This packet is designed to give you guidance in preparing assignments for the course. I give tips and suggestions that I think may help you succeed, but ultimately the responsibility lies with you to be fully prepared for class, as well as to get questions clarified.

You may have noticed that although Human Anatomy is a suggested prerequisite for the class, it currently is not required for you to register for Biology 225. Although you will not be punished for not having this class, I strongly suggest you consider taking it before Biology 225. I will try to give enough background for you to understand concepts in lecture and lab, but there will be instances where I simply cannot, and the onus will lie with you to be prepared with the proper background.

Many assignments and concepts contained herein were the result of discussions with colleagues Cherryl Baker, Diane Sullivan, Sharon Daniel, Marc Perkins (Orange Coast College), as well as Dale Deslauriers (Chaffey College).
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Grading Criteria and Grading:

Grading criteria: Accuracy, clarity, completeness, use of English, including grammar and spelling.

Exams & Quizzes:
Lecture Exams: 4 @ 100 400
Lab Final Exam: 100 100
Dynamic Study Modules: 10 @ 10 100

Writing:
Lab Reports: 3 @ 50 150
Term paper: (due 11/25/14) 100 100
Journal Article Summary: 2 @ 25 50

Presentations:
Self Design Presentation: 50 50
Journal Discussion Presentation: 25 25

Misc:
Pre-lab worksheets: 5 @ 5 25
Post-lab results write-ups: 5 @ 10 50
Other assignments: ???

Total: 1050 + other assignments

Your final grade in the class will be weighted such that lecture exams will comprise 60% of your grade, with the remainder of course work making up the additional 40%.

I grade on a straight scale:
90-100% = A
80-89% = B
70-79% = C
60-69% = D
50% and below = F

The grade you earn is the grade you get!
Lecture and Reading topics:

Build your comprehension of each topic in stages, simple and broad at first. Add details later.

**Stage 1:**
What does it do? Physiology is about mechanisms. The way biological systems work. For each topic, the most basic level of understanding is to be able to state in clear, simple statements, what a system does. What does an automobile do? It moves people and small objects around on land. What does a skeletal muscle do? It shortens when stimulated, producing forces that draw two bones (or other structures) closer together. You should be able to say something simple and clear like this about every item in the course.

**Stage 2:**
How does it work? This is usually a longer answer. How does an automobile move? The complete answer is many pages of details about engines and transmissions. How does a muscle move? Likewise, a long story involving membrane excitability, calcium, ATP, and proteins with names like actin, myosin, etc. Learning to tell a story at this level is a lot like learning to play music. You cannot prepare to perform music by looking at the sheet music. You have to pick up your instrument and practice. Rereading your notes or your text won't help. You have to say it yourself and write it many times to be sure you can do it on a test. It helps to practice with a friend once you get pretty good. (P.S. This is how you learned to do everything you ever learned. Did you learn to write, or tie your shoe, or sew, or hit a tennis ball, or program computers by watching others do it? Humans don't learn passively. We learn by actively doing the thing as many times as it takes to get it right.)

**Stage 3:**
How is it regulated? Living systems control their activities. Once you can explain how a muscle contracts, your next level of understanding is to be able to explain how the organism regulates which muscle contracts, and when, and how strongly. This will usually be the most complex level of comprehension, involving multiple systems, feedback loops, and so on. Once again, you learn this kind of complex idea by repeatedly explaining it yourself, verbally and in writing.
Exams:

Lecture content: You must be able to explain in words and diagrams every concept presented in lecture. No topic is off limits. Learn everything, Stages 1, 2, and 3 for every concept.

Reading content: Each exam will feature questions from the reading assignment that were not addressed at all in the lecture. My expectations of you will increase with each exam.

Exam 1: Stage 1. You will be expected to be able to state in short, clear sentences what various items do in a biological system. ATP? Mitochondrion? Transcription? Can you state in one sentence what these things do? Can you do it with topics that were in the book but not in the lecture?

Exam 2: Stage 2. You will be expected to be able to explain in words and diagrams how systems work. Second messengers? Chemical synapses? Spinal reflexes? Can you tell a detailed story about how each of these items works? Can you do it with topics that were in the book but not in the lecture?

Exams 3 and 4: Stage 3. You will be expected to be able to explain in words and diagrams the regulatory mechanisms, feedback loops, etc. that control physiological systems. Heart rate? Ventilation rate? Urinary excretion of sodium? Can you explain the feedback loops that regulate each of these? Can you do it with topics that were in the book but not in the lecture?
Term paper:

This assignment is designed to allow you to explore in-depth an area of physiology that is of interest to you, as well as to give you experience conveying scientific thoughts and ideas in writing.

• You may write on any topic you choose, as long as it can be classified as physiology. Students often choose to write about topics that are largely anatomical or psychological; if yours fits better into these categories, consider realigning your thesis. You may always ask me for guidance if you are unsure of your topic.

• Your paper should be prepared as a review manuscript for *The Journal of Experimental Biology*. To format it correctly, get a copy of a review paper from *J Exp Biol* and copy the formatting (you do not need to do 2-column presentation--1 column is fine). The body of your paper should be about 3000-3500 words long.

• You may include as many references as you wish, but five of your references must be from reputable scientific journals or books and they must be less than two years old.

• This assignment is worth 100 points. Of those 100 points:
  
  o Correct formatting = 10pts
  o Appropriate topic (again, almost anything physiological is okay) = 10pts
  o Content = 50 pts
  o Spelling/grammar = 20 pts
  o References = 10 pts

• A hard copy of this paper is due no later than week 14 at the beginning of the first lecture. You may also be asked to turn in an electronic copy of the paper (instructions will be given in class).

I shouldn't have to say this, but I will. You absolutely must give your references credit for their work: be ultra-careful about in-text citations and your reference list. Make sure to rewrite thoughts in your own words, and remember that using quotes are almost never acceptable in an assignment like this.
Experimental Design: a (really) quick primer

In lab, we will be talking about and using the scientific method a lot this semester. While it may seem intimidating at first, the scientific method—and science in general—is just a way a knowing, a way of making inquiries about the natural world. A core principle in the scientific method is experimental design.

Any good experiment will have several things in common:

1. **Control.** In an ideal world, everything would be the same between treatment groups except for the treatment itself. We know this is not true simply by looking around us—variability exists between individuals. However, a carefully designed experiment will make every effort to minimize variability between test subjects. What this means is that you do your best to treat every test subject as similarly as possible. For instance, if you were doing an experiment on mice, you would give every mouse the same food, water, bedding, etc. **In this context, ‘control’ does not refer to having a “control group.” That may not necessarily always be an ingredient of good experimental design.**

2. **Replication.** If we could exercise perfect control over our experimental subjects, we would only need one individual in each group. Any differences we observed between them would be due to the experimental treatment. Again, we know this is not the case; not even identical twins are perfectly controlled. Replication refers to having multiple independent experimental units in each treatment group. This does not refer to repeating the experiment multiple times, although you should be able to do that and get identical results.

3. **Randomization.** So, we have controlled our experimental units and have multiple units in each treatment group. What if there are “invisible” traits that have congregated in one group? Back to the mouse experiment, what if there is some unknown mouse trait that ends up congregating in one experimental group? Would this skew our results? In order to minimize this, we must randomly allocate the mice to experimental groups to try and ensure that the experimental groups are not inherently different.
There are several terms associated with experimental design you should define and understand the definition of.

Hypothesis
Independent variable
Dependent variable
Control group
Experimental groups
Constants
Trials
Variables

Practice: Write a hypothesis for each of the statements and identify the variables, control group, and experimental group.

1. Eating breakfast increases performance in school.

Hypothesis: If _______________________, then ____________________________.

Independent variable: ________________________  Dependent variable: ________________________

Control group: __________________________  Experimental group: ____________________________

2. Hummingbirds are attracted to the color red.

Hypothesis: If _______________________, then ____________________________.

