Laboratory Manual for Physiology 12

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Writing Hypotheses: a student lesson

Purpose: to learn when and how to write hypotheses.

Most students believe that they are going to be experimenting anytime they are given a laboratory assignment in science. However, more often than not, students are doing something other than experiments. This is not necessarily bad. A good deal of science is observational and descriptive. For example, the study of bio-diversity usually involves looking at wide variety of specimens and maybe sketching and recording their unique characteristics. However, there are other times when we science teachers are trying to teach students how scientists work and how we can verify things which others may say or believe is so without any proof.

To learn about what is not known or to verify a notion, the so-called "scientific method" might be carried out and an actual experiment may be conducted. It does not matter that your experiment has been done a thousand times before or that your teacher already knows the results. What matters is that you don't know the results and that you can independently find a verifiable answer. In real experiments, real hypotheses should be written before the actual experiment.

What Is a Real Hypothesis?

A hypothesis is a tentative statement that proposes a possible explanation to some phenomenon or event. A useful hypothesis is a **testable** statement which may include a prediction. A hypothesis should not be confused with a theory. Theories are general explanations based on a large amount of data. For example, the theory of evolution applies to all living things and is based on wide range of observations. However, there are many things about evolution that are not fully understood such as gaps in the fossil record. Many hypotheses have been proposed and tested.

When Are Hypotheses Used?

The key word is **testable**. That is, you will perform a test of how two variables might be related. This is when you are doing a real experiment. You are testing variables. Usually, a hypothesis is based on some previous observation such as noticing that in November many trees undergo color changes in their leaves and the average daily temperatures are dropping. Are these two events connected? How?

Any laboratory procedure you follow without a hypothesis is really not an experiment. It is just an exercise or demonstration of what is already known.

How Are Hypotheses Written?

1. Chocolate may cause pimples.
2. Salt in soil may affect plant growth.
3. Plant growth may be affected by the color of the light.
4. Bacterial growth may be affected by temperature.
5. Ultra violet light may cause skin cancer.
6. Temperature may cause leaves to change color.

All of these are examples of hypotheses because they use the tentative word "may". However, their form is not particularly useful. Using the word “may” does not suggest how you would go about proving it. If these statements had not been written carefully, they may not have even been hypotheses at all. For example, if we say "Trees will change color when it gets cold." we are making a prediction. Or if we write, "Ultraviolet light cause’s skin cancer." could be a conclusion. One way to prevent making such easy mistakes is to formalize the form of the hypothesis.

Formalized Hypotheses example: If ***skin cancer*** is related to **ULTRAVIOLET LIGHT**, then <u>people with a high exposure to ultraviolet light will have a higher frequency of skin cancer.</u>

If ***leaf color change*** is related to **TEMPERATURE**, then exposing plants to low temperatures will result in changes in leaf color.

Notice that these statements contain the words, if and then. They are necessary in a formalized hypothesis. But not all if-then statements are hypotheses. For example, "If I play the lottery, then I will get rich." This is a simple prediction. In a formalized hypothesis, a tentative relationship is stated. For example, if the ***frequency of winning*** is related to **FREQUENCY OF BUYING LOTTERY TICKETS**. "Then" is followed by a prediction of what will happen if you increase or decrease the frequency of buying lottery tickets. If you always ask yourself that if one thing is related to another, then you should be able to test it.

Formalized hypotheses contain two variables. One is "independent" and the other is "dependent." The independent variable is the one you, the "scientist" control and the dependent variable is the one that you observe and/or measure the results. In the statements above the ***dependent variable is italicized and bolded;*** and the **INDEPENDENT VARIABLE IS IN CAPS, BOLDED AND UNDERLINED**.

The ultimate value of a formalized hypothesis is it forces us to think about what results we should look for in an experiment.

Rewrite the first four hypotheses using the formalized style shown above. Single underline the dependent variable and double underline the independent variable in the If clause of each hypothesis. When you are done, write one more original hypothesis of your own using this form.

Data Analysis; Means and Standard Deviations

One of the most unique and beautiful things about human beings is that we are all different. We have different opinions, beliefs, tastes and outlooks on life. It should come as no surprise then that our bodies and our internal biology are unique and individual as well. Just as no two snowflakes are exactly alike so are no two human bodies exactly identical. We have different sizes, shapes, skin colors, and fingerprints. This raises a problem for those of us wanting to study physiology or practice healthcare. If our goal is to study or treat all people and all people are unique we need to figure out some way to accept the individual differences and still recognize that there are some things we all have in common.

One thing we are not trying to do in this lab is to document the individual differences in all our subjects. While doing this may make our subjects feel unique or special it does not help us in our pursuit of a greater understanding of general human physiology. When we study human physiology we want to know how all human bodies function. To do so we look to the similarities in our subjects not the differences.

Averages

One of the ways to look at the general trend in groups of people or subjects is to measure some dependent variable and then take the average of all your measurements to determine what the overall group average measurement is. You should know before taking this course that the average is calculated by adding up all the observations and dividing the total by the number of observations. Or to put it in symbols:

$$\bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$$

where x with a line over it stands for *average*, N stands for the number of observations, and x_i stands for each observation, (which is just a fancy mathematical way of saying add all observations up and divide by the number of observations.

For example if I were to measure the height of five of my physiology students I might get the following table

Student	Height in cm
#1	159
#2	180
#3	171
#4	165
#5	160

To find the average just add up the individual heights:

159+180+171+165+160 = 835cm

And divide by the number of observations which is 5:

835 / 5 = 167cm

This number (167cm) is the average of this group of five students and is a much more useful number to study when trying to learn things about student's heights in general. In fact, if the students in this group were representative of most students in the class their average height may be very close to the average height of all students in the class. Many times when we want to learn something about an entire population but do not have time to measure them all, we take a random sample of the population and calculate the average of that sample. If we truly took a random sample and did not employ any bias in how we picked our subjects than our subjects averages should be close to that of the population (there by

saving us a lot of measuring time and allowing us to discover things in the lab that apply to whole populations of people by only measuring a few subjects).

This technique of taking measurements from a sample group and comparing group averages is something you will be doing a lot of in the lab. Many times in lab we will not have enough time to measure everybody in the class and so we will rely on the average of a smaller sample of subjects. When you write up your lab reports averages are more important than individual measurements. Averages tell us about changes and trends that we would expect to see in all people, and this is the kind of information we are interested in discovering.

You might ask, how many subjects should I measure before the average of my subjects accurately represents the average of the entire population of subjects I am interested in? One?…. Two?… Five?…. More subjects is always better because the more subjects you measure the more accurately their average will mimic the average of the whole population. When it comes to your final projects your instructors will be looking for how many subjects you have in your experiments. More subjects = more accurate averages = more accurate conclusions = **better grade**!!! (*As a rule of thumb you should always have at least 6 subjects in every group you are interested in comparing if you are going to see any meaningful results to your experiments*).

What is The Standard Deviation?

So averages are better than individual measurements, but they do not tell the whole story. As an example, let's look at a few more measurements. Say repeated the example above of measuring the heights of students in lab only this time I measured two lab groups. Each lab group had five students in it, and from my measurements I got the following data:

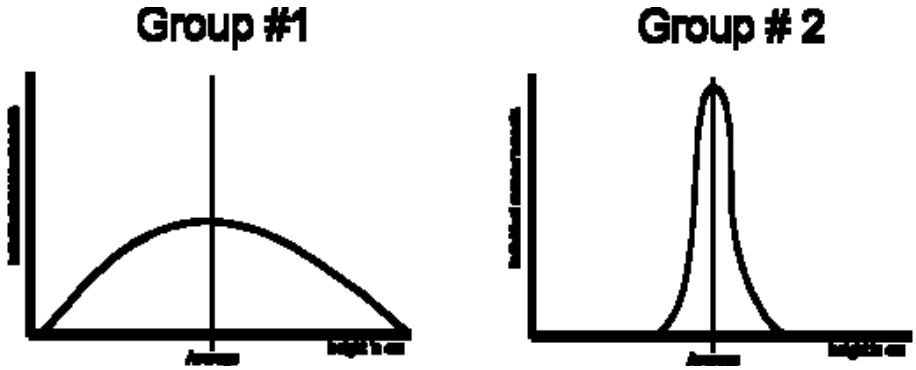
Student	Height in cm	
	Lab group #1	Lab group # 2
#1	149	167
#2	190	167
#3	176	167
#4	165	167
#5	155	167

Let's calculate the averages for these two groups:

Group #1 = **149+190+176+165+155 /5 = 167cm**
Group #2 = **167+167+167+167+167 /5 = 167cm**

So according to the averages the two groups are identical, … Right? (Both have an average height of 167cm)

The problem is both groups are not identical everybody in group #2 falls exactly on the average height while the heights in group #1 ranged from 149 cm to 176 cm tall. If we want to tell the whole story of how these two groups of students look when compared to each other we need to include something else in our measurements, we need to include some measurement of how much **variance** there was in our measurements. **Variance** is a measure of how spread out the individual measurements are from the group average. If all the individual measurements are close to the average then they don't vary much. If on the other hand, the average is made up of observations that are spread out, with some measurements falling far above the mean and some falling far below it than that group has a large variance. If we were to draw a picture of our observations plotting where they fell around the calculated average they might look something like this:



This type of drawing is called a histogram and it just shows in a picture where individual measurements fall in relationship to each other. In the figures above we can see that the average or mean score is represented by a vertical line. The bell shaped line represents where the individual measurements fell in relation to that average. The measurements from Group #1 fall both above and below the average line producing a flat bell shape. Measurements from Group #2 however fall closer (or on top of the) average line producing a tall thin bell shape. This shape of this bell graphically represents how close your scores are to the average or in other words these graphs show the variance in your measurements. If you have a lot of variance your individual measurements then any individual measurement may not fall anywhere near the calculated average while if you have very little variance, then you can be sure most of your individual measurements will fall on or near the average.

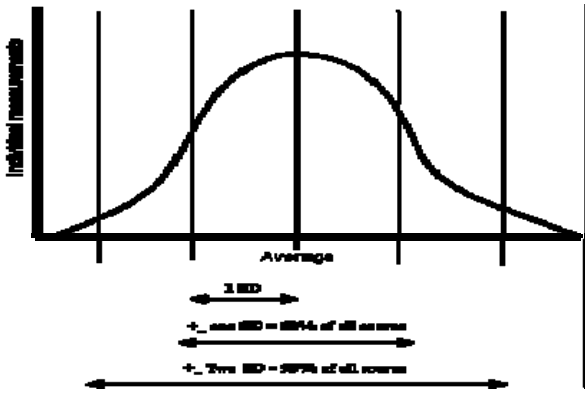
This concept of how much things vary and how close they are to an average is more than just some academic statistical concept. In biology everything has variance and our ability to distinguish differences between one set of measurements and another depends greatly upon our understanding of the variance in both our measurements and the naturally occurring variance in the dependent variable we are trying to measure.

To aid us in measuring variance there are many statistical tools and complex numeric comparisons available to the research scientist. This however is a course in physiology not statistics so in this course we will confine ourselves to one relatively simple statistical measurement to aid us in comparing the numbers that we measure in this course. The **Standard Deviation** (SD) is a statistical calculation that represents how much variation there is in a group on numbers. (*Technically the standard deviation is a calculation of the square root of the variance in a group on numbers, but even that may be too much info for this course*) Suffice it to say the standard deviation is a standardized measurement of how much variance there is in what you are measuring. Any time you can calculate an average you can and should also calculate the Standard Deviation. The Standard Deviation is a single number that represents variance, so if you have a large standard deviation then you know that there was a lot a variance in your measurements (the larger the SD the flatter the bell shape of the histogram above will be). If on the other hand your Standard Deviation is small then you know most of your measurements were very close to the calculated average. (*In the example above, group two would have a Standard Deviation of 0 because all there individual measurements were the same as the calculate average*).

When listing a group of numbers you will often see the average score followed by the "$\pm$" symbol and then another number. This second number represents the standard deviation. In our example above we could represent our measurements in writing by saying the average height in Group one was **167$\pm$16cm** and Group two was **167$\pm$0cm**. The number following the $\pm$ tells us roughly where our scores fell in regards to the average of 167cm.

In the figure below I have drawn vertical lines on a histogram to illustrate the spacing on one Standard Deviation. The reason for the $\pm$ (which stands for plus, minus) symbol has to do with the standardization part of the Standard Deviation. If I have a normal distribution of measurements (*meaning they form a normal bell shaped histogram*) and I calculate my standard deviation, then I know by the rules of statistics that 68% of all my population measurements will fall between + one and – one standard deviation of my average, and 95% of all my population measurements will fall between +2 and -2 standard deviations of my average.

Almost all statistical tests and numeric comparisons of groups of measurements are based on this principle. For the purposes of this course we will not be getting into complex statistical analysis but we do expect you to be able to calculate and represent standard deviation in your results. Calculating standard deviation is rather complex you must first calculate the variance by using the formula:



$$\text{Variance} = \frac{\Sigma(x - \bar{x})^2}{n - 1}$$

Where **X** is the individual measurement **X** (with a line over it) is the average. ∑ means sum and **n** represents the number of measurements.

Finally in order to get the Standard Deviation you must square the entire result. Resulting in the formula:

$$SD = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

Example using Group #1 scores:
Scores =149,190,176,165,155
Average = 167
Observations (n) = 5

Sum and square each scores difference from the average
$(149\text{-}167)^2 + (190\text{-}167)^2 + (176\text{-}167)^2 + (165\text{-}167)^2 + (155\text{-}167)^2 =$
$(-18)^2 + (23)^2 + (9)^2 + (-2)^2 + (-12)^2 =$
$324 + 529 + 81 + 4 + 144 =$
1082

Then divide by n-1
1082 / (5-1) =
1082 / 4 =
270.5

And take the Square root
√270.5 = 16

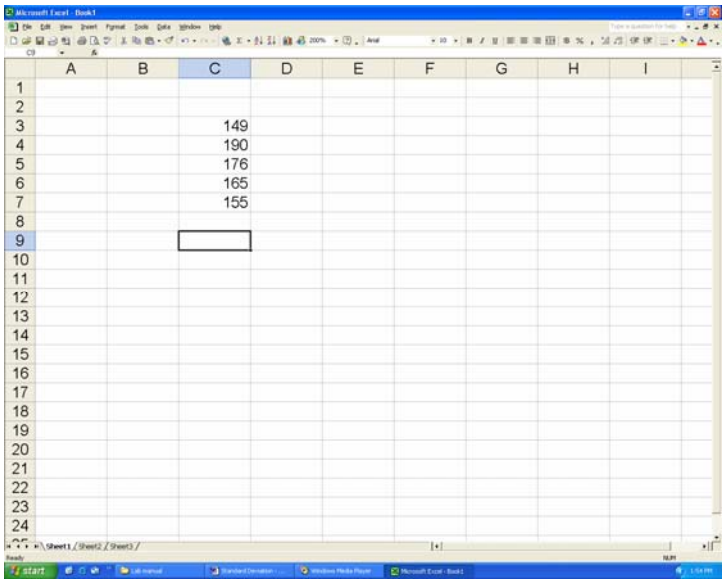
This formula may seem rather daunting but most scientific calculators have special functions that will calculate it for you as do the computers you will be working on in the lab.
Calculating Means and Standard Deviation using Excel

One of the tools that will be making life in physiology lab much easier this semester is the lab computers. They will save us all much time by making it easier to measure certain variables compile our numbers and calculate statistics. For many of the experiments we will be using the computers to pull together all the data from the class experiments and deliver it to you electronically. Rather than taking

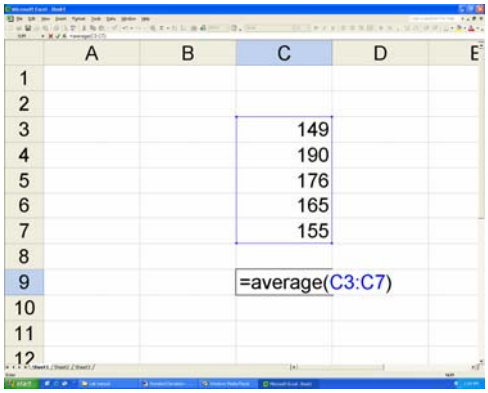
your data from the computer and calculating the averages and standard deviations by hand and then entering it back into the computer, there are a few simple commands within Microsoft Excel that will allow you to calculate averages and Standard Deviations very quickly and efficiently. Microsoft Excel is a basic spreadsheet program for organizing numeric data and can be found on all computers in the physiology lab as well as on all campus computers in the library and computer labs.

If you are not familiar with Excel or computers in general you are welcome to do all your calculations on paper but most students who encounter how much time and effort this takes quickly decide to learn a few basic computer skills to improve their lives. (After all once you leave this class and start working in your chosen field you are still going to have to master computer assisted measurement of data)

When you open the Excel program you are presented with what looks like an infinitely expanding table organized into columns and rows. The columns are organized by letters at the top of the page and the rows are organized by numbers on the left of the page (*See figure below*). The entire page is then cut up into cells or square in which you can input data. In the figure below I have entered the data from the Group # 1 height example above in the "C" Column starting in row three and ending at row 7. Each Cell or square on the spreadsheet is then identified by a letter and a number. For example the first height value of 149cm is in the cell "C3". The next value of 190cm is in "C4" etc. until the final value of 155cm in cell "C7". When you click on a cell it highlights the perimeter of that cell as is currently being done in the figure below for the cell "C9".



If you wanted to you could type a number into any empty cell and Excel would hold if there for you, as I have done for the heights in the figure above. You can however, instead of typing in a number type in a formula or calculation into a blank cell and Excel then produce the answer to that formula or calculation in the cell. For example if I wanted cell "C9" to list the average of all the numbers above it I would just have to type into it the equation:



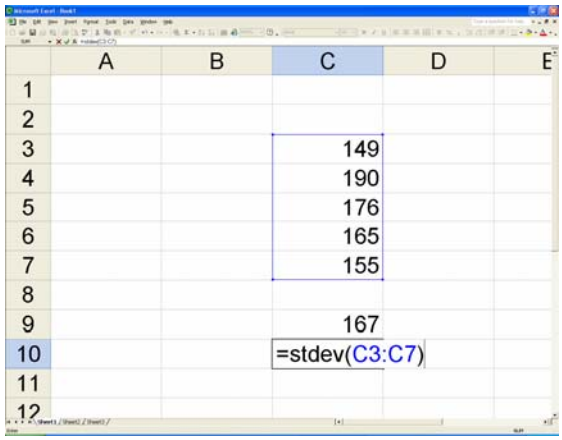
= AVERAGE(C3:C7)

By typing this equation into the cell excel will automatically calculate the average of the values found in cells C3 to C7 and produce their numeric average in cell C9.

In order to calculate Standard Deviation you only have to use the formula

=STDEV(C3:C7)

As I have done in the figure to the right in cell “C10”



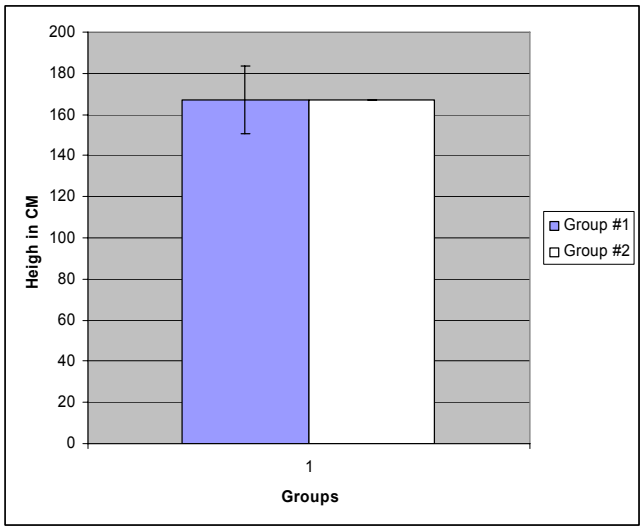
In these formulas it is important that you start them off with the = sign as that lets the program know that it is to solve the equation and put the answer in the cell. You will also see that there is a *fx* or function window at the top of the page that shows your formula or data for each cell.

There are many more tricks and time saving tools to be learned but even knowing just these two commands will greatly help you get through the data part of the lab write ups.

Feel free to ask your instructors or fellow classmates for more tips and tricks on using the computer programs as there are many helpful tricks and tools to use in the lab. The computer use is hard to explain in writing, but as soon as we get to work in the lab the computers usefulness, benefit, and convenience, will become apparent.

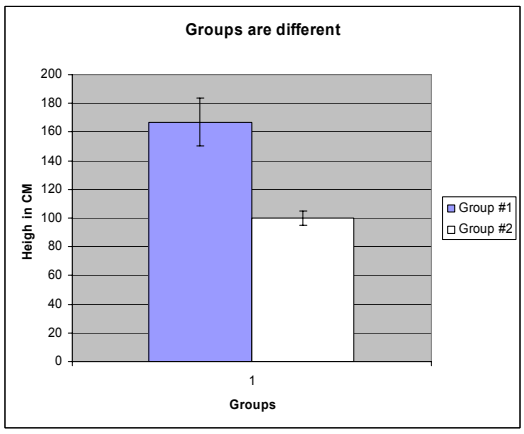
Graphing the Standard Deviation

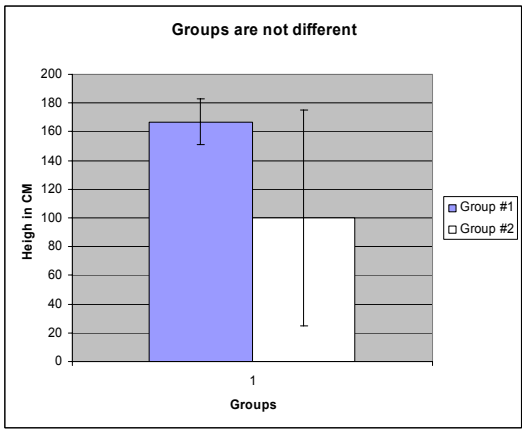
One of the many challenges of this lab is to speak with numbers, which means answering your experimental hypothesis by presenting the results of your experiments in a graphical format. There is no one graph or format that will fit all forms of data. Each experiment will require you to analyze what you measured and determine what will be the best format in which to present your findings. As read other scientific papers in your background research for each experiment, pay close attention to the graphs and figures presented in other papers. It is our hope that as you progress in this course you will be increase your familiarity and skill in dealing with analysis of numeric data and graphical representation of numeric data.



When making a relevant comparison of two averages, Standard Deviation should be included in your graph as error bars. Error bars are T shaped lines that project either above or below the average on the graph and indicate how much variance there was in the measurement. The height of the T Shaped error bar is usually 1 Standard Deviation. Below is a graph showing the height data we used in the previous section. Note that there is a small bar sticking above and below the average for group #1. The height of this bar is 1 Standard Deviation. (Group two does not have a bar because SD for this group was 0)

Error bars in your graphs and figures will give you a quick visual way to check how two groups of numbers compare to each other. If the error bars overlap each other then the averages really aren’t that much different (see lower graph on the left) if on the other hand the error bars do not overlap each other (as in the lower graph on the right) then we can be fairly confident that the averages will be statistically different.





(*For purposes of our class, including error bars in your figures and analysis will avoid any further complex statistical analysis*)
In your readings start looking for examples of how error is represented in scientific graphs and figures.

References:

Lovejoy, E.P. “Statistics for math haters” Harper & Row, publishers New York. 1975

Kenney, J. F. and Keeping, E. S. "The Standard Deviation" and "Calculation of the Standard Deviation." §6.5-6.6 in *Mathematics of Statistics, Pt. 1, 3rd ed.* Princeton, NJ: Van Nostrand, pp. 77-80, 1962.

Eric W. Weisstein. "Standard Deviation." From *MathWorld*--A Wolfram Web Resource. Retrieved on 8-05-2004 from http://mathworld.wolfram.com/StandardDeviation.html

Physio 12: Human Physiology Laboratory Write-ups

One goal of this laboratory is to equip you with the skills necessary to make rational decisions based scientific facts. This is no easy task given that most of what we read, listen and watch in our everyday lives (newspapers, television, the internet etc.) is based on opinion or personal experience. One of the basic principles of scientific methodology is that the outcome of scientific experimentation should not be dependent upon the personal experience or opinions of the scientists doing the experiments. This unique perspective is represented in the unique style of scientific communication, which is based on the assumption that, once an experiment has been completed the data should speak for itself. The primary way that scientists communicate their data to others is through laboratory reports.

Reading scientific literature is quite unlike reading Tolstoy, Grisham or Anne Landers. Scientist write in a language designed to be explicit, declarative and unbiased. Unlike newspaper editorials where the point of the writing is to sway or convince the reader of an opinion, good scientific writing should let the reader clearly know what was done and why. It takes more effort on the part of the reader to understand a scientific paper. It is our goal that by the time you finish this course you will have a greater ability to read scientific literature and evaluate it. To this end it's important to know the formats and conventions of scientific writing. We'll start by dissecting a scientific paper.

Although reviewing the scientific literature and writing a research paper is hard work, if well done the experience may be one of the most satisfying accomplishments that you have as a biology student. A well written scientific paper must fulfill two objectives. First, it must clearly and completely describe the procedures that were followed and the results that were obtained. Second, it must place these results in perspective by relating them to the existing state of knowledge and by interpreting their significance for future study.

To place your research in perspective requires:

1. **summarizing the state of knowledge on the general topic**
2. **relating your work to general body of knowledge on the topic**
3. **stating the critical hypotheses toward which the study is addressed**
4. **interpreting the results of the study in relation to these hypotheses and to the general state of knowledge**
5. **Identifying the scientific questions and procedural weaknesses that need to be addressed in the future.**

While you need to do all of this, you also need to write clearly and as concisely as possible. Superfluous verbiage is a hindrance to the reader. Some examples of unclear and excessive writing include "The rats, numbering six, were...", "It can be noted that fewer...", or "The tapeworm, when in adulthood, can,...". These should simply be "Six rats were...", "Fewer...", or "The adult tapeworm can....". When editing, read your paper out loud, making sure your grammar is complete and your statements make sense!

Anatomy of a Lab Report

Modified from Christopher Watters and Peter Wimmer Middlebury College, Middlebury, Vermont
Lab Manual by Edward Simon Hanson, PhD and Joe A. Reyes, PhD City College of San Francisco

Laboratory Reports

Biology, like any other human endeavor, requires effective communication, and certain conventions have evolved among scientists to facilitate such communication. One major convention concerns the acceptable format for a scientific paper, and the majority of biologists (and journals which publish their work) use a format that breaks the report into seven sections or parts:

- Title
- Abstract
- Introduction
- Methods & Materials
- Results
- Discussion
- Bibliography

Your lab reports *must* conform to this same format and should contain each one of these sections clearly marked. These seven sections and their contents are described below. At the end of this section we will discuss our grading policy for lab reports (which will make no sense if you don't understand what should go in each section).

TITLE.

The title of a laboratory write up seems straightforward but a good scientific title should be descriptive and informative. In many cases when searching a scientific database for information the title is the only part of the paper that will be available to the reader. In order for the reader to clearly know what the paper is about the title should describe what was compared or examined in the experiment. The title should also be short and to the point. These two properties of a good title stand in contrast to each other. The best titles are both short and descriptive. Here are some examples:

Expression of Fully Functional Tetrameric Human Hemoglobin in *Escherichia coli*
Renewal of Blood Cell Cholesterol
Osmosis in Bovine and Dog Erythrocytes
The Effect of Feeding and Fasting on Glycogen Content in Rat Liver Hepatocytes

Examples of bad lab report titles:

My lab report
Lab report #1
How the kidney works
The awesome EKG experience

Titles on your lab reports should be followed by your name and lab section time.

ABSTRACT.

The abstract is a *brief* description of the project and its *major* results and conclusions. Most readers familiar with scientific reports use the abstract of a scientific paper to decide if the paper warrants further study. If you are searching for information in a public database such as PUBMED, or MEDLINE the abstract is often the most information you can get online about the paper. Based on the summary of the paper in the abstract readers will decide whether it is worth the trip to the local medical library to find the paper. Because reader may be searching through hundreds of abstracts in any given time it is useful to be brief and to the point in your abstract as well. The abstract should summarize the methods, results, and conclusions of the experiment in **100 words or less.** One way to organize an abstract concisely is to include one or two sentences summarizing each of the major sections of the paper: the Introduction, Methods and Materials, Results, and Discussion. Importantly, the conclusions must be clearly stated at the end of the abstract. The abstract is indented five spaces on both the right and left margin, does not have a heading and is single-spaced.

The following abstracts are taken from a professional journal and show a variety of styles. Notice the organization and content of each.

> The fusion of endoplasmic reticulum (ER) membranes in yeast is an essential process required for normal progression of the nuclear cell cycle, karyogamy, and the maintenance of an intact organellar compartment ***(Background/significance of project)***. We showed previously

that this process requires a novel fusion machinery distinct from the classic membrane docking/fusion machinery containing Sec 17p and Sec 18p ***(Previous work by authors)***. Here we show that Cdc48p, a cell-cycle protein with homology to Sec 18p, is required in ER fusion. A temperature-sensitive cdc48 mutant is conditionally defective in ER fusion *in vitro.* Addition of purified Cdc48p restores the fusion of isolated cdc48 mutant ER membranes ***(Results and Methods combined)***. We propose that Cdc48p is part of an evolutionary conserved fusion/docking machinery involved in multiple homotypic fusion events. ***(Discussion and Conclusion)***.

(from Cell 82:885-89)

As a general form for abstracts, include at least one sentence from each part of the lab paper; *Introduction,* **Methods & Materials**, Results, and ***Discussion,*** as illustrated below:

> Red blood cells are used in many experiments to analyze transport across a *simple membrane.* **In this investigation, using phase contrast and bright field imaging, the effect of distilled water, a physiological saline solution (PSS) and concentrated PSS (2X) on the shape, size and degree of hemolysis of bovine and dog red blood cells was observed. Also the osmotic principles responsible for these changes in the red blood cells were explored.** It was found that in distilled water, both bovine and dog cells swelled and some burst and resealed to form membrane sacks called "ghosts" that were visible only in phase contrast imaging. Most bovine and dog erythrocytes in PSS maintained a normal size and shape. Most bovin erythrocytes in PSS (2X) were crenated. *We concluded that the changes in erythrocyte shape, size, and degree of hemolysis were a result of basic osmotic principles.*
>
> *Glycogen content in liver hepatocytes may be used to estimate the relative nutritional well-being of the studied organism. In this investigation, the effect of feeding and fasting on the relative glycogen content in rat hepatocytes was studied.*
> **Glycogen** was **stained and identified by comparing slides of hepatocytes that were treated with Schiff's reagent with or without periodic acid or alpha-amylase.** By comparing the Glycogen content of the slides, ***we concluded that fed hepatocytes contained relatively more glycogen than fasted hepatocytes*.**

Because it is easiest to write the abstract using one or two sentences from each of the subsequent sections of the report it follows that the abstract is easiest to write as the last section of the paper after you have written all the rest of the sections. Insert the abstract at the beginning of the report after the tilte and authors but before the introduction. Skip one line after the abstract before beginning the introduction

INTRODUCTION.

The introduction to a laboratory report serves a couple of functions, it outlines the problem or question under investigation and provides background information useful in understanding the rest of the paper. After reading the introduction the reader should know why you are doing the experiment. In addition the introduction usually includes research of others which lead to your own experimental design.

In short, this section discusses the results and conclusions of previously published studies, to help explain why the current study is of scientific interest. Explain exactly what the objectives of the study are, and why it's a worthwhile effort. In the end you should be able to defend each sentence if I ask **"why did you include this information?"**

The introduction should address the following questions:

1) **why** did you undertake this study?
2) **what** is the state of existing knowledge?
3) **what** specifically are you going to do (clearly state your hypotheses and/or objectives)

The introduction is organized to move from general information to specific information. This background must be summarized succinctly, but it should not be itemized. Take care not to go too far away from your original idea in providing background information; limit the introduction to studies that relate directly to the present study. Emphasize your specific contribution to the topic.

One important aspect of science is that it is seldom if ever done in a vacuum. In fact the best scientific reports are those that clearly present what was known before and put the experiment being reported clearly in context of what is known about the subject. For this reason introduction sections of laboratory reports are filled with citations referencing other information sources. A report citation is a small number or annotation in the text that corresponds to another scientific report or information source listed in the reference section at the end of the paper. Citations let the reader know that the information being discussed is based on previous scientific studies or sources. Rather than include too much detailed information in a paper, citations allow the reader choose whether they want to know more about the subject by looking up the reference. As mentioned citations within the text can be either numerical [1] or an in text inclusion of the referencing authors name and year of publication (Smith et al. 2002).

A report can never have too many references but don't just list references for the sake of making references they should direct the reader to more useful background information. In fact when researching a subject, a published papers references are sometimes the best places to find more useful information on the subject.

Finally the introduction should end by clearly stating what the hypothesis for the experiment is. The hypothesis is a clearly stated question that can be proved or disproved by the experiments preformed. **To train you in developing a hypothesis, read the section in your lab manual titled "What is a Hypothesis".** In the introduction you do not need to answer the question, (that will be done in the Discussion section later in the paper) It is enough at this point to let the reader know what the question is so that they have some way to put your experimental results in context. The Introduction should not be any longer than **2** paragraphs.

METHODS & MATERIALS.

This section of the lab report includes a thorough description of **how** you obtained your results. Ideally, a reader should be able to duplicate the experiments using your description. **This description should be a narrative of the steps you took in your experiment or study, not a list of instructions such as you might find in a cookbook .** With this in mind it is useful to be specific in your descriptions in the method section. "We tested some guys" is not as useful as "Four males ages 27-34 were tested". How specific your descriptions are depends a lot on what you are testing and why. Methods that have already been published are usually documented with a citation (same format as in the introduction) and **not** described in details. Your own report may refer to the laboratory handouts for methodological detail, but it should describe carefully any deviation from the standard protocol. An important part of writing a scientific paper is deciding what bits of information needs to be given in detail. **Do not quote or cite your laboratory manual!**

If in later sections you find yourself explaining in detail how certain results were obtained, then *this* section was not well written.

RESULTS.

The results section is where you report your data. In scientific investigations the data is what you measured and it should stand alone, without any interpretation. You should present a verbal description of your results but do not offer any interpretation of their significance. This division allows a peer to read your results and make their own conclusions. **Then** they can read your opinions of what it means in the **"Discussion"** section and compare their conclusions with yours.

The results section is devoted to presenting the data. It should include all the relevant data related to your experiments. The data should be presented so that it is easy for the reader to see the relevant comparisons. (The relevant comparisons are the ones that answer your experimental question). Your graphs, tables, and figures should be accompanied by text that describes what each figure or graph or table represents. All figures, graphs or tables should be clearly labeled, as should every axis in a graph so the reader knows exactly what he or she is looking at.

Generally, results include only **summarized data** and **observations** obtained in the study. **Do not present raw data**. If you want to include actual raw data sheets add them as part of an appendix to the paper. In addition, all figures and tables included in your paper must be **cited** in the results description. The results of statistical tests can be presented in the "Results" section but not explained.

It is extremely important in this section to verbally describe accurately what you obtained and to illustrate

that description with an appropriate table, graph or drawing. If a figure can be used to show the data, use the figure (e.g. a graph) instead of a table. Most people understand graphs more quickly than tables. Don't present the same data in several ways, choose the one best way. While tables are good for presenting some kinds of data, consider the options. Do not present tables in text form! Both tables and figures should be clearly labeled Figure 1 or Table 1 in order of being referred to in the text, and should include a descriptive caption so they "stand alone" without needing reference to the text of the results section. Table captions are always above a table, while figure captions are placed below a graph, picture, or sketch. This section is not simply a collection of tabulated and plotted data, however. ***It is also important that you avoid interpreting data here. Allow the reader the opportunity to make an unbiased examination of your results.***

DISCUSSION.

The purpose of the "Discussion" section is interpretation of data. Use your data (making reference to specific tables and graphs) to build a logical argument for your interpretation. Statistical tests should be interpreted at this point to support the validity of the conclusions you draw. Also, point out any general trends in the class data or data from outside of class. It is also useful to include a brief discussion of sources of data variation including experimental error. In doing so, it is helpful to point out possible error, but you should also explain how the results were specifically affected. Finally, proposing further experimentation or a different set of related experimental questions is often an effective way to broaden the context of the lab paper.

Your Discussion should integrate the material in the earlier sections into a **coherent whole.** How do the data answer the question asked in the Introduction? Utilize YOUR results to answer questions posed in the lab and to create arguments for or against your hypothesis. Are there problems with the data which possibly reflect the methods used? Are there possibly better methods? How would you do the experiment differently were you to do it again? How do your results compare with those of others? Not all these questions will be appropriate for every report; but, you should include in your discussion the answers to the questions raised in the laboratory handout. **The discussion section has not been well written if it contains only (or *mainly)*** a summary of textbook or library findings.

In your discussion, address the following:

1) reach conclusions about the initial hypotheses and/or objectives
2) compare how your conclusions agree or contrast with previously published works
3) identify sources of error and inadequacies of your research
4) speculate upon broader meaning of the conclusions
5) identify needed next steps in research on the problem

REFERENCES, LITERATURE CITED OR BIBLIOGRAPHY.

Footnotes are rarely used in the scientific literature. Rather, the work of others is cited briefly in the text and then a corresponding, more detailed entry is included in this separate, final section of the paper. References may be cited sequentially by number in your report: **e.g.,** "as indicated by our textbook author (1)"; in which case, the complete citation would be entered after that number in the **Literature Cited** section. Alternatively, the citation may include the surname of the author and the publication date, **e.g.** "(Schliwa, 1982)"; in this instance, it would be listed in the **Literature Cited** section in alphabetical order. In the latter convention a paper co-authored by more than two investigators would usually be cited as, e.g., "(Sabatini, et al, 1982)".

In any event, the overriding consideration in the use of citations should be the acknowledgment of the work of others (including earlier work by yourself, or that of fellow students), and the literature cited section should provide enough information for the reader to find the article cited.

Whichever convention you adopt should be used consistently. Complete citations should be entered in the "Literature Cited", using the format adopted by the **Journal of Cell Biology** presented below and at the end of this handout. A "Bibliography" is an exhaustive listing of readings relating to the subject and would be used only in a monograph on the subject, but the same format rules apply.

1. **Book Reference**

Last Name, First and middle initials. Year. **Title.** Edition. Volume. Publisher,City. Pages referred.

Examples:

Bradley, J.V. 1972. **Elementary Microstudies of Human Tissues.** Charles C. Thomas, Springfield, IL. pp. 194-195.

Pearse, A.G. 1968. **Histochemistry.** 3rd ed., Vol. 1. Churchill Livingston, Edinburgh. p. 309.

Whittam, R. 1964. **Transport and Diffusion in Red Blood** Cells. Williams & Wilkins Company, Baltimore. pp. 2-3.

2. Journal Articles

Last Name, First and middle initials. Year. Title of article. **Journal abbreviated. Volume:** page numbers.

Examples:

Brantley, R.E., Jr., S.J. Smerdon, A.J. Wikinson, E.W. Singleton, and J.S. Olson. 1993. The Mechanism of Autooxidation of Myoglobin. **J. Biol. Chem.** 268:6995-7010.

Carver, T.E., R.E. Brantley, Jr., E.W. Singleton, R.M. Arduini, M.L. Quillin, G.N. Phillips, Jr., and J.S. Olson. 1992. A Novel Site-directed Mutant of Myoglobin with an Unusually High 02 Affinity and Low Autooxidation Rate. **J. Biol. Chem.** 267:14443-14450.

If you cite work you have **not** read yourself, the following format should be used:

author(s). date. Title of article. **Journal Title.** volume: pagination. "cited in" author(s). date. , etc.

Awkward as it may seem at first, this format for writing lab reports does provide for the clear and concise presentation of complex material, and you will find the organization improves your thinking skills as well. Van Norman (1971) provides a thoughtful discussion of the scientific manuscript; the sections entitled "The Job of Writing" and "Presentation of Results" are especially useful. Tobin and Morel (1997) offer on pages 17-29 some useful information on hypothesis creation, scientific inquiry, asking critical questions, how to read a scientific paper and the importance of peer-reviewed publications to the advancement of science, which you should read carefully several times during this course. A more technical and thorough guide to the preparation of a scientific manuscript has been prepared by the Council of Biology Editors (1978), which is shelved in the Reference section of the Science Library. A **Short Guide to Writing About Biology** by Pechenik (1993) is an excellent, highly readable source of information on writing, reading and speaking about biology.

3. Article in an Internet-only journal

Fredrickson, B. L. (2000, March 7). Cultivating positive emotions to optimize health and well-being. *Prevention & Treatment, 3,* Article 0001a. Retrieved November 20, 2000, from http://journals.apa.org/prevention/volume3/pre0030001a.html

4. Article in an Internet-only newsletter

Glueckauf, R. L., Whitton, J., Baxter, J., Kain, J., Vogelgesang, S., Hudson, M., et al. (1998, July). Videocounseling for families of rural teens with epilepsy -- Project update. *Telehealth News,2*(2). Retrieved from http://www.telehealth.net/subscribe/newslettr4a.html1

- Use the complete publication date given on the article.

- Note that there are no page numbers.
- In an Internet periodical, volume and issue numbers often are not relevant. If they are not used, the name of the periodical is all that can be provided in the reference.
- Whenever possible, the URL should link directly to the article.
- Break a URL that goes to another line after a slash or before a period. Do not insert (or allow your word-processing program to insert) a hyphen at the break.

Nonperiodical documents on the Internet

5. Stand-alone document, no author identified, no date

GVU's 8th WWW user survey. (n.d.). Retrieved August 8, 2000, from http://www.cc.gatech.edu/gvu/usersurveys/survey1997-10/

- If the author of a document is not identified, begin the reference with the title of the document.

6. Document available on university program or department Web site

Chou, L., McClintock, R., Moretti, F., Nix, D. H. (1993). *Technology and education: New wine in new bottles: Choosing pasts and imagining educational futures.* Retrieved August 24, 2000, from Columbia University, Institute for Learning Technologies Web site: http://www.ilt.columbia.edu/publications/papers/newwine1.html

- If a document is contained within a large and complex Web site (such as that for a university or a government agency), identify the host organization and the relevant program or department before giving the URL for the document itself. Precede the URL with a colon.

Preparing & Writing a Lab Report

We suggest you adopt the following procedure when you write a laboratory report:

1. **Soon** after the laboratory work has been completed, **preferably no later than that evening,** collate the results and write a **concise description** of each experiment. This exercise need not take any more than 30 minutes. ***(The lab handouts and laboratory worksheets*** *assignments are intended to help you focus this effort.)*

2. Then, or later, when more time is available, expand the brief descriptions into the "Results" section. Organize the data into tables and graphs, as appropriate, and perform any necessary statistical tests. Next, **think** about the results and the questions raised in the lab hand-out (and in the paragraph on the Discussion above) and write a draft of the "Discussion" section. Rework these two sections to your satisfaction; then, **and only then,** compose the "Methods" section, "Introduction" and "Bibliography". **Finally,** write the "Abstract".

If this tactic is adopted, writing reports will be easier and more instructive. The reports themselves should be double-spaced, with one inch margins, and **should not exceed 4** typewritten pages (excluding drawings, tables and graphs).

REMEMBER: A REPORT IS WRITTEN TO BE READ. Your assignment is to communicate what you know and what you did to an audience that has no idea of what you are doing in the lab. It is not acceptable to assume the reader is your instructor who gave you the assignment. The reader should be someone unfamiliar with your work and the Physiology 12 course. Present and discuss your results, especially the graphs and figures, with this goal in mind. Ask your roommate or a friend to read your rough draft, and **proofread your final draft before submitting it.**

For an example for **how** a paper should be written, you might want to examine some of the following articles for format and writing tips.

Literature Cited

Council of Biology Editors. 1994. **Scientific Style and Format.,** 6th ed. Cambridge University Press, NY. 825 pp. (Reference section of Science Library). (REF T 1 1 S386 1994)

Gopen, **G. D.,** and **J. A.** Swan. 1990. The Science of Scientific Writing. **American Scientist. 78(6):550-558.** (A useful evaluation of methods for clear, concise written communication.)

Lobban, C. S. and M. Schefter. 1992. **Successful Lab Reports: A Manual for Science Students.** Cambridge University Press, NYC. (Q 183.Z I L63)

Pechenik, Jan A. 1993. **A Short Guide to Writing About Biology.** Harper Collins College Publishers, NYC. i-xvi, pp. 1-240.

Sabatini, D. D. , G. Kreibich, T. Morimoto, and M. Adesnik. 1982. Mechanisms for the incorporation of proteins in membranes and organelles. J. **Cell Biol. 92:1-22.** (In Science Library stacks; does not circulate)

Schliwa, **M.** 1982. Action of cytochalasin **D** on cytoskeletal networks. J. **Cell Biol. 92:79-91.** (In Science Library stacks; does not circulate; or ask for author at reserve desk.)

Tobin, Allan J. and Richard E. Morel. 1997. **Asking About** Cells. Saunders College Publishing, Harcourt Brace College Publishers, Fort Worth, TX. i-xxiv, 698 pp, Gl-27, 11-20.

Van Norman, R. W. 1971. **Experimental Biology,** 2nd ed. Englewood Cliffs, NJ. P. 249-258. (QH 324 V3 197 1)

Key points to writing the lab report:

The following is a bullet outline of the important points you should be including in your lab report. As graders of these reports we ask ourselves these questions when we are grading your papers. We will also be referring to this page when we talk to you about your paper(s). **Know this page!!** Each section is worth 5 points for a total of 25 overall points. It is important to remember that each section affects the others. All the pieces should fit together; create a story; demonstrate to us that you have a good understanding of the processes involved in the experiment.

Introduction: 0 – 5 points

- Is there a clearly stated hypothesis? (see section on hypothesis development)
- Is the relevance of the hypothesis clearly explained?
- Are there proper citations linking relevance to supporting scientific evidence/literature? (Citations: see section on citations and literature)
- Quality and quantity of citations (journal vs. website, ect.)

Methods: 0 – 5 points

- Does the paper clearly explain who or what were the subjects?
- Does the paper clearly explain what equipment was used?
- Does the paper clearly explain procedures used?
- Is information in method of sufficient detail for replication?
- Is the methods section written in the proper grammar and tense?
- From the methods is it clear how the data used for the results were collected?

Results: 0 – 5 points

- Are all the dependent variables clearly summarized? (Look at section on “Hypothesis”)
- Are graphs/figures/tables clearly labeled?
- Do the graphs/figures/tables clearly illustrate answer to hypothesis?
- Are the graphs/figures/tables accompanied by text that clarifies the numeric relationship, without interpretation?

