they continue to grow if you add EGF. (Without EGF they can't make cyclin.)

Note: p53 does not recognize individual mutations -- if it did, there would be no mutations at all and all genomes would be identical. (Either all the mutations would be repaired or the mutant cells would die by apoptosis) p53 does not stop growth if there is a single base change or even a large, local one in the DNA. p53 is stabilized when there is widespread DNA damage (or other large changes in stress to the cells). DNA damage alone must be insufficient to stop cell growth -- otherwise you wouldn't need p53.

15-18. A. When you add GGF, this is what should happen:

A-1. The proportion of Ras bound to GDP should decrease. (GDP should be replaced by GTP.)
A-2. Phosphorylation of the GGF receptor should increase.
A-3. Cyclin synthesis should increase.
A-4. Binding of E2F to DNA should increase. (This assumes E2F is not bound to DNA in its inactive state. If it is already bound, binding will not change but transcription will.)
The order of events should be A-2, then A-1, then A-3, then A-4.

B. After UV treatment, this is what should happen:

B-1. Level of active E2F should be lower. (It should be blocked by rb, which is not phosphorylated, since the start kinase is inactive.)
B-2. Level of p53 should be higher. (It is always made, but usually degraded. DNA damage causes stabilization of the p53, so more builds up.)
B-3. Level of cyclin/CDK complex should be the same. (But it should be inactive instead of active.)
Explanation: p53 is stabilized, so levels rise; start kinase (CDK/cyclin complex) is made, but cannot be activated -- the activation is the part blocked (indirectly) by p53.

C-1. NF-1 protein catalyzes hydrolysis of GTP.
C-2. NF-1 protein inactivates Ras protein (converts active Ras-GTP to inactive Ras-GDP). Since NF-1 is a tumor suppressor, it must block activity of Ras, not the reverse.
C-3. GGF inactivates NF-1 (thus allowing/prolonging activation of Ras).
Explanation: How Ras is inactivated or inactivated -- inactivated by hydrolysis of bound GTP to GDP; activated by exchange of bound GDP for bound GTP. How GFF, Ras and/or NF-1 (proteins) affect activity of MAPK. If NF-1 is active, Ras is inactivated, and can't start the MAP kinase cascade that leads to activation of MAPK. When GFF is added, NF-1 is inactivated, & Ras is activated. Therefore Ras can activate the MAPK cascade.

D-1 & D-2. After GGF is added, cells will synthesize mRNA and use it to make cyclin.
D-3 & D-4. GGF receptor will be phosphorylated first, then MAP kinase, then TF's, and last, rb. (raf is a kinase activated by binding to ras, not by phosphorylation. You can figure this out from the handout, because ras is not a kinase.)

Key to Problem Set 15R

15R-1. A-1. B cells; A-2. Soluble Antibody.
Explanation: Either the immune response is cell mediated, and the damage is caused by cytotoxic T cells, or the response is humoral, in which case the damage is done by soluble antibodies. If you discard the plasma, but return the cells to the patient, the symptoms improve. These results indicate that the plasma contains the material that destroys the receptors; the damage is not done by the cells in the blood, their surface proteins, or the locally acting substances that the cells secrete (such as perforin). It must be soluble antibody that is destroying the receptors, and antibodies are made and secreted by B cells.

B: CD4+ and cell surface TCR.
Explanation: The T cells specific for the AChR must be helper T cells.
How do we rule out cytotoxic Ts? From the results in A, we know that the immune response that destroys the receptors is mediated by antibody, not by cytotoxic T cells. So from that alone, we know that cytotoxic T cells are not involved. (Alternatively, you can argue that the cells with the AChR are not being destroyed, so cytotoxic Ts are not involved.)
How do we know helper Ts must be involved? If B cells are making antibody, you need helper T cells to allow the B cells to multiply and secrete antibody.
Helper T cells carry CD4 (while cytotoxic cells carry CD8.) Part credit for matching the T cells and their respective CDs.
You did not have to explain the following, but it is included here FYI. The TCR is the protein made by T cells (both helpers or cytotoxic Ts) that combines with the epitopes on the surface of target cells. The TCR is always on the T cell surface; it is never secreted. The epitope that binds to the TCR must be bound to MHC, but the MHC is on the target/presenting cell surface, not on the T cell.