Independent variable: ________________________  Dependent variable: ________________________

Control group: __________________________  Experimental group: ____________________________
3. *MacBook Pro* batteries last for 5 hours.

Hypothesis: If ______________________, then ________________________________.

Independent variable: ________________________ Dependent variable: ________________________

Control group: __________________________ Experimental group: __________________________

4. The deer population decreases in winter due to a lack of food.

Hypothesis: If ______________________, then ________________________________.

Independent variable: ________________________ Dependent variable: ________________________

Control group: __________________________ Experimental group: __________________________
Lab exercises:

I have several objectives for each lab exercise:

1. You will learn how to study some process. This means you must learn some method of measurement. In physiology, this almost always means hardware, buttons and dials, chemicals and measurement, etc. *I expect you to know how to run every piece of equipment and perform every measurement by yourself.*

2. You will execute some measurements, perhaps in the form of a miniature experiment, perhaps simply to make some measurements and observations. *You are expected to record all data from these measurements in your lab notebook.*

3. You will learn to analyze and interpret the data you collect. This will include some graphing, some algebraic or geometric computation, some computerized statistical analysis, etc. *I expect you to understand how to interpret the results of every measurement we do.*

4. We will spend time discussing further possibilities with each technique. You should keep your imagination alive, and try each week to see what new questions can be devised and answered with the methods you have learned.
Lab Reports:

TITLE: Write a title that describes the nature of the exercise. For example: “A Comparison of the Buffering Capacities of Bicarbonate, Protein, Serum, and Blood.”

INTRODUCTION: Introduce concepts that underlie the events to be measured and the reasons for wanting to measure them. Provide a logical framework for the reader to understand the physiological principles under investigation, and what kind of information is expected to come out of the experiment. **Make clear the connections between the principles you have explained, the measurements you intend to make, and the question you are about to propose.** The last sentence of your introduction must be a specific statement of the experimental question at hand.

MATERIALS AND METHODS: This section must be a complete description of everything you did. The best structure for the M&M section is a chronological narrative of the procedures and measurements. Equipment used, measurements made, experimental conditions, etc. should be included. Lists of equipment and materials are generally unnecessary if the narrative is complete. There should be enough information here that a peer could follow the directions and do exactly what you did. When you use information published elsewhere (e.g. in the lab handout or some other text) you must cite the source in the text AND include the source in your Reference section. Generally, you need not explain every move you make on a computer or a lab instrument. A one liner such as “pH was measured with a Corning Model 10 pH meter.” is usually sufficient. This way, you don't have to write three pages of instructions that are in print elsewhere.

RESULTS: This section contains the data. All the data. It also contains any graphs, charts, statistical analysis, etc. that comprise the analysis of the original data. This section specifically does not contain any interpretation of the data, nor any conclusions.

DISCUSSION: This section begins with interpretation of the data. Refer to the results section to specifically document the conclusions you draw. Discuss the relationship of the results to the principles you presented in the Introduction. This is the part of your report where you make sense of the data, and relate it to the physiological concepts that you learned in the process of carrying out the study. You have some freedom here to speculate on possible explanations for observations you made. You may also want to discuss sources of error, ideas for further experimentation, etc. I MOST CERTAINLY DO NOT WANT TO READ that the lab exercise was fun and you liked it. You are learning a formal style of writing here. If you liked it or disliked it, tell me in person, but don't put it in your lab report.
REFERENCES: In composing your lab reports you are almost certainly going to have to refer to some resources. At the very least, you will want to read the appropriate sections of your textbook. For your Methods section, you will have used your lab handout. List these resources in correct literature citation format. Use the guidelines below to help you cite references correctly:

**Introduction: the basics of literature citation**

Citing literature properly is key to writing well. While I’m sure that you’ve seen literature citation discussed in English classes, I’ve created this document to describe how literature citation applies to scientific writing (and this course). The basic idea behind literature citation is this: when you put your name on a paper, readers assume that you came up with every idea, and all the wording, found in your paper. Anytime you didn’t come up with an idea or specific wording in your paper, you need to indicate this to your reader by properly citing the information.

There are two basic requirements of scientific literature citation:
- Anytime you take information from another source you must cite that source at the end of the sentence that uses the source.
- Anytime you take exact wording from a source you must put the copied wording in quotes and include a citation at the end of the quotation.

These two requirements apply regardless of the source of your information: your source could be a friend helping you, a website, a textbook, a magazine article, a comic book, or an Abyssinian tablet.

In the next couple of pages, I’ll describe the literature citation format that your instructor expects you to use in this course. This citation format is based on those used in biological journals, and is similar to that used in all scientific writing. As a side note, biological writing has no standard format used by all journals; the style I describe is based largely on Pechenik’s (1997) guide (with some additions from Hacker 1992). Note, however, that different disciplines may have extremely different formats for references, and thus you should always check with someone in the field (or check a field-specific journal) to determine an appropriate style.
How to cite references in the body of your paper

When citing sources within the text of your paper you need to list the author and year of publication of the work you’re citing. This information is typically put in parentheses at the end of a quotation or at the end of a sentence that contains information taken from a source.

For example if Dr. Adolph was the author of a 1999 article you are referencing, you should write the sentence mentioning his work followed by (Adolph 1999). If there were two authors on the article, you list both authors (Kirkton and Greenlee, 1999), while if there were more than two authors you should state the name of the first author followed by et al. (Harrison et al., 1999). If you have multiple references for a single sentence, list them all at the end of the sentence and separate them by a semicolon (Adolph, 1999; Harrison et al., 1999; Kirkton and Greenlee, 1999).

If you discuss the author(s) of the reference you are citing in the text of the sentence, you can simplify your citation by simply following the author’s name with the year in parentheses. For example, “Frazier (1999) found something really cool.” Note that this style of in-text citation puts the focus of the sentence onto the people who did the work, rather than the work itself. In general, it’s better to focus on the actual work rather than the people who did the work, so don’t over-use this style of citation.

If you are citing information that has not been published, but which you learned directly from a person (e.g., it’s something you learned from a friend, or something a professor told you), you can cite the person by putting “pers. comm.” after their name. For instance, if Art Woods told you something you needed for your report, you might describe it and then say (Woods pers. com.). Note, however, that “pers. comm.” references are not a good way of citing information, and whenever possible you should find the same information in a published source and cite that source instead of using a “pers. comm.”

This table shows the proper format for in-text citations:

<table>
<thead>
<tr>
<th>Number of authors</th>
<th>Style</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>(Author, year)</td>
<td>(Russell, 2006)</td>
</tr>
<tr>
<td>Two</td>
<td>(Author 1 and Author 2, year)</td>
<td>(Russell and Elliott, 2006)</td>
</tr>
<tr>
<td>Three +</td>
<td>(Author 1 et al., year)</td>
<td>(Russell et al., 2006)</td>
</tr>
</tbody>
</table>
How to list references at the end of your paper

In addition to listing the author and year of your source in the body of the paper, you must have a “Literature Cited” or “References” section at the end of your paper that includes the full citation of all sources used in your paper. The full citation must include enough information so that any person with access to a reasonable library could easily find the exact source (or portion of a source) that you used.

Always organize the references section alphabetically (by first author’s last name) and then chronologically. If you have multiple sources from the same author in the same year, append lowercase letters to the year to differentiate them (e.g., Benford, 1999a; Benford, 1999b).

Below are details on how to cite common types of materials. For each type of material I’ve listed the general format, an example of how to cite a specific reference in the body of your paper, and a specific reference as it would appear in a literature cited section.

Journal Article

*General format:* Author last name, initials, other authors. (Year). Title of article. Journal name. *Volume,* pages.

*In-text example:* A neat paper a few years ago looked at lizard growth (Sinervo and Adolph, 1994).


Note: This format is also used for citing popular magazine articles, the only change being the addition of the issue of the magazine in parentheses after the volume.
Book

**General format:** Author last name, initials, other authors. (Year). Title of Book. Publisher: place of publication.

*In-text example:* I might want to cite my favorite college biology textbook (Purves et al., 1995).

