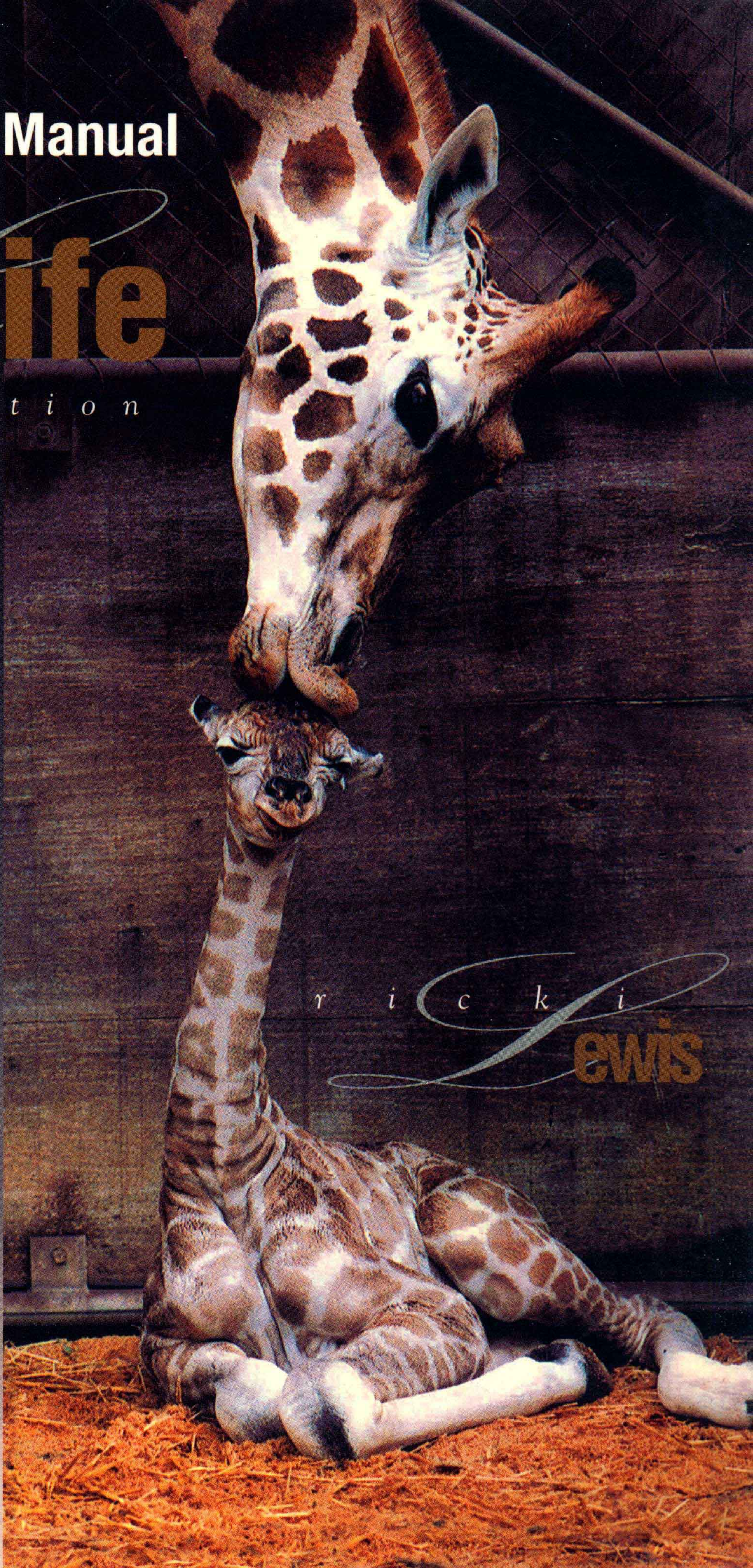
Laboratory Manual

Life

third edition

Prepared by
Alice Jacklet



ricki Lewis

McGraw-Hill

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McGraw-Hill

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Preface

This laboratory manual is designed to accompany *Life* by Ricki Lewis but can easily be adapted for use with most introductory biology textbooks. I have made a vigorous attempt to reduce the intimidation factor that afflicts many beginning science students. The text is written in simple language and is punctuated by poems and cartoons that enliven the material while emphasizing major concepts or important terms. Investigative experiments replace old-school cookbook memorization labs, providing students with an opportunity to learn information via the excitement of independent discovery. The basic premise is that not only is a lab experience integral to a biology curriculum, it also constitutes an essential part of a liberal education by encouraging ingenuity, accuracy, and common sense.

Fifteen years ago, when I became the Coordinator of General Biology at The University at Albany, I searched diligently through the commercially available lab manuals. They fell into two major categories: (1) those that were inexpensive and easy to prepare but dealt with the subject in a dry, mechanical way and (2) those that emphasized investigations but required complicated solutions and technical apparatus unavailable to many institutions. Dissatisfied, I created my own manual.