15R 2. A: be of the same class; B: have the same constant region of the H chain. (The variable regions will be different -- see below.)
Explanation: A. All the antibodies are IgG, so they are the same class (by definition of class). They are probably not all the same and not all directed at the same epitope. There are many epitopes (separate antigenic sites) on the AChR. The IgG against the AChR is probably a mix of antibodies -- the different antibodies in the mix combine with different epitopes. Why are there multiple antibodies? Many different B cells were stimulated by the AChR. The different B cells made different BCR's that combined with different parts of the AChR. Therefore the antibody is "polyclonal" -- you got clonal expansion of multiple B cells and each clone made a different antibody (to a different part of the AChR). That's why the soluble antibody to AChR (or just about any protein) is a mix.

B. All the antibodies are IgG, so they all have the same constant region of the heavy chain. (See note at # on next page.) That's what makes them IgG. Class switching is not involved here. If the DNA were rearranged, you'd get a different class of antibody. Somatic mutation is not involved either, as it does not affect the constant region. Also all IgG here are secreted, so there is no difference in the constant region due to alternative splicing.
Why will the variable regions be different? Because the DNA rearrangements in each B cell are different -- different B cell clones will be expanded in the two individuals.
Details: Each antibody that matches a particular epitope must fit the epitope in 3D. However there is more than one way to build a peptide that matches a particular epitope. Different amino acid sequences can produce peptides that bind the same epitope. Therefore not all variable regions directed against the same epitope have to be the same. Because of DNA rearrangements and clonal selection, antibodies from different individuals are unlikely to be the same, even if directed against the same epitope.
Each B cell rearranges the gene segments that code for the variable region of the light chain and the gene segments that code for the variable region of the heavy chain. Each B cell rearranges its DNA independently, and so each B cell generally comes up with a unique combination of V's, D's & J's (especially since joining is not exact). Therefore each B cell codes for a different BCR. If you inject the same antigen into two different animals, you stimulate two different sets of B cells and get two different collections of antibodies. Each collection usually contains antibodies that are directed against the same epitopes, but the antibodies in each collection are different. In each individual, the BCR's that best match a particular epitope will be selected, and the clones of cells with those BCR will be expanded and secrete antibody to the epitope. However the starting BCR's in the two individuals will probably be different, so the antibodies in the two individuals will be different too.

[#]Note: There are really several types of IgG that differ slightly in their heavy chain constant region, because the respective heavy chain constant regions are encoded by different segments of the DNA. We are ignoring this difference. If you brought this up and explained it correctly, that was fine.

15R-3. A. The transmitter is usually not acetyl choline. B. The receptor for Ac Ch is different. C. Inside ganglia.
Explanations: The sympathetic system almost always uses norepinephrine (adrenaline) not acetyl choline as a transmitter at the synapse between the post-ganglionic neuron and its effector (smooth muscle in this case).
(Note: in the rare cases where the sympathetic system uses ACh as a transmitter, the receptor is muscarinic.)
The parasympathetic system uses acetyl choline at the synapse between the post-ganglionic neuron and its effector, but the receptor is different than the one at the neuromuscular junction between the somatic nervous system and skeletal muscle. In the PS system the synapse uses a metabotropic muscarinic receptor, not an ionotropic nicotinic receptor.
The endplate structures and the mode of release of NT are different in the ANS and the somatic nervous system, but that won't explain why MG doesn't affect the ANS.
The synapses between the pre and post ganglionic neurons of the ANS use the same receptor and NT as the neuromuscular junction. So why don't antibodies in MG interfere with transmission at these synapses? The most plausible explanation is that the gross anatomy is different -- the synapse structure encloses the synaptic endings and protects them from antibodies.

