

Experiments in Human Anatomy and Physiology



Department of Biology

Eight Edition

WCU
WEST CHESTER
UNIVERSITY

Forward

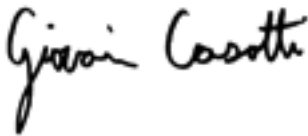
Welcome to Human Anatomy and Physiology II (Bio 269). This laboratory manual is designed to act as a guide through experiments in human physiology. Laboratory in Human Anatomy and Physiology II has three aims:

1. To learn physiological concepts,
2. To develop an understanding of the scientific approach (i.e., how scientists approach a problem and attempt to answer their questions using the scientific method), and
3. To engage in creative and critical thinking.

Some laboratory exercises in this manual are more structured (i.e., give more direction to you) than others. This has been done on purpose so that once you have an idea of how to perform the experiment, you will be asked (challenged) to come up with some novel experiments to test on your own (with the instructors guidance of course). In this manner you will become more involved in the decision making process, and hopefully will become creative and critical in your thinking towards physiology.

I hope that you find this laboratory course both challenging and interesting. Above all else have fun. I created this lab manual and as such any errors that you may find are mine alone. If you do find an error, or if in your opinion something is unclear, please do not hesitate to point it out to me, so that I may correct it for future students.

Again, welcome to the course and good luck.

A handwritten signature in black ink that reads "Giovanni Casotti". The signature is written in a cursive, flowing style.

Dr. Giovanni Casotti

Table of Contents

Table of Contents	3
Timetable and Assessment	4
Introduction to inquiry-based labs and oral presentations	5
Introduction to LabChart.....	9
Graphing Assignment	24
Psychophysiology I.....	26
Psychophysiology II: Independent investigation.....	37
Hematology	38
Electrocardiography I and Heart Sounds.....	48
Electrocardiography II	64
Respiratory Physiology I.....	65
Respiratory Physiology II	80
Renal Physiology.....	101
Digestive Physiology.....	111

All experiments using PowerLab® are Copyright ADInstruments 2016.

Reprinted and modified with permission.

Timetable and Assessment

Week **	Topic	Assessment
1	Introduction to inquiry-based learning	
2	Psychophysiology I	
3	Psychophysiology II	SmartBoard presentation
4	Hematology	
5	Electrocardiography I	
6	Electrocardiography II	SmartBoard presentation
7	Lab exam 1 **	
8	Respiratory Physiology	
9	Respiratory physiology I	
10	Renal Physiology	
11	Digestive Physiology (spring only)	
12	Lab exam 2 **	

**** For an accurate display of lab dates and exam dates please consult the Human Anatomy and Physiology II web site.**

Laboratory assessment will be as follows:

1. Introductory exercise		Total
2. Lab quizzes	(5 pts each)	10
3. Lab exams	(2 @ 60 pts)	30
4. Oral presentations	(2 @ 20 pts)	120
		40
		200

Introduction to inquiry-based labs and oral presentations

In lab, you will have several opportunities to design and conduct independent investigations with your lab group. Each group project lasts for two weeks and is worth 20 points (40 points for 2 projects). In the first week, you will gain experience in using the equipment, test specific variables, decide what question you will ask, what methods you will use, and possibly gather some preliminary data. In the second week, you need to come to lab prepared with some background material that relates to your question, finish collecting data, organize those data into graphs or tables, make conclusions, and prepare and make your presentation. You will have about 1 hour to complete your experiment and then your group will have 5 min to present the results to the class. We feel that it is important for you to report the results of your work orally because you will 1) improve your communication skills, 2) learn from the other groups, and 3) experience the ways in which scientists explain, and sometimes defend, their work.

Because inquiry and problem-solving labs run more than one week, you will need to communicate with each other between lab sessions. In the space below, **exchange full names, telephone numbers, and e-mail addresses with your group members.**

Oral Presentation Rubric (20 points total):

Team Members: _____

Project title: _____

Date: _____

1) Background material (2 points)

- Does the background material (at least 2 prior experiments) relate to the topic of the investigation?
- Highly relevant 2 pts Relevant 1.5 pts Somewhat relevant 1 pt Moderately relevant 0.5 pts Not relevant 0 pts
- How could this be improved upon?
- _____
- _____

2) Variable tested (1 point)

- Were the dependent and independent variables tested, clearly articulated to the audience?
- Clearly stated 1 pt Vaguely stated 0.5 pts Not stated 0 pts
- How could this be improved upon?
- _____
- _____

3) Hypothesis and prediction (2 points)

- Was a hypothesis presented and a prediction made, based on the background information?
- Highly relevant 2 pts Somewhat relevant 1 pt Not relevant/stated 0 pts
- How could this be improved upon?
- _____
- _____

4) Method (3 points)

- The methods were clearly outlined and appropriate for the proposed question:
- Clearly stated 2 pt Vaguely stated 1 pts Not stated 0 pts
- The experiment was properly controlled with replicates:
- Yes 1 pt No 0 pts
- How could this be improved upon?
- _____
- _____

5) Results (4 points)

- Figures or tables were clear, appropriately labeled, and well prepared:
- Excellent 2 pts Very good 1.5 pts Good 1pt Fair 0.5 pts Poor 0 pts
- This presentation style was an effective way to summarize the results:
- Yes 2 pts Needs improvement 1 pt No 0 pts
- How could this be improved upon?
- _____
- _____

6) Discussion (4 points)

Results were explained well using physiological principles:

- Excellent 2 pts Very good 1.5 pts Good 1pt Fair 0.5 pts Poor 0 pts

Reflection on the original hypothesis was made and discussed:

- Excellent 2 pts Very good 1.5 pts Good 1pt Fair 0.5 pts Poor 0 pts

How could this be improved upon?

7) Future Directions (1 point)

Future directions the research should go were presented and appropriate based on the study:

- Clearly stated 1 pt Vaguely stated 0.5 pts Not stated 0 pts

How could this be improved upon?

8) References (1 point)

At least 3 books or journal articles were presented using the correct formatting style:

- Complete 1 pt Incomplete 0.5 pts Not done 0 pts

How could this be improved upon?

9) Evidence of teamwork (see Supplemental rubric) (1 point)

The group worked cooperatively and shared responsibility:

- Yes 1 pt Needs improvement 0.5 pts No 0 pts

How could this be improved upon?

10) Presentation style (1 point)

The presentation was clear, well organized, and well presented:

- Well done 1 pt Needs improvement 0.5 pts Poor 0 pts

How could this be improved upon?

Oral Presentation Supplemental Rubric

Lab members (full first/last names)

Date _____

Title of lab project:

In the table below, sign your initials in the boxes on the top row and check off the areas for the oral presentation that you performed.

Lab member signature				
Background research				
PowerPoint presentation				
Experimental design				
Data collection				
Data analysis				
Preparation of tables or graphs				
Summary of results				
Making connections to lecture material				
Ideas for future experiments				

Identify at least 3 things that your group learned from this project:

- 1.

- 2.

- 3.

Introduction to LabChart

In this experiment, you will learn how to acquire data with the PowerLab Data Acquisition Unit and analyze the data using the LabChart software. You will make simple recordings and measurements using the Finger Pulse Transducer.

Background

The purpose of the PowerLab system is to acquire, store, and analyze data. The raw input signal is in the form of an analog voltage whose amplitude varies continuously over time. This voltage is monitored by the hardware, which can modify it by amplification and filtering processes called *signal conditioning*. Signal conditioning may also include zeroing, the removal of an unwanted steady offset voltage from a transducer's output. After signal conditioning, the analog voltage is sampled at regular intervals. The signal is then converted from analog to digital form before transmission to the attached computer. *Figure 1* shows a summary of the acquisition.

The LabChart software usually displays the data directly; it plots the sampled and digitized data points and reconstructs the original waveform by drawing lines between the points. Digital data can be stored for later retrieval. The software can also easily manipulate and analyze the data in a variety of ways.

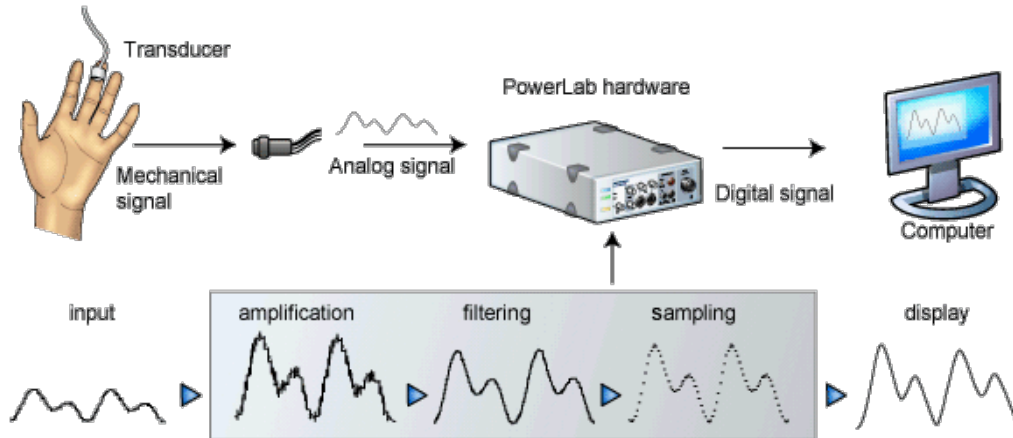


Figure 1. A Summary of Data Acquisition Using a PowerLab System

The basic hardware is a PowerLab, a recording instrument that measures electrical signals, usually through the inputs on its front panel. It can also generate output signals. Added hardware, such as the Finger Pulse Transducer, can extend its capabilities. There are various PowerLab models, but PowerLab 26T, which is recommended for this experiment, is designed especially for teaching experiments. It is a four-channel recording instrument with built-in front-ends that allow optimal recording of biological signals (through Bio Amplifiers) and provide safe stimuli for humans (through the Isolated Stimulator). Students are able to record physiological measurements such as finger pulse, blood pressure, respiration, and even more complicated measurements like an EMG, or electromyography, recording. The front of PowerLab 26T is shown in Figure 2. The back is shown in Figure 3.

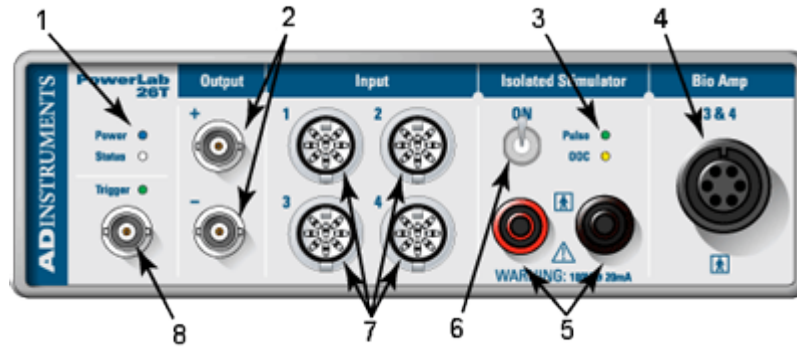


Figure 2. The Front Panel of PowerLab 26T

1. Power indicator light: illuminates when the PowerLab is turned on
2. Analog output connections: provide a voltage output in the 10 V range
⚠️ This is NOT safe for direct connection to humans
3. Isolated Stimulator status light: indicates if the Isolated Stimulator is working properly (green) or out of compliance (yellow)
4. Dual Bio Amp input: connects a 5 lead Bio Amp cable to the PowerLab; reads as inputs 3 and 4
5. Isolated Stimulator outputs: for connecting stimulating electrodes to the Isolated Stimulator
6. Isolated Stimulator switch: turns on/off the Isolated Stimulator
7. Pod ports: 8-pin connectors for attaching pods and certain transducers to Input; these supply a DC Power to the pods and transducers
8. Trigger input: can be used to start or stop a recording event

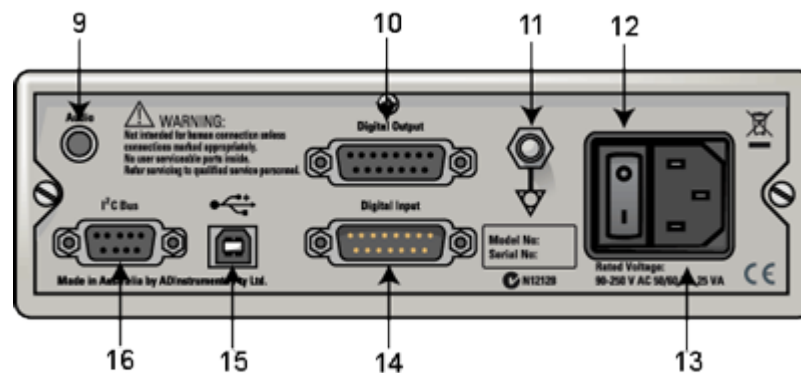


Figure 3. The Back Panel of PowerLab 26T

9. Audio output connector: standard $\frac{1}{8}$ " (3mm) phono jack for sound output of recordings from the Bio Amp
10. Digital Output Connector
11. Earthing post: used to ground the PowerLab, if grounded power supply if unavailable
12. Power switch
13. Power cord connector
14. Digital Input Connector
15. USB connector: connects a computer to the PowerLab
16. I²C connector: connects the PowerLab to special ADInstruments signal conditioners called front-ends

The PowerLab should be connected to your computer and turned on. The hardware is controlled through the software, so there are no knobs or dials on the PowerLab. As the LabChart software controls the PowerLab hardware, it displays the electrical signals measured by the PowerLab on the computer screen. The display format resembles a traditional chart recorder with a scrolling area of the window acting as the paper.

Required Equipment

- LabChart software
- PowerLab Data Acquisition Unit
- Finger Pulse Transducer

Procedure

Words appearing in **bold** are items to click in LabChart. If the word appears in **bold** and a **color**, it is referred to in the Student Quick Reference Guide. Use the color dividers in the guide to find the appropriate section for your topic. All **blue** text appears in Part One: Acquisition, all **green** text appears in Part Two: Data Analysis, and all **red** text appears in Part Three: Troubleshooting. An introduction to the PowerLab and LabChart appears in the **purple** section.

Exercise 1: Equipment Setup and Starting the Software

Connecting the Finger Pulse Transducer

1. Connect the Finger Pulse Transducer to Input 1 on the front panel of the PowerLab (Figure 4).
2. Place the pressure pad of the Finger Pulse Transducer on the tip of the index or middle finger of either hand of the volunteer. Use the Velcro strap to attach it firmly but without cutting off circulation.
 - If the strap is too loose, the **signal will be weak**, intermittent, or noisy. If the strap is too tight, blood flow to the finger will be reduced causing a weak signal and discomfort. You may need to adjust the strap in the next stage of the exercise.
3. Have the volunteer face away from the monitor. In most experiments, you do not want the volunteer to see the data while it is being recorded. Make sure the Finger Pulse Transducer cable can still reach the PowerLab while allowing the volunteer to sit comfortably. The volunteer should sit in a comfortable position and relax. The Finger Pulse Transducer **cannot** rest on any surface. The volunteer should support your wrist with your leg and have your fingers hang freely.

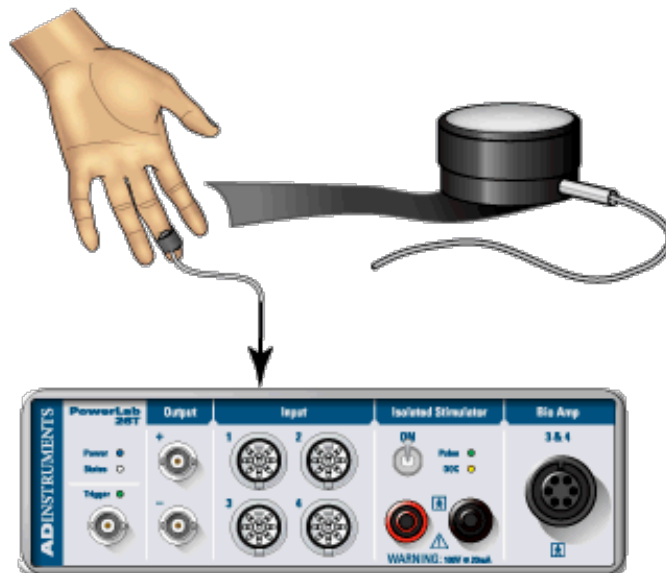


Figure 4. How to Connect the Finger Pulse Transducer with the PowerLab and Finger

Starting the LabChart Software

1. Start the LabChart software as you would any other computer program. LabChart will appear with either an empty Chart Window (Figure 5) or the Experiments Gallery dialog. If the Welcome Center appears, close it to see the Chart Window.
2. The Chart Window is divided up into a number of recording channels shown as horizontal strips across the screen. Various controls are located around the window. Take time to locate the ones shown and learn their functions.

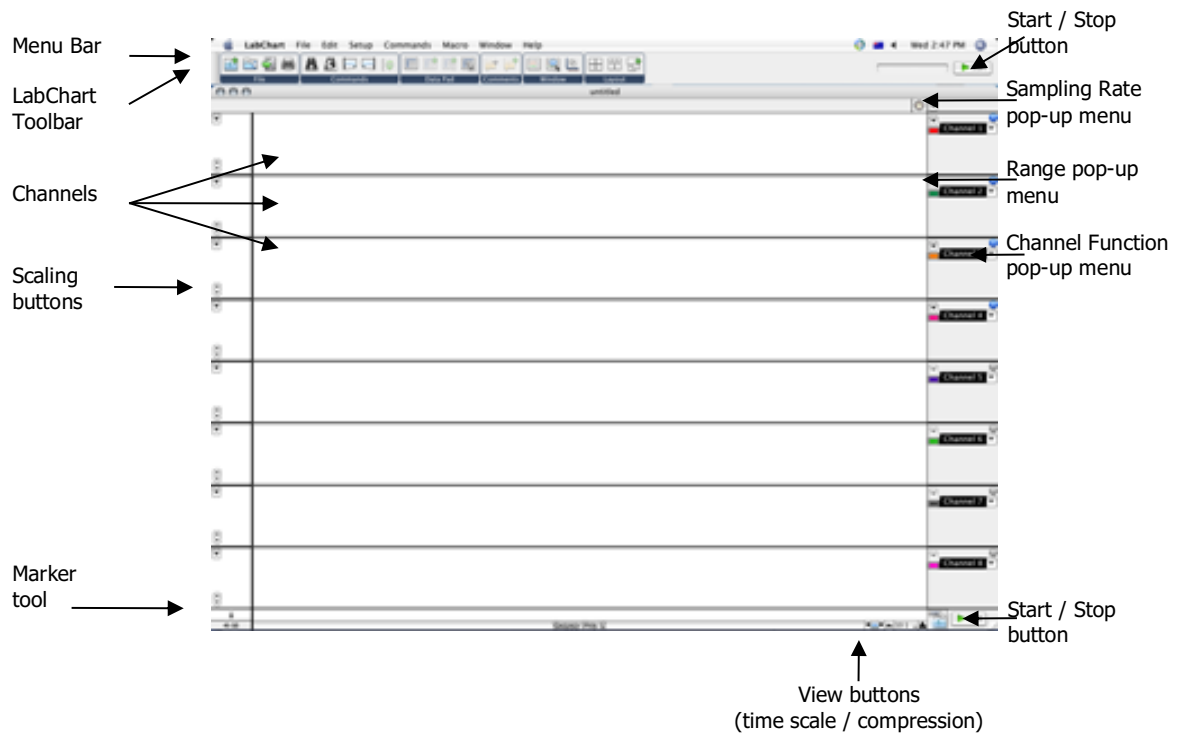


Figure 5. LabChart Interface with Default Windows and Settings

Opening a Settings File

1. Close the Chart Window. Do not save the file if asked. Under the File menu go to Welcome Center. Open An Introduction, Settings File, then choose Pulse Settings.
2. The Chart Window you originally saw when you first opened LabChart should now be replaced by a modified version of the window with only one channel visible.



Figure 6. Welcome Center

Using the Input Amplifier Dialog

Through the Input Amplifier dialog, you can modify signals so they are displayed optimally when you start recording.

1. Select **Input Amplifier** from the Channel 1 Channel Function pop-up menu (Figure 7). The Input Amplifier dialog will appear with a scrolling signal in the display area on the left side of the dialog (Figure 8).

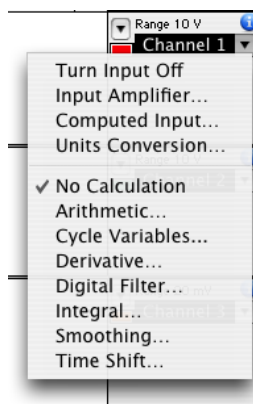


Figure 7. Channel Function Pop-up Menu

2. The signal from the Finger Pulse Transducer is much smaller than 10 V, so you have to **adjust the range** to view the signal. To adjust the sensitivity of the channel, choose an appropriate range setting from the Range pop-up menu in the Input Amplifier dialog. The number displayed in the range menu indicates the maximum input voltage currently selected.

Note: Make sure the Finger Pulse Transducer is not touching any surface and is still attached to the volunteer. Refer to "Connecting the Finger Pulse Transducer" above.

3. Select the **500 mV range**. You will notice the vertical scale changes and the small rhythmic deflections that appear on the signal trace. Select **200 mV** and note the signal trace now has a much larger deflection. Continue to adjust the range setting until the deflection fills about one half to two-thirds of the data display area (as shown in Figure 8) and press **OK**.

- The signal from the Finger Pulse Transducer has not changed, only the sensitivity of the recording system has. If the rhythmic signal is a series of downward deflections, click in the Invert checkbox to reverse the direction.

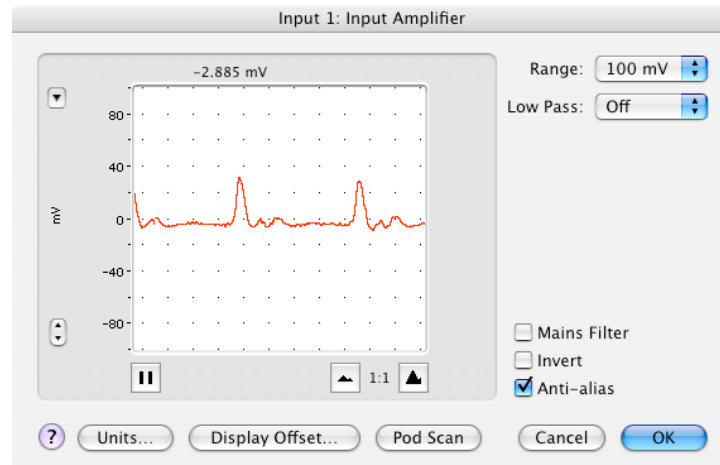


Figure 8. Input Amplifier Dialog

Creating Digital Voltmeter Mini-windows (DVMs)

It is possible to create mini-windows of the Rate/Time and Range/Amplitude. This allows you to see the numbers clearly if you are recording data away from the monitor.

- Position the cursor over the Rate/Time. Click-and-drag this area to create the DVM. You can then do the same for Range. Figure 9 shows where to position the cursor. Once created, you can move the DVMs anywhere on the screen.
 - When the cursor is in the data channels, the DVMs will display the time and amplitude. When the cursor is elsewhere in the window, the DVMs will be blank. An example of these DVMs is shown in Figure 10.

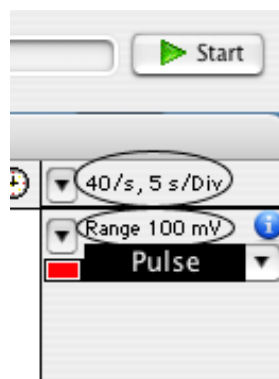


Figure 9. The Circles Indicate Where to Position the Cursor to Create DVMs

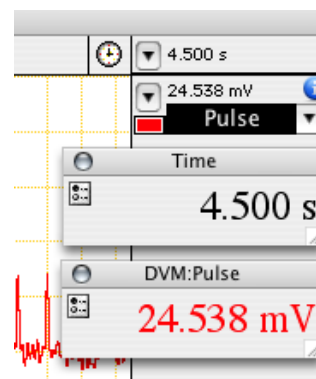


Figure 10. DVMs

Saving the File

It is wise to save work frequently when working with any computer. Saving files in LabChart is the same as saving any file you would on your personal computer. If you choose to save your files, the Save As dialog will appear so you can save the file under a suitable name and location.

Closing the File

In this experiment, you can leave the file open between exercises. In other experiments, when you are finished with a file, you can close it the same way you would files in other programs. If you have any unsaved changes, an alert box will appear asking if you want to save them. If your instructor requires you to save the file, or if you wish to analyze the results at a later time, click **Yes** to save the recording as a LabChart data file. If you accidentally close the file or program, click **Cancel** to go back to LabChart.

Exercise 2: Recording Data

This exercise teaches you how to record data, make adjustments to the file, and add notes to it. Remind the volunteer to face away from the monitor and keep their hand and fingers still.

1. **Start** recording. Record the finger pulse waveform for 20 seconds. Your record should resemble that shown in Figure 11. Note that **Start** changes to **Stop**.

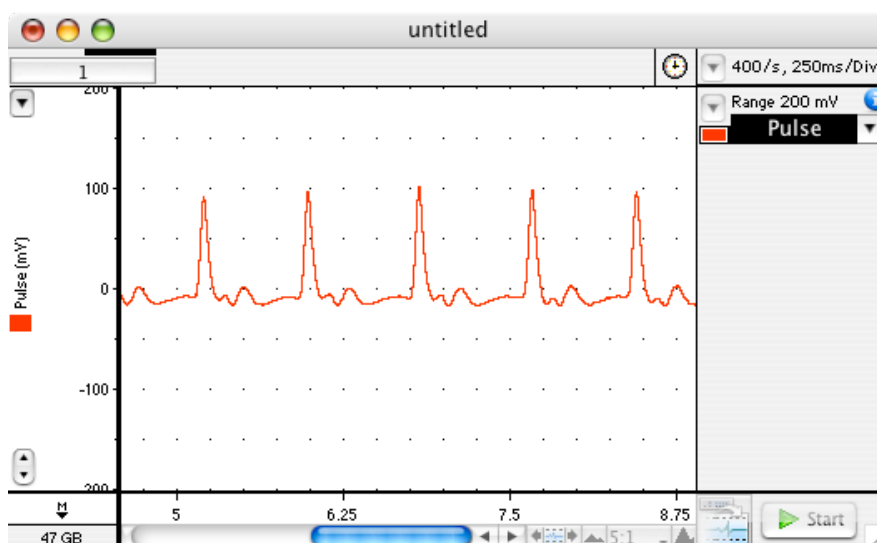





Figure 11. Example of Waveform Seen with Finger Pulse Transducer

2. Move the mouse pointer about the Chart Window and observe what happens. The values in the Rate/Time and Range/Amplitude displays change with the location of the pointer and the **Waveform Cursor**.
3. Move the pointer over the scale at the left of the Chart Window. The pointer changes to point to the right and small arrows appear beside it. When the pointer is over the scale, you can either stretch or move the scale by dragging the scale numbers or the scale between them. The small arrows beside the pointer indicate what will happen. 
4. The Scaling buttons are on the left side of each channel's Amplitude axis. The up  button will double the vertical scale shown, and the down button will halve the vertical scale  shown.
5. Right-clicking in the channel will show several options for displaying data. **Auto Scale Channel** will automatically adjust the amplitude axis so the maximum value is just larger than the maximum value of visible data in this channel. **Show Points as Dots** and **All Channels with Dots** will show you the individual points the PowerLab is sampling. If you changed the size of the data channel, **Equalize Channel Heights** will make all the data channels the same size again. "Add Comment, Set Marker, and Add Channel" will be covered later in this exercise. "Split Window" is an advanced feature and may or may not be covered by your instructor.

Note: **AutoScale** is also found in the LabChart Toolbar under the Command **Auto Scale All Channels**.

Adjusting the Sampling Rate

Look at your data trace in the Chart Window. The peaks may not look quite the same as they did in the Input Amplifier dialog. This can be explained in terms of sampling rate. A digital recording system, like the PowerLab system, records the value of the signal at regular time intervals, rather than continuously. This is called sampling. When sampling occurs too slowly, some of the faster parts of the waveform, like the pulse peak, may not be recorded causing the recorded signal to inaccurately represent the real one. To record a signal accurately using this technique, the sampling rate must be set high enough that the signal does not vary too much between samples.

You will be running a macro to adjust the sampling rate.

Running Macros

A macro is a recorded set of commands and operations which can be executed with a single command. If you want settings to change while you are recording, you can create a macro to automatically change the settings for you at a specific time. The settings file you opened for this experiment contains one macro to automatically adjust the sampling rate for you. Macros are used in many other LabChart experiments.

1. Select **Macro** from the **Menu** Bar and scroll down to "Sampling Rate." This is the title of the macro. Have the volunteer relax and wear the Finger Pulse Transducer as before. The macro will do everything for you – it will even stop recording.

Your recording should look something like the one shown in Figure 12. The block boundaries separate the segments of data recorded at different rates. Note the difference between the waveforms in the blocks and how the signal looks quite different. Essentially, information about the signal shape has been lost at the slowest sampling rate. This demonstrates the need to sample fast enough to adequately represent the signal you record.