*Full citation example:*

**Lab manual or course handout**

Treat most non-published course handouts and lab manuals as books written by their respective authors, replacing the publisher and place of publication with the academic institution of their author(s).

*In-text example:* If you wanted to cite a handout I gave in my spring 2014 Biology 225 class, you would cite it this way (Russell, 2014).

*Full citation example: Russell, G. (2014). Biology 225 course handout. Orange Coast College: Costa Mesa, CA.*

**Person**

**General format:** Person’s name. Personal Communication - venue. Institution. Date.

*In-text example:* If I told you something interesting you should reference it like this (Russell, pers. com.).

Items from the web

There is currently no biological standard for citing information from websites, primarily because journals do not allow most web citations. The web can also be difficult to cite because at times no author or date of publication is listed, and pages can change frequently. Thus we have two possible citation formats for web-based material:

Web pages with author and publication date information:

*General format:* Author last name, first name. Date. “Title of source page.” Title of Site. Complete source URL. Accessed date.

*In-text example:* Ned Flanders is a character on the Simpsons (Paakkinen, 2001).


Web pages without author and publication date information:

*General format:* Title of site, accessed date. “Title of source.” Complete source address.

*In-text example:* True bugs are also called hemipterans (Tree of Life web project, 2006).

Notes for web references

- Always include the URL for the specific page you got information from; do not list the URL of the base page of the site.

  To clarify: Your reader should be able to type the URL you include in your literature cited section into a web browser and be taken directly to the page you used.

- If you use multiple pages from a single site, include each page as a separate citation (unless the pages are all clearly linked together, such as in a magazine article that’s been split onto multiple web pages).

- You can always find the title of a webpage; it’s either the most prominent text at the top of the page, or the text that appears above the menu bar of your web browser. If a webpage doesn’t have a title, it’s been coded very badly.

- The title of the site can often be found on the base page of the website; if it’s not clear what the title is, choose the closest thing you can find on the site.

- Since webpages can change frequently, include more specific date information than you would for other sources (e.g., include at least the month and year of publication, and always include the date you accessed the material).

- If you are citing a peer-reviewed journal, magazine, newspaper, or other source that can also be found in print, but you found the information online, cite the source as though it were obtained in print. If you’re not sure if the information can be obtained offline, cite it as a web source.
Instructions for brief review of Journal Paper:

Find a journal paper from the last two years (2009-2011) on a topic that has been covered (or at least mentioned), or will be covered, in class. Any peer-reviewed scientific journal is fine, but the paper must involve at least some physiology. Also, the paper must be an original study, not a review paper.

You may not use the same paper that you have used elsewhere in this course!


Turn in a copy of the entire paper and answers to the following questions:

1. Full citation of the paper (author(s), year, title, journal, volume, pages).
2. What specific hypothesis/hypotheses were the authors testing?
3. Why did they think this was an interesting or important issue (relevant background information and their rationale)?
4. Briefly describe their experimental design and methods (Don’t worry about minor details—e.g., time of day, drug doses, sample sizes).
5. Briefly describe their results.
6. What did they conclude?
7. Do you agree with their conclusions? Why or why not?
8. What would be two interesting follow-up questions to ask? Don’t just say to try the same thing in the other sex or in another species, unless you can offer a really compelling reason to do so.

Your answer to each question should be no more than 3-4 sentences, and your entire report must be no more than one page. Please make sure to discuss the journal paper in your own words! That is, do not just copy sentences directly from the paper.

*****Your report must be typed, not hand-written!*****

Points will be deducted for spelling errors, poor grammar, and sentence structure, etc.
Journal Article Discussions:

In any discussion of science, it is important to be able to understand scientific literature.

We will be discussing (as a class) several papers this semester. Each discussion will have two components:

1. One lab group will make a short (15-20 min) presentation to the class, summarizing the major points of the paper. That group will then facilitate a discussion on the paper, attempting to answer questions from the class, and relating the material to what we’ve been talking about in lecture. While the discussion will be mediated by your instructor, the content and direction are largely up to you. The content and quality of your presentation are worth 25 points; you will be graded as a group.

2. In order for this to be a learning experience, you simply must be an active participant. Choosing not to be involved with the discussion will result in the loss of participation points.

The journal articles will be chosen by your instructor, and will be available for download before the date of the discussion.

How can you be an active participant in the discussion?

1. Read the paper. This should go without saying, but even if you don’t understand it, having read it will help you to gain a better appreciation of what was done.
2. Make notes. Underline parts of the paper you understand and highlight the parts you don’t (for example). Make notes in the margins, or use a separate sheet of paper. Don’t just summarize the paper (the authors have already written an abstract), but try to make connections between it and things you know already. Try to make connections to the bigger picture.
3. Ask questions. It’s okay to not understand something. If your other classmates say they understand it completely, they’re lying to you. The point of these discussions is to help us all arrive at a better (but not complete) understanding of a topic.
4. Contribute to the discussion. Even if it’s something you know only a little bit about, contribute it. Really.

You will have an online quiz after each discussion, and participation points may be assigned based on how much you contribute. I would expect that everyone would have at least two separate items to contribute to the discussion, whether it’s an opinion, question, or a connection to another aspect of physiology. Asking a question that you can look up the answer to (e.g. What was their sample size in this experiment?) won’t get you very far—that is childish, and reflects a lack of preparation.
Enzyme Kinetics of Salivary Amylase

Enzymes accelerate chemical reactions. The speed of an enzyme catalyzed reaction is influenced by a great many factors, for example, concentrations of enzyme and substrate, temperature, pH, presence of assorted coenzymes and cofactors, inhibitors of various types. The study of an enzyme's kinetics is the measurement of the enzyme's activity (that is, its rate of reaction) and its response to the influence of factors such as those mentioned above.

Salivary amylase (also called ptyalin) hydrolyzes various starches, such as amylose and amylopectin. These two starches are polymers of glucose. Amylose is a long unbranched chain of glucoses. Amylopectin contains many branch points in the chain. These molecules are so large that they cannot be directly absorbed by the digestive system. Amylase begins the digestion of starch by splitting the sugars off these starches in pairs. The pairs of glucoses become a disaccharide called maltose. The enzyme cannot get close enough to the branch points to completely dismantle amylopectin. The resulting fragments containing the branch points are called dextrins.

The goal of this lab exercise is to measure the rate of this process, and to try to discover the rules that govern the rate. Factors that may be of interest include enzyme concentration, starch concentration, temperature, pH, regulatory responses, to mention a few.
Week 1: Principles of colorimetry, and generation of a standard curve.

Physiologists often need to know the concentrations of materials in solution: sugar, lipids, proteins, etc., in blood or urine, or concentrations of reactants or products in an experimental chemical system. The colorimeter (Spectronic 20) measures concentrations of lots of substances. If a substance is colored, or if I am a smart enough chemist to give it color, then the intensity of the color will depend on the concentration of material. For example, I can make starch green by adding iodine to it. The more starch, the greener it gets. The Spec 20 can measure this precisely.

Since there is no magical way to state how green a given concentration of starch would be, we must make a set of starch specimens whose concentrations we know. If we then read the intensity of their green in the Spec 20 they can be used to create a scale by which to judge the concentrations of other starch specimens. Such a scale is called a standard curve. Then we can use this set of standards for comparison to any starch specimen we want. You will use the Spec 20 to watch starch disappear under the action of salivary amylase. The standard curve will permit you to precisely measure how fast it disappears.

Each group needs the following materials:

- Starch solution: 0.5 g/l
- Iodine solution
- Buffer pH = 7.0
- Distilled water
- Pipettes: 1 ml capacity
- Test tubes: 7 clean ones

Take three of your test tubes and get about 5 ml of starch, iodine and buffer from the containers on the side counter. (Not 5.000 ml! Just pour about two inches worth of each so you can have a supply at your desk.)

Preparation of starch standards: serial dilution

Take four clean test tubes and number them 1-4. Add the ingredients exactly the sequence listed below.