Discussion: 0 – 5 points

- Is the relevance of the data to the hypothesis clearly explained?
- Are the results explained in the context of the current scientific knowledge (citations, ect.)
- Are the further implications of this experiment discussed?
- Is the writer aware of the experimental shortcomings?

General: 0 – 5 points (Overall paper quality, this section could influence scores above)

- Use of language
- Objectivity
- Grammar
- Quality of science writing
- Depth of paper
- Title, bibliography, Section formatting

Pathfinder for
PHYS 1 and PHYS 12, Introduction to Human Physiology
City College of San Francisco, San Francisco, CA
Maria Capucciati and Joe Reyes, Ph.D., 11/13/02

Subject Area
Medical research to support the study of human physiology, and secondarily, human anatomy.

Audience
This pathfinder is designed to assist the students of PHYS 1 and 12 in performing research beyond the scope of the required textbook in order to complete the outside report and problem set assignments. Students need to be able to locate three things:

- primary research articles in peer-reviewed medical journals
- articles in popular literature about health and medical topics
- reference sources to assist in understanding medical terminology

Scope
The pathfinder will cover sources available on the Internet, through the CCSF library website, in the CCSF library, and at other medical libraries in the Bay Area.

Caveats

- Where a website URL is listed as is, the site can be accessed by anyone; where a source can only be accessed through the CCSF library website, it is noted.
- This pathfinder's goal is to list as many free (no cost) sources for medical literature as possible. It is likely, however, that even on some of these sites, certain articles may require payment or there may be associated printing charges. It will be up to the student to determine the value of the article, or to keep on searching.

Web-Based Introductory Sources

A Guide to Boolean Searching
http://library.albany.edu/internet/boolean.html
This guide is a primer on how to combine terms for more effective searching on the Internet or in databases. If you are not familiar with the methodology of Boolean searching, start here.

Primary Articles vs. Secondary Sources in the Sciences
http://library.sjsu.edu/subject/biology/primary.htm
This page lays out the criteria for a primary research article. Use it as a guide to determine whether articles you want to use for your reports are valid primary research.

Evaluating Information – Peer Reviewed Journals
http://library.sjsu.edu/subject/biology/primary.htm
This page contains a good definition of a peer-reviewed journal.

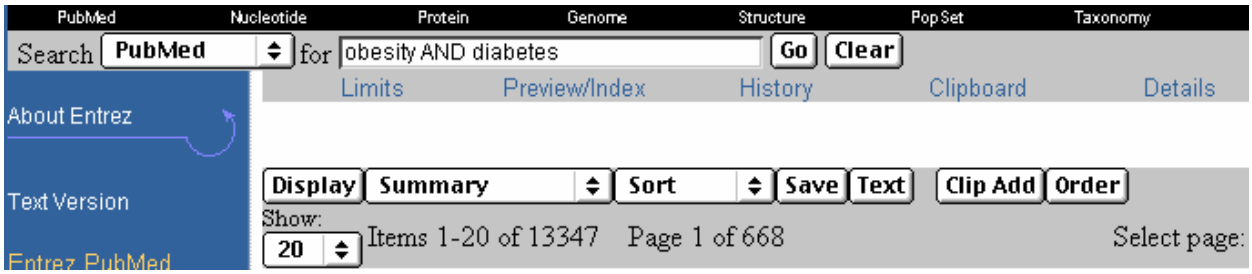
Google Web Directory: Journals
http://directory.google.com/Top/Health/Medicine/Journals/
http://directory.google.com/Top/Health/Education/Patient_Education/Journals/
http://directory.google.com/Top/Science/Biology/Publications/Journals/
These directories contain names and websites for medical journals broken down by field or specialty. They will help you become familiar with journal names that you will encounter in your searches. It might also give you some ideas for narrowing searches to a particular field.

Web-Based Sources for Primary Research Articles

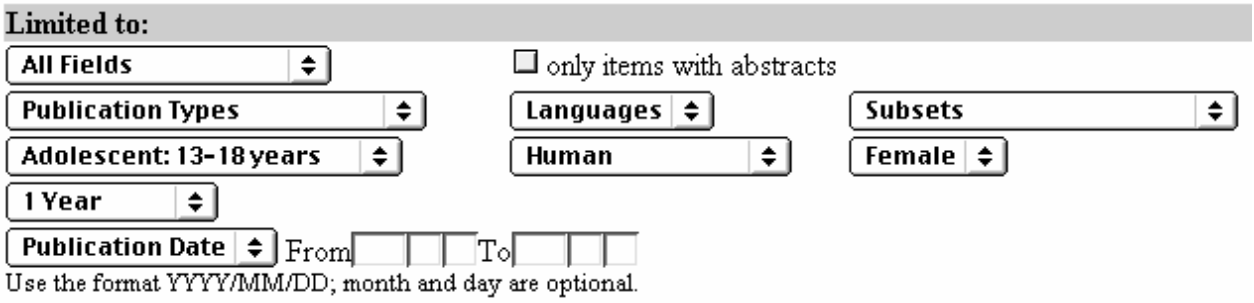
PubMed

http://www.ncbi.nih.gov/entrez/query.fcgi
PubMed is the public interface to the databases at the National Library of Medicine, part of the National Institutes of Health. It is one of the largest sources of primary medical research n the world, with 12 million citations. All articles in this database are peer-reviewed. (N.B. In your Web browsing, you may see references to MEDLINE. MEDLINE is the same thing as PubMed.)

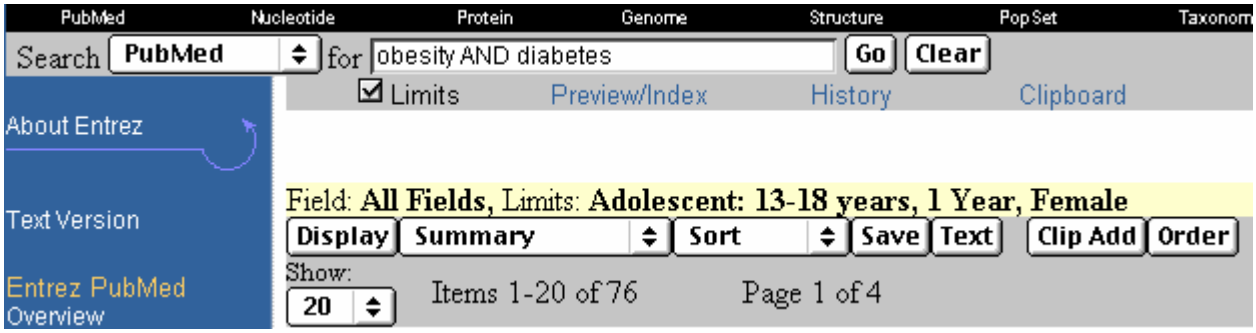
Performing a Basic Search in PubMed
Let's say you have found an article in a health magazine about the link between obesity and diabetes in adolescent girls. You want to search for some of the primary medical research on this topic. You access PubMed, type some criteria and click "go". (N.B. Boolean operators such as "AND" must always be all caps in PubMed.)



Your search for "obesity AND diabetes" returns 13,347 citations. Which of those have to do with adolescent girls? You could try typing "obesity AND diabetes AND girls", which will get your results down to 74. However, you are specifically looking for studies on adolescent girls, not eight-year olds. One way to get more accurate results is to set limits. Click the "Limits" link right under the search field to see the following screen:



Here, the drop-downs allow you to specify adolescent human females. The "1 year" is the date range limit, so you will get pretty recent studies. Here's the result. You can see the extra criteria that have been applied.



The other useful links under the search field are:

Preview/Index: type your criteria, then click this link to see how many results you will get before you execute the search.
History: shows you a list of the searches you have already performed. This is useful when you can't remember, or want to run one again.

Clipboard: if you find articles you want to keep track of, but want to continue searching, click on the checkboxes next to the article names and click the “Clip Add” button. This will save the articles to a list, which you can then see by clicking “Clipboard”.
Details: this will show you what MeSH terms PubMed has used in executing your search. (See below for an explanation of MeSH terms.)

MeSH Terms
MeSH stands for Medical Subject Headings. This is the controlled vocabulary used by the content managers of PubMed to index the citations. Searching using the proper terminology can hugely improve the accuracy of your searches. You can find “MeSH Browser” in the left-hand frame under “PubMed Services”. When you click it, you will see a search box.

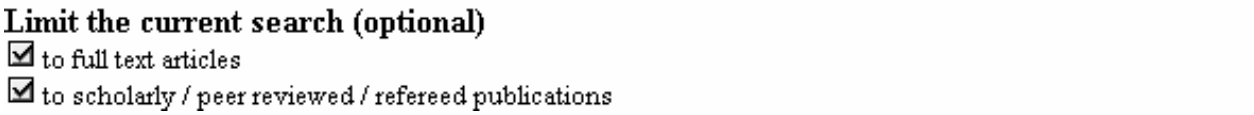
To try this out, type in “diabetes,” from the previous example. While “diabetes” is the commonly used term for the disease, it is not the exact medical term. The MeSH browser will show you the correct term, plus all the associated terms used in PubMed. You can add the MeSH term to your search, or look at the detailed display for associated subheadings.

For more help in understanding MeSH, click the “Help” link in the left-hand frame.

InfoTrac Health Reference Center Academic and Expanded Academic ASAP
For CCSF students only; a student ID with a library bar code is necessary to access this database.
http://www.ccsf.edu/library/period.htm
These are two of the databases under the InfoTrac umbrella. Health Reference Center Academic has a narrower scope; it is limited to medical journals, periodicals and reference sources. Expanded Academic Index covers most of the same sources, but also includes more general publications that might contain information from different contexts, not necessarily medical. You can use either one to search, but will probably be able to get more targeted results with Health Reference Center Academic. The search interfaces are virtually identical.

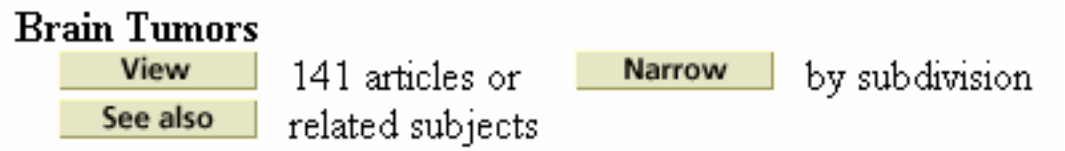
There are two ways to search these databases: by subject guide or keyword search. In either case, the first thing to do is to turn ON the two checkboxes under “Limit the current search”: to full text articles, and to scholarly / peer reviewed / refereed publications. Do this every time and you will not have to worry about the immediate availability or the validity of the results.

Subject guide search
Use the subject guide search when you have a broad topic and you want to narrow it down to something manageable. Let’s say you have read an article in a science magazine about advances being made in the treatment of malignant brain tumors, and you want to see some data on studies. Click “Subject guide” in the left-hand frame.



This isn’t a place for Boolean searching, or for specific terminology; in fact, the broader the term, the better. Turn on the limit checkboxes (see above) and type in “brain.”

In the results, you will see all the subjects associated with the term “brain”. Scroll down and you have:



The search has yielded three things: specific articles on the topic, subdivisions of the topic, and related subjects. In “subdivisions,” you will find 8 articles on “case studies,” which might be a good place to start.

Keyword search
Use keyword search when you have a very specific idea of what you are looking for. Here, you can use Boolean searching. As before, turn on the limit checkboxes. By default, you are searching through title, citation and abstract. To continue our example, try a keyword search for “brain AND tumor AND treatment.”

Medscape
http://www.medscape.com/px/urlinfo
Medscape is the health care professional’s version of the popular WebMD consumer health site. The site requires registration, but registration is free. Once you register, the Medscape library (click “Library” on the horizontal toolbar) contains the journals listed at http://www.medscape.com/pages/public/publications. To check whether a publication is peer-reviewed, click on the journal name, then follow the “Publication Information” links on the left-hand side.

To search Medscape journals, type keywords into the search field at the top of the page. In the search results, click on the link at the top right to show only articles. Most of the articles within Medscape are free, full-text versions. You can also run the same search in MEDLINE by switching tabs at the top of the page. (Remember, MEDLINE = PubMed.)

BioMed Central
http://www.biomedcentral.com/start.asp
BioMed Central is a medical publishing company whose philosophy is to make the journals they publish freely and immediately available on the web for wide dissemination. All their journals are peer-reviewed. To see a list of their journals, click “Journals A-Z” or “Journals by Subject” in the horizontal toolbar. There is a quick keyword search field in the left-hand frame and an advanced search link on the toolbar. The quick search can also search PubMed.

HighWire Press
http://highwire.stanford.edu/lists/freeart.dtl
HighWire is a medical publications division of Stanford University. They make 342 of their own journals freely available online after a certain time period has elapsed. This page shows the list of journals and what issues are available. There is a quick search by author and by keyword at the top of the page. You can search HighWire journals only, or MEDLINE/PubMed also. For advanced search, click the “advanced” link underneath “quick search.”

Free Medical Journals
http://www.freemedicaljournals.com/
This site contains free articles from 900 medical journals. The collection is not very deep (it does not go back more than a few years, and some journals have limited article access) but it is worth looking at. The downside of this site is that it is not searchable. You can browse the journals by specialty, alphabetically by title, and see a list of new journals. To get to articles, click on the journal name. (This would be a good site to check when you are looking for a specific journal article that you cannot get for free elsewhere. See if the journal is listed here.)

Web-Based Sources for Popular Articles
InfoTrac OneFile and Health and Wellness Resource Center
For CCSF students only; a student ID with a library bar code is necessary to access this database.
http://www.ccsf.edu/library/period.htm
The OneFile database contains newspaper and periodical articles on health issues, and the Health and Wellness Resource Center also has full-text articles in addition to all the reference materials. The same instructions apply as for the other InfoTrac databases discussed earlier, except you don't have to search for peer-reviewed articles only.

Google Web Directory: Magazines
http://directory.google.com/Top/Science/Publications/Journals_and_Magazines/
http://directory.google.com/Top/Health/Publications/Magazines_and_E-zines/
These directories contain names and websites for popular health and science magazines.

Health and Medical Topics in the News
http://www.lib.berkeley.edu/PUBL/news.html
The top half of this page contains links to current health-related news from reputable newspaper and television sources.

Mednews
http://www.newswise.com/menu-med.htm
Mednews is primarily a press release site for journalists. It is useful in this context because almost all the press releases have to do with conclusions drawn from recent medical research. (The press releases are often from the university doing the research.) Stories on this site with the little red "lock" icon are unavailable to the public, but most are open access.

Newsbank
For CCSF students only; a student ID with a library bar code is necessary to access this database.
http://www.ccsf.edu/library/period.htm
Newsbank contains articles from 500 US and Canadian newspapers and newswires. Both basic and advanced searches are available. You can limit your search by source, and by date range. This would be a good database for finding very recent health-related articles, but it would be good to have a specific topic or source in mind before trying it.

Web-Based Reference Sources

InfoTrac Health and Wellness Resource Center
For CCSF students only; a student ID with a library bar code is necessary to access this database.
http://www.ccsf.edu/library/period.htm
This InfoTrac database contains searchable full-text for *The Gale Encyclopedia of Medicine*, *The PDR Family Guide to Prescription Drugs*, and *Mosby's Medical, Nursing and Allied Health Dictionary*.

HealthFinder Library
http://www.healthfinder.gov/library/
There are links on the right-hand side of this page for medical dictionaries, MEDLINE Plus's medical encyclopedia, and prescription drug information through DrugDigest.

The Merck Manual
http://www.merck.com/pubs/mmanual/
This is the online, searchable, full-text version of *The Merck Manual of Diagnosis and Therapy*, widely recognized as the most commonly used medical text for clear explanations of diseases and their treatment.

MEDLINE Plus
http://medlineplus.gov/

The links on the left-hand side of the home page will lead you to the medical encyclopedia, prescription and non-prescription drug information, and medical dictionaries.

PDR.net
http://www.gettingwell.com/drug_info/index.html
The online, searchable version of the *Physician's Desk Reference*, a standard guide to prescription drugs.

On-Line Medical Dictionary
http://cancerweb.ncl.ac.uk/omd/
This dictionary was written by a British physician and has not only A-Z entries, but a listing by subject area and a list of entries by non-alphabetical characters.

AMA Atlas of the Body
http://www.ama-assn.org/ama/pub/category/7140.html
The American Medical Association's website of anatomical illustrations. Also includes illustrations showing the effects of adverse conditions on certain parts of the body.

Gray's Anatomy
http://www.bartleby.com/107/
An online, searchable, full-text version of the seminal anatomy book. Includes all illustrations.

CCSF Rosenberg Library Print Sources

Periodical Holdings List (password is NOT necessary)
http://www.ccsf.edu/Library/periodicals/main.html
This is the online catalog for the periodicals and journals owned in hard copy by the CCSF libraries. If the journal article you want is unavailable online, it is worth checking to see if the library has it in hard copy.

Reference Books
The Rosenberg Library has a selection of medical reference books on the first floor. I am giving general call number sections to encourage browsing, since there are too many titles to give specific recommendations. If you have trouble locating these sections, don't hesitate to ask a reference librarian for help.

Topic	Found in reference section
Human Anatomy	QM to QP
Medical Dictionaries	R121
Medical Terminology	R123
Diagnostics	RB38
Diseases and Treatment	RC41 to RC76
Medical Encyclopedias	RC81
Prescription Drug Guides	RS

Other Medical Libraries

University of California at San Francisco (UCSF)
http://www.library.ucsf.edu/ (Galen II)
The closest medical library to the CCSF campus is at UCSF, which allows public access to most of their onsite collection. If you really want to track down an article you can't get elsewhere, chances are UCSF will have it. However, you are somewhat limited in accessing their website off-campus. If you have a journal title, you can find out if they own it, but access to the actual contents of the journal (either hard copy or electronic) is available only at the library.

Galen II is the web interface to the UCSF library. At the top of the home page, these are the two primary links to pay attention to:

Find Books and Journals. This is where you would search to see if UCSF has a journal in hard copy. This will work best if you know the exact title of the journal, since searching for keywords will also give you book titles in the results.
When you find the journal, there will often also be a link to the electronic resource. Those links will not work if you are off campus. If the journal is available in hard copy, see http://www.library.ucsf.edu/library/locations.html to determine its location in the library.

Electronic Journals. Here, you can search for a specific keyword and find related journal titles, or browse electronic journals by category. When searching, remember that only the journal titles, not contents, are being searched. The contents of electronic journals are available only from terminals located in the UCSF library. The search will also allow you to limit the results to availability by UC campus. Leave UCSF selected by default, unless you happen to be interested in going over to UC Berkeley instead.

If you actually locate an article you want at UCSF, there are several ways to obtain it from the library:

- If it's in print, locate it in the stacks and copy it at a copy machine (paid copies).
- If it's electronic, you can print it at the library only if you have set up a Galen account with UCSF. This is fairly easy; instructions are at http://www.library.ucsf.edu/eservices/. You create an account, deposit money into it, and printing fees are deducted from it.
- Another way to get electronic articles without paying to print them is to bring a floppy or ZIP disk to the library with you and copy off what you want to the disk.

University of California at Berkeley Public Health Library
http://www.lib.berkeley.edu/PUBL/
Since Berkeley is another school in the UC system, their policies are quite similar to UCSF's. Library services and collections are largely open to the public, but you must be on the premises. The library home page has some very helpful links under the title "Internet Resources."

Stanford University Lane Medical Library
http://lane.stanford.edu/index.html
Stanford has the third major medical library in the Bay Area. I was unable to ascertain from the Lane Library website how open they are to public access. Unlike the UC system, Stanford is entirely private. If you are interested in using their resources, I suggest you e-mail or call them first to see what their policies are.

The Health Library at Stanford
http://healthlibrary.stanford.edu/index.html
Stanford has set up a consumer health library at the shopping center. Since it is consumer-oriented, there are more books and videotapes available than journal articles. What databases they have are only accessible at the library. The one section worth looking at on this site is under "Health Info." Once you click on that, the first item in the subsequent toolbar is "Selected Internet Resources," which has some useful links.

Medical/Health Sciences Libraries on the Web
http://www.lib.uiowa.edu/hardin/hslibs.html
A listing of medical libraries on the web by state; also, some libraries outside the United States.

MedLib
http://www.medlib.netfirms.com/
An index of more than 750 medical libraries around the world, broken down by continent, then country.

Tips for Searching

- Begin to familiarize yourself with medical terminology. If your search terms aren't working, look in medical dictionaries or PubMed's MeSH browser for synonyms. If a drug is involved in the study, look for the generic name as well as the brand name in *Physician's Desk Reference*. Use anatomy resources to find the Latin term for a body part. Sometimes the information is readily available, but you need the correct term in order to find it. Try as many variants as possible.

- Many articles will talk about studies having been done at a particular university. Sometimes the research is published on the university website itself. In "Other Medical Libraries," there are several links to listings of medical library websites, including most university libraries. This is worth a chance if you cannot find the published report elsewhere.

- Usually, research scientists work in the same field of specialty for years. If you find an article that is not quite what you are looking for, but covers the correct field, try searching the database using the author names to find their other publications. Also, if your article is available for a fee in one database, use the author name to search in other databases where it might be free.

Experiments in Respiratory Physiology:

The Respiratory Cycle:

The respiratory cycle consists of alternating processes of inspiration and expiration. During inspiration, skeletal muscles such as the diaphragm and external intercostals contract, thereby increasing volume within the thorax and lungs. As volume within the air spaces of the lung (intrapulmonic volume) increases, air pressure within the lung (intrapulmonic pressure) falls below atmospheric pressure and air rushes into the lung. During expiration, the inspiratory muscles relax, causing the volume of the thorax and lungs to be reduced. The reduction in intrapulmonic volume is accompanied by an increase in intrapulmonic pressure above atmospheric pressure, forcing air out of the lungs. Normally, unlabored expiration at rest is a passive event determined by relaxation of inspiratory muscles. When an increase in pulmonary ventilation is required, such as during exercise, expiration becomes an active event dependent upon contraction of expiratory muscles that pull down the rib cage and compress the lungs beyond the resting end expiratory level.
During one respiratory cycle, a specific volume of air is drawn into the respiratory system and then pushed back out. This volume, first inspired, then expired, is known as tidal volume (TV). The actual value of tidal volume varies in direct proportion to the depth of inspiration. During normal quiet breathing (eupnea) at rest, adult tidal volume is about 500 ml.

A normal rate of breathing at rest is about 15 respiratory cycles per minute (RR = 15 cpm). The respiratory rate varies with changes in body activity. During exercise, respiratory rate increases, but it decreases, for example, when a person concentrates on certain tasks, such as attempting to thread a needle.

The product of tidal volume and respiratory rate (TV x RR) equals the rate of pulmonary ventilation, also known as minute respiratory volume. During conditions of body rest, an adult rate of pulmonary ventilation is approximately 7.51/min (500 ml x 15 cpm/1000).

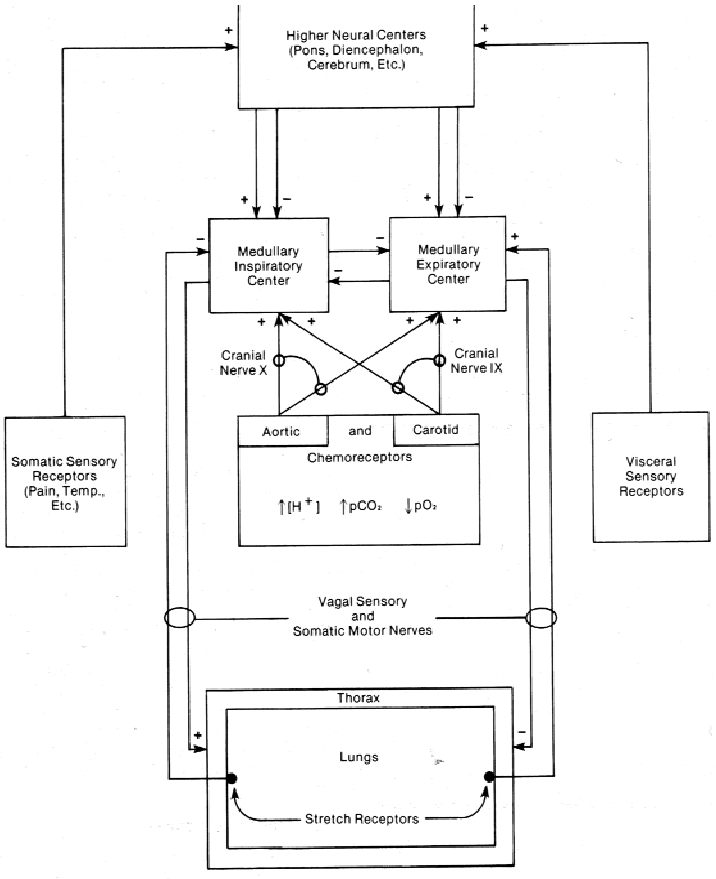


FIGURE **B10.1** Regulation of pulmonary ventilation

The rate and strength of contraction of respiratory muscles, and hence the rate and depth of respiration, are controlled by primary respiratory centers (inspiratory and expiratory) located in the medulla oblongata at the base of the brain stem. The primary centers are inherently rhythmic, alternating their activity to produce inspiration and then expiration. During normal quiet breathing at rest (eupnea), the expiratory center acts to limit and then inhibit the inspiratory center, thereby producing a passive expiration. In contrast, the inspiratory center always acts to produce an active inspiration. When respiratory depth increases, as in exercise, both inspiration and expiration are active processes controlled by their respective medullary centers.

To adjust respiratory rate and depth according to the body's

needs, the medullary centers receive inputs from higher neural centers (e.g., pons, cerebellum, cerebral cortex) and from peripheral receptors such as chemoreceptors in aortic and carotid bodies; stretch receptors in joints, muscles, and tendons; and somatic sensory receptors for pain and thermal stimuli (figure B 10.1). For example, cerebral control of the medullary respiratory centers may be evidenced by observing the modification of the respiratory cycle as a subject attempts to thread a needle. The cycle temporarily ceases in order to minimize body movement so that the needle may be threaded more easily.

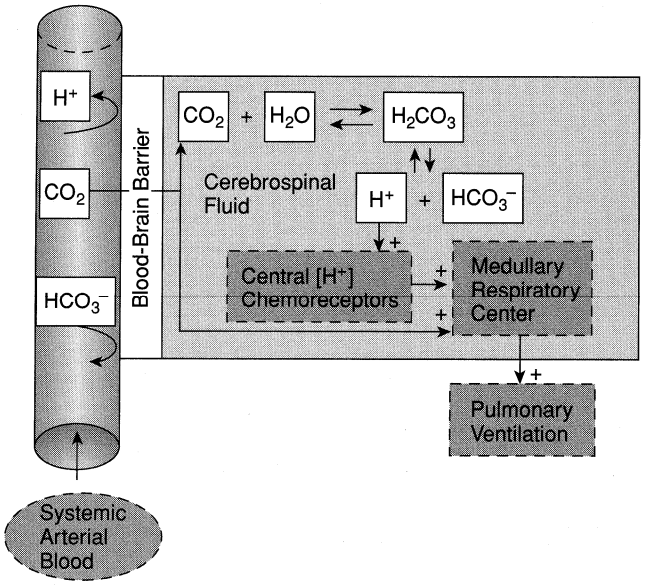


FIGURE B10.2 Direct and indirect effects of carbon dioxide on pulmonary ventilation

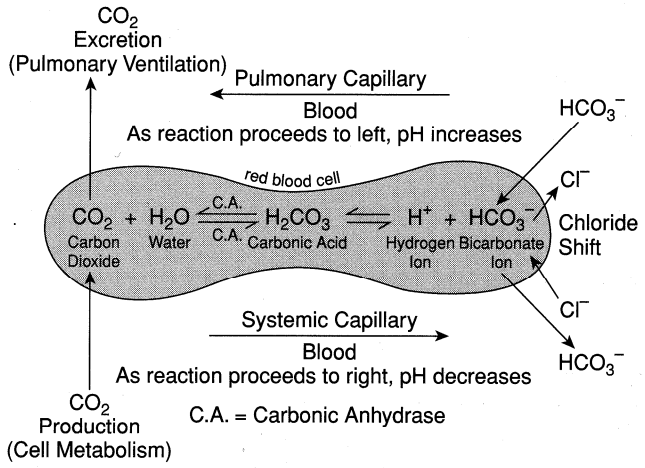


FIGURE B10.3 Influence of CO_2 excretion rate on blood pH

Other modifications of the respiratory cycle occur as a result of changes in oxygen, carbon dioxide, or hydrogen ion levels in plasma and cerebrospinal fluid. Respiratory rate and depth is a function of chemical regulation. Blood bathes chemoreceptors located in the aortic and carotid bodies. Chemoreceptors sense changes in systemic arterial PCO_2 H+, and $P0_2$, and send impulses to the respiratory centers in the medulla oblongata.

Increased $PC0_2$, increased H+, and/or Decreased $P0_2$ increase rate and depth of respiration.

Decreased PCO_2, decreased H+, and/or Increased $P0_2$ tend to decrease respiratory rate and depth.

Changes in arterial carbon dioxide content exert by far the strongest influence on respiratory drive. $C0_2$ by virtue of its solubility in body fluids is able to influence respiratory activity by directly affecting chemoreceptors on the ventral surface of the medulla oblongata which are bathed by cerebrospinal fluid. Also, being lipid-soluble and crossing the blood-brain barrier (BBB), $C0_2$ can exert its effect by changing the hydrogen ion concentration of the brain cerebrospinal fluid (figure B10.2).

$P0_2$ changes are important in regulation of respiration mainly because such changes affect the relative sensitivity of chemoreceptors to $PC0_2$. If the arterial $P0_2$ is very low, chemoreceptors become more sensitive to changes in arterial $PC0_2$.

One of the functions of the respiratory system is to eliminate carbon dioxide from body fluids. Carbon dioxide is being continually produced during cellular metabolism. Carbon dioxide diffuses into systemic capillary blood where some of it reacts with water to form carbonic acid, which dissociates into hydrogen ion and bicarbonate ion (figure B10.3). These reactions are reversible and are accelerated by an enzyme within the red blood cell called carbonic anhydrase.

In the systemic capillary, $C0_2$ is added to the reaction, driving the reaction to the right, forming more H^+ and lowering the pH of the blood.

In the pulmonary capillary, $C0_2$ is removed from the reaction, pulling the reaction to the left, reducing the amount of H+ and elevating the pH of the blood.

Normally, the lungs eliminate $C0_2$ at the same rate as it is being produced by cells. Under such conditions, the reaction sequence (figure B 10.3) moves to the right and then back to the left an equal amount (a state of equilibrium) and no net change in hydrogen ion concentration or carbon dioxide content occurs.

Elimination of carbon dioxide from body fluids at a rate faster than it is being produced would drive the reaction sequence more to the left, thereby reducing the amount of H^+ in the body fluids and raising the pH. This would occur in alveolar hyperventilation. The process is called respiratory alkalosis, and the resultant elevated blood pH is called alkalemia.

If the hyperventilation is voluntary, excess $C0_2$ will be removed from blood (hypocapnia), tending to depress breathing until normal $C0_2$ and H+ levels are restored. The temporary cessation of breathing after voluntary hyperventilation is known as apnea vera.

Elimination of carbon dioxide from the body fluids at a rate slower than it is being produced would drive the reaction sequence more to the right, thereby increasing the amount of H+ in body fluids and lowering the pH. This would occur in alveolar hyperventilation. The process is called respiratory acidosis, and the resultant decreased blood pH is called acidemia.

If the hypoventilation is voluntary, excess $C0_2$ will accumulate in the blood (hypercapnia), tending to stimulate breathing until normal $C0_2$ and H+ levels are restored.

Volumes and Capacities:

The volume of air a person inhales (inspires) and exhales (expires) can be measured with a spirometer (spiro = breath, meter = to measure). A bell spirometer (figure B 11. 1) consists of a double-walled cylinder in which an inverted bell filled with oxygen-enriched air is immersed in water to form a seal. The bell is attached by a pulley to a recording pen that writes on a drum rotating at a constant speed. During inspiration, air is removed from the bell and the pen rises, recording an inspired volume. As expired air enters the bell, the pen falls and an expired volume is recorded. The resultant record of volume change versus time is called a spirogram.

The volume of air inspired or expired during a single breath is called tidal volume (TV). When a resting person breathes normally, tidal volume is approximately 500 ml. During exercise, tidal volume can be more than 3 liters.

Tidal volume is one of four nonoverlapping primary compartments of total lung capacity (see figure B11. 1). The other three primary lung volumes are inspiratory reserve volume, expiratory reserve volume, and residual volume.

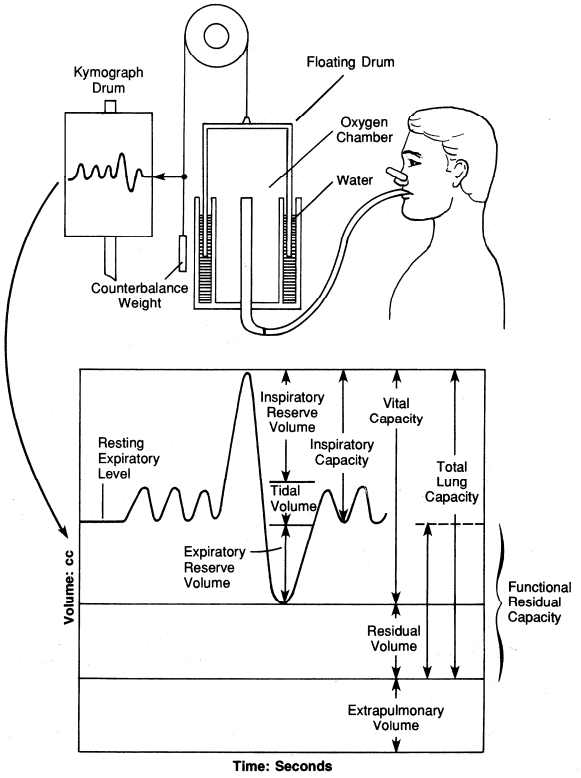


FIGURE B11.1 Spirometer and spirogram

TABLE B11.2 Components of lung volume (adult)

	Males	*Females*
Tidal volume (TV)	500 ml	500 ml
Inspiratory reserve volume (IRV)	3300 ml	1900 ml
Expiratory reserve volume (ERV)	1000 ml	700 ml
Residual volume (RV)	1200 ml	1100 ml
Inspiratory capacity (IC)	3800 ml	2400 ml
Functional residual capacity (FRC)	2200 ml	1800 ml
Total lung capacity (TLC)	6000 ml	4200 ml

Inspiratory reserve volume (IRV) is the volume of air that can be maximally

inhaled at the end of a tidal inspiration. Resting IRV is approximately 3300 ml and 1900 ml in young adult males and females, respectively.

Expiratory reserve volume (ERV) is the volume of air that can be maximally exhaled at the end of a tidal expiration. Resting ERV is approximately 1000 ml and 700 ml in young adult males and females, respectively.

Residual volume (RV) is the volume of gas remaining in the lungs at the end of a maximal expiration. In contrast to IRV, TV, and ERV, residual volume does not change with exercise. Average adult RV values for males and females are 1200 ml and 1100 ml, respectively. Residual volume reflects the fact that after we take our first breath at birth and inflate the lungs, we never completely empty the lungs during any subsequent respiratory cycle.

A pulmonary capacity is the sum of two or more primary lung volumes. There are five pulmonary capacities: inspiratory capacity, expiratory capacity, functional residual capacity, vital capacity, and total lung capacity.

Inspiratory capacity (IC) =	**TV + IRV**
Expiratory capacity (EC) =	**TV + ERV**
Functional residual capacity (FRC) =	**ERV + RV**
Vital capacity (VC) =	**IRV + TV + ERV**
Total lung capacity (TLC) =	**IRV + TV + ERV + RV**

Each of these capacities is represented graphically in figure B11.1.

Table B11.1 summarizes the terms, symbols, and definitions for the standard divisions of lung volume, and table B11.2 lists normal adult values.

Pulmonary volumes and capacities are generally measured in the clinical assessment of a variety of pulmonary disorders. In general, chronic pulmonary diseases may be classified into two physiologic categories: (1) obstructive pulmonary disorders, such as emphysema and bronchial asthma, and (2) restrictive pulmonary disorders, such as pulmonary fibrosis and other chronic diseases of the lung interstitium.

In a chronic obstructive pulmonary disease (COPD) such as bronchial asthma, excessive mucus secretion partially blocks airways, increasing airway resistance and thus making breathing more difficult. The asthmatic may take longer to inspire and expire, but pulmonary volumes may be normal or near normal.

In a restrictive pulmonary disease, the ability to change lung volume is decreased. For example, in silicosis (grinder's disease), a disorder caused by chronic inhalation of stone dust, sand, or flint, the lungs lose distensibility and become stiffer.

TABLE B11.1 Pulmonary volumes and capacities

Standardized term	*Symbol*	*Definition*
Inspiratory reserve volume	IRV	Maximal volume of gas that can be inspired from end tidal inspiration
Tidal volume	TV	Volume of gas inspired or expired during each respiratory cycle
Expiratory reserve volume	ERV	Maximal volume of gas that can be expired from resting expiratory level
Residual volume	RV	Volume of gas remaining in the lungs at the end of a maximal expiration
Total lung capacity	TLC	Amount of gas contained in the lungs at the end of a maximal inspiration
Vital capacity	VC	Maximal amount of gas that can be expelled from the lungs following a maximal inspiration
Inspiratory capacity	IC	Maximal amount of gas that can be inspired from the resting expiratory level
Functional residual capacity	FRC	Amount of gas remaining in the lungs at the resting end-expiratory level

In restrictive pulmonary diseases, lung capacities and volumes are generally reduced (e.g., decreased vital capacity), and in obstructive pulmonary diseases, pulmonary airflow is generally reduced. Both obstructive and restrictive pulmonary diseases often coexist (e.g., combined pulmonary emphysema and fibrosis).

Restrictive pulmonary diseases may be diagnosed, in part, by determining the lung capacities and volumes that you will measure in the experiments in this BIOPAC lab experiment. Obstructive pulmonary diseases usually require measurements of pulmonary flow rates, which will be measured in BIOPAC lab experiment B12.

Functional Expiratory Volume and Maximal Voluntary Ventilation:

Pulmonary volumes, pulmonary capacities, and pulmonary airflow rates are often measured in diagnosing and assessing chronic disease of the respiratory system. In general, chronic pulmonary diseases may be classified into two physiologic categories: chronic obstructive pulmonary disease (COPD) and chronic restrictive pulmonary disease.

In **restrictive pulmonary disease**, the subject's ability to inflate and deflate the lungs is reduced, and as a result, some lung volumes and capacities are below normal. For example, in pulmonary fibrosis, such as occurs in coal miner's disease, or in silicosis or other chronic diseases of the lung interstitium in which the lungs become less distensible, vital capacity is reduced because of reductions in inspiratory and expiratory reserve volumes.

Airflow into and out of the lungs is reduced in a **chronic obstructive pulmonary disease (COPD),** such as asthma or emphysema. In asthma, inflammation of the linings of the airways and heavy mucus secretion reduce airway diameters and increase airway resistance. This results in a wheezing sound characteristic of asthmatic breathing and a reduction in the volume of air flowing per minute into and out of the lungs.

It is not uncommon for a person to have both restrictive and obstructive pulmonary disease at the same time. For example, a person may suffer from emphysema and fibrosis of the lung at the same time, even though each disease may have a different origin and begin at a different time.

Restrictive pulmonary diseases are diagnosed, in part, by determining lung volumes and capacities as in the previous chapter. However, obstructive pulmonary diseases usually require measurements of pulmonary flow rates. In this BIOPAC lab experiment you will perform two tests to measure pulmonary flow rates: (1) forced expiratory volume (FEV) and (2) maximal voluntary ventilation (MVV).

TABLE B12.1 Forced expiratory volumes (adult)

$FEV_{1.0}$ = 83% of vital capacity in one second
$FEV_{2.0}$ = 94% of vital capacity in second second
$FEV_{3.0}$ = 97% of vital capacity in third second

Forced expiratory volume, known also as forced vital capacity and timed vital capacity, is a test in which a limit is placed on the length of time a subject has to expel vital capacity air. The normal adult is able, with maximal effort, to expire about 83% of their vital capacity in one second (FEV1.0), 94% of vital capacity in the second second (FEV2.0), and 97% of vital capacity by the end of the third second (FEV3.0). In restrictive pulmonary diseases, FEV is reduced. A person with asthma may have a normal or nearnormal vital capacity as measured in a simple one-stage test in which the subject can take as long as necessary to maximally inhale and exhale; however, when an asthmatic exhales vital capacity with maximal effort, FEV measurements are all reduced because heavy mucus secretion reduces airway diameter and it takes longer to completely exhale vital capacity against increased airway resistance. Normal FEV values are tabulated in table B12. 1.

Maximal voluntary ventilation, also known as maximal breathing capacity, measures both volume and flow rates and is used to assess overall function with respect to pulmonary ventilation. In performing this test, the subject inspires and expires as deeply and as rapidly as possible while the tidal volume and the respiratory rate are measured. The product of the average volume per respiratory cycle (liters) times the number of cycles per minute equals MVV (liters/min). Normal values vary with sex, age and body size (see tables B12.2 and B12.3). MVV tends to be reduced in both restrictive and obstructive pulmonary diseases.

TABLE B12.2 Predicted maximal voluntary ventilation—males, 1/min (Cournand and Richards)

Age years	Body surface area—m^2 1.40	1.45	1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10
16–19	103	112	116	120	124	128	132	135	139	143	147	151	155	159	163
20–24	105	109	113	116	120	124	128	131	135	139	143	146	150	154	158
25–29	101	105	109	112	116	120	123	126	130	134	138	141	145	148	152
30–34	98	101	105	108	112	115	119	122	126	129	133	136	140	143	147
35–39	94	97	101	104	108	111	114	117	121	124	128	131	134	137	141
40–44	91	94	97	100	103	106	110	113	116	119	123	126	129	132	136
45–49	87	90	93	96	99	102	105	108	112	115	118	121	124	127	130
50–54	83	86	89	92	95	98	101	104	107	110	113	115	118	121	124
55–59	79	82	85	88	91	93	96	99	102	105	108	110	113	116	119
60–64	76	78	81	83	86	89	92	94	97	100	103	105	108	111	113
65–69	72	74	77	79	82	84	87	90	92	95	98	100	103	106	108
70–74	68	70	73	75	78	80	83	85	88	90	93	95	98	100	102
75–79	64	67	69	71	74	76	78	80	83	85	88	90	92	94	97

TABLE B12.3 Predicted maximal voluntary ventilation—females, 1/min (Cournand and Richards)

Age years	Body surface area—m^2 1.40	1.45	1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10
16–19	88	91	94	98	101	194	197	110	113	116	120	123	126	129	132
20–24	85	88	92	95	98	101	104	106	109	112	116	119	122	125	128
25–29	82	85	88	91	94	97	100	102	105	108	111	114	117	120	123
30–34	79	81	84	87	90	93	96	98	101	104	107	109	112	115	118
35–39	75	77	80	83	86	89	91	94	97	99	102	105	108	111	113
40–44	72	74	77	79	82	85	87	90	93	95	98	101	103	106	108
45–49	68	70	73	75	78	81	83	85	88	91	93	96	98	101	103
50–54	65	67	70	72	74	77	79	81	84	86	88	91	93	96	98
55–59	62	64	66	68	71	73	75	77	79	81	84	86	88	91	93
60–64	59	61	63	65	67	69	71	73	75	77	79	81	84	86	88
65–69	55	57	59	61	63	65	67	69	71	73	75	77	79	81	83
70–74	52	54	56	58	59	61	63	65	67	69	71	72	74	76	78
75–79	48	50	52	54	55	57	59	61	62	64	66	68	69	71	73

Experiments I: Measurements of Volumes and Capacities

Objectives:

1. To observe experimentally, record, and/or calculate selected pulmonary volumes and capacities.
2. To compare the observed values of volume and capacity with predicted normals.
3. To compare the normal values of pulmonary volumes and capacities of subjects differing in sex, age, weight, and height.

Questions to think about:

1. Explain the difference between a pulmonary volume and a pulmonary capacity.

2. Vital capacity and expiratory reserve volume tend to decrease with age, but functional residual capacity normally remains constant (ages 20-65). Why?

3. Explain the difference between obstructive pulmonary disease and restrictive pulmonary disease.

4. Bronchial asthma is an obstructive pulmonary disease in which vital capacity (single-stage) may be normal. Explain.

5. Adult vital capacity gradually decreases with age. Give two reasons why.

6. Which pulmonary volumes would change during exercise and which would not? Explain your answer.

Materials

BIOPAC SS11LA airflow transducer
BIOPAC disposable mouthpiece and bacteriological filter BIOPAC AFT8
autoclavable reusable mouthpiece (optional)
BIOPAC calibration syringe
nose clip

EXPERIMENTAL METHODS: Setup

1. Turn on your computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC100A transducer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.

3. Plug the airflow transducer (SS11LA) into channel 1 (figure B11.2).

4. Place a filter onto the end of the calibration syringe and insert the calibration syringe/filter assembly into the airflow transducer (figure B 11.3).

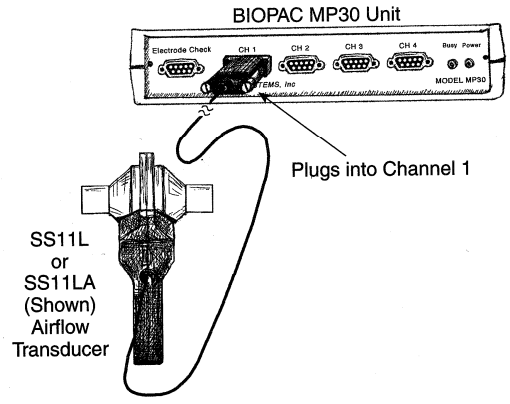


FIGURE B11.2 Used with permission from BIOPAC

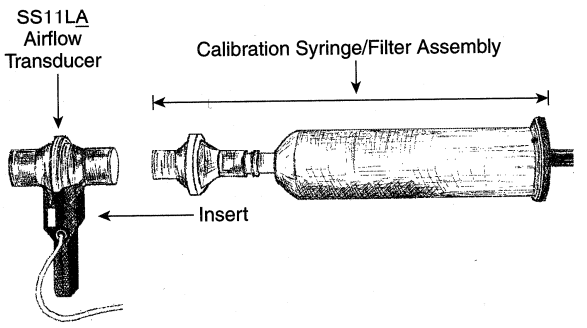


FIGURE B11.3 Used with permission from BIOPAC

NOTE: If using the SS11LA transducer with nonremovable head, insert the syringe assembly into the large diameter port. If using the SS11LA transducer with removable, cleanable head, insert the syringe assembly so that the transducer cable exits on the left, as shown in figure B11.3.