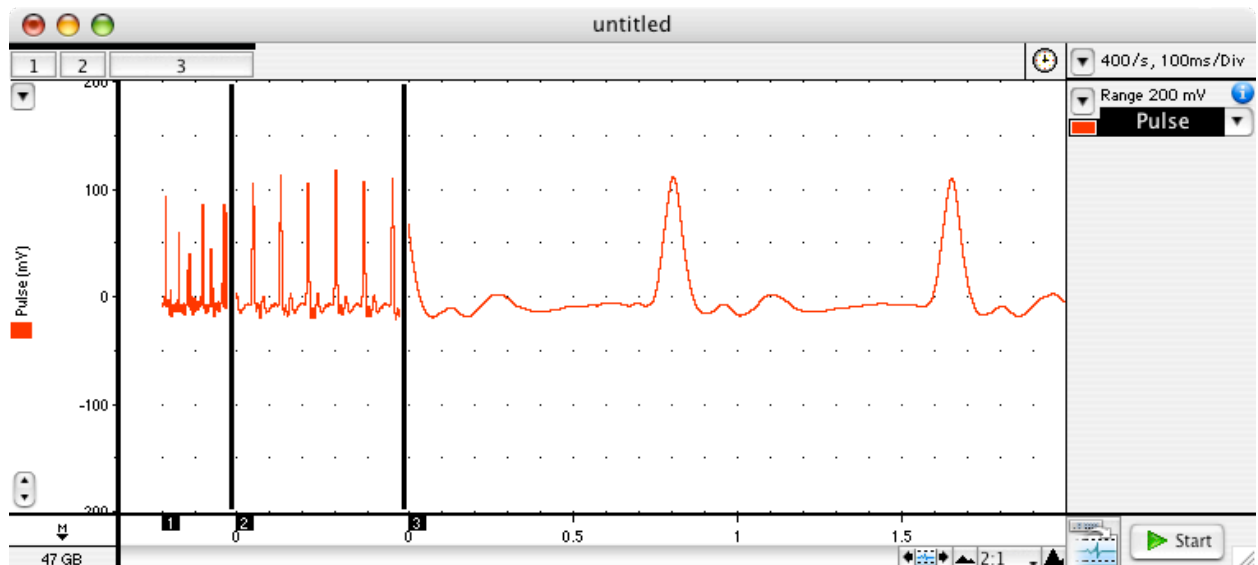


Figure 12. Waveforms Produced From Different Sampling Rates

The last waveform recorded at 400 samples per second may have a different height than that of the slower rate recordings. This is because at the faster rate more sample points are taken thereby giving a more accurate reproduction of the signal, including the peak value. Right-click on the data channel and select **Show Points as Dots** to help you visualize the different sampling rates. Right-click on the data channel and select **Join Points with Line** to see the original waveform.

Annotating a Record

This experiment is divided into a series of exercises. It is convenient to annotate each exercise, using a comment, to determine what was done at any particular stage during subsequent review. In many experiments, adding comments will be part of the procedure. You can **add comments** while you are still recording and after you have finished.

1. Set the sampling rate to 400 samples per second (400/s) and **Start** recording.
2. Type "comment 1" or something similar on the keyboard. The words appear in the Comments bar at the bottom of the Chart Window. Add the comment by pressing Return/Enter or by **Add** at the right of the Comments bar.

The vertical dotted line marks when you added your comment to the recording. If there is enough room, the comment appears along the dotted line. There is a numbered comment box at the bottom of the vertical dotted line. You can right-click this box in the Time axis to change the comment (Figure 13).

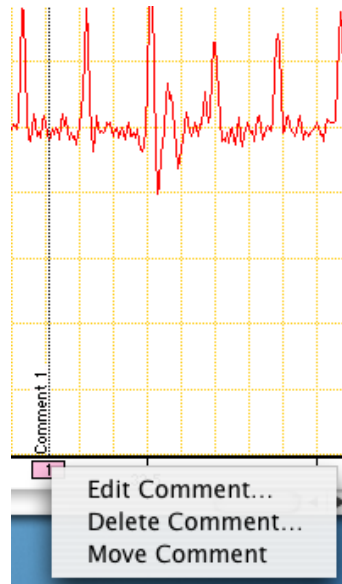


Figure 13. Comment Number Pop-up Menu

To add a comment after recording, right-click the data channel on the point you want to annotate. Select **Add Comment** and a dialog like the one in Figure 14 will appear. Use the pop-up menu to select in which channels you want the comment located.

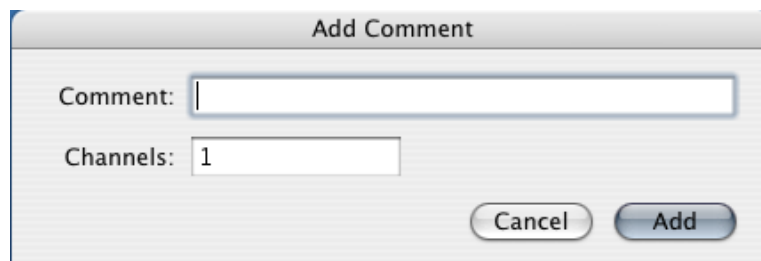


Figure 14. Add Comment Dialog

Note: If you want to enter comments quickly while recording, it is possible to press Return/Enter to insert blank comments. You can go back after you have finished recording to add the description using the "Edit Comment" feature described above.

Exercise 3: Analysis

The LabChart program is not only used to record waveforms but also to analyze them. This exercise shows you how to use more features of LabChart: navigating the Chart Window to find data, measuring amplitude and time values from the waveform, using the Zoom Window for a more detailed view, and creating channel calculations.

Navigating in the Chart Window

There are a variety of ways to view a LabChart data file and to navigate around it. You can use the scroll bar to scroll to different parts of the recording, compress the Time axis so more of a waveform can be seen, and locate specific sections of the recording by searching for the comment inserted there.

Scrolling

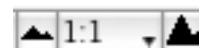
The scroll bar provides the simplest way of moving backwards and forwards through your file and works the same as it would in any other computer program. You can think of your recording as a large strip of paper of which only one part can be seen at any one time.



Note: If your mouse is equipped with a scroll wheel, rolling the wheel forward will scroll your data to the right and rolling the wheel to the rear will scroll to the left.

View Buttons

By using the **View Buttons** at the bottom of the Chart Window, you can compress or expand the Time axis to see more or less of a waveform. The left button will compress your data. The right button will expand it. If you select the right button, a pop-up menu appears in which you can choose the new compression directly.



right

Locating Specific Sections by Comments

The **Commands** menu has some other ways you can navigate around your recording. **Find** brings up the "Find and Select" dialog. Each type of find is based on an initial selection or active point, which serves as a starting point for the find. **Find Next** will perform the find a second time in the direction chosen in the Find and Select dialog. **Go to Start** will take you to the beginning of the Data file. **Go to End** will take you to the end of the data file.

1. Select **Find** in the LabChart Toolbar and go to **Find Comment**. Choose the search direction and enter text from the comment you wish to find. (If the comment is already in the Chart Window, the data trace will not move.)

Alternately, you can go to **Comments List** in the LabChart Toolbar and select the comment you want to see in the data trace. LabChart will automatically make the comment visible.

Making Measurements with the Waveform Cursor

The **Waveform Cursor** is a tool that can be used to read amplitude and time values directly from a waveform on screen.

1. Move the pointer over the data trace in the Chart Window and move it left to right. A small cross-hair **Waveform Cursor** appears on the waveform at the same time value as the pointer. As you do this, the Rate/Time shows the time at the Waveform Cursor, and the Range/Amplitude shows the amplitude of the signal at that time (Figure 15).

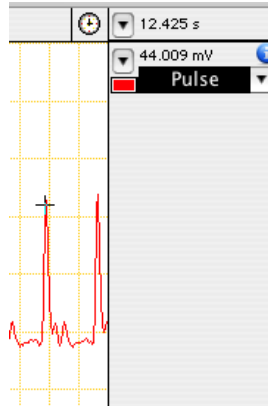


Figure 15. Waveform Cursor and Pointer

Using the Marker

The **Waveform Cursor** is often used in conjunction with the **Marker**. The Marker is located at the bottom left of the Chart Window and can be dropped on any part of the waveform to allow relative measurements.

1. Drag the **Marker** from the Marker Box to a location on the trace and release. The Marker does not have to be placed exactly on the waveform; it will attach itself to the waveform at the time position you dropped it.
2. Move the pointer away from the Marker. When the Marker is in use, the amplitude and time values displayed are relative to the marked reference point. This means the time and amplitude values are now displayed as differences (Δ) between the Waveform Cursor and the Marker. This is very useful for measuring the time between events or measuring the relative amplitudes of parts of a waveform.
3. As an exercise, measure the amplitude of your finger pulse signal and the time in seconds from peak-to-peak (as shown in Figure 16).

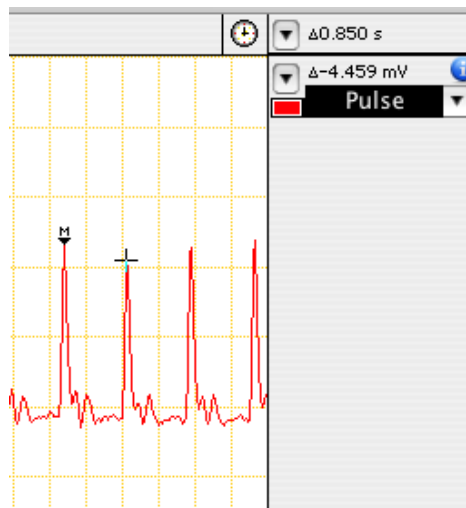


Figure 16. Making a Peak-to-peak Measurement

- What is your heart rate based on the peak-to-peak measurement? Enter your measured value below.

60 s _____ =

The heart rate calculated from the time difference shown in Figure 16 would be $60 \text{ s} / 0.850 \text{ s} = 70.59$ beats per minute (BPM).

If you want to remove the Marker from the data trace, either click in the Marker Box to return the Marker home or drag the Marker back to its home location.

Using the Zoom Window

A convenient feature of LabChart is the ability to zoom in on a selected region of data. This allows you to select a specific area of a signal and look at it much more closely. It also allows accurate measurements to be made more easily. With **Zoom Window**, you can copy the image onto the Clipboard so it can be pasted into a word-processor or graphics file. (The Copy command from the Edit menu changes to Copy Zoom Window when the Zoom Window is in front.) You can print the image on a connected printer.

1. Select a rectangular area of data by dragging across the waveform. The selection will be highlighted.
2. Now select **Zoom Window** from the LabChart Toolbar. The Zoom Window will appear in a new window with the data you have selected (both the vertical and horizontal extents).
3. Use the **Marker** and **Waveform Cursor** to measure pulse amplitude and time interval – these values appear under the title bar in the Zoom Window, as shown in Figure 17.

Note: If the Marker was not included in your selection, note that it is duplicated at the bottom left of the Zoom Window.

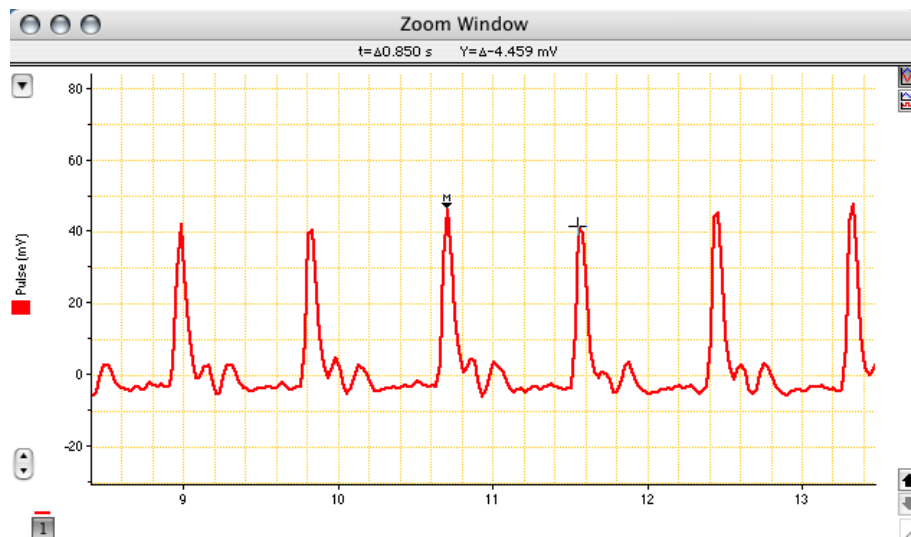


Figure 17. Zoom Window

Creating Channel Calculations

LabChart has the ability to automatically detect cycles in a waveform and calculate cyclic measurements such as rate and amplitude. These calculations can be done in any unused channel.

2. Right-click anywhere in the data channel and select **Add Channel**. A new channel will appear in the display (Figure 18).

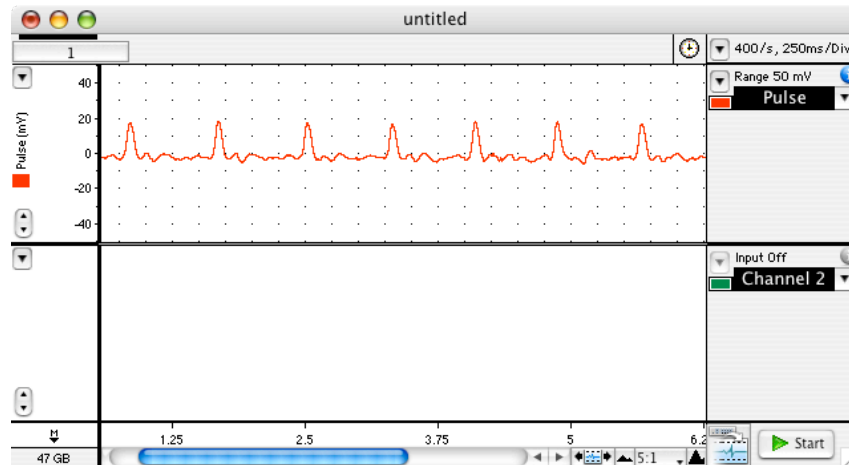


Figure 18. Chart Window with Two Channels

4. From the Channel 2 Channel Function pop-up menu, select Cyclic Measurements.
5. In the Cyclic Measurements dialog (Figure 19), set the Source to Channel 1, the Measurement to Rate, and the Detection Settings Preset to Cardiovascular – Finger Pulse. Small circles, known as event markers, will appear above the detected beats (also referred to as pulse peaks).
 - If all of the beats from the source channel are not detected, alter the Minimum Peak Height by moving the slider to the left. You want the minimum sensitivity necessary to detect beats but want to avoid smaller secondary peaks or noise being detected mistakenly. If too many peaks from the source channel are detected (event markers appear where they should not), move the slider to the right.

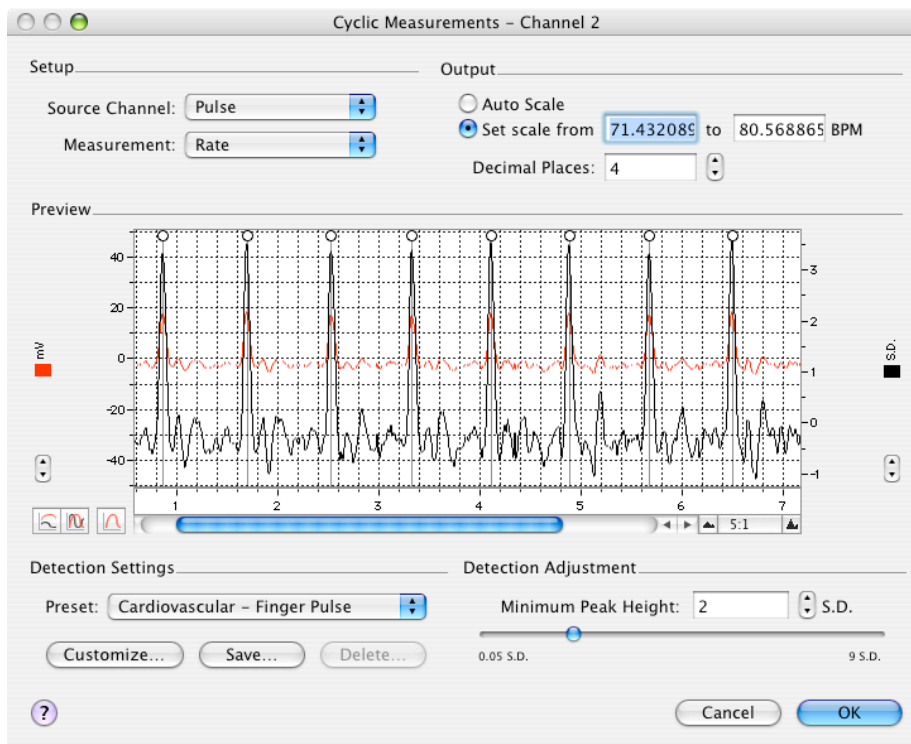


Figure 19. Cycle Variables Dialog with Proper Event Markers Shown

Using the Zoom Window with Multiple Channels

3. Drag along the Time axis to select the same portion of all active channels. If all the data is of interest, you can double-click the Time axis to select the entire data trace. Then select **Zoom Window**.

Alternately, there is an advantage to the following method in that it permits you to select only the trace and its immediate area, not the empty portions above and below it, thus producing as large an image as possible in the Zoom Window.

6. Highlight an area in one of the channels, and while holding down Shift, drag across a second channel to highlight an area of data in it. You can only highlight data from the same period of time as chosen in the first channel. Repeat as required for different traces. Once you have highlighted everything you want, select **Zoom Window**.

For either method, once the Zoom Window appears, use the stacked and overlay buttons in the upper left corner of the window to change the way the data traces are displayed. Refer to Figure 17 if you cannot find these buttons.

Deleting Data

Occasionally you may want to discard a segment of your data trace or delete some noisy data.

Note: If you delete data, this action cannot be undone.

1. Scroll through your data and find a section that appears excessively noisy. Click-and-drag over the section to **highlight** this part of the data trace.
2. You can either press the delete key or go to the **Edit** menu and select **Clear Selection**.

You will be asked if you want to delete the data from all the channels; choose **OK**. If you delete data from one channel, it will automatically be deleted from all the channels to keep the data trace uniform.

It is possible to delete an entire block of the data trace by double-clicking on the Time axis and pressing the delete key.

If you delete a portion of the data trace from the middle of the recording, LabChart will insert a black vertical line into the trace, indicating that the data has been broken into separate records (Figure 20). You may want to insert a **comment** at this break to indicate that data was deleted.



Figure 20. The Vertical Line Denotes Data Has Been Deleted (With Comment Added)

Once you have completed the tutorial complete the graphing assignment on the following page.

Graphing Assignment

Names (work in groups of 2): _____

Lab day and time: _____

Hypothesis

1. What do you predict will happen to pulse amplitude when you raise your hand? _____
lower your hand? _____ .

Procedure

1. Place the pulse transducer on the index finger of a subject and record the pulse for 1 min with the hands (palm up) **resting** on the table.
2. While still recording have the subject raise their hand **high** over their head and place the comment "hand high". Record for 1 min.
3. Have the subject **lower** their hand as far as possible along their side and place the comment "Hand low". Record for 1 min then **stop** the recording.

Data Analysis

1. Use the waveform cursor to analyze the amplitude of the pulse in all 3 situations listed above.
2. Use Microsoft Excel to make a table and draw one graph of the data showing changes in amplitude of the pulse with the hand at different positions. Make sure the graph has an appropriate title (it should be descriptive of the experiment undertaken), labels with units, and enough information so that the effect can be clearly seen. Print one copy of the table and graph for your instructor.

Questions

1. Which axis on the graph represents the independent versus the dependent variable?

Independent variable = _____ on the _____ axis

Dependent variable: = _____ on the _____ axis

2. What can you conclude about the effect of altering hand position on pulse amplitude?

Question #3 on next page.

3. Based on the results of the experiment what types of experiments might you do in the future to investigate what other factors may effect pulse amplitude?

I.

II.

III.

Psychophysiology I

In this experiment, you will examine techniques that are intended to measure psychological responses to stimuli. You will measure skin conductance, skin temperature and pulse rate to see if you can use these signals to interpret autonomic responses.

Introduction

Psychophysiology is the study of physiological responses to behavioral stimuli. Physiological parameters commonly measured by psychophysicists include electrodermal activity (called the galvanic skin response, GSR), heart rate, skin temperature and respiration rate. These variables are of interest because they respond to signals from the autonomic nervous system, which is not under conscious control.

During a GSR, eccrine sweat glands present on the palms of the hands and on the bottom of the feet, are innervated by the sympathetic branch of the autonomic nervous system. These glands act as variable resistors; as they fill with fluid, skin resistance (i.e., resistance to an electrical current) decreases. Sympathetic activity causes an increase in sweat production, thereby increasing skin conductance (i.e., an increase in conductance allows electricity to pass freely through a structure). Skin conductance is expressed in microsiemens (μS), a common unit of conductance.

Skin temperature is another physiological parameter often studied by psychophysicists. Circulation in the microvasculature is under autonomic control, and can be varied in order to regulate temperature and oxygen supply to tissues. Absolute skin temperatures differ among individuals as a function of metabolism, cutaneous fat, and health factors. However, a change in skin temperature can be brought about by a change in emotional state. Stress responses that activate the sympathetic nervous system usually result in a reduced peripheral circulation, causing a lowered skin temperature. On the other hand, a strong signal from the parasympathetic nervous system can cause a localized increase in peripheral circulation: blushing.

Pulse rate is beyond your conscious control and is therefore under the control of the autonomic nervous system. Pulse increases when norepinephrine is released by an upregulation sympathetic nerve fibers. These fibers are connected to the heart at specific areas known as nodes. A stress response is one situation when you would expect to see an increase in the rate of the peripheral pulse.

In these series of experiments you will examine the ANS response to various stimuli by measuring a change in skin conductance, skin temperature and peripheral pulse rate.

Experimental Equipment

A computer system
LabChart 7.0 or higher
PowerLab 4/26T
GSR Amplifier
GSR electrodes

Thermistor Pod
Nasal thermistor bead
Medical tape
Pulse transducer

Procedures

A. Set up and calibration of equipment

1. Make sure the PowerLab is turned off and the USB cable is connected to your computer.
2. Connect the GSR Amplifier to the PowerLab by first attaching the I²C cable from the “I²C Bus” on the back panel of the PowerLab to the “Input” socket on the back of the GSR Amplifier (Figure 1). Then connect the BNC to BNC cable from the “Signal Output” port on the back of the GSR Amplifier to the BNC socket of Input 1 on the front panel of the PowerLab.
3. Connect the red and black GSR finger electrodes to the front of the GSR Amplifier (Figure 2). Do not attach the electrodes to the volunteer.
4. Turn on the PowerLab. **Note:** The GSR Amplifier must be connected to the PowerLab before you turn on the PowerLab.
5. Launch LabChart 7 and select Human Experiments, Psychophysiology, Setting Files, ANS Settings. After a few seconds, a blank data file will appear.

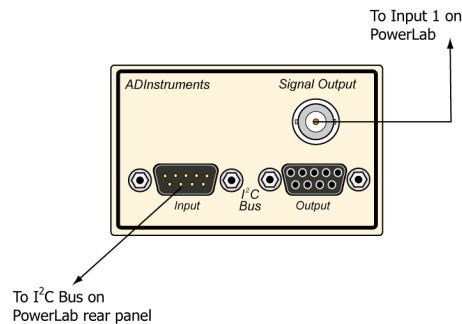


Figure 1. The rear panel of the GSR Amplifier, showing connections for the I²C cable and BNC cable.

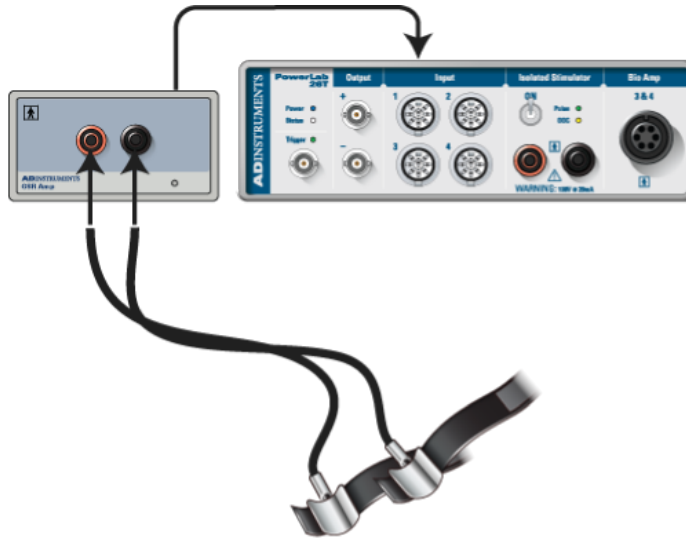


Figure 2. PowerLab and GSR Amplifier set up.

Exercise 1: Skin conductance and the startle response

In this exercise, you will examine changes in skin conductance associated with the startle response. If possible, conduct this exercise in a quiet room, away from distractions.

Zeroing the GSR Amp

The GSR Amplifier must be zeroed before you start this exercise:

1. Open the settings file called “ANS Settings”. A new blank data file will appear in a few seconds, with 3 channel. You are only interested in the first channel for now.
2. From the GSR Channel Function pop-up menu in LabChart, select **GSR Amplifier**. A dialog box will appear. Make certain the finger electrodes are not touching one another. If you get an out of range error, change the range to eliminate the error.
3. Make sure that the GSR finger electrodes are disconnected from the volunteer, and click the **Open Circuit Zero** button in the GSR Amplifier dialog box.
4. After you perform the open circuit zero, attach the GSR electrodes to the volunteer as shown in Figure 3. Make sure that the volunteer removes any jewelry and has clean, dry hands.
5. Click the **Subject Zero** button in the GSR Amplifier dialog box. When the zeroing process finishes, click **OK** to close the dialog box and return to the LabChart View.

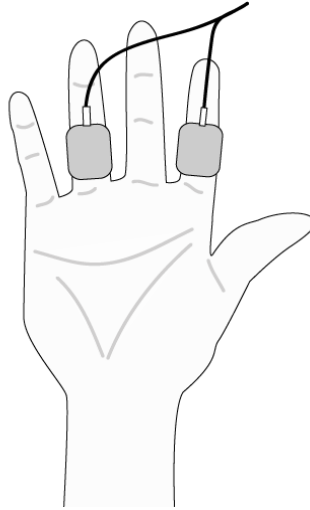


Figure 3. Placement of the GSR electrodes on the hand. The hands should be clean and dry, and all jewelry should be removed prior to recording.

Examining the startle response

1. Click **Start** to begin recording.
2. Add a **comment** with the volunteer's name into the LabChart window.
3. The volunteer should be sitting relaxed, with their hands in their lap, facing away from the computer monitor.
4. Prepare a **comment** with "breathing." Do not enter the comment, yet.
5. Ask the volunteer to take several deep breaths. When they do so, enter the comment by pressing the Return/Enter key on the computer keyboard.
6. Next, ask the volunteer to close their eyes and relax. Prepare a **comment** with "startle," but do not enter it into the LabChart window.
7. Allow the volunteer to relax for two or three minutes. Without warning the volunteer, make a sound near the subject's ear such as whispering or clicking a finger.
8. Immediately press the Return/Enter key on the computer keyboard to enter the comment into the LabChart window.
9. Continue recording for one minute.
10. Click **Stop** to end the recording.
11. **Save** the trace to the desktop of the computer.
12. Remove the GSR finger electrodes from the volunteer.
13. If you have time, repeat this exercise for each member of your lab group.

Analysis

Exercise 1: The startle response

1. Examine your data in the LabChart View window.
2. Place the marker on the data trace where the comment was added, signifying a stimulus.
3. Place the waveform cursor on the peak in the skin conductance tracing.
4. Record the change in amplitude and the latency (time from stimulus onset to peak response) in Table 1 of your Data Notebook.
5. Repeat steps 2-4 for the other stimulus events in the recording.
6. Calculate the mean skin conductance change and mean latency and record the values in Table 1.

If you performed this exercise on other people in your group, record their average response and latency separately from other people's data.

Table 1. Skin conductance and the startle response.

Individual	Change in skin conductance after stimulus (μS)	Latency (s)
1		
2		
3		
4		
5		
	Average skin conductance response (μS)	Average latency (s)

Exercise 2: Skin conductance, skin temperature and peripheral pulse during mild stress

In this exercise, you will examine skin conductance, skin temperature and peripheral pulse during mild stressful activities. You will need to work in groups of three or more students for this exercise.

1. Attach the Skin Temperature Probe to the back of the Thermistor Pod (see Figure 4 on the next page).
2. Attach the GSR electrodes back onto the hand.

3. Connect the 8-pin DIN cable from the rear of the Thermistor Pod to the Input on the front panel of the PowerLab. Note Fig. 4 shows the 4/25T not the 4/26.
4. Connect the Pulse Transducer to input channel 3 on the PowerLab 4/26.
5. Open the settings file called “ANS Settings.” Make sure you have saved your data from Exercise 1. A new blank data file will appear in a few seconds, with 3 channels. Channel 1 measures GSR, channel 2 measures skin temperature and channel 3 measures the peripheral pulse.
6. Choose a volunteer from your group to participate in this exercise.
7. Attach the Skin Temperature Probe to the palmar surface of one of the volunteer’s fingers using medical tape.
8. Attach the pulse transducer to the tip of the middle finger.
9. Follow the “Zeroing the GSR Amplifier” procedure from Exercise 1. Make sure that the GSR electrodes, Skin Temperature Probe and Pulse Transducer are on the same hand.
10. Ask the volunteer to relax, and place their hands in their lap.
11. Click **Start** to begin recording.
12. Add a **comment** with the volunteer’s name into the LabChart window.

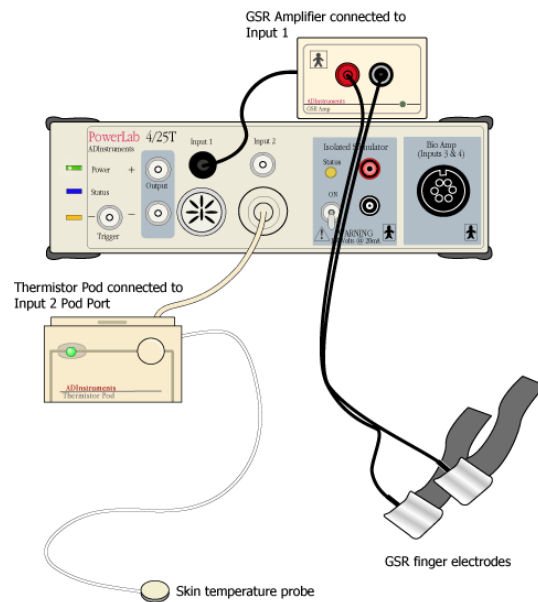


Figure 4. The PowerLab set-up for measuring skin conductance and temperature.