1. Tubes 2, 3, and 4: Add 1.0 ml of buffer to each.
2. Tube 1: Add 1.0 ml of 1 g/l starch.
3. Tube 2: Add 1.0 ml of 1 g/l starch. Mix.
4. Remove 1.0 ml from tube 2 and transfer it to tube 3. Mix.
5. Remove 1.0 ml from tube 3 and transfer it to tube 4. Mix.
6. Remove 1.0 ml from tube 4 and discard it.
7. Tubes 1, 2, 3, 4: Add 1.0 ml of iodine to each.
8. Tubes 1, 2, 3, 4: Add 8.0 ml of distilled water to each. Mix.

Now think about what you just made. What are the concentrations of starch in each tube at the end of step 6? What do the colors of the tubes mean to you? Why do you suppose I had you dilute it with all that water in step 8?

Setting up the Spec 20:

1. Turn on the Spec 20 if it isn't already on. The dial on the left front controls the power switch. Warm up for at least 15 minutes.
2. Set wavelength dial to 660 nm. This is the dial on the top right.
3. See that the sample chamber is empty, and close the lid.
4. Use the LEFT hand dial on the front of the machine to set % Transmittance to 0.0
5. Insert a cuvette containing water into the chamber. This is called your blank. Close the lid.
6. Use the RIGHT hand dial on the front of the machine to set % Transmittance to 100.0 (Absorbance to 0) (Repeat steps 3-6 at least once to verify your settings. Keep at it until it reads 0% and 100% with no adjustments.) A little drift is unavoidable. If you can't get 100.0%, 99.8% is good enough.
7. Insert a cuvette containing your specimen into the chamber and close the lid. DO NOT ADJUST ANY DIALS. If you make a mistake and turn any dials, you must go back and repeat steps 3-6 to recover.
8. Read the Absorbance number from the analog display.
Read the absorbances of your standards:

1. Pour some of tube 1 into the empty cuvette. Fill it about half full. Insert this into the machine, close the lid and read the absorbance. Write down the result.
2. Pour the liquid back into tube 1 and gently tap to get the big drops out.
3. Recheck your blank. It is good practice to check the blank before and after each reading. It may require readjustment. If it never changes, you may omit this step once you trust your machine a lot. Just verify it after the last reading. If it changed, you have to repeat them all.
4. Read absorbances on tubes 2, 3, and 4 just as you did for tube 1. Record the number of the machine you used. Use the same one every time.

The standard curve:

You will make a graph of your results: [Starch] vs Absorbance. It will look like the one shown here. This graph will be your tool for estimating starch concentrations next week. If you have a sample of starch whose concentration is unknown, you can mix it with iodine and water as you did the standards, read its absorbance in the Spec 20, and find its starch concentration using the graph. Next week we’ll use this to find out how fast starch disappears under the action of amylase.
**Week 2: Kinetics of Salivary Amylase**

Now that we have a standard curve, we can measure starch concentration at any time we choose. This makes it possible to measure the rate that starch is digested by amylase. The reaction rate is generally so fast that we must dilute the saliva in order to slow it down to a rate we can measure. Typical dilutions might be 1:5, 1:10, 1:20, 1:100. Since the amylase activity of saliva is unpredictable, the dilution that works best for this experiment will not be the same between individuals—you will need to experiment.

**Rate measurements:**
1. Get six test tubes and label them 1 through 5, and label the remaining tube “control”.

2. Pipet 1.0 ml of iodine solution into all six test tubes. Do not use this pipet for anything else.

3. Pipet 7.0 ml of starch solution into a clean test tube labeled “rxn”. Also pipet 1.0 ml of starch solution into the control tube. Set the control tube aside, so it does not get in your way for the next steps.

4. Get two 1 ml pipets, a sample of the diluted saliva, and a clock with a second hand.

5. This is a timed run. Be ready. Add 1 ml diluted saliva to the rxn tube. Note the time. Stir quickly.

6. At 15 seconds, draw 1.0 ml of the saliva-starch mix into a pipet. Watch the clock. At 30 seconds, quickly drain the pipet into tube #1. Do not use this pipet for anything else.

7. Working as a precision team, repeat step 7 to deliver 1 ml of the saliva-starch mix into each of the remaining tubes at 60 sec, 2 min, 4 min, and 8 min. Use the same pipet for all five samples. *(NOTE: These times are approximate. Use your brain. If tubes # 1, 2, and 3 are the same color as the control, you might want to delay tubes 4 and 5 to give the reaction more time. If you miss your intended time, just record the actual time. Also, note that the times are total elapsed time from the beginning, when you added saliva to the starch, not times from the previous sample. So the entire run, start to finish, normally takes 8 minutes.)* When the sampling is done, pipet 8.0 ml of water into each of the five tubes, and into the control tube.

8. Take your tubes to the Spec 20. Calibrate the scale the same way you did last week, and read the absorbance at 660 nm for each of your five samples. Record the data.

9. Using the standard curve you made last week, find the concentration of starch in each sample.

10. Make a graph of starch concentration vs. time.

**Interpretation:**

A large dilution factor means a low enzyme concentration. Look at your results. What is the relationship between total elapsed time and the rate the starch disappears? What is the relationship between enzyme concentration and reaction rate? Is the answer clear-cut? Can you think of variables we did not control that may affect the reaction rate? Can you see how to invent an experiment to measure the importance of these other variables? Can you see ways to improve the quality of repeatability of the results?
Using Neurosim to Understand Functional Properties of Neurons

Neurosim is a computer program that will help us understand neuronal function in a more hands-on way than simply learning about neuronal properties in lecture.

During week one, we'll look at some basic properties of neurons.

The Passive Conduction Model simulates the passive, non-spiking conduction properties of a neuron into which four microelectrodes have been placed along the length of an axon. You’ll be visualizing a graded response here that varies with the intensity and time course of the stimulus and spreads without regenerating itself.

You can alter the position of electrodes, the amplitude and duration of the current pulse, as well as axon parameters such as membrane characteristics or diameter of the axon.

1. From the start menu, start Neurosim and select the Passive Conduction Model.
2. Press start to run the experiment. Acquaint yourself with the position of the current stimulus, the amplitude of the response, and the resulting pulses at each of the recording electrodes. There will be two pulses shown.
3. We want to observe just one pulse for the first part of this exercise. On the “Setup” screen, set the amplitude of pulse 2 0.0 nA. Press enter.
4. Press start to run the experiment again. You will now see just one response. Using the “Measure” tool on your “Results” screen, measure the voltage of the response, and the time base of the response using your cursor. You’ll see the results popping up on the screen as you click your mouse.
5. Now, change the pulse amplitude to 2.0 nA and press start to run the experiment again. Note the superimposition of the two traces. Measure the differences in the amplitude between the various recordings. What are the effects of changing the pulse amplitude?
6. Now press clear to clear the results of this experiment. Change your pulse width to 10.0 ms. Press start to run the experiment. What are the effects of changing the pulse width (duration)? Use the cursor to make your measurements.
7. Return to the default values and run the program with two stimuli. Is there any effect on the second pulse when amplitude or duration of the stimulus are altered? Why?
8. Change the position of the electrodes by increasing the distance from the initial stimulus to the last electrode. Run the simulation. **What does this tell you about a graded response? Would this response be indicative of an axon that can transmit stimuli over a long distance?**

9. Build your own experimental axon by altering the membrane characteristics and dimensions. Change the axoplasmic resistance, membrane resistance, membrane conductance, and axon radius. Run the simulation changing each parameter separately. **Which of these parameters affects the response of the axon more? Why?**

For the next part of this exercise, you’ll use the Hodgkin-Huxley Model, which looks specifically at the voltage-gated channels present on the neuron and examines their function.

The simulation runs in two modes: unclamped and voltage clamped. In the former mode, two pulses of specified amplitude and duration can be injected into the neuron. The membrane voltage response, as well as current and conductance changes are displayed. You can use this mode to observe the relationship between threshold stimulus strength and duration and the refractory period. Similarly you can also use this mode to understand the role of each ion in conducting a nerve impulse.