15R-4. A: Too slow a heart beat; B: SA node; C. pacemaker potential; D. decrease.
Explanation: Heart beat is determined by the pacemaker cells that generate their own internal rhythm. The cells do not have a steady resting potential -- after an AP they repolarize and then slowly depolarize spontaneously (without neuronal input). The slow depolarization is known as the pacemaker potential (it is not considered part of the AP). When the cells depolarize to threshold they fire an AP that triggers the contractile cells of the heart to "beat."
Acetyl choline slows the heart beat -- it changes the pacemaker potential so that it takes longer for the pacemaker cells to reach threshold and fire an AP*. (You didn't have to explain the details of the channels and ions involved. See handout if you are interested.)
When you add ACh esterase, the ACh builds up and has a greater effect.

*ACh decreases the slope of the pacemaker potential, and has additional effects on other aspects of the pacemaker potential. All slow the time between AP's in pacemaker cells.

15R-5. A-1. EPP should be proportional to NT released. A-2. EPP should be significantly above threshold. E-3. All AP's should be the same size.
Explanation: EPP is a graded potential -- the more NT released from the neuron, the more post synaptic channels open, the more Na^+ enters the muscle cell and the more depolarization occurs at the end plate. When the EPP exceeds threshold, an AP is fired in the membrane of the muscle cell. The size of the AP is always the same. The EPP is variable in size, but the AP is all or nothing. Either the EPP is large enough to open voltage gated channels and an AP occurs, or there is no AP. (The size of the AP depends on the relative concentrations of ions on both sides of the membrane, and this is normally kept at a constant level.)
One AP traveling down a motor neuron always triggers a twitch in the target skeletal muscle cell. This means that the neuron must release enough NT to trigger an AP in the muscle cell every time. To do that, the NT must generate an EPP that is always over threshold, even if the amount of NT released is less than average. This will not work unless the average EPP is significantly above threshold. If the EPP is close to threshold, then every AP in the neuron will not trigger an AP in the muscle cells. Some of the time, the 10% variation in the amount of NT released will cause the EPP to be below threshold, and no muscle AP will result.

Answer to 15R-5, cont.

B-1. Smaller EPP. B-2. AP from some EPP's but not others. B-3. Close to threshold. B-4. Increase depolarization.

Explanation: The EPP is reduced, because there are less receptors to respond. The same amount of NT is released, but less channels are opened, and less depolarization occurs. When esterase inhibitor is added, the NT stays around in the synaptic cleft for a longer time, more channels are opened, and the response (depolarization) is larger.

Why do some muscle cells respond with a twitch while others do not? As the autoimmune response progresses, more and more receptors are destroyed, and the average EPP gets smaller and smaller, until it is close to threshold. This is the point at which symptoms are detected. Once the EPP is close to threshold, some EPP's are over threshold and fire an AP while some EPP's are below threshold and fail to fire an EPP. (Remember that the EPP varies by about 10%.) The result is muscle weakness -- the muscles still respond, but there are fewer contracting fibers per muscle. If nothing is done, the EPP gets even smaller until most EPP's are under threshold, most muscle fibers do not respond, and the patient dies (usually because the muscles that allow breathing fail). If the patient is treated to inhibit ACh esterase and/or to reduce the immune response, the size of the EPP increases and muscles function more normally. (Note that one treatment increases the effectiveness of the receptors while the other increases the number of receptors.)

An alternative acceptable explanation: From the results given, you might suspect that some synapses are seriously damaged and generate a very low EPP that is below threshold and so those muscle fibers do not respond. Other synapses have enough receptors left to generate an EPP over threshold, so those muscle fibers do respond. According to this solution, there are two classes of synapses -- those that are seriously damaged and those that are relatively normal. Therefore you should get either an EPP over threshold or a greatly reduced EPP at any one synapse. For all the synapses over all, the average EPP should be lower than normal. This is not the actual situation -- in MG the same muscle fibers respond to some neuronal AP's and not to others. All the synapses are significantly damaged. Each muscle fiber generates an EPP (in response to an AP) that is close to threshold every time -- sometimes the EPP is sufficient to trigger an AP and the muscle responds; at other times the EPP is below threshold and the same muscle fiber doesn't respond. It's not that some muscle fibers continue to respond and some never respond -- because some synapses are seriously damaged and some aren't. (This isn't the situation in MG, but from the information given in the problem, you couldn't rule it out.)