13. Record baseline skin temperature, skin conductance and pulse rate for one minute.
14. Ask a member of your group (not the volunteer) to pick a number between 500 and 800.
15. Ask another member of the group to choose an odd number between seven and thirteen.

16. Each time the volunteer calls out a number, add a **comment** to the data trace by pressing the Return/Enter key on the computer keyboard.
17. Instruct the volunteer that when you say “go,” to start with the first chosen number and subtract the second chosen number from it, aloud. Have the volunteer continue to subtract the second chosen number from the resulting value until you say, “stop.”
18. When you say, “go,” add a **comment** with “stress” to the LabChart window.19. Enter a **comment** with “end arithmetic” to the LabChart window.
20. Allow the volunteer to perform the mental arithmetic exercise for one minute. At the end of one minute, instruct the volunteer to stop, and allow them to relax.
21. Continue to record for one minute, then, click **Stop**.
22. **Save** the trace to the desktop of the computer.

Analysis

Exercise 2: Skin conductance, skin temperature and peripheral pulse during mild stress

1. Examine your data in the LabChart View window.
2. Select data in all channels from the first minute of the trace, before the subject was asked to perform mental arithmetic.
3. Click the Data Pad button from the LabChart Toolbar.
4. The Data Pad will appear, and display the average values for skin conductance, skin temperature and peripheral pulse** in columns one, two and three.
5. Record these values in Table 2 under the heading “Baseline Values”.
6. Return to the LabChart View window, and examine the data in the section of the trace during which the volunteer was performing mental arithmetic.
7. Each blank comment represents when the subject announced a number. Select several seconds of data around the first blank comment. Return to the Data Pad by clicking the Data Pad button from the LabChart Toolbar. Record the average skin conductance, skin temperature and pulse rate in Table 2 under the heading “Stress”.
8. Repeat steps 6-7 for each of the blank comments in the mental arithmetic section of the data trace.
9. Select the last thirty seconds of the recording (the relaxation period), and determine the mean skin temperature, skin conductance values and the pulse rate. Record them in Table 2 under the heading “Recovery.”

10. If you performed this exercise on multiple group members, each person in the group should record their own results and then share them with the other members of your group or class.

**** A better way to analyze the peripheral pulse is to use the Cycle Variables option as you learned about in the Tutorial on page 24 of this manual.**

Table 2. Skin conductance, skin temperature and peripheral pulse in response to mild stress.

Skin conductance (μS)			Skin Temp. ($^{\circ}\text{C}$)			Pulse (bpm)		
Baseline	Stress	Recovery	Baseline	Stress	Recovery	Baseline	Stress	Recovery

SmartBoard Discussion

Provide data for Table 1 and 2 to your laboratory instructor. The instructor will go over the data with you using the SmartBoard, using the format of an oral presentation session to demonstrate to you how to disseminate information to your classmates. In subsequent weeks you will be asked to do the same in front of the class using the SmartBoard.

Study Questions

1. Did the subjects' skin conductance increase or decrease in response to being startled? What does your result suggest is occurring in the skin?

2. Was there a noticeable change in the volunteer's skin temperature during mild stress?

3. Did skin temperature increase or decrease during stress? What branch of the autonomic nervous system do your data suggest was active during stress?

4. Did pulse rate increase or decrease during stress? Explain why physiologically.

5. In many countries, polygraph (lie detector) results are not admissible as evidence in a court of law. Based on your results, why do you suppose this is?

Group members _____

- 1) What is your idea for your independent experiment?

- 2) What are your independent and dependent variables?

- 3) What is your testable hypothesis?

- 4) Draw pictures of the graphs in the space below showing the expected results for each dependent variable.

Approved by lab instructor (1/group) _____

Psychophysiology II: Independent investigation

This week you will design your own experiments based on the parameters you measured last week in lab, namely skin conductance, temperature and pulse.

Introduction

You will spend the first 45 minutes of the laboratory conducting your experiments, the next 30 minutes analyzing your data, then presenting your data in an oral presentation during the remainder of the class time.

SmartBoard presentation

Once you have completed the experiment and analyzed the data you will be required to present the results to the rest of the class using the **Smart Board** in an oral presentation session. You should plan on talking for **5 minutes**. You should summarize your data by drawing a graph(s) or placing the data in a table.

In your presentation you should mention the following points:

- (1) What experiment you performed (what variable you tested)
- (2) The hypothesis you tested (your expectation)
- (3) What data you obtained (your results)
- (4) Whether your results were consistent with your predications
- (5) What you think the data mean (i.e., conclusions/significance of the results)
- (6) If the data were not as you expected and you could perform the experiment again, how might it be improved or done differently.

Follow the grading rubric closely.

Hematology

Introduction

Blood provides a medium for homeostasis in the cells' environment. Blood bathes cells within the human body and transports O₂ as well as nutrients to body cells while removing CO₂ and waste products. This transport system also serves to link the various organ systems of our body together, integrating them through the action of hormones. Blood also performs other not so obvious functions such as providing buffers for acid-base balance, destroying foreign organisms by phagocytosis and antibody action, distributing and conserving body heat, and preventing its own loss through hemostatic (coagulation) mechanisms. The average amount of blood in the human body is about 5 – 6 L in adult males and 4 – 5 L in adult females.

Blood is composed of a liquid portion, the plasma, and formed elements (red, white cells and platelets). In humans, **red blood cells** (RBC's) or erythrocytes make up about 45% of total blood volume, this figure is called the *hematocrit*. Leukocytes or **white blood cells** and **platelets** make up 1% of total blood volume, and the rest (about 54%) consists of **plasma**. Normal blood volume is approximately 8% of a person's weight. In a 70 Kg human, this equates to 5.6 L in volume. RBC's contain the protein **hemoglobin** which enables them to carry O₂ to body cells and CO₂ away from the cells. In male humans, the average amount of hemoglobin per 100 ml of red cells is 16 g. For females the average is around 13 g.

Blood contains 5 types of white cells (leukocytes), divided into **granulocytes** and **agranulocytes**. Granulocytes are named because they contain heavy pigmented granules in their cytoplasm, whereas agranulocytes contain no visible granules. Table 1 summarizes the different types of cells within human blood, how to identify them under a microscope, the number of cells you might expect to find, and the function of each cell type.

Human blood is characterized by **blood typing**. Blood typing is a system of classification based on the presence of specific glycoproteins on the outer surface of the RBC plasma membrane. Such proteins are called **agglutinogens**, and are genetically determined. In many cases these antigens are accompanied by plasma proteins, **antibodies** or **agglutinins**, that react with RBC's bearing different antigens, causing them to clump, agglutinate and even hemolyze. It is due to this phenomenon that a person's blood must be carefully typed before a blood transfusion. Several blood typing systems exist, based on a variety of possible antigens, but the factors routinely typed are for the **ABO** and **Rh blood groups**. The basis of the ABO typing system is shown in Table 2.

TABLE 17.2

Summary of Formed Elements of the Blood







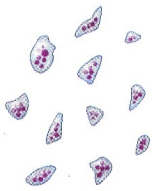
CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/ μL (mm^3) OF BLOOD
Erythrocytes (red blood cells, RBCs)		Biconcave, anucleate disc; salmon-colored; diameter 7–8 μm	4–6 million
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800
Granulocytes			
▪ Neutrophil		Nucleus multilobed; inconspicuous cytoplasmic granules; diameter 10–12 μm	3000–7000
▪ Eosinophil		Nucleus bilobed; red cytoplasmic granules; diameter 10–14 μm	100–400
▪ Basophil		Nucleus bilobed; large purplish-black cytoplasmic granules; diameter 10–14 μm	20–50
Agranulocytes			
▪ Lymphocyte		Nucleus spherical or indented; pale blue cytoplasm; diameter 5–17 μm	1500–3000
▪ Monocyte		Nucleus U or kidney shaped; gray-blue cytoplasm; diameter 14–24 μm	100–700
Platelets		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 μm	150,000–400,000

Table 2 ABO Blood Typing

ABO blood type	Antigens present on RBC membranes	Antibodies present in plasma	% of U.S. population		
			White	Black	Asian
A	A	Anti-B	40	27	28
B	B	Anti-A	11	20	27
AB	A and B	None	4	4	5
O	Neither	Anti-A and anti-B	45	49	40

Objectives

- To be able to identify the different types of leukocytes (WBC’s) under the microscope.
- To be able to determine the hematocrit from a sample of blood and to understand what is meant by the term hematocrit.
- To understand the role played by hemoglobin in blood and be able to determine the concentration of hemoglobin using the Tallquist method.
- To know how to test a person’s blood for the number of erythrocytes (RBC’s) and leukocytes (WBC’s), and to know what numbers are normal and abnormal.
- To understand what is meant by the term blood typing, and be able to determine blood types in commercially available blood samples.

Experimental Equipment

- | | |
|--|----------------------------|
| Sheep’s blood | Distilled water |
| Red and white blood cell counting blood | Kimwipes |
| Hematocrit tubes | Counters |
| Hematocrit reader | Compound microscopes |
| Hematocrit centrifuge | Prepared WBC slides |
| Critoseal | Coverslips |
| Reagents and instructions for blood typing | Pasteur pipettes and bulbs |
| Tallquist paper | |

Experimental procedure

White blood cell identification

Go to the microscopes on the back table and view the prepared slides of leukocytes. Be able to identify the different types of white blood cells in the microscopes using characteristics given to you in Table 1. If you have trouble with the identification ask your laboratory instructor for help.

(1) Hematocrit

Place a hematocrit tube in a tilted test tube of sheep’s blood. The blood will move into the tube by capillary action. Fill the hematocrit tube 3/4 full. Use critoseal to seal one end of the tube. Your instructor will show you how to place the tube inside the hematocrit centrifuge. Remember to place the sealed end towards the *outside* of the centrifuge. Once centrifuged, use the hematocrit reader to read the percentage of RBC’s in the sample. Again, your instructor will show you how to read the sample.

The hematocrit for my sample is: _____

(2) Hemoglobin determination

Tallquist method

You should perform this test in pairs. This test uses a book of Tallquist blotting paper and a color comparison chart having different intensities of red. These intensities correspond to different concentrations of hemoglobin (Hb) found in blood. Instructions for performing the test are given on the first page of the booklet.

Obtain a drop of blood from the test tubes on the front table and place it on a piece of blotting paper. *Before* the blood dries, match its color with the closest color on the comparison Chart. The number by each color represents the percentage of Hb in the blood. Read this number directly off the chart. The Hb concentration is: _____ g/dL for my sample.

(3) Red Blood Cell counting

RBC's are so numerous that they must first be diluted before counting. The procedure below describes how scientists perform a **dilution** using a device called a Thoma pipette. Your blood sample has been diluted for you, therefore you do not have to dilute your blood sample. **However, you do need to remember what the dilution factor is (i.e., by how much the blood is diluted).**

PROCEDURE FOR BLOOD SAMPLE DILUTION

Red and white blood cells are too numerous to count, therefore a dilution of the blood needs to be performed. This has already been done for you. Your instructor will explain how this operates during the pre-laboratory discussion. Basically a known amount of blood is aspirated into the pipette and an appropriate diluent is added. The resulting solution is mixed and pipetted into a hemocytometer (Fig. 20.2).

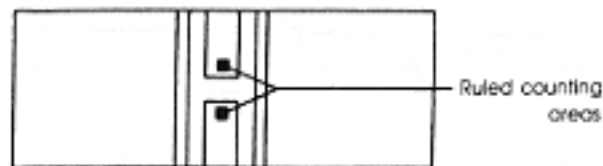


FIGURE 20.2. Hemocytometer.

STEPS YOU MUST DO

1. Place the hemocytometer on the microscope stage and see if you are able to see the counting grid (Fig. 20.2). If not, consult your lab instructor. Use low power (x100) to find the grid and high power (x400) to identify and count the RBC's.

2. Take the hemocytometer out from the microscope and place it on the bench top. Place a coverslip on the hemocytometer, then using a Pasteur pipette, aspirate a small volume of sample into wells directly beneath the coverslip as shown in Figure 1 below. Your lab instructor will show you how to perform this step.

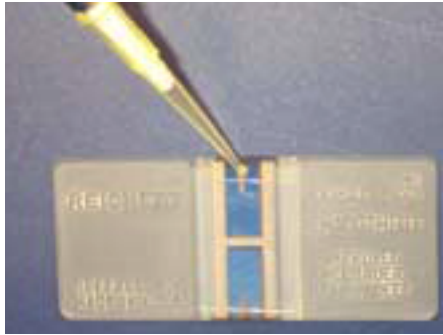


Figure 1. Loading of the hemocytometer.

3. Place the hemocytometer back on the microscope stage and see if you are able to see the counting grid and tiny red blood cells. If not, consult your lab instructor. They might appear green. Remember, use low power (x100) to find the grid and high power (x400) to identify and count the RBC's.

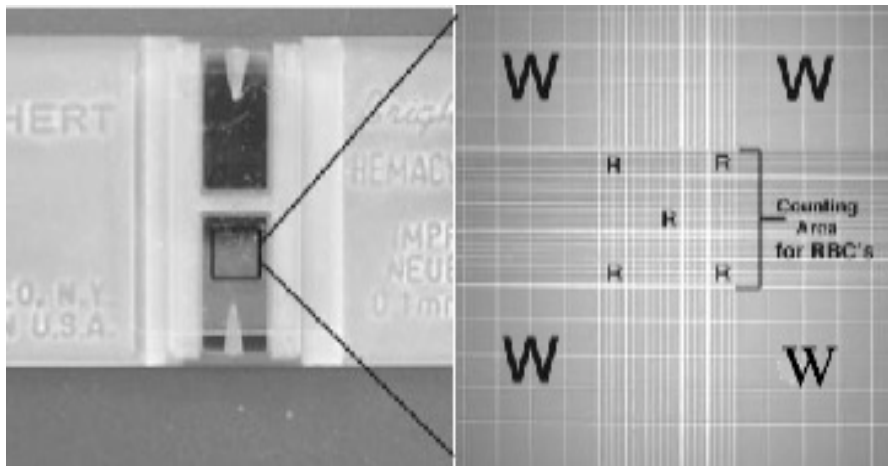


Figure 2. Counting grid for RBC's and WBC's

4. Count the number of RBC's in 5 squares (Fig. 2 above), and add these numbers together. In your counting you will notice that some cells touch the boundary lines around the squares. Count the cells that touch on two adjacent sides of the square and omit those that touch the other two sides. Which two sides you decide to count is up to you.

5. Calculate the number of RBC's per cubic mm of blood by taking into account the following multiplication factor:

$$\text{Multiplication factor} = \text{Number of RBC's} \times 10,000$$

For example, if your total number of RBC's is 600, then your RBC count is $600 \times 10,000 = 6,000,000$ RBC's/ mm^3 .

Your total RBC count is: _____ RBC's/ mm^3 .

In humans, the average number of RBC's for males is between 5.4 ± 0.8 million/ mm^3 , and for females 4.8 ± 0.6 million/ mm^3 .

6. Using your hematocrit and hemoglobin values, calculate the mean corpuscular hemoglobin concentration (MCHC) for your erythrocytes. **The normal value for humans is 32 - 36%.** The MCHC is a measure of the concentration of hemoglobin within a red blood cell. This measurement is useful in evaluating the clinical response of an anemic patient to therapy. Elevated MCHC is associated with spherocytosis (the production of spherical RBCs that are destroyed by the spleen). Diminished MCHC can be associated with iron deficiency, chronic blood loss or thalassemia.

$$\text{MCHC}(\%) = \frac{\text{Hb (g/dL blood)}}{\text{hematocrit}(\%)} \times 100$$

Your value is: _____.

7. Use your RBC count and hematocrit to calculate the average volume of your RBC's, i.e., the mean corpuscular volume (MCV). MCV is a measure of the average red blood cell volume (i.e. size). **The average value for humans is $87 \mu\text{m}^3$ with a range from 80 – 96 μm^3 .** The MCV is elevated if RBCs are larger than normal (macrocytic), for example in anemia caused by vitamin B12 deficiency. When the MCV is decreased, RBCs are smaller than normal (microcytic) as is seen in iron deficiency anemia or thalassemia.

$$\text{MCV}(\mu\text{m}^3) = \frac{\text{Hematocrit (\%RBC)} \times 10}{\text{RBC count (millions}/\text{mm}^3)}$$

Your value is: _____.

The above measures are useful because anemia may be caused by several factors, such as RBC frailty, maturation deficiency and hemorrhage. The above measures allow one to classify the types of anemia more precisely.

(4) White blood cell differential count

The white blood cell differential is often used as part of a complete blood count (CBC) as a general health check. It may be used to help diagnose the cause of a high or low white blood cell (WBC) count, as determined with a CBC. It may also be used to help diagnose and/or monitor other diseases and conditions that affect one or more different types of WBCs.

The differential totals the number of each WBC type and determines if the cells are present in normal proportion to one another, if one cell type is increased or decreased, or if immature cells are present. This information is useful in helping to diagnose specific illnesses.

Using the WBC image provided in the lab, count the number of different types of WBCs and place the information in the table below.

WBC Identity	Total WBC number	Differential count (Percent)
Neutrophil		
Eosinophil		
Monocyte		
Basophil		
Lymphocyte		

(5) Blood typing: Crime Scene Investigation

Purpose

Demonstrate your knowledge of blood typing process by completing this exercise.

Crime Scenario: You are a criminologist working for the Department of Justice crime lab for your county. You are called to the scene of a murder in town. The victim, Bill Ding, had apparently walked in on a burglary in progress. It is believed that the criminal, startled by the presence of the victim, attacked Mr. Ding. In his haste, the criminal cut his arm on the broken window that he had used to enter the apartment.

A small piece of clothing stained with the criminal's blood was taken from the scene of the crime. Four suspects have been identified who either knew the victim or were seen in the area before the body was discovered. Your task is to identify the blood type found on the weapon, to see if it indeed was used to kill Mr. Doe, and match the crime scene sample to one of the four suspects.

Before performing the blood typing, answer this question.

List all of the different blood types present in humans. What makes these blood types different from one another? Be specific.

2. Complete the table below to ensure you understand the theory behind blood typing. For a positive reaction write agglutination, for a negative reaction write -.

Blood Type	Antigens Present	Plasma antibodies present	Reaction w/ Anti-A Serum	Reaction w/ Anti-B Serum	Reaction w/ Anti-Rh Serum
A+					
A-					
B+					
B-					
AB+					
AB-					
O+					
O-					

Observations:

Set up your well plates as illustrated below.

Place 1 drop of each of the suspect's blood in each of 3 wells. In a separate well plate, place 1 drop of the crime scene blood in each of 3 wells.

Add 1 drop of the appropriate antiserum as shown below to each well and stir with a toothpick. Wait 2-3 min then read the samples.

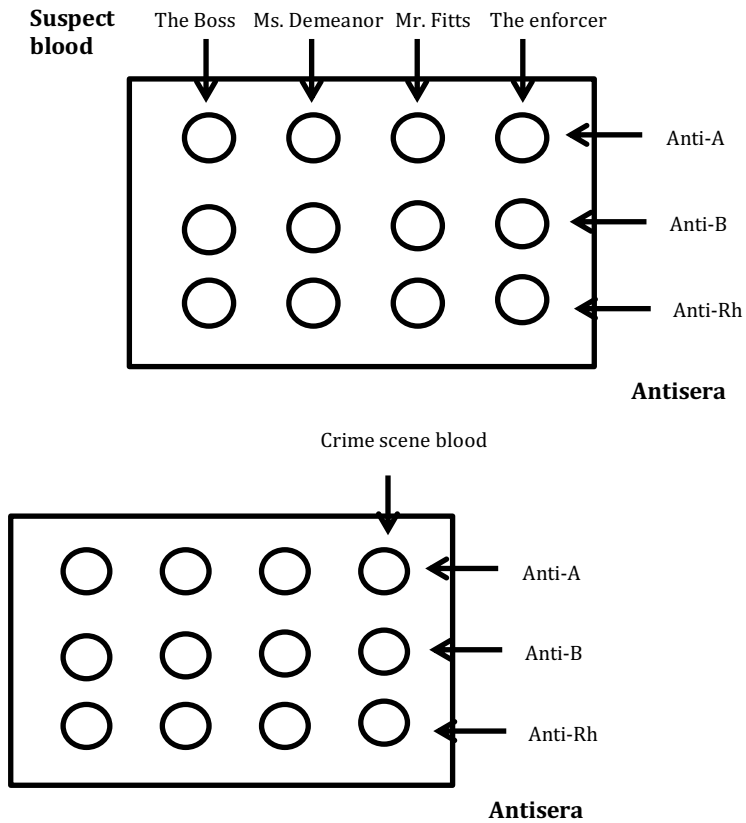


Table 1. Blood Agglutination (clumping) results

If the blood sticks together (agglutinates) after an anti-serum is added, then the suspect has that blood type.

Source	Observations			
	Anti-A Serum	Anti-B Serum	Rh Serum	Blood Type
Crime Scene and weapon sample				
Suspect #1 – John “The Boss” McLittle				
Suspect #2 – Miss Demeanor				
Suspect #3 – J.R. Fitts				
Suspect #4 – Ed “The enforcer”				

Conclusion

Based on the blood typing results, which of the four suspects most likely committed the burglary? Why?

Study Questions

1. What is anemia? Which blood measurements provide information on a possible anemic condition?

2. Polycythemia occurs in patients with chronic emphysema. Explain the mechanism responsible for this response.

3. How does hemoglobin carry both oxygen and carbon dioxide?

4. Why is inhaling automobile exhaust fumes (carbon monoxide) life threatening? Explain the physiological mechanism involved.

5. Why are hematocrits, hemoglobin concentrations and erythrocyte counts generally lower for females than for males?

Electrocardiography I and Heart Sounds

Introduction

The cardiovascular system consists of the heart and blood vessels. The heart is a multi-chambered organ that provides enough pressure to pump blood throughout the body. Along its route through the body, blood receives oxygen in the lungs (*pulmonary circuit*) and distributes it to the body tissues (*systemic circuit*). At the same time as blood becomes oxygenated it also collects carbon dioxide from the body tissues and transports it back to the lungs for expulsion from the body.

The beating of the heart is accompanied by both electrical activity and sound. The pattern of electrical activity produced by each heart beat cycle is called the **electrocardiogram** or ECG. The events that occur during a normal heartbeat, including electrical events as well as heart sounds, and changes in pressure due to changes in blood volume within the heart are all part of what is referred to as the *cardiac cycle*.

Background

The heart is a dual pump that pushes blood around the body and through the lungs. Blood enters the **atrial** chambers of the heart at a low pressure and leaves the **ventricles** at a higher pressure; it is this high arterial pressure that provides the energy to force blood through the circulatory system. Figure 1 shows the organization of the human heart and the circulatory system, in schematic form. Blood returning from the body arrives at the right side of the heart and is pumped through the lungs to pick up oxygen and release carbon dioxide. This oxygenated blood then arrives at the left side of the heart, from where it is pumped back to the body.

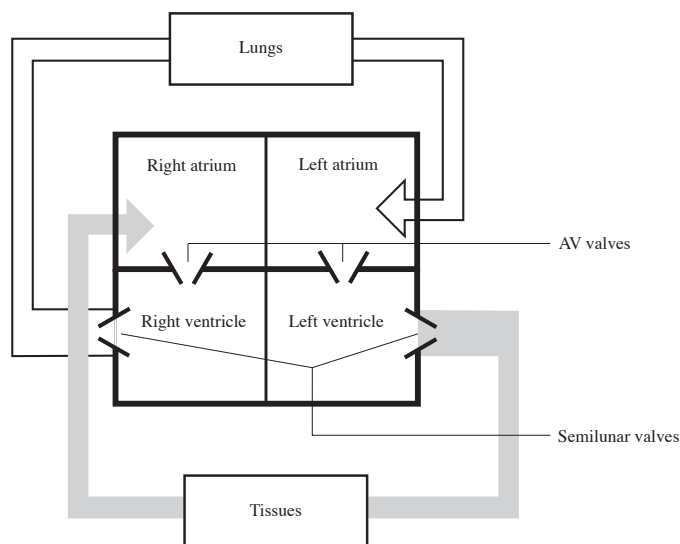


Figure 1. A schematic diagram of the human heart and circulatory system.

Cardiac contractions are not dependent upon a nerve supply. A group of weak muscle cells (sinoatrial or sinoatrial node, SA node) acts as the pacemaker for the heart (Fig. 2). These cells rhythmically produce action potentials that spread through the fibers of the atria. The resulting contraction pushes blood into the ventricles. The only electrical connection between the atria and the ventricles is via the atrioventricular (AV) node. The action potential spreads slowly through the AV node (thus giving a time delay for ventricular filling) and then rapidly through the AV bundle and Purkinje fibers to excite both ventricles. The large muscle mass of the ventricles allows powerful contractions.

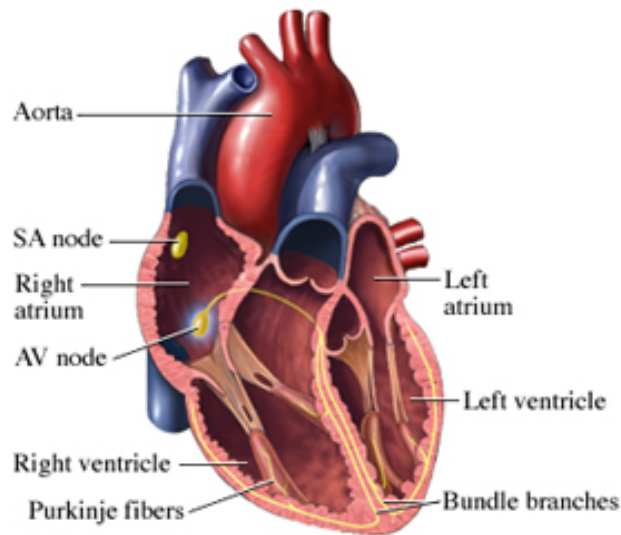


Figure 2. Components of the human heart involved in conduction.

Each side of the heart is provided with two valves, to convert rhythmic contractions into a unidirectional pumping. The valves close automatically whenever there is a pressure difference across the valve that would cause backflow of blood. Closure gives rise to audible vibrations (heart sounds). **Atrioventricular (AV) valves** between the atrium and ventricle on each side of the heart prevent backflow from ventricle to atrium. **Semilunar valves** are located between the ventricle and the artery on each side of the heart, and prevent backflow of blood from artery to ventricle. The cardiac cycle involves a sequential contraction of the atria and the ventricles. The combined electrical activity of the different myocardial cells produces electrical currents that spread through the body fluids. These currents are large enough to be detected by recording electrodes placed on the skin. The regular pattern of peaks produced by each heart beat cycle is called the electrocardiogram or ECG (Fig. 3).

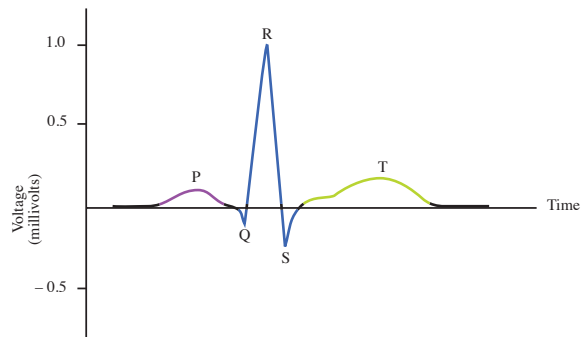


Figure 3. A typical ECG showing the fundamental parts that make up the signal.

The action potentials recorded from atrial and ventricular fibers are different from those recorded from nerves and skeletal muscle. The cardiac action potential is composed of three phases: a rapid depolarization, a plateau depolarization (which is very obvious in ventricular fibers), and a repolarization back to resting membrane potential. The components of the ECG can be correlated with the electrical activity of the atrial and ventricular muscle:

- the **P-wave** is produced by atrial depolarization
- the **QRS complex** is produced by ventricular depolarization; atrial repolarization also occurs during this time
- the **T-wave** is produced by ventricular repolarization.

The characteristic sound produced by the heart is usually referred to as a ‘lub-dub’ sound. The lower-pitched ‘lub’ sound occurs during the early phase of ventricular contraction and is produced by closing of the atrioventricular valves (the mitral valve and tricuspid valve), which prevent blood from flowing back into the atria. This represents the **onset of systole**. When the ventricles relax, the blood pressure drops below that in the artery and the semilunar valves (aortic and pulmonary) close, producing the higher-pitched ‘dub’ sound. This represents the **onset of diastole**.

Objectives

1. To be understand the gross anatomy of the human heart.
2. To understand the difference between the pulmonary and systemic circuit.
3. To be able to undertake an electrical recording of the human heart (i.e., ECG) and understand what causes each of the phases of an ECG.
4. To be able to hear heart sounds (auscultation) and correlate these with phases of the ECG.
5. To be able to measure blood pressure under different conditions.

Required Equipment

A computer system
LabChart software version
PowerLab (4/26T)
Five-lead Shielded Bio Amp Cable & snap-connect Lead Wires
Heart models
Disposable adhesive electrodes
Cardiomicrophone
Stethoscope
Alcohol swabs (70% ethanol on cotton wool)
Step stool

Assignment for the laboratory

Follow the instructions as outlined in the sections below to complete the experiments in this laboratory.

- Identify the structures outlined on Figures 4 and 5 on the heart models on display.
- Using Figure 6, understand the difference between the pulmonary and systemic circuit.
- Follow the directions to listen to auscultating heart sounds. You can hear heart sounds wearing a shirt.
- Follow the PowerLab procedures to record an ECG and heart sounds for ONE person in your group.
- Measure and record a patient's pulse from BOTH the position of the common carotid artery and radial artery when the person is quiescent, and compare these data to those after the patient has performed some degree of exercise. Place the data in the Table 3.
- Using the sphygmomanometers, measure blood pressure under conditions of different postures. Place the data in Table 4.