1. Briefly scan the experimental parameters. As with the previous experiment, we want to make our observations on a single pulse. Achieve this by changing the stimulus amplitude of the second pulse to 0.0 μA. Run the experiment.

2. Observe the various parameters defining the action potential generated.

3. **What are the ionic properties of the above parameters? Where do the voltage gated sodium and potassium channels become activated? Why do they become activated?**

4. Return to the experimental parameters and try changing some of the parameters affecting the action potential (e.g., the concentrations of the external ions or the strength of the stimulus). What are the effects of these changes?

5. Return the parameters to the initial values displayed when you first entered the Hodgkin-Huxley Model. Run the experiment.

6. Measure the amplitude of the first and second evoked action potentials, using resting membrane potential as the baseline.

7. Why might the second action potential have a lower amplitude than the first? What does this tell you about the inter-stimulus interval? Is it within the absolute or refractory periods?
8. Using a maximum stimulus pulse of 500 μA for the second pulse, determine the absolute refractory period.

9. Change the value of the second stimulus pulse to 50 μA (the same as the first) and determine the relative refractory period.

10. Turn off the second stimulus again, this time testing the effects of the three different drugs separately. For each one, describe the effect on the action potential waveform and then determine the specific effect of each drug. Remember that voltage-gated sodium channels have separate activation gates (these are called m gates) and inactivation gates (h gates), while potassium channels have only activation gates (n gates).

11. Remove the drugs and test the effect of doubling the concentration of extracellular potassium (from 10 to 20 mM). Did the stimulus evoke multiple action potentials? Why?


For week two, we’ll follow the directions below. By now you should be familiar with the program, but I still include some tips to make things a bit easier. These exercises will all be done using the Hodgkin-Huxley Model you used in the second part of week one.

First, you are interested in the role that sodium and potassium play in the conductance of an action potential.

1. Start Neurosim and select “Hodgkin-Huxley” model.
2. With the “Setup” window active, do the following:
   a. Set pulse 1 to 100 microamps, and pulse 2 to 0 microamps.
   b. Set the duration of pulse 1 to 0.5 ms.
   c. Change the temperature of your bath to 25°C
3. With the “Results” window active, do the following:
   a. Scale the x-axis to 3 ms by clicking on the red “10,” and keying in “3.”
   b. Click on View → Trace Display and uncheck the axis boxes for “Stimulus,” “Conductance,” as well as the box for Potassium current.
4. Hit “Start” and run the experiment.

What do you see here? On the voltage axis does the trace look familiar? How does the movement of sodium across the membrane (as indicated by current) correspond with your knowledge of an action potential? Note that in a real experiment sodium current cannot be directly measured, but here it is calculated from underlying equations.
5. The next thing we want to do is put a horizontal datum line at the Equilibrium Potential for sodium (ENa+). You will need to calculate ENa+ using the Nernst Equation in your book. However, we are doing this experiment at 25°C instead of 37°C so you will need to replace the “61” with the constant using a temperature of 25°C. You’ll need the information contained in Appendix B of your book to do this. Ask your instructor for assistance if you need it. You can get the internal and external sodium concentrations from the “Setup” screen.

Tip: watch your units here!

Write your calculated ENa+ here: ______________ mV.

6. To add a horizontal datum line, go to View → Datum Lines → Add horz. Your cursor will now appear as an arrow and you can place your datum line near ENa+. Using the “Measure” tool, you can place your datum line exactly on ENa+.

Where does your datum line appear compared with the peak of the action potential? Are they close to one another? From an ionic point of view, what is happening at the peak of the action potential?

7. Now, return to your Setup View without clearing the Results View. Change the external sodium concentration from 418 to 836 mM (double it), and run an experiment. Now halve the value (209 mM), and repeat, halving the concentration in steps to a level of 52 mM.

8. At each sodium concentration, calculate the ENa+. What do you notice about the relationship between the peak of the action potential and ENa+?

9. Finally, you can use the “Measure” tool in your Results View to measure the voltage at the peak of each of your action potentials.

Plot a graph of both the peak action potential amplitude AND ENa+ against the log (base 10) of sodium concentration. For every 10-fold change in sodium concentration what changes do you observe in peak action potential amplitude and ENa+?
Interpretation:

Your graph of ENa+ versus log [Na+] is what the Nernst equation predicts what would happen if your neuron were only permeable to sodium. The fact that peak action potential amplitude comes very close to ENa+ tells you that the action potential is largely sodium dependent. **However, the peak is always lower than the predicted value. Why?**

Finally, repeat the experiment above by using the default sodium concentration (418 mM) but by varying extracellular potassium concentration. What do you note that's different? **Would you say an action potential is primarily sodium- or potassium-dependent?**

An excellent resource to help you understand all of the neuronal properties we've investigated here can be found here at http://neuroscience.uth.tmc.edu/index.htm
Endocrine Physiology

The endocrine system uses chemical messengers known as hormones to allow cells within the body to communicate with one another. Hormones are secreted by endocrine glands into the bloodstream where they travel to target cells, binding receptors and effecting a response.

The hypothalamus is critical to our endocrine system because it produces hormones that regulate the anterior pituitary gland (the “master gland” of the body) as well as hormones that are secreted via the posterior stalk of the pituitary gland (oxytocin and vasopressin). The hormones that regulate the function of the anterior pituitary are thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and adrenocorticotropic hormone (ACTH); they are all secreted into a specialized blood vessel system called the hypophyseal-hypothalamic portal system.

The figure to the right shows the relationship between these organs and hormones.
This lab exercise focuses on the control pathways of three hormones: thyroid hormone, cortisol, and testosterone. The pathways are shown below; make sure to review your lecture notes and textbook to fully understand each of these pathways.

A surplus or deficiency of hormones can result in disease conditions, but it is important to remember that a constant stimulation of a gland will result in hypertrophy of that gland, and a lack of stimulation will result in atrophy. Also, remember from lecture that hormones are regulated by negative feedback (illustrated above). Positive feedback loops are rare, but present in our biology.
Endocrine Physiology Question set #1

1. Describe the relationship between the hypothalamus and anterior pituitary gland.

2. Why is the anterior pituitary gland called the master gland of the body?

3. What is negative feedback?

4. Describe the effects of thyroid hormone.

5. Describe the effects of cortisol.

6. Describe the effect of LH in both males and females.

7. Describe the difference between hypertrophy and atrophy.

8. Consider the differences between hyperthyroidism and hypothyroidism. What are some characteristics of each?

This concept map may help you in completing this exercise.
Endocrine Lab experimental data

The data for this lab were compiled from seven sets of male lab rats, two rats per set; one set was the control group and the remaining six were experimental groups. The rats were all male to simplify the study of the relationship between the reproductive system and endocrine system. In each set of rats there was an “intact” rat and a “castrate” rat. The castration involved removal of the testes to eliminate testosterone production. The two rats (normal and castrate) of each group were treated alike in all other aspects. All rats, except for those in the control group were injected with a hormone on a daily basis for two weeks. Autopsies were performed on the animals at that time.

The group of students performing this exercise were very disorganized and rushed through the work, making errors in labeling the bottles of hormone. The students obtained the following results for organ weights after the autopsies were performed. They noted amazing changes in the size of certain organs when they compared the experimental group of rats with the control group. Using the flowchart (previous page), the table below, and the autopsy data, match the unknown rat groups with their respective hormones. The bottles on the refrigerator shelf were ACTH, cortisol, LH, TSH, TRH, and testosterone.

To help you determine the identity of the unknown hormones, look for changes between the control values and the values of the unknown hormones (both the intact and castrate animals). The changes in organ mass between the control and experimental rats should be > 20% if they are to be considered biologically significant. If the change is < 20%, it is attributed to experimental or biological error.