My pedagogical philosophy and the manual have evolved simultaneously as a result of the experiences of the thousands of students who have passed through my course. I now know that if students are to truly understand and be captivated by science, they must participate in it actively and intimately.

For example, the conventional mitosis lab that relies on observing commercially prepared slides, plastic models, and videos may instruct in the facts, but the wonder of cell division is missing. However, if the students are responsible for germinating seed root tips themselves, they become personally involved in the outcome. They spend the first week worrying: Will it germinate? Then, when it does and the root begins to grow, they feel relief, satisfaction, and increasing interest. Having invested time and emotion, they fix, stain, and observe the dividing cells with a special concern. This intense and personal involvement throughout the entire process engenders an understanding of cell division they simply cannot get from ancillaries.

Each of the topics includes more than enough material to fill 2–3 hours of laboratory work, giving the instructor the option of choosing what best suits the students. To keep preparation time to a minimum and to facilitate a smooth and trouble-free experience, I have written a Laboratory Resource Guide detailing the materials involved, preparations, and how to avoid possible problems.

I think your students will enjoy these lab exercises, and I welcome your comments or suggestions. Sincere thanks to the following reviewers whose input helped improve the third edition of this laboratory manual.

William H. Chrouser
Warner Southern College

Sheila M. Schneider
Cuyahoga Community College

Roger Schmidt
Columbia College

To the Student

The purpose of your laboratory experience is to introduce you to the meaning and method of science by having you perform investigations designed to answer fundamental questions in biology. Attention to the following suggestions will increase your enjoyment of the labs.

1. Always read the laboratory work and fill out the prelab proposal before you come to your laboratory session so you can perform the experiments in a calm and thoughtful manner. Advance preparation can prevent frenzied and aimless activity.
2. Always know what you are doing in the laboratory and *why* you are doing it. If you are confused about instructions, ask your lab instructor.
3. Many individuals are involved in this course. Without consideration for all the others, the lab experience would quickly degenerate into chaos. You are expected to cooperate in leaving the laboratory room as neat as possible, with equipment and glassware properly returned to the correct storage areas, and the lab benches thoroughly cleaned.
4. The cornerstone of science is the ability to approach a new problem with an open-minded and objective attitude. Although you may have a hypothesis before beginning the experiment, never assume that you know what is going to happen until you see your results.
5. We do not expect every experiment to work in textbook fashion for every student. However, we do expect you to record your data faithfully and honestly even if it is different from everyone else's. Do *not* falsify your data or record results you have not actually obtained.
6. The records of your laboratory investigations will take several forms, including written observations, calculations, and data analyzed in tabular and graphic form. All of these records are to be entered on the laboratory reports. They constitute a written record of your observations and conclusions, which is essential for a thorough and meaningful understanding of the subject.
7. Biology is the study of life, not of death. These exercises give you an opportunity to work with living organisms in situations where unnecessary death is eliminated. At the conclusion of the experiments, do not throw the organisms down the drain or dump them casually in the garbage. Place them in the designated "recovery" areas. This simple procedure underscores the premise that all living organisms should be treated with respect.
8. Our aim is to create a healthy environment not only for the experimental organisms, but also for the students. Please pay attention to the safety instructions given in the lab manual and by your instructor.

Most important of all, do not be limited by this book. Learn to observe the world around you and to ask questions. Use the knowledge you gain from this course concerning the complexities of living organisms and their relationships with one another to extend your horizons. The planet Earth depends upon people like you.

Contents

The Scientific Method

1

Prelab Proposal

Objective(s): To learn the scientific method by investigating the effects of drugs (alcohol and caffeine) on the rate of heartbeat in *Daphnia.*

Procedures(s): Record the heartbeat after adding drops of increasing concentrations of alcohol, then drops of increasing concentrations of caffeine. Also perform a control experiment.

Hypothesis: The alcohol will slow the heartbeat rate, and caffeine will increase it. Addition of drops of water should not affect the heartbeat rate.

The Prelab Proposal is a useful tool for preparing yourself for the lab. In future labs you will be required to fill out the Prelab Proposal before attending lab.

Biology is more than the descriptions of life forms. It is a dynamic field whose aim is to unravel the mysteries of life itself. Throughout history, humans have been curious about the world around them. They have observed natural phenomena and wondered "why?" Those who have contributed the most to our knowledge, whether Aristotle 2,000 years ago or today's molecular geneticists, have certain traits in common. They have inquiring minds and great powers of observation, and they use a systematic approach for testing those phenomena that particularly intrigue them.

In this course you will have the opportunity to develop your potential as a scientist. The laboratory exercises are designed to stimulate your curiosity, heighten your powers of observation, and introduce you to the scientific method.

The scientific method is neither complicated nor intimidating—nor is it unique to science. It is a powerful tool of logic that can be employed any time a problem or question about the fundamental nature of something arises. In fact, we all use elements of the scientific method to solve little problems every day, but we do it so quickly and automatically that we are not conscious of the methodology. In brief, the scientific method consists of observing, predicting, testing, and interpreting.