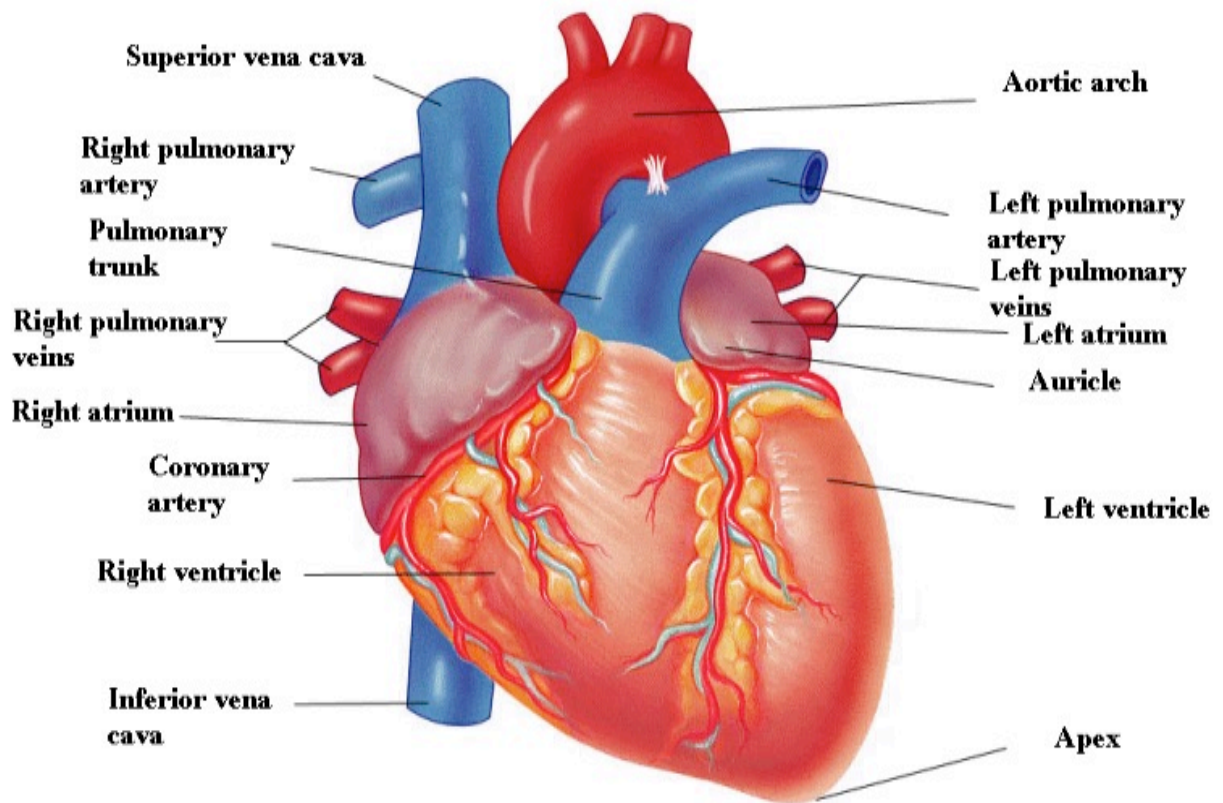


Figure 4. External anterior view of the human heart.

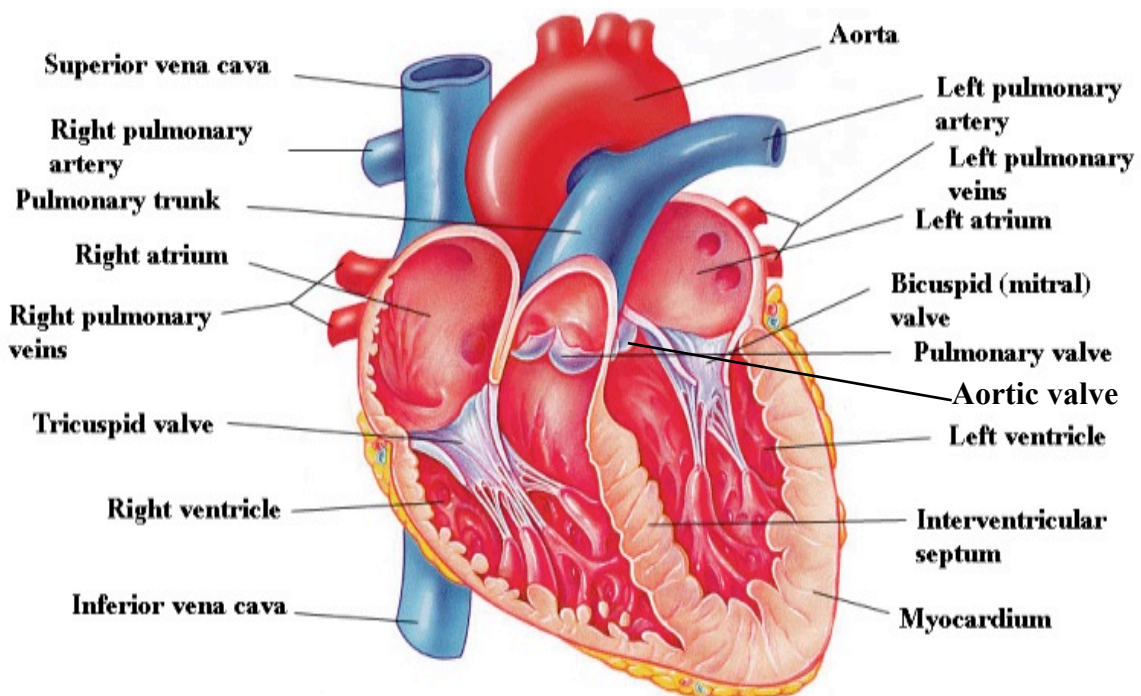


Figure 5. Frontal section of the human heart.

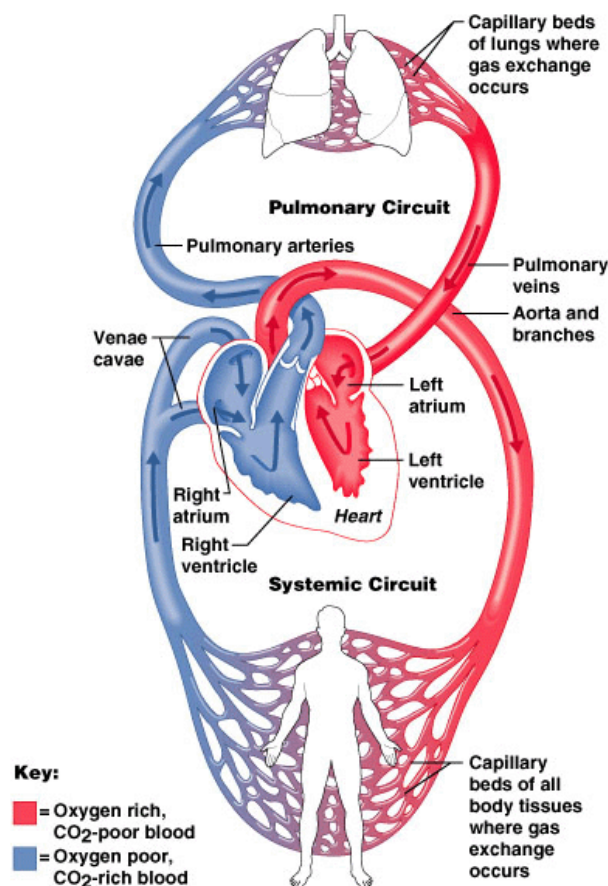


Figure 6. Systemic and pulmonary circuits in humans.

PowerLab Procedures

Subject preparation

The student volunteering for the experiment should remove any watch, jewelry and so on from his or her wrists and ankles.

1. Connect the cardiomicrophone to Input 1 (Fig. 7).
2. Plug the Bio Amp cable into the Bio Amp socket (Fig. 7).
3. Connect the leads to Earth, CH1 negative and CH1 positive, on the Bio Amp cable.
4. If you are using the disposable electrodes (which have electrode gel on them already), just attach the electrodes to the subject as shown in Fig. 8, and connect the electrodes to the leads.
5. Ensure the volunteer is relaxed and sits as still as possible to minimize any signal disturbance.

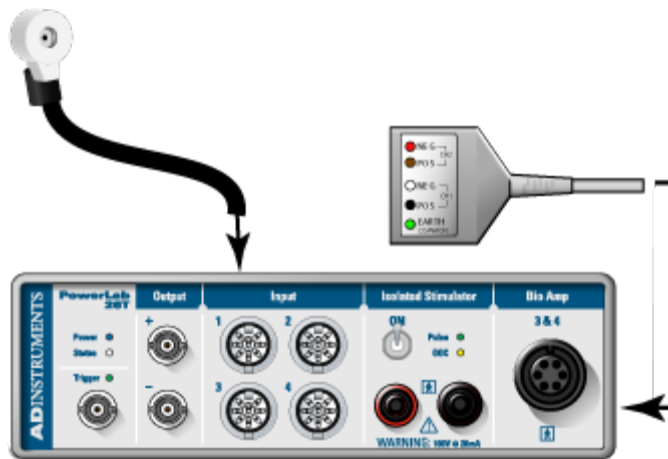


Figure 7. The equipment setup for this experiment.

Attaching the electrodes

6. Attach the positive electrode to the left wrist, the negative to the right wrist, and the ground to the right leg (Fig. 8).

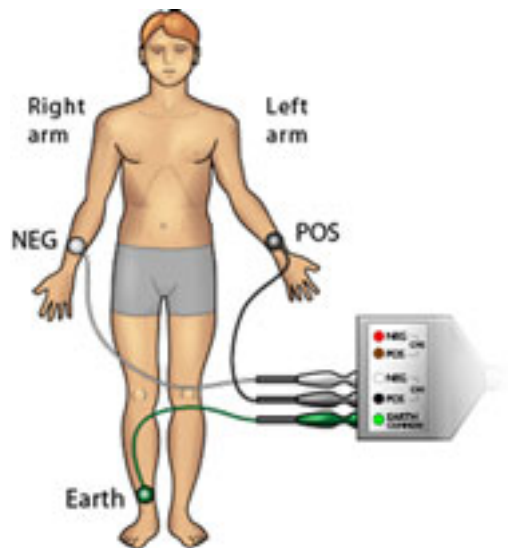


Figure 8. Connecting the electrodes to the volunteer.

Starting the software

To set up recording for this experiment, you load a settings file from the Experiments Gallery.

1. Locate LabChart on your computer and start the software in the usual way. If the Experiments Gallery dialog box does not appear in front of the LabChart window, choose the Experiments Gallery... command from the File menu.

2. In the Experiments Gallery dialog box, select this experiment (Go to Human Experiments, Cardiovascular Physiology, ECG and Heart Sounds, Settings Files). Select “Heart Sounds Settings” in the right-hand list, then click the Open button to apply those settings.

3. After a short time, the LabChart window on the computer screen should be set up for the experiment. Channel 1 should be named ‘Audio’ and Channel 3 should be named ‘ECG’. Ignore Channel 2.

4. You are now ready to begin the exercises. Remember to ensure that the volunteer is relaxed and sits as still as possible, to minimize any signal disturbance. The Bio Amp cable has a clip that can be used to fasten the cable and leads to the volunteer’s clothing.

Exercise 1: ECG in a resting volunteer

Objectives

To measure the ECG in a resting volunteer, and analyze the resultant signal. Also, to observe the effects of slight movement on the signals.

Procedure

Everything should be set up as described in the general notes above.

1. Choose the Bio Amplifier... item from the Channel 3 (ECG) Channel Function pop-up menu. Observe the signal.

If the ECG cannot be seen, check that all three electrodes are correctly attached. Adjust the range if necessary. If the signal is noisy and indistinct, make sure that the volunteer is relaxed.

2. Ask the volunteer to open and close their hands, and then move both arms across the chest. Note that the trace moves all over the place, and the ECG becomes distorted. This should show you why it is necessary to keep still and relaxed when recording the ECG.

3. Click the OK button to return to the LabChart window.

4. With the volunteer sitting quietly, click the Start button. When you have a suitable trace, type ‘Resting ECG, ’ and the volunteer’s name, and press the Return key on the keyboard to enter the comment. There should be a flat line in Channel 1 (Sounds) at this stage.

5. After 1 minute, click the Stop button to stop LabChart recording.

Analysis

Exercise 1: ECG in a resting volunteer

1. Use the View buttons in the LabChart window to set the horizontal **compression** to 50:1.
2. Scroll through your data and observe the regularly occurring ECG cycles.
3. Using the **Marker** and **Waveform Cursor**, measure the amplitudes (i.e., height) of three P waves, QRS complexes and T waves from the ECG trace. Place the data in Table 1. Now obtain an average of these measurements for each wave and place the result also in Table 1.
4. You will now measure the duration (i.e., time period) for different parts of the ECG. To do this, place the Marker on the ECG trace immediately before the wave of interest, move the Waveform Cursor to the end of the waveform, and read off the time from the Rate/Time display. The ' Δ ' symbol indicates that the value is the difference in time between the positions of the Marker and Waveform Cursor. Place the individual durations for each interval in the first row of Table 2. Collect data from other lab groups and add these to the remaining rows in Table 2.
5. Place the Marker before a QRS complex and use the View buttons in the LabChart window to compress the view horizontally to 50:1. Move the Waveform Cursor to the right until roughly a 15-second difference is shown in the Rate/Time display (this should appear as ' $\Delta 15s$ '). Count the number of QRS complexes between the Marker and the Waveform Cursor. Multiply this number by four to calculate resting heart rate in beats per minute (bpm). Using Table 2 copy down data for your entire class.

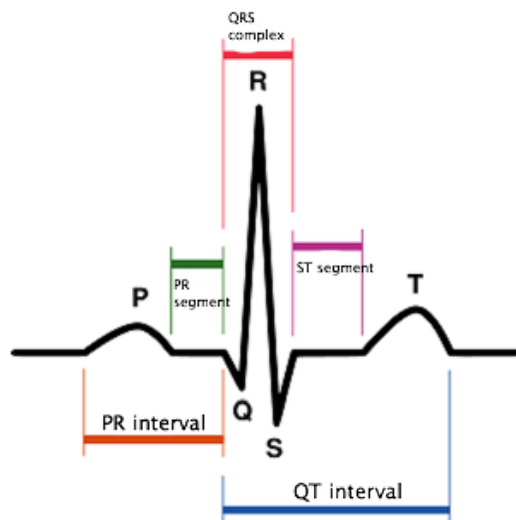


Figure 9. Use this Figure to help you fill out Table 2. You will need at least 2 ECG's to calculate the R-R interval.

Table 1. Results for the data analysis for Exercise 1.

Component	Amplitude (mV)
P wave	
QRS complex	
T wave	

Table 2. Group data results for Exercise 1.

Person's name	PR Interval (sec)	QT Interval (sec)	RR Interval (sec)	Heart rate (bpm)

Exercise 2: ECG and heart sounds

Objectives

To measure and correlate the ECG and heart sounds in a resting volunteer.

Using the Cardiomicrophone

Your tutor will briefly demonstrate how to use the cardiomicrophone. The microphone is not sensitive enough to pick up signals directly from the heart. Therefore, instead of placing the microphone over the heart, you will place the microphone over the carotid artery. The artery is close enough to the heart that the heart sounds can be recorded.

To record the sounds, have the patient place their index and middle finger over the carotid artery as shown in Figure 10a. Once the pulse is found, have another person place the microphone directly over the artery and hold it in place securely. It is very important to place the microphone over the middle of the carotid artery.



Figure 10a. Location of the carotid artery.

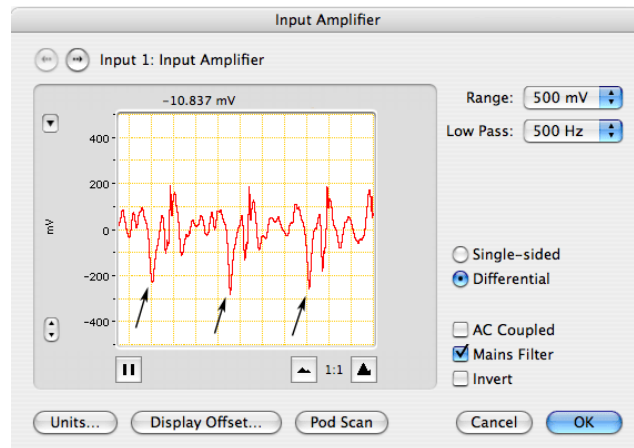


Figure 10b. Settings file on the input amplifier.

Procedure

You will be recording the ECG and heart sounds simultaneously. During the experiment a person (other than the volunteer) will hold the cardiomicrophone over the carotid artery to hear the heart sounds.

1. To open a settings file choose Human Experiments, Cardiovascular Physiology, ECG and Heart Sounds, Settings Files, Heart Sounds Settings.
2. Choose the Input Amplifier... item from the Channel 1 (Audio) Channel Function pop-up menu. You are interested in data collected from the Audio and ECG Channels.
3. Place the microphone directly over the carotid artery and move it until you begin to see a regular pattern of activity such as is seen in Fig. 10b. Arrows on the figure indicate the regular pattern. You may need to adjust the Range and low pass settings other than those

suggested on Fig. 10b to get the signal to work for the volunteer. **** It is very important that the volunteer and anyone close by does not talk or move during this procedure.****

4. Once you have a regular pattern close the Input Amplifier box by, clicking the OK button to return to the LabChart window.
5. Click the Start button to start recording both the ECG and heart sounds simultaneously. Record for 2 minutes. Remember during this time the patient must remain absolutely still and must not talk.
6. After two minutes click the Stop button to stop recording. Your tracing should resemble a regular pattern. You will now use PowerLab to smooth the tracing.
7. Choose the Digital Filter ... item from the Channel 1 (Sounds) Channel Function pop-up menu and type in the settings seen in Figure 11. You will see the Heart Sounds recording change to a more regular recording.
8. Choose Save from the File menu, and save the recording to the Desktop then onto a Flash drive.
9. Find a representative portion of the tracing that highlights both the ECG and heart sounds. Highlight both channels, go to the Zoom Window and print out the tracing, one for each group member. **Make certain you are able to correlate the timing of each sound with the electrical events from the tracings.**

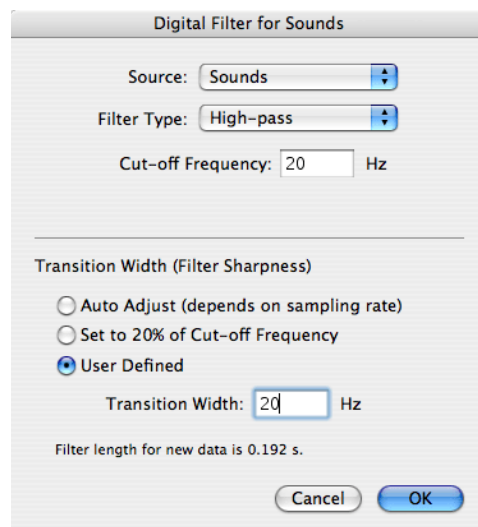


Figure 11. Settings for the Digital Filter.

Analysis

Exercise 2: ECG and heart sounds

1. **Select** a 5 – 6 second region of heart sound data using the mouse. Then highlight a region of the ECG simultaneously. To do this, use and highlight the same region on the ECG channel.
2. Select Zoom Window from the Window menu. The Zoom window appears with the Event and ECG signals overlaid. If not, click the small icon on the top right of the view window to overlay them.
3. Note the correlation between the Sounds and ECG signals.

The first ‘Sounds’ signal should be present (signaling the ‘lub’ sound) very soon after the QRS complex, and the second signal (signaling the ‘dup’ sound) at or shortly after the T wave.

What do you notice about the timing of the ECG and heart sounds? Why?

Measuring Blood Pressure

Measure blood pressure using the automatic blood pressure cuffs. Measure blood pressure in three different positions: sitting, standing and the supine (laying down) position. Wait at least 3 min. after laying down, 1 minute after sitting before taking their blood pressure. For the standing position take the BP immediately upon standing. Record your measurements in Table 4.

Table 4. Class data for blood pressure changes with posture.

Name	Supine (wait 3 min)	Sitting (wait 1 min)	Standing (immediately)

Study Questions

1. Describe the amplitude of the various waves in different cardiac cycles within the same individual?

2. Are the amplitudes and durations of the various waves in different individuals similar or very different? WHY?

3. The P wave and the QRS complex represent depolarization of the atrial and ventricular muscle respectively. Why does the QRS complex have the largest amplitude?

4. The range for a normal resting heart rate is 60 to 90 bpm. A trained athlete could have a resting heart rate of 45 to 60 bpm. Why might a very fit person have a slower heart rate than someone of average fitness?

Group members _____

- 1) What is your idea for your independent experiment?

- 2) What are your independent and dependent variables?

- 3) What is your testable hypothesis?

- 4) Draw pictures of the graphs in the space below showing the expected results for each dependent variable.

Approved by lab instructor (1/group) _____

Electrocardiography II

This week you will design your own experiments based on the parameters you measured last week in lab. You may either, measure heart rate from the ECG and the amplitude of the pulse using the PowerLab) OR and measure blood pressure (systolic and diastolic pressures) and pulse rate using the automatic BP cuffs.

Introduction

You will spend the first 45 minutes of the laboratory conducting your experiments, the next 30 minutes analyzing your data, then presenting your data in an oral presentation during the remainder of the class time.

SmartBoard presentation

Once you have completed the experiment and analyzed the data you will be required to present the results to the rest of the class using the **Smart Board** in an oral presentation session. You should plan on talking for **5 minutes**. You should summarize your data by drawing a graph(s) or placing the data in a table.

In your presentation you should mention the following points:

- (1) What experiment you performed (what variable you tested)
- (2) The hypothesis you tested (your expectation)
- (3) What data you obtained (your results)
- (4) Whether your results were consistent with your predications
- (5) What you think the data mean (i.e., conclusions/significance of the results)
- (6) If the data were not as you expected and you could perform the experiment again, how might it be improved or done differently.

Follow the grading rubric closely.

Respiratory Physiology I

Introduction

The two phases of **pulmonary ventilation** or breathing are **inhalation**, during which air is taken into the lungs, and **expiration**, during which air is expelled from the lungs. Inhalation is caused as a result of contraction of the external intercostal muscles and diaphragm. The diaphragm, normally a dome-shaped muscle, flattens as it moves inferiorly while the external intercostal muscles between the ribs lift the rib cage. These cooperative actions increase thoracic volume. The increase in volume causes a partial vacuum allowing air to rush into the lungs. During normal expiration, the inspiratory muscles relax, causing the diaphragm to rise and the chest wall to move inward. The thorax returns to its normal shape due to the elastic properties of the lung and thoracic wall. Although expiration is normally a passive process, abdominal muscles and the internal intercostal muscles can contract to force air from the lungs. Blowing up a balloon is an example where such **forced expiration** would occur.

Objectives

A. Computer-based portion

• Define the following terms:

Ventilation, inspiration, expiration, forced expiration, tidal volume, expiratory reserve volume, inspiratory reserve volume, residual volume, vital capacity, forced expiratory volume, minute respiratory volume, and surfactant.

- Understand that the lungs are not made of muscle and that respiration is therefore caused by external forces.
- Explore the effect of changing airway resistance on breathing.
- Study the effect of surfactant on lung function.
- Examine factors that cause lung collapse.
- Understand the effects of hyperventilation, rebreathing, and breath holding on the CO₂ level in the blood.

B. Anatomy-based portion

To be able to identify the anatomical structures comprising the human respiratory system.

Computer simulations using the program PhysioEx

Select the blue-colored Apple icon on the Dock on the computer. Choose the file **Start Here.html**. Select **Enter**, then **Exercise 7: Respiratory System Mechanics** from the drop down menu and click **Go**. Before you perform the activities, watch the **Water-**

filled **Spirometer** video to see the experiment with a human subject. Then click **Repirometry Volumes**. The opening screen should look like Figure 1 below.

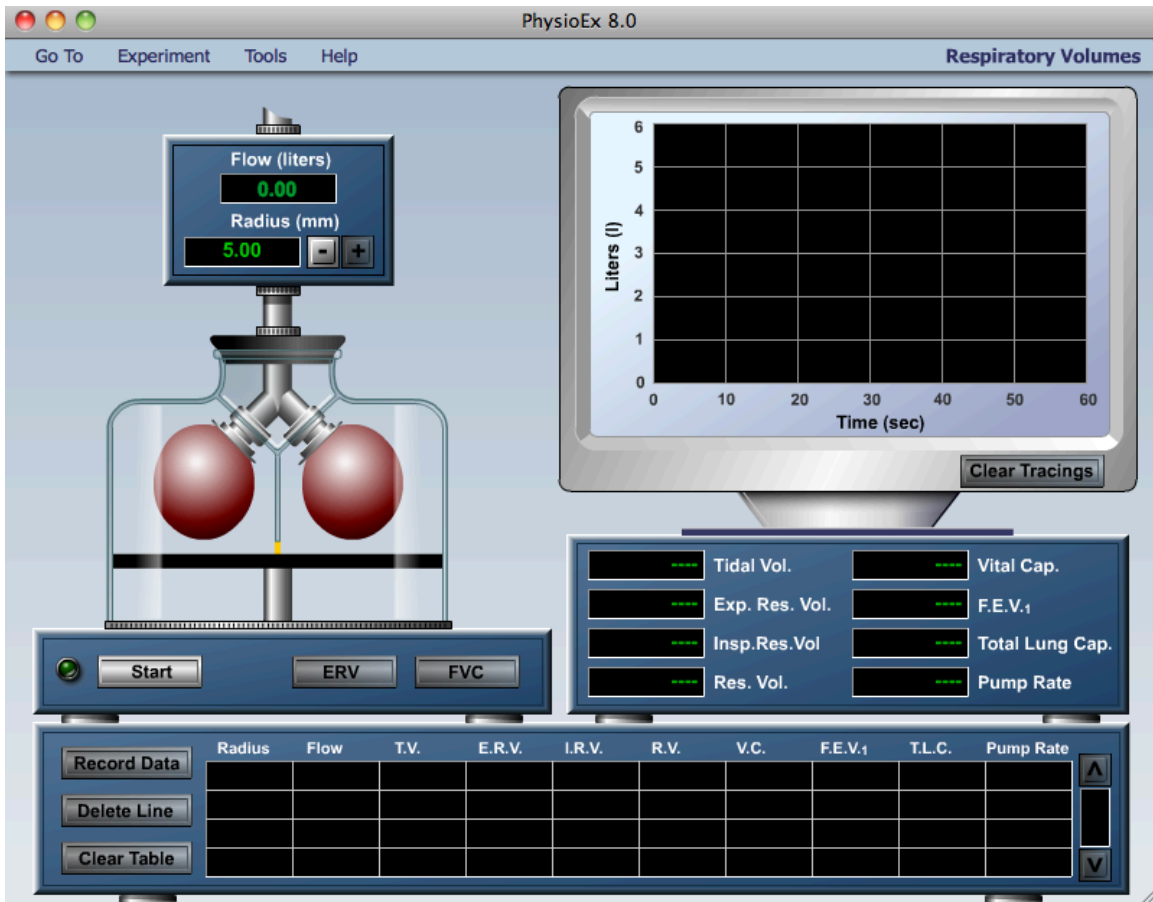


Figure 1.

The main features on the screen are the pair of *simulated lungs* inside the bell jar to the left, the *oscilloscope* to the right, the *data display area* to the right beneath the oscilloscope, and the *data control unit* at the bottom of the screen. The black rubber “diaphragm” sealing the bottom of the bell jar is attached to a rod in the pump below the jar. The rod moves the rubber diaphragm up and down to change the pressure within the bell jar (comparable to intrapleural pressure in the body). As the diaphragm moves inferiorly, the resulting volume increase creates a partial vacuum inside the jar because of lowered pressure. This vacuum causes air to be sucked into the tube at the top of the jar, and then into the simulated lungs. Conversely as the diaphragm moves up, the rising pressure inside the bell jar forces air out of the lungs. The partition between the two lungs compartmentalizes the bell jar into right and left sides. The lungs are connected to an inflow tube in which the diameter is adjustable by clicking the (+) and (-) buttons next to the Radius window the equipment atop the bell jar. The volume of each breath that passes through the single airflow tube above the bell jar is displayed in the Flow window.

Clicking **Start** below the bell jar begins a trial run in which the lungs will “breathe” in normal tidal volumes and the oscilloscope will display the tidal tracing. When **ERV** is clicked, the program will simulate forced exhalation utilizing the contraction of the internal intercostals and abdominal muscles, and the expired reserve volume will be displayed in the Exp. Res. Vol. window below the oscilloscope. When **FVC** is clicked, the lungs will inhale maximally then exhale fully to demonstrate forced vital capacity. After ERV and FVC have been measured, the remaining lung values will be calculated and displayed below the oscilloscope. The data control equipment displays the data collected. When you click **Record Data** the data will be recorded in the data grid. Clicking **Delete Line** allows you to delete the highlighted line and **Clear Table** the contents of the entire table.

Activity 1: Measuring Respiratory Volumes

1. Adjust the radius of the airways to 5.00 mm by clicking the appropriate button next to the Radius window.
2. Click **Start** and allow the tracing to complete. Watch the lungs begin to breathe. At the same time the oscilloscope will display a tracing of the tidal volume for each breath. The flow window atop the bell jar indicates the tidal volume for each breath, and the tidal volume window below the oscilloscope shows the average tidal volume. The pump rate displays the number of breaths.
3. Click **Clear Tracings**.
4. Click **Start** again. After a second or two click **ERV** wait 2 seconds and then click **FVC** to complete the measurement of respiratory volumes. ERV, IRV and RV will be displayed from the tests you have performed thus far. TLC is also calculated.
5. Compute the **minute respiratory volume (MRV)** using the formula:

$$\text{MRV} = \text{TV} \times \text{BPM (breaths per minute)}$$

$$\text{MRV} = \text{_____ ml/min.}$$

Does ERV include tidal volume? _____
 Explain your answer: _____

6. Click **Record Data** to record the current experimental data in the data grid. Then click **Clear Tracings**.

Activity 2: The effect of changing airway resistance on respiratory volumes.