<table>
<thead>
<tr>
<th>TRH</th>
<th>TSH</th>
<th>ACTH</th>
<th>Cortisol</th>
<th>Testosterone</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Castrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Castrate</td>
<td></td>
</tr>
</tbody>
</table>

A + denotes an increase in size. A − denotes a decrease in size. Place the letters NC in the box where no change occurs. TRH, thyroid-releasing hormone; TSH, thyroid stimulating hormone; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone.

Comparison of hormonal effects on different organs.

Use this table to help you, but complete it before looking at the experimental data.
<table>
<thead>
<tr>
<th></th>
<th>Control Rat</th>
<th>Hormone 1</th>
<th>Hormone 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>12.9 mg</td>
<td>10.1 mg</td>
<td>9.8 mg</td>
</tr>
<tr>
<td>Thyroid</td>
<td>250 mg</td>
<td>245 mg</td>
<td>250 mg</td>
</tr>
<tr>
<td>Thymus</td>
<td>475 mg</td>
<td>250 mg</td>
<td>480 mg</td>
</tr>
<tr>
<td>Adrenals</td>
<td>40 mg</td>
<td>100 mg</td>
<td>40 mg</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>500 mg</td>
<td>Seminal vesicles: 490 mg</td>
<td>Seminal vesicles: 900 mg</td>
</tr>
<tr>
<td>Prostate</td>
<td>425 mg</td>
<td>430 mg</td>
<td>800 mg</td>
</tr>
<tr>
<td>Testes</td>
<td>3200 mg</td>
<td>3000 mg</td>
<td>5700 mg</td>
</tr>
<tr>
<td>Body weight</td>
<td>300 g</td>
<td>200 g</td>
<td>385 g</td>
</tr>
</tbody>
</table>

**Castrate**

<table>
<thead>
<tr>
<th></th>
<th>Control Rat</th>
<th>Hormone 1</th>
<th>Hormone 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>12.9 mg</td>
<td>10.1 mg</td>
<td>13 mg</td>
</tr>
<tr>
<td>Thyroid</td>
<td>250 mg</td>
<td>250 mg</td>
<td>250 mg</td>
</tr>
<tr>
<td>Thymus</td>
<td>480 mg</td>
<td>250 mg</td>
<td>480 mg</td>
</tr>
<tr>
<td>Adrenals</td>
<td>40 mg</td>
<td>95 mg</td>
<td>42 mg</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>450 mg</td>
<td>Seminal vesicles: 410 mg</td>
<td>Seminal vesicles: 412 mg</td>
</tr>
<tr>
<td>Prostate</td>
<td>387 mg</td>
<td>380 mg</td>
<td>375 mg</td>
</tr>
<tr>
<td>Body weight</td>
<td>270 g</td>
<td>195 g</td>
<td>275 g</td>
</tr>
<tr>
<td>Hormone 3</td>
<td>Hormone 4</td>
<td>Hormone 5</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Pituitary:</strong></td>
<td><strong>Pituitary:</strong></td>
<td><strong>Pituitary:</strong></td>
<td></td>
</tr>
<tr>
<td>10.2 mg</td>
<td>25 mg</td>
<td>9.8 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Thyroid:</strong></td>
<td><strong>Thyroid:</strong></td>
<td><strong>Thyroid:</strong></td>
<td></td>
</tr>
<tr>
<td>252 mg</td>
<td>490 mg</td>
<td>245 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Thymus:</strong></td>
<td><strong>Thymus:</strong></td>
<td><strong>Thymus:</strong></td>
<td></td>
</tr>
<tr>
<td>470 mg</td>
<td>462 mg</td>
<td>150 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Adrenals:</strong></td>
<td><strong>Adrenals:</strong></td>
<td><strong>Adrenals:</strong></td>
<td></td>
</tr>
<tr>
<td>38 mg</td>
<td>39 mg</td>
<td>30 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Seminal vesicles:</strong></td>
<td>1400 mg</td>
<td><strong>Seminal vesicles:</strong></td>
<td>480 mg</td>
</tr>
<tr>
<td><strong>Prostate:</strong></td>
<td><strong>Prostate:</strong></td>
<td><strong>Prostate:</strong></td>
<td></td>
</tr>
<tr>
<td>900 mg</td>
<td>400 mg</td>
<td>410 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Testes:</strong></td>
<td><strong>Testes:</strong></td>
<td><strong>Testes:</strong></td>
<td></td>
</tr>
<tr>
<td>2400 mg</td>
<td>3150 mg</td>
<td>3200 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Body weight:</strong></td>
<td><strong>Body weight:</strong></td>
<td><strong>Body weight:</strong></td>
<td></td>
</tr>
<tr>
<td>490 g</td>
<td>160 g</td>
<td>150 g</td>
<td></td>
</tr>
</tbody>
</table>

**Hormone 3 (intact)**

- Pituitary: 10.2 mg
- Thyroid: 252 mg
- Thymus: 470 mg
- Adrenals: 38 mg
- Seminal vesicles: 1400 mg
- Prostate: 900 mg
- Testes: 2400 mg
- Body weight: 490 g

**Hormone 3 (castrate)**

- Pituitary: 10.1 mg
- Thyroid: 250 mg
- Thymus: 470 mg
- Adrenals: 41 mg
- Seminal vesicles: 1200 mg
- Prostate: 800 mg
- Body weight: 485 g

**Hormone 4**

- Pituitary: 25.7 mg
- Thyroid: 495 mg
- Thymus: 460 mg
- Adrenals: 38 mg
- Seminal vesicles: 450 mg
- Prostate: 375 mg
- Body weight: 144 g

**Hormone 5**

- Pituitary: 25.7 mg
- Thyroid: 495 mg
- Thymus: 460 mg
- Adrenals: 38 mg
- Seminal vesicles: 450 mg
- Prostate: 375 mg
- Body weight: 144 g

**Hormone 5 (castrate)**

- Pituitary: 9.7 mg
- Thyroid: 247 mg
- Thymus: 140 mg
- Adrenals: 29 mg
- Seminal vesicles: 440 mg
- Prostate: 380 mg
- Body weight: 135 g
Hormone 6

What were each of the 6 hormones? Explain your answers.

(intact)

Pituitary: 8 mg
Thyroid: 500 mg
Thymus: 455 mg
Adrenals: 37 mg
Seminal vesicles: 480 mg
Prostate: 405 mg
Testes: 2790 mg
Body weight: 152 g

(castrate)

Pituitary: 7.8 mg
Thyroid: 505 mg
Thymus: 461 mg
Adrenals: 37 mg
Seminal vesicles: 445 mg
Prostate: 375 mg
Body weight: 135 g
### Blood Physiology

#### A. Determination of the ABO and Rh blood types

The ABO and Rh blood type of each student will be determined in this exercise. The antigens (agglutinogens) that determine blood type are located on the plasma membrane of red blood cells, and the antibodies (agglutinins) in the plasma. The antibodies cause the agglutination (clumping) of the red blood cells carrying the corresponding antigen.

In order to determine your ABO blood type, Anti-A and Anti-B sera (which contain the A and B antibodies, respectively) will be used. If the blood is type A, there should be agglutination of the RBCs by Anti-A serum; if type B, agglutination by Anti-B serum; if type AB, agglutination by both antisera; if type O, there should by no agglutination by either antiserum.

The Rh blood type will be determined in a similar manner. Although there are several Rh agglutinogens, the blood will be tested only for the presence of Anti-D (Rh\(_0\)), the most common antigen. If the blood reacts with the serum, the blood is Rh positive (Rh\(^+\)); if the antiserum does not cause agglutination of the cells, the blood is Rh negative (Rh\(^-\)). Approximately 85% of all Caucasian are Rh\(^+\) and approximately 15% are Rh\(^-\).

**Procedure:**

1. Place one drop of Anti-A serum in the A circle, one drop of Anti-B serum in the B circle, and one drop of anti-Rh serum in the Rh\(_0\) circle.
2. Clean your finger with an alcohol swab; let dry.
3. Puncture your finger tip with a sterile lancet.
4. Place one drop of blood next to each drop of antiserum.
5. Mix the blood with the antiserum by means of a toothpick. To avoid contamination, use a separate toothpick for each well.