For more details on MG there is a review paper from 1994 at
http://content.nejm.org/cgi/content/full/330/25/1797#R158.

15R-6. A. *All of these*. All of these components are either attached to the plasma membrane of the cell (TCR, attached antibody, CD4, MHC's) or secreted by the cell (secreted antibody, perforins, cytokines, complement). Therefore all of these should be synthesized on ribosomes attached to the ER, co-translationally imported into the ER, and transported through the endomembrane system (ER --> Golgi --> secretory or default vesicles --> fusion with plasma membrane) to the surface of the cell. If the proteins are inserted into the ER membrane, they end up on the cell surface as part of the plasma membrane; if the proteins are released into the lumen of the ER they end up outside the cell.

B. *Cytokines* are the only paracrines listed. A paracrine is a secreted protein that acts locally as a signaling molecule -- it affects neighboring cells. It is not carried through the blood to its target (unlike an endocrine).

Perforins act locally, but are not considered signaling molecules. They are considered effectors -- molecules that actually do something, such as make pores in their target cells. (A paracrine might bind to a neighboring cell and trigger a series of events that leads to formation or opening of pores in the membrane, but the paracrine itself would not form the pores, unlike a perforin.)

C. MHC I, MHC II, & attached antibody. All nucleated cells have MHC I on their surfaces. All antigen presenting cells (& almost all cells of the immune system, but not necessarily all T cells) have MHC II on their surface. The MHC II (+epitope) on the surface of the B cell matches up with the TCR and other CDs on the surface of the corresponding T cell. Note that CD4 (& CD8) are made by T cells, not B cells. Naive B cells -- those not yet exposed to antigen -- make attached antibody, also called the B cell receptor or BCR. The point of the clonal selection theory is that B cells make antibody first, *before* exposure to antigen. Antigen binds to the BCR and triggers the cells to multiply and secrete antibody.

Answer to 15R-6, cont.

D. *MHC I and TCR.* The TCR on the surface of a cytotoxic T cell binds to the MHC I (+ viral epitope) on the surface of an infected cell. The result will be apoptosis of the infected cell triggered by either (a) perforins (plus enzymes) from the T cell or (b) juxtacrine signaling from the T cell. Juxtacrine signaling involves contact between a receptor on the surface of one cell and a ligand on the surface of another cell. Both the receptor and the ligand here (TCR & MHC) are surface proteins -- neither one is soluble, so surface-surface contact is required for receptor-ligand binding. As in this case, it is sometimes arbitrary which protein is the "ligand" and which protein is the "receptor." The T cell may "respond" to the MHC signal by secreting perforins; the infected cell may "respond" to the TCR signal by carrying out apoptosis.

Perforins are involved in the interaction between lymphocytes and infected cells, but they are not considered signaling molecules for the reasons explained above. They are also different from juxtacrines in that they are secreted by one cell before they are inserted into the membrane of another.

E. *Complement and secreted antibody*. Complement (in its inactive form) is always present in the liquid part of the blood (plasma) -- in the same way that inactive clotting factors are always present, waiting to be activated and used as needed. Secreted antibodies are also always present in the blood, even if a person is not in the midst of an acute immune reaction. The antibodies are left over (and/or produced at a low level by memory cells) from previous exposures.

Perforins and cytokines are soluble, secreted proteins, but they would not be found in the blood in this case. Perforins act locally, so they would not normally be found in the blood at all. In addition, they are only released as part of an immune response, which is ruled out here. Cytokines generally act as paracrines, but can sometimes be released into the blood and act as endocrines. However, their release into the blood is triggered by infections and other acute immune responses which are ruled out here.