Lung diseases are often classified as obstructive or restrictive. When obstructive, expiratory flow is affected, when restrictive there might be a reduction in inspiratory volume. Pulmonary function tests such as FEV1 help to clinically determine the difference between obstructive and restrictive problems. In obstructive disorders such as bronchitis and asthma, airway resistance is increased and FEV1 will be low. We will first examine the effect of a change in diameter of the airway on pulmonary function.

1. Do *not* clear the data table from the previous experiment.
2. Adjust the radius of the airways to 4.5 mm by clicking the (-) button next to the Radius window.
3. Click **Start** to begin the respirations.
4. Click **FVC**.
5. When the lungs stop respiring. Click **Record Data** to record the current data in the data grid.
6. Decrease the airway radius in 0.5 mm decrements and repeat steps 4 and 5 until the minimum radius of 3.0 mm is achieved. Click **Clear Tracings** between trials. Write down the data in the table below.
7. A useful way of expressing FEV1 is as a percentage of the forced vital capacity. Copy the FEV1 and vital capacity values from the computer screen to the table below, and then calculate the FEV1 (%) by dividing the FEV1 volume by the vital capacity volume and multiply by 100. Record the FEV1 (%) in the table below.

FEV1 as % of FVC			
Radius (mm)	FEV1 (ml)	Vital Capacity (ml)	FEV1 (%)
5.00			
4.50			
4.00			
3.50			
3.00			

What happened to the FEV1 (%) as the radius of the airways was decreased? _____

Explain your answer: _____

Simulating factors affecting respirations

This part of the simulation allows you to examine the action of surfactant on pulmonary function and the effect of changing the intrapleural pressure.

Choose **Factors Affecting Respirations** from the **Experiment** menu. The screen should look like Figure 2.

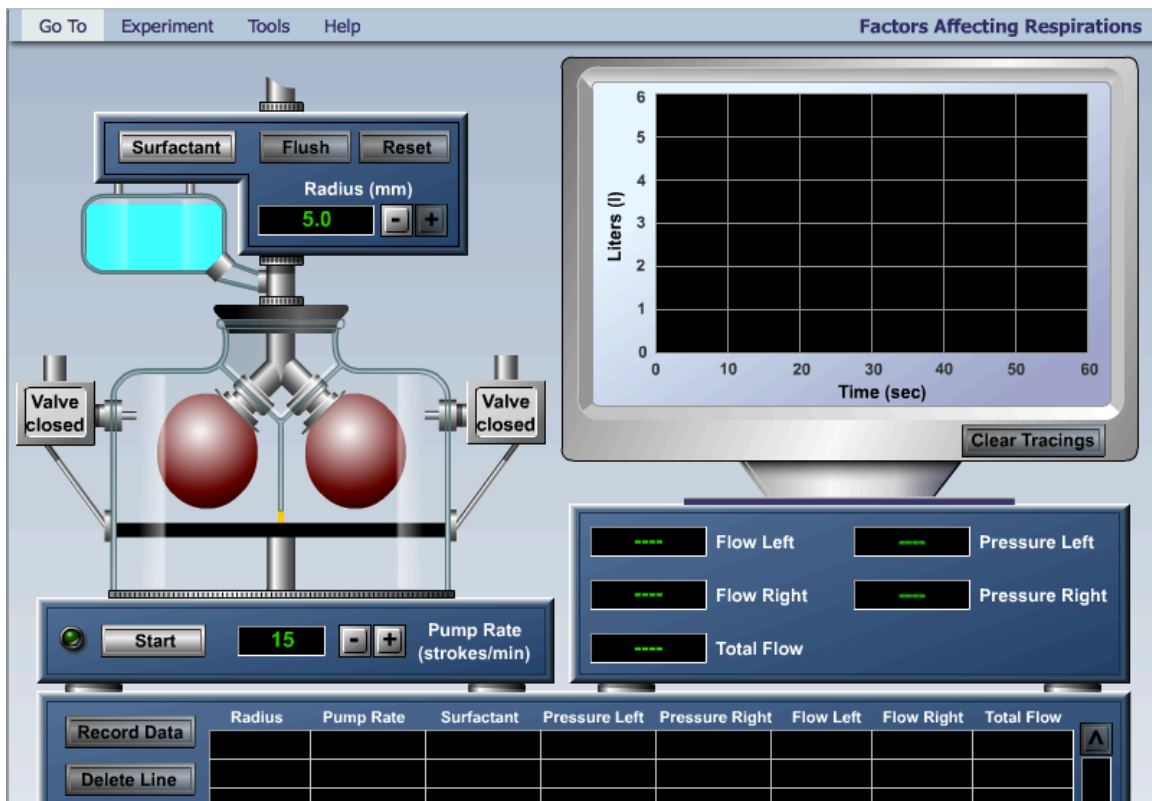


Figure 2.

The basic features on the screen when the program starts are the same as before. Additional equipment includes a surfactant dispenser atop the bell jar and valves on each side of the jar. Each time **Surfactant** is clicked, a measured amount is sprayed onto the lungs. Clicking **Flush** washes surfactant from the lungs to prepare for another run.

Clicking the valve button (currently reads closed) allows the pressure on each side of the bell jar to equalize with atmospheric pressure. When **Reset** is clicked, the lungs are prepared for another run.

Data accumulated during a run is displayed below the oscilloscope. When you click **Record Data**, that data are recorded.

Activity 3: Examining the effect of surfactant.

At any gas-liquid boundary, the molecules of the liquid are attracted more strongly to each other than they are to the air molecules. This unequal attraction provides tension at the liquid surface called *surface tension*. Because the surface tension resists any force that tends to increase surface area, it acts to decrease the size of the hollow spaces, such as the alveoli. The inner air sacs of the lungs are lined with surfactant, a detergent like lipoprotein that decreases surface tension by reducing the attraction of water molecules for each other. In this experiment you will examine the action of surfactant.

1. Adjust the airway radius to 5.00 mm.

2. If necessary click **Flush** to clear the lungs of existing surfactant.
3. Click **Start**, and allow the baseline run without surfactant to complete.
4. When the run completes, click **Record Data**.
5. Now click **Surfactant** twice.
6. Click **Start** to begin the surfactant run.
7. When the lungs stop respiring, click **Record Data** to display the data in the grid.

How has the airflow changed compared to the baseline run? _____

Premature infants often have difficulty breathing. Explain why this might also be so.

Activity 4: Investigating intrapleural pressure.

The pressure within the pleural cavity, **intrapleural pressure**, is less than the pressure within the alveoli. This negative pressure condition is caused by two forces, the tendency of the lung to recoil due to its elastic properties and the surface tension of the alveolar fluid. These two factors act to pull the lungs away from the thoracic wall, creating a partial vacuum in the pleural cavity. Because the pressure in the intrapleural space is lower than atmospheric pressure, any opening caused by the thoracic wall equalizes the intrapleural pressure with the atmospheric pressure by allowing air to enter the pleural cavity, a condition called **pneumothorax**. Pneumothorax allows lung collapse, a condition known as **atelectasis**.

In the simulated respiratory system, the intrapleural space is the space between the wall of the bell jar and the outer wall of the lungs it contains. The pressure difference between inspiration and expiration for the left lung is displayed in the Pressure Left window below the oscilloscope: the Pressure Right window gives the data for the right lung.

1. Do *not* discard your previous data.
2. Click **Clear Tracings** to clean up the screen, and then click **Flush** to clear the lungs of surfactant from the previous run.
3. Adjust the radius of the airways to 5.00 mm by clicking the appropriate button next to the Radius window.
4. Click **Start**, and allow one screen of respirations to complete. Notice the negative pressure condition displayed below the oscilloscope when the lungs inflate.
5. When the lungs stop respiring, click **Record Data** to display the data in the grid.
6. Now click the valve button (which currently reads **Valve Closed**) on the left side of the bell jar above the Start button to open the valve.
7. Click **Start** to begin the run.

8. When the run completes, click **Record Data** again.

What happened to the lung in the left side of the bell jar? _____

How did the pressure in the left lung differ from that in the right lung? _____

Explain. _____

How did the total airflow in this trial compare with that in the previous trial in which the pleural cavities were intact? _____

What do you think would happen if the two lungs were in a single cavity instead of separate cavities? _____

9. Now close the valve you opened earlier by clicking it again, and then click **Start** to begin a new trial.

10. When the run completes, click **Record Data** to display the data in the grid.

Did the deflated lung re-inflate? _____

Explain. _____

11. Click the **Reset** button atop the bell jar. This action draws the air out of the intrapleural space and returns it out of the normal resting condition.

12. Click **Start**, and allow the run to complete.

13. When the run completes, click **Record Data** to display the data in the grid.

Why did lung function in the deflated (left) lung return to normal after you clicked Reset?

Simulating variations in breathing

This part of the simulation allows you to examine the effects of hyperventilation, rebreathing, and breath holding on CO₂ level in the blood.

Choose **Variations in Breathing** from the **Experiment** menu. The opening screen will appear below (Fig. 3). The buttons beneath the oscilloscope control the various possible breathing patterns. Clicking **Hyperventilation** causes the lungs to breath faster than normal. A small bag automatically covers the airway tube when **Rebreathing** is clicked. Clicking **Breath holding** causes the lungs to stop respiring. Click **Normal Breathing** at any time to resume normal tidal cycles. The window next to the start button displays the breathing pattern being performed by the simulated lungs.

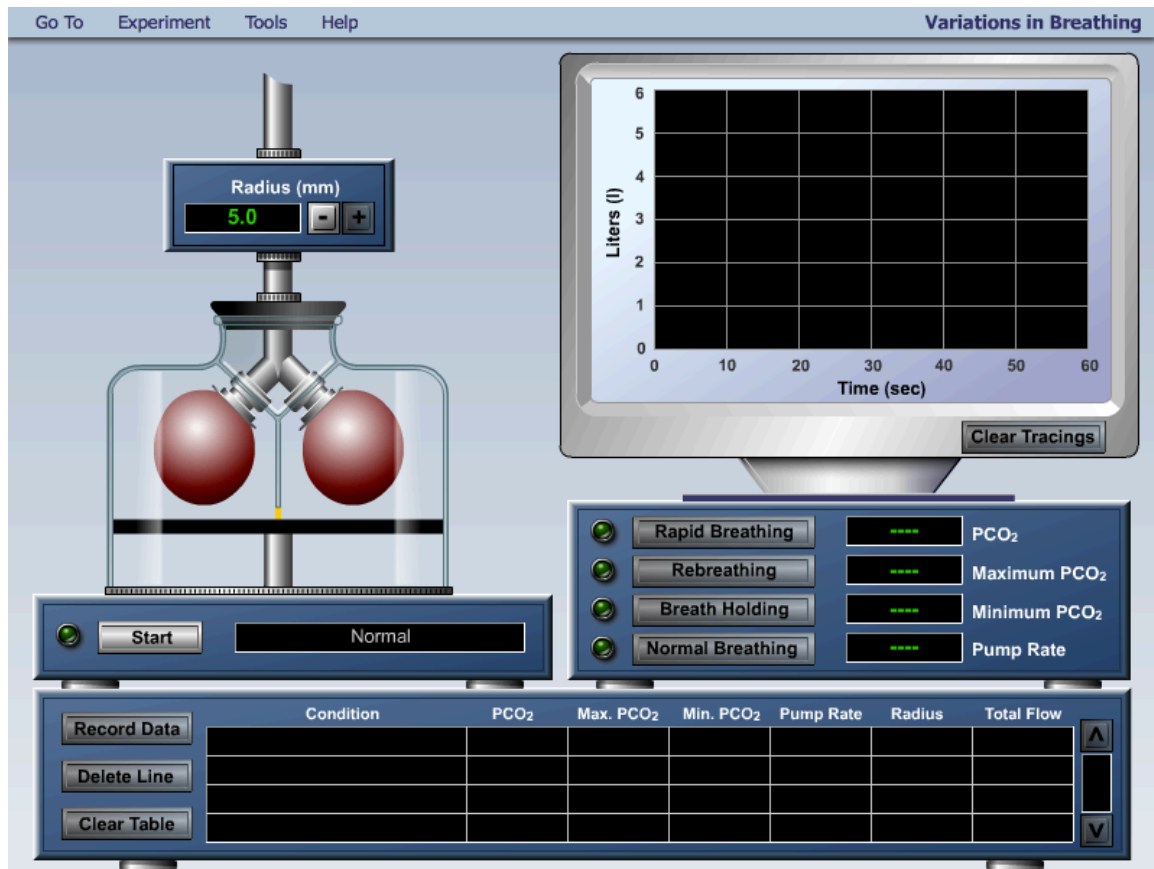


Figure 3.

The windows below the oscilloscope display the PCO₂ of the air in the lungs, Maximum PCO₂, Minimum PCO₂, and Pump Rate.

Data cumulated during the run are displayed in the window below the oscilloscope. When you click **Record Data**, data appear in the data grid. **Delete Line** and **Clear Table** delete a line of data or the entire table respectively.

Activity 5: Exploring Various Breathing Patterns

You will establish the baseline respiratory values in the first part of this experiment.

1. Adjust the radius of the airways to 5.00 mm by clicking the appropriate button next to the Radius window. Now read through steps 2 – 4 before attempting to execute them.
2. Click **Start**, and notice that it changes to **Stop** to allow you to stop the respiration. Watch the simulated lungs being to respire as a result of external mechanical forces supplied the pump below the bell jar. Simultaneously, the oscilloscope will display a tracing of the tidal volume for each breath.
3. After 2 seconds, click the **Rapid Breathing** button and watch the PCO_2 displays. The breathing pattern will change to short, rapid breaths. The PCO_2 of the air in the lungs will be displayed in a small window to the right of the Hyperventilation button.
4. Watch the oscilloscope display and the PCO_2 window, and click **Stop** before the tracing reaches the end of the screen.
5. Click **Record Data**.

What happens to the PCO_2 during rapid breathing? _____

Rebreathing

When **Rebreathing** is clicked, a small bag will appear over the end of the air tube to allow the air within the lungs to be repeatedly inspired and expired.

1. Click **Start**, wait 2 seconds, and then click **Rebreathing**.
2. Watch the breathing pattern on the oscilloscope, and notice the PCO_2 during the course of the run. Click **Stop** when the tracing reaches the right edge of the oscilloscope.

What happens to PCO_2 during the entire time of the rebreathing activity? _____

Did the depth of the breathing pattern change during rebreathing? Carefully examine the tracing for the rate and depth changes, as the changes can be subtle). Explain your observations. _____

3. Click **Record Data**, and then click **Clear Tracings** to prepare for the next run.

Breath Holding

Breath holding can be considered an extreme form of rebreathing in which there is no gas exchange between the atmosphere and the air within the lungs.

1. Click **Start**, wait a second or two, and then click **Breath Holding**.
2. Let the breath holding activity continue for 5 seconds, and then click **Normal Breathing**.
3. Click **Stop** when the tracing reaches the right edge of the oscilloscope.
4. Click **Record Data**.

What happened to the PCO_2 during breath holding? _____

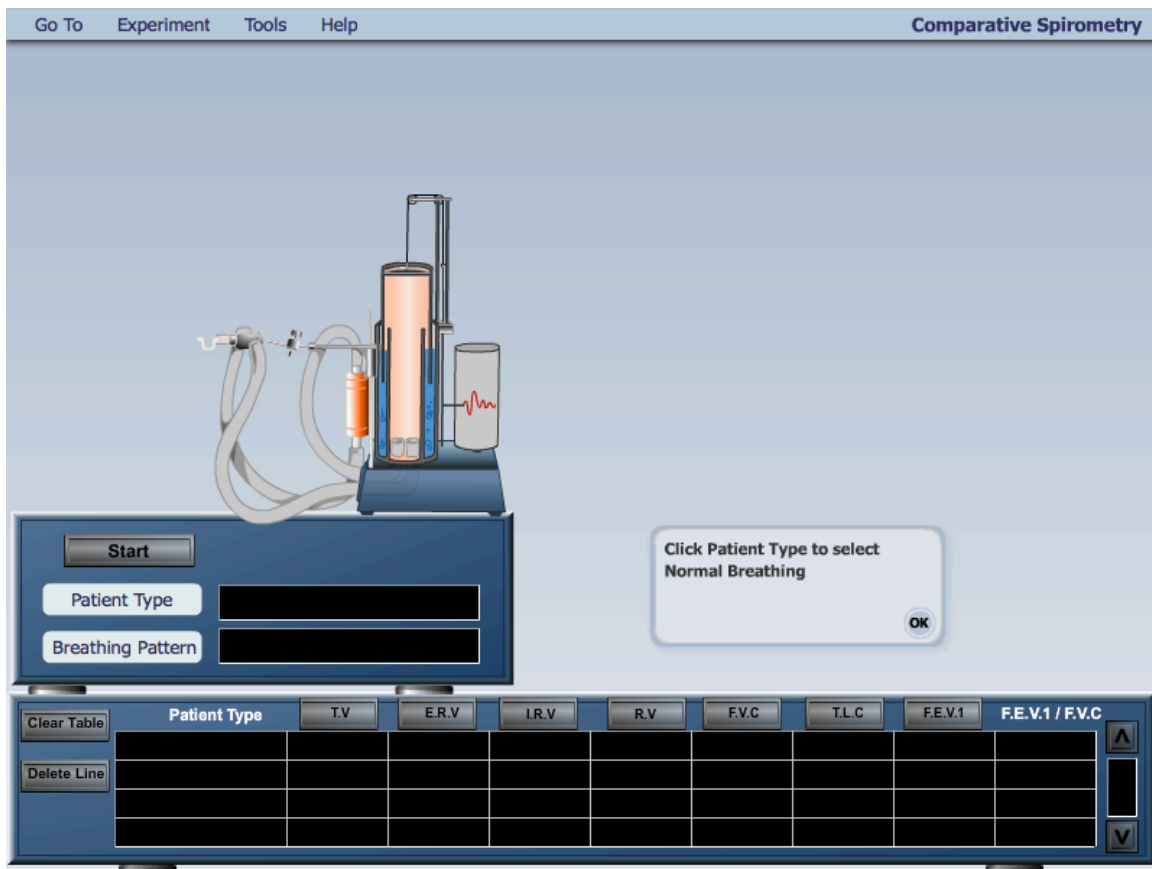
What happens to the breathing pattern when normal respiration resumes? _____

Activity 6: Comparing Spirometry

In activity 1, normal respiratory volumes and capacities were measured. In this activity, you will explore what happens to these values when pathophysiology develops or during episodes of aerobic exercise. Using a water-filled spirometer and knowledge of respiratory mechanisms, changes to these values in each condition can be predicated, documented, and explained.

Normal Breathing

1. Click the **Experiment** menu, and then click **Comparative Spirometer**. The opening screen (Fig. 4) will appear in a few seconds.



2. For the patient's type of breathing, select the **Normal** option from the drop down menu in the **Patient Type** box. These values will serve as a basis of comparison in the diseased conditions.
3. Select the patient's breathing pattern as **Unforced Breathing** from the **Breathing Pattern Option** box.
4. Click the **Start** button and watch as the drum starts turning and the spirogram develops on the paper rolling off the drum across the screen, left to right.
5. When half the screen filled with unforced tidal volumes and the trace has paused, select the **Forced Vital Capacity** button in the Breathing Pattern Options box.
6. Click the **Start** button and the trace will continue with the FVC maneuver. The trace ends as the paper rolls to the edge of the screen.
7. Now click on the individual measure buttons that appear in the data table above each data column to measure the lung volume and lung capacity data. Note that when a measure button is selected, two things happen simultaneously: (1) a bracket appears on the spirogram to indicate where the measurement originates on the spirogram, and (2) the value in ml appears in the data table. Also note that when the FEV₁ measure button is selected, the final column labeled FEV₁/FVC will automatically be calculated and appear in the data table. The calculation is (FEV₁/FVC) x 100%, and the result will appear as a percentage in the data table.
8. Place the data in your table.

What do you think is the clinical importance of the FVC and FEV₁ values? _____

Why do you think the ratio of these values is important to clinicians when diagnosing respiratory diseases? _____

FEV₁/FVC x 100% = _____

Emphysema Breathing

In a person with emphysema, there is a significant loss of intrinsic elastic recoil in the lung tissue. This loss occurs as the disease destroys the walls of the alveoli. Airway resistance is also increased as the lung tissue becomes more flimsy and exerts less mechanical tethering on the surrounding airways. Thus the lung becomes overly compliant and expands easily. Conversely a great effort is required to exhale as the lungs can no longer passively recoil and deflate. A noticeable and exhausting muscular effort is required for each exhalation. Thus a person with emphysema exhales slowly.

1. Using this information, predict what lung values will change in the spirogram when a patient with emphysema breathing is selected. Assume that significant disease has developed, and thus a loss of elastic recoil has occurred in the patient's lungs.
2. Select **Emphysema** from the drop down menu in the **Patient Type** box.
3. Select the patient's breathing pattern as **Unforced Breathing** from the **Breathing Pattern** box.
4. After the selections are made and the existing spirogram screen clears, click the **Start** button and watch as the drum starts turning and a new spirogram develops on the paper rolling of the drum.
5. Repeat steps 5 – 7 of the Normal Breathing section in this activity.
6. Place the data in the data grid.
7. Now consider the accuracy of your predictions (what changed compared to what you expected).

Compared to values of normal breathing:

Is the FVC reduced or increased? _____

Is the FEV₁ reduced or increased? _____

Which of these two changed more? _____

Explain the physiological reasoning for the lung volumes and capacities in the spirogram for this condition. _____

Acute Asthma Attack Breathing

During an asthma attack, bronchiole smooth muscle will spasm and thus airways become constricted. They also become clogged with thick mucous secretions. This leads to significantly increased airway resistance. This inflammatory response can be brought about by triggers such as allergens (dust, pollen etc.) and even exercise. During an asthma attack the airways collapse and pinch closed before a forced expiration is completed. Thus the volume and peak flow rates are significantly reduced during an attack. However, the elastic recoil is not diminished during an attack.

1. Using this information, predict what lung values will change in the spirogram when a patient who is having an asthma attack is selected. Assume that significantly decreased airway radius and increased airway resistance have developed in the patient's lungs.
2. Select **Asthma** from the drop down menu in the **Patient Type** box.
3. Select the patient's breathing pattern as **Unforced Breathing** from the **Breathing Pattern** box.
4. After the selections are made and the existing spirogram screen clears, click the **Start** button and watch as the drum starts turning and a new spirogram develops on the paper rolling of the drum.
5. Repeat steps 5 – 7 of the Normal Breathing section in this activity.
6. Place the data in the data grid.
7. Now consider the accuracy of your predictions (what changed compared to what you expected).

Compared to values of normal breathing:

Is the FVC reduced or increased? _____

Is the FEV₁ reduced or increased? _____

Which of these two changed more? _____

Explain the physiological reasoning for the lung volumes and capacities in the spirogram for this condition. _____

How is this condition similar to having emphysema? How is it different? _____

B. Anatomy-based portion of lab

Objective

To be able to identify the anatomical structures comprising the human respiratory system.

Procedures

Identify the anatomical structures below on the models on display. Use Figs 2 - 4 to identify the structures.

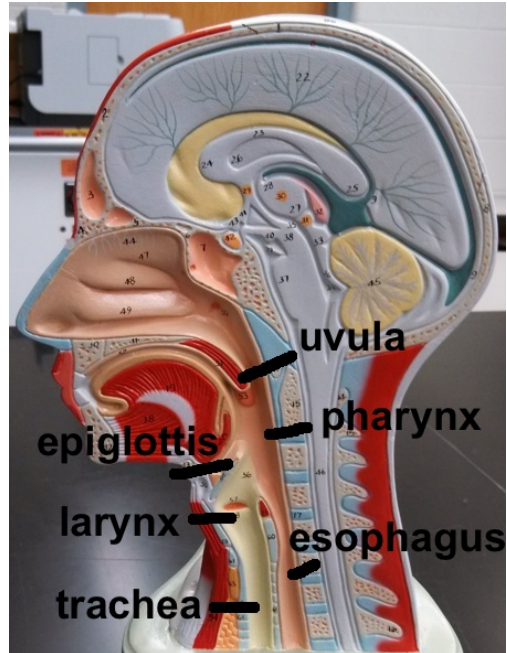


Figure 2. Structures of the upper respiratory tract.

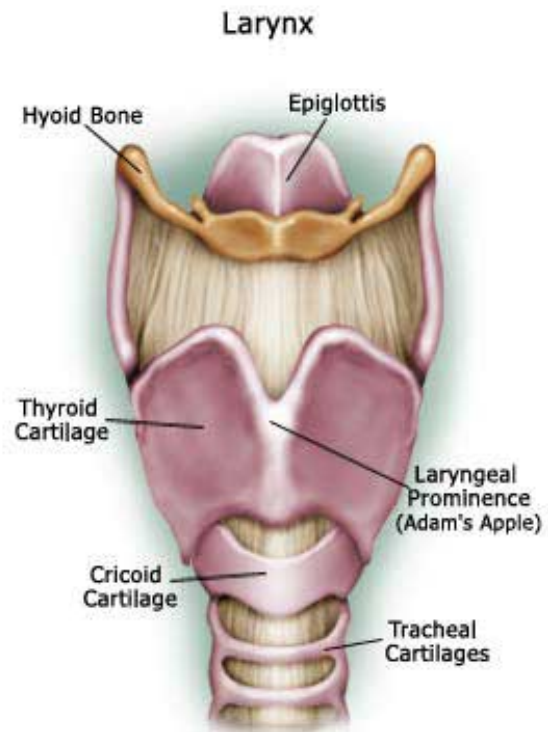


Figure 3. Structure of the larynx (anterior view).

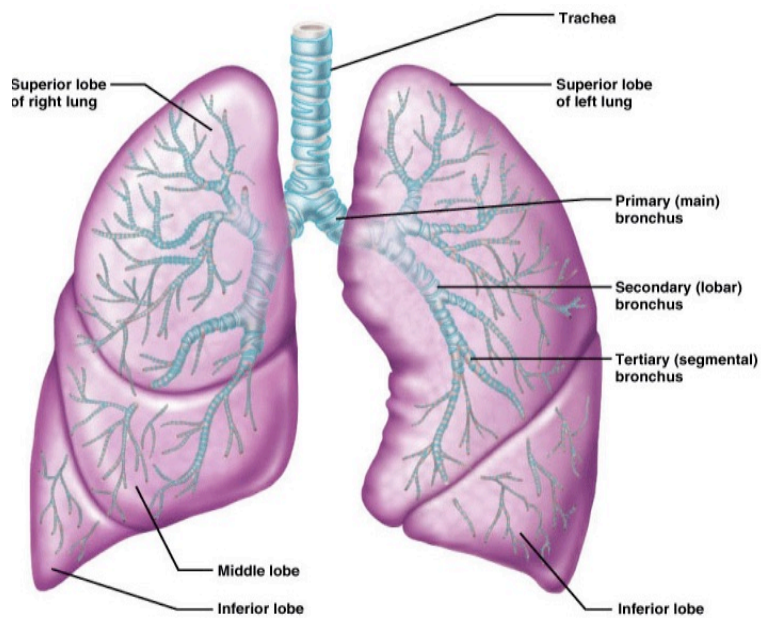


Figure 4. Structures of the lower respiratory tract.

Respiratory Physiology II

In this experiment, you will be introduced to spirometry as a technique for recording respiratory variables and you will analyze a recording to derive respiratory parameters. You will examine lung volumes and capacities, as well as basic tests of pulmonary function.

Background - Spirometry

Gas exchange between air and blood occurs in the alveolar air sacs. The efficiency of gas exchange is dependent on ventilation; cyclical breathing movements alternately inflate and deflate the alveolar air sacs. Inspiration fills the alveoli with fresh atmospheric air and expiration removes stale air, which has reduced oxygen and increased carbon dioxide concentrations.

Many important aspects of lung function can be determined by measuring airflow and the corresponding changes in lung volume using spirometry. Spirometry allows many components of pulmonary function (Fig. 1) to be visualized, measured and calculated. Respiration consists of repeating cycles of inspiration followed by expiration. During the respiratory cycle, a specific volume of air is drawn into and then expired out of the lungs; this volume is the Tidal Volume (V_T). In normal ventilation, the breathing frequency (f) is 12 - 15 respiratory cycles per minute. This value varies with the level of activity. The product of f and V_T is the **Expired Minute Volume** (\dot{V}_E), the amount of air exhaled in one minute of breathing. All these parameters may change according to a person's height, weight and level of activity.

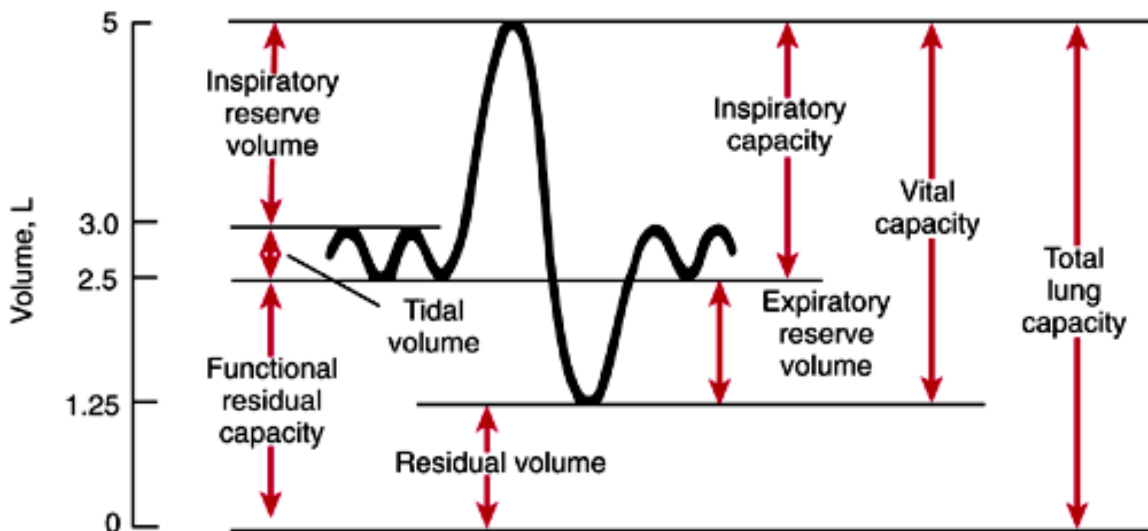


Figure 1. Lung volumes and capacities. **Tidal volume** (V_T): the amount of air inspired or expired during normal, quiet respiration, **Inspiratory reserve volume** (IRV): The amount of air forcefully inspired above and beyond normal inspiration, **Expiratory reserve volume** (ERV): The amount of air forcefully expired beyond normal expiration, **Residual volume** (RV): The amount of air remaining trapped in the lungs after

a forceful expiration, **Total lung capacity (TLC)**: The sum total of air the lungs can contain, **Vital capacity (VC)**: The maximal amount of air that can be forcefully expired after a maximal inspiration (some texts view this as the same measurement), **Functional residual capacity (FRC)**: The amount of air remaining in the lungs after a normal expiration, **Inspiratory capacity (IC)**: The maximal amount of air that can be inspired after a normal expiration.

For Quiz: Go to pages 91 and 92 to read background information on Chest Movement.

Objective

To define, understand and measure V_T , IRV, ERV, EC, VC, FVC, FRC, EMV, and FEV₁.

Experimental Equipment

A computer system

LabChart software

Spirometry extension for LabChart

PowerLab

Spirometer Pod

Respiratory flow head (1000 L/min)

Disposable filters

Re-useable mouthpieces

Nose clip

PowerLab procedure

Set up and calibration of equipment

A. Connecting the equipment

1. Connect the Spirometer Pod to the Pod Port for Input 1 on the PowerLab.
2. Since the Spirometer Pod is sensitive to temperature and tends to drift during warm-up, we recommend that the PowerLab and Spirometer Pod be turned on for at least 5-10 minutes before use. To prevent temperature drift, place the Spirometer Pod on a shelf or beside the PowerLab, away from the PowerLab power supply to avoid heating.
3. Connect the two plastic tubes from the respiratory flow head to the short pipes on the back of the Spirometer Pod, as shown in Figure 6.

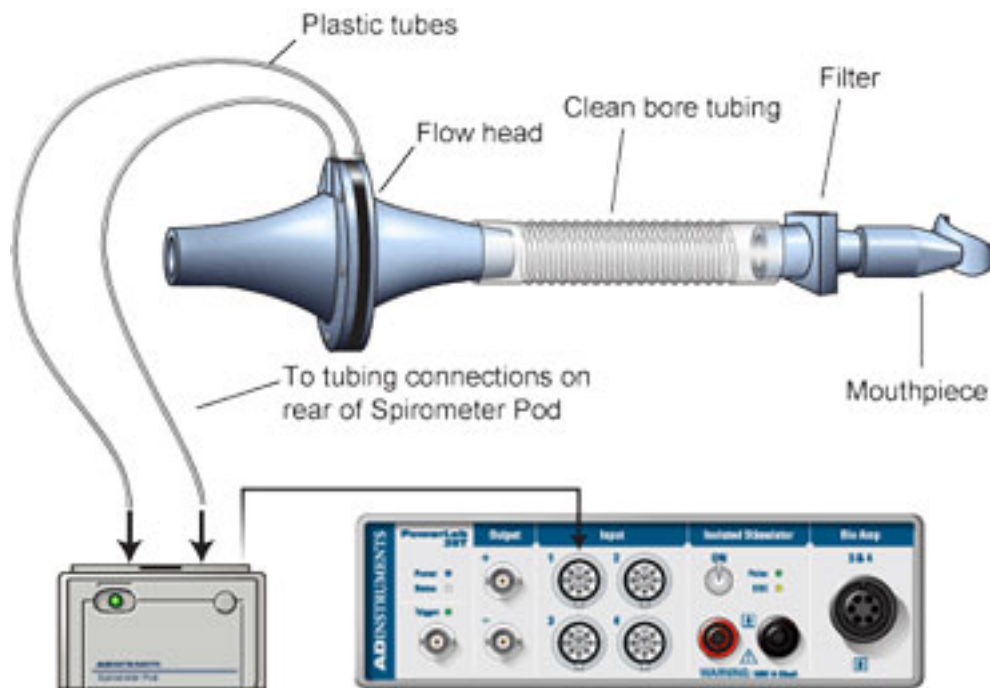


Figure 6. Setting up the spirometry experiment: connecting the flow head and attachments to the Spirometer Pod.

B. Hygiene

A clean mouthpiece and air filter should be supplied for each volunteer. The vinyl mouthpiece can be cleaned between uses by soaking it in disinfectant. **If you are suffering from a respiratory infection, we suggest that you do not volunteer for this experiment.**

C. Starting the software

1. Locate LabChart on your computer and start the software.
2. In the Experiments Gallery dialog box, select Human Experiments, Respiratory Physiology, Respiration, Setting Files from the left-hand list. Select Respiratory Settings from the right-hand list, and click the Open button to apply those settings. If the Experiments Gallery dialog box does not appear in front of the LabChart View, choose the **Experiments Gallery...** command from the File menu.
3. After a short time, the LabChart View on the computer screen should be set up for the experiment. Channels 1 and 2 are visible, with Channel 2 turned off; Channel 1 is named "Flow" and Channel 2 "Volume".

D. Calibrating the Spirometer Pod

**** WARNING: Any movement in the flow head prior to and during step 2 will cause the equipment to malfunction ****

1. The flow head must be left undisturbed on the bench during the zeroing process.
2. Choose **Spirometer Pod** from the Flow Channel Function pop-up menu. The Spirometer Pod dialog box appears, as shown in Figure 7. Click the Zero button.
3. When zeroing has finished, have the volunteer breathe out gently through the flow head, and note the recorded signal in the data display area (Fig. 7). If the signal shows a downward deflection (that is, negative), proceed to Step 5.
4. If the signal deflects upward, you need to invert it. Click the Invert checkbox once to toggle its state.
5. Click OK to close the dialog box and return to the LabChart View.
6. You are now ready to begin the exercises.

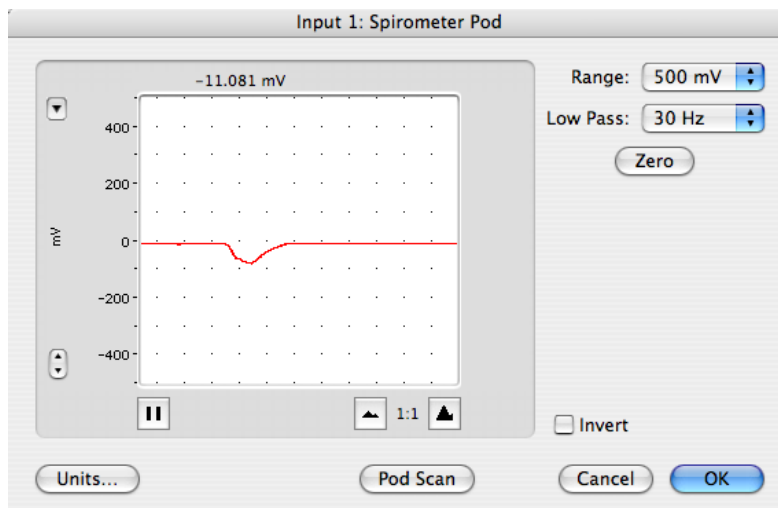


Figure 7. The dialog box for the Spirometer Pod, showing an exhaled breath.

Exercise 1: Becoming familiar with the equipment

Objectives

In this exercise, you will learn the principles of spirometry, and how integration of the flow signal gives a volume.

Software Procedure

1. Two channels appear on the screen: **Flow** and **Volume**. Go to the pull down menu for the flow channel and select “**Spiro. Flow**” (Fig. 8). Click OK.

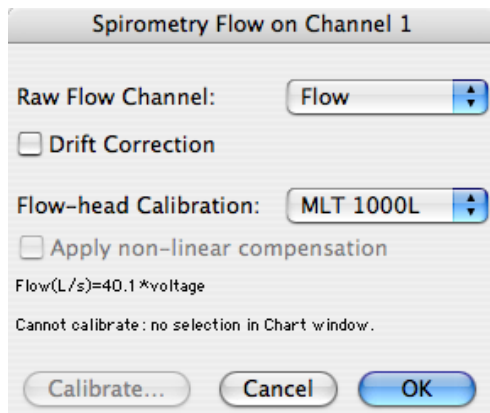


Figure 8. The Spirometry Flow dialog box.

2. Go to the pull down menu for the volume channel and select “**Spiro. Volume**”. Click OK.

Breathing Procedure

1. The volunteer should put the mouthpiece in his or her mouth, and hold the flow head carefully with both hands. **The two plastic tubes should be pointing upwards.**
2. Put the nose clip on the volunteer’s nose. This ensures that all air breathed passes through the mouthpiece, filter and flow head (Fig. 9).

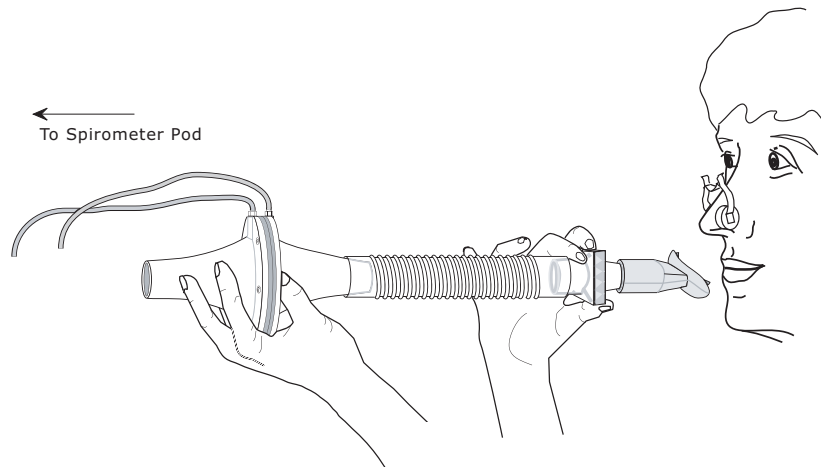


Figure 9. The volunteer should hold the flow head as shown here.

3. After the volunteer becomes accustomed to the apparatus (this normally takes 1 -2 minutes) and begins breathing normally, you are ready to begin.
4. Click the Start button to begin recording.
5. Have the volunteer perform a full expiration and then breathe normally. Record the volunteer’s tidal breathing for one minute. **At the end of one minute**, have the volunteer

perform another full expiration. You should observe data being recorded in the Flow channel, but not in the Volume channel.

6. Click Stop to end the recording. The volunteer can stop breathing through the flow head and remove the nose clip.

Setting up the Spirometry Extension

The Spirometry Extension processes the raw voltage signal from the Spirometer Pod, applies a volume correction factor to improve accuracy, and displays calibrated Flow (L/s) and Volume (L) traces. It takes over from units conversion. The trace that you recorded in this exercise will provide reference points for the Spirometry Extension that allow it to calculate and perform corrections on the trace.

1. **Select the entire recording of tidal breathing data including the two forced expirations by double-clicking in the Time axis beneath the trace (which selects a block of data).**

2. Choose **Spirometry Flow...** from the Flow (Channel 1) Channel Function pop-up menu. The Spirometry Flow dialog box appears (Fig. 8).

3. Flow (Channel 1) should be selected in the Raw Flow Channel pop-up menu; MLT 1000L should be selected in the Flow Head Calibration pop-up menu. When you are finished and the settings are the same as in Figure 8, click the OK button to close the dialog box.

4. Choose **Spirometry Volume...** from the Volume (Channel 2) Channel Function pop-up menu. The Spirometry Volume dialog box appears (Fig. 10).

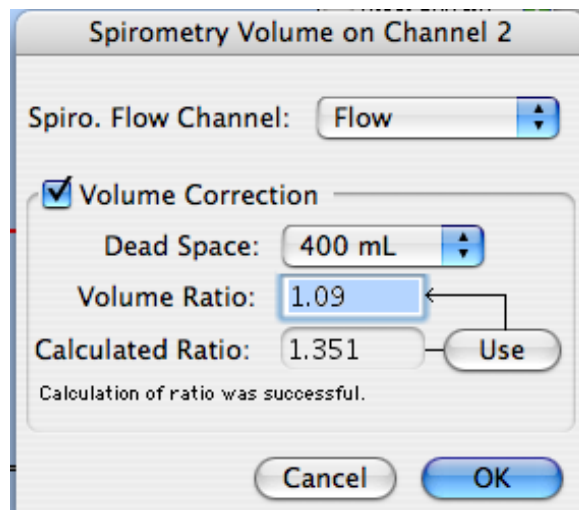


Figure 10. The Spirometry Volume dialog box.

5. Flow (Channel 1) should be selected in the Spirometry Flow Channel pop-up menu. Ensure that Volume Correction is on. Click the Use button to allow the extension to use the volume correction ratio that it has calculated from your data. **If the Use button cannot be selected go back to your tracing (step 1) and selected only a small portion of your data trace that includes the expiration instead of the entire tracing.**

6. When you are finished, click the OK button to close the dialog box. The LabChart View should now appear with calculated volume data on Channel 2.

7. Choose **Set Scale...** from the **Scale** pop-up menu in the Amplitude axis for the Flow channel. Make the top value 10 L/s and the bottom value -10 L/s, and click OK.

8. Choose **Set Scale...** from the **Scale** pop-up menu in the Amplitude axis for the Volume channel. Make the top value 5 L and the bottom value -5 L, and click OK.

Exercise 2: Lung volumes and capacities

Objectives

In this exercise, you will examine the respiratory cycle and measure changes in flow and volume.

Procedure

Note: It is important when recording normal respiration that the volunteer is facing away from the computer screen, and is not consciously controlling breathing.

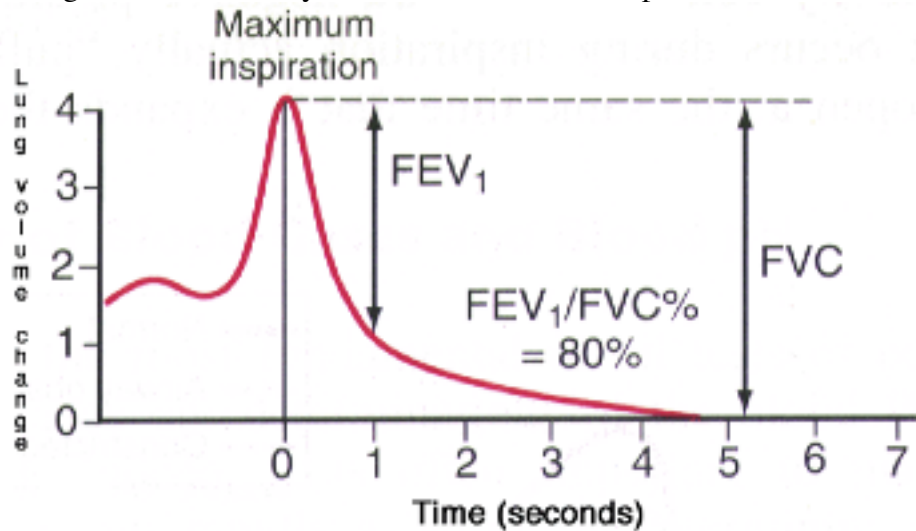
1. The flow head must be left undisturbed on the bench during the zeroing process.
2. Choose the **Spirometer Pod...** item from the Flow channel Function pop-up menu. Click the Zero button to re-zero the Spirometer Pod. When zeroing has finished, click the OK button to return to the LabChart View.
3. Note the time and click Start to begin recording. Ask the volunteer to replace the nose clip and breathe normally through the flow head. Record normal tidal breathing for at least 20 seconds. Add the **comment** "Normal tidal breathing" to the LabChart trace.
4. Click Stop to end the recording.
5. Click Start to restart the recording.
6. Prepare a **comment** called "IRV procedure", but do not press the Return key. At the end of a normal tidal inspiration ask the volunteer to breathe in as deeply as possible and then to breathe normally. Press the Return key to add the comment to the LabChart trace.

7. Prepare a **comment** called “ERV procedure”. At the end of a normal tidal expiration ask the volunteer to exhale as deeply as possible and then to breathe normally. Press the Return key to add the comment.

8. Select some normal breaths as well as the IRV and ERV tracing and go to the Zoom Window. **Print the volume channel only from the Zoom Window and save the file onto a flash disk to take away and view in LabChart Reader.**

Exercise 3: Forced Expiratory Volume Objectives

Two useful clinical pulmonary tests are *forced expiratory volume* and *forced expiratory vital capacity*. (*FVC*). In this exercise, you will measure forced expiratory volume (FEV_1) (the amount of air forcibly expired in 1 second) and compare this to forced expiratory vital capacity using the equation, FEV_1/FVC . **Average FEV_1/FVC in humans is 75 – 80%.** The figure below shows you how to measure these parameters.



Procedure

Note: It is important when recording normal respiration that the volunteer is facing away from the computer screen, and is not consciously controlling breathing.

1. Click Start to begin the recording.
2. Record a normal tidal volume for 5 breaths.
3. Prepare a **comment** called “FEV procedure”, but do not press the Return key. At the end of a normal tidal expiration ask the volunteer to breathe in as deeply as possible, hold this breathe for 1-2 seconds, then breathe out as fast and quickly as possible, then to breathe normally again for 10 seconds. Press the Return key to add the comment to the LabChart trace.

4. Select some normal breaths plus the FEV tracing and go to the Zoom Window. **Print the volume channel from the Zoom Window and save the file onto a flash disk to take away and view in LabChart Reader.**

Analysis: Lung volumes and capacities

1. Examine the first part of the data trace. Observe the number of times the volunteer breathes over 15–20 seconds. Calculate how many breaths there would be in a one-minute period (f). Record f (/min) in Table 1 provided at the end of the lab.

2. Drag the **Marker** from its box at the bottom left of the LabChart View to the Volume trace at the start of a quiet inspiration (Fig. 11). Move the **Waveform Cursor** to the next peak of the Volume trace (this should be 0.5 to 1.5 s to the right of the **Marker**). Read off the numerical value of Volume from the Range/Amplitude display at the right.

3. The number in the Range/Amplitude display should have a “ Δ ” symbol in front of it, indicating that it is the difference between the volume at the **Waveform Cursor** position and the volume at the **Marker** position. If you have both the **Marker** and the pointer in the right places, the value shown is the Tidal Volume (V_T) for that breath. Record this value in Table 1.

4. Return the **Marker** to its box at bottom left of the LabChart View, by double-clicking the **Marker**, dragging it back, or clicking its box.

5. Using the value for V_T and the number of breaths, f (/min), observed over a one-minute period, calculate the Minute Volume using Equation 3. Record your value in Table 1.

$$\dot{V}_E = V_T f \quad (\text{L/min}) \quad \text{Equation 3}$$

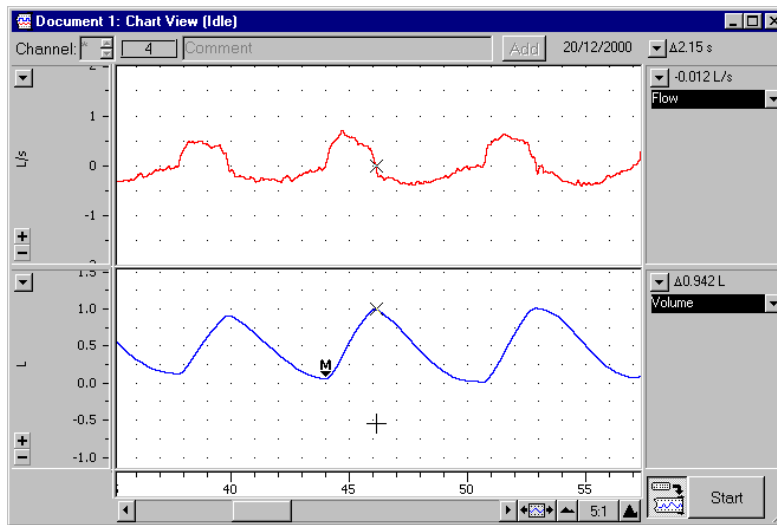


Figure 11. A typical tidal breathing record, displayed at 5:1 horizontal compression. The Marker and Waveform Cursor are positioned to measure the Tidal Volume of a single breath.

6. Find the “IRV procedure” comment in your data trace. Place the **Marker** on the peak of the inspiratory volume of the previous tidal breath and move the **Waveform Cursor** along to the peak of the volume trace from the full-deep breath (Fig. 12). The difference displayed in the Range/Amplitude display is the Inspiratory Reserve Volume (IRV). Record this value in the Table 1.

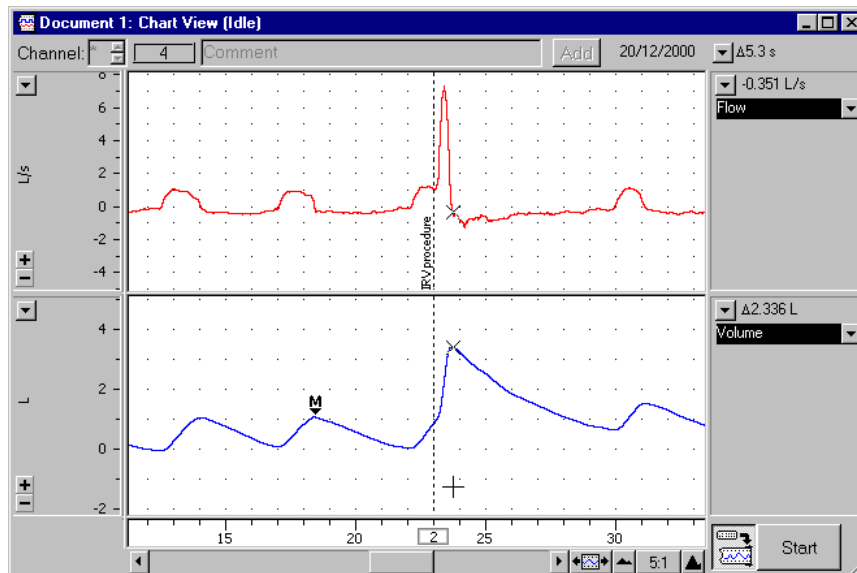


Figure 12. Record of full inhalation, with the Marker and Waveform Cursor positioned to measure IRV.

7. Calculate the Inspiratory Capacity (IC) using Equation 4.

$$IC = V_T + IRV \quad (L) \quad \text{Equation 4}$$

8. Return the **Marker** to its box at bottom left, by double-clicking the **Marker**, dragging it back, or clicking its box.
9. Find the comment containing “ERV procedure”. Place the **Marker** on the trough of the expiratory volume of the previous tidal breath and move the **Waveform Cursor** along to the trough of the volume from the forceful exhalation. Figure 13 shows where to make the measurement. The difference that will be displayed in the Range/Amplitude display is the Expiratory Reserve Volume (ERV). Disregard the delta symbol and the negative sign.

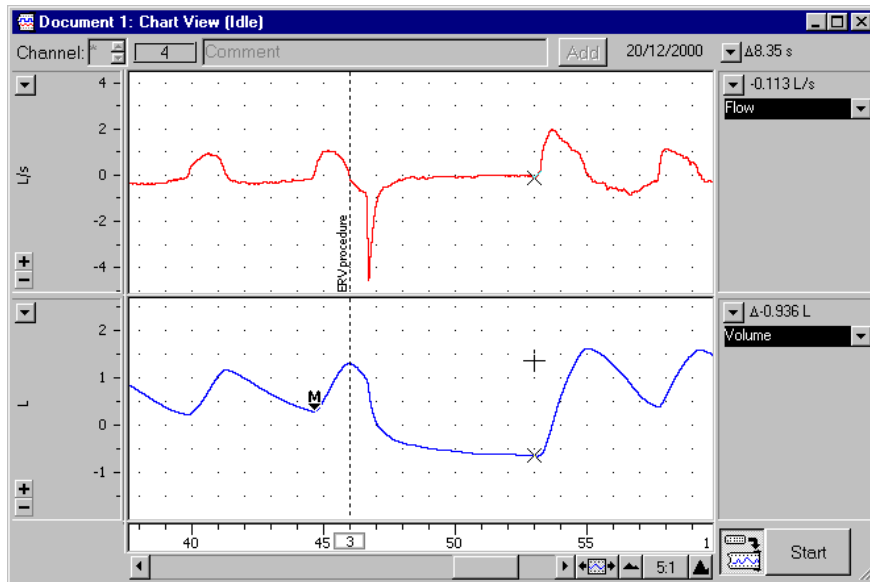


Figure 13. Record of full exhalation, with the Marker and Waveform Cursor positioned to measure ERV.

10. Calculate the Expiratory Capacity (EC) using Equation 5.

$$EC = V_T + ERV \quad (L) \quad \text{Equation 5}$$

11. Use the tables provided at the end of the laboratory to determine the volunteer’s predicted Vital Capacity (VC). The predicted value varies according to the volunteer’s sex, height and age.

12. Calculate the volunteer’s measured VC using the experimentally derived values for IRV, ERV and V_T (Equation 6).

$$VC = IRV + ERV + V_T \quad (L) \quad \text{Equation 6}$$

13. Residual Volume (RV) is the volume of gas remaining in the lungs after a maximal expiration. The RV cannot be determined by spirometric recording. Using Equation 7, determine the predicted RV value for the volunteer. This equation predicts RV for 16–34 year-old subjects of either sex.

$$RV = VC \times 0.25 \quad (L) \quad \text{Equation 7}$$

14. The Total Lung Capacity (TLC) is the sum of the vital capacity and residual volume. Calculate the predicted TLC for the volunteer (Equation 8) using the predicted values for VC and RV.

$$TLC = VC + RV \quad (L) \quad \text{Equation 8}$$

15. Functional Residual Capacity (FRC) is the volume of gas remaining in the lungs at the end of a normal tidal expiration (the sum of the RV and ERV). Calculate the FRC value for the volunteer using Equation 9.

$$FRC = ERV + RV \quad (L) \quad \text{Equation 9}$$

16. Forced Expiratory Volume (FEV_1) is the volume of air expelled forcibly expelled from the lungs in 1 second. Find the “FEV” comment in your data trace. Place the **Marker** on the peak of the inspiratory volume and move the **Waveform Cursor** along the tracing until 1 second past the peak of the volume trace from the full-deep breath. You may need to increase the scales on the X- and Y-axes. Record the FEV_1 value in Table 1.

Background – Chest movement

The metabolic activities of tissues consume oxygen and produce carbon dioxide. Oxygen and carbon dioxide are exchanged between tissues and blood. Breathing movements pump air in and out of the lungs, where close contact between air and blood occurs, allowing interchange of oxygen and carbon dioxide between air and blood.

The internal structure of the lungs consists of a series of branching tubes that carry air to the alveoli. Alveoli, sometimes called air sacs, are tiny thin-walled, highly vascularized structures where respiratory gas exchange occurs.

The principal muscle activity in quiet breathing is rhythmic contraction of the diaphragm, a dome-shaped sheet of muscle that separates the thorax from the abdomen. As contraction of the diaphragm pulls the lower surface of the lungs down, air is inspired. In quiet breathing, expiration is mainly passive, and results from the elastic recoil of the lungs. Rib movements also occur in quiet breathing because of the activity of the intercostal muscles, but are of small amplitude.

In forceful breathing, rib movements are obvious, and greatly expand and contract the volume enclosed by the ribcage. In addition, other muscles are recruited. The sternomastoid muscles of the neck assist in raising the sternum in forceful

inspiration. Abdominal muscles raise the pressure in the abdomen and push the diaphragm up, providing a powerful expiratory force.

Breathing movements are unusual in that they are under dual control from the central nervous system. Breathing movements can be made voluntarily in the same way as arm and leg movements. However, if no conscious attention is focused on breathing, rhythmic muscle contractions will occur spontaneously. Spontaneous breathing is controlled by the respiratory center in the medulla of the brain. The respiratory center ensures that gaseous exchange at the lung matches the requirements of the body. In times of increased demand, the rate and depth of breathing are increased to bring more fresh air into the lungs. The respiratory center is powerfully stimulated by a rise in the partial pressure of carbon dioxide in the blood, and more weakly stimulated by a fall in oxygen partial pressure.

In this experiment, you will record breathing movements with a respiratory belt transducer fastened around the chest. You will investigate various aspects of breathing (i.e., how breathing is affected under various conditions). Each group will conduct the standard “normal breathing” experiment.

Safety Note: Subjects suffering from upper respiratory problems or asthma should not act as subject during these experiments.

Experimental Equipment

Macintosh PowerBook
LabChart software, version 6.0

PowerLab
Respiratory belt transducer

Procedures

A. Set up and calibration of equipment

1. **This step may already be done for you. Check to see before proceeding further.** Turn off the PowerLab. Connect the BNC plug on the respiratory belt transducer cable to the BNC connector for Input 2 on the front of the PowerLab (Figure 14).

2. Locate LabChart on your computer and start the software.

3. From the Experiments Gallery dialog box, select Human Experiments, Respiratory Physiology, Beathing, and Setting files from the left-hand list. Select “Breath Settings” from the right-hand list, and click the Open button to apply those settings. If the Experiments Gallery dialog box does not appear in front of the LabChart window, choose the **Experiments Gallery...** command from the File menu.



4. The LabChart window on the computer screen will now be set up for the first exercises. Two channels, one titled called “Breath” and the other titled “Rate” should appear.

5. Fasten the respiratory belt around the middle of the sternum of a volunteer (Fig. 14). The transducer should be at the front of the body, and the belt should fit snugly.