#### B. Determination of the Hemoglobin Content of Blood

Each hemoglobin molecule contains four heme groups, which are each made of a porphyrin ring and an iron molecule. Since 70% of our body’s iron is found in hemoglobin, a low hemoglobin value is often indicative of anemia. Here, you will use two methods to determine the amount of hemoglobin in a sample of your blood.

**Determination of the Hemoglobin Content of Blood using the Tallquist scale.**

The Tallquist scale is used to estimate the amount of hemoglobin in blood (g/100 ml) by comparing the color of a drop of blood with a standard color chart.

**Procedure:**

1. Remove one square of absorbent paper from the Tallquist test booklet.
2. Clean your finger with 70% alcohol, letting it evaporate.
3. Puncture your finger tip with a sterile lancet.
4. Wipe away the first trip of blood with the edge of an absorbent paper.
5. Place the second drop of blood in the center of the paper. (Do not squeeze the finger; blood must be free flowing)
6. In a few seconds, the blood stain will lose its glossiness, and comparison should then be made immediately with the Tallquist color chart outside, in the sunlight. Do not let the blood dry to a brown color, or inaccurate values will be obtained.
7. Locate the color on the Tallquist scale that most closely matches the color of the blood stain. Since the color differences on the chart represent 10% variations in hemoglobin content, it will be necessary to estimate the intermediate percentages.
Determination of the Hemoglobin Content of Blood using a Hemoglobinometer

The hemoglobinometer is an instrument that can read the hemoglobin content of whole blood by spectrophotometric measurements of the blood.

Procedure:

1. Turn on the hemoglobinometer
2. Insert a blank (new) card
3. Place a drop of blood in the small well at the middle of the card
4. Take the reading; the machine will count down for approximately 2 minutes before giving you your value.

**The machine must be turned on, with the blank card inserted, before adding blood!!!**

C. Blood Hematocrit (Hct)

The hematocrit is the percent volume of whole blood that is occupied by red blood cells. It is determined by centrifuging the blood in special capillary tubes, that are coated with heparin to prevent the blood from clotting. The percent of whole blood made up of cells is determined by the height of the red cells in the tube, compared to the height of the total column of blood.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>46%</td>
<td>43-49%</td>
</tr>
<tr>
<td>females</td>
<td>41%</td>
<td>40-45%</td>
</tr>
</tbody>
</table>

The hematocrit may fall as low as 15% in severe anemias or rise to as high as 70% in a condition known as polycythemia. Under what conditions might such a rise be seen?

Procedure:

1. Puncture your finger with a sterile lancet to obtain a drop of blood. Wipe off that drop and allow a second drop to form.
2. Touch the red-circled end of a heparinized capillary tube to the drop. Hold the tube in a horizontal position to allow the blood to enter the tube until it is ~2/3 - 3/4 full.
3. Seal one end of the tube.
4. Place the capillary tube in a micro-hematocrit centrifuge with the plugged end to the outside, and centrifuge.
5. After centrifugation, measure (in mm) the height of the red cell column and the height of the cells plus the plasma. Calculate the hematocrit.
D. Blood Smear Preparation

1. Follow the instructions in the figure at the right to prepare your blood smear.

2. Allow the slide to air dry completely before staining.

3. Using the sink, flood your slide with the Wright Stain Solution. Let stand for 1-3 minutes (using the tray).

4. Using the sink, flood your slide with buffer and let stand for 2 minutes.

5. Rinse your stained slide with deionized (DI) water.

6. Air dry completely before viewing your slide underneath the microscope.

Image from: http://www.ruf.rice.edu/~bioslabs/studies/sds-page/bloodcytology.html
Blood Physiology

THE FOLLOWING THINGS SHOULD BE IN YOUR LAB NOTEBOOK FOR THIS LAB

1. Record the results of the entire class in the following tables.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Number of individuals</th>
<th>Percent</th>
<th>Theoretical percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>45</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>A</td>
<td>41</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>AB</td>
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<td></td>
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</tr>
<tr>
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</table>

<table>
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<th>Percent</th>
<th>Theoretical percent</th>
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</thead>
<tbody>
<tr>
<td>Rh+</td>
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</tr>
<tr>
<td>Rh-</td>
<td>15</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

2. Explain any differences in the percentages obtained in your class and the theoretical percentages.
3. What was your ABO blood type? Your Rh blood type?
4. Which agglutinins are contained in your blood plasma?
5. Which agglutinogens are contained in your blood cells?
6. What was the hemoglobin content of your blood using the Tallquist scale? The hemoglobinometer?
7. What would account for some differences between the methods of determining hemoglobin? What are some sources of error using the Tallquist scale?
8. What was your hemoglobin value? How does this compare to the class average for your gender?
9. What causes the characteristic difference in hematocrit value between the sexes?
Urinalysis

Examination of the urine is a common clinical procedure. None of the tests are difficult, but all may yield results that are of considerable diagnostic importance. The purpose of this exercise is to demonstrate some of the more common tests.

Obtain materials and execute just one test at a time to avoid contaminating reagent tablets, etc. Each student will complete all of the tests on a sample of their own urine. Approximately 100 ml of urine is adequate.

1. Specific Gravity

Examine the urinometer float carefully and note the scale on the stem. Understand how the scale is read before proceeding with the test, then check the accuracy of the float using distilled water. It should read 1.000 at 15°C (make temperature correction as described below). Fill the urine cylinder 2/3 to 3/4 full with urine then gently drop the float into it. After it has settled, carefully read the scale at the fluid meniscus. The scale on the urinometer stem is calibrated to be correct at 15°C. Measure the temperature of your specimen. For every three degrees above 15°C add 1 to the last decimal place (thousandths) of the urinometer reading. This gives an accurate determination. For example:

Original specific gravity reading = 1.017
Fluid temperature reading = 24°C

24°C is 9°C or 3 x 3°C above 15°C; therefore add 0.003 to the reading:

1.020 = specific gravity, corrected value.

According to several sources, the normal range for specific gravity of human urine is 1.002 to about 1.030. However, in the average individual, the value will usually fall between 1.015 and 1.025, depending on diet, fluid intake, and other factors.
2. **Albuminuria**

This test looks for the presence of a common plasma protein, albumin, in the urine. Reagent strips commercially called “Albustix” are employed. Obtain a reagent strip but do not touch the colored end of it; dip the colored end into the urine specimen, then compare the dipped end with the color scale on the bottle. Record your result in the number of milligrams of albumen. Normally, there is little or no albumen in the urine. Small amounts are regarded as normal; stress and other factors can influence this value.

3. **pH**

Using a strip of pH paper, dip the colored end into your urine sample. Compare the dipped end with the color scale on the package. Urine pH is generally slightly acidic, but may vary from 4.8 to 8.0 under different circumstances.

4. **Glucose**

The test for glucose will be performed using reagent strips called “Clinistix.” Repeat the procedure for #2 above. Normally the urine should contain no glucose; the presence of glucose in the urine is most commonly due to diabetes mellitus.

5. **Occult Blood (Occult hematuria)**

Occult blood is simply that present in such minute quantities that it can be detected only by chemical means. We will use “Hemastix” to test for blood in our urine. Follow the procedures for #2 above. Normally no blood is found in the urine in males; it may be found during menstruation in females.

6. **Ketonuria**

This is a test for ketones in the urine. We will use “Ketostix” for this test. Follow the procedures for #2 above. Normally no acetone is found in the urine; the presence of them are often seen during starvation, or in diabetes mellitus.
7. **Bilirubinuria**

Obtain an ictotest tablet, and mat. Put 5 drops of urine on the mat, place the tablet on top of it and add 1 drop of water. After 5 seconds, add a second drop of water. If the drops remain on the tablet, add another drop of water. The test is positive when the mat around the tablet turns bluish purple (ignore tablet color). The amount of bilirubin is proportional to color and time of reaction. Negative test is when mat shows no color or is only pink or red. Normally bile acids and bilirubin are not found in the urine.