Observation

Observation is the most basic tenet of the scientific method. All biological knowledge is based on situations in which an individual made an observation of a particular event and recorded that observation. Scientists can rely only on their own sense organs, or they can use technological aids that extend their perceptual limits. These aids might be gel electrophoresis to see protein molecules, oscilloscopes to see electrical nerve impulses, or microscopes to see the very small.

Today you will use the stereoscopic (dissecting) microscope. It is much simpler than the compound microscope, which you will study in detail in exercise 2. Think of the microscope as a pair of powerful magnifying glasses. You can adjust the magnification by turning the zoom knob (on top). As with a pair of binoculars, you can adjust the two eyepieces for your eyes so that both fields are viewed as one. Place a coin in the center of the stage. Next, turn the focusing knob (on the side) so that the coin comes into focus.

You will base today's experiment on observations of twentieth-century American life-styles. You have probably observed that when people drink too much coffee, they are often hyperactive. They may be jittery, nervous, and complain about being unable to relax. On the other hand, when people drink beer, their speech often slurs, they may lose control of their muscular coordination, and their reactions may slow down. Too much beer may even cause them to pass out.

Hypothesis

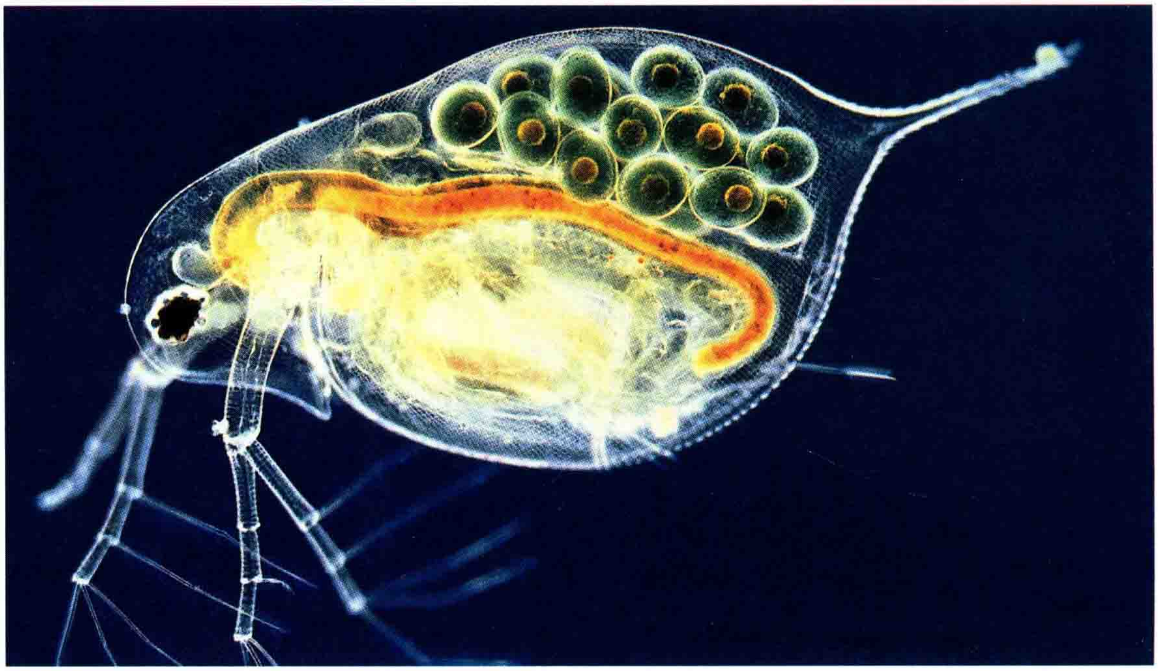
The next step in the scientific method is to make an educated guess (more formally called a hypothesis) based on your observations. A possible hypothesis could be that the alcohol present in beer causes a decrease in reaction time, whereas the caffeine in coffee causes a speeding up of reactions.

Designing an Experiment

Since it is neither wise nor ethical for untrained scientists to experiment on humans, you will instead use a living water flea, *Daphnia magna,* to test your hypothesis (fig. 1.1).

All organisms are classified by names that identify them. Something is not a "bug" simply because it is small.

Figure 1.1 *Daphnia.*



You must identify an organism by its proper scientific name so that other scientists know what you are talking about.

The advantage of studying *Daphnia* is that it is almost transparent. You can see the heart beating, the squeezing action of the intestine, muscular movements, and occasionally, babies in the brood pouch. Also, because *Daphnia* is a small, aquatic organism, it makes an excellent subject for studying the effects of drugs on circulation.