Note: The respiratory belt transducer can be used over clothing, and it doesn't matter whether the volunteer is sitting or standing, as long as they are comfortable. Because everyone's breathing patterns differ, you may need to change the position of the transducer to get the best signal.

6. Place a nose clip over the subjects' nose so that they can only breathe from their mouth.

7. Choose the **Input Amplifier...** command from the Breath Channel Function pop-up menu.

8. Ask the volunteer to take deep, strong breaths and observe the signal in the Input Amplifier dialog box (Figure 15).

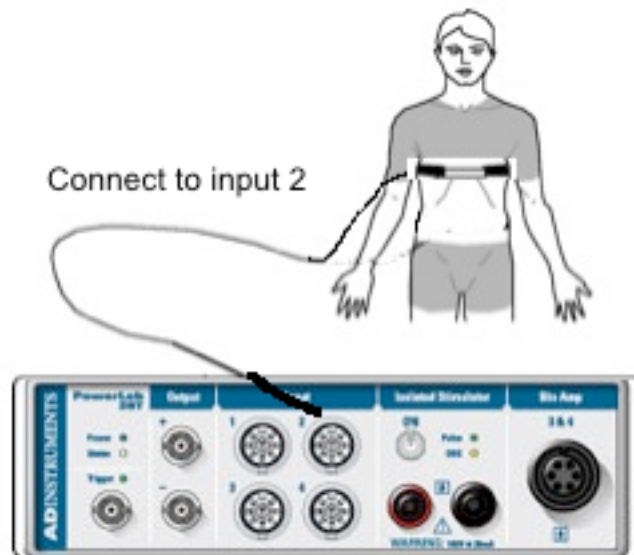


Figure 14. Connecting the respiratory belt to the PowerLab.

8. Adjust the **Range** pop-up menu of the Input Amplifier dialog box so that the breathing signal occupies about a half to two thirds of full scale. Click OK to close the dialog box.

9. It is important when recording normal respiration that the volunteer is facing away from the computer screen, and is not consciously controlling breathing.

The volunteer may have to stare out a window or read a book to avoid conscious control of respiration.

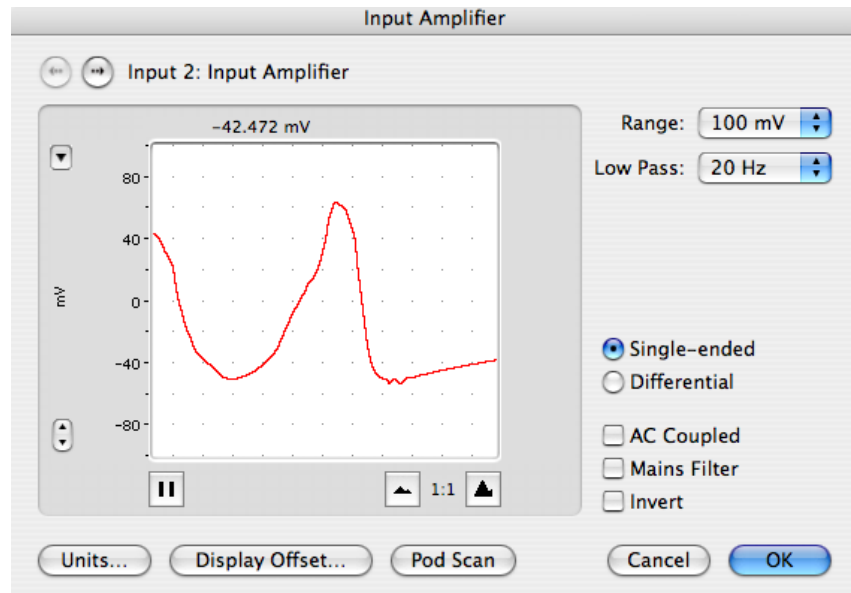


Figure 15. The Input Amplifier dialog box for the “Breath” channel. The Range has been adjusted so that the signal is the correct size, about 1/2 of the window height.

Exercise 1: Normal respiration

Objectives

In this exercise, you will investigate the characteristics of normal respiration and your ability to hold your breath after inspiration and expiration.

Procedure

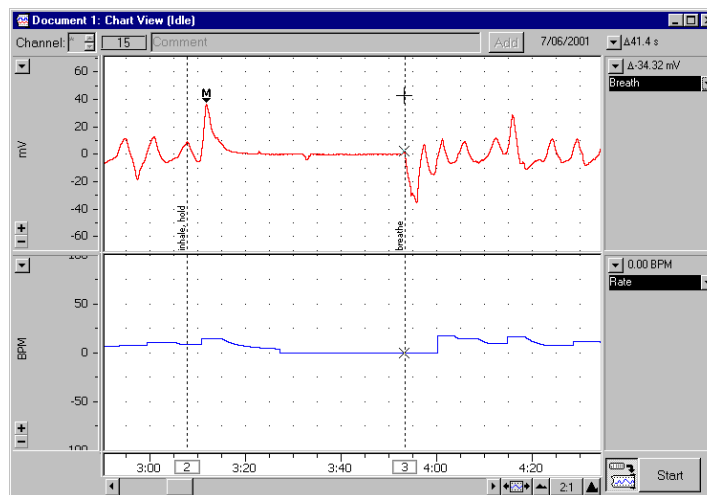
1. Click Start to begin recording. Enter a **comment** called “Baseline 1” into your recording.
2. Record 2–3 minutes of normal, quiet breathing and observe the trace. Once you have recorded the baseline signal, prepare a **comment** called “inhale, hold” but do not press the Return key.
3. Press the Return key to enter the comment, then immediately ask the volunteer to take a deep breath and hold it in for as long as possible.
4. Prepare a **comment** called “breathe”, and when the volunteer begins to breathe again, press the Return key to enter the comment.
5. Continue recording for another 2–3 minutes. Prepare a comment called “exhale, hold”.

6. Press the Return key to enter the **comment**, then immediately ask the volunteer to breathe out fully and hold the breath for as long as possible.
7. Prepare another comment called “breathe”, and when the volunteer begins breathing press the Return key to enter it.
8. **Continue recording for another 2 minutes, then click Stop.** The volunteer can now relax and breathe normally.

Analysis

Exercise 1: Normal breathing

1. Drag the **Marker** to the large peak following the comment “inhale, hold”. Move the **Waveform Cursor** to the start of the first breath afterwards, also preceded by a comment. Record the duration breath was held (as shown in the Rate/Time display, Fig. 16), and the respiration rate for 1 minute following return to normal breathing in Table 3.
2. Drag the **Marker** to the large (negative) peak straight after the comment “exhale, hold”. Move the **Waveform Cursor** to the start of the first breath afterwards, also preceded by a comment. Record the duration breath was held, as shown in the Rate/Time display, in Table 3.



Rate/Time display

Figure 16. Determining the duration of breath holding using the Marker and Waveform Cursor.

Table 1.
Respiratory parameters from your lab group

Respiratory parameter	Abbreviation	Units	Experimental & calculated value
Frequency	f	(breaths/min)	
Tidal Volume	V_T	L/breath	
Expired Minute Volume	$\dot{V}_E = V_T \cdot f$	L/min	
Inspiratory Reserve Volume	IRV	L	
Inspiratory Capacity	$IC = V_T + IRV$	L	
Expiratory Reserve Volume	ERV	L	
Expiratory Capacity	$EC = V_T + ERV$	L	
Vital Capacity	$VC = IRV + ERV + V_T$	L	
Residual Volume	$RV = VC \times 0.25$	L	
Total Lung Capacity	$TLC = VC + RV$	L	
Functional Residual Capacity	$FRC = ERV + RV$	L	
Forced Expiratory Volume	FEV_1	L	
Forced Vital Capacity	FVC		
Forced Expiratory Volume/Capacity	FEV_1/FVC	%	

Table 2.
Respiratory parameters from the entire lab

Respiratory parameter	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
Frequency					
Tidal Volume					
Expired Minute Volume					
Inspiratory Reserve Volume					
Inspiratory Capacity					
Expiratory Reserve Volume					
Expiratory Capacity					
Vital Capacity					
Residual Volume					
Total Lung Capacity					
Functional Residual Capacity					
Forced Expiratory Volume					
Forced Expiratory Volume/Capacity					

Predicted Vital Capacities in Healthy Individuals

Table 1. Males

Age (years)	Height (cm)											
	145	150	155	160	165	170	175	180	185	190	195	200
15	3.482	3.802	4.122	4.443	4.763	5.083	5.403	5.723	6.043	6.363	6.683	7.003
20	3.327	3.647	3.967	4.288	4.608	4.928	5.248	5.568	5.888	6.208	6.528	6.848
25	3.172	3.492	3.812	4.133	4.453	4.773	5.093	5.413	5.733	6.053	6.373	6.693
30	3.017	3.337	3.657	3.978	4.298	4.618	4.938	5.258	5.578	5.898	6.218	6.538
35	2.862	3.182	3.502	3.823	4.143	4.463	4.783	5.103	5.423	5.743	6.063	6.383
40	2.707	3.027	3.347	3.668	3.988	4.308	4.628	4.948	5.268	5.588	5.908	6.228
45	2.552	2.872	3.192	3.513	3.833	4.153	4.473	4.793	5.113	5.433	5.753	6.073
50	2.397	2.717	3.037	3.358	3.678	3.998	4.318	4.638	4.958	5.278	5.598	5.918
60	2.087	2.407	2.727	3.048	3.368	3.688	4.008	4.328	4.648	4.968	5.288	5.608
70	1.777	2.097	2.417	2.738	3.058	3.378	3.698	4.018	4.338	4.658	4.978	5.298
80	1.467	1.787	2.107	2.428	2.748	3.068	3.388	3.708	4.028	4.348	4.668	4.988

Table 2. Females

Age	Height (cm)											
	145	150	155	160	165	170	175	180	185	190	195	200
15	2.911	3.171	3.431	3.691	3.951	4.211	4.471	4.731	4.991	5.251	5.512	5.772
20	2.821	3.081	3.341	3.601	3.861	4.121	4.381	4.641	4.901	5.161	5.422	5.682
25	2.731	2.991	3.251	3.511	3.771	4.031	4.291	4.551	4.811	5.071	5.332	5.592
30	2.641	2.901	3.161	3.421	3.681	3.941	4.201	4.461	4.721	4.981	5.242	5.502
35	2.551	2.811	3.071	3.331	3.591	3.851	4.111	4.371	4.631	4.891	5.152	5.412
40	2.461	2.721	2.981	3.241	3.501	3.761	4.021	4.281	4.541	4.801	5.062	5.322
45	2.371	2.631	2.891	3.151	3.411	3.671	3.931	4.191	4.451	4.711	4.972	5.232
50	2.281	2.541	2.801	3.061	3.321	3.581	3.841	4.101	4.361	4.621	4.882	5.142
60	2.101	2.361	2.621	2.881	3.141	3.401	3.661	3.921	4.181	4.441	4.702	4.962
70	1.921	2.181	2.441	2.701	2.961	3.221	3.481	3.741	4.001	4.261	4.522	4.782
80	1.741	2.001	2.261	2.521	2.781	3.041	3.301	3.561	3.821	4.081	4.342	4.602

Table 3. Breath holding duration during the respiratory cycle.

Condition	Breath hold duration (sec)	Respiration rate before (bpm)	Respiration rate after 1 min. (bpm)	Respiration depth before (mV)	Respiration depth after 1 min. (mV)
Breath hold after inhalation					
Breath hold after exhalation					

Study questions

1. Define each of the following terms.

A. Tidal volume:

B. Inspiratory reserve volume:

C. Expiratory reserve volume:

D. Vital capacity:

2. An athlete and non-athlete both take a pulmonary function test to measure their Forced Expiratory Volume (FEV). Which person do you think would have the higher FEV and WHY?

3. What might the value of pulmonary function tests be in exercise science?

4. Examine your data trace and describe the normal respiratory movements. Note the characteristics of the trace such as the rate, and the relative durations of inspiration (breathing in) and expiration (breathing out).

5. In your own words, explain the effect of holding your breath on the respiratory pattern (i.e., how does breathing change after holding your breath?). Why?

6. During which phase of respiration can the breath be held the longest? Why?

Renal Physiology

Introduction

The urinary system in humans consists of two kidneys and ureters and a single urinary bladder and urethra. The human kidney consists of an outer area called the *cortex* and an inner *medulla*. Major arteries and veins infiltrate deep within the kidney and these branch off to form smaller vessels the size of capillaries.

The functional unit of the kidney, the *nephron*, enables a concentrated or dilute urine to be excreted from the body. The human kidney contains about 1 million nephrons. The nephron structure is complex and includes a proximal portion, a loop of Henle, and a distal portion. The distal portions of all nephrons combine their fluid into a common collecting duct. The collecting duct eventually empties urine into the ureter that terminates in the urinary bladder. Urine is stored in the urinary bladder temporarily before being voided from the body.

The kidneys function to regulate the consistency of the internal environment (i.e., the body) with respect to such variables as pH, plasma glucose levels and blood volume to name only a few. Another major function of the kidneys is to maintain water and ionic balance. This is achieved by producing a concentrated urine in times when water is lacking and a dilute urine in times when water is in abundance. The kidneys achieve this role using the organic solute *urea*. In addition, hormones play a pivotal role in regulating water and ionic balance. The most important of these hormones are *antidiuretic hormone (ADH)* that decreases urine production and *aldosterone* that increases sodium reabsorption.

The human kidney filters blood plasma. Water from the plasma is filtered through the glomerular filtration membrane and enters the nephron. Approximately 180 L of water per day is filtered into the kidneys. Once in the nephron, most of the filtered water (178.5L) is reabsorbed from different portions of the nephron and back into the blood. Thus 99% of all the water that enters into the nephron is reabsorbed back into the blood.

Water reabsorption along the nephron is passive and occurs as a result of the reabsorption of sodium and chloride in the cortex, and an ionic gradient in the medulla created by the loop of Henle and the collecting duct. The ionic gradient in the medulla is caused by the reabsorption of sodium and chloride ions along the length of the thick ascending limb of Henle, and the reabsorption of urea in the collecting duct. These ionic gradients in both the cortex and medulla allow water to be reabsorbed by osmosis through the proximal and distal convoluted tubules, the descending limb of Henle and the collecting duct. The stronger the ionic gradient the greater the osmotic pressure difference between the tubular lumen and the interstitium, and thus the more water that is reabsorbed across the nephron.

In this lab, the water reabsorption experiment is designed to demonstrate how water is reabsorbed along different portion of the nephron. In the medulla that water is reabsorbed in two areas of the medulla; the descending limb of Henle and in the collecting duct. Unlike the descending limb of Henle cells that are always permeable to the movement of water, the cells of the collecting duct are only permeable to water in the presence of arginine vasopressin (antidiuretic hormone – ADH). For this experiment we will assume ADH is present.

Objectives

- To be able to identify structures given a longitudinal section of a human kidney.
- To be able to identify structures on a human nephron.
- To understand how water is reabsorbed along the nephron and the importance that solutes play in producing a concentrated urine in the renal medulla.
- To understand and describe the roles of the hormones ADH and aldosterone.
- To perform routine tests on urine samples to determine if samples are within normal range.

Experimental Equipment

Models of the kidney and nephron
Simulated urine from different patients
Pasteur pipettes and bulbs
5 ml test tubes
5 x 200 ml glass beakers
Dialysis tubing
NaCl solutions: 300 (250 ml), 500 (150 ml), 600 (200 ml), 700 (200 ml), 900 (150 ml) and 1,200 mOsm (750 ml)

Vapor pressure osmometer
Weigh boats
Top loading balance
Paper clips
Paper towels
20% potassium chromate
2.9% silver nitrate
Refractometers
Labstix test strips

Experimental procedure

Identification of kidney and nephron structures

View each of the different models on display in the laboratory and be able to identify the structures outlined in Table 1. Use Figures 1 and 2 to help you locate each of the structures.

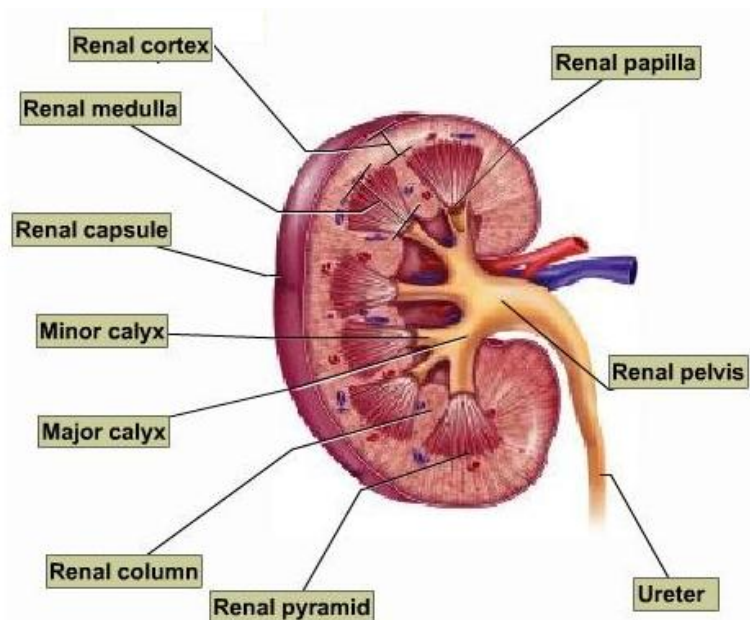


Figure 1

Figure 2

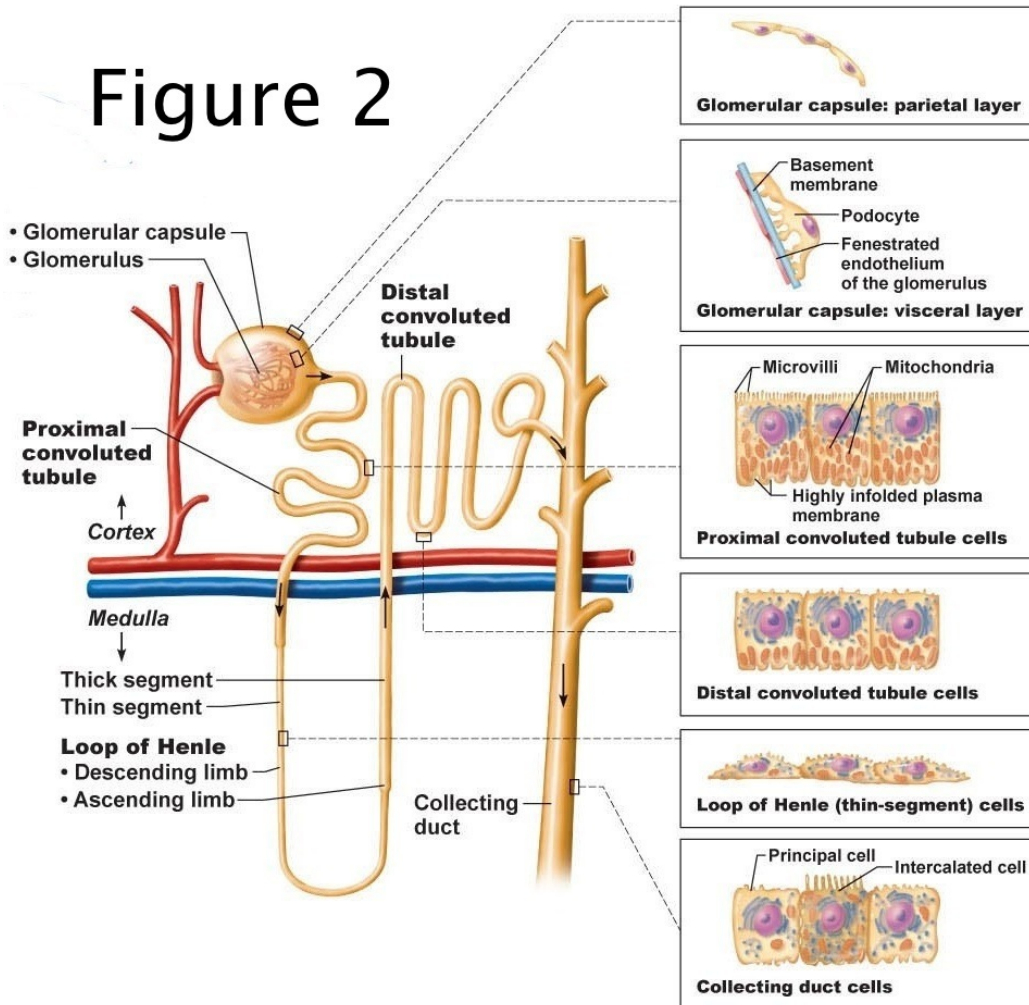


TABLE 1

Kidney structures	Nephron structures
Cortex	Renal corpuscle
Medulla (renal) pyramid	Afferent/efferent arterioles
Renal capsule	Glomerulus
Renal pelvis	Podocyte
Renal artery	Proximal convoluted tubule
Renal vein	Loop of Henle (ascending and descending limbs)
Ureter	Loop of Henle (thin and thick segments)
Calyx (major and minor)	Distal convoluted tubule
Renal papilla	Macula densa
Urinary bladder *	Collecting tubule/duct
Adrenal gland *	Peritubular capillaries
	Vasa recta

* not part of the kidney.

Water reabsorption along the nephron

Aims

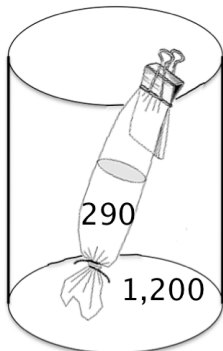
1. The apparatus for groups 1 and 2 are designed to show the effect of interstitial solute concentration on urine concentrating ability.
2. The apparatus for group 3 - 5 are designed to show the effect of ADH, aldosterone and atrial natriuretic peptide respectively.

Procedure

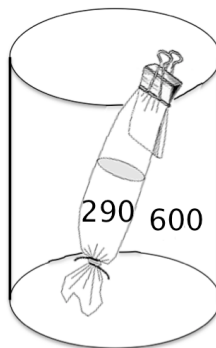
Each group should choose one of the following procedures to complete. The dialysis bag represents the filtrate inside the nephron. For groups 1 and 2, the solution in the beaker the concentration of the interstitium. For groups 3-5 the solution in the beaker represents ADH, aldosterone and atrial natriuretic peptide respectively.

Before the experiment

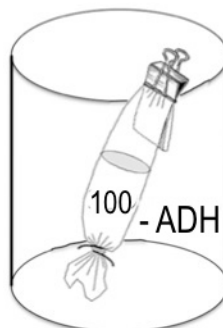
1. Take a sample of the solution in the 290 mOsm container on the front table of the lab. Place 10 ml of the solution inside the dialysis bag. Blot any excess fluid on the outside of the bag with a paper towel then weight dialysis bag with the solution in it BEFORE the start of the experiment.
2. Depending on which group you have, add 100 ml of the appropriate solution in the glass beaker. Place the solutions are seen on the illustrations below.



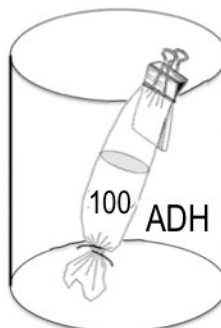
Group 1



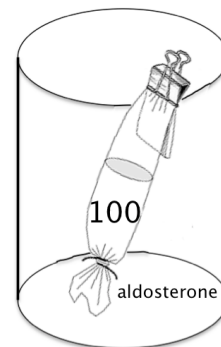
Group 2



Group 3



Group 4



Group 5

Hypothesis

For each of the groups hypothesize which direction the water will move and why?

Group 1: _____

Group 2: _____

Group 3: _____

Group 4: _____

Group 5: _____

Run the experiment for 30 minutes. At the END of the experiment, **remove the bag from the beaker and blot it completely dry with a paper towel then weight the bag**. Then measure the osmolality of the solution inside the dialysis bag using the osmometer. Place the results in the table below.

At the conclusion of the experiment dispose of the solutions in the dialysis tubes in the sink and dispose of the bags in the rubbish bin. Dispose of the solutions in the beaker down the sink. Rinse out all of the beakers with water and leave them on your lab bench to dry. Return the paper clips to the front bench.

Experimental results

Group 1 – 290mOsm in bag, 1,200mOsm in interstitium

Parameter	Before	After	Change
Bag osmolality (mOsm)			
Weight (g)			

Group 2 – 290mOsm in bag, 600mOsm in interstitium

Parameter	Before	After	Change
Bag osmolality (mOsm)			
Weight (g)			

Group 3 – 100mOsm in bag, without ADH

Parameter	Before	After	Change
Bag osmolality (mOsm)			
Weight (g)			

Group 4 – 100mOsm in bag, with ADH

Parameter	Before	After	Change
Bag osmolality (mOsm)			
Weight (g)			

Group 5 – 100mOsm in bag, with aldosterone

Parameter	Before	After	Change
Bag osmolality (mOsm)			
Weight (g)			

Conclusions

Based on the results from groups 1 and 2, what can you conclude about the effect of interstitial solute concentration on the kidneys' ability to produce a concentrated urine?

Based on the results from groups 3 – 5, what can you conclude about the effect of ADH and aldosterone?

Information for instructors

Solutions are made from NaCl (except the deionized water)

100 mOsm: 2L for 10 lab sections

290 mOsm: 500ml for 10 sections

600 mOsm: 2.5L for 10 sections

1,200 mOsm: 2.5L for 10 sections

Testing of simulated urine samples

Each group of students (i.e., table) should perform all of the tests on one sample. Combine class data to complete the Table 2 below.

Perform standardized tests on samples of unknown simulated urine such as measuring chloride concentration, specific gravity and tests for color, pH, blood, glucose and ketones. Your laboratory instructor will outline how to perform these in detail. Place class data in the table below.

HOW TO PERFORM TESTS

A. Color

Look at the the simulated urine sample in the flask and score the color as being either clear or cloudy, bright or dark and describe the color.

B. Chloride concentration

Measure the *chloride concentration* of the urine by placing 10 drops of simulated urine into a 5 ml test tube using a Pasteur pipette. Add 1 drop of 20% potassium chromate. Add 2.9% silver nitrate solution drop by drop, shaking the mixture continuously while adding the drops (count the number of drops), until the mixture changes color from a bright yellow to a brown color. Each drop of 2.9% silver nitrate is equivalent to 61 mg of Cl⁻ per 100 ml (1 deciliter – dL) of urine. Therefore to obtain a chloride concentration in mg/100 ml, simply multiply the number of drops by 61. For example if 10 drops of 2.9% silver nitrate were required, then $10 \times 61 \text{ mg of Cl}^-/\text{dL} = 610 \text{ mg Cl}^-/\text{dL}$. Record the amount of NaCl in the urine in mg/dL in the table below. **Normal chloride content in humans ranges from 50 – 750 mg/dL. High values indicate a high salt diet.**

C. Specific gravity (S.G.)

Measurement of urine specific gravity is important in determining the ability of the kidney to concentrate or to dilute the urine. The inability of the kidneys to perform this function may be indicative of renal structural damage, metabolic disorders or endocrine disturbance. The specific gravity of a liquid is defined as the ratio of the density of the substance being measured compared to the density of water at a specific temperature

Measure the *specific gravity* of the urine by placing 1-2 drops of simulated urine on the prism of the urinometer then take a reading by looking through the ocular. Once finished clean the prism with a little water and wipe it dry with a kimwipe tissue. **The normal range of urine specific gravity in humans is 1.001 to 1.035. Low values indicate a dilute urine and high values a concentrated urine.**

D. Tests for glucose, bilirubin, ketones, blood, pH and proteins using LabStix strips.

Take out a single test strip being carefully not to touch the colored portion of the strip. Place the top back on the test strip bottle tightly. Immerse the entire colored portion of the test strip in the simulated urine and wipe off any excess with a paper towel. Use the legend on the test strip bottle read off the concentrations for the pH, blood, glucose and protein tests. **Normal pH range for human urine is 4.5 – 8.0. Normally you should not find blood, glucose or protein in human urine (see Table 3).**

Table 2. Summary table for class results.

Patient	Color	Chloride	S.G.	Glucose	Bilirubin	Ketone	Blood	pH	Protein
A									
B									
C									
D									
E									

Table 3 below summarizes some commonly occurring abnormal urinary constituents. You should know these conditions for the final laboratory exam.

Abnormal Urinary Constituents		
SUBSTANCE	NAME OF CONDITION	POSSIBLE CAUSES
Glucose	Glucosuria	Nonpathological: excessive intake of sugary foods. Pathological: diabetes mellitus.
Proteins	Proteinuria	Nonpathological: excessive physical exertion, pregnancy, high protein diet. Pathological: heart failure, severe hypertension, damage to glomerular filtration membrane - glomerulonephritis
Bilirubin	Bilirubinemia	Pathological: Liver disease. Non-pathological: gall stone blocking the common bile duct.
Ketone bodies	Ketonuria	Excessive formation and accumulation of ketone bodies, as in starvation and untreated diabetes mellitus.
Erythrocytes	Hematuria	Bleeding urinary tract

Study questions

1. You are on a 2 day hiking trip to the mountains. You pack adequate provisions including plenty of water because you know that no water is available on the trail. About 4 hrs into the hike you fall on loose rock and your backpack falls, breaking the water bottles which soaks the ground. You decide to go back due to the lack of water. Explain what will happen to your urine concentration and why? Mention the specific events that occur along different parts of the nephron.

2. If you are shipwrecked on an island with no access to fresh water, should you drink seawater? Explain your answer.

3. You like drinking water, all day and everyday. Explain the events in the nephron (and the related hormones) that enable you to remain in osmotic equilibrium.

Digestive Physiology

Introduction

The digestive system enables nutrients to be broken down and assimilated by the human body. The digestive process is both chemical and physical. Chemical breakdown is due to the effects of **enzymes** released by the alimentary tract epithelium. These enzymes include: **amylase** that breaks down complex carbohydrates, **pepsin** that aids in protein digestion, and **lipase** that aid in fat digestion. Non-enzymatic substances such as **HCl** for protein degradation and **bile** for fat emulsification help some of these enzymes. Amylase is secreted by the salivary glands and pancreas, pepsin and HCl by the stomach mucosa, lipase by the pancreas and bile by the liver.