IMPORTANT: If your lab sterilizes the airflow heads after each use, make sure a clean head is installed now.

5. Locate the BIOPAC Student Lab program. A prompt will appear (figure B 11.4) asking you to choose a lesson. Choose Lesson 12 ("L12-Lung-1") by clicking on it to highlight it, then clicking "OK:'

6. A prompt should appear asking you to "Please type in your file name." Enter a unique identifier so that you can locate and retrieve your data for analysis after data recording.

7. After you log on, a window similar to figure B11.5 will appear. This ends the setup procedure.

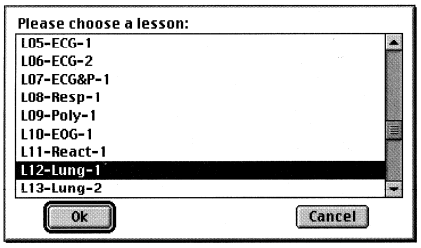


FIGURE **B11.4**

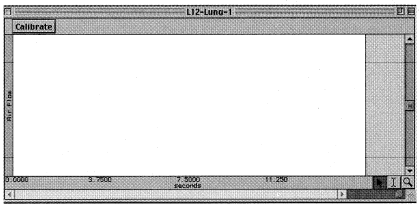


FIGURE **B11.5**

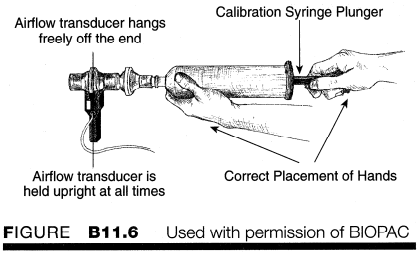


FIGURE **B11.6** Used with permission of BIOPAC

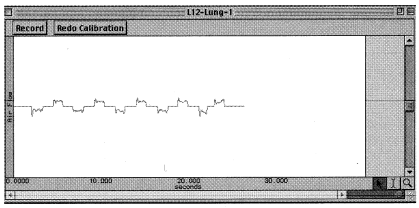


FIGURE **B11.7**

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1. Pull the calibration syringe plunger all the way out and hold the calibration syringe/filter assembly upright
(figure B11.6). The airflow transducer is sensitive to gravity so it needs to be held upright throughout the calibration and recording.

IMPORTANT: Never hold on to the airflow transducer handle when using the calibration syringe or the syringe tip may break.

2. Click on "Calibrate." Leave the plunger extended and hold the assembly steady and upright during the entire calibration procedure. Do not touch the plunger, because any pressure at this stage will cause inaccurate results.
The first stage of calibration will run for 8 seconds and automatically end with an alert box. Read step 3 before proceeding.

3. In the second stage of calibration, you will cycle the syringe plunger completely in and completely out for 5 consecutive cycles (10 strokes). Use a rhythm of about 1 second per stroke with 2 seconds of rest between strokes.

NOTE: The calibration procedure may seem a little strange, but it is required because of the complexity of the airflow-to-volume calculation. The accuracy of this conversion is aided by analyzing the airflow variations occurring over 5 complete cycles of the calibration syringe.

4. Click "Yes" to start the second stage of calibration. Cycle the syringe plunger as stated above in step 3. At the end of the fifth cycle, stop the calibration procedure by clicking on "End Calibration:'

5. At the end of the calibration recording, your screen should resemble figure B11.7. If your data shows 5 downward deflections and 5 upward deflections, proceed to the Data Recording section. If the data recording shows any large spikes, then you must redo the calibration by clicking on "Redo Calibration" and repeating the entire calibration sequence.

Data Recording

In order to work efficiently, read this entire section so you will know what to do for each recording segment. The subject should remain seated and continue to relax while you review the lesson. Following the procedure precisely is very important, as the calculation from airflow to volume is very sensitive.

Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not use an excessive amount of time (time is memory).

NOTE: Residual volume (RV) cannot be determined using a normal spirometer or airflow transducer, so the BIOPAC Student Lab software uses a default of 1L. If 1L of RV is not desired, see your instructor.

Hints for Obtaining Optimal Data

1. Keep the airflow transducer upright at all times (figure B11.11).
2. If you start the recording on an inhale, try to end on an exhale, and vice versa. This is not absolutely critical, but it does increase the accuracy of the airflow-to-volume calculation.
3. Subject should not look at the screen during recording.

Procedure

1. Insert a clean mouthpiece (and filter, if applicable) into the airflow transducer.
Important: If your lab sterilizes the airflow heads after each use, make sure a clean head is installed now. To be safe and to avoid contamination, have the subject personally remove the filter and mouthpiece from the plastic packaging. This mouthpiece will become the subject's personal mouthpiece. It is advisable to write the subject's name on the mouthpiece and filter with a permanent marker so they can be reused later.

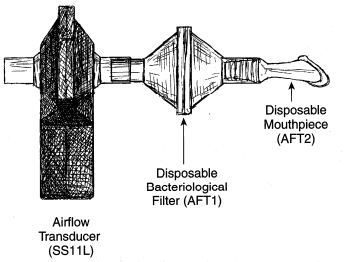


FIGURE **B11.8** Used with permission of BIOPAC

If using the SS11LA transducer with nonremovable head, insert a new filter and mouthpiece into the larger diameter port (figure B 11.8).

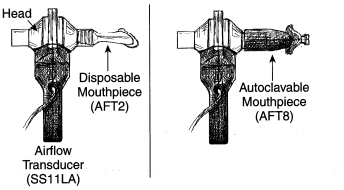


FIGURE **B11.9** Used with permission of BIOPAC

If using the SS11LA transducer and sterilizing the head after each use, insert a disposable mouthpiece (BIOPAC AFT2) or an autoclavable mouthpiece (BIOPAC AFT8) into the airflow transducer (figure B11.9).

If using the SS11LA transducer and not sterilizing the head after each use, insert a filter and mouthpiece into the airflow transducer (figure B11.10).

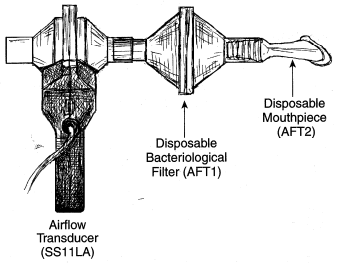


FIGURE **B11.10** Used with permission of BIOPAC

2. Ask the subject to place a nose clip on his or her nose and begin breathing normally through the airflow transducer (figure B11.11). The subject must hold the airflow transducer upright at all times.

3. Click on "Record." Ask the subject to:

(a) breathe normally for 3 breaths, then, after a resting expiration
(b) inhale as deeply as possible, and then

(c) exhale just to the point of normal resting expiration, and then
(d) breathe normally for 3 breaths and then after a normal resting inspiration, maximally exhale, and then
(e) resume breathing normally for 3 breaths.

4. Click on "Stop." As soon as the Stop button is pressed the BIOPAC Student Lab software will automatically calculate volume data based on the recorded airflow data. At the end of the calculation, both waveforms will be displayed on the screen (figure B11.12).

5. If all went well, your data should look similar to figure B 11.12. The data would be incorrect and you will need to repeat the recording if you feel you did not follow the procedure precisely, (for example, if the subject coughed or air escaped). In this case, you should redo the recording by clicking on "Redo" and repeating step 3. Note that once you press "Redo ", the data you have just recorded will be erased.

6. Click on "Done." After you press Done, your data will automatically be saved in the "Data Files" folder on your hard drive. A pop-up window with four options will appear. Make your choice, and continue as directed.

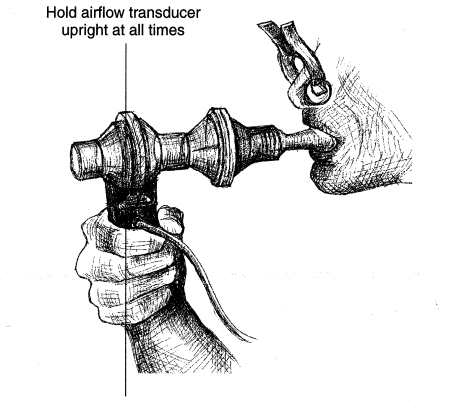


FIGURE B11.11 Used with permission of BIOPAC

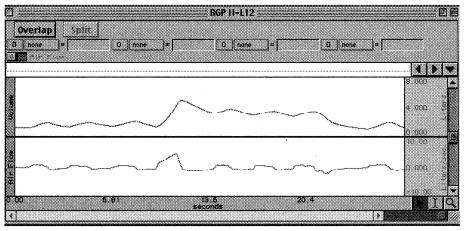


FIGURE B11.13

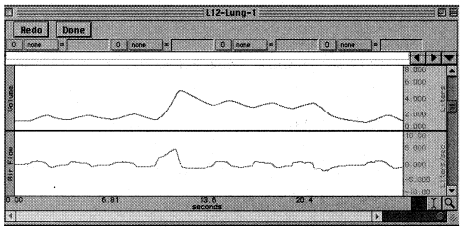


FIGURE B11.12

If choosing the "Record from another subject" option:

(a) You will not need to recalibrate the airflow transducer. For this reason, we recommend that all recordings be completed before your proceed to Data Analysis.
(b) Remember to have each person use his or her own mouthpiece, bacteriological filter, and nose clip.
(c) Repeat recording steps 1-6 for each new subject.
(d) Each person will need to use a unique file name.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file, and open it. The data window should be similar to figure B11.13. Note the channel number designations:

CH 0 = volume
CH 40 = airflow

2. Although you will not use the airflow data, it actually contains an interesting perspective on the recording. Looking at the airflow waveform, note that the vertical scale is in liters per second ("Liters/sec.") and that the data recording is centered on zero. Looking at the graph, you can see that with

each exhale, a downward pointing curve appears. The deeper an inhale, the larger the positive peak; the more forceful an exhale, the larger the negative peak.

3. Turn off channel 40 (airflow). To toggle a channel on/off, click on the channel number box and hold down the option key (Mac) or the Ctrl key (PC).

4. Set up the measurement boxes as follows:

CH 0 = p-p
CH 0 = max
CH 0 = min
CH 0 = delta

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. Here are some brief descriptions of the specific measurement boxes used for this experiment:

p-p: This measurement reflects the maximum value in the selected area minus the minimum value found in the selected area.

max: This measurement is the maximum value in the selected area.

min: This measurement is the minimum value in the selected area.

Δ: The "delta amplitude" measurement computes the difference in amplitude between the last point and the first point of the selected area.

The "selected area" is the area selected by the I-beam tool (including the endpoints).

5. Use the I-beam tool to select the region of the first three breaths (figure B11.14). The p-p measurement represents tidal volume (TV). Record the value in table B11.3 in the report.

6. Use the I-beam tool to select an appropriate area for measurement and the Δ measurement to determine values for IRV (figure B11.15), ERV (figure B11.16), and VC (figure B11.17). Record the values in table B11.3 in the report.

7. You may save the data to a diskette, save notes that are in the journal, or print the data file.

8. Exit the program. Turn off the MP30 unit and shut down the computer.

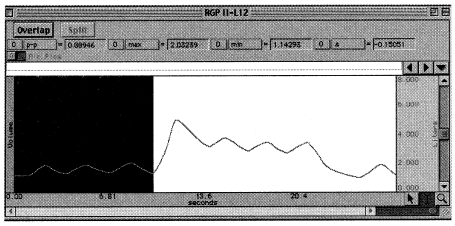


FIGURE **B11.14**

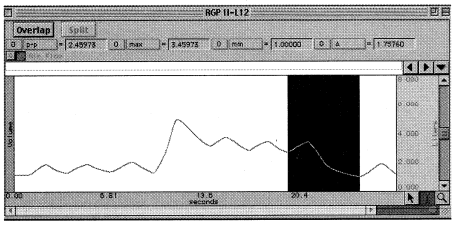


FIGURE **B11.16**

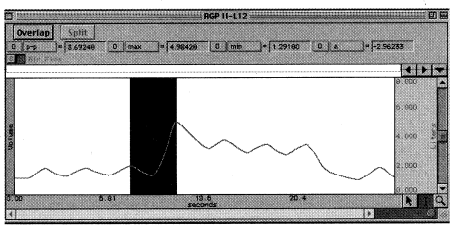


FIGURE **B11.15**

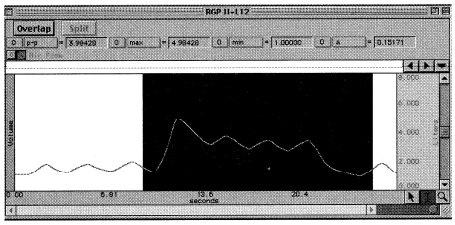


FIGURE **B11.17**

Volumes and Capacities Data:

Experimenter Name:_____

Date:_____

1. Volume measurements:

(a) Subject

Name_____Age_____Height_____Weight_____

Gender: Male/Female

(b) Predicted vital capacity (Kory, Hamilton, Callahan, 1960): Use the equation below to calculate the subject's predicted vital capacity.

Equations for predicted vital capacity

Male **VC = 0.052H - 0.022A - 3.60**

Female **VC = 0.041H - 0.018A - 2.69**

VC = vital capacity in liters
H = height in centimeters
A = age in years

Work space for calculating predicted vital capacity:

Predicted vital capacity:_____liters

Notes:

(c) Observed volumes and capacities:

TABLE B11.3

Volume titles	*Measurement (liters)*
Tidal volume (TV)	
Inspiratory reserve volume (IRV)	
Expiratory reserve volume (ERV)	
Vital capacity (VC)	

Residual volume (RV) used:_____liters (default is I liter)

Notes:

Using data obtained above, calculate the capacities indicated in table B11.4.

TABLE B11.4

Capacity	Formula	Your calculation
Inspiratory (IC)	IC = TV + IRV	
Expiratory (EC)	EC =TV+ ERV	
Functional residual (FRC)	FRC = ERV + RV	
Total lung (TLC)	TLC= IRV+TV+ ERV+ RV	

Compare the subject's lung volumes with the average volumes presented in the introduction to this **BIOPAC** lab experiment.

	Subject	*Average*
Tidal volume	_____	_____
Inspiratory reserve volume	_____	_____
Expiratory reserve volume	_____	_____

Notes:

(d) Observed capacity versus predicted capacity:

What is the subject's observed vital capacity compared to predicted vital capacity?

$$\frac{\text{\_\_\_\_\_ liters obs}}{\text{\_\_\_\_\_ liters pred.}} \times 100 = \text{\_\_\_\_\_}\%$$

NOTE: Vital capacities are dependent on other factors besides age and height. Therefore, 80% of predicted values are still considered "normal."

Notes:

Data Analysis:

Can you answer these questions?

1. Explain the difference between a pulmonary volume and a pulmonary capacity.

2. Vital capacity and expiratory reserve volume tend to decrease with age, but functional residual capacity normally remains constant (ages 20-65). Why

3. Explain the difference between obstructive pulmonary disease and restrictive pulmonary disease

4. Bronchial asthma is an obstructive pulmonary disease in which vital capacity (single-stage) may be normal. Explain

5. Adult vital capacity gradually decreases with age. Give two reasons why.
(a

(b

6. Which pulmonary volumes would change during exercise and which would not? Explain your answer.

(a) TV

(b) IRV

(c) ERV

(d) RV

Experiments II: Measurements of Forced Expiratory Volume and Maximal Voluntary Ventilation

Experimental Objectives

1. To observe experimentally, record, and/or calculate forced expiratory volume (FEV) and maximal voluntary ventilation (MVV).
2. To compare observed values of FEV and MVV with predicted normals.
3. To compare normal values of pulmonary flow rates of subjects differing in sex, age, and body surface area.

Questions to think about:

2. Is it possible for a subject to have a vital capacity (single stage) within the normal range but a value for FEV1.0 below normal range? Why?

3. Maximal voluntary ventilation decreases with age. Why?

4. According to tables B 12.2 and B 12.3, for any given age and body surface, men have a greater MVV than women. Give one anatomic or physiologic reason why.

5. What effect would you expect smoking to have on a subject's FEV and MVV? Explain.

6. Asthmatics tend to have their smaller airways narrowed by smooth muscle constriction, thickening of the walls, and mucous secretion. How would this affect vital capacity, FEV,, and MVV?

7. Define forced expiratory volume (FEV).

8. Define maximal voluntary ventilation (MVV).

9. What would you predict the FEV, MVV, and VC would be for someone with emphyzcema? Someone who is an olympic swimmer? Someone who smokes six (6) cigarrettes a month?

Materials

BIOPAC SS11LA airflow transducer
BIOPAC disposable mouthpiece and bacteriological filter
AFT8 autoclavable reusable mouthpiece
BIOPAC calibration syringe

EXPERIMENTAL METHODS
Setup

1. Turn on your computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC100A transducer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.

3. Plug the airflow transducer (SS11LA) into channel I (figure B12.1).

4. Place a filter onto the end of the calibration syringe and insert the calibration syringe/filter assembly into the airflow transducer (figure B12.2).

NOTE: If using the SS11L transducer with nonremovable head, insert syringe assembly into the large diameter port.

If using the SS11LA transducer with removable, cleanable head, insert the syringe assembly so that the transducer cable exits on the left, as shown in figure B12.2.

IMPORTANT: If your lab sterilizes the airflow heads after each use, make sure a clean head is installed now.

5. Locate the BIOPAC Student Lab program. A prompt will appear (figure B12.3) asking you to choose a lesson. Choose Lesson 13 ("L13Lung-2") by clicking on it to highlight it, then clicking "OK."

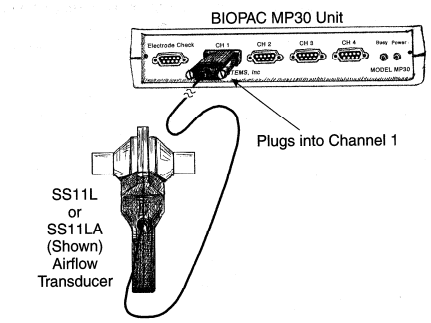


FIGURE B12.1 Used with permission of BIOPAC

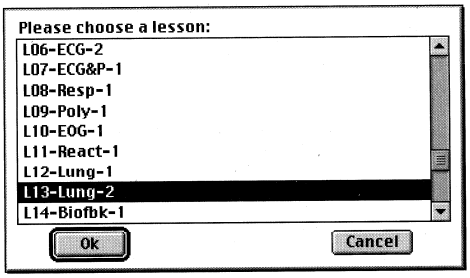


FIGURE B12.3

6. A prompt should appear asking you to "Please type in your file name." Enter a unique identifier so that you can locate and retrieve your data for analysis after data recording.

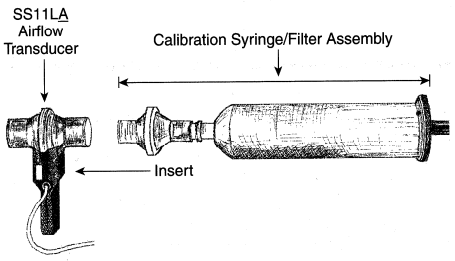


FIGURE B12.2 Used with permission of BIOPAC

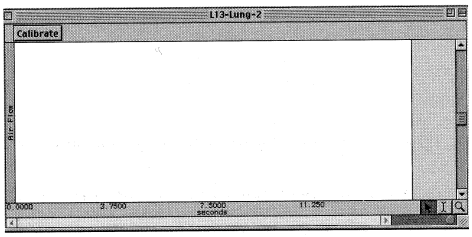


FIGURE B12.4

7. After you log on, a window similar to figure B12.4 will appear. This ends the setup procedure.

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1. Pull the calibration syringe plunger all the way out and hold the calibration syringe/filter assembly upright (figure B12.5). The airflow transducer is sensitive to gravity so it needs to be held upright throughout the calibration and recording.

IMPORTANT: Never hold on to the airflow transducer handle when using the calibration syringe or the syringe tip may break.

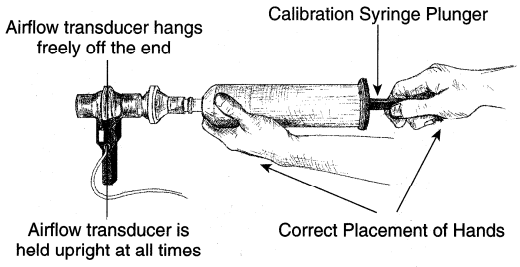


FIGURE B12.5 Used with permission of BIOPAC

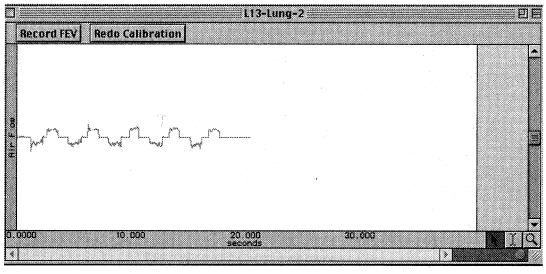


FIGURE B12.6

2. Click on "Calibrate," Leave the plunger extended and hold the assembly steady and upright during the entire calibration procedure. Do not touch the plunger because any pressure at this stage will cause inaccurate results.
The first stage of calibration will run for 8 seconds and automatically end with an alert box. Read step 3 before proceeding.

3. In the second stage of calibration, you will cycle the syringe plunger completely in and completely out for 5 consecutive cycles (10 strokes). Use a rhythm of about 1 second per stroke with 2 seconds of rest between strokes.

NOTE: The calibration procedure may seem a little strange, but it is required because of the complexity of the airflow-to-volume calculation. The accuracy of this conversion is aided by analyzing the airflow variations occurring over 5 complete cycles of the calibration syringe.

4. Click "Yes" to start the second stage of calibration. 7.
Cycle the syringe plunger as stated above in step 3. At the end of the fifth cycle, stop the calibration procedure by clicking on "End Calibration:'

5. At the end of the calibration recording, your screen should resemble figure B12.6. If your data shows 5 downward deflections and 5 upward deflections, proceed to the Data Recording section. If the data recording shows any large spikes, then you must redo calibration by clicking on the "Redo Calibration" and repeating the entire calibration sequence.

Data Recording

In order to work efficiently, read this entire section so you will know what to do for each recording segment. The subject should remain seated and continue to relax while you review the lesson. Following the procedure precisely is very important, as the calculation from airflow to volume is very sensitive.

Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not use an excessive amount of time (time is memory).

Hints for Obtaining Optimal Data

1. The subject should wear loose clothing so clothing does not inhibit chest expansion.

2. Always use the nose clip to ensure that there is no loss of air through the nose during recording.

3. The subject must try to expand the thoracic cavity to its largest volume during maximal inspiratory efforts.

4. During recording of FEV, the subject should attempt to exhale as quickly as possible into the mouthpiece.
During recording of MVV, the subject should attempt to exhale and inhale as quickly as possible. Breathing rates should be faster than 60 breaths/minute or greater than I breath/second for the best results. The breathing needs to be maintained for 12-15 seconds.

6. The subject should always begin breathing into the airflow transducer before the recording begins and continue breathing into the airflow transducer until after the recording ends.

If you start the recording on an inhale, try to end on an exhale, and vice versa. This is not absolutely critical,
but it will increase the accuracy of the airflow-to-volume calculation.

Procedure

1. Insert a clean mouthpiece (and filter, if applicable) into the airflow transducer.

Important: If your lab sterilizes the airflow heads after each use, make sure a clean head is installed now. To be safe and to avoid contamination, have the subject personally remove the filter and mouthpiece from the plastic packaging. This mouthpiece will become the subject's personal mouthpiece.

It is advisable to write the subject's name on the mouthpiece and filter with a permanent marker so they can be reused later. If using the SS11L transducer with nonremovable head, insert a new filter and mouthpiece into the larger diameter port (figure B12.7).

If using the SS11LA transducer and sterilizing the head after each use, insert a disposable mouthpiece (BIOPAC AFT2) or an autoclavable mouthpiece (BIOPAC AFT8 into the airflow transducer (figure B12.8). If using the SS11LA transducer and not sterilizing the head after each use, insert a filter and mouthpiece into the airflow transducer (figure B12.9).

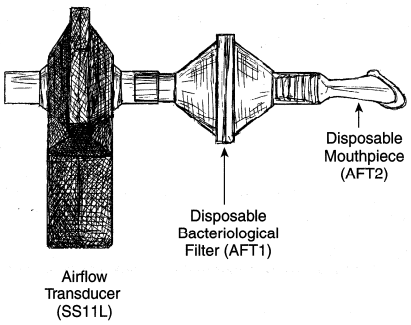


FIGURE B12.7 Used with permission from BIOPAC

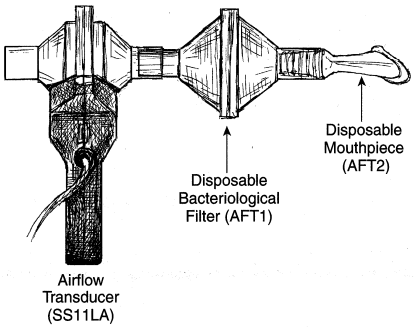


FIGURE B12.9 Used with permission from BIOPAC

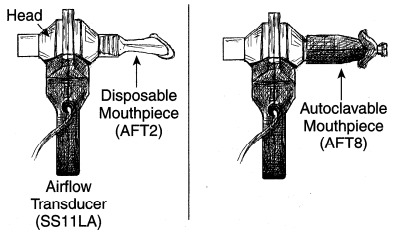


FIGURE B12.8 Used with permission from BIOPAC

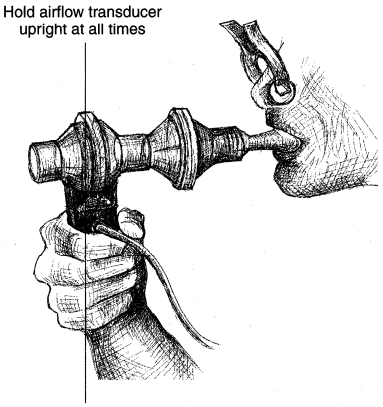


FIGURE B12.10 Used with permission from BIOPAC

2. Ask the subject to place a nose clip on his or her nose and begin breathing normally through the airflow transducer (figure B12.10). The subject must hold the airflow transducer upright at all times.

3. Click on "Record." Ask the subject to:

(a) Breathe normally for 3 cycles (1 cycle inspiration + expiration).
(b) Following a normal expiration, inhale as deeply as possible (maximal inspiration), hold the breath for 6 about a second, then forcefully and maximally exhale (maximum expiration).
(c) Resume normal breathing for 3 more cycles.

4. Click on "Stop." As soon as the Stop button is pressed the BIOPAC Student Lab software will automatically convert the airflow data to volume data. At the end of the calculation, just the volume data will be shown on the screen, as in figure B12.11.

5. Your data should look similar to figure B12.11. Your data are incorrect if you cannot clearly define the start of maximal expiration or you feel you did not follow the procedure precisely. If your data are incorrect, you should repeat the recording by clicking on "Redo" and repeating steps 3 and 4. .

6. Use the I-beam tool to select the area from the beginning of maximal expiration to the end of maximal expiration (figure B12.12). The selected area must be at least 3 seconds in length.

The first measurement box will automatically record Δ**T** so you can make sure the selected area is longer than 3 seconds.

7. When you have selected the correct area, click on "Setup FEV." The program will cut out your selected area, invert it, and paste it into a new channel (figure B12.13). The volume waveform originally recorded

will be hidden from view so you can concentrate on the data needed to calculate FEV. The plot shows the cumulative expired volume over time.

8. Compare your data to figure B12.13. If you wish to reselect an area in figure B12.12, click on "Redo" and repeat steps 6 and 7.

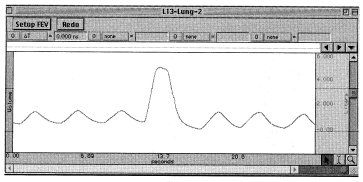


FIGURE **B12.11**

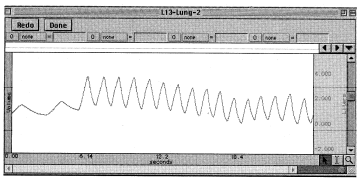


FIGURE **B12.14**

9. Click on "Begin MVV." The current FEV data on the screen will be automatically saved to disk for later analysis and the screen display will change to include a "Record MVV" button.

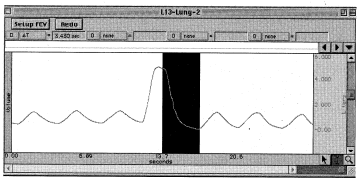


FIGURE **B12.12**

10. Ask the subject to place a nose clip on and begin breathing through the airflow transducer. It is important that the subject begin breathing through the airflow transducer before you click on the "Record MVV" button. Make sure air does not leak through the mouthpiece or nose clip.

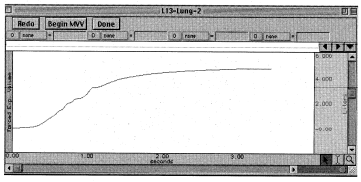


FIGURE **B12.13**

11. Click on "Record MVV." Instruct the subject to perform the following procedure:

(a) Breathe normally into the airflow transducer for 5 cycles.
(b) Breathe as quickly and as deeply as possible for 12-15 seconds. For optimal results the emphasis should be on speed more than depth of breathing.

WARNING
This procedure can make the subject dizzy and lightheaded. The subject should stop maximally ventilating if sickness or excessive dizziness is experienced.

(c) Breathe normally for 5 additional cycles.

12. Click on "Stop." As soon as the Stop button is pressed, the BIOPAC Student Lab software will automatically convert the airflow data to volume data. At the end of the calculation, just the volume data will be shown on the screen (figure B12.14).

13. Your data should resemble figure B12.14. Your data would be incorrect if you did not follow the procedure precisely or if the subject failed to breathe as rapidly and as deeply as possible for at least 12 seconds. If your data are incorrect, click on "Redo" and repeat steps 10, 11, and 12. Note that once you press "Redo," the data you have just recorded will be erased.

14. Click on "Done." After you press "Done," your data will automatically be saved in the "Data Files" folder on your hard drive (with an FEV and an MVV extension after the file name). A pop-up window with four options will appear. Make your choice, and continue as directed.

If choosing the "Record from another subject" option:

(a) You will not need to recalibrate the airflow transducer. For this reason, we recommend that all recordings be completed before your proceed to Data Analysis.
(b) Remember to have each person use his or her own mouthpiece, bacteriological filter, and nose clip.

(c) Repeat recording steps 1-13 for each new subject. (d) Note that each person will need to use a unique file name.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file (your file name with the extension "FEV-L13"), and open it.

2. Turn grids on by selecting "Display preferences" from the File menu; then choose "Grids' " select "Show Grids," and click "OK:' The data window should resemble figure B 12.15.

3. Set up the measurement boxes as follows:

CH 1 =∆T
CH 1 =p-p

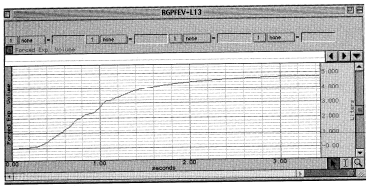


FIGURE **B12.15**

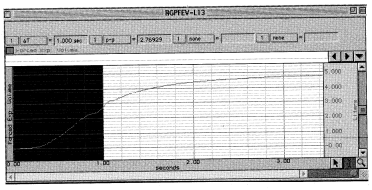


FIGURE **B12.16**

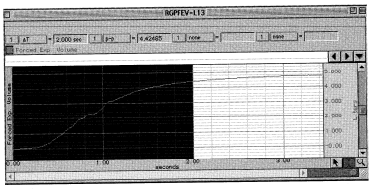


FIGURE **B12.17**

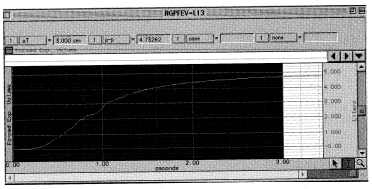


FIGURE **B12.18**

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. Here are some brief descriptions of the specific measurements used for this experiment:

∆**T:** The "delta time" measurement is the difference in time between the end and beginning of the selected area.

p-p: This measurement reflects the maximum value found in the selected area minus the minimum value found in the selected area.

The "selected area" is the area selected by the I-beam tool (including the endpoints).

4. Use the I-beam tool to select the first 1 -second interval (figure B12.16). The selected area should be from time 0 to the 1 -second reading as displayed in the ∆**T** measurement.

Record the volume expired at the 1-second mark (p-p value) in table B 12.4 in the report.

5. Use the I-beam tool to select the 2-second interval from time 0 to the 2-second mark (figure B12.17). Record the volume expired at the 2-second mark (p-p value) in table B12.4 in the report.

6. Use the I-beam tool to select the 3-second interval from time 0 to the 3-second mark (figure B12.18). Record the volume expired at the 3second mark (p-p value) in table B12.4 in the report.

7. Use the I-beam tool to select the entire record from time 0 to the end of the recording. The p-p value is now the value for vital capacity (in liters). Record the vital capacity in table B12.4 in the report.

8. Pull down the Lessons menu, select "Review Saved Data," choose your data file from the MVV recording (your file name with the extension "MVV.L13"), and open it.

9. Use the zoom tool to select the deep, fast-breathing segment for analysis (figure B 12.19).

10. Set up the measurement boxes as follows:

CH0 = ∆T
CH0 = p-p

11. Use the I-beam tool to select a 12-second area that is convenient to count the number of cycles in the interval (figure B12.20). Start at the beginning of an inspiration and drag the cursor to the right until the **∆T** measurement value is 12 seconds.

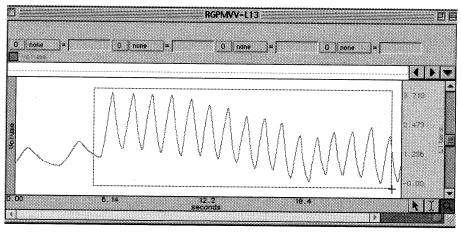


FIGURE **B12.19**

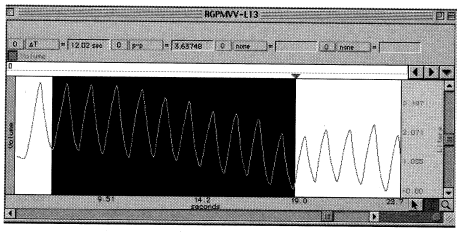


FIGURE **B12.21**

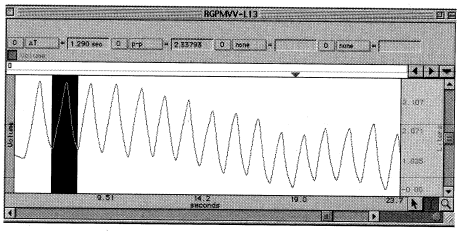


FIGURE **B12.20**

12. Count the number of complete cycles within the selected area and record the number in the report.

13. Leave the selected area darkened, click on the cursor tool, and use it to place a marker at the end of the selected area (figure B12.20). To insert a marker where the cursor tool is located along the marker bar, press the Esc key (Mac) or the F9 key (PC).

14. After placing the marker, click on the I-beam tool and use it to select each complete individual cycle in the 12second interval defined in step 11. Select one cycle at a time (figure B12.21). Record the volume (p-p) per cycle in table B12.5 in the report.

15. You may save the data to a diskette, save notes that are in the journal, or print the data file.

16. Exit the program. Turn off the MP30 unit and shut down the computer.

17. Use the subject's height and weight to determine body surface area (BSA) from the nomogram in figure B12.22. Record the value in the report.

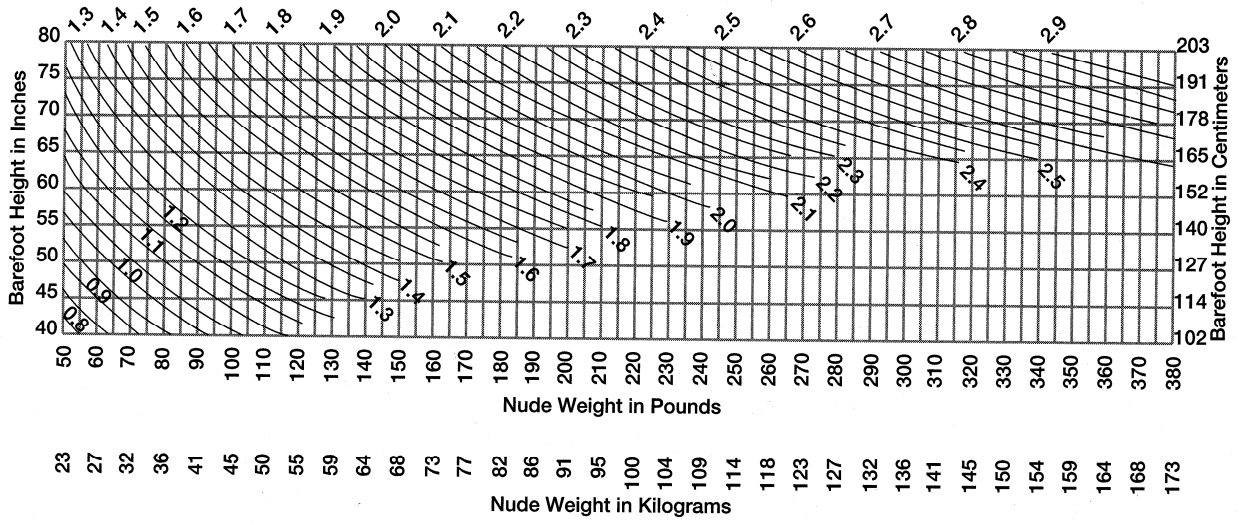


FIGURE B12.22 Body surface area nomogram

Forced Expiratory Volume and Maximal Voluntary Ventilation Data:

Experimenter Name:_____

Date:_____

1. Data and calculations:

(a) Subject

Name_____Age_____Height_____Weight_____

Gender: Male/Female_____Body Surface Area (BSA)_____(use nomogram in figure B12.22)

(b) Comparison of % (FEV/VC) to normal values:

TABLE B12.4

		p-p Measurement	
	(liters)	*FEV/VC*	*(FEV/VC) x 100*
FEV1.0	_____	_____	_____**%**
FEV2.0	_____	_____	_____**%**
FEV3.0	_____	_____	_____**%**

Vital capacity

Subject's (FEV/VC)* X *100	***Averages***
FEV1.0_____%	**66-83%**
FEV2.0_____%	**75-94%**
FEV3.0_____%	**78-97%**

How does the subject's FEV values compare to the average?
FEV1.0_____
FEV2.0_____
FEV3.0_____

(c) MVV measurements (all volume measurements are in liters):
Number of cycles in 12-second interval_____
Calculate the number of cycles per minute:

Cycles/min = number of cycles in 12-second interval x 5

Number of cycles in 12-second interval (from above): _____x 5=_____cycles/min

Notes:

Complete table B12.5 with a measurement for each individual cycle. If the subject had only 5 complete cycles per 12-second period, then only fill in the volumes for 5 cycles. If there is an incomplete cycle, do not record it. (The table may have more cycles than you need.)

TABLE B12.5

Cycle	*Measurement [CH 0 p-p]*
Cycle 1	
Cycle 2	
Cycle 3	
Cycle 4	
Cycle 5	
Cycle 6	
Cycle 7	
Cycle 8	
Cycle 9	
Cycle 10	
Cycle 11	
Cycle 12	
Cycle 13	
Cycle 14	
Cycle 15	

Calculate the average volume per cycle (AVPC):

(1) Add the volumes of all counted cycles from table B12.5:_____liters

(2) Divide the above sum by the number of counted cycles. The answer is the average volume per cycle (AVPC).

AVPC =_____liters

C***alculate the MVV.***

(1) Multiply the AVPC times the number of respiratory cycles per minute (RR) as calculated earlier.
MVV = AVPC X RR =_____liters/min

(2) How does the subject's MVV compare to his or her expected normal in table B12.2 or table B12.3?

Notes:

Data Analysis:

Can you answer these questions?

1. Is it possible for a subject to have a vital capacity (single stage) within the normal range but a value for FEV1.0 below normal range? Why?

2. Maximal voluntary ventilation decreases with age. Why?

3. According to tables B 12.2 and B 12.3, for any given age and body surface, men have a greater MVV than women. Give one anatomic or physiologic reason why.

4. What effect would you expect smoking to have on a subject's FEV and MVV? Explain.

5. Asthmatics tend to have their smaller airways narrowed by smooth muscle constriction, thickening of the walls, and mucous secretion. How would this affect vital capacity, FEV,, and MVV?

6. Define forced expiratory volume (FEV).

7. Define maximal voluntary ventilation (MVV).

Experiments III: Measurements of the Respiratory Cycle

In the experiments that follow, physiologic modification of the respiratory cycle as it occurs under a variety of conditions will be observed. You will measure ventilation by recording the rate and depth of the breathing cycle using a pneumograph transducer. This transducer converts changes in chest expansion and contraction to changes in voltage, which will appear as a waveform. One respiratory cycle will then be recorded as an increasing voltage (ascending segment) during inspiration and as a decreasing voltage (descending segment) during expiration. Also, you will record the temperature of the air flowing in and out of one nostril with a temperature probe. The temperature of the air passing by the temperature probe is inversely related to the expansion or contraction of the subject's chest. During inspiration when the chest expands, the subject breathes in relatively cool air (compared to the subject's body temperature). The air is warmed in the body. During expiration, when the chest contracts, the warmer air is compressed out of the lungs and out the respiratory passages.

Experimental Objectives

1. To observe and record normal respiratory rate and depth utilizing pneumograph and air temperature transducers.

2. To observe and record modifications in the rate and depth of the normal respiratory cycle due to cerebral influence and chemoreceptor influence on the medullary control centers.

Questions to Think about:

1. If the subject had held his or her breath immediately after hyperventilation and hypoventilation, would the subject have been able to hold his or her breath longer after hyperventilation or after hypoventilation? Why?

2. After a brief period of hyperventilation, apnea vera occurs.

   (a) Define hyperventilation and apnea vera.

   (b) Describe the feedback loop causing apnea vera

3. (a) What changes occur in the body with hypoventilation?

   (b) How does the body adjust rate and depth of ventilation to counteract the effects of hypoventilation?

4. In which part of the respiratory cycle is temperature the highest? Lowest?
Explain why the temperature varies with the respiratory cycle.

5. Describe or define cough in terms of modification of the breathing cycle.

6. What modifications of the breathing cycle occur when reading aloud? Why?

Materials

BIOPAC respiratory transducer SS5LA or SS51

BIOPAC temperature transducer SS6L

EXPERIMENTAL METHODS
Setup

1. Turn on your computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC100A transformer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.

3. Attach the respiratory transducer (SS5L or SS5LA) around the chest below the armpits and above the nipples (figure B 10.4). The correct tension is critical. The respiratory transducer must be slightly tight at the point of maximal expiration. The transducer can be worn over a thin shirt or blouse. IMPORTANT USE NOTE: If using the SS5LA transducer, you must be very careful not to pull or yank on the rubber bowtie portion that contains the sensor element.

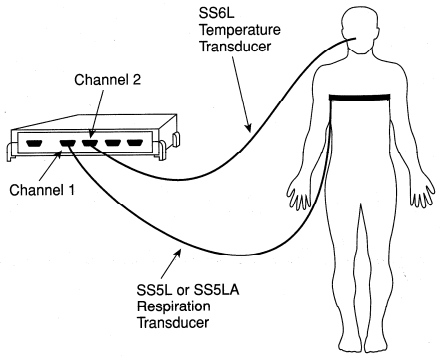


FIGURE B10.4 BIOPAC setup for measuring airflow and respiration

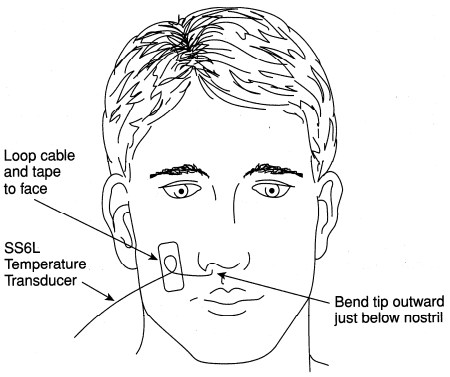


FIGURE B10.5 Placement of airflow sensor

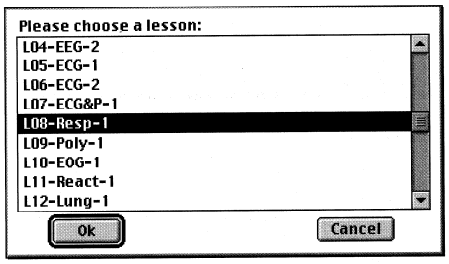


FIGURE B10.6

4. Attach the temperature transducer (thermistor) to the subject as shown in figure B10.5. The thermistor should be firmly attached so it does not move. The thermistor should be positioned below the nostril, directly in the airflow pathway, and should not be touching the face. It is usually best to make a small loop in the cable about 2 inches from the temperature probe tip and tape the loop to the subject's face.
Plug the respiratory transducer into channel 1 of the MP30 and plug the temperature transducer into channel 2.

5. Locate the BIOPAC Student Lab folder, open it, and start the BIOPAC Student Lab program. A prompt will appear (figure B10.6) asking you to choose a lesson. Choose Lesson 8 ("L08-Resp-1") by clicking on it to highlight it, then clicking "OK:'

6. A prompt should appear asking you to "Please type in your file name."

7. After you log on, a window similar to figure B10.7 will appear. Check to make sure the transducers are secure and plugged into correct channels. This concludes the setup procedures.

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

The program needs a reading of the subject's maximum volume and temperature changes to perform autocalibration. The calibration will run for 8 seconds and then stop automatically, so let it run its course.

1. The subject should sit in a relaxed state and breathe normally.

2. Click on "Calibrate," wait 2 seconds, and then instruct the subject to breathe deeply for one cycle and then breathe normally.

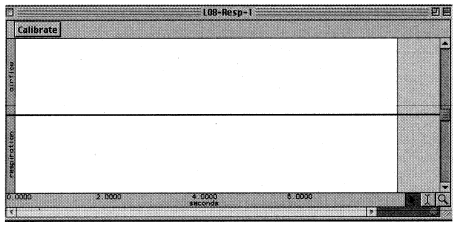


FIGURE **B10.7**

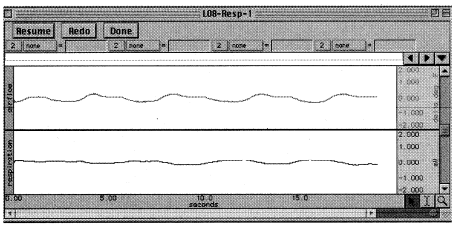


FIGURE **B10.9**

3. At the end of the calibration recording your screen should resemble figure B10.8.

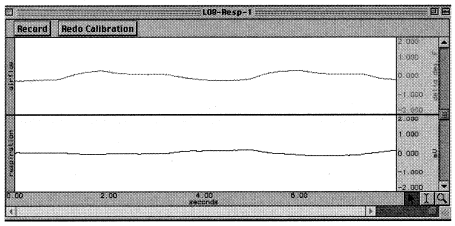


FIGURE **B10.8**

4. Neither channel ("respiration" or "airflow") should show a flat straight line. If there is not any fluctuation, then it is possible that a transducer is not connected or positioned properly and you must redo the calibration. Redo the calibration by clicking on "Redo Calibration" and repeating the entire calibration sequence. Before recalibrating, check the transducer position and connection.

Data Recording

You will record airflow on one channel and chest expansion (respiration) on another channel from the subject under four conditions:

Segment 1: Sitting at rest, normal breathing
Segment 2: Sitting, hyperventilation, and recovery
Segment 3: Sitting, hypoventilation, and recovery
Segment 4: Coughing, reading aloud

In order to work efficiently, read this entire section so you will know what to do for each recording segment.
Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not use an excessive amount of time (time is memory).

Hints for Obtaining Optimal Data

1. Subject should stop hyperventilation or hypoventilation if dizziness develops.

2. The respiration transducer should fit snugly around the chest prior to inspiration.

3. The temperature transducer should be firmly attached so it does not move. The transducer thermistor should be positioned below the nostril and not touching the face.

4. The subject should be sitting for all segments.

5. The recording should be suspended after each segment so that the subject can prepare for the next recording
segment.

Segment 1

1. The subject should be sitting in a chair, breathing normally, and not facing the monitor screen. Click on "Record" and record normal resting respiration for 15 seconds.

2. Click on "Suspend:" Your data should resemble figure B10.9. The data would be incorrect if:

(a) The pneumograph (respiration) data recording has "plateaus" instead of waveforms. If there are plateaus, adjust the pneumograph.
(b) The waveforms in the temperature (airflow) data are not offset from the respiration data. If there is no offset, adjust the respiration transducer.
(c) The temperature thermistor moved and is no longer directly under the nostril.
(d) The pneumograph slipped.
(e) The Suspend button was pressed prematurely.
(f) Any of the channels have flat data, indicating no signal.

3. If the data recording is incorrect, you should redo it by clicking on "Redo" and repeating steps 1 and 2. Note that once you press "Redo," the data you have just recorded will be erased.

Segment 2

1. Ask the subject to hyperventilate (breathe rapidly and deeply) through the mouth for a maximum of 30 seconds. Click on "Resume" at the beginning of hyperventilation.

WARNING
The director should watch the subject and stop the procedure if the subject starts to feel sick or excessively dizzy.

2. At the end of the 30-second hyperventilation period (about the 45-second mark), ask the subject to stop hyperventilating and resume breathing nasally until a normal breathing pattern is reestablished. Record recovery for 30 seconds, then click on "Suspend:'

3. If all went well, your data should resemble figure B10.10. Use the horizontal scroll bar to look at different portions of the waveform.

4. If your data recording is incorrect, for reasons given in step 2 of the segment 1 instructions, redo segment 2 by clicking on "Redo" and repeating the recording of steps I and 2. Note that once you press "Redo," the data you have just recorded will be erased.

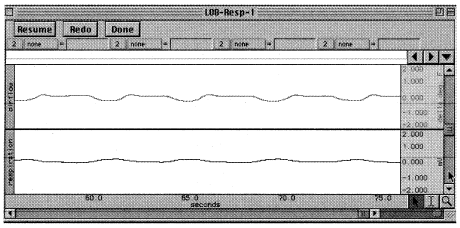


FIGURE **B10.10**

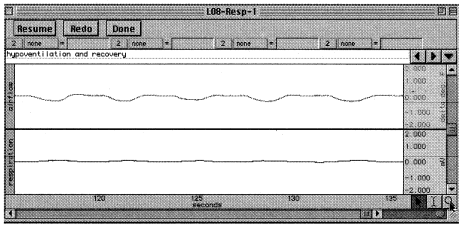


FIGURE **B10.11**

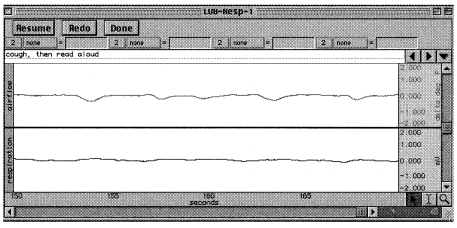


FIGURE **B10.12**

Segment 3

1. Allow time for the subject's breathing to return to normal resting level.
2. Ask the subject to hypoventilate. The subject should breathe slowly and shallowly through the nose. Click on "Resume" and record hypoventilation for 30 seconds.

3. At the end of the 30-second hypoventilation period, ask the subject to resume normal breathing. Continue to record 30 seconds of recovery, then click on "Suspend" (at the 135-second mark).

4. Your data should resemble figure B10.11. If the data recording is incorrect, for reasons given in step 2 of the segment 1 instructions, redo segment 3 by clicking on "Redo" and repeating steps 2 and 3. Note that once you press "Redo," the data you have just recorded will be erased.

Segment 4

1. Click on "Resume" and ask the subject to cough once and then begin reading aloud.

2. Record for 30 seconds, then click on "Suspend."

3. Your data should resemble figure B10.12. If necessary, repeat the recording of segment 4 data by clicking on "Redo:'

4. Click on "Done." A pop-up window with four options will appear. Make your choice, and continue as directed.

5. Carefully remove the respiration and temperature transducers.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file, and open it. The data window should be similar to figure B10.13. Note the channel number (CH) designations:

CH 2 = airflow
CH 40 = respiration

2. Set up the measurement boxes as follows:

CH 40 = ∆ T
CH 40 = BPM
CH 40 = p-p
CH 2 = p-p

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. Here are some brief descriptions of the measurement boxes used for this experiment:

∆T: The "delta time" measurement is the difference in time between the end and beginning of the selected area.

BPM: The beats per minute measurement first calculates the difference in time

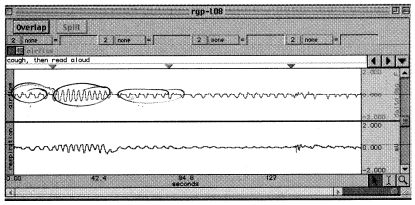


FIGURE **B10.13**

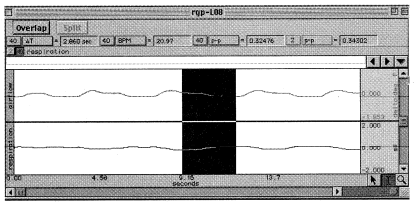


FIGURE **B10.14**

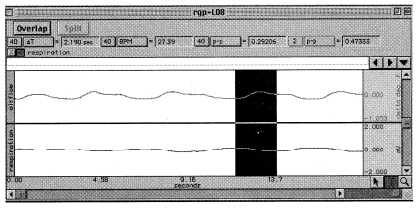


FIGURE **B10.15**

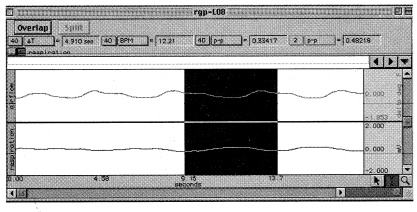


FIGURE **B10.16**

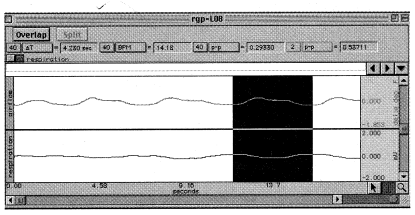


FIGURE **B10.17**

between the end and beginning of the area selected by the I-beam tool (just like ΔT), and then divides this value into 60 seconds/minute. Because the BPM only uses the time measurement of the selected area for its calculation, the BPM value is not specific to a particular channel.

p-p: This measurement reflects the maximum value found in the selected area minus the minimum value found in the selected area. The "selected area" is the area selected by the I-beam tool (including the endpoints).

3. Use the zoom tool to select about four cycles of respiration in segment I data.

4. Use the I-beam tool to select an appropriate area for the measurement of the duration (ΔT) of inspiration (figure B10. 14), expiration (figure B10.15), and cycle duration (figure B10.16). Record the measurements in table B10.1 in the report. Use the I-beam tool to select an appropriate area from the peak of one cycle to the peak of the next cycle (figure B10.17) for the measurement of respiratory rate (BPM) and record the measurement in table B 10.1 in the report.

5. Repeat step 4 for two other respiratory cycles in the segment I data and calculate mean values.

6. Repeat steps 4 and 5 on each of the three remaining data segments (segment 2, segment 3, and segment 4) and record the measurements in table B 10.2 of the report. Calculate mean values where indicated in table B10.2

7. Use the I-beam tool to select three individual cycles in each of the four data segments and determine the respiration amplitude for each. Record measurements in table B10.3 in the report.
Figure B10.18 shows an example of selecting an area in the cycle that captures the minimum and maximum amplitude values. The p-p measurement will display the amplitude. Note that segment 4 (cough) requires only one measurement.

8. Use the I-beam tool to select the interval between the maximal inspiration and the maximal temperature change (figure B10.19) in data from segment 1, segment 2, and segment 3. Record the AT (time interval) between the two peaks and the p-p (temperature amplitude) in table B10.4 in the report.

9. You may save the data to a diskette, save notes that are in the journal, or print the data file.

10. Exit the program. Turn off the MP30 and shut down the computer.

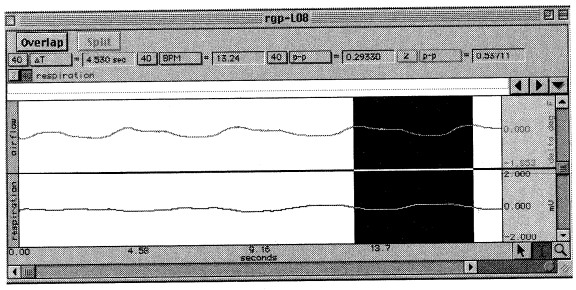


FIGURE B10.18

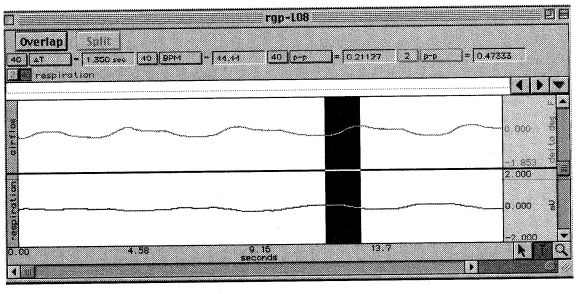


FIGURE B10.19

The Respiratory Cycle Data

Experimenter Name:

Date:

1. Data and calculations:

(a) Subject :

Name Age Height Weight

Gender: Male/Female

(b) Eupnea (normal breathing-segment 1):

Complete table B10. 1 with values for each cycle and calculate the means. Remember what a respiratory cycle is? Make sure before you work on this data!!

TABLE B10.1

Rate	***Measurement***	*CH #*	***Cycle 1***	***Cycle 2***	***Cycle 3***	***Mean***
Inspiration duration	∆T	CH 40				
Expiration duration	∆T	CH 40				
Total duration	∆T	CH 40				
Breathing rate	BPM	CH 40				

Notes:

(c) Comparison of ventilation rates (segments 2-4):

Complete table B10.2 with measurements from CH 40 for three cycles of each segment and calculate the means where indicated. *(Note:* ∆T *is* cycle duration, BPM is breaths per minute, and Cough has only one cycle.)

TABLE B10.2

Measurement	***Hyperventilation Segment 2***		***Hypoventilation Segement 3***		***Cough Segment 4***		***Read aloud Segment 4***	
	∆T	***BPM***	***∆T***	***BPM***	***∆T***	***BPM***	***∆T***	***BPM***
Cycle 1								
Cycle 2								
Cycle 3								
Mean								

Notes:

(d.) Relative ventilation depths (segments 1-4):

TABLE B10.3

Depth	p-p [CH 40] Cycle I	Cycle 2	Cycle 3	Calculate Mean
Eupnea *Segment 1*				
Hyperventilation *Segment 2*				
Hypoventilation *Segment 3*				
Cough *Segment 4*				

Notes:

(e) Association of respiratory depth and temperature (segments 1-3):

TABLE B10.4

Measurement	Channel	Eupnea Segment 1	Hyperventilation Segment 2	Hypoventilation Segment 3
Peak ∆ Temp	CH 2 *p-p*			
∆T between max inspiration and peak ∆ Temp	CH 40 ΔT			

Notes:

Data Analysis:

Can you answer the questions?

1. Would the subject been able to hold his or her breath longer immediately after hyperventilation and hypoventilation? Why?

2. After a brief period of hyperventilation, **APNEA VERA** occurs.

(a) Define **hyperventilation** and **apnea vera**.

(b) Describe the feedback loop causing **apnea vera**.

4. (a) What changes occur in the body with **hypoventilation**?

(b) How does the body adjust rate and depth of ventilation to counteract the effects of **hypoventilation**?

5. In which part of the respiratory cycle is temperature the **highest**?:

Lowest?

Explain why the temperature varies with the respiratory cycle.

6. Describe or define cough in terms of modification of the breathing cycle.

7. What modifications of the breathing cycle occur when reading aloud? Why?

Electrocardiography:

Components of the ECG

The normal heart contracts regularly and continuously throughout life at a rate of 60-100 times each minute. Although the cardiac muscle is supplied with motor nerves that can influence either the rate of contraction or the strength of contraction, the extrinsic nerves play no role in the genesis of the heartbeat. If the extrinsic nerves (sympathetic and parasympathetic) were cut or even if the heart were to be removed from the body, it would continue to beat rhythmically as long as it was supplied with oxygen and vital nutrients. The heart possesses the unique ability to contract by itself without any stimulation from the rest of the body. This property of cardiac muscle is called inherent rhythmicity or automaticity.

The control and coordination of cardiac muscle's inherent rhythmicity is dependent on a specialized system of conductive tissue within the heart. Before each contraction of the heart can occur, an electric current must first pass through the myocardial fibers. The conduction system of the heart is responsible for generating these electric currents and conveying them in an orderly sequence to all parts of the heart. The conduction system, or pacemaker system, consists of the following areas of specialized conducting tissue: the sinoatrial (SA) node, internodal and interatrial pathways, the atrioventricular (AV) node, the bundle of His, right and left bundle branches, and the Purkinje fiber network. Figure B7.1 illustrates each of the components of the pacemaker system.

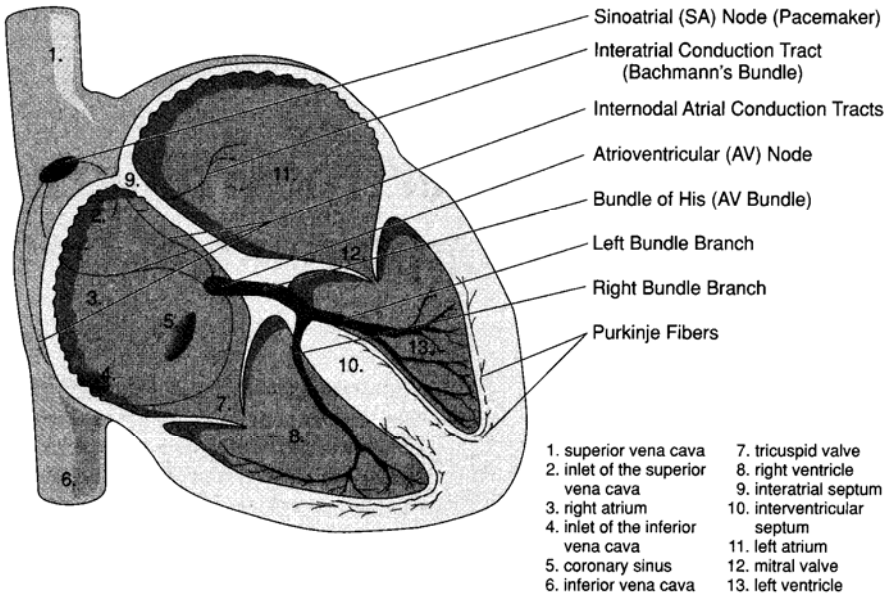


FIGURE B7.1 Conduction system of the heart

The sinoatrial (SA) node is located near the junction of the right atrium and superior vena cava. The electric impulse that initiates each contraction of the heart normally originates from this node. The SA node, without neural or endocrine stimulation, spontaneously depolarizes at a rate of more than 80 times per minute. Normally, its frequency of depolarization is between 60 and 80 times per minute because of vagal inhibition. Because the SA node discharges electric impulses at a higher frequency than does any other part of the conduction system, it paces the electrical and mechanical activities of the entire heart. Therefore, the SA node is commonly called the pacemaker.

Once an impulse has been initiated by the SA node, it is transmitted through both atria along the internodal and interatrial pathways, stimulating atrial muscle to contract. The impulse also spreads to another specialized area of the conduction system the atrioventricular (AV) node-which is part of the junctional tissue between the right atrium and ventricle. The AV node, driven by the rate of the SA nodal firing, relays the electric impulse toward the ventricles after a slight delay. The delay allows the atria time to contract before excitation of the ventricles occurs. The delay also helps protect the ventricles from rapid atrial impulses.

After passing through the AV node, the impulse is carried to the ventricles through the bundle of His, a common bundle of specialized conductive fibers lying along the upper part of the interventricular septum. The bundle of His runs down within the upper interventricular septum and branches into a right and left bundle. The right bundle branch carries the impulse to the right ventricle; the left bundle branch

carries the impulse to the left ventricle. Each bundle branch further subdivides into numerous small conducting fibers called Purkinje fibers, which relay the electric impulse directly to ventricular muscle, stimulating the ventricles to contract.

Note that any of the cells of the conduction system may act as pacemaker cells, but atrial and ventricular muscle cells do not normally do so. In a case of damage to the SA node, for example, the AV node may take over as the primary pacemaker for the ventricles, although the intrinsic rate of firing of the AV node (40-60 cycles per minute) is less than the normal firing rate of the SA node (80-100 cycles per minute).

In summary, the contraction of cardiac muscle is associated with an electric impulse initiated at the sinoatrial node, which sweeps over the conduction path of the heart, preceding the mechanical change in the muscle. In each normal cardiac cycle, the electrical events follow a sequence: (1) depolarization and repolarization of the sinoatrial (SA) node; (2) depolarization and repolarization of atrial muscle; (3) depolarization and repolarization of the atrioventricular (AV) node and bundle; (4) depolarization and repolarization of the Purkinje network; (5) depolarization and repolarization of ventricular muscle.

The electric current associated with the cardiac cycle may be detected at the surface of the body, amplified, and recorded as a time record of the electrical events occurring during each cardiac cycle. Thus, heart rate can be accurately determined, and abnormalities of rhythm and conduction can be identified. The electrical and mechanical device that records the electrical activity of each cardiac cycle is called an electrocardiograph. The study of electrocardiograph applications and the interpretation of electrocardiograms (the records made by an electrocardiograph) is called electrocardiography.

The electric current associated with and generated during the cardiac cycle is detected by placing a positive electrode and a negative electrode on selected areas of the skin surface and recording the electric current changes occurring between the electrodes as the heart beats. The particular arrangement of two electrodes, one positive and the other negative, with respect to a third electrode, the ground electrode, is called a lead. The standard bipolar limb leads (figure B7.2) used in electrocardiography by convention, with the right leg electrode serving as ground, are as follows:
lead I = right arm (-) to left arm, lead II = right arm to left leg, lead III = left arm to left leg.

For diagnostic work in cardiology, other leads have been developed to broaden the scope and utility of the electrocardiograph. The electrocardiogram (ECG) of the chest leads (VI, V2, V3, V4, V5, and V6) is obtained by uniting the standard limb electrodes to a single negative pole called a common terminal (CT). A chest electrode is attached to the positive pole and moved through six standard positions on the chest surface (figure B7.3) The chest leads provide additional information relative to the detection and location of an abnormality in the conduction system of the heart or in cardiac muscle.

Recording from the unipolar limb leads (aVF, aVR, aVL) involves uniting two of the standard limb electrodes to a negative pole (CT), and the remaining standard limb electrode to a positive pole (figure B7.4). The unipolar limb leads are as follows:

aVF = left arm and right arm (-) to left leg
aVR = left arm and left leg (-) to right arm
aVL -_ right arm and left leg (-) to left arm

Additional leads are the bipolar chest leads (CR, CL, CF), obtained by making the chest electrode positive and one of the limb electrodes negative. The bipolar chest leads are as follows:

CR = chest lead to right arm
CL = chest lead to left arm
CF = chest lead to left leg (-)

The electrocardiograph records electrical activity of the heart on special graph paper of the following standard dimensions: the horizontal lines represent amplitude in fractions of millivolts (uV), and the vertical lines represent time in fractions of seconds. The recording paper travels at a standard speed of 25 mm/s. The sensitivity of the recorder is set so that a 1 uV input results in a pen deflection of 10 mm. on the paper. The interval between two vertical lines is, therefore, 0.04 second; the interval between two horizontal lines is 0.1 uV.

Figure B7.5 is a normal electrocardiogram (ECG), associated with a single cardiac cycle, as recorded from lead 11.

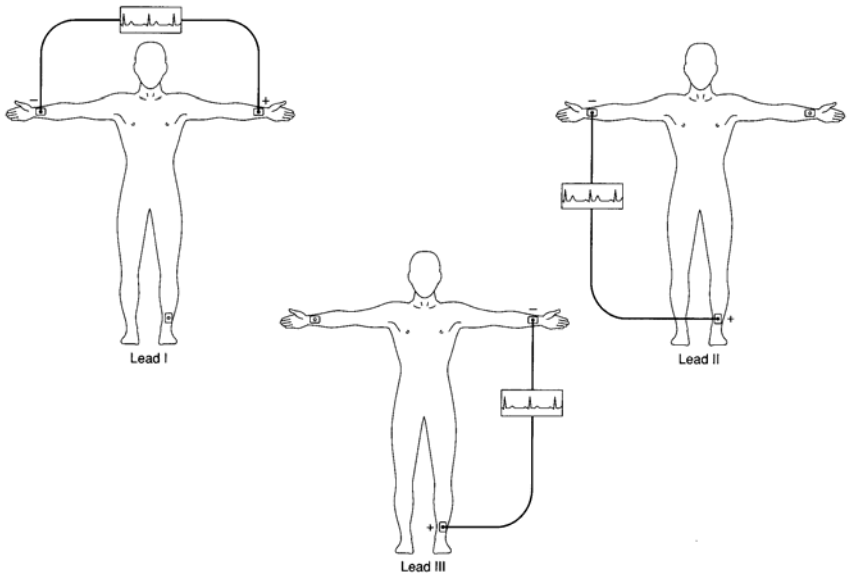


FIGURE B7.2 The standard (bipolar) limb leads I, II, and III

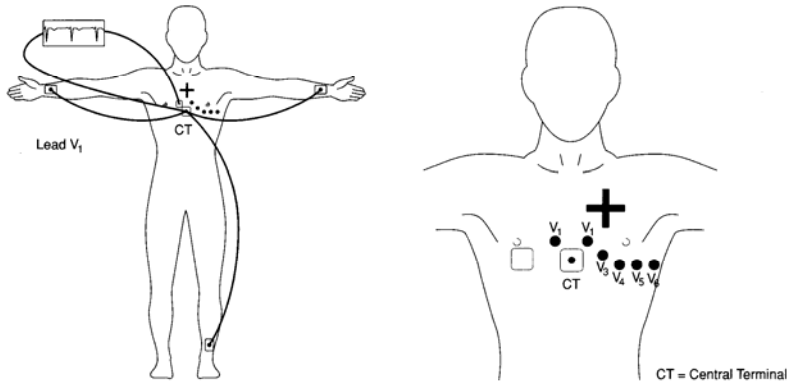


FIGURE B7.3 Precordial (unipolar) leads

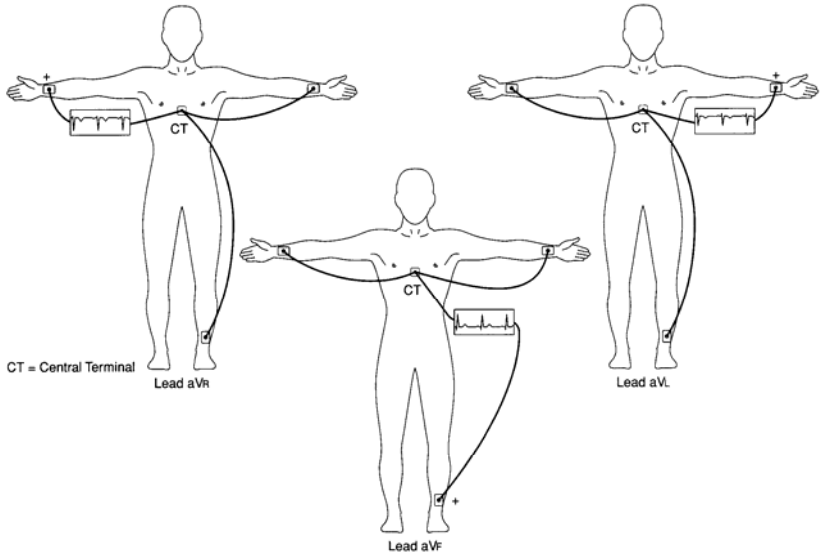


FIGURE B7.4 The augmented (unipolar) leads aV_R, aV_L, and aV_F

The following phases of the ECG complex may be recognized:

1. The isoelectric line (baseline) is the point of departure for the P, Q, R, S, and T waves.

2. The P wave represents the depolarization of atrial muscle as a wave of negativity spreads from the SA node toward the ventricles. It is normally upright in all three standard limb leads.

3. The P-R interval is measured from the beginning of the P wave to the beginning of the QRS complex; it represents the interval between the activation of the SA node and the AV node. An abnormal lengthening of the P-R interval suggests interference with conduction to the ventricles.

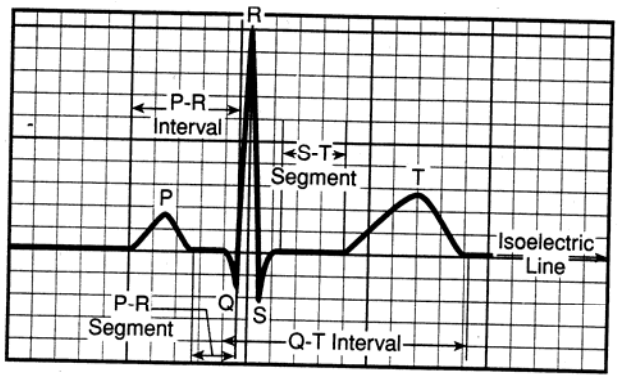


FIGURE B7.5 Lead II electrocardiogram

4. P-R segment is measured from the end of the P wave to the beginning of the QRS complex; it represents the interval between atrial depolarization and ventricular depolarization. Although AV nodal delay and depolarization of the AV node, AV bundle, and Purkinje network occur during this segment, no external potentials are recorded.

5. The QRS complex represents the spread of excitation through the ventricular myocardium, resulting in depolarization of ventricular muscle. Repolarization of atrial muscle also occurs during this phase of the ECG. The record is complex; its shape, amplitude, and direction depend on the position of the heart in the chest, its size relative to body mass, and the time relationship between right and left ventricular activity. An abnormal lengthening of the duration of the QRS complex suggests interference with the spread of Setup excitation through ventricular muscle, as may occur in Purkinje failure or myocardial infarction.

TABLE B7.1

Phase	*Duration (second)*	*Amplitude (millivolt)*
P wave	0.06–0.11	<0.25
P-R interval	0.12–0.20	
P-R segment	0.08	
QRS complex (R)	<0.12	0.8–1.2
S-T segment	0.12	
Q-T interval	0.36–0.44	
T wave	0.16	<0.5

6. The S- T segment represents the interval between the end of the S wave and the beginning of the T wave, the period during which the ventricles are more or less uniformly excited. Normally, it indicates an isoelectric state. Its position and shape are important in the diagnosis of abnormalities of ventricular repolarization.

7. The T wave represents the restoration of ventricular myocardium to the resting or excitable state. The Q-T interval is measured from the beginning of the QRS complex to the end of the T wave; it represents the time of electrical systole when the ventricular beat is generated. It varies with the heart rate.

Values within normal ranges for the duration and voltage of the different phases of the ECG complex as seen in lead II are indicated in table B7.1.

Bipolar Limb Leads and Vectorcardiography:

The anatomic axis of the heart is the angle of the heart in the body from base to apex. The normal anatomic axis is around 55. That is, if an imaginary cross is drawn through the center of the heart with the horizontal arms parallel to the ground representing 0' left and +130' right, and +90' at the bottom of the cross, the apex or tip of the heart would be located along the 55' radial. In other words, the apex of the heart normally points toward the lower left rib cage.

The electrical axis of the heart (mean electrical axis) is the preponderant direction of current flow during the cardiac cycle. Typically, the mean electrical axis is around +60' for a 70 kg adult. The normal range is

0' to +90'. The mean electrical axis is influenced by the size of the heart, the anatomic position of the heart, and electrical activity of the conduction system and cardiac muscle.

Willem Einthoven developed a "string galvanometer" in 1901 that could record the electrical activity of the heart. Although it was not the first such recorder, it was a breakthrough in that it was accurate enough to allow anybody to duplicate the results on the same patient. His work established a standard configuration for recording the EKG and won him the Nobel Prize in 1924. Since that time, the EKG has become a very powerful tool in diagnosing disorders of the heart. It should be noted that the clinical interpretation of the ECG is quite empirical in practice and has evolved from a long history of reference to and correlation with known cardiac disorders.

Recall that the cardiac cycle, or heartbeat, begins with an electric impulse (a wave of depolarization followed by a wave of repolarization) generated by and conducted away from the sinoatrial (SA) node. This impulse spreads throughout the atria stimulating atrial muscles to contract. Depolarization of the atria is recorded as the P wave of the electrocardiogram. The impulse spreading through the right atrium reaches the atrioventricular (AV) node, where impulse conduction is much slower, thereby delaying conduction of the impulse to the ventricles so that the atria have time to complete their contraction. After AV node delay, the impulse is rapidly conducted down the AV bundle, bundle branches, and Purkinje fibers, which stimulate ventricular muscles to contract. Depolarization of the ventricles is recorded as the QRS complex of the electrocardiogram. Repolarization of the ventricles is recorded as the T wave.

Because the current spreads along specialized pathways and depolarizes in sequence, the electrical activity has a spatial orientation or electrical axis. Because the amount of electric signal generated is proportional to the amount of tissue being depolarized, and the ventricles make up the majority of the mass, the largest potential difference reflects the depolarization of the ventricles. Furthermore, since the left ventricle is larger than the right, more of the QRS complex reflects the depolarization of the left ventricle.

The body contains fluids with ions that allow for electric conduction. This makes it possible to measure electrical activity in and around the heart from the surface of the skin (assuming we can make good electrical contact with the body using electrodes) and also allows the legs and arms to act as simple extensions of points in the torso. Measurements from the leg approximate those occurring in the groin and measurements from the arms approximate those from the corresponding shoulder (figure B 8.1).

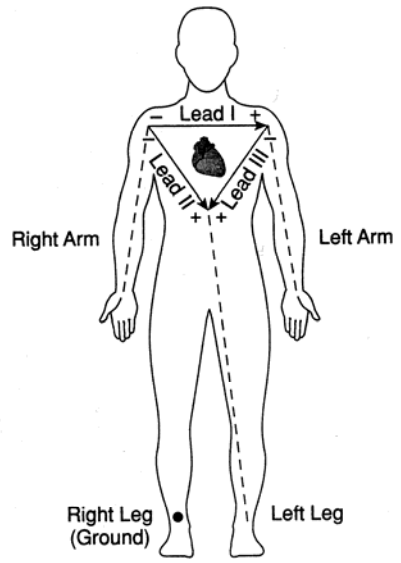


FIGURE B8.1 Einthoven's triangle

It is desirable to place the electrodes on the ankles and wrists for convenience to the subject undergoing the ECG evaluation. In order for the electrocardiograph to work properly, a ground reference point on the body is required. This ground is obtained from an electrode placed on the right leg above the ankle.

The electrocardiogram is a record of the overall spread of electric current through the heart as a function of time in the cardiac cycle. The direction of polarity (+ or -) of the waveforms obtained depends upon the location of the recording electrodes on the surface of the body and whether the electrical activity is directed toward or away from the surface electrode. In general, if a wave of depolarization is approaching a positive electrode, a positive voltage will be seen by that electrode relative to a grounded reference electrode. If the wave of depolarization is traveling toward a negative electrode, a negative voltage will be seen. The opposite is true for repolarization; i.e., repolarization waves approaching a positive electrode produce negative voltages.

Recall that the term "lead" is defined as a spatial arrangement of two electrodes on the body. One lead is labeled + and the other -. The electrode placement defines the recording direction of the lead, which is called the lead axis or angle. The axis is determined by the direction when going from the negative to positive electrode. The electrocardiograph computes the voltage difference (magnitude) between the positive and negative electrodes and displays the changes in voltage difference with time.

The standard bipolar limb leads are:

lead I = right arm (-), left arm (+)

lead II = right arm (-), left leg (+)

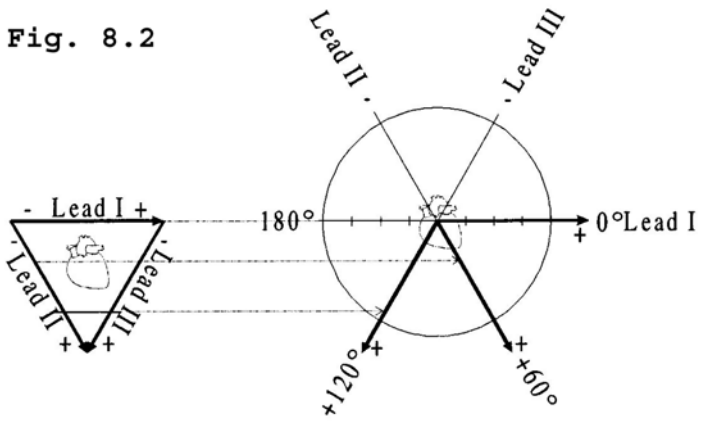
lead III = left arm (-), left leg (+)

The three standard bipolar limb leads may be used to construct an equilateral triangle, called Einthoven's Triangle at the center of which lies the heart figure B8.1. Each side' of the triangle represents one of the bipolar limb leads, and each lead forms a 60' angle with the two opposite leads.

At any given moment during the cardiac cycle (during the QRS complex, for example), the net electrical activity seen by a lead may be represented by a vector. A vector is an entity represented by an arrow that has both magnitude and direction. The vector for a particular lead is plotted on the axis for that lead, with the arrowhead pointing in the positive direction. The length of the arrow is proportional to the magnitude of the lead. Figure B8.2 shows another way to look at Einthoven's triangle. You can move each axis horizontally or vertically and still have the same representation. This makes it a little easier to visualize the mean electrical axis of the heart.

The mean electrical axis of the heart is the summation of all the vectors occurring in a cardiac cycle. Since the QRS complex caused by ventricular depolarization represents the majority of the electrical activity of the heart, we can approximate the mean electrical axis by looking only at the Q, R, and S waves.

Fig. 8.2



A further approximation can be made by looking only at the peak of the R wave, which makes up the largest magnitude in the cardiac cycle. To define the mean electrical axis precisely, you would need to define it in three dimensions (X, Y, and Z). This is done in practice by using a standardized set of 12 leads. Three of these leads are the ones previously defined and allow the mean electrical axis to be calculated in the frontal plane. For this chapter we will only be interested in the frontal plane axis

One way to approximate the mean electrical axis in the frontal plane is to plot the magnitude of the R wave from lead I and lead III (figure B8.3).

1. Draw a perpendicular line from the ends of the vectors (right angles to the axis of the lead).

2. Determine the point of intersection of these two perpendicular lines.

3. Draw a new vector from point 0,0 to the point of intersection.

The direction of this resulting vector approximates the mean electrical axis of the heart. The length of this vector approximates the mean potential of the heart.

A more accurate method of approximating the mean electrical axis is to algebraically add the Q, R, and S potentials for each lead instead of using just the magnitude of the R wave to plot the lead vectors. This method will be discussed later.

Clinically, using sophisticated equipment, vectorcardiography involves the continuous recording of electrical activity of the heart and plotting of electrical vectors in two and even three dimensions. It allows a continuous display of the depolarization-repolarization process as it sweeps over the heart and is used to reveal abnormalities of the conduction process. In this experiment, we will focus on the determination of the mean electrical axis and mean potential of the ventricles.

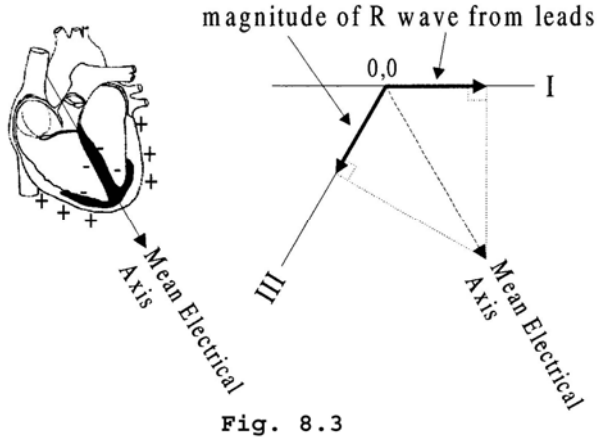


Fig. 8.3

Experiment I: Measurement of the components of the ECG

Objectives:

1. To become familiar with the electrocardiograph as a primary tool for evaluating electrical events within the heart.

2. To correlate electrical events as displayed on the electrocardiogram with the mechanical events that occur during the cardiac cycle.

3. To observe changes in the electrocardiogram associated with body position, exercise, body size, and age.

4. To anticipate the nature of changes in the electrocardiogram associated with pathology of the heart.

Questions to think about:

2. What components of a *single* ECG complex change between resting and exercise states? For example, do amplitudes increase or decrease? Do intervals become longer or shorter? Are there no changes at all?

3. Is heart rate higher when the subject is sitting than it is when the subject is supine? Give a physiologic reason for your observation.

4. Did you observe heart rate to vary with inspiration and expiration during deep breathing at rest? Explain your observation in terms of baroreceptor reflex control of heart rate.

5. How does the ratio between the durations of ventricular systole and ventricular diastole change from resting to exercise states? (See tables B7.4 and B7.7.)

6. What changes in lead II ECG would you expect to see associated with the following conditions? (a) 2:1 heart block; (b) Premature ventricular contractions (PVC); (c) Increased AV node delay; (d) Bradycardia

Materials:

BIOPAC electrode lead set (SS2L)
BIOPAC disposable vinyl electrodes (EL503), 3 electrodes per subject
cot or lab table and pillow
BIOPAC electrode gel (GELI) and abrasive pad (ELPAD) or skin cleanser or alcohol prep

EXPERIMENTAL METHODS

1 . Turn on your computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC100A transformer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.

3. The subject must remove all clothing, jewelry, and other accessories from the areas of electrode placement (figure B7.6). With the subject resting comfortably in a supine position on the laboratory table, use a cotton ball or paper towel soaked in alcohol (or an ELPAD) to cleanse the skin on the anterior aspect of the right and left wrists and the medial aspect of the right and left ankles where the ECG electrodes will be placed (figure B7.6). Attach the electrodes and the colored electrode leads to the subject as shown in figure B7.6, and plug the SS2L electrode lead set into channel 2 of the MP30. Position the electrode cables such that they are not pulling on the electrodes. Connect the electrode

cable clip (where. the cable meets the three individual colored wires) to a convenient location (can be on the subject's clothes). This will relieve cable strain.
Note that the subject should not be in contact with nearby metal objects (faucets, pipes, etc.).

4. Locate the BIOPAC Student Lab folder, open it, and start the BIOPAC Student Lab program. A prompt will appear asking you to choose a lesson. Choose Lesson 5 ("L05-ECG-1") by clicking on it to highlight it, then clicking "OK!'

5. A prompt should appear asking you to "Please type in your file name." Enter a unique identifier so that you can locate and retrieve your data for analysis after data recording.

6. After you log on, a window similar to figure B7.9 will appear. Check to make sure the electrodes and electrode leads are secure and the electrode assembly is plugged into channel 2. This concludes the setup procedures.

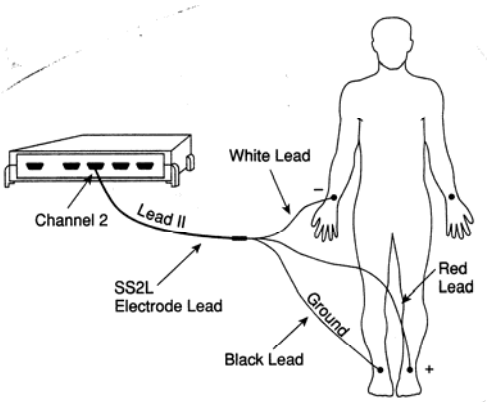


FIGURE **B7.6** Electrode placement lead II

Calibration
The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1. Make sure the electrodes adhere securely to the skin. If they are being pulled up, you will not get a good ECG
signal. The subject must be relaxed and as still as possible during the calibration procedure. The electrocardiograph is very sensitive to small changes in voltage caused by contraction of skeletal muscles, so the subject's arms and legs need to be relaxed so that the muscle (EMG) signal does not corrupt the ECG signal.

2. Click on “Calibrate." The calibration procedure will begin and stop automatically after 8 seconds. At the end of the calibration recording there should be a greatly reduced ECG waveform with a relatively flat baseline.

3. If the data recording shows any large spikes, jitter, or large baseline drafts, you should redo the calibration by clicking on the "Redo Calibration" button and repeating the entire calibration sequence.
Data Recording Lead II ECG will be recorded from the subject in four conditions or recordings segments:

Segment 1: At rest, lying down.
Segment 2: Immediately after sitting up.
Segment 3: At rest, breathing deeply.
Segment 4: Immediately after light exercise.

In order to work efficiently, read this entire section so you will know what to do for each recording segment.

The subject should remain in a supine position and continue to relax while you review the lesson.

Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not use an excessive amount of time (time is memory).

Hints for Obtaining Optimal Data

1. The subject should not talk or laugh during any of the recording segments.

2. The subject should be in a relaxed state for each recording segment and in the position noted for each segment.

3. When the subject is asked to sit up, he or she should do so in a chair, with arms relaxed on the armrest (if available) or with arms relaxed at the side if sitting on the edge of a bench or table.

4. For recording segment 2, click on "Resume" as soon as possible after the subject sits up in order to capture the heart rate variation, but do not click on "Resume" while the subject is in the process of sitting up or there will be excessive motion artifact.

5. The subject should be as still as possible during the recording segment. The electrocardiograph is very sensitive to small changes in voltage caused by contraction of skeletal muscles, and the subject's arms and legs need to be relaxed so that the muscle (EMG) signal does not corrupt the ECG signal.

Segment 1

1. Click on "Record," and record data 20 seconds then click on "Suspend" at the 20-second mark.

2. If all went well, your data should look similar to figure B7.11. The data would be incorrect if: (a) The Suspend button was pressed prematurely. (b) An electrode peeled up, causing a large baseline drift, spike, or loss of signal. (c) The subject has too much muscle (EMG) artifact. In this case, you should redo the recording by clicking on "Redo" and repeating step 1. Note that once you press "Redo," the data you have just recorded will be erased.

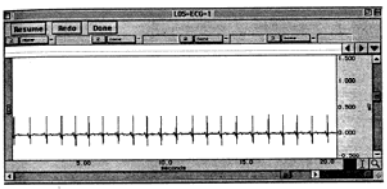


FIGURE **B7.11**

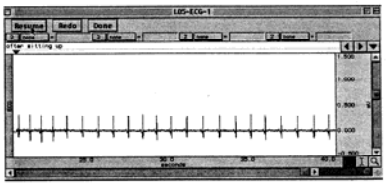


FIGURE **B7.12**

Segment 2

1. In order to capture the heart rate variation, it is important that you click on "Resume" to continue recording as quickly as possible after the subject sits up. However, it is also important that you do not click on "Resume" while the subject is in the process of sitting up or you will capture motion artifact.
2. Have the subject sit up quickly. Click on "Resume:' Record for 20 seconds. The recording will continue from the point where it last stopped, and a marker labeled "sitting up" will automatically come up when "Resume" is pressed. Click on "Suspend" at the end of the 20-second recording segment (at the 40-second mark).
3. If all went well, your data should resemble figure B7.12. The data would be incorrect for the reasons given in step 2 of the instructions for segment 1. If it is incorrect, redo the recording by clicking on "Redo" and repeating step 2. Note that once you press "Redo," the data you have just recorded will be erased.

Segment 3

1. Click on "Resume." The recording will continue from the point where it last stopped, and a marker labeled "5 deep breaths" will automatically come up when "Resume" is pressed.

2. Record for (20 seconds and have the subject take in 5 deep breaths during recording. The 5 deep breathing cycles should be deeper and slower than normal breathing at rest and should follow one another. During this time, the person who is the recorder should insert a marker at the beginning of an inhale and insert another marker at the corresponding exhale. The recorder should label these markers "inhale" and "exhale."

3. At the end of the 20-second recording segment (60 second mark), click on "Suspend:' If all went well, your data should resemble figure B7.13. Note that deep breathing may produce a baseline drift. This is normal and does not necessitate redoing the recording. The data would be incorrect for reasons given in step 2 of the instructions for segment 1. If it is incorrect, redo the recording by clicking on "Redo" and repeating step 2. Note that once you press "Redo," the data you have just recorded will be erased.

Segment 4

1. Disconnect the electrode leads (do not remove the electrodes) and have the subject move to a safe area and perform an exercise that will elevate his or her heart rate fairly rapidly (e.g., push-ups, jumping jacks, etc.)

2. Immediately after the exercise, have the subject assume a sitting position as in recording segment 2 and reconnect the electrode leads (RA = white, LL = red, RL = black).
In order to capture the heart rate variation, it is important that you resume recording as quickly as possible after the subject has performed the exercise. However, it is also important that you do not click "Resume" while the subject is exercising or you will capture motion artifact.
Click on "Resume." The recording will continue from the point where it last stopped, and a marker labeled "after exercise" will automatically come up when "Resume" is clicked on.

3. Record the postexercise ECG for 60 seconds, then click on "Suspend" at the 120-second mark

4. Note that the baseline may drift. This is fairly normal, and unless it is excessive, it does not necessitate redoing the recording. If incorrect, you should redo the recording by clicking on "Redo" and repeating steps 1-3. Note that once you press "Redo," the data you have just recorded will be erased. Click on "Done." A pop-up window with four options will appear. Make your choice, and continue as directed. If choosing the "Record from another subject" option: (a) Attach electrodes per the setup instructions and repeat the entire lesson, beginning with calibration.
(b) Note that each person will need to use a unique file name.

5. Remove the electrode cable pinch connectors, and peel off the electrodes. Throw out the electrodes (BIOPAC electrodes are not reusable). Wash the electrode gel residue from the skin, using soap and water. The electrodes may leave a slight ring on the skin for a few hours, which is quite normal.

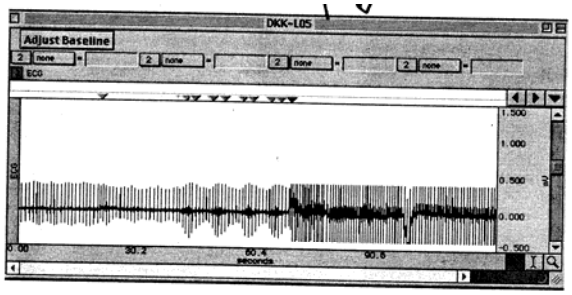


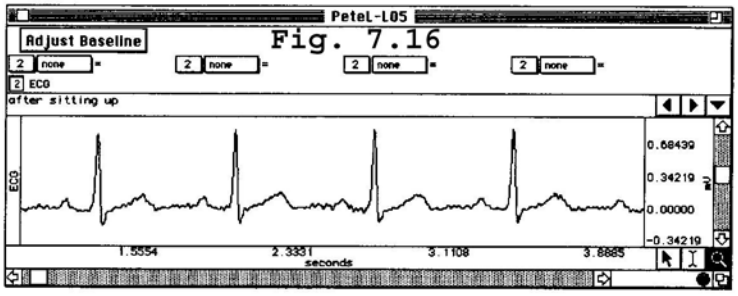
FIGURE **B7.15**

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file, and open it. The data window should be similar to figure B7.15. Note the channel number (CH) designation: CH 2=ECG lead II.

2. Use the zoom tool to enlarge four successive beats from segment 1 data (figure B7.16). Note that you may turn Grids ON and OFF by choosing "Display: Preferences" from the File menu. The "Adjust Baseline" button allows you to position the waveform up or down in small increments so that the baseline can be exactly zero. Such positioning is not necessary to get accurate amplitude measurements, but it may be desired before making a printout or when using grids. Once the "Adjust Baseline" button has been pressed, two more buttons, Up and Down, will be displayed. Simply click on these to move the waveform up or down.

3. Set up the measurement boxes as follows:



CH2 = ΔT
CH2 = BPM
CH2 = Δ
CH2 = max

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. Here are some brief descriptions of the specific measurement boxes used for this experiment:

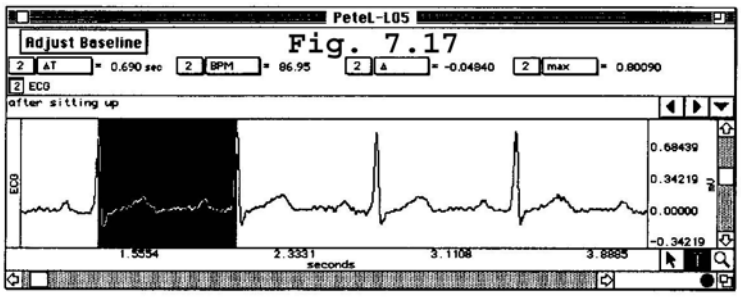
ΔT: The "delta time" measurement is the difference in Time. Time between the end and the beginning of the selected area.

BPM: The beats per minute measurement first calculates the difference in time between the end and the beginning of the area selected by the I-beam tool just like ΔT), and then divides this value into 60 seconds/minute.

Δ: "Delta" computes the difference in amplitude between the last point and the first point of the selected area.

max: This measurement is the maximum amplitude value within the area selected by the I-beam tool (including the endpoints). The "selected area" is the area selected by the I-beam tool (including the endpoints).

4. Use the I-beam tool to select the area between two successive R waves. Try to go from R-wave peak to R-wave peak as precisely as possible (figure B7.17). Record the R-R interval time (ΔT) and the heart rate (BPM) in the report in table



5. Repeat step 4 for two other RR intervals in the current waveform display.

6. Using the time scroll bar, look at segment I data and examine lead II for regularity of rhythm. Mark the distance between two R waves, and compare this R-R interval to other R-R intervals in lead H. Is there a slowing-down-speeding-up rhythm that appears to be correlated with the respiratory cycle? This is commonly and normally seen in young people and is known as sinus arrhythmia

The range for the normal resting heart rate is 60-100 beats per minute. Heart rates above 100 beats per minute are known as tachycardias, and those below 60 beats per minute are known as bradycardias. Under resting conditions, the heart rate is usually 70-90 beats per minute. The conditioned athlete may have a resting heart rate of 46-60 beats per minute due to a more efficient heart pumping a larger stroke volume with a corresponding decrease in rate.

7. Use the time scroll bar to inspect for the presence of regularly occurring P-QRS-T complexes during segment 1. Examine the P waves. Are they present or absent-visible in some cases, but absent in others? Normal P waves are small, smoothly contoured upright waves in all three limb leads; they indicate an SA node pacemaker. P waves that are peaked, toothed, upside down, absent, or in other ways different from the normal waveform may indicate that some other area (instead of the SA node) is in command as the pacemaker. Variations of P wave shape also occur in some types of arrhythmias (irregular rhythms).
Examine lead II relative to the QRS complex. Is each P wave followed by or related to a QRS complex? In some arrhythmias, P waves are not followed by QRS complexes for each cycle (e.g., heart block).

8. Choose an ECG complex near the 10-second recording mark from segment 1 data. Use the I-beam tool to select, and then use ΔT to determine the duration of the following:

P wave duration (figure B7.18)
P-R interval
QRS interval
Q-T interval

T wave duration
End of T wave to beginning of next R wave

Record the data in the report in tables B7.3 and B7.4

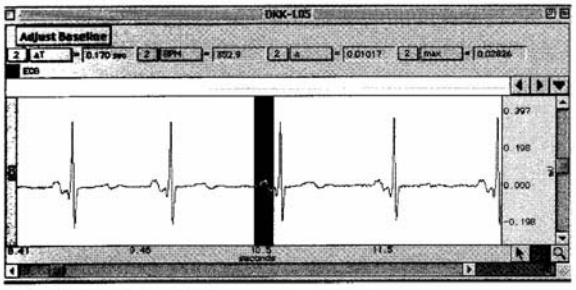


FIGURE **B7.18**

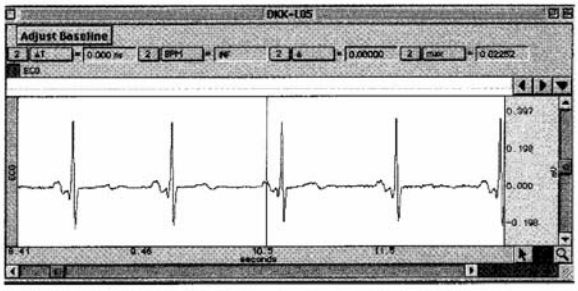


FIGURE **B7.19**

9. Repeat step 8 for two other ECG complexes in the current waveform display.

10. Normally the P-R interval does not exceed 0.20 second. A prolonged P-R interval indicates an abnormal delay in the spread of the impulse from the SA node to and through the AV node. If the P-R interval exceeds 0.21 second, AV block exists and beats will be dropped.
Examine the QRS complexes to determine whether the conduction of the impulse through the ventricles is normal. Prolongation of the QRS interval beyond 0.09 second generally indicates a defect or delay in the conduction of the impulse through the ventricles (e.g., Purkinje failure). Examine the shape of all QRS complexes for lead H. Are they uniform?
Examine the S-T segment. Its position should be horizontal along the isoelectric line, or slightly ascending. Its duration is normally 0. 12 second. The position and shape of the S-T segment are important in the diagnosis of abnormalities of ventricular repolarization, but it is beyond the scope of this book to present an analysis.

11. If necessary, adjust the isoelectric line to 0.00 mV. Using the same P-QRS-T complex as in step 8, use the I-beam tool and the max function to measure the following amplitudes: P wave (figure B7.19), Q wave, R wave, S wave, and T wave. Compare measured values with normal values. Record data in table B7.3 in the report.
To determine waveform amplitude (+ is above the isoelectric line, - is below the isoelectric line), position the I-beam cursor on the peak of the waveform, click once, and read the max measurement value.

12. Scroll or use the marker buttons to display segment 2 data. Measure heart rate (BPM) and R-R ΔT at the beginning, at the middle, and at the end of segment 2. Record the data in table B7.5 in the report.

13. Repeat step 12 measurements for data in recording segment 3. Make measurements during inspiration and during expiration. Record the measurements in table B7.6 in the report.

14. Scroll or use the marker buttons to display segment 4 data. Make the following measurements at the beginning, middle, and end of segment 4:

(a) Q-T interval (approximates the period of ventricular systole).

(b) End of T wave to subsequent R wave (approximates the period of ventricular diastole). Record the measurements in table B7.7 in the report.

15. You may save the data to a diskette, save notes that are in the journal, or print the data file.
16. Exit the program. Turn off the MP30 and shut down the computer.

Components of the ECG Data:

Name:

Date:

1. Data and calculations:

(a) Subject

Name Age Height Weight

Gender: Male/Female

Resting Phase Data:

Data recorded from the Resting (supine) phase:

TABLE B7.2

		Cardiac cycle				
Measurement	**From channel**	**1**	**2**	**3**	**Mean**	**Range**
ΔT (R-R)	CH 2					
BPIVI	CH 2					

TABLE B7.3

ECG component	Duration Cycle 1	Cycle 2	Cycle 3	Mean	Ampl. (mV) Cycle 1	Cycle 2	Cycle 3	Mean
P wave								
P-R interval								
P-R segment								
QRS complex								
Q-T interval								
S-T segment								
T wave								

TABLE B7.4

Ventricular readings	Cycle I	Cycle 2	Cycle 3	Mean
Q-T interval (corresponds to ventricular systole)				
End of T wave to subsequent R wave (corresponds to ventricular diastole)				

Notes: Compare the information recorded in this table to data recorded in table B7.7 (Postexercise Data)

Sitting Phase Data:

Data recorded from the Sitting up phase:

TABLE B7.5

Heart rate	*Ch. #*	*Cycle I*	*Cycle 2*	*Cycle 3*	*Mean*
ΔT	CH2				
BPM	CH 2				

Notes:

Deep Breathing Phase Data:

Data recorded from the Breathing phase:

TABLE B7.6

Rhythm	*Ch. #*	*Cycle I*	*Cycle 2*	*Cycle 3*	*Mean*
Inspiration					
ΔT	**CH2**				
BPM	**CH 2**				
Expiration					
ΔT	**CH 2**				
BPM	**CH 2**				

Notes:

Postexercise Data:

Data recorded from the Postexercise phase:

TABLE B7.7

Ventricular readings	*Cycle 1*	*Cycle 2*	*Cycle 3*	*Mean*
Q-T interval (corresponds to ventricular systole)				
End of T wave to subsequent R wave (corresponds to ventricular diastole)				

Notes: Compare this data to table B7.4 (Resting phase data)

Data Analysis:

Can you answer these questions:

1. What components of a *single* ECG complex change between resting and exercise states? For example, do amplitudes increase or decrease? Do intervals become longer or shorter? Are there no changes at all?

2. Is heart rate higher when the subject is sitting than it is when the subject is supine? Give a physiologic reason for your observation.

3. Did you observe heart rate to vary with inspiration and expiration during deep breathing at rest? Explain your observation in terms of baroreceptor reflex control of heart rate.

4. How does the ratio between the durations of ventricular systole and ventricular diastole change from resting to exercise states? (See tables B7.4 and B7.7.)

5. What changes in lead II ECG would you expect to see associated with the following conditions?

(a) 2:1 heart block

(b) Premature ventricular contractions (PVC)

(c) Increased AV node delay.)

(d) Bradycardia

Experiment II: Bipolar Limb Leads, Vectorcardiography

Experimental Objectives:

1. To record ECG from standard bipolar limb leads I and III when the subject is supine, sitting, and breathing deeply while sitting.
2. To determine the mean electrical axis of the ventricles using vectors derived from the amplitude and polarity of the QRS complex in two of the three bipolar limb leads.
3. To determine the mean electrical potential of the ventricles.

Questions to think about:

1. Define Einthoven's law.

2. What factors affect the orientation of the mean electrical axis?

3. How did the amplitudes of lead I and lead III change between inhalation and exhalation? Did the heart's axis and magnitude change?

4. What factors affect the amplitude of the R wave recorded on the different leads?

5. Compare the mean electrical axis and magnitude obtained when (a) Using just the amplitude measurement of the R wave versus net potentials (b) Lying down versus sitting up.

Materials

BIOPAC electrode lead set (SS2L), quantity of 2
BIOPAC disposable vinyl electrodes (EL503), 6 electrodes per subject
cot or lab table and pillow
two different-colored pens or pencils BIOPAC electrode gel (GELl) and abrasive pad (ELPAD) or skin cleanser or alcohol prep

EXPERIMENTAL METHODS

Setup
1. Turn on the computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC100A transformer (which supplies power to the MP30) is plugged into an electrical outlet.

3. The subject must remove all clothing, jewelry, and other accessories from areas of electrode placement (figure B8.4). With the subject resting comfortably in a supine position on the laboratory table, use a cotton ball or paper towel soaked in alcohol (or an ELPAD) to cleanse the skin on the anterior aspect of the right and left wrists and the medial aspect of the right and left ankles where the ECG electrodes will be placed.
Attach the electrodes and the colored electrode leads to the subject as shown in figure B8.4. Note that for the two electrodes on the right ankle and the left wrist, lead I should go to the upper of the two electrodes. Plug the SS2L electrode lead set for lead I into channel 1 of the MP30 and plug the SS2L electrode lead set for lead III into channel 3 of the MP30. Position the electrode cables such that they are not pulling on the electrodes. Connect the electrode cable clip (where the cable meets the three individual colored wires) to a convenient location (can be on the subject's clothes). This will relieve cable strain.
Note that the subject should not be in contact with nearby metal objects (faucets, pipes, etc.).

4. Locate the BIOPAC Student Lab folder, open it, and start the BIOPAC Student Lab program. A prompt will appear asking you to choose a lesson. Choose Lesson 6 ("L06-ECG-2") by clicking on it to highlight it, then clicking "OK:'

5. A prompt should appear asking you to "Please type in your file name." Enter a unique identifier so that you can locate and retrieve your data for analysis after data recording.

6. After you log on, a window will appear. Check to make sure the electrodes and electrode leads are secure, the colored leads are clipped to the proper electrode, and the electrode assemblies are plugged into the correct channels on the MP30. This concludes the setup procedures.

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1. Make sure the electrodes adhere securely to the skin. If they are being pulled up, you will not get a good ECG signal. The subject must be relaxed and as still as possible during the calibration procedure. The electrocardiograph is very sensitive to small changes in voltage caused by contraction of skeletal muscles, so the subject's arms and legs need to be relaxed so that the muscle (EMG) signal does not corrupt the ECG signal.
2. Click on "Calibrate." The calibration procedure will begin and then stop automatically after 8 seconds. At the end of the calibration recording. There should be a greatly reduced ECG waveform with a relatively flat baseline.
3. If the data recording shows any large spikes, jitter, or large baseline drifts, you should redo the calibration by clicking on "Redo Calibration" and repeating the entire calibration sequence.

Data Recording

You will record two segments of data:
Segment 1: Subject supine, resting (20 seconds)
Segment 2: (1) Subject sitting, at rest (10 seconds); (2) subject sitting, breathing deeply (10 seconds)

In order to work efficiently, read this entire section so you will know what to do before recording.

Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not use an excessive amount of time (time is memory).

To minimize muscle (EMG) corruption of the ECG signal and baseline drift:

1. The subject should not talk or laugh during any of the recording segments.

2. When lying down or sitting up, the subject should be completely relaxed.
3. When sitting up, the subject's arms should be supported on an armrest or resting at the side.
4. The recording should be suspended before the subject prepares for the next recording segment.
5. The subject should breathe normally during the recording, unless otherwise directed, to minimize EMG from the chest area.
6. Electrodes should not peel up.

Segment 1

1. Click on "Record" and record segment I data as the subject continues to be supine in a relaxed state. Record the data for 20 seconds.
2. Click on "Suspend:' A marker will automatically mark the end of segment 1 data.
3. Note that a little baseline drift when the subject breathes in and out is normal and does not indicate incorrect data. The data would be incorrect if:

(a) The Suspend button was pressed prematurely.

(b) An electrode peeled up, giving a large baseline drift.
(c) The subject has too much muscle (EMG) artifact.

If your data recording is incorrect, click on "Redo" and repeat steps I and 2. Note that once you click on "Redo," the data you have just recorded will be erased.

Segment 2

1. Have the subject sit up with arms relaxed. Click on "Resume" as soon as possible **after** the subject sits up. Do not click on "Resume" while the subject is in the process of sitting up or there will be excessive motion artifact.

2. After about 10 seconds of recording, ask the subject to increase breathing by about 70%. The subject should not breathe in too deeply as that will cause excessive EMG or baseline drift.

Insert markers **(for Mac, use the Esc key; for PC, use the F9 key)** at the beginning of an inhale and at the beginning of an exhale. Record deep breathing data for about 10 seconds.

3. Click on "Suspend." Your data should resemble figure B8.9. If the data recording is incorrect (for the same reasons mentioned in step 3 of the segment 1 instructions), you will need to repeat segment 2 data recording by clicking on "Redo:' Before clicking on "Redo," have the subject assume a resting supine position for about 5 minutes. Note that once you click on "Redo," the data you have just recorded will be erased.

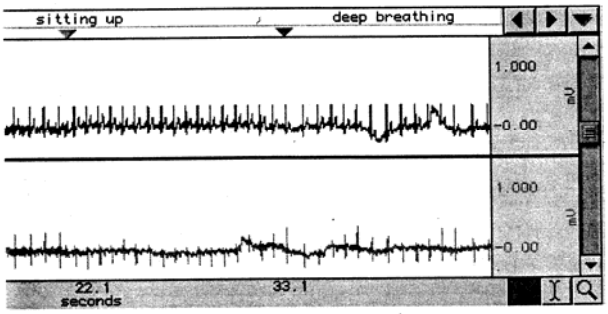


FIGURE **B8.9**

4. If the data recording looks OK, click on "Done." After you press "Done," a pop-up window with four options will appear. Make your choice, and continue as directed.

If choosing the "Record from another subject" option:

(a) Attach electrodes per step 6 of the setup instructions and then continue the entire lesson from step 11 of the setup instructions.
(b) Note that each person will need to use a unique file name.

Remove the electrode cable pinch connectors, and peel off the electrodes. Throw out the electrodes (BIOPAC electrodes are not reusable). Wash the electrode gel residue from the skin, using soap and water. The electrodes may leave a slight ring on the skin for a few hours, which is quite normal.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file, and open it. Note that when you clicked on "Done" in the last step of the data recording, you automatically entered the Review Saved Data mode, but only for the last subject. If you have data from only one subject, continue with step 2. Otherwise, open the appropriate file as directed. The data window should resemble figure B 8.10.

Note that following the click on the “Done” button to terminate data recording, the software automatically calculated lead II from leads I and III using Einthoven's law. The calculated lead II is now displayed with leads I and III in the Review Saved Data mode.

2. Note the following channel number (CH) designations:

CH 1 = lead I
CH 3 = lead III
CH 40 = lead II (calculated)

3. Set up your display window for optimal viewing of lead I and lead III. To hide a channel, click on the channel box and hold down the option key on a Mac or the Ctrl (control) key on a PC. This will toggle between hiding and showing the data. You also have the option of showing grids on the screen. Turn on or turn off grids from the Display Preferences option under the File menu. Hide channel 40; display channel 1 and channel 3.

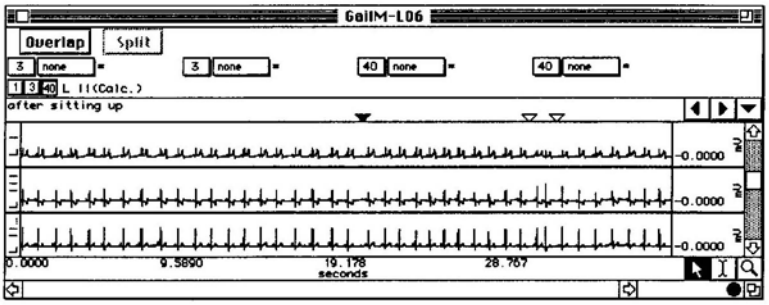


Fig. 8.10

4. Set up the measurement boxes as follows:

CH 1 = max
CH 3 = max

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. The measurement box used in this part of the experiment, called max, provides the maximum amplitude value within the area selected by the I-beam tool (including the endpoints).

5. Use the zoom tool to focus on one of the ECG complexes in the recording of segment 1 data.

6. Use the I-beam tool to select the QRS interval (figure B8.11). Record the R-wave amplitude (max measurement) values for lead I and lead III in table B8.1 in the report.

7. Insert a marker at the R-wave peak to indicate where the QRS measurement was taken (for Mac, use the Esc key; for PC, use the F9 key). To place a marker after the data has been recorded, click in the marker region (the region above the top channel). Place the marker directly above the selected R wave. An arrow should appear. Type in "supine"

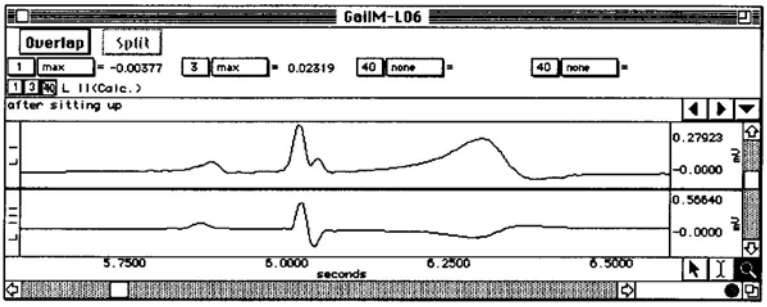
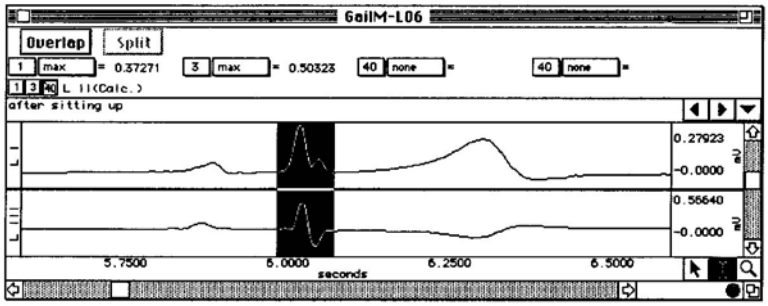


Fig. 8.11a & b

8. Scroll to segment 2 and repeat steps 6 and 7 on one of the ECG complexes prior to deep breathing. Type in "sitting" next to the inserted marker.

9. Repeat step 6 for a cardiac cycle in segment 2 and for a cardiac cycle in segment 2



10. Set up the measurement boxes as follows:

CH 1 =Δ
CH 3 =Δ

The delta amplitude (Δ) measurement computes the difference in amplitude between the first point and the last point of the selected area. It is particularly useful for taking ECG measurements, because the baseline does not have to be at zero to obtain quick, accurate measurements.

NOTE: Make sure you pay attention to the polarity of the A measurement as it is based on the result of the first point minus the last point. Thus, if the point at the start of the selected area is larger than the last point in the selected area, the polarity will be positive. If the reverse is true, the polarity will be negative.

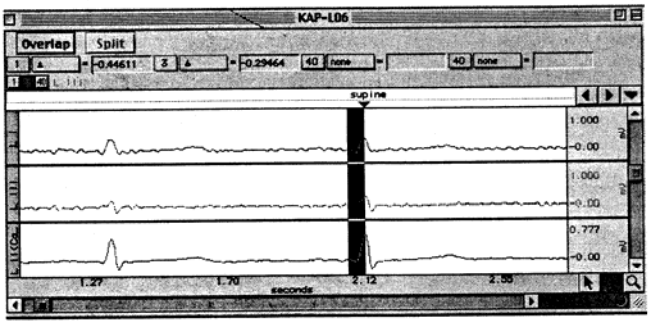


FIGURE **B8.12**

11. Scroll back to segment I data and the marker labeled "Supine" This was the same QRS region you used the first time you did step 6. (Note that instead of scrolling, you can use the marker tools at the far right of the marker bar to go to different markers.)

12. Use the I-beam tool to select an area from baseline (isoelectric line) to peak (+ or -) of the wave and record the amplitudes (Δ) of the Q, R, and S waves individually for both lead I and lead III. A sample measurement of R-wave amplitude is shown in figure B8.12. Record measurement values in table B8.2 in the report.

13. You may save the data to a diskette, save notes that are in the journal, or print the data file.

14. Exit the program. Turn off the MP30 and shut down the computer.

Electrocardiography II: Bipolar Limb Leads, Vectorcardiography Report

---

Experimenter Name:

Date:

1. Data and calculations:

(a) Subject

Name Age Height Weight

Gender: Male/Female

(b) Mean electrical magnitude and axis-graphical estimate: Use table B8.1 to record measurements from the Data Analysis section, step 6.

TABLE B8.1 R-wave amplitudes

Condition	***Lead I [CH 1] max***	***Lead III [CH 3] max***
Lying down (Supine)		
Sifting up		
Inhale		
Exhale		

One way to approximate the mean electrical axis in the frontal plane is to plot the magnitude of the R wave from lead I and lead III.

(1) Start at 0,0 on the vectorgraph (figure B8.13 and place a dot at the amplitude value of the R wave (from tableB 8. 1) on the lead I axis.

(2) Start at 0,0 on the vectorgraph and place a dot at the amplitude value of the R wave on the lead III axis.

(3) Draw a line perpendicular to the lead axis through the dot on lead I. Repeat for lead III.

(4) Determine the point of intersection of the two perpendicular lines.

(5) Draw a new vector from point 0,0 to the point of intersection. The direction of the resulting vector (in degrees) approximates the mean electrical axis of the heart. The length of this vector (subdivided into mV units identical to those of lead I or lead III) approximates the mean electrical potential of the ventricles. Create two plots on each of the following graphs (figures B 8.13 and B 8.14), using data from the table B 8. 1. Use a different color pencil or pen for each plot.

From the graph in figure B8.13, find the following values:

Condition	***Mean Electrical Magnitude***	***Mean Electrical Axis***
Lying down	_____	_____
Sitting up	_____	_____

Explain the difference (if any) in mean electrical magnitude and axis under the two conditions:_____

_____

_____

_____

_____

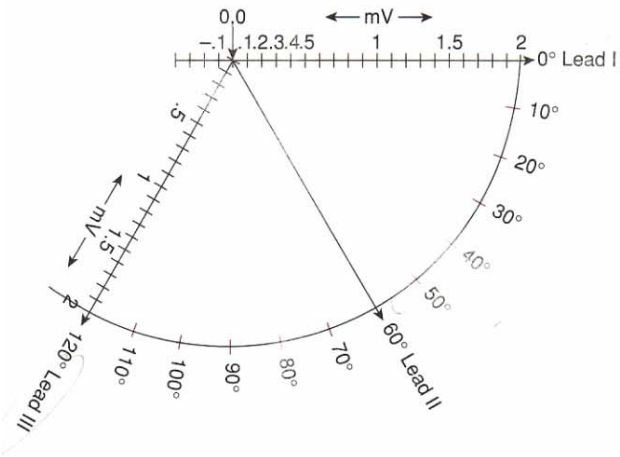


FIGURE B8.13 Vectorgraph: Lying down and sitting up

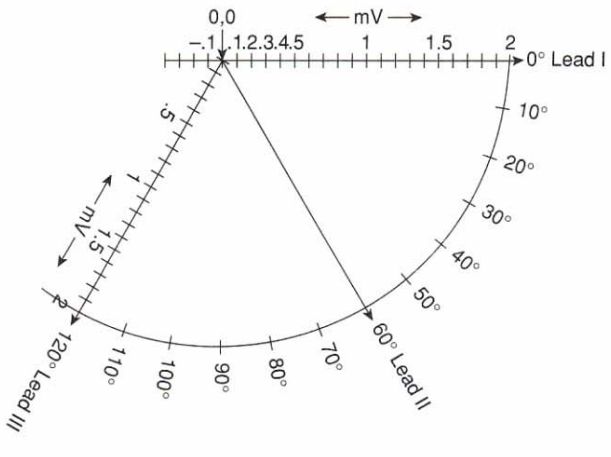


FIGURE B8.14 Vectorgraph: Breathing in/breathing out

From the graph in figure B8.14, find the following values:

Condition	*Mean Electrical Magnitude*	*Mean Electrical Axis*
Breathing in	_____	_____
Breathing out	_____	_____

Explain the difference (if any) in mean electrical magnitude and axis under the two conditions:_____

_____

_____

_____

_____

_____

_____

TABLE B8.2 Lying down

Lead I	*Lead II*
Q_____	**Q**_____
R_____	**R**_____
S_____	**S**_____
QRS net_____	**QRS net**_____

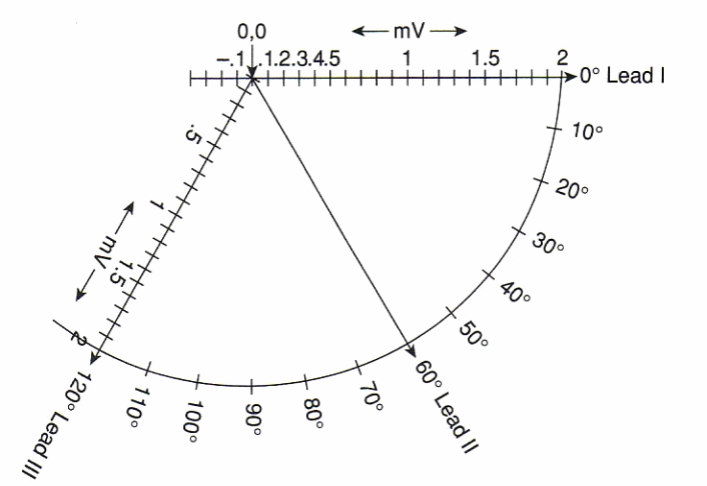


FIGURE B8.15 Vectorgraph: Lying down

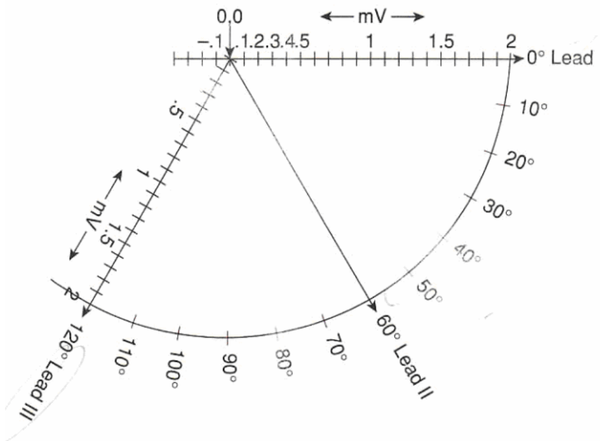
(c) Mean electrical axis and magnitude-more accurate approximation:

Algebraically add the Q, R, and S potentials to obtain net potentials. Plot the net potentials in figure B8.15.

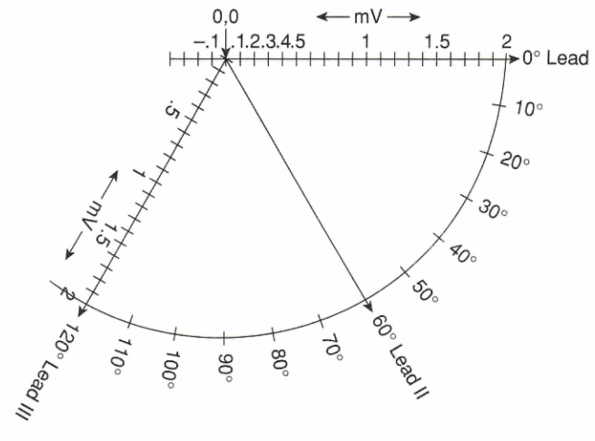
From the graph in figure B8.15, find the following values:

Condition	***Mean Electrical Magnitude***	***Mean Electrical Axis***
Lying down	_____	_____

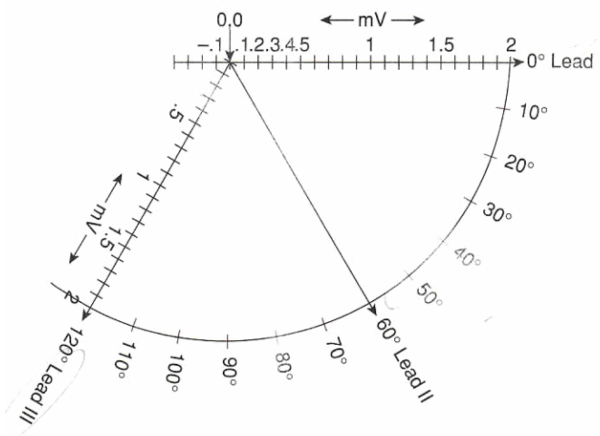
Explain the difference between the mean electrical magnitude and axis for the lying down data in this plot (figure B8.15) and in the first plot (figure B8.13).



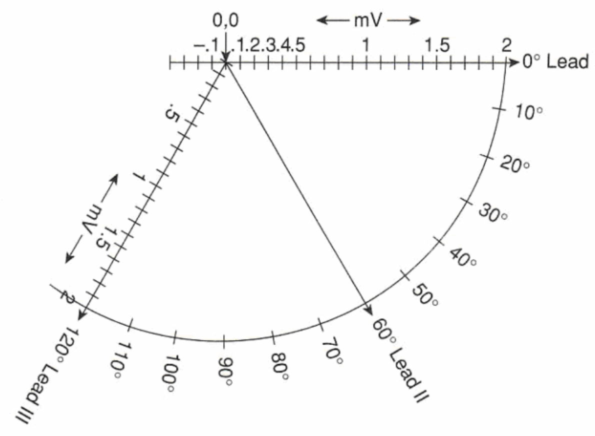
Vectorgraph:



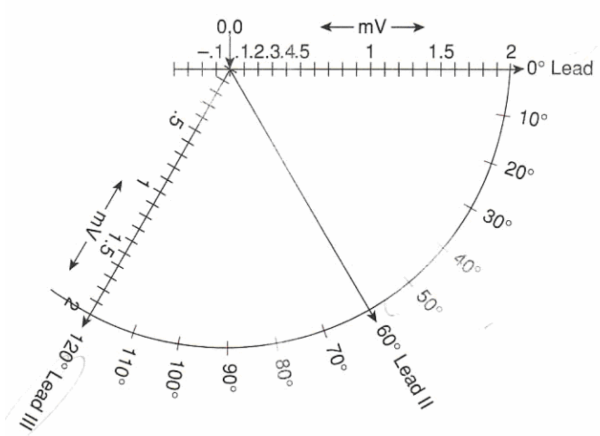
Vectorgraph:



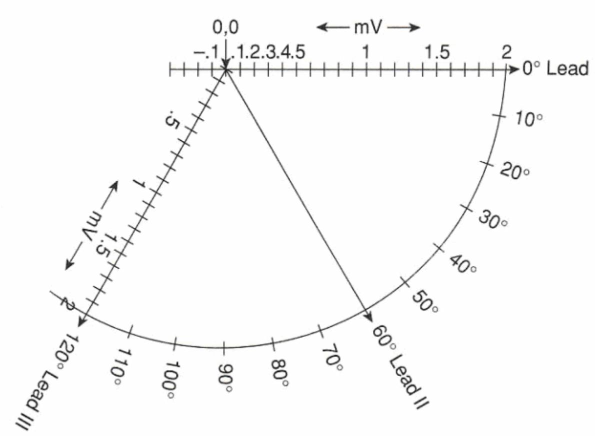
Vectorgraph:



Vectorgraph:



Vectorgraph:



Vectorgraph:

Data Analysis:

Can you answer these questions:

1. Define **Einthoven's** law.

2. What factors affect the orientation of the **mean electrical axis**?

3. How did the amplitudes of **lead I and lead III** change between inhalation and exhalation? Did the heart's axis and magnitude change?

4. What factors affect the amplitude of the **R wave** recorded on the different leads?

5. Compare the **mean electrical axis** and **magnitude** obtained when:

   (a) Using just the amplitude measurement of the **R wave** versus **net potentials**

   (b) Lying down versus sitting up

Renal System

INTRODUCTION

The kidneys play an essential role in regulating the volume and chemical composition of the plasma, interstitial fluid, and lymph. They are primarily concerned with the regulation of extracellular concentrations of water, electrolytes, and certain end products of metabolism; therefore, they regulate not only the concentrations of chemicals in the extracellular fluids but also the pH and osmotic pressure.

The primary functional unit of the kidneys is the **nephron (figure** 30. 1). Each kidney contains approximately 1 million nephrons. Urine is formed in the nephron by the combined processes of glomerular filtration, tubular reabsorption, and tubular secretion. Excretion by the kidney refers to the process of eliminating urine into the ureter, a thin-walled tube that conveys urine to the urinary bladder, where urine is stored until it can be voided from the body (micturition). The glomerular filtrate is an ultrafiltrate of the plasma. This means that the filtrate contains substances of small molecular weight (glucose, amino acids, urea, water, etc.) in essentially the same concentrations as the plasma, but colloidal material and substances of high molecular weight (plasma proteins, erythrocytes, leukocytes, etc.) are normally absent from the filtrate. As the filtrate passes through the nephron, most of the constituents of the glomerular filtrate are reabsorbed to varying degrees, depending on the kidneys' need to adjust and maintain the chemical concentrations of the extracellular fluids. In addition, some substances (e.g., potassium, hydrogen ions, and drugs) are secreted by tubular cells into the urine. The quantity of urine formed and its chemical composition varies, depending on such factors as exercise, dietary intake, and pregnancy.

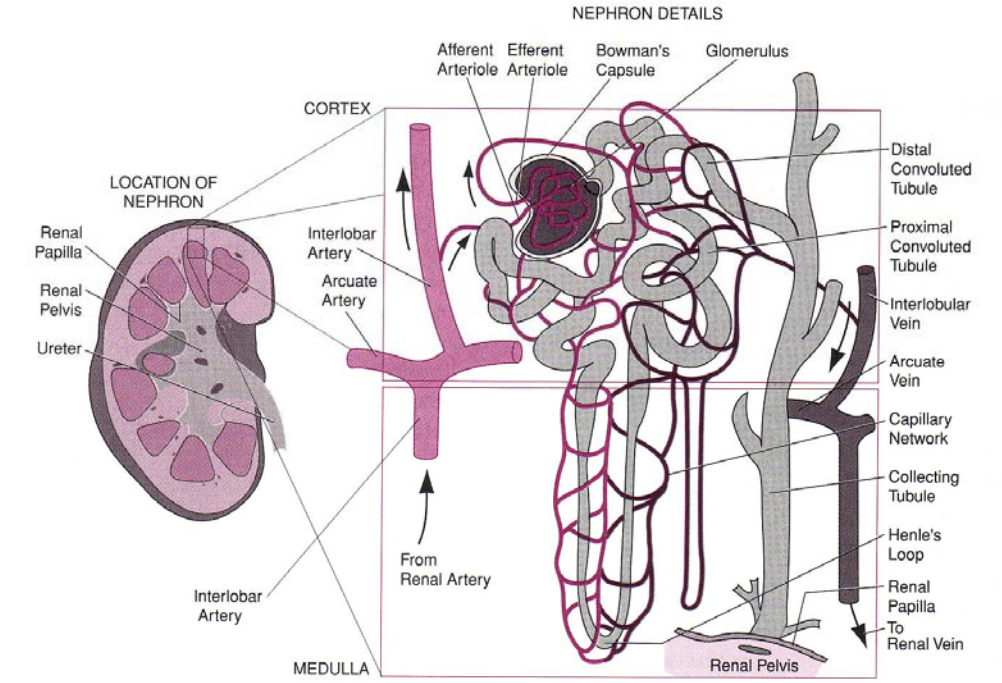


FIGURE **30.1**

A typical 70 kg adult normally forms about 1500 ml of urine per 24-hour period. This 1500 ml of urine normally contains about 60 g of solids. Approximately 30 g of this is *urea* (a nitrogenous waste product) and about 15 g is sodium chloride. The remaining 15 g consists of various organic and inorganic constituents, such as creatinine, amino acids, uric acid, ammonia, sulfates, phosphates, potassium, and magnesium. Under unusual and/or pathologic conditions, substances normally absent or present in only trace amounts in the urine may be detected. These include proteins, glucose, ketone bodies, bile, hemoglobin, and blood cells. Table 30.1 indicates the chemical composition of normal urine.

What the kidneys contribute to homeostasis cannot be understood by examination of the urine alone. However, examination of the urine *(urinalysis)* is useful when performed in conjunction with other diagnostic methods for assessing renal function and diagnosing various types of pathophysiologies involving not only the kidneys but other organs and tissues as well.

EXPERIMENT I: Routine Laboratory Screening of Urine

1. To become familiar with routine laboratory tests for screening urine.

2. To examine urine for the presence of normal and abnormal constituents.

3. To compare urine samples of normal persons as to specific gravity, pH, and chemical constituents.

4. To acquire an appreciation for the value of urinalysis as a diagnostic aid.

5. To understand the process of urine clearance.

6. To understand the effects of differing solute concentrations in solutions on urine output.

Materials

Equipment
compound light microscope
water bath
watch with second hand
test-tube rack
centrifuge

Supplies
clean microscope slides
forceps
hydrion paper
six disposable test tubes (16 x 25 mm)
wire test-tube grippers
Pasteur pipettes and bulbs
filter paper
small plastic funnel
droppers
Icotest tablet and mat
Acetest tablets and chart
Hemastix
Albustix and chart
Bili-labstix and chart
Clinistix and chart
urine specimen jars and lids

Solutions
50 nil of fresh urine (subject's own)
concentrated nitric acid
methylene blue solution
Sulkowitch reagent (2.5 g oxalic acid + 2.5 g ammonium oxalate + 5 ml glacial acetic acid + distilled water to make a total of 150 ml)
Fouchet's reagent (10 g ferric chloride + 100 ml distilled water) + (25 g trichloroacetic acid + 100 ml distilled water)
3% silver nitrate solution
concentrated ammonium hydroxide solution
concentrated hydrochloric acid
10% barium chloride solution
Benedict's reagent
sodium nitroprusside
powder saturated ammonium sulfate solution

EXPERIMENTAL METHODS CAUTION:

In this experiment you will obtain and analyze a sample of your own urine. Handle only your own urine. Dispose of all supplies (disposable test tubes, microscope slides, coverslips, etc.) that come in contact with your urine by placing them in properly marked containers. Always treat all body fluids and supplies as infectious. Nondisposable items that come in contact with your urine are to be washed with a detergent and tap water, then rinsed successfully with tap water, bleach solution, distilled water, and acetone. Disinfect your laboratory table or laboratory bench top by wiping it clean with a bleach solution prior to obtaining your urine sample and again at the end of the experiment.

Quantity of Urine

Increase your fluid intake the day before and the day of urinalysis so as to increase urine output. If possible, avoid emptying the urinary bladder 2-4 hours prior to obtaining a urine sample.

Obtain a urine specimen jar and lid from the laboratory instructor. Go the restroom and collect a midstream urine sample in the container. (Void the first part of the urine sample before collecting the urine specimen.) Return to the laboratory bench, and mark the specimen jar for identification.

The average adult voids approximately 1500 ml of urine per day. The exact amount varies between 800 and 2300 ml per day, depending on the intake of liquid and food. The elimination of an increased quantity of urine (beyond the normal range) is called polyuria and occurs in a number of diseases, such as diabetes mellitus, diabetes insipidus, adrenal diabetes, and chronic nephritis. The elimination of a decreased quantity of urine (oliguria) occurs in fever, diarrhea, thermal stress, and other states.

Color, Transparency, and Odor of Urine

Normally, the color of urine is amber (yellow) with the intensity of the color (light to dark) varying with the amount of urine voided. The color is due to the presence of pigments, such as urochrome, urobilin, and hematoporphyrin, which are normally present in urine. The presence of abnormal constituents may change the color drastically. For example, the presence of hemoglobin will give the urine a brown to red color. Freshly voided urine is transparent, but after a few hours it becomes slightly opaque because of the separation and settling of epithelial cells, leukocytes, and mucus. After standing in the air, urine becomes alkaline, due to the conversion of urea to ammonia, and cloudy, due to the precipitation of substances such as phosphates. Cloudiness in freshly voided urine may indicate the presence of pus, blood, or bacteria from urinary tract infections.

The odor of urine varies with dietary intake. The excretion of waste products associated with the metabolism of certain foodstuffs (e.g., asparagus, cabbage) or the excretion of drugs by the kidneys often causes the urine to have an offensive odor. The decomposition and evaporation of urine usually results in an ammonia-like odor.

Examine the urine specimen for color, transparency, and odor. Record your observations in the report.

pH of Urine

The chemical reaction of normal urine varies from pH 4.8-7.5, depending primarily on dietary intake. Usually the pH is slightly acid (about 6) because the metabolism of the foodstuffs contained in the average diet results in the production of excess acids, which must be eliminated from the body fluids. The acidity of urine also increases in acidosis (metabolic and respiratory) and during fever. An alkaline urine may be produced by letting it stand or by storing it in the urinary bladder. In both cases, the alkaline pH is due to the conversion of urea to ammonia. Other causes of alkaline urine include excessive dietary intake of certain foods (e.g., fruits), the ingestion of alkaline substances (e.g., sodium bicarbonate), and various states of alkalosis (metabolic and respiratory).

Test the chemical reaction (acidity or alkalinity) of the specimen by using hydrion papers. Hydrion paper contains chemical indicators that change color when wetted with solutions of various pH: red to orange colors indicate pH 1-6; green indicates neutral, pH 7; and dark green to blue colors indicate pH 8-11. Compare the color reaction with the color chart on the side of the hydrion paper dispenser. Record the

data in the report and on the chalkboard. Compare the reaction of your urine sample with the results reported by others in the class, and note the variation.

Specific Gravity of Urine

The specific gravity of a liquid is determined by comparing the weight of a given volume of the liquid to the weight of an identical volume of pure water. Specific gravity is expressed as a simple number-the ratio of two other numbers. By definition, the specific gravity of water is 1.000. The normal range for the specific gravity of urine is from 1.010 to 1.030. Higher values for the specific gravity indicate a more concentrated urine, and lower values indicate a more dilute urine.

The specific gravity of urine tends to be low in diabetes insipidus and after drinking excessive quantities of liquid. It may be high during fever and in various diseases, such as diabetes mellitus and adrenal diabetes.

Adhering to the precautions outlined by the laboratory instructor, determine the specific gravity of the specimen by using the urinometer (figure 30.2). Fill the urinometer cylinder to three-fourths capacity with the urine specimen and insert the float. If the float does not float, add more urine until it does. Read the specific gravity from the stem of the float where the urine sample intersects. Record the specific gravity in the report and on the chalkboard. **Empty the urine back into the specimen jar, and then carefully** wash the urinometer with soap and rinse it with distilled water, bleach solution, and acetone. Compare the specific gravity of your urine sample with the results of others in the class, and note the variation.

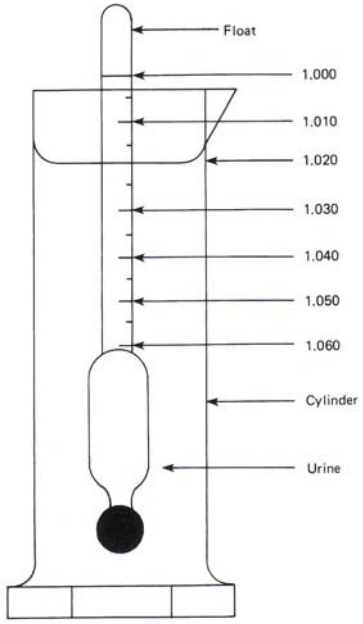


FIGURE 30.2 The urinometer

Estimation of Amount of Urinary Solids

The concentration of urinary solids in grams per liter can be approximated by multiplying the last two digits of the specific gravity by 2.66 (Long's coefficient). For example, if the specific gravity of the sample is 1.022, the approximate concentration of urinary solids would be 22 x 2.66 = 58.5 g/l. Estimate the amount of urinary solids in the sample, and record the data in the report.

Normal Inorganic Constituents of Urine

1. *Chlorides:* Next to urea, chlorides are the most abundant substances found in normal urine. They are derived primarily from the metabolism of food, and their quantity in the urine fluctuates, depending on dietary intake. The principal chloride in urine is sodium chloride. Fevers and various forms of nephritis tend to decrease the renal output of chloride.

Test for the presence of chlorides in the sample by pouring approximately 10 ml of urine into a clean test tube. Add 3 drops of nitric acid to prevent the precipitation of phosphates, and then add 3 drops of 3% silver nitrate solution. If chlorides are present, a white, curdy precipitate will form. If the chloride content of the urine is low, the urine will appear only milky. Record your observations in the report.

2. *Phosphates:* Phosphates are derived chiefly from food, although small amounts are produced during cellular metabolism. Large amounts of phosphates are present in the mineral complex of bone; hence, in bone diseases, such as rickets or osteomalacia, there is an increase in the renal excretion of phosphates.

Test for the presence of phosphates by pouring approximately 10 ml of fresh urine into a clean test tube. Add ammonium hydroxide until the urine is alkaline. If phosphates are present in the urine, precipitates of calcium and magnesium phosphate will form. Record your observations in the report.

3. *Sulfates:* Most of the sulfur present in urine originates from the dietary intake of protein and from the cellular metabolism of protein compounds. Ninety percent of it is present in an inorganic form combined with sodium, potassium, calcium, and magnesium.

Test for the presence of sulfates by pouring approximately 10 ml of fresh urine into a clean test tube. Add I drop of hydrochloric acid and shake well.
Add 3 drops of barium chloride. If sulfates are present in the urine, a white precipitate will form. Record your -observations in the report.

4. Calcium: Calcium is involved in a number of important physiologic processes in the body, such as blood coagulation, nerve conduction, and muscle contraction. Thus, the extracellular concentration of calcium must be maintained within fairly narrow limits. Approximately 99% of the total body calcium is located in bone. There, in the form of mineral complexes, it helps give bone its rigidity. Total plasma calcium is normally about 10 mg/dI.

Two primary forms of calcium in plasma are filterable calcium and protein-bound calcium. Filterable calcium consists of a free calcium ion (Cat') and calcium complexed with an anion such as phosphate. Free calcium is the physiologically active forin of calcium.

Approximately 99% of calcium filtered by the kidneys is reabsorbed, mostly in the proximal tubule and loop of Henle. Normal urinary excretion of calcium is 0.1-0.3 g every *24* hours. An increase in urinary calcium is observed in conditions associated with hypercalcemia (above-normal plasma calcium levels), such as hyperparathyroidism and hypervitaminosis D. A decrease in urinary calcium is observed in conditions associated with hypocalcemia (below-normal plasma calcium levels), such as hypoparathyroidism and hypocalcemic tetany of skeletal muscle.

Urinary excretion of calcium can be easily and rapidly assessed by Sulkowitch's test, often used to quickly diagnose hypocalcemic tetany.

Mix equal volumes of urine and Sulkowitch's reagent. A white precipitate of calcium oxalate is formed in a quantity proportionate to the urine calcium present. A fine white precipitate is indicative of a normal blood level; a heavy precipitate suggests hypercalcemia; and the absence of a precipitate indicates hypocalcemia.

Abnormal Constituents of Urine

I. Glucose: Normally, all of the glucose filtered out of the glomerulus is reabsorbed by the proximal convoluted tubule. The reabsorption of glucose is an active process requiring energy and involving a carrier-mediated transport system in the tubular cell. The transport system has a maximum capacity (T.), which is normally not exceeded. However, when the glucose level in the plasma exceeds 180 mg/dI (glucose threshold), the transport capacity is exceeded and glucose begins to appear in the urine *(glucosuria).* The normal blood level of glucose is 60-120 mg/dI, well below the renal threshold for glucose. When the glucose level exceeds the normal range *(hyperglycemia),* such as occurs in diabetes mellitus, adrenal diabetes, or the excessive intake of sugar, glucosuria will result if the renal threshold is exceeded.

Glucose may also be excreted even at a normal plasma glucose concentration. Renal glucosuria is a benign, inherited abnormality in which the proximal tubules' transport capacity for glucose is reduced. **Benedict's Test:** Test for the presence of glucose by adding 5 ml of Benedict's reagent to a clean, dry test tube. Heat the test tube contents to boiling by placing the test tube in a boiling water bath. Now add 8 drops of fresh urine, and allow the contents to boil for 5 minutes. Remove the test tube from the water bath, and set it aside to cool. If glucose is present in concentrations of 0.05% or higher, the reagent is reduced and a greenish to red-brown precipitate forms, depending on the concentration of glucose. Record your observations in the report. **Clinistix:** Remove a test strip from the bottle and replace the cap. Dip the test area of the strip in urine and remove it immediately. Draw the edge of the strip against the rim of the urine container to remove excess urine. Compare the test area to the Clinistix color chart exactly 10 seconds after it is moistened. Ignore color changes that occur after 10 seconds. Record your observations in the report. *Albumin* and *globulin:* Under normal circumstances, only a small amount of protein is present in the glomerular filtrate, and most of any filtered protein is reabsorbed. The "albumin"

of the urine is actually a mixture of serum albumin and serum globulin. Excess protein in the urine *(proteinuria)* reflects an abnormal leakiness and/or severe damage of the glomerular membrane. Various types of nephrosis and nephritis due to infection, vascular degeneration, and other causes may result in proteinuria. **Nitric Acid Test:** Test for the presence of protein in the urine by adding 10 in] of fresh urine to a clean test tube. Heat to boiling by placing the tube in a boiling water bath. Now add 5 drops of concentrated nitric acid. If a white precipitate forms on boiling and does not disappear when nitric acid is added, protein is present in the urine. Record your observations in the report. **Albustix:** Remove a test strip from the bottle and replace the cap. Dip the test area of the strip in urine and remove immediately. Draw the edge of the strip against the rim of the urine container to remove excess urine. Immediately compare the test area to the Albustix color chart. Record your observations in the report.

3. Ketone bodies: Ketone bodies, such as acetic acid and Beta-hydroxybutyric acid, are normally present only in trace amounts in the urine. However, the excessive metabolism of fats due to a high dietary intake of fat or a dependence of the cells on lipid metabolism to produce energy because of an inadequate glucose uptake will result in the presence of larger amounts of ketone bodies in the urine. **Rothera's Test:** Test for the presence of ketone bodies in the urine by placing about I g of powdered sodium nitroprusside crystals in a test tube. Add 5 ml of urine, then 5 ml of saturated ammonium sulfate. Mix. Carefully "layer" concentrated ammonium hydroxide on the mixture. If a purple ring appears at the junction of the two fluids, acetoacetic acid is present in the urine. **Acetest:** Test for the presence of acetone bodies by placing an Acetest tablet on a clean laboratory napkin. Put a drop of fresh urine on the tablet. Compare the color change at exactly *30* seconds with the color chart. The test result is negative if the tablet color does not change or turns cream-colored from wetting. The test is positive if the tablet color changes from lavender to purple. Record your observations in the report.

4. Bilirubin: Approximately 250 billion erythrocytes are destroyed and replaced each day in a healthy adult human. Aged erythrocytes are destroyed by the reticuloendothelial tissues of the spleen, liver, and bone marrow. In the process, hemoglobin is broken down and bilirabin is formed as an end product of heme metabolism. Bilirubin is released into the plasma, bound to albumin, and transported to the liver. The hepatic cells conjugate bilirubin with glucuronic acid and excrete it in the bile as bilirubin glucuronide. In the large intestine, it is converted to stercobilinogen and urobilinogen by bacteria. Some of the urobilinogen is then reabsorbed and eventually eliminated into the urine by the kidney. The remainder is excreted in the feces. Unconjugated bilirubin is not excreted by the kidney. However, conjugated bilirubin is normally excreted in very small amounts, since there is usually only a small amount in the serum. Bilirubin appears in the urine *(bilirubinuria)* when there is partial or complete obstruction of the extrahepatic biliary ducts, hepatitis, cirrhosis, or other types of destructive liver disease. **Fouchet's Test** Test for the presence of bilirubin by mixing 5 ml of urine with 5 ml of 10% barium chloride solution. Filter the mixture. The precipitated bile will remain on the filter paper. Allow the filter paper to dry. Add 2 drops of Fouchet's reagent to the area of the filter paper containing the residue.

A green color appears in the presence of bilimbin. Record your observations in the report. **Icotest** Place an Icotest mat on a paper towel and place 10 drops of urine on the mat. Use forceps to remove an Icotest tablet (do not touch reagent tablet with your fingers) from the bottle and place the tablet in the center of the moistened area on the mat. Immediately recap the bottle. Add one drop of water to the tablet, wait 5 seconds, then add another drop so that the water runs off onto the mat. Wait 1 minute, then observe the color of the mat around the tablet. A blue or purple color indicates the presence of bilirubin. A pink or light red color indicates negative for bilirubin. Record your observations in the report. *Hemoglobin* and *myoglobin:* Aged erythrocytes are destroyed by the cells of the reticuloendothelial system, but some of the hemoglobin molecules escape metabolism and are released into the plasma (free hemoglobin). Normally, the amount of free hemoglobin in the plasma is very small; however, when the plasma level of free hemoglobin exceeds 100 mg/dl, hemoglobin appears in the urine *(hemoglobinuria).* Hemoglobinuria occurs when there is an extensive or rapid destruction of erythrocytes at a rate that is too fast to allow for the adequate storage or metabolism of free hemoglobin. The causative factors include several types of hemolysis, bums, crushing injuries, transfusion reactions, and poisons (e.g., snake venoms, mushrooms). *Myoglobin* is a red respiratory pigment found in skeletal muscle, where the pigment performs in a manner similar to hemoglobin of the blood. Normally, myoglobin is absent from the plasma and, therefore, from the urine. However, free myoglobin may appear in the plasma and, hence, in the urine, following an extensive crushing injury to muscle, necrotic diseases of muscle, certain types of infections (e.g., *Clostridium),* and occasionally after severe exercise. **Hemastix** Test for the urinary presence of hemoglobin and/or myoglobin by dipping the test end of a Hemastix into a fresh specimen of urine and

immediately removing the dipstick. While removing the dipstick, run the edge of it against the rim of the urine container to remove excess urine. Wait 60 seconds and compare the test area with the color chart provided. The absence of a blue color in the test area after I minute indicates a negative test. A positive test is indicated by the appearance of a blue color on the test area in 1 minute or less. The intensity of the color change is proportional to small, moderate, or large amounts of excreted hemoglobin/myoglobin. Record your observations in the report. The Hemastix test does not differentiate hemoglobinuria from myoglobinuria; however, such a differentiation can be done by spectrophotometry. **Bifi-labstix** The Bili-labstix reagent strip is a dip-and-read test for pH, protein, glucose, ketones, bilirubin, and blood in urine. Basically, the reagent strip is a composite of the individual reagent pads (e.g., Albustix, Clinistix) used previously. Note the time intervals to be followed in reading the test strip.

Remove a test strip from the bottle and replace the cap. Dip the test areas of the strip in urine and remove immediately. Tap the edge of the strip against the rim of the urine container to remove excess urine. Note the time, then compare the test strip to the Bili-labstix color chart, and make the following determinations at the designated time lapses:

Immediately:

15 seconds:

20 seconds:

25 seconds:

30 seconds:

pH and protein

ketones

bilirubin

blood (hemoglobin) glucose

Record your observations in the report.

Microscopic Examination of Urine Sediment

A complete microscopic examination of urine involves both a visual assessment of the sediment and a bacteriologic analysis. In health the urine contains small numbers of cells and other formed elements from the whole length of the genitourinary tract: casts and epithelial cells from the nephron; epithelial cells from the pelves, ureters, bladder, and urethra; mucus threads and spermatozoa from the prostate. A few erythrocytes and leukocytes may also be present from any part of the urinary tract. We will perform only a simple qualitative analysis of urine using the compound light microscope.

Place 5 ml of fresh urine in a clean centrifuge tube and centrifuge for 5 minutes. Pour off the clear urine, and mix the remaining sediment by shaking the test tube. Place a drop of the sediment on a clean microscope slide, cover it with a clean coverslip, and examine it under the microscope while it's still wet. Always use subdued light; use the condenser and diaphragm to reduce the lighting. Examine the slide using first the low-power objective and then the high-power objective. Scan the sample for various cells, casts (protein formations), crystals, and mucus threads (figure 30.3).

Casts may be distinguished from other contaminants, like mucus fibers or crystals, by their regular parallel walls and "squared" ends. They consist of densely packed cells and/or cellular elements and include hyaline casts, red cell casts, leukocyte casts, epithelial cell casts, granular casts, fatty casts, and others.

A variety of *crystals* may be found in normal urine, and generally the type of crystal depends upon the urinary pH. Clinically, the presence of crystals in urine is of little significance, except in certain metabolic diseases and drug intoxications.

Red blood cells come from anywhere in the genitourinary tract. They are hemolyzed (laked) in dilute urine and crenated in concentrated urine. Crenated red cells and red cell casts come from the kidney and indicate serious renal disease. Persistent finding of even small numbers of erythrocytes should be thoroughly investigated.

Leukocytes may come from anywhere in the genitourinary tract. Leukocyte casts always come from the kidney and suggest an infective or noninfective inflammatory disease of the kidney. Urine cultures are used to differentiate infective from noninfective disease.

After scanning the sample with low- and high-power objectives, add a drop of methylene blue to the sample and scan again. Methylene blue helps to delineate the formed elements. Record your observations in the report.

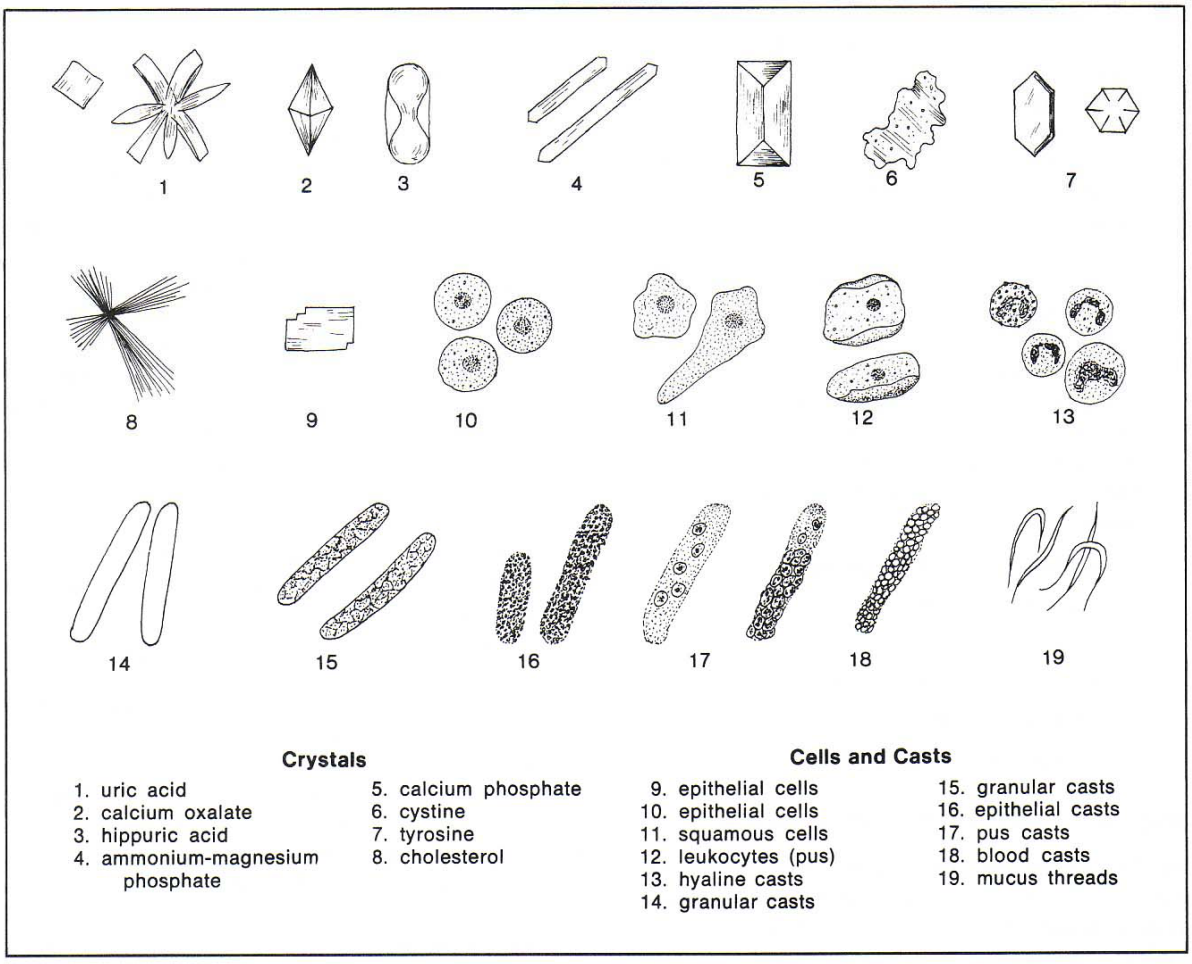


FIGURE 30.3 Urine sediment

Analysis of Urine Data:

Experimenter Name:_____

Lab Section: _____

1. Data:
   Subject_____Age _____Sex_____

   Time of sample_____Time of urinalysis_____

   Gross analysis:_____

   Color _____

   Specific gravity_____pH_____

   Clarity _____

   Urinary solids_____

Biochemical analysis (0, 1+, 2+, 3+, 4+)

Chlorides Sulfates

Phosphates Calcium

Glucose
- Benedict's Clinistix
- Bili-labstix

Albumin-globulin:
- Nitric acid Albustix
- Bili-labstix

Ketones
- Rothera's test Acetest
- Bili-labstix

Bilirubin
- Fouchet's test Icotest
- Bili-labstix

Hemoglobin/myoglobin:
- Hemastix Bili-labstix

Microscopic analysis of urine sediment:
- Squamous RBCs Crystals
- Transitional WBCs Casts
- Renal Bacteria

Data Analysis:

Can you answer these questions:

1. Under what conditions would bilirubin be present in the urine?

2. Does the presence of sugar in the urine always indicate sugar diabetes? Why or why not?

3. Does the specific gravity of urine ever fall below 1.000? Explain:

4. Define the following:
   a. Glucosuria

   b. Alburninuria

   c. Ketonuria

   d. Glucose threshold

   e. Diuresis

5. Why is the chemical reaction of urine usually acid?

6. Under what nonpathologic conditions might protein appear in the urine?

7. What is phenylketonuria? How is it detected?

Experiment II: Renal Clearance Exercise

Protocol for Renal Clearance Exercise

1. Note time last emptied bladder.

2. Upon entering lab void bladder as completely as possible. **(Note time for a control sample).**

3. Calculate amount of test fluid to drink.

mls of fluid intake = body weight (lbs) times 7 mls/lb.

4. Drink fluid and jot down time when taken.

5. **Flow rate determination:** Take control sample and measure void volume by pouring it into a graduated cylinder. Record amount.

Flow Rate = volume voided/minutes from last void.

6. Void bladder every 30 minutes from the time you finished drinking the solution for a period of 120 minutes. Divide each void volume by 30 to determine the new flow rate (ml/min).

7. Calculate flow rate for T0, T30, T60, T90, & T120.

8. Record your flow rate in your notes.

9. You will use this data to make a graphical representation to show changes in flow rate during the test period of 120 minutes. Your graph will show mean values ± standard errors.

Lab Report: Your discussion should include the role of the antidiuretic hormone in renal function as well as the mechanisms involved in stimulating and inhibiting its release.

Data Analysis:

Electromyography

Standard and Integrated EMG Motor Unit Recruitment:

The human body contains three kinds of muscle tissue, each performing specific tasks to maintain homeostasis. Cardiac muscle is found only in the heart. When it contracts, blood circulates, delivering nutrients to cells and removing their wastes. Smooth muscle is located in the walls of hollow organs such as the intestines, blood vessels, and lungs. Contraction of smooth muscle changes the internal diameter of hollow organs and is thereby used to regulate the passage of material through the alimentary canal, control blood pressure and flow, and regulate airflow during the respiratory cycle. Skeletal muscle derives its name from the fact that it is usually attached to the skeleton. Contraction of skeletal muscle moves one part of the body with respect to another part, as in flexing the forearm. Contraction of several skeletal muscles in a coordinated manner moves the entire body in its environment, as in walking or swimming

Regardless of kind, the primary function of muscle is to convert chemical energy to mechanical work, and in doing so, the muscle shortens or contracts. In this chapter we will investigate some properties of skeletal muscle. In other chapters we will study physiologic phenomena associated with other kinds of muscle, such as electrophysiology of the heart.

Human skeletal muscle consists of hundreds of individual cylindrically shaped cells, called fibers, bound together by connective tissue. In the body, skeletal muscles are stimulated to contract by somatic motor nerves, which carry signals in the form of nerve impulses from the brain or spinal cord to the skeletal muscles (figure B2.1). Nerve cells that innervate skeletal muscle are called somatic motor neurons. They are located in the gray matter of the spinal cord and brain. Axons, or nerve fibers, which are long cylindrical extensions of the neurons, leave the spinal cord or brain via spinal or cranial nerves and are distributed to appropriate skeletal muscles in the form of a peripheral nerve, a cable-like collection of individual nerve fibers. Upon reaching the muscle, each nerve fiber branches and innervates several individual muscle fibers.

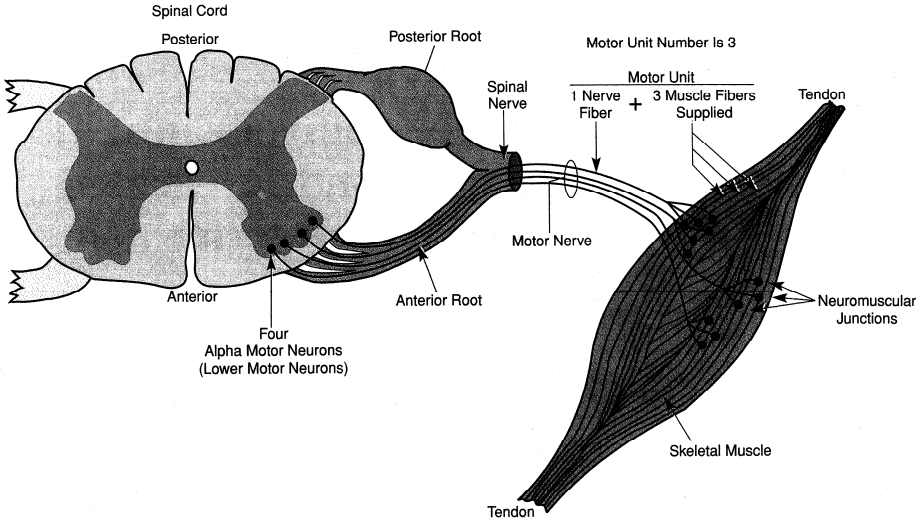


FIGURE B2.1 Organization of skeletal muscle and nerve

Although a single motor neuron can innervate several muscle fibers, each muscle fiber is innervated by only one motor neuron. The combination of a single motor neuron and all of the muscle fibers it controls is called a motor unit (figure B2.1). When a somatic motor neuron is activated, all of the muscle fibers it innervates respond to the neuron's impulses by generating their own electric signals that lead to contraction of the activated muscle fibers. The strength of contraction of the motor unit muscle fibers is directly proportional to the frequency of the motor neuron impulses.

The size of the motor unit arrangement (e.g., 1:10, 1:50, or 1:3000) of a skeletal muscle varies, depending on its function (flexion, extension, etc.) and location in the body. The smaller the size of a muscle's motor units, the greater the number of neurons needed for control of the muscle and the greater the degree of control the brain has over the extent of shortening. For example, muscles that move the fingers have very small motor units to allow for precise control, as when operating a computer keyboard.

Muscles that maintain posture of the spine have very large motor units because precise control over the extent of shortening is not necessary.

Smooth controlled movements of the body, such as walking, swimming, or jogging, are produced by graded contractions of skeletal muscle. Grading means changing the strength of muscle contraction or the extent of shortening in proportion to the load placed on the muscle. Skeletal muscles are thus able to react to different loads accordingly. For example, the effort of muscles used in walking on level ground is less than the effort those same muscles expend in climbing stairs.

Physiologically, the degree of skeletal muscle contraction is controlled by (1) activating a desired number of motor units within the muscle and (2) controlling the frequency of motor neuron impulses in each motor unit.
The strength of skeletal muscle contraction is directly proportional to the number of motor units that are simultaneously active. When an increase in the strength of a muscle's contraction is necessary to perform a task, the brain increases the number of simultaneously active motor units within the muscle. This process is known as motor unit recruitment.

Resting skeletal muscles in vivo exhibit a phenomenon known as tonus, a slight state of constant tension that serves to maintain the muscle in a state of readiness. Tonus is due to alternate periodic activation of a small number of motor units within the muscle by motor centers in the brain and spinal cord.

When a motor unit is activated, the component muscle fibers generate and conduct their own electric impulses, which ultimately result in contraction of the fibers. Although the electric impulse generated and conducted by each fiber is very weak (less than 100 uV), many fibers conducting simultaneously induce voltage differences in the overlying skin that are large enough to be detected by a pair of surface electrodes. The detection, amplification, and recording of changes in skin voltage produced by underlying skeletal muscle contraction is called electromyography. The recording thus obtained is called an electromyogram (EMG).

Motor Unit Recruitment and Fatigue Using the BIOPAC Hand Dynamometer:

Mechanical work in the physical sense refers to the application of a force that results in the movement of an object. Skeletal muscle performs mechanical work when the muscle contracts and an object is moved, as in lifting a weight.

Physiologically, skeletal muscle is stimulated to contract when the brain or spinal cord activates **motor units** of the muscle (figure B4.1) A discussion of motor units and their control was presented in chapter B2. Most human skeletal muscles are composed of hundreds of motor units. When a skeletal muscle is called on to perform mechanical work, the number of motor units in the muscle activated by the brain is *proportional* to the amount of work to be done by the muscle; the greater the amount of work to be done, the greater the number of motor units activated. Thus, more motor units are simultaneously active when a skeletal muscle lifts 20 kg than when the same muscle lifts 5 kg.

The brain determines the number of active motor units required for a muscle to perform a given task by utilizing sensory information from stretch receptors in the muscle and associated tendons and joint capsules. For example, as part of the process of lifting a bucket of water from the ground, the brain first activates several motor units in the requisite skeletal muscles. If sensory information returning from the muscles indicates the muscles are contracting but not developing adequate power to lift the bucket, the brain activates additional motor units until the sensory information indicates the bucket is being lifted. Appropriately, this stepwise activation of motor units up to a number adequate to perform a designated task is called **motor unit recruitment.** Once you have lifted a light object, the brain recruits approximately the same *number* of motor units to keep the object up, but cycles between *different* motor units. The muscle fibers consume stored energy available in the muscle and generate a force by contracting. As the muscle fibers deplete this fuel source, more energy must be created in order to continue contracting. When the brain recruits different motor units, formerly active motor units can relax and replenish their fuel sources.

Skeletal muscles performing acute maximal work or chronic submaximal work of a repetitive nature eventually fatigue. **Fatigue** is caused by a reversible depletion of the muscle's fuel supply. If the muscle uses adenosine triphosphate (ATP) faster than it can be generated by cellular metabolism, fatigue occurs. During contraction, skeletal muscle cells convert chemical energy into thermal and mechanical energy and, in the process, produce chemical waste products. Normally the waste products are removed from the muscle by the circulatory system as the blood brings nutrients to the muscle for energy transformation. If certain waste products (metabolites) are not removed at an adequate rate, they will accumulate and chemically interfere with the contractile process, thereby hastening the onset of fatigue. Some accumulated waste products also stimulate pain receptors in surrounding connective tissue and induce cramping of skeletal muscle, a general sign of inadequate blood flow to the muscle.

In this lesson, we will examine motor unit recruitment and skeletal muscle fatigue by combining electromyography with dynamometry. **Dynamometry** means the measurement of power *(dyno = power, meter = measure),* and the graphic record derived from the use of a dynamometer is called a **dynagram.** Experimentally, one way to measure the power of skeletal muscle contraction is by determining the rate at which the muscle shortens when it performs a prescribed amount of work, such as lifting a weight. In this lesson, the power of contraction of grip muscles will be determined by a hand dynamometer equipped with an electronic transducer for recording.

The BIOPAC system will simultaneously record three bands of information:

1. The force you exert on the transducer

2. The electric signal produced by the muscle during contraction

3. The integrated waveform, which is an indication of the activity levels of the muscle

Experiment 1: Skeletal muscle tonus

Experimental Objectives

1. To observe and record skeletal muscle tonus as reflected by a basal level of electrical activity associated with the muscle in a resting state.

2. To record maximum grip strength for right and left hands and compare differences between male and female.

3. To observe, record, and correlate motor unit recruitment with increased power of skeletal muscle contraction.

4. To listen to EMG "sounds" and correlate sound intensity with motor unit recruitment.

Questions to think about:

1. Does there appear to be any difference in tonus between the two forearm grip muscles?

2. Are the EMG grip clusters (mean measurement) the same for the right and left hand? Which hand suggests the greater grip strength?

4. What factors in addition to gender contribute to observed differences in grip strength?

5. Explain the source of signals detected by the EMG electrodes.

6. What does the term motor unit recruitment mean?

7. Define skeletal muscle tonus.

8. Define electromyography.

Materials

BIOPAC SS2L electrode lead cables
BIOPAC GEL1 electrode gel and ELPAD BIOPAC EL503 disposable vinyl electrodes BIOPAC OUT1 headphones

EXPERIMENTAL METHODS

Set Up

1. Turn on your computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC 100A transformer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.

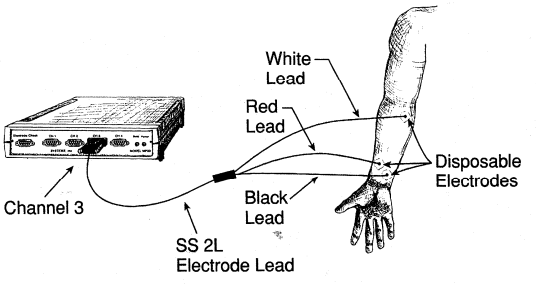


FIGURE **B2.2**

3. Select a subject for electromyography. Using a cotton ball or paper towel soaked in alcohol, or an ELPAD, cleanse the skin on the medial aspect of the anterior forearm (figure B2.2) where the EMG

electrodes will be attached. For the first recording segment, select the subject's dominant forearm (generally the right forearm if the subject is right-handed or the left forearm if the subject is left-handed) and attach the electrodes as shown. This will be Forearm 1. Use the subject's other forearm for the second recording segment. This will be Forearm 2. Following are recommendations regarding the use of electrodes.

4. Electrodes: The disposable electrodes used by the BIOPAC Student Lab are standard disposable electrodes and are widely used in clinical, research, and teaching applications. Although they sound complex, electrodes are very simple devices that consist of a small piece of metal designed to make indirect contact with the skin and a larger adhesive plastic disk. Each electrode is about 1 inch in diameter and is sticky on one side so it will adhere to skin. If you look closely at the electrode, you can see that there is a small piece of plastic mesh filled with a -gel. Get conducts electricity better than skin and is more flexible than the metal part of the electrode; this allows the subject's skin to flex and change shape somewhat without losing the electrical connection with the metal
part of the electrode. These electrodes come in strips of 10, and you should not remove an electrode from the backing until you are ready to use it. The purpose of an electrode is to act as a "connector" between the SS2L cable and the subject's skin, where electric signals are easiest to detect. Electrodes have no moving parts, so there is nothing you have to do to get an electrode to "work," although the key to working with electrodes is making sure that everything is connected properly.

If an electrode is able to make a good connection with the subject's skin, the signals that appear on the BIOPAC Student Lab will be relatively accurate. However, if an electrode is not adhering well to the skin, then the signal plotted on the screen can appear "fuzzy." Some refer to this as "noise," and although it is always present to some degree, it is desirable to reduce it as much as possible.

There are several things you can do to reduce noise when electrodes are connected. A common problem is that something on the surface of the skin is coming between the electrode and the skin. For instance, if there is too much hair between the outer layer of skin and the electrode, then you may not be able to sense the electrical activity taking place below the surface of the skin. Since in this experiment we do not recommend shaving the arm for the sake of getting a good signal, the best you can do is to try to place the electrodes where there is the least amount of hair.

One way you can improve electrode connections is to gently rub the area where the electrode is to be placed with an ELPAD electrode pad (included with the BIOPAC Student Lab). The ELPAD abrades the skin, removing a thin layer of dead skin from the skin's surface. Since dead skin does not conduct electricity very well, this improves the connection between the electrode and the skin.

It is also a good idea to attach the electrodes a few minutes before you are going to use them. The best results are achieved by putting the electrodes in place about 5 minutes before you begin recording data. This gives the electrodes time to establish contact with the surface of the skin.

Attaching electrodes: To attach an electrode, peel the electrode from its backing and place it on the area indicated in the lesson. You may want to squeeze a drop or two of electrode gel onto either the surface of the skin or onto the electrode to help ensure that the electrode will make good electrical contact with the skin. Once it is in place, press down firmly on the electrode with two fingers and rock the electrode back and forth for a few seconds. This will ensure that it is adhering to the skin as much as possible. With the electrode in place, attach the electrode lead from the SS2L to the snap connector on top of the electrode. Each color lead is different, so it is important that you attach each cable to the appropriate electrode site.

Removing electrodes: Once you have finished recording data, disconnect the lead, peel the electrode off the skin, and dispose of the electrode.

5. Plug the electrode assembly (SS2L) into channel 3. Attach the color-coded electrode leads to the subject according to figure B2.2. The subject is to be seated at the laboratory bench with the selected forearm resting on the table top, palm facing upward.

6. Locate the "BIOPAC Student Lab" folder, open it, and start the BIOPAC Student Lab program. A prompt (figure B2.3) will appear asking you to choose a lesson. Choose Lesson I ("L01- EMG-1") by clicking on it to highlight it, then clicking on "OK"

7. A prompt (figure B2.4) should appear asking you to "Please type in your Me name:' The program does this so that it can store all the data files you create in one place, and making it easier for you to retrieve data later on.

You can enter your real name, a nickname, or the name of your group, if you are working with other students. However, the BIOPAC Student Lab software does not allow for duplicate names, so if there are a lot of other students using your computer and you try to log on as "John," for example, there is a good chance the BIOPAC Student Lab software will ask you to use a different name. The best approach is to select and type in a unique identifier, such as the subject's nickname or student ID number, your full name, or some combination of your name and other letters and/or numbers (like "JohnF" or "John3"). It is a good idea to use the same log-on name for each lesson. Whichever log-on name you choose, be sure to write it down so that you can keep track of where your data is stored.

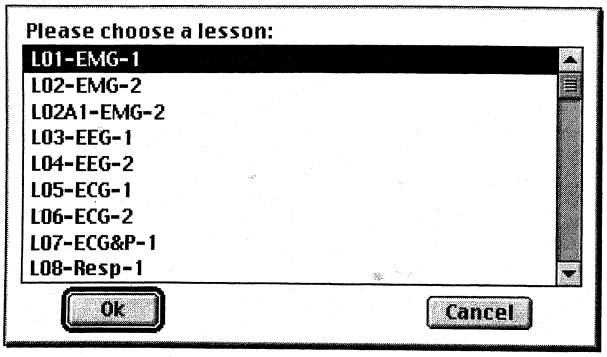


FIGURE B2.3

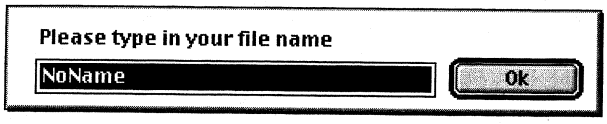


FIGURE B2.4

Once you enter your name and choose "OK," the BIOPAC Student Lab software creates a folder inside the "Data Files" folder, which is inside the "BIOPAC Student Lab" folder on your computer. This is where all your data will be stored. If you choose the same file name for other lessons, they will also be stored in this folder. However, if you try to use the same file name when you repeat a lesson or if someone else tries to choose the same name, the program will insist that you choose a different name. The files inside your folder can be moved, copied, duplicated, and deleted just like any other files. If you wish, you can copy them to a diskette as a backup or in case you want to view them later. Check with your instructor or lab assistant for more information on how to do this.

8. After you log on, a window similar to figure B2.5 will be displayed. Check to ensure the proper placement of electrodes and electrode leads and make certain that the electrode assembly is plugged into channel 3. This concludes the setup procedures.

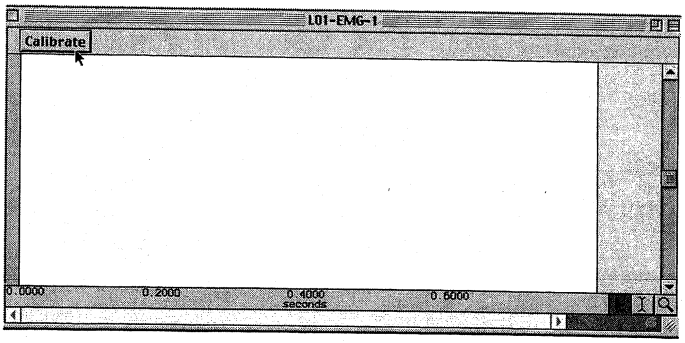


FIGURE B2.5

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1 Click on "Calibrate:' This will start the calibration recording.

2. A dialog box pops up when you click the Calibrate button, telling you to wait about 2 seconds, clench the fist as hard as possible, then release.

3. When ready, click "OK" and perform the calibration procedure as given above. The calibration procedure will last 8 seconds and stop automatically, so let it run its course. At the end of the 8-second calibration recording, the screen should resemble figure B2.6.

4. If your calibration recording did not begin with a zero_ baseline (subject clenched fist before waiting 2 seconds), you need to repeat calibration to obtain a reading similar to figure B2.6. If necessary, click on "Redo Calibration" to repeat the calibration procedure.

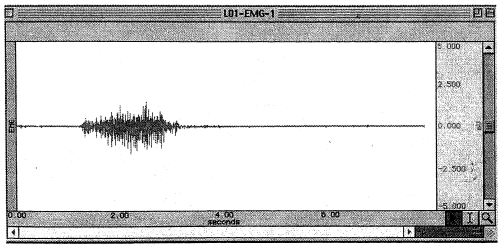


FIGURE **B2.6**

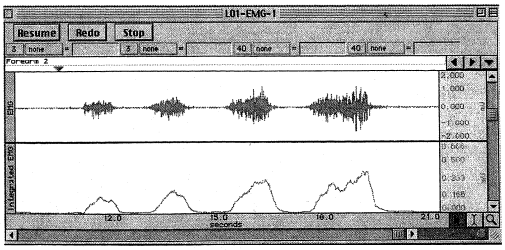


FIGURE **B2.8**

Data Recording

You will record two segments of data per subject: segment 1 data will be recorded from Forearm 1 (the dominant forearm), and segment 2 data will be recorded from Forearm 2.

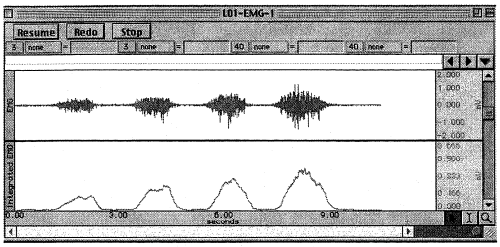


FIGURE **B2.7**

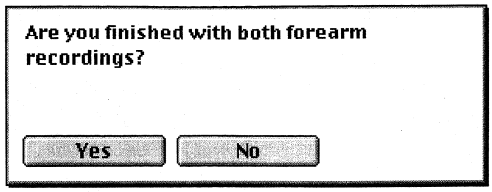


FIGURE **B2.9**

In order to work efficiently, read this entire section so you Segment 2 will know what to do before recording.

Check the last line of the journal and note the total amount of time available for recording. Stop each recording
segment as soon as possible so you do not waste recording time (time is memory).

Segment 1

1. When you begin to record, the subject will repeat a cycle of clench-release-wait, holding the clench for 2 seconds, and waiting for 2 seconds after releasing before beginning the next cycle. The subject should try to increase clench strength in equal increments such that the fourth clench is the maximum clench force. In this part of the experiment, we will record an increase in the number of active grip muscle motor units (motor unit recruitment) as grip force increases.

2. When ready, click on "Record.' The subject can begin the clench-release-wait cycles.

3. After the fourth clench (maximum clench) is released, click on "Suspend.' The recording should halt, giving you time to review the data and prepare for the next recording segment. If all went well, your data should resemble figure B2.7.

4. The data would be different if (1) the Suspend button was pressed prematurely or (2) the instructions were not followed. If the data is not similar to the data in figure B2.7, click "Redo" and repeat steps 2 and 3. Note that once you click on "Redo," the data you have just recorded will be erased.

Segment 2

1. Attach three new electrodes to the subject's opposite (non-dominant) forearm. Again refer to figure B2.2 for proper placement of electrodes.

2. When you resume recording, a marker labeled "Forearm 2" will automatically be inserted in the marker bar. The subject will repeat a cycle of clench release-wait, holding the clench for 2 seconds and waiting for 2 seconds after releasing before beginning the next cycle. The subject should try to increase clench strength in equal increments such that the fourth clench is the maximum clench force.
3. When ready, click on "Resume.' The subject can begin the clench-release-wait cycles.

4. After the fourth clench (maximum clench) is released, click on "Suspend.' If all went well, your data should look similar to figure B2.8.

5. If the data are not similar, click "Redo" and repeat steps 3 and 4. Note that once you click on "Redo" the data you just recorded for segment 2 will be erased.

6. Click on "Stop:' When you click on "Stop," a dialog box comes up asking if you are finished with both forearm recordings (figure B2.9). Clicking "Yes" will end the data recording segment, and automatically save the data. Clicking "No" will bring you back to the "Resume" or "Stop" options.

7. If you are finished with both forearm recordings and you want to listen to the EMG signal, click on "Yes:'

Listening to the EMG Signal:

Listening to the EMG can be a valuable tool in detecting muscle abnormalities; it is performed here for general interest. Listening to the EMG signal is optional, and the screen data will not be saved for later analysis.

When listening to the EMG signal, it is possible that the volume through the headphones may be very loud due to system feedback. The volume cannot be adjusted, so you may have to position the headphones slightly off the ear to reduce the sound.

1. Plug the OUT I headphone set into the output port in the back of the MP30. The subject puts on the headphones.

2. When ready, click on "Listen.' Experiment by changing the clench force as you watch the screen and listen. Note the increase in sound intensity as grip strength increases.

When finished listening, click "Stop." If others in your lab group would like to hear the EMG signal, switch the headphones from the subject to the new person and click "Redo:'

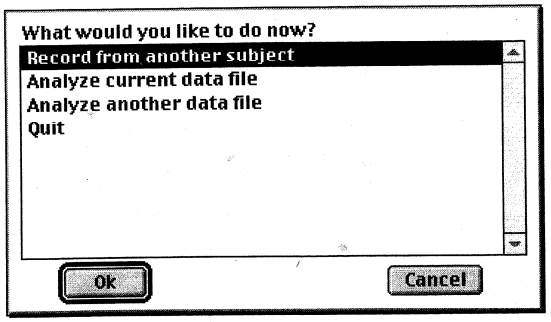


FIGURE **B2.10**

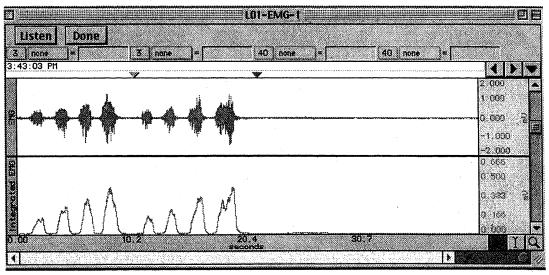


FIGURE **B2.11**

4. When finished listening, click "Done." A pop-up window with four options will appear (figure B2.10). Make your choice and continue as directed.

If choosing the "Record from another subject' option, attach new electrodes as per the setup procedures given earlier in this chapter and repeat the steps outlined in the calibration section and the data recording section.
Remember: Each person will need to use a unique file name.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu and choose the correct file.

2. When you open the file, the data window should resemble figure B2.1 1. Note that the complete data file is displayed (Forearm 1 data and Forearm 2 data), and therefore the horizontal time scroll bar is inactive.

The first marker indicates the beginning of Forearm 2 data, and the second marker indicates the end of data recording. Click on the marker tools to become more familiar with their use.

3. Note the channel number (CH) designations: CH 3 displays standard EMG data and CH 40 displays the integrated EMG data. As you can tell from the record in CH 3, the standard EMG waveforms are centered around zero and contain both positive and negative deflections. The baseline activity between EMG clusters represents tonus.

To assess the amount of work done by a muscle (or a group of muscles, in this experiment), it is often useful to plot the integrated EMG which is the envelope of the standard waveform. This is also known as contour-following and is generally like taking the absolute value of the EMG signal. An integrated EMG signal is always greater than zero and appears to "trace" an imaginary upper edge (or contour) of the standard EMG signal. In this experiment, the BIOPAC Student Lab calculates and plots an integrated EMG as the raw EMG data is being received. Calculations performed while the data is being recorded are sometimes referred to as "real-time" calculations, since the results are displayed as data is being collected.

4. Notice that the integrated EMG trace is different in color from the standard EMG. You may superimpose the integrated EMG on the standard EMG by clicking on the "Overlap" button. Your screen should resemble figure B2.12. Click on the "Split" button to restore the split screen display.

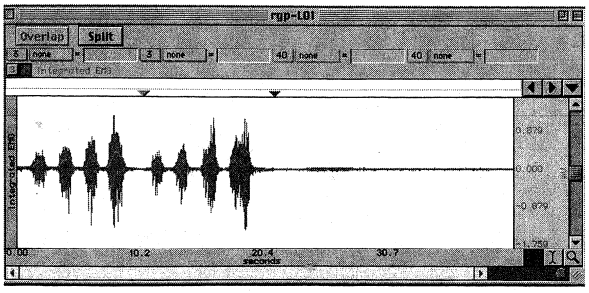


FIGURE **B2.12**

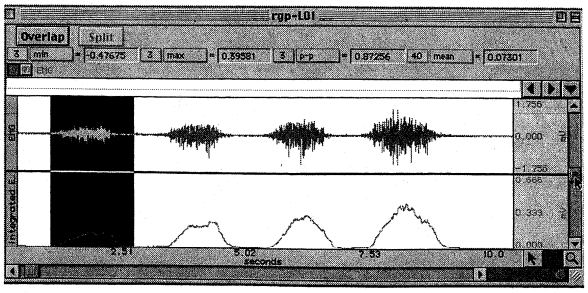


FIGURE **B2.13**

5. Use the zoom tool to select data from Forearm I for optimal viewing. Select the first four clusters of standard EMG.

6. Set up the measurement boxes as follows (refer to chapter B 1 if necessary):

Channel	**Measurement**
CH3	min
CH3	max
CH3	p-p
CH 40	mean

min displays the minimum value in the selected area.
max displays the maximum value in the selected area.
p-p finds the maximum value in the selected area and subtracts the minimum value in the selected area.
mean displays the average value in the selected area.

The "selected area" is the area selected by the I-beam tool (including the endpoints).

7. Using the I-beam tool, select an area enclosing the first EMG cluster (figure B2.13).

8. Record the measurement values in the journal and in the report.

9. Repeat steps 7 and 8 on each successive EMG cluster for Forearm 1.

10. Scroll to the Forearm 2 EMG clusters and repeat steps 7 and 8.

11. Scroll back to the Forearm 1 EMG clusters and examine the baseline activity between the clusters. The baseline electrical activity in a resting muscle reflects tonus. Scroll to the Forearm 2 EMG clusters and compare tonus levels with Forearm 1. Are the levels about the same? Record observations in the journal and in the report.

12. You may save the data to a diskette, save notes that are in the journal, or print the data file.

13. Exit the program. Turn off the MP30 and shut down the computer.

Standard and Integrated EMG Motor Unit Recruitment Data:

Experimenter Name:

Date:

1. Data and Calculations

(a) Subject profile:

Name: **Gender: Male/Female**

Age **Height** **Weight**

(b) EMG measurements:

	Dominant Forearm				*Other Forearm*			
	Min	***Max***	***P-P***	***Mean***	***Min***	***Max***	***P-P***	***Mean***
Cluster #	***[3 min]***	***[3 max]***	***[3 p-p]***	***[40 area]***	***[3 min]***	***[3 max]***	***[3 p-p]***	***[40 area]***
1								
2								
3								
4								

Notes:

(c) Use the mean measurement from the table above to compute the percentage increase in EMG activity recorded between the weakest grip and the strongest grip of Forearm 1 (Dominant).

Calculation:

Answer (%):

Data Analysis:

Can you answer these questions:

1. Does there appear to be any difference in tonus between the two forearm grip muscles?

Yes **No**

Would you expect to see a difference? Explain._____

2. Compare the mean measurement for the right and left maximum grip EMG cluster. Are they the same or different?

Same **Different**

Which one suggests the greater grip strength?

Right **Left** **Neither**

Explain_____

3. What factors in addition to gender contribute to observed differences in grip strength?_____

4. Explain the source of signals detected by the EMG electrodes._____

5. What does the term motor unit recruitment mean?_____

6. Define skeletal muscle tonus._____

7. Define electromyography._____

Experiment II: Maximal strength and fatigue

Experimental Objectives

1. To determine the maximum grip strength for right and left hands and compare differences between male and female.
2. To observe, record, and correlate motor unit recruitment with increased power of skeletal muscle contraction.
3. To record the force produced by grip muscles, EMG, and integrated EMG when inducing fatigue.

Questions to think about:

1. Is the strength of the subject's right arm different from that of the subject's left arm?

2. Is there a difference in the absolute values of force generated by males and females in your class?

3. When holding an object, does the number of activated motor units remain constant? Are the same motor units used for the duration of holding the object?

4. As you fatigue, the force exerted by your muscles decreases. What physiologic processes explain the decline in strength?

6. Define motor unit

7. Define motor unit recruitment

8. Define fatigue

9. Define EMG

10. Define dynamometry

Materials

BIOPAC electrode lead set (SS2L)
BIOPAC disposable vinyl electrodes (EL503), 6 electrodes per subject
BIOPAC electrode gel (GEL1) and abrasive pad (ELPAD) or skin cleanser or alcohol prep
BIOPAC SS25 hand dynamometer
BIOPAC OUT1 headphones

EXPERIMENTAL METHODS

Setup

1. Turn on your computer. The desktop should appear on the monitor. If it does not appear ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, make sure the AC 100A transformer is plugged into an electrical outlet on the wall or laboratory bench. Select a subject for electromyography. Using a cotton ball or paper towel soaked in alcohol, or an ELPAD, cleanse the skin on the medial aspect of the anterior forearm (figure B4.2) where the EMG electrodes will be attached.

3. Select the right forearm if the subject is right-handed, or the left forearm if the subject is left-handed. The dominant forearm will be designated Forearm 1 and will be used for the first recording segment. The other forearm (Forearm 2) will be used for the second recording segment. Attach the electrodes by peeling each electrode from its backing and placing it on the area indicated. You may want to squeeze a

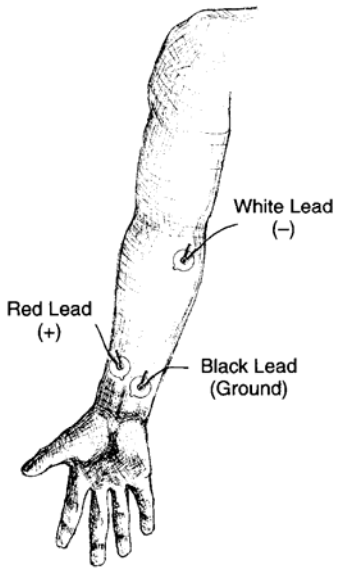


FIGURE **B4.2**

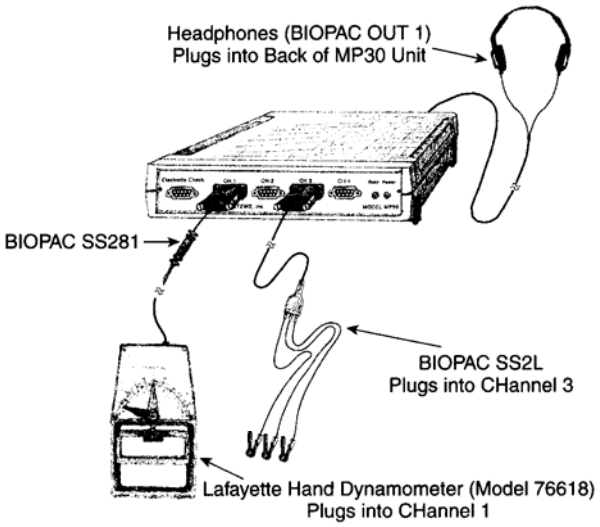


FIGURE **B4.3**

drop or two of electrode gel onto either the surface of the skin or onto the electrode to help ensure that the
electrode will make good electrical contact with the skin. Once it is in place, press down firmly on the electrode with two fingers and rock the electrode back and forth for a few seconds. This will ensure that it is adhering to the skin as much as possible. With the electrode in place, attach the electrode lead from the SS2L cable to the snap connector on top of the electrode. Each color lead is different, so it is important to attach each cable to the appropriate electrode site. Once you have completed the lesson, peel the electrode off of the skin and dispose of the electrode.

4. Plug the electrode assembly (SS2L) into channel 3. Attach the color-coded electrode leads to the subject according to figure B4.2. The subject is to be seated at the laboratory bench with the selected forearm resting on the tabletop, palm facing upward.

5. Plug the hand dynamometer (figure B4.3) into channel 1. The subject is to grasp the hand dynamometer, as shown in figure B4.4.

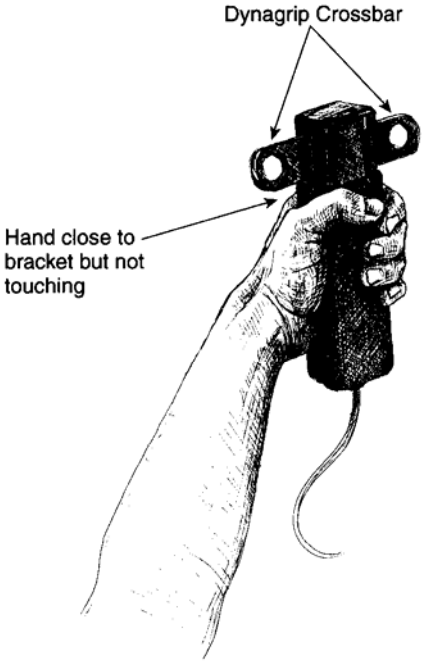


FIGURE **B4.4**

6. Locate the "BIOPAC Student Lab" folder, open it, and start the **BI0PAC Student Lab program.** A prompt (figure B4.5) will appear asking you to choose a lesson. Choose Lesson 2 **("L02- EMG-2")** by clicking on it to highlight it, then clicking on "OK:'

7. A prompt (figure B4.6) should appear asking you to “Please type in your file name.” The program does this so that it can store all the data files you create in one place, making it easier for you to retrieve data later on. You can enter your real name, a nickname, or the name of your group, if you are working with other students. However, the BIOPAC Students Lab software does not allow for duplicate names, so if there are a lot of other students using your computer and you try to log on as “John”, for example, there is a good chance the BIOPAC Student Lab software will ask you to use a different name. The best approach is to select and type in a unique identifier, such as the subject’s

nickname or student ID number, your full name, or some combination of your name and other letters

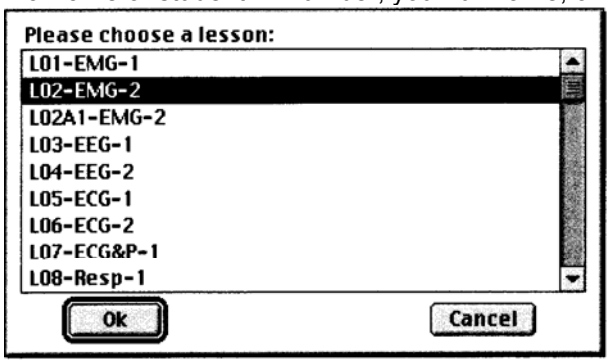


FIGURE **B4.5**

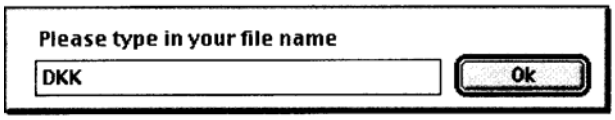


FIGURE **B4.6**

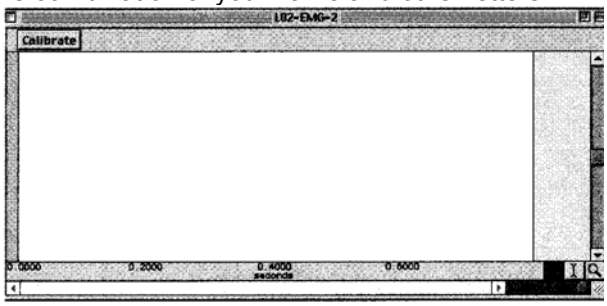


FIGURE **B4.7**

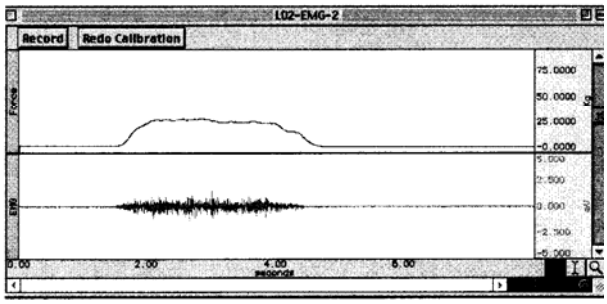


FIGURE **B4.8**

and/or numbers (like "JohnF" or "John3"). It is a good idea to use the same log-on name for each lesson. Whichever log-on name you choose, be sure to write it down so that you can keep track of where your data is stored.

Once you enter your name and choose "OK," the BIOPAC Student Lab software creates a folder inside the "Data Files" folder, which is inside the "BIOPAC Student Lab" folder on your computer. This is where all your data will be stored. If you choose the same file name for other lessons, they will also be stored in this folder. However, if you try to use the same file name when you repeat a lesson or if someone else tries to choose the same name, the program will insist that you choose a different name. The files inside your folder can be moved, copied, duplicated, and deleted just like any other files. If you wish, you can copy them to a diskette as a backup or in case you want to view them later. Check with your instructor or lab assistant for more information on how to do this.

8. After you log on, a window similar to figure B4.7 will be displayed. Check to ensure the proper placement of electrodes and electrode leads. Make sure the electrode assembly is plugged into channel 3 and the dynamometer assembly is plugged into channel 1. This concludes the setup procedures.

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. **Pay close attention to the calibration procedure.**

1. Click on **"Calibrate." A** pop-up window will tell you to remove any grip force from the hand dynamometer. To do so, the subject sets the hand dynamometer down. Hands must be removed from the transducer to make sure there is no force on the transducer. This establishes a zero-force calibration before you continue the calibration sequence. Click "OK:' The subject now grasps the BIOPAC hand dynamometer with the hand as close to the dynagrip crossbar as possible **without actually touching the crossbar** (figure B4.4). When ready, click **"OK:'**

IMPORTANT: The subject needs to hold the dynamometer in the same position for all measurements from each arm, so note the grip position with respect to the crossbar for the first segment and try to repeat it for the other segments.

3. A pop-up window will prompt as follows: "After clicking on OK, subject should wait two seconds, then clench the hand dynamometer as hard as possible, then relax grip." When ready, click "OK" and have the subject perform this maneuver. The calibration procedure will last 8 seconds and stop automatically, so let it run its course.

4. At the end of the 8-second calibration recording, the screen should resemble figure B4.8. The signal in the force channel should increase and you should see a corresponding increase in the EMG signal on the bottom channel. If your calibration recording did not begin with a zero baseline (subject clenched before waiting 2 seconds), you need to repeat calibration to obtain a reading similar to figure B4. 10.

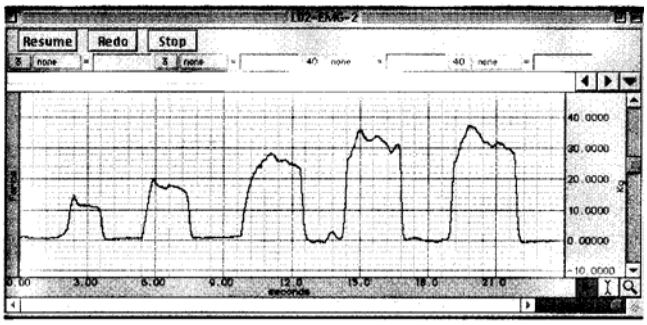


FIGURE **B4.9**

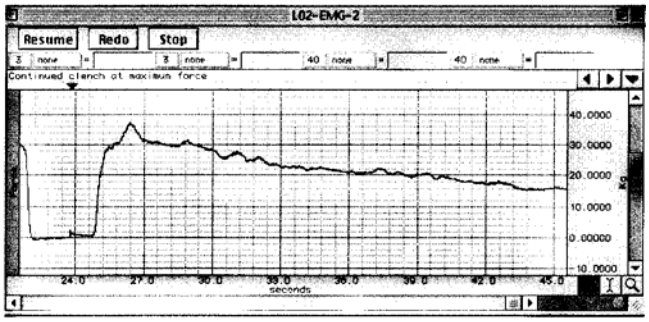


FIGURE **B4.10**

Data Recording

You will record two segments on each forearm:

1. Segment I records motor unit recruitment.

2. Segment 2 records fatigue.

In order to work efficiently, read this entire section so you will know what to do before recording. Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not waste recording time (time is memory).

Segment 1

1. Click on **"Record." After you** click on record, the screen will change to display only the hand dynamometer channel, and a grid with a scale showing 10 kg per division will appear so that you can visually review the force level. You will begin to record data.

2. Repeat a cycle of **clench-release-wait, with** the subject holding the clench for 2 seconds and waiting for 2 seconds after releasing before beginning the next cycle. Begin with 10 kg of clench force, and watch the horizontal grid lines on the screen so the subject can increase clench force in 10 kg increments for each cycle until maximum clench force is obtained (see figure B4.9).

3. Click on **"Suspend:'** The recording should halt, giving you time to review the data and prepare for the next recording segment of Forearm 1.

4. If all went well, your data should look similar to figure B4.9. The important aspect for you to check for is multiple peaks in your data (indicating the clench cycles). The data shown is from a subject who has experience with the dynamometer and was able to maintain an even force throughout the clench. Your data may be correct even if the peaks are not as smooth as those shown.

The data would be *incorrect* if the Suspend button was pressed prematurely or the instructions were not followed. Click on **"Redo" if your** data was incorrect and repeat steps 1-3. Note that once you press "Redo," the data you have just recorded will be erased.

Segment 2

1. Click on **"Resume." A** marker labeled "Continued clench at maximum force" will automatically be inserted when you click the Resume button, and the recording will continue from the point it left off. The subject should clench the hand dynamometer with maximum clench force. Note this force and have the

subject try to maintain it. As the subject tries to maintain the maximum clench force, forearm muscles will fatigue and the clench force will decrease.

2. When the maximum clench force displayed on the screen has *decreased by more than 50%, click* on **"Suspend." The** time to fatigue to 50% of maximal clench force will vary greatly among individuals.

3. If all went well, your data should look similar to figure B4.10. Note that the peak found immediately following the start of segment 2 represents the maximal clench force. This example shows the point of fatigue to 50% maximal clench force captured on the same screen, but in your data maximum clench force may scroll out of view. You may use the horizontal (time) scroll bar to view your entire recording.

The data would be *incorrect* if (1) you didn't record to the point of 50% maximal clench force, (2) the Suspend button was pressed prematurely, or (3) the instructions were not followed.

4. If the data is incorrect, click **"Redo"** and have the subject rest so the arm muscles recover and the fatigue data will be meaningful. When ready, repeat steps 1-3. Note that once you press "Redo' " the data you have just recorded will be erased.

5. Click on **"Stop:' When** you click on **"Stop'** " a dialog box comes up asking if you are sure you want to stop the recording. Clicking "Yes" will end the data recording segment, and automatically save the data. Clicking "No" will bring you back to the Resume or Stop options. This is simply one last chance to confirm that you do not need to redo the last recording segment.

When you click "Yes," the program automatically saves the data just recorded with an added file name extension:

Forearm I = "1-LO2" Forearm 2 = "2-LO2"

NOTE: You will need to know the file name extensions when you enter the **Review Saved Data mode.**

Listening to the Signal

Listening to the EMG is optional. Listening to the EMG can be a valuable tool in detecting muscle abnormalities; it is performed here for general interest only. When listening to the EMG signal, it is possible that the volume through the headphones may be very loud due to system feedback. The volume cannot be adjusted, so you may have to position the headphones slightly off the ear to reduce the sound.

1. Plug the OUT1 headphone set into the output port in the back of the MP30. The subject puts on the headphones. You will hear the EMG signal through the headphones as it is being displayed on the screen. The screen will display three channels: CH 1, Force-, CH 3 EMG; and CH 40, Integrated EMG. The data on the screen will not be saved. The signal will run until you press "Stop."

2. When ready, click on **"Listen:'** Experiment by changing the clench force as you watch the screen and listen. Note the increase in sound intensity as more motor units are recruited to make the grip stronger.

3. When finished listening, click **"Stop:'** If others in your lab group would like to hear the EMG signal, switch the headphones from the subject to the new person and click **"Redo:'**

4. When finished, click **"Forearm 2."** When you click on "Forearm 2," the program returns you to the calibration sequence.

5. Attach new electrodes to the subject's other forearm. Refer to the setup section earlier in this chapter for proper placement of electrodes and electrode leads.

6. Complete the entire calibration sequence outlined in the preceding section, and then follow the entire data recording procedure so as to record the same kind of date from Forearm 2 as was previously recorded from Forearm 1.

7. When finished recording, click **"Done:'** A pop-up window with four options will appear (figure B4.11). Make your choice and continue as directed.

If choosing the "Record from another subject" option, attach new electrodes as per the setup procedures and repeat the steps outlined in the calibration section and the data recording section.

REMEMBER: Each person will need to use a unique file name.

8. Remove the electrode cable pinch connectors, and peel off the electrodes. Throw out the electrodes (BIOPAC electrodes are not reusable). Wash the electrode gel residue from the skin, using soap and water. The electrodes may leave a slight ring on the skin for a few hours, which is quite normal.

Data Analysis

1. Enter the **"Review Saved Data"** mode from the **Lessons menu** and choose the correct file. For the first part of the analysis, choose the data file from the subject's first forearm (Forearm 1), saved with file name extension " 1 -L02." For the second part of the analysis, choose the data file from the subject's second forearm (Forearm 2), saved with the file name extension "2-LO2."

2. When you open the file, the data window should resemble figure B4.12. The first data segment is the recording before the first marker. The second data segment is the recording after the first marker.

Note the channel number (CH) designations: CH 1, Force; CH 3, Standard EMG; and CH 40, Integrated EMG.

3. Pull down the Display menu and select “Autoscale waveforms” so as to center the waveforms in their recording windows.

4. Set up the measurement boxes as follows (refer to chapter B 1 if necessary):

Channel	**Measurement**
CH 1	mean
CH 3	P-P
CH 40	mean

Recall:

mean displays the average value in the selected area.

p-p finds the maximum value in the selected area and subtracts the minimum value in the selected area.

The **"selected area"** is the area selected by the I-beam tool (including endpoints).

5. Using the **I-beam tool** select an area on the plateau phase of the first clench (figure B4.13). Record the measurement values in table B4.1 in the report.

6. Repeat step 5 on the plateau of each successive clench in segment I of the Forearm I data.

7. Examine the second recording segment. Segment 2 begins after the first marker and represents the repeated maximum clench (figure B4.14). Set up the measurement boxes as follows:

Channel	**Measurement**
CH 1	**Value**
CH 40	**ΔT**

Recall:

Value displays the amplitude value for the channel at the point selected by the I-beam cursor. If a single point is selected, the value is for that point, if an area is selected, the value is the endpoint of the selected area.

ΔT displays the amount of time in the selected segment (the difference in time between the endpoints of the selected area).

8. Use the **I-beam tool** to select a point of maximal clench force immediately following the start of segment 2. The point selected should represent the maximal clench force at the *start* of segment 2 as shown in figure B4.14. Calculate 50% of the maximum clench force measured in step 8. Enter the result here. You will need this number to complete step 10.

10. Beginning with the **I-beam cursor** positioned on the plateau of maximal clench (figure B4.15), drag the cursor to the right while looking at the "value" measurement box. Stop dragging the cursor when it reaches a clench plateau closest to the 50% of maximum clench value calculated in step 9. The selected area should resemble the selected area in figure B4.15. It represents the area from maximum clench strength to 50% of maximum clench strength.

Note the time to fatigue, measured by the ∆T function (CH 40). Record the time to fatigue in table B4.2 in the report.

11. You may save the data to a diskette, save notes that are in the journal, or print the data file.

12. Repeat the entire Data Analysis section, beginning with step 1, with the data file for Forearm 2.

13. Exit the program. Turn off the MP30 and shut down the computer.

Motor Unit Recruitment and Fatigue Using the BIOPAC Hand Dynamometer Data:

Experimenter Name:

Date:

1. (a) Motor unit recruitment:

Name **Age** **Height** **Weight**

Gender: Male/Female **Dominant forearm: Right/Left**

Complete table B4.1 using segment 1 data. ***(Note:*** **You may not need nine peaks to reach max.)**

TABLE B4.1

		Forearm 1 (Dominant)			***Forearm 2 (non-Dominant)***		
		Force at peak	***Raw EMG***	***Int. EMG***	***Force at peak***	***Raw EMG***	***Mt. EMG***
Peak #	***Targeted force***	***[CH 1] mean***	***[CH 3] P-P***	***[CH 40] mean***	***[CH 1] mean***	***[CH 3] P-P***	***[CH 40] mean***
1							
2							
3							
4							
5							
6							
7							
8							
9							

Notes:

(b) Fatigue

Complete table B4.2 using segment 2 data from each arm.

TABLE B4.2

Forearm 1 (Dominant)

Maximum clench force	**50%** *of* max **clench force**	**Time to reach 50**% *of* max	Total Clench Time (CCF)
***CH* 1 value**	**calculate**	***CH 40* ΔT**	

Notes:

Forearm 2 (non-Dominant)

Maximum clench force	**50**% *of* max **clench force**	**Time to reach 50**% *of* max	Total Clench Time (CCF)
***CH* 1 value**	**calculate**	***CH 40* ΔT**	

Notes:

Data Analysis:

Can you answer these questions?

1. Is the strength of the subject's right arm different from that of the subject's left arm?
Yes **No**

2. Is there a difference in the absolute values of force generated by males and females in your class?
Yes **No**

What might explain the difference?_____

3. When holding an object, does the number of activated motor units remain constant? Are the same motor units used for the duration of holding the object?_____

4. As you fatigue, the force exerted by your muscles decreases. What physiologic processes explain the decline in strength?_____

5. Define motor unit_____

6. Define motor unit recruitment._____

7. Define fatigue._____

8. Define EMG._____

9. Define dynamometry._____

Electroencephalography

Relaxation and Brain Rhythms:

The brain is encased by the cranium, bones of the skull that immediately cover and protect brain surfaces. A thin cover of skin, called the scalp, covers most of the cranium. The largest part of the brain, located immediately beneath the cranium, is the cerebrum (figure B5.1). The cerebrum is divided into hemispheres and each hemisphere is divided into frontal, parietal, temporal, and occipital lobes. The outer cell layers of the cerebrum form the cerebral cortex, the "gray matter" of the brain often referred to in popular literature. The cerebral cortex contains billions of nerve cells (neurons), many of which are functionally connected to each other, and connected to other parts of the brain.

Functions of the cerebral cortex include abstract thought, reasoning, memory, voluntary and involuntary control of skeletal muscle, and the recognition and differentiation of somatic, visceral, and special sensory stimuli. Specific regions of the cerebral cortex (figure B5.2) process or generate various kinds of information. For example, the frontal lobe generates nerve signals that voluntarily control skeletal muscle contractions such as in walking or riding a bicycle. The occipital lobe processes visual (sight) information, and the temporal lobe processes auditory (hearing) information. Cutaneous pain and temperature information and other somatosensory information is processed in the parietal lobe. Electrical activity in the form of nerve impulses being sent and received to and from cortical neurons is always present, even during sleep or other states when the level of consciousness is reduced. In a legal sense as well as a medical or biological sense, absence of electrical activity in the human cerebral cortex signifies death.

In 1929, an Austrian physician named Hans Burger discovered that electrodes placed on the scalp could detect various patterns of electrical activity and that this detected electrical activity was not due simply to artifacts of scalp musculature. Burger recorded the patterns of electrical activity and called the record an electroencephalogram (electro = electric, encephalo = brain, gram = record). Soon after Burger's discovery, scientists began to study these
"brain waves," and the detection, amplification, recording, and interpretation of the patterns of electrical activity associated with functioning of the cerebral cortex became known as electroencephalography. The hardware used to record such patterns is called an electroencephalograph, and the record obtained from its use is called an electroencephalogram, or EEG.

Today, the EEG is still medically useful recording for brain function. In medical and basic research, the correlation of particular brain waves with sleep phases, emotional states, psychological profiles, and types of mental activities is ongoing.

EEG signals are recorded as a series of complex waveforms. Basic knowledge regarding waveform terminology and analysis is useful and therefore is reviewed here to assist you as you record, examine, and analyze an EEG.
Two fundamental characteristics of a regular,repeating waveform are its amplitude and its frequency.

Amplitude refers to the "height" or "depth" of a waveform as measured from a reference point called the **baseline**. Amplitude values above the baseline are considered positive (+), and this part of the waveform appears as a "hill" or "peak." Amplitude values below the baseline are considered negative (-), and this part of the waveform appears as a "trough" or "valley" (figure B5.3). The amplitude of an electrical waveform may be measured in volts (V), millivolts (mV), or microvolts (uV).

Frequency refers to the number of times a waveform repeats itself in a given interval of time, such as one second or one minute. A waveform repeating itself 60 times in one second has a frequency of 60 **cycles per second (cps)** or 60 hertz (Hz). One hertz equals one cycle per second.

Frequency is measured by counting the number of peaks or troughs (but not both) within one second (cps) or one minute (cpm).

A waveform with a constant interval of time between peaks is called periodic. A waveform with variable intervals of time between peaks is termed nonperiodic. The waveforms shown in figure B5.3 are periodic sine waves.

Waveforms may have identical amplitudes but differ in frequencies, as shown in figure B5.3 or they may have identical frequencies but differ in amplitudes. A complex waveform results when two or more waveforms, each with different amplitudes and frequencies, are added together. Figure B5.4 shows the complex waveform that represents the sum of the 2 Hz, 4 Hz, and 16 Hz sine waves shown in figure

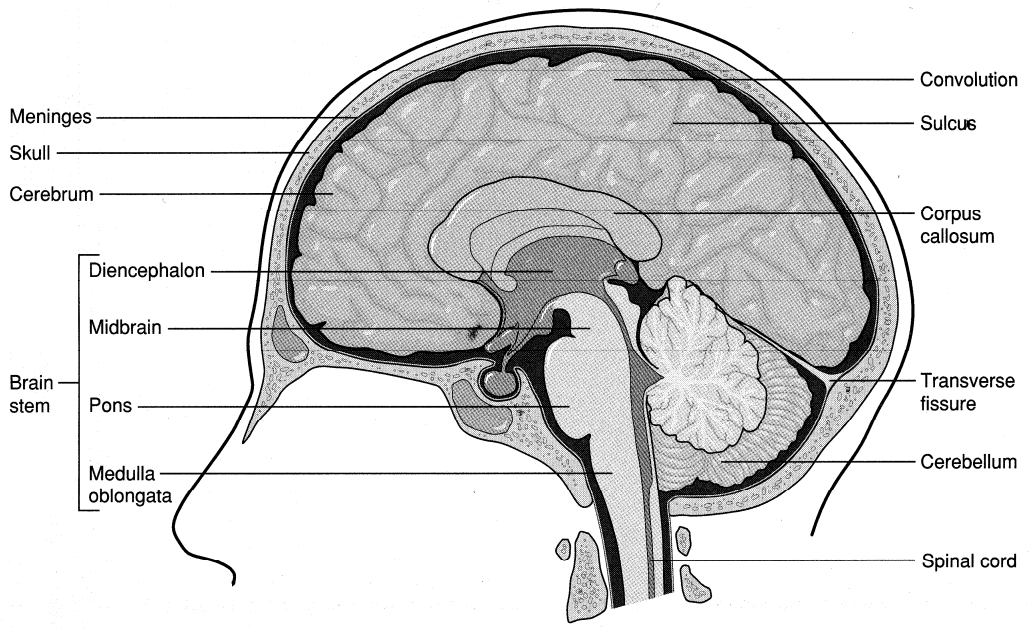


FIGURE B5.1 The major portions of the brain: the cerebrum, cerebellum, and brain stem

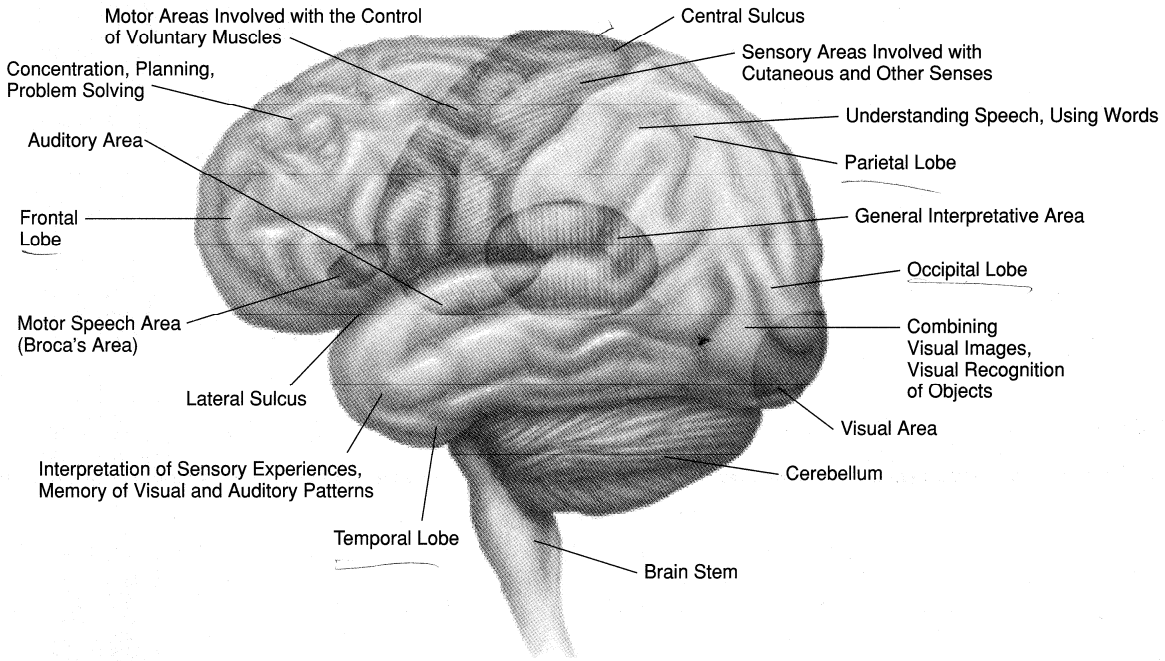


FIGURE B5.2 Some motor, sensory, and association areas of the cerebral cortex

B5.3. If we were to mathematically remove (by waveform subtraction) the 2 Hz and 4 Hz waveforms, we would be left with a 16 Hz waveform as shown in figure B5.3. Similarly,
when an EEG is analyzed, dominant waveforms or rhythms are separated from the rest of the record so as to make the examination easier.

Four simple periodic rhythms recorded in the EEG are alpha, beta, delta, and theta (table B5.1). These rhythms are identified by frequency (Hz, or cps) and amplitude. The amplitudes recorded by scalp electrodes are in the range of microvolts (uV, or 1/1,000,000 of a volt). The amplitude measurements shown in table B5.1 are those values reported for clinical settings; in a classroom setting, the amplitudes may be much lower.

Alpha

In general, the alpha rhythm is the prominent EEG wave pattern of an adult who is awake but relaxed with eyes closed. Each region of the brain has a characteristic alpha rhythm, but alpha waves of the greatest amplitude are recorded from the occipital and parietal regions of the cerebral cortex. Results from various studies indicate that:

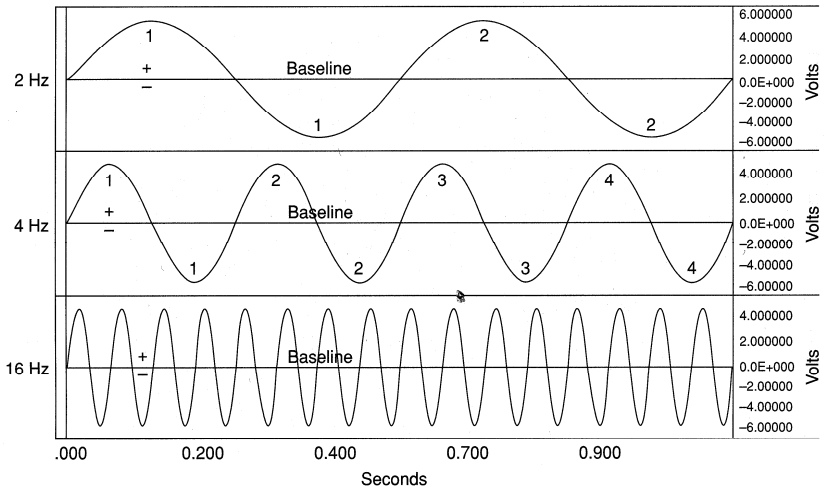


FIGURE B5.3 Waveforms with identical amplitudes but different frequencies

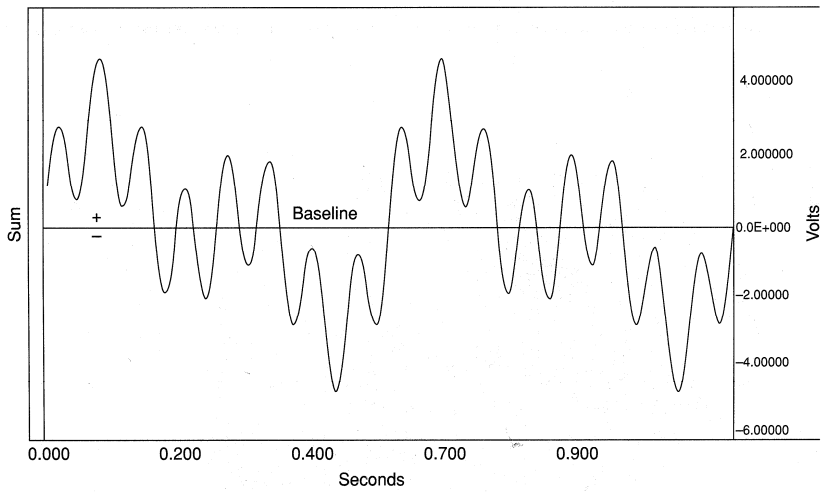


FIGURE B5.4 A complex waveform representing the sum of 2 Hz + 4 Hz + 16 Hz (sine waves shown in figure B5.3)

1. Females tend to have higher mean frequencies of alpha waves than males.
2. Alpha wave amplitudes are likely to be higher in "outgoing" subjects.
3. Alpha wave amplitudes vary with the subject's attention to mental tasks performed with the eyes closed.

In general, amplitudes of alpha waves diminish when subjects open their eyes and are attentive to external stimuli, although some subjects trained in relaxation techniques can maintain high alpha amplitudes even with their eyes open.

Beta

When the eyes are open and the individual becomes alert and attentive to external stimuli or exerts conscious mental effort such as when performing mental computations, the alpha rhythm is replaced by the lower, faster beta rhythm. This transformation is known as desynchronization of the alpha rhythm and represents arousal of the cortex to a state of alertness. Paradoxically, beta rhythms also occur during deep sleep, that is, rapid eye movement (REM) sleep, when the eyes rapidly move back and forth beneath closed eyelids. The beta rhythm is best recorded from precentral regions of the frontal cortex. Figure B5.5 illustrates various patterns of the EEG associated with the awake state. Notice that the amplitudes of beta waves tend to be lower than those of alpha waves. This does not mean that there

TABLE B5.1 Typical frequencies and amplitudes of synchronized brain waves

Rhythm	*Typical frequencies (Hz)*	*Typical amplitude (μV)*
Alpha	8–13	20–200
Beta	13–30	5–10
Delta	1–5	20–200
Theta	4–8	10

is less electrical activity; rather, it means that the "positive" and "negative" waveforms are starting to offset one another so that the sum of the electrical activity is less.

Delta and Theta

Delta and theta rhythms are low-frequency EEG patterns associated with drowsiness and sleep in the normal adult. As a person becomes drowsy, the alpha rhythm is gradually replaced by the lower-frequency theta rhythm. As sleep deepens, the slow-wave delta rhythm becomes dominant. Periodically during slow-wave sleep, the delta rhythm is interrupted by episodes of paradoxical sleep during which the subject appears to be asleep but has an EEG pattern similar to the beta rhythm of an alert individual.

During episodes of paradoxical sleep, twitching of muscles in the face and limbs and rapid eye movements behind closed lids occur. As a result, paradoxical sleep is also to as rapid eye movement (REM) sleep.

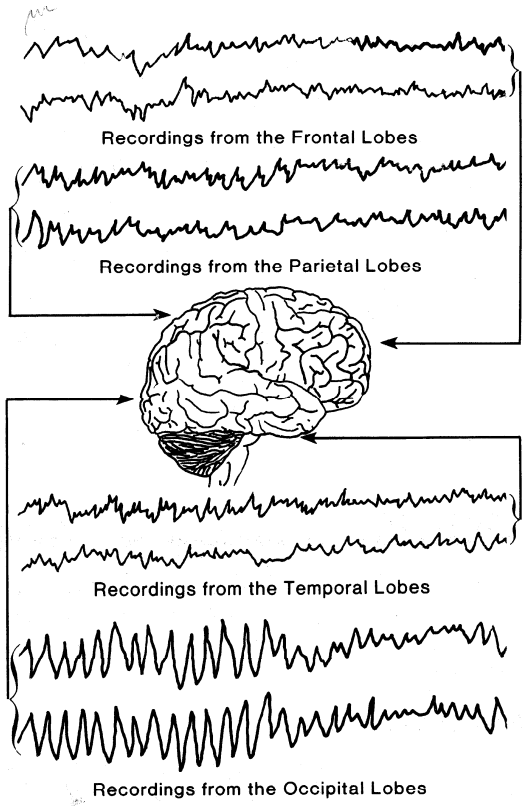


FIGURE B5.5 EEG patterns associated with each lobe of the cerebrum in the awake state

Electrode Positions

Electrode positions have been named according to the brain region below that area of the scalp: frontal, central (sulcus), parietal, temporal, and occipital. There are two basic methods of electrode placement when recording the EEG. In a monopolar recording, an active electrode is placed over the cortical region of interest and a "reference" electrode is attached to the earlobe or a more distant part of the body. In a bipolar recording, the voltage difference between two electrodes placed over the cortical region of interest is measured with respect to a third "reference" electrode. Bipolar recording of the EEG is more localized. Figure B5.6 illustrates standard and commonly employed electrode positions.

Analysis of the EEG involves determination of the dominant frequency or rhythm, measurement of the amplitudes of different frequencies; calculation of the percentage of time that each frequency is present; and consideration of waveform, synchronism, and topographical distribution. Detailed analysis of the EEG is beyond the scope of this experiment. Instead, you will record an EEG using the bipolar method and perform some simple experiments and observations.

Occipital Lobe Alpha Rhythms:

The cerebrum is divided into hemispheres and each hemisphere is divided into frontal, parietal, temporal, and occipital lobes (figure B6.1). Each lobe has functions that are unique, but each lobe also shares functions with other lobes, and indeed, other parts of the brain. For example, as a child, we may see (occipital lobe) a flame and touch (frontal lobe) it to see what it is like, experiencing heat and pain (parietal lobe), and remembering (temporal lobe) not to repeat the experience. These functions and many

others, such as reasoning and abstract thought, occur in the cerebral cortex, the thin covering of gray matter forming the surface of the cerebrum.

Electrical activity in the cerebral cortex is continuous from formation of the cerebrum in utero to death. As demonstrated in the preceding chapter, electrical activity of the cerebral cortex can be detected and recorded using scalp electrodes and an electroencephalograph. The record obtained, called an electroencephalogram (EEG), is complex and variable between subjects, although under certain conditions, the EEG exhibits simpler, rhythmic activity.

Your EEG changes as you grow. The development of EEG is rapid with newborns. As neural development proceeds, the EEG recorded from the posterior regions of the brain of an infant of 3-4 months begins to resemble EEGs recorded from the posterior region of adults. The difference is that the 3-4-month-old infants have EEGs in the frequency range of 3-4 Hz, whereas adults tend to have average frequencies of 10 Hz. By the time the infant is I year old, the posterior region EEG is approximately 6 Hz; by 3 years it is 8 Hz; and by puberty (13-14 years old) the average frequency is similar to adults at 10 Hz.

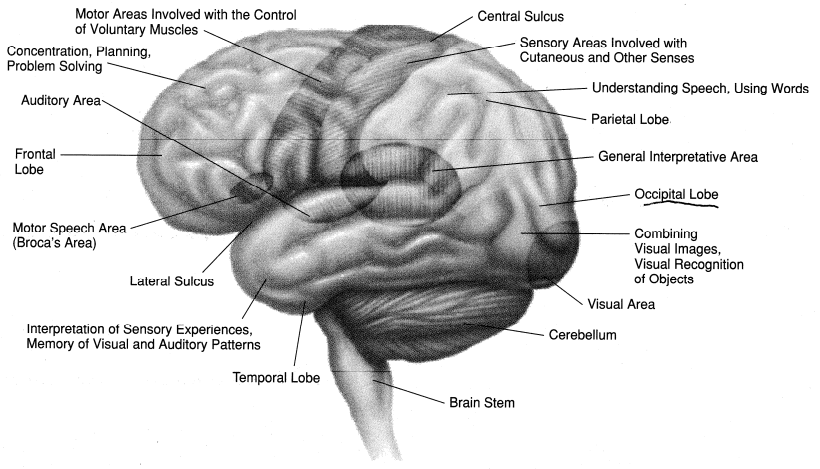


FIGURE B6.1 Some motor, sensory, and association areas of the cerebral cortex

Recall from BIOPAC lab experiment B5 that one of the simpler patterns is the alpha rhythm. The alpha rhythm is characterized by a frequency of 8-13 Hz and amplitudes of 20-200 uV. Each region of the brain has a characteristic frequency of alpha rhythm. Alpha waves of the greatest amplitude tend to be recorded from the occipital and parietal regions of the cerebral cortex.

The EEG is variable, depending on the mental state of an individual, and the frequency and amplitude of
alpha rhythms within an individual change. In general, the alpha rhythm is the prominent EEG wave pattern of a relaxed, inattentive state in an adult with eyes closed; however, specific conditions can influence the alpha rhythm, for example:

1. **Hyperventilation**: Breathing abnormally quickly and deeply causes carbon dioxide levels of the blood and cerebrospinal fluid to fall and pH levels to rise. These effects increase electrical activity of cortical nerve cells, often increasing amplitude of the alpha waves.

2. **Gender**: Females tend to have higher mean frequencies of alpha waves than males, although the differences are small.

3. **Memory:** Frequency may affect the speed of "remembering" and may be approximately 1 Hz higher during memory tests for high-scoring subjects than for subjects who scored lower.

4. **Personality:** Amplitudes tend to be higher in subjects who are extroverted.

5. **Mental stress:** Amplitudes vary with the difficulty of mental tasks performed with the eyes closed.

6. **Visual attention:** Amplitudes of alpha waves diminish when subjects open their eyes and are attentive to external stimuli. Thus, instead of getting the wavelike synchronized pattern of alpha waves, desynchronization occurs.

7. **Time of day:** Amplitudes increase when subjects are less alert and tend to be higher from 1:30-4:30 P.m.

In the following experiments you will record the EEG and alpha rhythm under several conditions. At the same time, the root-mean-squared of the alpha rhythm (alpha-rms) and an "alpha thermometer" will be displayed. Alpha-rms and the "alpha thermometer" are visual indices of the activity levels of the alpha rhythm.

Relaxation and Brain Rhythms Data:

Experimental Objectives

1. To record a frontal lobe EEG from an awake, resting subject with eyes open and eyes closed.
2. To identify and examine alpha, beta, delta, and theta components of the EEG complex.

Questions to think about:

1. List and define two characteristics of regular, periodic waveforms.

2. Compare and contrast synchrony and alpha block.

3. Examine the alpha and beta waveforms for change between the “eyes closed” state and the “eyes open” state.

   a. Does desynchonization of the alpha rhythm occur when the eyes are open?

   b. Does the beta rhythm become more pronounced in the “eyes open” state?

4. The amplitude measurements (stdev) are indicative of how much alpha activity is occurring in the subject. However, the amplitude values for beta do not truly reflect the amount of mental activity occurring with the eyes open. Explain:

5. Examine the delta and theta rhythms. Is there an increase in delta and theta activity when the eyes are open? Explain your observation.

6. Define the following terms:

   a. Alpha rhythm

   b. Beta rhythm

   c. Delta rhythm

   d. Theta rhythm

Materials

BIOPAC electrode lead set (SS2L)
BIOPAC disposable vinyl electrodes (EL503), 3 electrodes per subject
cot or lab table and pillow
BIOPAC electrode gel (GEL1) and abrasive pad (ELPAD)
or skin cleanser or alcohol prep
BIOPAC SS25 hand dynamometer
BIOPAC OUT1 headphones

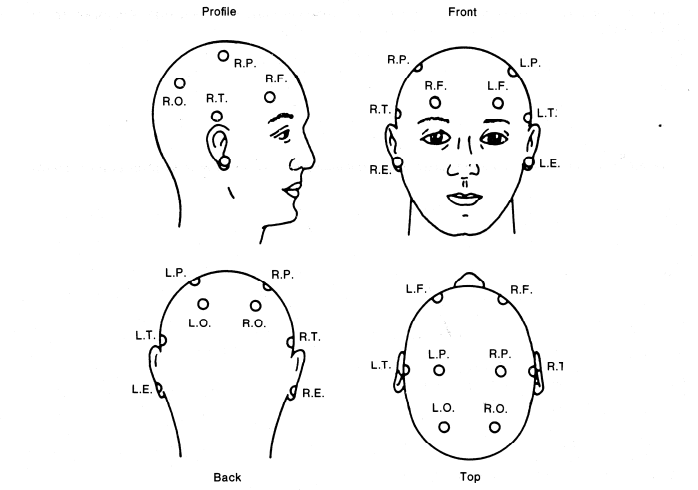


FIGURE B5.6 Standard EEG electrode positions

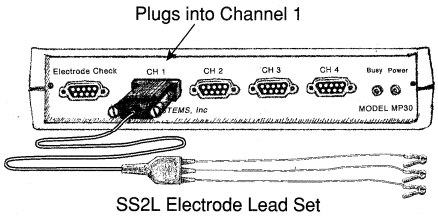
EXPERIMENTAL METHODS

Setup

1. Turn on the computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory

instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC I 00A transformer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.



SS2L Electrode Lead Set

3. Select a subject for electroencephalography. Have the subject assume a relaxing position. A supine position with the head resting comfortably but tilted to one side is recommended. The best recordings occur when the subject is relaxed throughout the session. Read the section below carefully before attaching electrodes to the subject. Electrode adhesion to the scalp is crucial for obtaining a meaningful EEG recording.

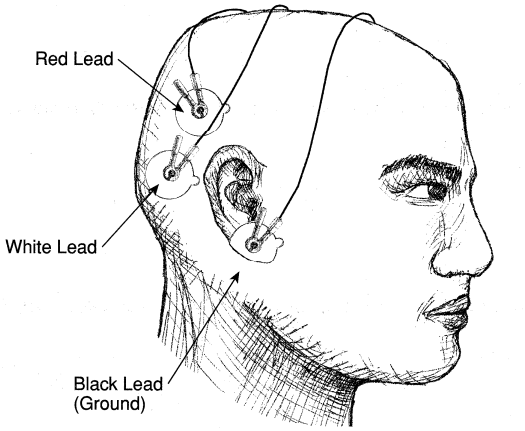


FIGURE B5.7 Suggested electrode placement
Used with permission of BIOPAC

Hints for Obtaining Optimal Data

(a) As much as possible, move the hair away from the electrode adhesion area. Otherwise, the hair will pull the electrodes up, away from the scalp.

(b) Apply pressure to the electrodes for about I minute after the initial placement.

(c) Subject should try to remain still because blinking and other movement will affect the recording of all four rhythms.

(d) Despite your best efforts, electrode adhesion may not be strong enough to record data; if so, try another subject or different electrode placement.

Guidelines for Electrode Placement

(a) The placement of the scalp electrodes can vary (within limits) depending on your instructor's or the subject's preference. A suggested electrode placement is shown in figure B5.7.

(b) Keep the electrodes on one side (right or left) of the head.

(c) The third electrode is the ground electrode and is connected to the earlobe (position "c"). Although the adhesive collar is larger than the earlobe, it can be folded under the ear for proper adhesion. Alternately, the ground electrode can be placed on the facial skin behind the earlobe.

4. Attach the electrodes to the subject, then attach the electrode leads to the electrodes, following the color code in figure B5.7. Plug the electrode lead set (SS2L) into channel 1.
5. Ask the subject to relax completely, with eyes closed, for approximately 5 minutes before recording. Ideally, the room should be reasonably quiet.

6. Locate the BIOPAC Student Lab folder, open it, and start the BIOPAC Student Lab program. A prompt will appear (figure B5.8) asking you to choose a lesson. Choose Lesson 3 ("L03- EEG-1") by clicking on it to highlight it, then clicking on "OK."

7. A prompt (figure B5.9) should appear asking you to "Please type in your file name." By doing so you enable the computer to store all the data files you create in one place, making it easier for you to retrieve data later on.

8. After you log on, a window similar to figure B5.10 will be displayed. Check to make sure the electrodes and electrode leads are secure and the electrode assembly is plugged into channel 1. This concludes the setup procedures.

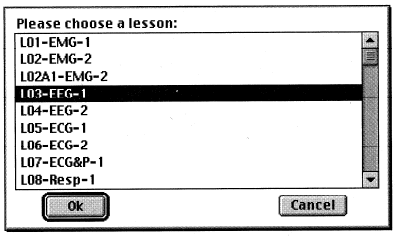


FIGURE **B5.8**

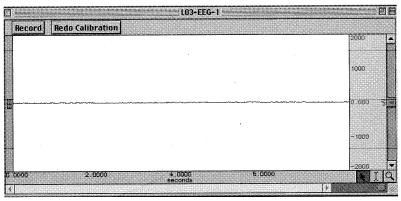


FIGURE **B5.11**

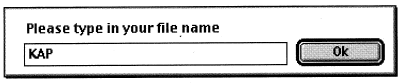


FIGURE **B5.9**

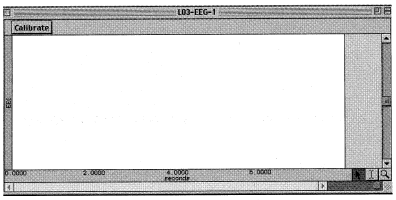


FIGURE **B5.10**

Calibration
The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1. Click on "Calibrate." A warning will pop up requesting that you check the electrode attachments.

2. Click "OK:' This will begin the calibration procedure. The BIOPAC Student Lab will begin recording data and use it to calculate optimal settings for the subject. The calibration procedure will stop automatically after 15 seconds. At the end of the calibration recording your screen should resemble figure B5.11.

3. If the data recording shows any large spikes, then you must do the calibration again. Click on "Redo Calibration" and repeat the entire calibration sequence.

Data Recording
You will record the "raw EEG" while the subject is relaxed with eyes closed, eyes opened, and eyes closed again. The recording should last about 30 seconds. After recording the "raw EEG" signal, you will then extract the four brain rhythms: alpha, beta, delta, and theta.

In order to work efficiently, read this entire section before starting to record. Here are some hints for obtaining optimal data:

1. Good electrode contact is essential to minimize "noise" and increase signal amplitude.
2. The subject should lie still and especially try to keep facial muscles still.
3. During the "eyes open" segment, subject should not blink.
4. The subject should try relaxation techniques, such as concentrating on breathing slowly or relaxing muscles.

Decide who, among those students in your lab group, will be director and who will be recorder. The director will give verbal commands to the subject, and the recorder will insert markers and marker labels at appropriate times during data recording.

To insert markers, use the Esc key (Mac) or F9 key (PC). Type in marker text (labels) after data recording has stopped.

1. Click on "Record" and follow the procedures below:
   (a) Time 0-10 seconds: Subject relaxes, eyes closed.

(b) Time 10-20 seconds: Subject relaxes, eyes open. The subject should not blink during this recording interval. Insert marker and marker label "eyes 'Open" at the beginning of this recording interval (10-second mark).

(c) Time 20-30 seconds: Subject relaxes, eyes closed. Insert marker and marker label "eyes closed" at the beginning of this record interval (20-second mark).

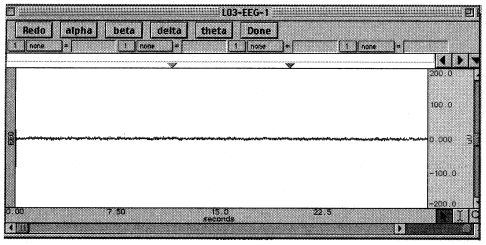


FIGURE **B5.12**

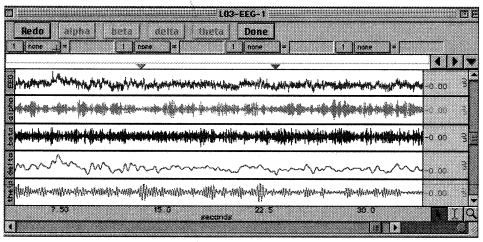


FIGURE **B5.13**

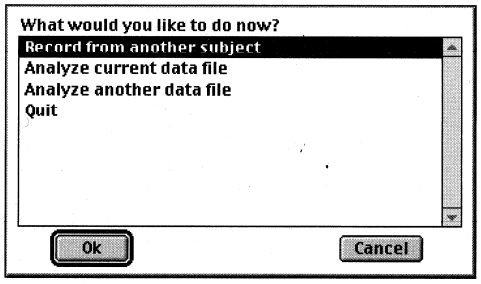


FIGURE **B5.14**

2. At the conclusion of the 30-second recording segment, your data should resemble figure B5.12. If you feel you made a mistake in the recording, or there are large spikes in your data (indicating the subject may have blinked or moved), you should redo the recording. You can redo the recording by clicking on "Redo" and repeating step 1. Note that once you click on "Redo," the data you have just recorded will be erased.

3. If your data look OK, click on the frequency buttons in the following sequence: alpha, beta, delta, theta. When you click each button, the program will compute and display the specific frequency bands listed in table 3.

4. Look at the alpha frequency band. Your data should look similar to figure B5.13, which shows a decrease in amplitude during the "eyes open" segment. If your data recording does not show any change, it is possible that the electrodes were not properly attached to the skin or the recording procedure was not properly followed. If this is the case, redo the recording by clicking on "Redo" and repeating steps 1, 2, and 3.

5 If your data look similar to Figure B5.13, click on "Done." After you click on "Done," a pop-up window (figure B5.14) with four options will appear. Make your choice and continue as directed. If choosing the "Record from another subject" option:

(a) Attach electrodes per the setup procedures given previously and continue the entire lesson from the
Calibration section through the Data Recording section.

(b) Note that each person will need to use a unique file name.

6. Remove the electrode cable pinch connectors, and peel off the electrodes. Throw out the electrodes (BIOPAC electrodes are not reusable). Wash the electrode gel residue from the skin, using soap and water. The electrodes may leave a slight ring on the skin for a few hours, which is quite normal.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file, and open it. The data window should be similar to figure B5.13.

2. Note the following channel number (CH) designations:

CH 1 = raw EEG
CH 2 = alpha rhythm
CH 3 = beta rhythm
CH 4 = delta rhythm
CH 5 = theta rhythm

3. Set up the measurement boxes as follows:

CH 2 = stddev
CH 3 = stddev
CH 4 = stddev
CH 5 = stddev

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. Here are some brief descriptions of the measurement boxes used in this experiment:

stddev: Standard deviation is a measure of the variability of data points. The data represent amplitudes of the brain rhythms. The advantage of the "stddev" measurement is that extreme values or artifacts do not unduly influence the measurement.
Freq: The frequency measurement converts the time segment of the selected area to frequency in cycles/sec.
None: No measurement tool has been selected.

The **"selected area"** is the area selected by the I-beam tool (including the endpoints).

4. Use the I-beam tool to select the area from time 0 to the first marker (figure B5.15). Record the standard deviation data in table B5.2 of the report.

5. Repeat step 4 for the second and third recording segments.

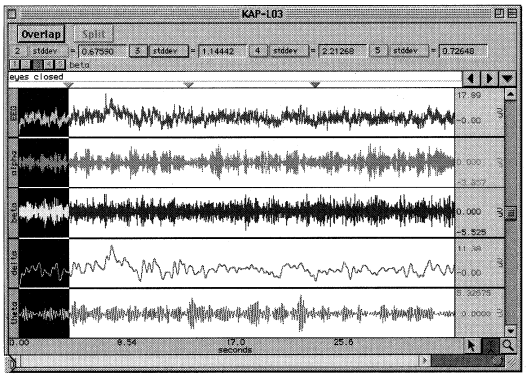


FIGURE **B5.15**

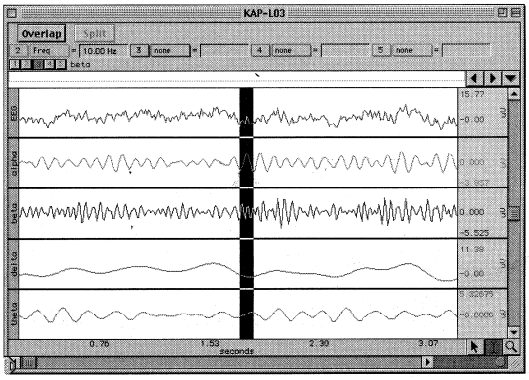


FIGURE **B5.16**

6. Set up the measurement boxes as follows:

CH 2 = Freq
CH 3 = none
CH 4 = none
CH 5 = none

7. Use the zoom tool to zoom in on a 3-4 second section of data from time 0 to the first marker.

8. Use the I-beam tool to select an area that represents one cycle in the alpha wave (figure B5.16). Record the frequency measurement in table B5.3 of the report.

9. Repeat step 8 for two other alpha wave cycles.

10. Repeat steps 8 and 9 for one cycle of the beta wave.

11. Repeat steps 8 and 9 for one cycle of the delta wave.

12. Repeat steps 8 and 9 for one cycle of the theta wave.

13. You may save the data to a diskette, save notes that are in the journal, or print the data file.

14. Exit the program. Turn off the MP30 and shut down the computer.

Relaxation and Brain Rhythms Data:

Experimenter Name:

Date:

1. Data and calculations:

Subject **Age:** **Height:** **Weight:** **Gender:**

(a) EEG Amplitude Measurements: Complete table 2 with standard deviation measurements:

Table 1

Rhythm	Channel	Eyes Closed	Open	Closed	Open	Closed	Open
Alpha	Ch2						
Beta	Ch3						
Delta	Ch4						
Theta	Ch5						

Notes:

(b) EEG frequency measurements: Complete table 3 with the frequencies for each rhythm and calculate the mean frequency.

Table 2a: Eyes Open

Rhythm	Cycle 1	Cycle 2	Cycle 3	Mean
Alpha				
Beta				
Delta				
Theta				

Notes:

Table 2b: Eyes Closed

Rhythm	Cycle 1	Cycle 2	Cycle 3	Mean
Alpha				
Beta				
Delta				
Theta				

Notes:

Data Analysis:

Can you answer these questions:

1. List and define two characteristics of regular, periodic waveforms.

2. Compare and contrast synchrony and alpha block.

3. Examine the alpha and beta waveforms for change between the “eyes closed” state and the “eyes open” state.

   a. Does desynchonization of the alpha rhythm occur when the eyes are open?

   b. Does the beta rhythm become more pronounced in the “eyes open” state?

4. The amplitude measurements (stdev) are indicative of how much alpha activity is occurring in the subject. However, the amplitude values for beta do not truly reflect the amount of mental activity occurring with the eyes open. Explain:

5. Examine the delta and theta rhythms. Is there an increase in delta and theta activity when the eyes are open? Explain your observation.

6. Define the following terms:

   c. Alpha rhythm

   d. Beta rhythm

   e. Delta rhythm

   f. Theta rhythm

Electroencephalography II: Occipital Lobe Alpha Rhythms

Experimental Objectives

1. To record an EEG from an awake, resting subject under the following conditions: relaxed with eyes closed; performing mental arithmetic with eyes closed; hyperventilating (breathing quickly and deeply) with eyes closed; relaxed with eyes open.
2. To examine differences in the level of alpha rhythm activity during mental arithmetic and hyperventilation, compared to the control condition of eyes closed and relaxed.

Questions to think about:

1. When was the general amplitude of the EEG highest?

2. When were the alpha wave levels highest?

3. How do you think your results will compare with the information presented in the introduction to this lab experiment?

4. Did you think the subject will need to concentrate during the math problem? Yes _____ No

5. Which conditions will produced the lowest alpha activity?

Materials

BIOPAC electrode lead set (SS2L)
BIOPAC disposable vinyl electrodes (EL503), 3 electrodes per subject
cot or lab table and pillow
BIOPAC electrode gel (GEL I) and abrasive pad (ELPAD) or skin cleanser or alcohol prep
BIOPAC SS25 hand dynamometer.
BIOPAC OUT1 headphones

EXPERIMENTAL METHODS

Setup

1. Turn on the computer. The desktop should appear on the monitor. If it does not appear, ask the laboratory instructor for assistance.

2. Turn on the MP30 data acquisition unit. The power switch is on the rear panel. An LED on the front panel indicates power on. If the LED does not light up when the power switch is turned on, check to make sure the AC100A transformer (which supplies power to the MP30) is plugged into an electrical outlet on the laboratory bench.

3. Select a subject for electroencephalography. Have the subject assume a relaxing position. A supine position with the head resting comfortably but tilted to one side is recommended. The best recordings occur when the subject is relaxed throughout the session. Read the section below carefully before attaching electrodes to the subject. Electrode adhesion to the scalp is crucial for obtaining a meaningful EEG recording

Hints for Obtaining Optimal Data

(a) As much as possible, move the hair away from the electrode adhesion area. Otherwise, the hair will pull the electrodes up, away from the scalp.

(b) Apply pressure to the electrodes for about 1 minute after the initial placement.

(c) Subject should try to remain still because blinking and other movement will affect the recording of all four rhythms.

(d) Despite your best efforts, electrode adhesion may not be strong enough to record data; if so, try another subject or different electrode placement.

Guidelines for Electrode Placement

(a) The placement of the scalp electrodes can vary (within limits) depending on your instructor's or the subject's preference. A suggested electrode placement is shown in figure B6.2.
(b) Keep the electrodes on one side (right or left) of the head.
(c) The third electrode is the ground electrode and is connected to the earlobe (position "c"). Although the adhesive collar is larger than the earlobe, it can be folded under the ear for proper adhesion. Alternately, the ground electrode can be placed on the facial skin behind the earlobe.

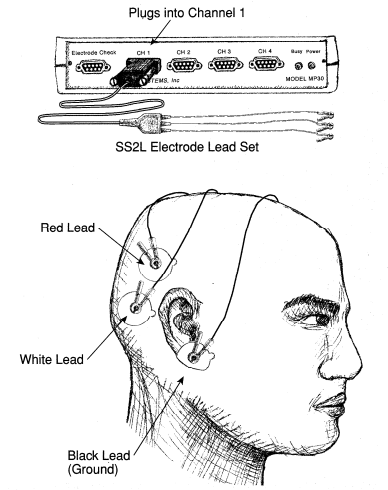


FIGURE **B6.2** Suggested electrode placement
Used with permission of BIOPAC

4. Attach the electrodes to the subject, then attach the electrode leads to the electrodes, following the color code in figure B6.2. Plug the electrode lead set (SS2L) into channel 1.

5. Ask the subject to relax completely, with eyes closed, for approximately 5 minutes before recording. Ideally, the room should be reasonably quiet.

6. Locate the BIOPAC Student Lab folder, open it, and start the BIOPAC Student Lab program. A prompt will appear (figure B6.3) asking you to choose a lesson. Choose Lesson 4 ("L04-EEG-2") by clicking on it to highlight it, then clicking on "OK:'

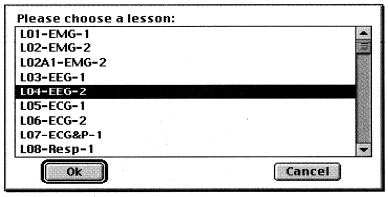


FIGURE **B6.3**

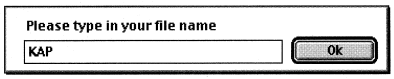


FIGURE **B6.4**

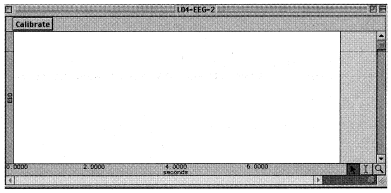


FIGURE **B6.5**

7. A prompt (figure B6.4) should appear asking you to "Please type in your file name," By doing so you enable the computer to store all the data files you create in one place, making it easier for you to retrieve data later on.

8. After you log on, a window similar to figure B6.5 will be displayed. Check to make sure the electrodes and electrode leads are secure and the electrode assembly is plugged into channel 1. This concludes the setup procedures.

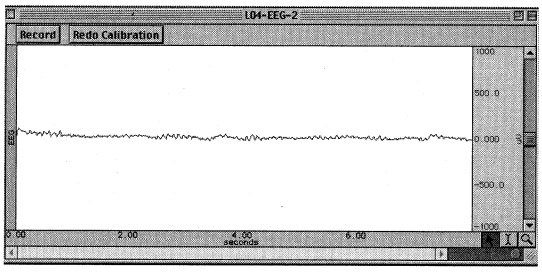


FIGURE **B6.6**

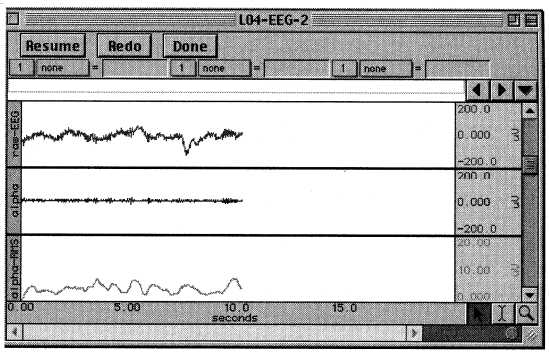


FIGURE **B6.7**

Calibration

The calibration procedure establishes the hardware's internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the calibration procedure.

1. Click on "Calibrate." A warning will pop up requesting that you check the electrode attachments.

2. Click "OK." This will begin the calibration procedure. The BIOPAC Student Lab will begin recording data and use the data to calculate optimal settings for the subject. The calibration procedure will stop automatically after 15 seconds. At the end of the calibration recording your screen should resemble figure B6.6.

3. If the data recording shows any large spikes, then you must redo the calibration. Click on "Redo Calibration" and repeat the entire calibration sequence.

Data Recording

Prepare for the recording and have the subject lie down and relax with eyes closed. Decide who, among those students in your lab group, will be director, and who will be recorder The director will give verbal commands to the subject; the recorder will insert markers and marker labels at appropriate times in the data record.

To insert markers, use the Esc key (Mac) or F9 key (PC). Type in marker text after data recording has stopped.

You will record the subject in four conditions:

Segment 1: Relaxed with eyes closed. 10
Segment 2: Performing mental math with eyes closed.
Segment 3: Recovering from hyperventilation with eyes closed.
Segment 4: Relaxed with eyes open.

The subject will perform tasks in the intervals between Segment 1 recordings.

In order to work efficiently, read this entire section so you will know what to do for each recording segment.

The subject should remain in a supine position and continue to relax while you review the lesson.

Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you do not use an excessive amount of time (time is memory).

Hints for Obtaining Optimal Data

1. It is important that you pay attention to the specific instructions for each recording segment.
2. Good electrode contact is essential to minimize noise and increase signal amplitude.
3. The subject should lie still and should not blink during the "eyes open" segment. Best results are obtained if the eyes remain still at all times.
4. The subject should not talk during any of the recording segments and should not verbalize answers to the mental arithmetic.
5. The alpha signal will be increased during the relaxation segment if the subject concentrates on breathing slowly and/or relaxing muscles.
6. For the mental math segment the director should prepare by coming up with a math problem before recording begins. The math problem should be challenging but not too difficult (e.g.: take the number 2 and double it, double again, double again, divide by 3, multiply by 15, divide by 7, multiply by 12). The point is to make the subject really work to get the answer, not to stump the subject or cause the subject to give up. The math problem should require a minimum of 20 seconds.
7. Before the recovery from hyperventilation segment the subject should breathe quickly and deeply for 2 minutes, as if he or she had just finished exercising and needed air. The subject should not be hyperventilating during-the actual recording.

Segment 1

1. Click on "Record" and have the subject continue to relax with eyes closed. Record for 10 seconds, then click on "Suspend:' Note that during data recording the graph window will resize and the "Input values" window will appear on the right side of the graph window. The "Input values" window displays the alpha-rms value in a thermometer-like bar display and can be used as a visual aid to determine fluctuations in alpha-rms activity. The bar is displayed only when data are being recorded and does not show in the Review Saved Data mode.

2. Review the data recording on the screen. It should look similar to figure B6.7. The data would be incorrect if

(a) The Suspend button was pressed prematurely.
(b) An electrode peeled up, causing a large baseline drift, spike, or loss of signal.
(c) The subject did not follow the proper procedure.
(d) The subject had too much EMG artifact.
If your data recording is incorrect, redo the recording by clicking on "Redo" and repeating segment 1. Note that once you press "Redo" the data you have just recorded will be erased.

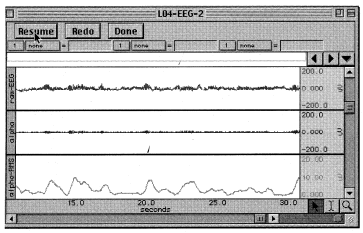


FIGURE **B6.8**

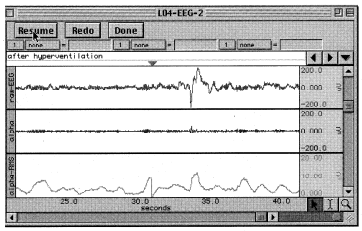


FIGURE **B6.9**

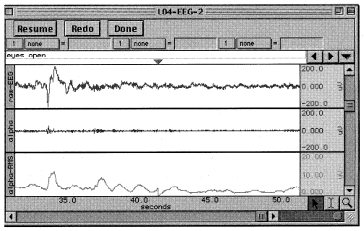


FIGURE **B6.10**

Segment 2

1. The director should verbally give the subject a set of mental math problems while the subject remains relaxed with eyes closed. As soon as the problems have been given, the recorder clicks on "Resume" and the subject silently begins to solve the problems.

2. Record for 20 seconds (seconds 11-30), then click on "Suspend." Review the data on the screen. It should resemble figure B6.8. If the data is incorrect, click on "Redo" and repeat the previous step. Note that once you press "Redo," the data you have just recorded will be erased.

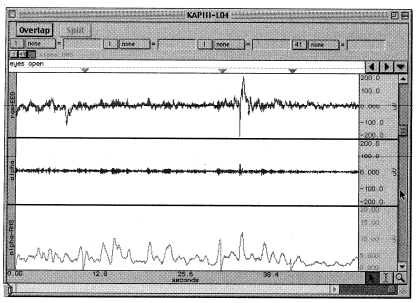


FIGURE **B6.11**

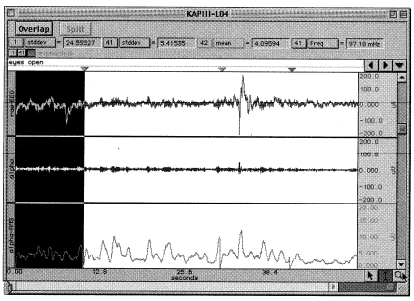


FIGURE **B6.12**

Segment 3
1. The director advises the subject to hyperventilate for 2 minutes while the subject remains in the supine position with eyes closed.

WARNING: Hyperventilation can make the subject dizzy and light-headed. Stop the procedure if the subject starts to feel sick or dizzy.

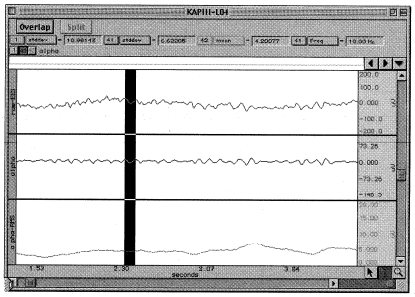


FIGURE **B6.13**

2. At the end of the 2-minute hyperventilation period (or as soon as stopped for sickness or dizziness click on "Resume:' It is important that you resume recording as quickly as possible after the subject has hyperventilated; however, it is also important that you do not click "Resume" while the subject is hyperventilating or you will capture EMG artifact.

3. Record for 10 seconds (seconds 31-40) while the subject is recovering from hyperventilation. The subject should be in a relaxed state with eyes closed. At the end of the 10-second recording segment, click on "Suspend,"

4. If all went well, your data should resemble figure B6.9. If it does not, click "Redo" and repeat segment 3.

Segment 4
1. The director should advise the student to open his or her eyes and remain relaxed and lying down.

2. Click on "Resume" and record for 10 seconds (seconds 41-50), then click on "Suspend:'

3. Review the data recording to make sure it is very similar to figure B6. 10. If the data recording is not correct, click on "Redo" and repeat segment 4. If the data recording is OK, click on "Done:'
A pop-up window with four options will appear.

Make your choice and continue as directed. If choosing the "Record from another subject" option:

(a) Attach electrodes per the setup instructions and repeat the entire lesson.
(b) Note that each person will need to use a unique file name.

4. Remove the electrode cable pinch connectors, and peel off the electrodes. Throw out the electrodes (BIOPAC electrodes are not reusable). Wash the electrode gel residue from the skin, using soap and water. The electrodes may leave a slight ring on the skin for a few hours, which is quite normal.

Data Analysis

1. Enter the "Review Saved Data" mode from the Lessons menu, choose the correct file, and open it. The data window should be similar to figure B6.11.

2. Note the following channel number (CH) designations:
CH 1 = raw EEG
CH 41 = alpha rhythm
CH 42 = alpha-rms

3. Set up the measurement boxes as follows:
CH 1 = stddev
CH 41 = stddev
CH 42 = mean
CH 41 = Freq

Recall that the measurement boxes are above the marker region in the data window and each measurement has three sections: channel number, measurement type, and result. The first two sections are pull-down menus that are activated when you click on them. Here are some brief descriptions of the measurement boxes used for this experiment:

stddev: Standard deviation will be higher if there is a lot of activity and lower if there is less. The advantage of the "stddev" measurement is that extreme values or artifacts do not unduly influence the measurement.

mean: This box displays the average value in the selected area.

Freq: This measurement converts the time segment of the selected area to frequency in cycles per second.

NOTE: The "Freq" measurement applies to all channels since it is calculated from the horizontal time scale.

The "selected area" is the area selected by the I-beam tool (including the endpoints).

4. Use the I-beam tool to select the first data segment (figure B6.12) from time 0 to the first marker. Record measurement box values in table B6.1 and table B6.2 in the report.

5. Repeat step 4 for data segments 2, 3, and 4.

6. Use the zoom tool to enlarge a small section of the first data segment so that you can easily measure the frequency of the alpha waves (CH 41). Use the I-beam tool to select an area from one peak to the next in CH 41 (figure B6.13). Record the measurement box value for frequency of the alpha rhythm in the report.

8. You may save the data to a diskette, save notes that are in the journal, or print the data file.

9. Exit the program. Turn off the MP30 and shut down the computer.

Occipital Alpha Rhythms Data:

Experimenter Name: _____

Lab Section: _____

Date:

Data and calculations:
Subject_____Age:_____Height:_____Weight:_____Gender:_____

Table 1: Complete table 1 with amplitudes of the recorded data in the control and experimental conditions.

Segment	Condition	Raw EEG [CH1-stdev]	Alpha [CH41-stdev]	Alpha-rms [CH42-mean]
1	**Eyes closed (control)**			
2	**Eyes closed (mental math)**			
3	**Eyes closed (hyperventilation)**			
4	**Eyes Open**			

1. What is the frequency of the alpha rhythm from segment 1 data? _____Hz.

2. Complete table 2 with the mean values of the alpha-rms channel from table 1. The "control" is the mean alpha-rms from the data in segment 1. You will need to calculate the difference between the experimental mean and the control mean. Summarize whether the experimental mean was larger (+), smaller (-), or the same (=) as the control mean.

Table 2:

Seg.	Experimental Condition	Experimental Mean	Control Mean	Difference (experimental – control)	Summary (+, -, =)
2	**Mental math**				
3	**Hyperventilation**				
4	**Eyes Open**				

3. Refer to table 1. When was the general amplitude of the EEG highest?_____
_____
_____

4. Refer to table 2. When were the alpha wave levels highest?_____
_____
_____

5. Refer to table 2. How do your results compare with the information presented in the introduction to this lab experiment?_____
_____

6. Did the subject need to concentrate during the math problem? Yes _____ No_____, Why?

7. Which conditions produced the lowest alpha activity?_____

Data Analysis:

APPENDIX:

Laboratory Safety Guidelines

The following is a brief list of safety guidelines for you to follow in the anatomy and physiology lab. More complete descriptions of safety procedures are found throughout the manual.

1. Read all of the lab material prior to coming to class. This is a safety issue. Failure to read or understand the lab can result in hazards. Unauthorized experiments are not allowed in the lab.

2. Locate the eyewash station, shower station, fire blanket, fire extinguisher, and other safety areas in the lab prior to beginning the first lab. Be familiar with how to use the equipment in the event of an emergency.

3. Clean up spills. Inform your instructor of any spill in the lab. Be careful if the material is toxic or caustic. If you are not sure if the material I's hazardous, ask your instructor for the proper procedure for the cleanup.

4. Assume all bodily fluids in the lab are infectious. Follow precautions when handling bodily fluids, such as wearing latex gloves, lab coats, and protective eyewear. Never use any instrument twice that comes into contact with bodily fluid! Once the instrument is used, dispose of it in either a biohazard bag or in a container of 10% -bleach. Clean all lab surfaces with a bleach solution at the end of a lab involving bodily fluids, even if you think no fluid has come 'in contact with the table surface.

5. Keep the lab clean and free of clutter. Place all backpacks, purses, and umbrellas in safe areas and not on the lab tables.

6. Do not eat or smoke in the lab. Many reagents in the lab are toxic, so do not drink them. Wash your hands after the lab and again prior to eating. Never pipette anything, by mouth. Use a pipette bulb or pipette pump.

7. Keep your hair secured so it does not catch fire or dip into the beakers of solutions. Never heat volatile material over an open flame. An explosion might occur.

8. Do not wear contact lenses in the lab.

9. Do not throw sharp material such as glass or cutting blades in the normal trash containers in the lab. They are to be disposed of in an appropriate container such as a "sharps" container.

10. Never point a test tube that is heating over a Bunsen burner in the direction of someone else. Never walk away from anything that is being heated. **Pay attention to material** on hot plates and remove material with appropriate mitts or tongs. Heat material only in appropriate heat-resistant containers.

11. Dissect with the blade cutting away from you and your lab partners. If you do cut yourself, make sure you wash the wound well with soap and water and notify your instructor.

12. If you have an allergic reaction to the preserving fluid (usually restricted breathing, a flushed feeling, or a skin rash), notify your instructor immediately. Notify your instructor if you are pregnant or have any medical condition.

Anatomy and Physiology Safety Guidelines

1 . Upon entering the laboratory, locate exits. fire extinguisher, fire blanket, chemical shower, eyewash station, first aid kit, broken glass containers, and cleanup materials for spills.

2. Do not eat, drink, smoke, store food, or apply cosmetics in the laboratory. Restrain long hair, loose clothing, and dangling jewelry.

3. Students who are pregnant, taking immunosuppressive drugs, or who have any other medical conditions (e.g., diabetes, immunological defect) that might necessitate special precautions in the laboratory must inform the instructor immediately.

4. Wearing soft contact lenses in the laboratory is inadvisable because of possible lens absorption of volatile chemicals. If possible, regular eyeglasses should be worn instead.

5. Use safety glasses in all experiments in which solutions or chemicals are heated over a Bunsen burner. Never leave heat sources unattended.

6. Decontaminate work surfaces at the beginning and end of every lab period, using a commercially prepared disinfectant or 10% bleach solution. After labs involving dissection of preserved material, use hot soapy water or disinfectant.

7. Keep all liquids away from the edge of the lab bench to avoid spills. Clean up spills of viable materials using disinfectant or 10% bleach solution.

8. Properly label glassware and slides.

9. Use mechanical pipetting devices; mouth pipetting is prohibited.

10. Wear disposable gloves when handling blood and other body fluids, mucous membranes, and nonintact skin, and/or when touching items or surfaces soiled with blood or other body fluids. Change gloves between procedures. Wash hands immediately after removing gloves. (Note: cover open cuts or scrapes with a sterile bandage before donning gloves.)

11. Place glassware and plasticware contaminated by blood and other body fluids in a disposable autoclave bag for decontamination by autoclaving, or place them directly into a 10% bleach solution before reuse or disposal. Place disposable materials such as gloves, mouthpieces, swabs, and toothpicks that have come into contact with body fluids into a disposable autoclave bag.

12. To help prevent contamination by needle stick injuries, use only disposable needles and lancets. Do not bend the needles and lancets; replace them in sheaths, or remove them from syringes following use. Needles and lancets should be placed promptly in a freshly prepared 10% bleach solution or placed in a puncture-resistant container and decontaminated, preferably by autoclaving.

13. Report all spills or accidents, no matter how minor, to the instructor.

14. Never work alone in the laboratory.

15. Wash hands and remove protective clothing before **leaving the laboratory.**

*Adapted from:
Biosafety in Microbiological and Biomedical Laboratories. 1988. *U.S.* Government Printing Office, Washington, D.C. *20402.*

Centers for Disease Control. *1989.* "Guidelines for Prevention of Transmission of Human Immunodeficiency Virus and the Hepatitis B Virus to Health-Care and Public-Safety Workers." *MMWR: 38 (S6).- . 1987.* "Recommendations for Prevention of HIV Transmission in Health-Care Settings." *MMWR: 36 (2s).*

Johnson, Ted, and Christine Case. *1992. Laboratory Experiments in Microbiology, Brief Version, Third Edition.* Redwood City, CA: Benjamin/Cummings Publishing Co.

School Science Laboratories: A Guide to Some Hazardous Substances. 1984. U.S. Consumer Product Safety Commission. Washington, *D.C. 20207.*

Are we really made of proteins?
Determining an unknown Protein Concentration

Objective:

- To make a quantitative determination of the amount of protein obtained from a swab of cheek cells.

Experimental physiology is based on the need to measure physiological variables in a quantitative manner. In order to compare the effects of various manipulations on concrete endpoints it is crucial that our observations are quantitative or measurable in numbers. The present challenge is to obtain a quantifiable number for the amount of protein obtained from a cheek swab. To do this we will employ a chemical reaction of a dye that changes color in the presence of proteins.

Materials needed

Each lab group should have:

- A test tube rack with ~ 15 (13x100mm) test tubes
- A small vial of protein assay reagent (~ 10mg/ml of protein)
- A test tube with 2ml protein standard
- A small beaker of deionized water
- Cotton swab
    - Acess to
        - Pipets (1ml and .1ml)
        - A mixer (vortex)
        - A spectrophotometer

Protocol:

You will first have to establish a standard curve.

1. Set up five test tubes containing 1ml of pure deionized water in each. Label these tubes # 2-6 *(Deionized water is just really clean water which has had all the salts removed, it's piped into the far right sink in the lab)*
2. Label your protein standard tube #1. Pipet 1ml of protein standard from tube #1 into tube #2. Once you have transferred the liquid pipet the liquid in tube #2 up and down slowly five or six times to thoroughly mix the protein and water. *(this will dilute the protein content in tube #2 resulting in a protein concentration in tube #2 that is ½ of that in tube #1)*
3. Repeat step 2 above taking 1ml from tube #2 an adding and mixing it with the water in tube #3. Repeat this dilution for tubes 4-6 so what you have is six tubes each one containing one half of the concentration of protein that was in the tube preceding it. *(you will use these protein dilutions to establish your standard curve for the assay)* Calculate the protein concentration in each tube. You will then assay a sample from each of these 6 tubes and record the absorbance at 595 nm. This will allow you to create a graph of protein concentration vs. absorbance for known protein values. You will then use this graph to tell you how much protein is in your unknown cheek swipe sample.

Obtaining a protein Sample from a cheek wipe.

1. Fill a test tube with 1ml of deionized water.
2. With a cotton swab and wipe the inside surface of your subjects cheek lightly.
3. Dip the swab into the test tube and swirl it gently but thoroughly to transfer anything it picked up from your subjects mouth into the water. Label this tube as your unknown and assay it..

Assaying protein content.

1. Pipet 50 µl (*that's .05 ml, so use the more accurate yellow tipped pipeters*). of each standard and sample solution into their own clean, dry test tube. Protein solutions are normally assayed in duplicate or triplicate but due to limited supplies try it first with only one assay tube per sample and see how it turns out.
2. Add 2.5 ml of diluted dye reagent to each tube and mix using the vortex mixers on the back bench.
5. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; so samples should incubate at room temperature at least 5 min but no more than 1 hour.
6. Measure absorbance on the spectrophotometer at 595 nm.

Using the Spectrophotometer

The spectrophotometer sends light of a particular wavelength through a cuvet or test tube and measures how much of it comes through the other side. If what is in the test tube absorbs light of the wavelength the machine is set to less light will make it to the reading sensor and the absorbance will read lower. Absorbance, therefore give us an indirect way to measure how much blue dye has formed with protein in the test tubes. In order to get the most accurate reading we should calibrate the machine for our test tubes before taking readings from it. Plug the spectrophotometer in and allow it to warm up until the red light comes on. With the cuvet holder empty adjust the zero dial so that absorbance is set to "0". T'hen insert a test tube containing at least 2ml of pure water into the reader. With this tube in place adjust the "slit" (*right hand)* knob so that the absorbance is reading at "100%". Remove the water containing tube and you are ready to read your samples.

Background
The Bio-Rad Protein Assay, based on the method of Bradford, is a simple and accurate procedure for determining concentration of solubilized protein. It involves the addition of an acidic dye to protein solution, and subsequent measurement at 595 nm with a spectrophotometer or microplate reader. Comparison to a standard curve provides a relative measurement of protein concentration.

The Bio-Rad Protein Assay is a dye-binding assay in which a differential color change of a dye occurs in response to various concentrations of protein.[1] The absorbance maximum for an acidic solution of
Coomassie® Brilliant Blue G-250 dye shifts from 465 nm to 595 nm when binding to protein occurs.[2,3,4] The Coomassie blue dye binds to primarily basic and aromatic amino acid residues, especially arginine.[5] Spector[6] found that the extinction coefficient of a dye-albumin complex solution was constant over a 10-fold concentration range. Thus, Beer's law may be applied for accurate quantitation of protein by selecting an appropriate ratio of dye volume to sample concentration. Interferences may be caused by chemical-protein and/or chemical-dye interactions.

References

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*Coomassie is a trademark of ICI.