The surface proteins of B and T cells will stick out into the plasma (the liquid portion of the blood) in whole blood, but isolated plasma does not contain any cells at all. So isolated plasma does not contain any cell surface proteins, only secreted proteins.

F. *a. MHC II; b. antibody; c. RME.* The sample that does not activate T cells has antigen attached to antibody -- the antigen has stuck to antibody on the cell surface, but since the cells are fixed (dead) the antigen cannot be internalized and digested. The antigen just sits on the outside of the cells, attached to antibody. In order to activate T cells, the antigen has to be "displayed" by MHC -- that is, it has to be attached to MHC on the cell surface. Antigen attached to antibody (as vs attached to MHC) cannot activate T cells directly. In order to get attached to MHC II, the antigen must first be internalized and digested. In B cells, internalization of antigen occurs by RME of the antigen-antibody complex. After internalization, the complex is digested and the pieces of antigen (epitopes or antigenic determinants) get attached to MHC II *inside* the cell. Then the MHC II-antigen complex gets sent back out to the cell surface. If the cells are fixed *before* antigen is added, RME cannot occur, the antigen cannot be displayed, and T cells cannot be activated.

The sample that does activate T cells is able to display pieces of digested antigen bound to MHC II. In this case, the cells were fixed later, so internalization, processing, and display of antigen occurred before the cells were fixed. Antibody bound whole antigen, and then the antibody-antigen complex was carried into the cell by RME. Inside the cell, the antigen was digested, the pieces bound to MHC II, and the MHC II-epitope complex sent back to the plasma membrane. Once the antigen was properly displayed, the cells could be fixed and still activate (bind to) T cells.

15R-7. A-1. surface immunoglobulin.
A-2. class(es) of immunoglobulin.
A-3. MHC I & MHC II.

B-1. Lolly.
B-2. a skin cell from Holly & a kidney cell from Polly.

C-1. be specific for the same antigen, & have the same amino acid sequence for at least half of the heavy chain.
C-2. none of these.

Explanation: A. B cells make surface immunoglobulins (BCR's) before they are exposed to antigen; they secrete immunoglobulins (antibodies) after exposure to antigen. Before meeting Ag, the B cells make surface antibodies of classes M & D. In any particular cell, both classes of antibody (M or D) have the same variable region. However each cell makes a unique variable region, by rearranging the DNA coding for the variable parts of the heavy and light chains. Therefore, the heavy and light chains of each cell are different. B cells make both MHC I (like all other nucleated cells) and MHC II (to display epitopes derived from Ag bound to the BCR).

B-1. The mitochondrial DNA will match that of the enucleated egg. Mitochondria are found in the cytoplasm, and the enucleated egg contributes all the cytoplasm.
B-2. The nuclear DNA sequence will match that of the donor nucleus. All the somatic cells in both Holly and the sheep that donated the nucleus will have the same DNA. (That is, have the same DNA sequence. We are ignoring any differences in telomere length or the conformation of the DNA.) However, the DNA in a naive B cell of Holly will be different from the DNA in the somatic cells of the same individual. That's because the DNA coding for the variable parts of the immunoglobulins will be rearranged prior to exposure to antigen.

C-1. All the antibodies you are isolating share the property that they bind to protein H. Therefore they are all said to be specific for the same antigen, namely protein H. Some may be less specific than others, that is, some may bind related or similar proteins better than others. But all share the ability to bind to protein H. However, the antibodies do not all bind to the same epitope, or piece, of protein H. The antibodies are specific for different epitopes of the protein.
Each cell will produce a different antibody because of DNA rearrangements. The variable regions will be different, so the heavy and light chains will not be the same. However the constant region of each heavy chain will be the same, because all the antibodies are of class M.
C-2. Holly and the nuclear donor have the same DNA. However, because of DNA rearrangements that have a random component, no two individuals are expected to produce exactly the same collection of antibodies, even if the starting genomic DNA is the same.