Once digested, nutrients are absorbed across the epithelium of the intestines. Eighty percent of all absorption occurs in the small intestine due to its modified anatomy that is enhanced for the role of nutrient uptake.

Objectives

- Identify the location of the major organs of the human alimentary tract and their parts.
- Identify structures that comprise the epithelium of the small intestine.
- Examine the role played by salivary amylase in the digestion of starch (carbohydrate).
- Examine the role played by pepsin in the digestion of protein.
- Examine the role played by lipase and bile in the digestion of fat.

Equipment available in lab

Models of the visceral organs

Models of the human intestine

Experimental procedure

Gross anatomy of the human alimentary tract.

Use the models on display in the laboratory and Figure 1 on the following page to help you identify the gross anatomy of the human alimentary tract.

Microscopic anatomy of the human small intestine.

Use the models on display in the laboratory and Figure 2 on the following page to help you identify all of the structures of the small intestine epithelium.

Figure 1

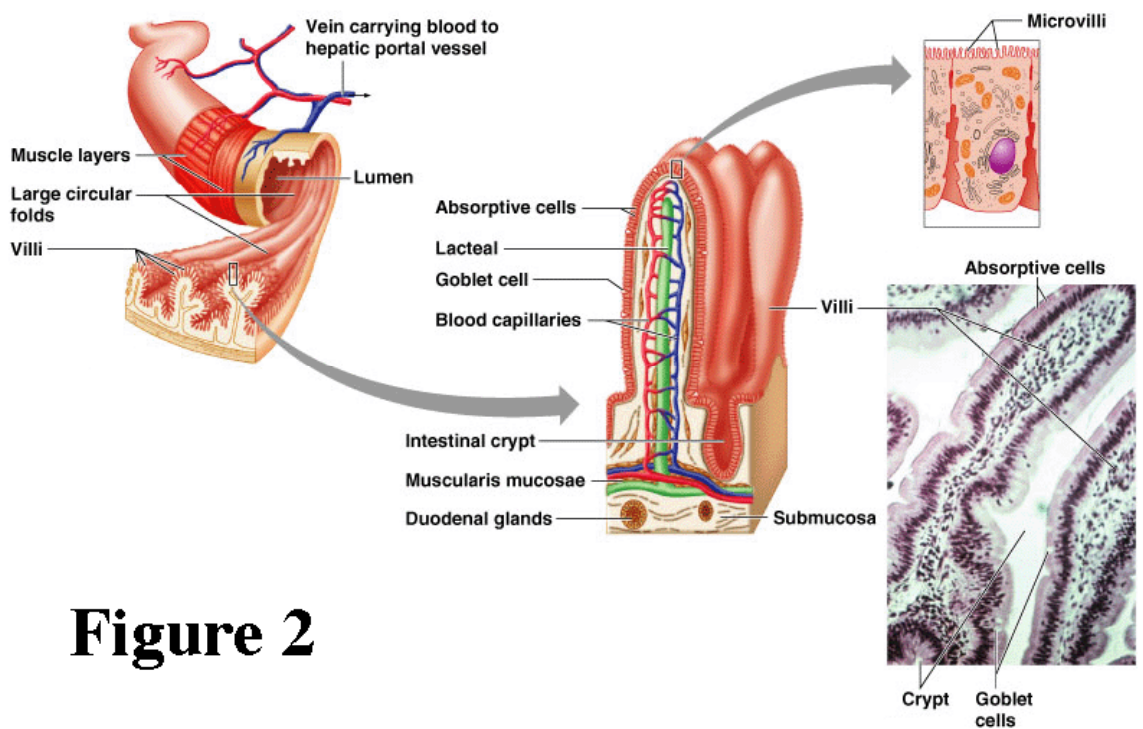
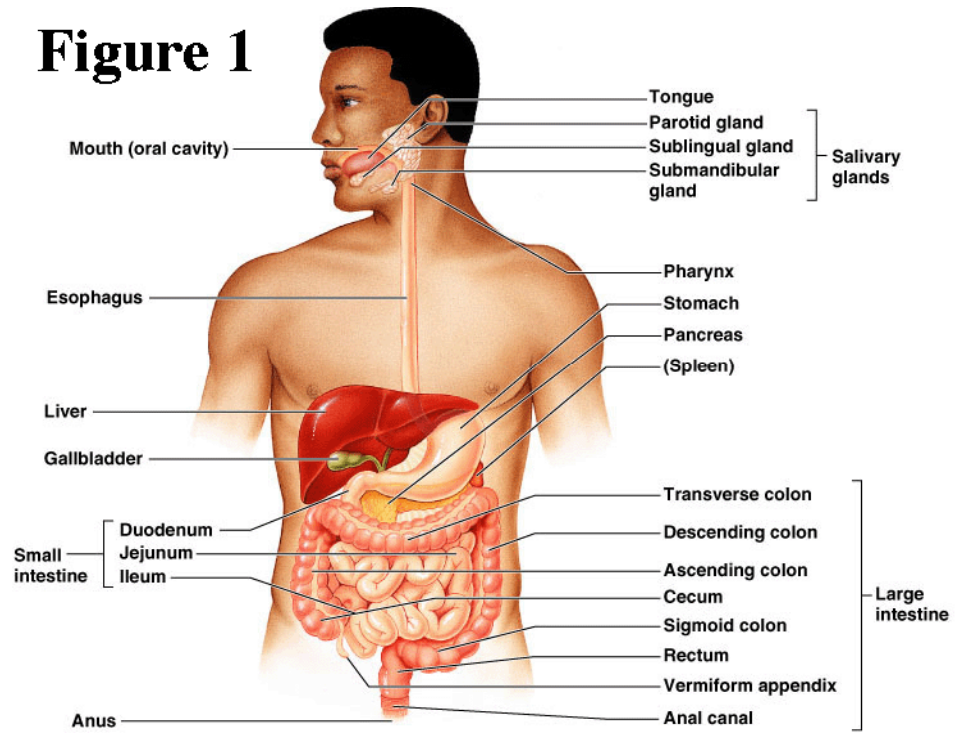


Figure 2

Computer simulations using the program PhysioEx

Introduction

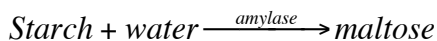
The digestive system is comprised of finely orchestrated chemical and physical activities. The food we ingest must be broken down to its molecular form for us to get the nutrients we need. Digestion involves a complex sequence of mechanical and chemical processes designed to achieve this goal. As food passes through the gastrointestinal tract, it is progressively broken down by the mechanical action of smooth muscle and the chemical action of enzymes until most nutrients have been extracted and absorbed into the blood.

Food is broken down into nutrients the body can absorb using **enzymes**. These large protein molecules act as biological **catalysts** that increase the rate of chemical digestion. Digestive enzymes are hydrolytic enzymes, or **hydrolases**, that break down organic food molecules, or **substrates**, by adding water to the molecular bonds, thus cleaving the bonds between the subunits.

Starch Digestion by Salivary Amylase

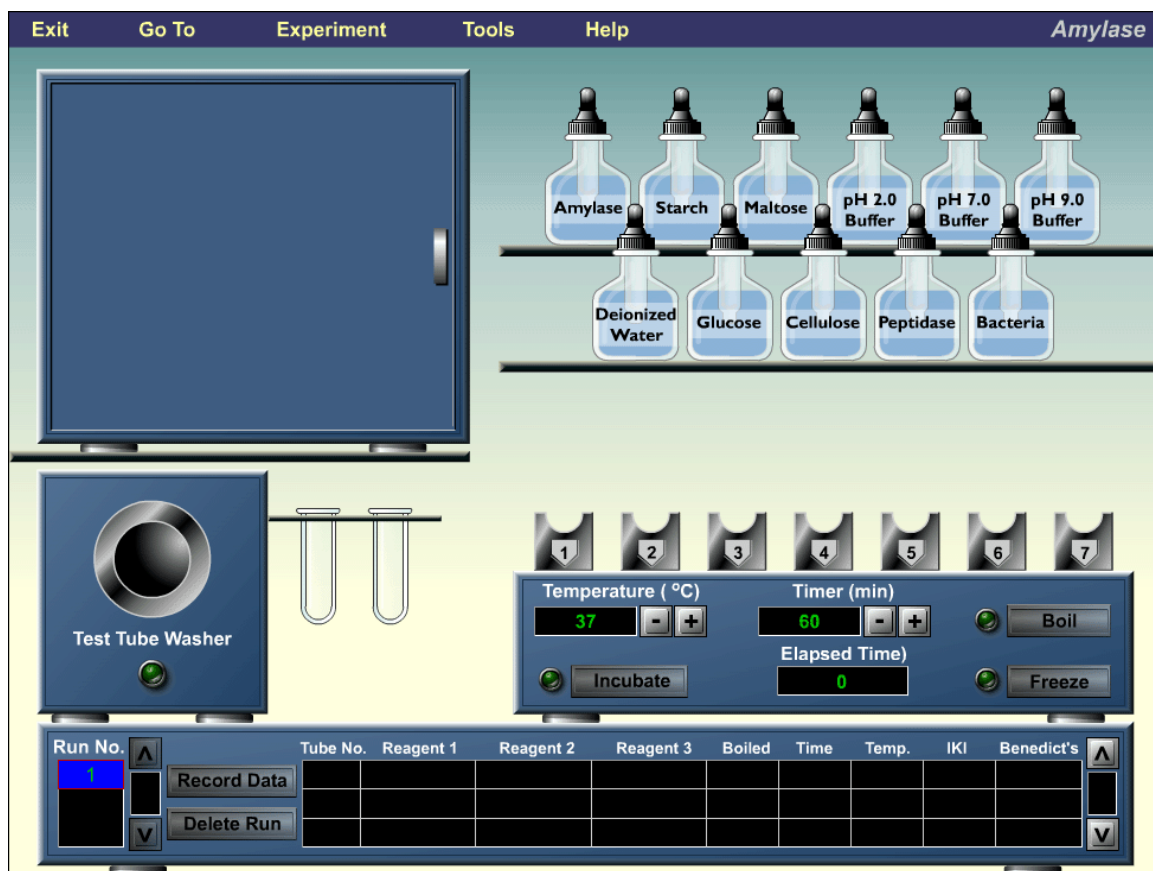
In this experiment you will investigate the hydrolysis of starch to maltose by the enzyme salivary amylase. For you to be able to detect whether or not the enzymatic action has occurred, you need to be able to identify the presence of these substances to determine to what extent hydrolysis has occurred. Thus **controls**, must be prepared to provide a known standard against which comparisons can be made. The controls will vary for each experiment and will be discussed in each enzyme section in this exercise.

Starch decreases and sugar increases as digestion proceeds according to the following equation:



The chemical changes that occur as starch is digested to maltose cannot be seen with the naked eye, thus you need to conduct an *enzyme assay*, the chemical method of detecting the presence of digested substances. You will perform 2 enzyme assays on each sample. The **IKI assay** detects the presence of **starch** and the **Benedict's** assay for the presence of reducing sugars, such as **glucose and maltose**. IKI turns blue-black in the presence of starch, and Benedict's solution turns changes from green to orange to reddish-brown with increasing amounts of maltose.

Choose the file **Start Here.html**. Select **Enter**, then **Exercise 8: Processes of Digestion**, then **Go**. Choose **Amylase** from the main menu. The screen is shown on the following page. The *solutions shelf* in the upper right part of the screen contains the substances to be used in the experiment. The *incubation unit* beneath the solutions shelf contains a rack of test tube holders and the apparatus needed to run the experiments. Test tubes from the *test tube washer* on the left part of the screen are loaded into the rack in the incubation unit by clicking and holding the mouse button on the first tube, and then releasing it into any position in the rack. The substances in the dropper bottles on the solutions shelf are dispensed by dragging-and-dropping the dropper cap to the position over any test tube in the rack and then releasing it. During the dispensing event, five drops of solution drips into the test tube; then the dropper cap automatically returns to its position in the bottle.



Activity 1: Assessing starch digestion by salivary amylase

Incubation

1. Individually drag 7 test tubes to the test tube holders in the incubation unit.
2. Prepare tubes 1 – 7 with the substances indicated in the Table 1 using the following approach.
 - Click and hold the mouse button on the dropper cap of the desired substance on the solutions shelf.
 - While still holding the mouse button down, drag the dropper cap to the top of the desired test tube.
 - Release the mouse button to dispense the substance. The dropper cap automatically returns to its bottle.
3. When all tubes are prepared, click the number **(1)** under the first test tube. The tube will descend into the incubation unit. All other tubes should remain in the raised position.
4. Click **boil** to boil tube 1. After boiling for a few moments, the tube will automatically rise.
5. Now adjust the incubation temperature to 37°C and the timer to 60 min (compressed time) by clicking (+) or (-) buttons. Click **Incubate** to start the run. The incubation unit will gently agitate the test tube rack, evenly mixing the contents of all tubes. When the incubation is complete, the tubes will automatically rise, and the doors to the assay cabinet will open.

TABLE 1. Salivary Amylase Digestion of Starch

Tube no.	1	2	3	4	5	6	7
Additives	Amylase Starch pH 7.0 buffer	Amylase Starch pH 7.0 buffer	Amylase Deionized water pH 7.0 buffer	Deionized water Starch pH 7.0 buffer	Deionized water Maltose pH 7.0 buffer	Amylase Starch pH 2.0 buffer	Amylase Starch pH 9.0 buffer
Incubation Condition	Boil, incubate at 37°C for 60 min.						
Benedict's Test							
IKI test							

Assays

After the assay cabinet doors open, notice two reagents in the assay cabinet. IKI tests for the presence of starch and Benedict's for reduced sugars such as glucose and maltose, the digested products of starch. Below the reagents are 7 small assay tubes into which you will dispense a small amount of test solution from the incubated samples in the incubation unit, plus a drop of IKI.

1. Click and hold the mouse on the first tube in the incubation unit. Notice that the mouse pointer is now a miniature test tube tilted to the left.
2. While still holding the mouse button down, move the mouse pointer to the first small assay tube on the left side of the assay cabinet. Release the mouse button. Watch the first test tube automatically decant approximately half of its contents into the first assay tube on the left.
3. Repeat steps 1 and 2 for the remaining tubes, moving to a fresh assay tube each time.
4. Next. Click and hold the mouse button on the IKI dropper cap and drag it to the first assay tube on the left. The computer will dispense the IKI reagent automatically to the remaining tubes. You will see IKI drop into the tube, which may cause a color change in the solution. A blue-black color indicates a **positive starch test**. If starch is not present, the mixture will look like diluted IKI, a **negative starch test**. Intermediate starch amounts result in a pale grey color.
5. Record your results (+ for positive, - for negative) in Table 1.
6. Dispense Benedict's reagent into the remaining mixture in each tube in the incubation unit by dragging-and-dropping the Benedict's dropper cap to the top of each test tube.
7. After the Benedict's reagent has been delivered to each tube in the incubation unit, click **Boil**. The entire test tube rack will descend into the incubation unit and automatically boil the tube contents for a few moments.
8. When the rack of tubes rises, inspect the tubes for color change. A green-to-reddish color indicates that a reducing sugar is present; this is a **positive sugar test**. An orange colored sample contains more sugar than a green sample. A reddish-brown color indicates even more

sugar. A negative sugar test is indicated by no color change from the original bright blue. Record your results in Table 1.

9. Click Record Data to display results in the grid and retain the data in the computer's memory for later analysis.

QUESTIONS

1. What do tubes 2, 6 and 7 reveal about pH and amylase activity?

2. Which pH buffer allowed the highest amylase activity?

3. Which tube indicates that the amylase did not contain maltose?

4. Would this be a valid experiment if we left out control tubes 3, 4 and 5. Why?

5. Would the amylase present in saliva work optimally in the stomach? Explain your answer.

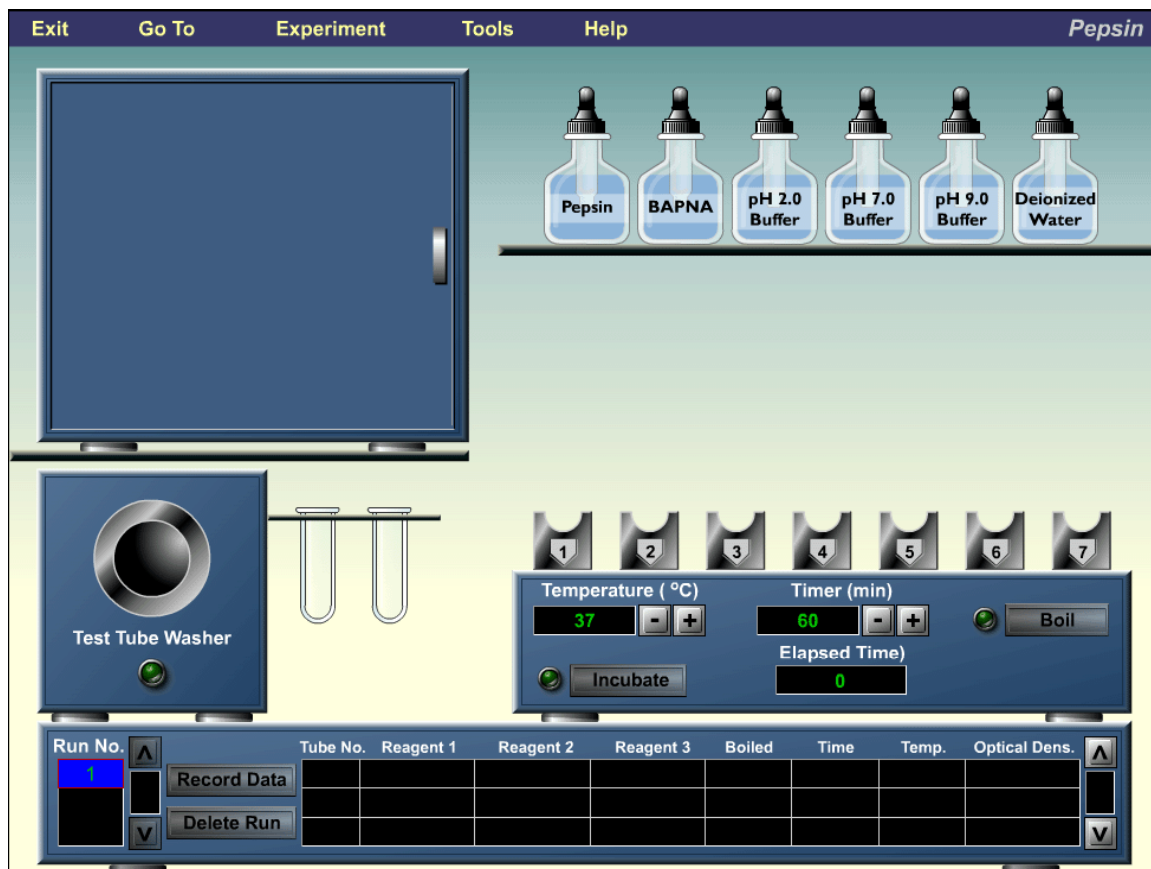
6. What effect does boiling have on enzyme activity? Why?

Protein Digestion by Pepsin

Introduction

The chief cells of the stomach mucosa produce pepsin, a protein digesting enzyme. Pepsin hydrolyzes proteins to small fragments (proteoses, peptones and peptides). In this experiment. You will use BAPNA, a synthetic 'protein' that is transparent and colorless when in solution. However, if an active protein-digesting enzyme such as pepsin is present, the solution will become yellow. You can use this characteristic to detect pepsin activity: the solution turns yellow if the enzyme digests BAPNA substrate; it remains colorless if pepsin is not active or not present.

Choose **Pepsin** from the upper pull down menu. You will see the following screen. The solutions self, test tube washer, and incubation equipment are the same as the amylase experiment; only the solutions have changed.



Activity 2: Assessing Protein Digestion by Pepsin.

Incubation

1. Individually drag 6 test tubes to the test tube holders in the incubation unit.
2. Prepare the tubes with the substances indicated in the Table 2 using the following approach.
 - Click and hold the mouse button on the dropper cap of the desired substance and drag the dropper cap to the top of the desired test tube.
 - Release the mouse button to dispense the substance.
3. When all tubes are prepared, click the number (1) under the first test tube. The tube will descend into the incubation unit. All other tubes should remain in the raised position.
4. Click **boil** to boil tube 1. After boiling for a few moments, the tube will automatically rise.
5. Now adjust the incubation temperature to 37°C and the timer to 60 min (compressed time) by clicking (+) or (-) buttons. Click **Incubate** to start the run. The incubation unit will gently agitate the test tube rack, evenly mixing the contents of all tubes. When the incubation is complete, the tubes will automatically rise, and the doors to the assay cabinet will open.

TABLE 2. Pepsin Digestion of Protein

Tube no.	1	2	3	4	5	6
Additives	Pepsin, BAPNA pH 2.0 buffer	Pepsin, BAPNA pH 2.0 buffer	Pepsin, Deionized water pH 2.0 buffer	Deionized water, BAPNA pH 2.0 buffer	Pepsin, BAPNA pH 7.0 buffer	Pepsin, BAPNA pH 9.0 buffer
Incubation Condition	Boil, incubate at 37°C for 60 min.	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.
Optical Density						

Assay

After the assay cabinet doors open, you will see an instrument called a spectrophotometer, which you will use to measure how much yellow dye was liberated by pepsin digestion of BAPNA. When a test tube is dragged to a holder in the spectrophotometer and the **Analyze** button is clicked, the instrument will shine a light through a specimen to measure the amount of light absorbed by the sample within the tube. The measure of the amount of light absorbed by the solution is known as its *optical density*. A colorless solution does not absorb light, whereas a colored solution has a relatively high light absorbance. If no light is absorbed the reading is 0. If some is absorbed then a number greater than 0 will be observed.

In this experiment, a yellow colored solution is a direct indication of the amount of BAPNA digested by pepsin.

1. Click and hold the mouse on the first tube in the incubation unit and drag it to the holder in the spectrophotometer.
2. Release the mouse button to drop the tube into the holder.
3. Click **Analyze**. The optical density of the sample will be displayed in the optical density window below the Analyze button.

4. Record the optical density in Table 2.
5. Drag the tube to its original position in the incubation unit and release the mouse button.
6. Repeat steps 1 - 5 for the remaining test tubes in the incubation unit.
7. Click **Record Data** to display your results in the grid and retain your data in the computer's memory for later analysis.

QUESTIONS

1. Which pH provided the highest pepsin activity?

2. Would pepsin be active in the mouth? Explain your answer.

3. How did the results of tube 1 compare with those of tube 2?

4. Tubes 1 and 2 contained the same substances. Explain why their optical density measurements were different.

5. Did the pepsin of deionised water contain any contaminating digested BAPNA? Which tubes confirm this?

6. What do you think would happen if you reduced the incubation time to 30 minutes? (Use the simulation to help you answer this question if you are not sure).

7. What do you think would happen if you decreased the temperature of incubation to 10°C? (Use the simulation to help you answer this question if you are not sure).

Fat Digestion by Pancreatic Lipase and the Action of Bile Introduction

The treatment that fats and oils undergo during digestion in the small intestine is a bit more complicated than that of carbohydrates or proteins. Fats and oils require pretreatment with bile to physically emulsify the fats. As a result, two sets of reactions must occur. First:

Fats/oils $\xrightarrow{\text{bile}}$ fat/oil droplets

Then:

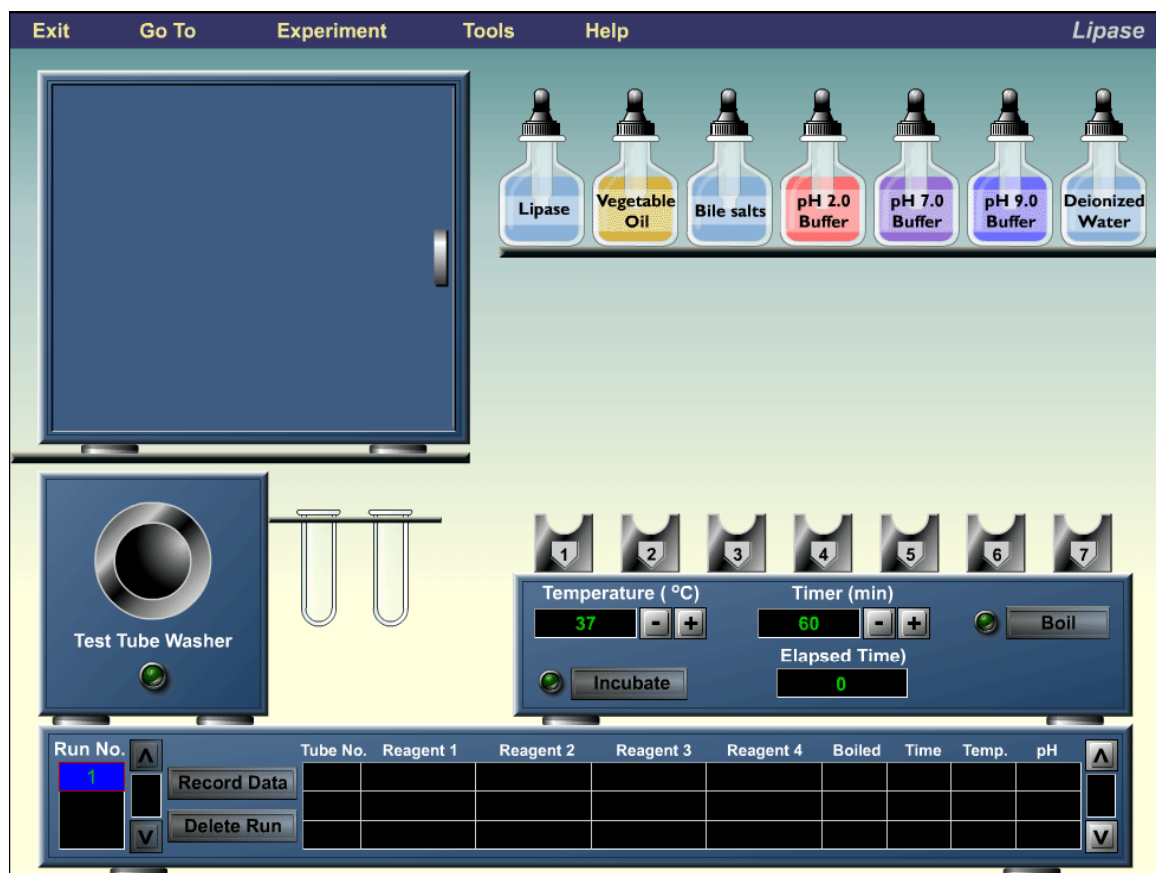
Fat/oil droplets $\xrightarrow{\text{lipase}}$ monoglycerides and fatty acids

Lipase hydrolyzes fats and oils to their component monoglycerides and two fatty acids. Occasionally lipase hydrolyzes fat and oils to glycerol and three fatty acids. Fatty acids decrease pH and provide an easy way to recognize that digestion is ongoing or completed. You will use a pH meter in the assay to record the drop in pH as the test tube contents become acid.

Choose Lipase from the Experiment menu. The screen will look as it does on the following page. The solutions self, test tube washer, and incubation equipment are the same as the other activities; only the solutions have changed.

INCUBATION

1. Individually drag 6 test tubes to the test tube holders in the incubation unit.
2. Prepare the tubes with the solutions indicated in Table 3 by using the following method.
 - Click and hold the mouse button on the dropper cap of the desired substance.
 - While holding the mouse button down, drag the dropper cap to the top of the desired test tube.
 - Release the mouse button to dispense the substance.
3. Adjust the incubation temperature to 37°C and the timer to 60 min (compressed time) by clicking (+) or (-) buttons. Click **Incubate** to start the run. The incubation unit will gently agitate the test tube rack, evenly mixing the contents of all tubes. When the incubation is complete, the tubes will automatically rise, and the doors to the assay cabinet will open.



Assay

After the assay cabinet doors open, you will see a pH meter that you will use to measure the acidity of your test solutions. When a test tube is dragged to the holder in the pH meter and the Measure pH button is clicked, a probe will descend into the sample, take a pH reading, and then retract. The pH of the sample will be displayed in the pH window below the Measure pH button. **A solution containing fatty acids liberated from fat by the action of lipase will exhibit a lower pH than one without fatty acids.**

1. Click and hold the mouse on the first test tube in the incubation unit, and drag it to the holder in the pH meter. Release the mouse button to drop the tube into the holder.
2. Click **Measure pH**.
3. Record the pH in Table 3.
4. Drag the test tube in the pH meter to its original position in the incubation unit, and release the mouse button.
5. Repeat steps 1 – 4 for the remaining test tubes.
6. Click **Record Data** to display your results in the grid and retain your data in the computer's memory for later analysis.

TABLE 3. Pancreatic Lipase Digestion of Fats and the Action of Bile

Tube no.	1	2	3	4	5	6
Additives	Lipase, Vegetable oil Bile salts pH 7.0 buffer	Lipase, Vegetable oil Deionized water pH 7.0 buffer	Lipase, Deionized water Bile salts pH 7.0 buffer	Deionized water Vegetable oil Bile salts pH 7.0 buffer	Lipase Vegetable oil Bile salts pH 2.0 buffer	Lipase Vegetable oil Bile salts pH 9.0 buffer
Incubation Condition	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.	37°C for 60 min.
** pH						
Change in pH						

** Look for a change in pH from the initial pH of the tube and compare it to the final pH of the tube.

QUESTIONS

1. Explain the difference in activity between tubes 1 and 2.

2. Can we determine if fat hydrolysis has occurred in tube 6? Explain your answer.

3. Which pH results in maximum activity in lipase activity?

4. Is this method of assay sufficient to determine if the optimum activity of lipase is at pH 7.0? Explain your answer.

5. In theory, would lipase activity be active in the mouth and/or stomach? Explain your answer.

6. Based on the enzyme pH optima you determined in each of the Activities above, where in the human body would you expect to find the enzymes in these experiments?

Amylase _____

Pepsin _____

Lipase _____