Procedure (Experimental)

1. Capture a living *Daphnia* and place it in a small drop of water on a slide. The most obvious structure is the *eye.* The *brain* is a light-colored organ lying above the eye. Two pairs of *antennae* protrude from the head. These are used for locomotion and to sense the environment. Inside the *exoskeleton* are five pairs of *legs.* Comblike *gills* are attached to some of the legs. When the legs kick forward, they bring a stream of water across the gills and wash bits of food up to the *mouth,* which lies just beneath the *beak.* From the mouth, the *esophagus* runs up into the head and then down into the body, where it widens into the *stomach,* which connects to the *intestine.* The *heart* lies in the upper part of the *Daphnia.* In females, a large *brood chamber* is located behind the heart. Usually it will contain eggs, but occasionally a fortunate student will find it filled with baby *Daphnia.* Label the drawing at the end of this lab, including as many of the italicized structures as you can find. Also write down any pertinent observations you have made.
2. Count the number of heartbeats for 15 seconds. The rate in a healthy *Daphnia* will be very rapid (2–5 beats per second). Record your data on the laboratory report at the end of this lab and calculate the number of beats per minute. The simplest way to do this is to use ratios. For example, if you count 10 beats in 15 seconds, the calculations are as follows:

   $$\frac{X}{60} = \frac{10}{15}; X = \frac{600}{15} \text{ or 40 beats/minute}$$

   Then remove the water by placing an edge of a tissue to it.
3. Place one drop of 2% alcohol on the *Daphnia.* Wait 1 minute and then again count the heartbeats.
4. Using the same procedure, monitor the effects of 4%, 6%, 8%, and 10% alcohol solutions. Record your results.
5. At the end of this series of tests, try the second series, substituting caffeine (1%, 2%, and 3%) for alcohol. Record your results. Note: if, during the alcohol series, your *Daphnia* looks like it is going to pass out or worse, immediately switch to the caffeine series.
6. Having revived the *Daphnia,* return it to the recovery tank.

Even if you performed all your experiments very carefully, you cannot be certain that the effect you see is due to the drugs. Perhaps the change in heartbeat rate is caused by the heat of the microscope light, or perhaps it is affected by the removal or additions of solutions. Without a control experiment, your data are meaningless.

Procedure (Control)

The control procedure must be performed exactly as the experimental procedure. The only difference is that the variable is omitted. In this case that means that alcohol and caffeine are not added. Again place a *Daphnia* on a slide. Using the same time intervals and following the same procedure, substitute one drop of water for each drop of alcohol or caffeine. Record the heart rate on the laboratory report.

Collecting Data

During these biology labs, you will occasionally have an experiment that does not "work." This does not necessarily mean that you have disproved the hypothesis. It does mean that the experiment must be repeated so that variations in technique or in an individual organism's response are put in perspective. For example, having used a different *Daphnia* for the control procedure, how can you be sure that its reactions compare adequately to the experimental procedure? The answer is to repeat the experiment many times. Instead of repeating it yourself, collect your class data and record it on the chart provided in the laboratory report (page 6). Then individual results can be compared to a larger sample.

Analyzing Data

Results from experiments must be presented in a clear, scientific way. The first lab of the year is the time to learn this. If tables and graphs are well constructed, they provide a concise summary and allow the reader to see at a glance the pertinent results of the experiment. Remember, a picture is worth a thousand words—unless, of course, the picture is messy, unclear, or inaccurate. Graph your data on page 7.

Interpretation and Conclusions

One of the most important features of scientific inquiry is the exchange of information. Scientists publish experimental results to make them known to others, and they include their interpretations of what those results mean. In the space provided at the end of this lab, write a brief discussion of this experiment, being careful not to let preconceived notions interfere with an objective analysis.

Further Experiments

The scientific method is a continuing cycle of questions and answers. A good experiment not only answers the question that was originally posed but also gives rise to further questions. As a final assignment, list several questions this experiment raised and the procedures that could be used to test them.

To learn the scientific method is your first task.
From preliminary observations, find a question to ask.
Design an experiment that has the potential
To answer the problem. A control is essential.
Carefully collect data for graphs or a table.
Interpret the results. Is the hypothesis true or a fable?
In your discussion be objective and clear.
Then ask more questions—to be tested next year.

Cleaning Up: The Final Stage of the Scientific Method

Return all materials and solutions, and clean up your work area as directed by your laboratory instructor.

Laboratory Report 1

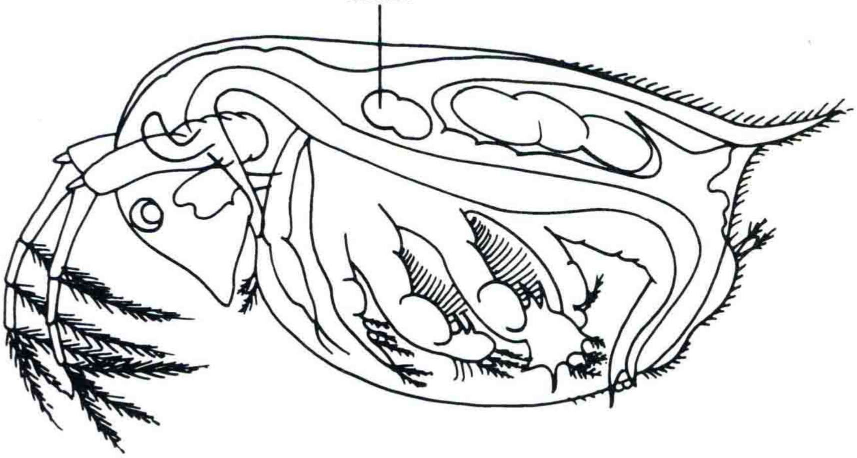
Student ______________________________

Date ______________________________

The Scientific Method

Observations

Label the following diagram of *Daphnia*.



Collecting Data

Experimental Results

Drop #	Drug %	Beats/15 sec	Beats/min

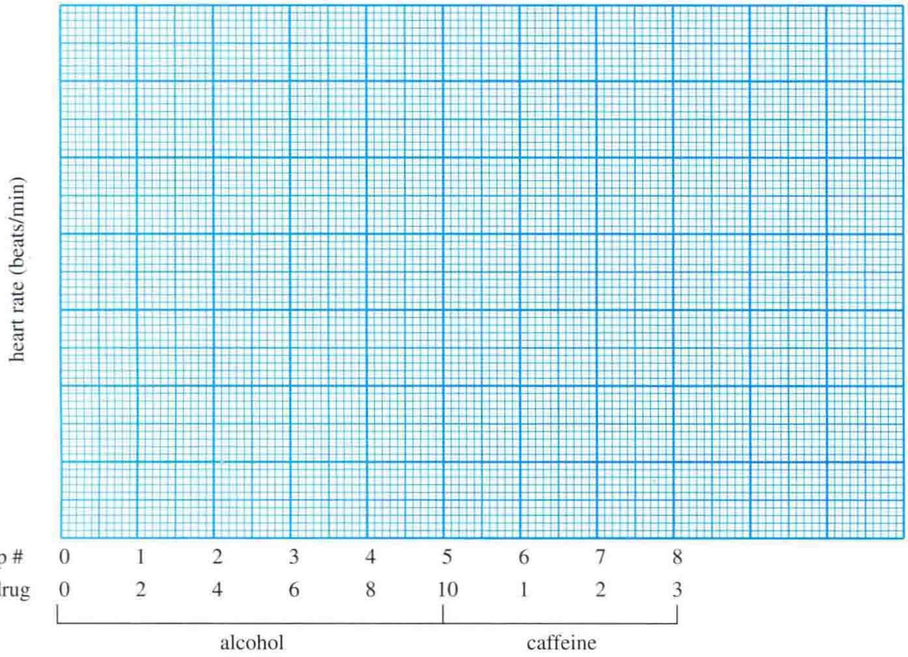
Control Results

Drop #	Water	Beats/15 sec	Beats/min

Sample Size—Compiled Class Data

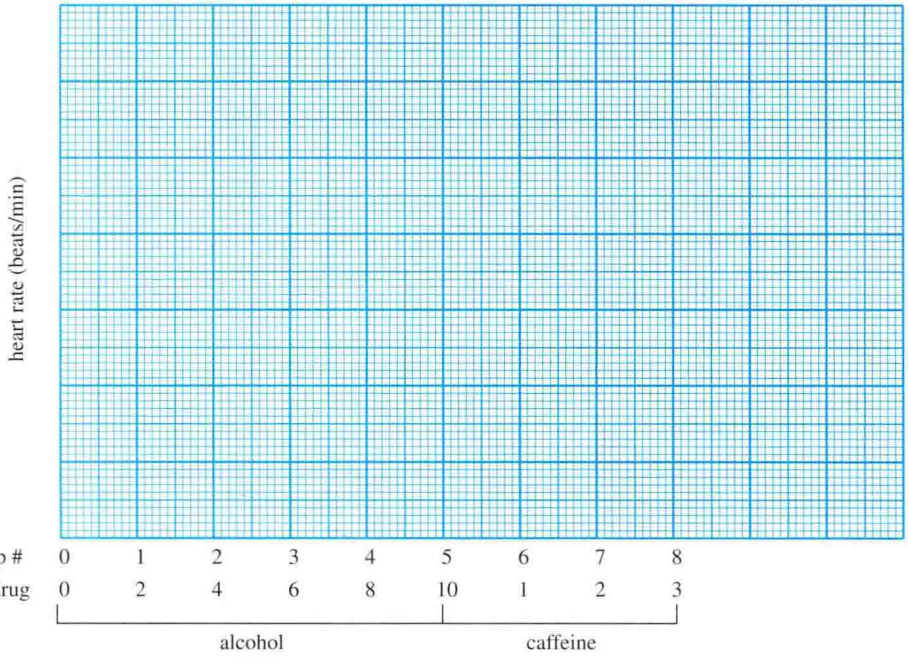
Experiment (beats/min)																							
% of Drug	Student Number																						Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
0% water																							
2% alcohol																							
4% alcohol																							
6% alcohol																							
8% alcohol																							
10% alcohol																							
1% caffeine																							
2% caffeine																							
3% caffeine																							
Water	Control (beats/min)																						Average
Drop 1																							
Drop 2																							
Drop 3																							
Drop 4																							
Drop 5																							
Drop 6																							
Drop 7																							
Drop 8																							

Individual Results



Key:
o = experiment
x = control

Compiled Results



Key:
o = experiment
x = control

Interpretation and Conclusions

Further Experiments

Practice with the Scientific Method

Read the following article and comment on the scientific method it uses:

THE DREAD TOMATO ADDICTION

(Adapted from Mark Clifton. "Astounding Science Fiction," February 1958.)

Ninety-two and four-tenths percent (92.4%) of juvenile delinquents have eaten tomatoes. Eighty-seven and one-tenth percent (87.1%) of the adult criminals in penitentiaries throughout the United States have eaten tomatoes. Eighty-four percent (84%) of all people killed in automobile accidents during the year 1990 had eaten tomatoes.

Those who object to singling out specific groups for statistical proofs require measurements within a total. Of those people born before 1850 and known to have eaten tomatoes, there has been a 100% mortality.

In spite of their dread addiction, a few tomato eaters born between 1850 and 1900 still manage to survive, but the clinical picture is poor—their bones are brittle, their movements feeble, their skin seamed and wrinkled, their eyesight failing, hair falling out, and frequently they have lost all their teeth.

Those born between 1900 and 1950 number somewhat more survivors, but the overt signs of the addiction's dread effects differ not in kind but only in degree of deterioration. Prognosis is not hopeful.

Exhaustive experimentation shows that when tomatoes are withheld from an addict, invariably his cravings will cause him to turn to substitutes such as oranges or steak and potatoes. If both tomatoes and all substitutes are persistently withheld, death invariably results within a short time!

The skeptic of apocryphal statistics or the stubborn nonconformist who will not accept the clearly proven conclusions of others may conduct his own experiment. Obtain two dozen tomatoes—they may actually be purchased within a block of some high schools, or discovered growing in a respected neighbor's backyard! Crush them to a pulp in exactly the state they would be in if introduced into the stomach, pour the vile juice and pulp into a bowl, and place a goldfish therein. Within minutes the goldfish will be dead!

Those who argue that what affects a goldfish might not apply to a human being may, at their own choice, wish to conduct a direct experiment by fully immersing a live human head into the mixture for a full 5 minutes.

Source: Adapted from Mark Clifton, "Astounding Science Fiction," February 1958.

Comments:

The Microscope

2

Prelab Proposal

Objective(s): ______________________________

____

____

Procedure(s): ______________________________

____

____

Hypothesis: ______________________________

____

____

____

The world is filled with an amazing diversity of living organisms. Some are made up of millions of cells, like *Daphnia,* and some are only one cell, like the protistan *Euglena.* (As you proceed through these labs, read the information in the text that pertains to the organism you are currently studying.) Even a tiny, one-celled organism exhibits behavior. Today you will test the reaction of *Euglena* to light (phototaxis). Since *Euglena* are tiny organisms, in order to perform this experiment you must be able to use a compound microscope.

If you have never used a compound microscope, or if you need a review, study this section in detail. Several laboratory exercises depend on your familiarity with the parts of the microscope and with their interactions. A little extra practice now will pay off later.

A microscope is a delicate and expensive instrument and must be treated gently. It allows you to enter the world of the very small, which is a fascinating experience. If your eyes and back are strained, however, or if the image is blurry, you will experience frustration rather than fascination. In order to enjoy your experience, you must be physically and mentally comfortable as you use the microscope. Feeling at ease will come with practice.

The Parts of a Compound Microscope

A microscope has three basic requirements: (1) magnifying lenses, (2) a means of focusing, and (3) light.

1. A compound microscope is so named because it has two sets of magnifying lenses. The *eyepiece* is the cylinder in the top of the microscope (fig. 2.1). It contains *lenses,* which magnify the object 10 times. The next set of lenses is found in the three small cylinders (*objectives*) located on the *revolving* nosepiece. Each one magnifies a different amount. The general rule is: the shorter the objective, the less the magnification. The total magnification is determined by multiplying the magnification of the eyepiece times the magnification of the objective. If the eyepiece magnifies 10 times (10×) and the objective you use magnifies 10 times, then the total magnification is 100 times. The object will appear 100 times larger. How much larger will an object appear if you use a 10× eyepiece and a 43× objective? ________Wipe the eyepiece lens and the lens of each objective carefully with lens paper. The major enemy of microscopes is dust. Each microscope has a dust cover, which should be kept on the instrument when not in use.
2. The microscope is focused by turning the two adjustment wheels on the sides. The outside wheel is the *coarse adjustment* and is used to bring the object into view. The *fine adjustment* (the inside wheel) is used for sharp focus. What happens to the objectives when you turn the coarse adjustment *toward* you?

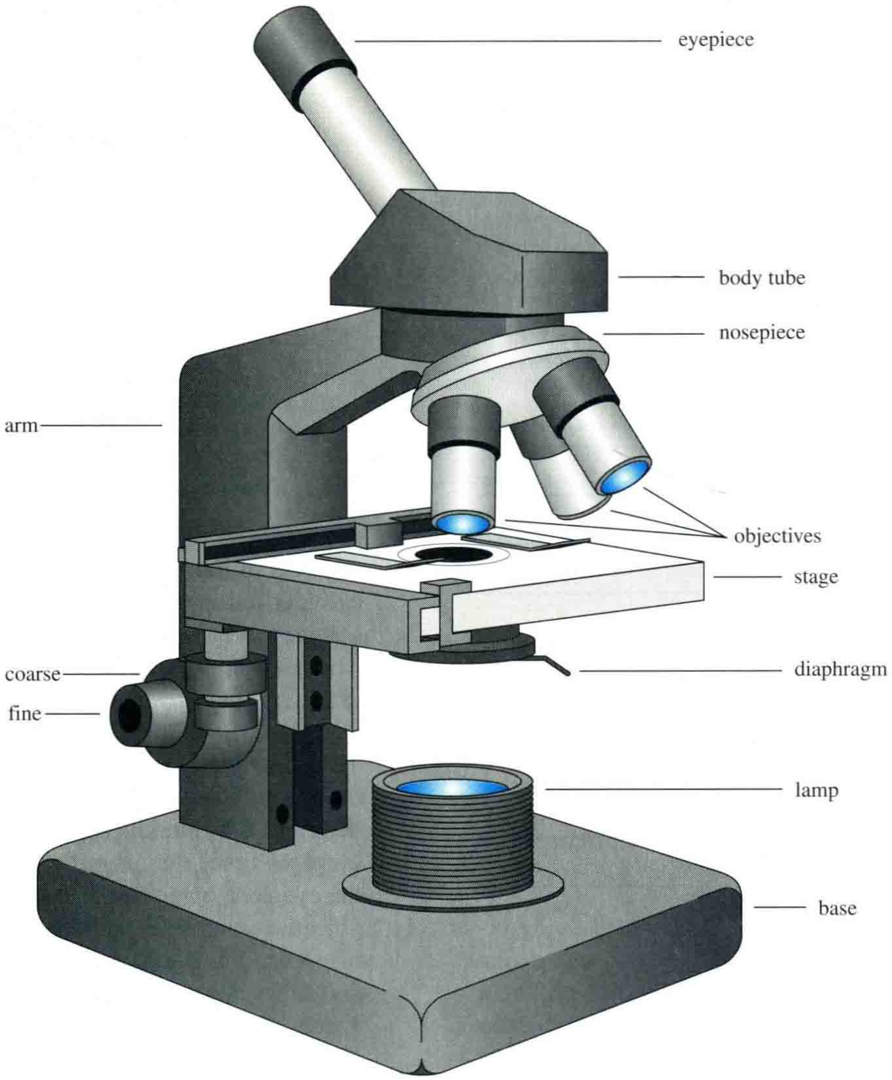
   ____

   Turning the fine adjustment wheels has the same effect but so slight that it can hardly be noticed. With the objectives raised, rotate the nosepiece to see how each objective clicks into position. Leave the lowest objective clicked into place.
3. Turn on the light. Move the *diaphragm lever* (below the stage) to the left and to the right. At which position is the light the brightest? ______________

   The dimmest? ______________________

   Leave the diaphragm open about halfway.

Figure 2.1 The microscope.



At this point, the lenses are clean, the objectives are safely raised with the lowest objective clicked into place, and the diaphragm lever is positioned in the middle. Now you are ready to use the microscope.

Use of the Microscope—Practice Session

1. At the bottom of this page is a group of printed letters. Cut it out and place this small piece of paper on a slide. Add a drop of water and place a coverslip on top. Position the slide on the stage of your microscope so that the light goes through the specimen and clip the slide into place.
2. Watching the bottom lens, and using the coarse adjustment knob, slowly turn the *low*-power objective down as far as it will go. Do not force it beyond the automatic stop. This stop prevents the low-power objective from hitting the slide.

Euglena

3. Place your eye to the eyepiece and turn the coarse adjustment slowly toward you, raising the objective. When in proper focus, a 4× objective is about 2 centimeters above the slide and a 10× objective is about 1 centimeter above. A 43× objective will almost touch the slide, which is why you should *always* focus by *raising* the objectives, not lowering them.
4. Slowly rotate the fine adjustment knob until specimen detail is in sharp focus. You may need to adjust the amount of light by moving the diaphragm lever. Does the object appear normal or upside down? ______________________
When you move the slide away from you a tiny bit, does the object appear to move away from you or toward you? ______________ It is good technique to keep both eyes open while looking through the eyepiece.
5. Once specimen detail is in sharp focus under the low-power objective, you can rotate to other objectives without changing the position of the coarse

adjustment knob. Very little refocusing is required. Center the image in the middle of the field of view and move the next highest objective into position. You may have to focus a little with the fine focus adjustment but *do not* touch the coarse focus adjustment. You may also have to change the amount of light in order to have a clear view.

Microscopes are designed to increase the apparent size
Of objects too tiny for human eyes.
Remember: It is a precision machine.
Handle it with care and keep the lenses clean.
They're in an eyepiece on top, and objectives at lower station;
Multiplication of both gives total magnification.
Adjust the light by moving the diaphragm lever.
Now find a Euglena *for your next endeavor.*

The Reaction of *Euglena* to Light

Today you will perform a simple experiment testing the reaction of *Euglena* (fig. 2.2) to light (phototaxis). Since *Euglena* are tiny organisms that can swim in and out of the field of view, this exercise will be a good test of your ability to use a microscope. Another purpose of this laboratory is to introduce you to the way scientists write about their experiments.

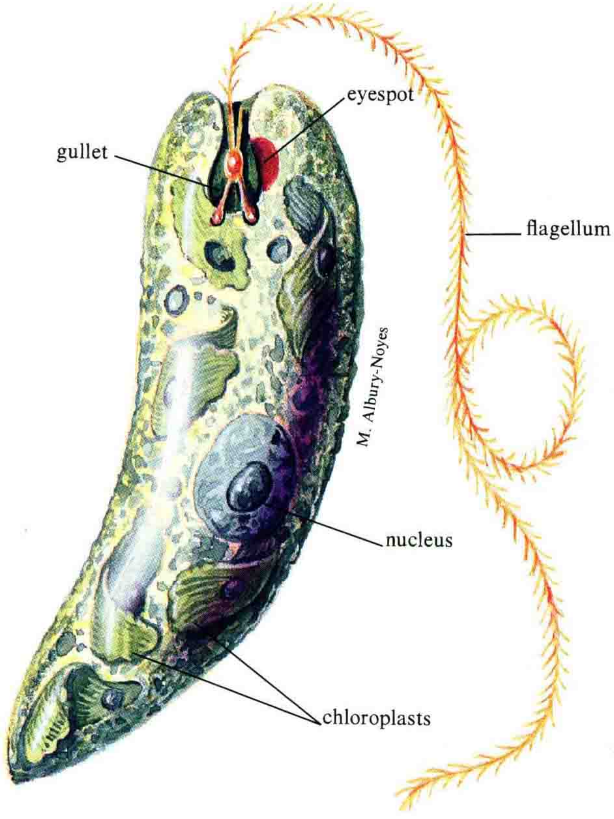
An essential component of the scientific method is communication. Scientists must not only do science, they must also write about it. A research project is not completed until it is published, because only then is new knowledge made available to other scientists. By tradition, a scientific paper follows a specific format: title, abstract, introduction, material and methods, results, discussion, and references. Your assignment will be to write a report on today's investigations in the correct format. To make it easier for you, the paper has been formatted and partially written. You need only fill in the blanks. After this exercise, you should have no trouble writing lab reports in the correct scientific format. (For more detail, see Appendix B.)

Title: The title should be short and informative and should accurately portray the scope of the paper: Phototaxis in *Euglena gracilis.*

Abstract: The abstract is a *short* summary paragraph at the beginning of the report that a scientist (in this case, your lab instructor) looks at to decide if he or she is interested in reading the rest of the paper—so make the abstract good! It should include the problem investigated, the hypothesis, the major methods employed, the major results obtained, and the major conclusion reached.

Introduction: The introduction gives the background of the paper, explaining the general problem being investigated and why it is of interest. In this section you should consult outside references. Be sure to keep track of where you get this information so that you can present your references accurately at the end of the paper.

Figure 2.2 *Euglena.*



1. State the purpose of the investigation.
2. Provide background information on the experiment. In this paper you might include the following: the scientific name of the organism, a brief description of its physical characteristics (size, single cell, eyespot), relevant behavior (euglenoid and flagellar movement), physiological processes (chloroplasts, photosynthesis), and information on the particular process you have investigated (phototaxis).
3. State your hypothesis and explain why it is a good one. Hint: since *Euglena* use photosynthesis (a light-requiring process) to make their food, do you expect positive phototaxis (a tendency to move toward the light) or negative phototaxis (a tendency to move away from the light)?

Methods: This section should include a *concise* description of the equipment, materials, and procedures used. Include enough information so the reader could repeat your experiments. Write this section in the past tense because you are describing the work that *was* done. Also, do not list the