8. **Amount of solids excreted**

Multiply the last two decimal places (hundredths and thousandths) of the specific gravity of your urine by 2.6 (Long’s coefficient). This will give, in grams, the approximate amount of solids in one liter of urine. What are the principle solids of the urine? The usual value for total solids is 50-70g in a 24-hour specimen.
Urinalysis Report Sheet

Some of the urine-related disorders are discussed in your text. For the others, you will need to refer to a book on clinical diagnosis or a medical physiology text; the Merck Manual may also be helpful.

Results:

Specific Gravity, corrected value: ______________

Color & Clarity: ______________

pH: ____________

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Trace</th>
<th>+</th>
<th>++</th>
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</thead>
<tbody>
<tr>
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<tr>
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<td></td>
</tr>
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<td>Bilirubin</td>
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</tbody>
</table>

Questions:

1. What is meant by the term osmolarity as it applies to urine?
2. Why is it necessary to make a correction for temperature using the urinometer?
3. What is the normal range of urine output, daily, for a healthy adult?

4. What conditions may be indicated by “cloudy” urine? Is this a normal finding? Why or why not?

5. The pH of urine normally ranges from what to what? What does a decrease in urine pH mean? An increase?

6. What are the principle solids found in urine? What is Long’s coefficient? How many grams of solids are present in your urine?

7. Discuss what an abnormal reading in the tests you performed today would indicate physiologically. Consider things such as filtration, reabsorption, damage to kidneys, or malfunction of other organs.
Appendix 1: How to avoid plagiarism

Introduction

According to Webster’s Dictionary, to plagiarize is “to take (ideas, writings, etc.) from (another) and pass them off as one’s own,”. Thus, if we as writers want to avoid plagiarizing, we must never use ideas from another source without giving that source proper credit. Additionally, if we use exact, or extremely similar, wording as the original source, we must both cite the source and put the words in quotation marks (or, for long passages, indent them) to indicate that the wording (in addition to the ideas) is not our own.

Below, I’ve taken a short passage from a biology textbook (see the “Original passage” section below) and have re-written it using both proper and improper literature citation. All of the examples included in the “Incorrectly cited passages” section are in fact plagiarized, and if I found work like this in a submitted paper I would consider the paper to be plagiarized. Also, note that these examples are not intended to be all-inclusive, as there are many ways of writing both plagiarized and non-plagiarized versions of any passage.

The three golden rules to avoid plagiarism

Avoiding plagiarism is as easy as following three rules:

1. If a sentence contains information you learned from another source (or sources), cite the source(s) at the end of that sentence.

2. If a sentence (or group of sentences) contains wording that you copied directly from a source (or wording that is nearly directly copied, i.e., you only changed a few words), you must put the entire portion of text that you copied from that source in quotes (in its original wording) and cite the original source at the end of the quotation.

3. Include a literature citation (“references”) section at the end of your paper that includes full citations for all references listed in your paper. All items listed in the literature citation section should have matching in-text citations.

Note that these rules also apply to non-textual information in your work: artwork, audio clips, video clips, or any other information that you take from another source must be cited appropriately.
Original passage

“Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation. In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte” (Johnson, 2003).

Correctly cited passages

This summary nicely captures the essence of the original:

Seeds are a combination of two plant gametes (haploid cells, like our sperm and eggs) completely covered with their parent's tissue (Johnson, 2003).

A bit close to the original, but it works:

Plants are much like animals: they create their equivalent of sperm and eggs (gametes, a haploid generation) from a diploid generation (Johnson, 2003). One difference, however, is that parent plants surround their developing gametes, which eventually become seeds, with their own cells (Johnson, 2003).

A mixture of summary and quote that works well:

Plants produce gametes much like animals do, by creating haploid cells from a diploid organism (Johnson, 2003). However, “in angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte” (Johnson, 2003).

If you wanted to quote the entire passage with a bit of introduction:

Animals aren't the only organisms that create haploid cells. “Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation. In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte” (Johnson, 2003).

If you simply wanted to quote the entire passage with no introduction of your own:

“Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation. In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte” (Johnson, 2003).
Incorrectly cited (plagiarized) passages

This example takes Johnson's exact wording, but doesn't put it in quotes:

Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation. In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte (Johnson, 2003).

Here we only slightly modify the text, but not nearly enough to call it our own. We need to un-edit the text (restore Johnson's exact wording) and put it in quotes:

Plant life cycles contain an alternation of generations, where a diploid sporophyte generation gives rise to a haploid gametophyte. In flowering plants, the developing gametophyte generation is completely enclosed within the tissues of the parent (Johnson, 2003).

This is completely wrong. We've used the exact wording and haven't cited it at all in-text. This would be incorrect regardless of whether we've included the reference at the end of the paper:

Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation. In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte.

Here we take one of the correct examples above and remove one of the citations. With only one citation it now appears that the first sentence is our own idea, even though it is clearly based on Johnson:

Plants are much like animals: they create their equivalent of sperm and eggs (gametes, a haploid generation) from a diploid generation. One difference, however, is that plants surround their fertilized gametes, which we call seeds, with their own cells (Johnson, 2003).

Here we take another correct example and, by removing the citation, make it incorrect. Even if we have cited Johnson at the end of the paper, it is not clear that this quote came from Johnson:

Animals aren't the online organisms that create haploid cells. “Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation. In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte.”
A simple reorganization of the sentence order doesn’t make the work our own. Besides being slightly confusing, since this uses Johnson’s exact words, this needs to be turned into a direct quote, appropriately cited.

In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte (Johnson, 2003). Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation (Johnson, 2003).

Here we properly cite the quote, but fail to cite the second sentence as being from Johnson.

“Plant life cycles are characterized by an alternation of generations, in which a diploid sporophyte generation gives rise to a haploid gametophyte generation” (Johnson, 2003). In angiosperms, the developing gametophyte generation is completely enclosed within the tissues of the parent sporophyte, which we call seeds.

References

Appendix 2: Example Results Section

It can be confusing to learn how to report results properly. One of the most common mistakes I see students make is that they only show tables and graphs, but don’t describe the data. I think this is probably because they confuse describing the data with interpreting it.

When you describe data, you’re taking what the graphs or tables show, and putting it into words. For instance, if two variables appear to be correlated with each other, you would say that: “Variable A and B appear to be positively correlated with one another.”

When you interpret data, you’re explaining what that means. This would be done in the context of your original hypothesis. You interpret data in the Discussion section of your paper.

Below is a completely fictional results section that illustrates how to describe data.

Results

In this experiment, we were interested in whether encountering unicorns makes a hiking trip more pleasant than encountering rattlesnakes. Our hypothesis was that unicorns are much more pleasant than angry rattlesnakes.

As described in the materials and methods, we sent 10 people on a hike, and when they finished we asked them how many unicorns and rattlesnakes they encountered, and then asked them to rate on a scale of 1-10 how pleasant their hike was, with 10 being sheer ecstasy and 0 being pure hell.

Figure 1 illustrates the correlation between the number of unicorns encountered and pleasantness. You can see that pleasant increases linearly with the number of unicorns encountered, but the correlation changes direction at around 6 unicorns. The implications of this will be discussed in the Discussion section.
When encountering rattlesnakes, there was a strong negative correlation between the number of snakes seen, and the pleasantness of the hike, as shown in Figure 2.

The stats for this fake experiment can’t be easily calculated with a t-test, but give me a break…this is all I could come up with on short notice. Hopefully you get the picture. Notice that I don’t interpret any data here (in regards to the hypothesis), but I do describe it all briefly AND point out important trends that I want the reader to pay attention to. I can come back to all of that in the context of the hypothesis in the discussion section of my paper.
Acknowledgments

Pages 9-14 (on literature citation) and Appendix I on avoiding plagiarism were used with permission of Marc Perkins, and were modified from his lab manual for Biology 185, Orange Coast College:
