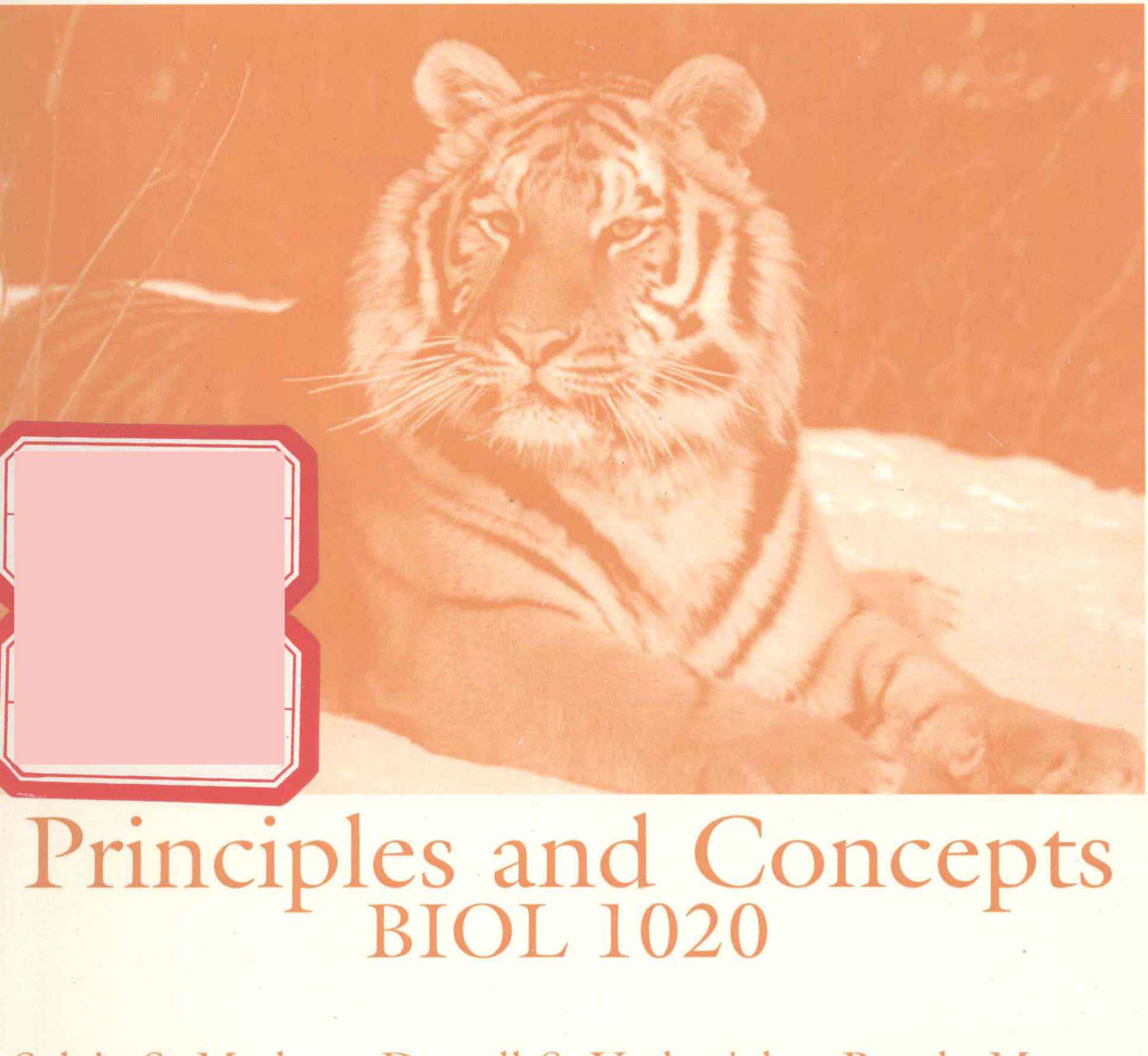
LABORATORY INVESTIGATIONS IN BIOLOGY



Principles and Concepts
BIOL 1020

Sylvia S. Mader • Darrell S. Vodopich • Randy Moore
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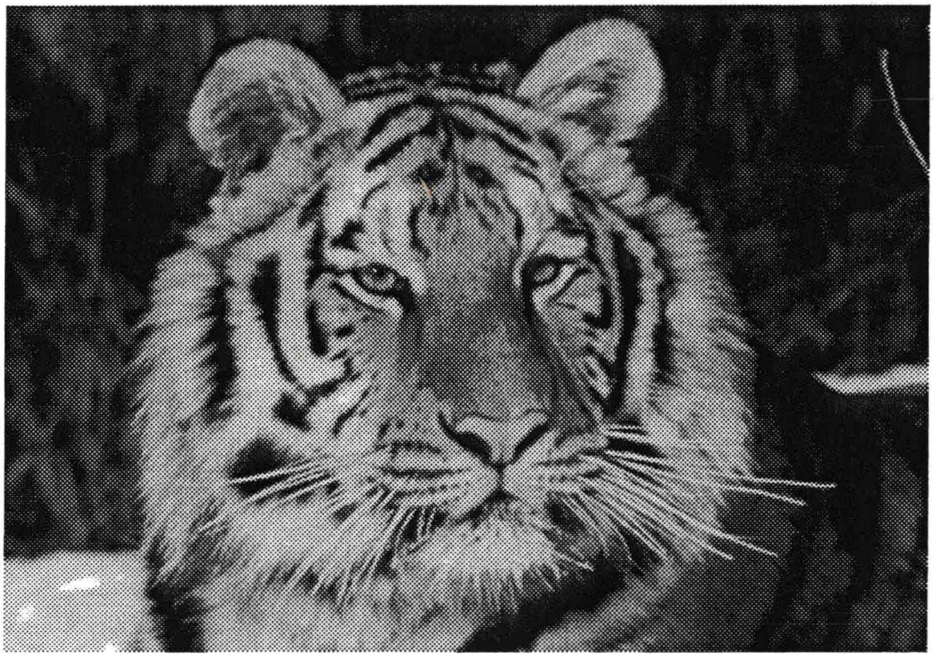

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Principles and Concepts
BIOL 1020

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Laboratory Safety

Many of the reagents (chemicals) and some equipment in a biology laboratory are potentially dangerous. Following rules of laboratory safety and using common sense throughout the course will enhance the learning experience by increasing the student's confidence in his or her ability to safely use chemicals and equipment.

The following rules of laboratory safety should be studied and should become a habit.

1. Wear safety glasses or goggles during exercises in which glassware and solutions are heated, or during which dangerous fumes may be present, creating possible hazard to eyes or contact lenses.
2. Assume that all reagents are poisonous and act accordingly. Read labels on chemical bottles for safety precautions and know the nature of the chemical you are using. If chemicals come into contact with skin, wash immediately with water.
3. **DO NOT**
   a. ingest any reagents;
   b. eat, drink, or smoke in the laboratory. Toxic material may be present, and some chemicals are flammable;
   c. carry reagent bottles around the room;
   d. pipette anything by mouth;
   e. put chemicals in sink or trash unless instructed to do so;
   f. pour chemicals back into containers unless instructed to do so;
   g. operate any equipment until you are instructed in its use.
4. **DO**
   a. keep work area neat, clean, and organized. Ask your instructor for assistance in cleaning up broken glassware and spills. Wash hands, desk area, and glassware at the end of each exercise and/or before leaving the laboratory.
   b. stopper all reagent bottles when not in use. Immediately wash reagents off yourself and your clothing if they spill on you, and immediately inform the instructor. If you accidentally get any reagent in your mouth, rinse mouth thoroughly and immediately inform your instructor.
   c. handle hot glassware with test-tube clamp or tongs. Use caution when using heat, especially when heating chemicals. Do not leave a flame unattended; do not light a Bunsen burner near a gas tank or cylinder; do not move a lit Bunsen burner; do keep long hair and loose clothing well away from the flame; do make certain gas jets are off when Bunsen burner is not in use. Use proper ventilation and hoods when instructed.
   d. use extra care when working with scalpels, knives, and glass tubing.
   e. wear clothing that, if damaged, would not be a serious loss, or use aprons or laboratory coats, since some chemicals may damage fabrics.
   f. wear shoes as protection against broken glass or spillage that may not have been adequately cleaned up.
   g. be familiar with the experiments you will be doing before coming to the laboratory. This will increase your understanding, enjoyment, and safety during exercises. Confusion is dangerous. Completely follow the procedure set forth by the instructor.
   h. note the location of emergency equipment such as a first aid kit, eyewash bottle, fire extinguisher, switch for ceiling showers, fire blanket(s), sand bucket, and telephone (911). Report all accidents immediately.
   i. report any condition that appears unsafe or hazardous to the instructor.

I understand the safety rules as presented above, and agree to follow them and all other instructions given by the instructor.

______________________________ ______________

Name   Date

____

Laboratory Class and Time

TABLE OF CONTENTS

WEEK

1

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.

THE SCIENTIFIC METHOD

Prelab Proposal

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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.

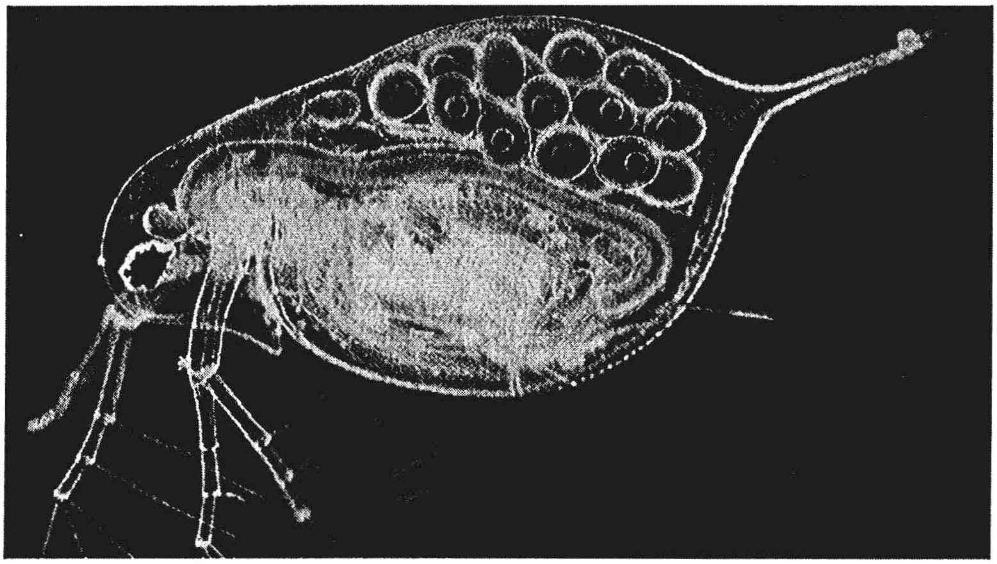


Figure 1.1 Daphnia

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:

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

   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. Then individual results can be compared to a larger sample.

Analyzing Data

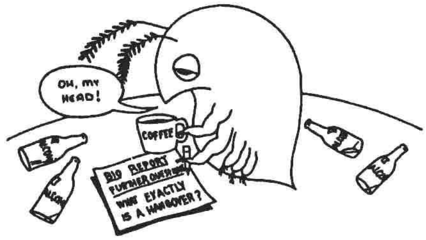
Results from experiments must be presented in a clear, scientific way. The first lab of the year is the time to learn this. If tables and graphs are well constructed, they provide a concise summary and allow the reader to see at a glance the pertinent results of the experiment. Remember, a picture is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

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

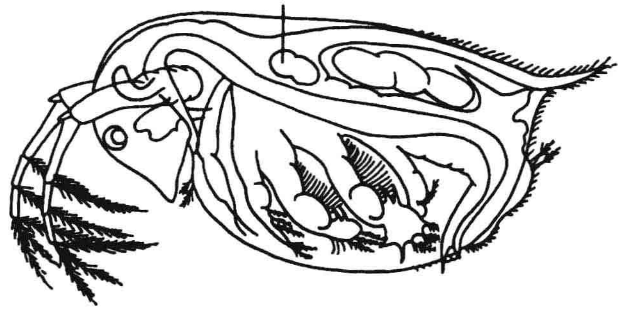
Return all materials and solutions, and clean up your work area as directed by your laboratory instructor.

LABORATORY REPORT I

The Scientific Method

Observations

Label the following diagram of *Daphnia*.



Collecting Data

Experimental Results

Drop #	Drug %	Beats/15 sec	Beats/min

Control Results

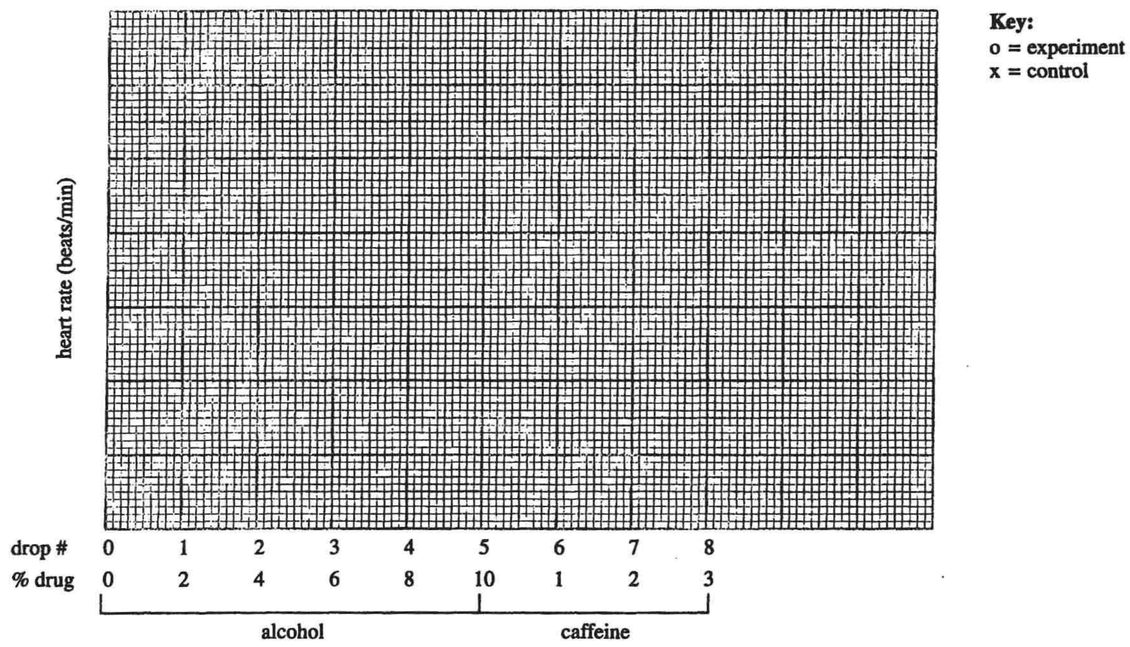
Drop #	Water	Beats/15 sec	Beats/min

Sample Size - Compiled Class Data

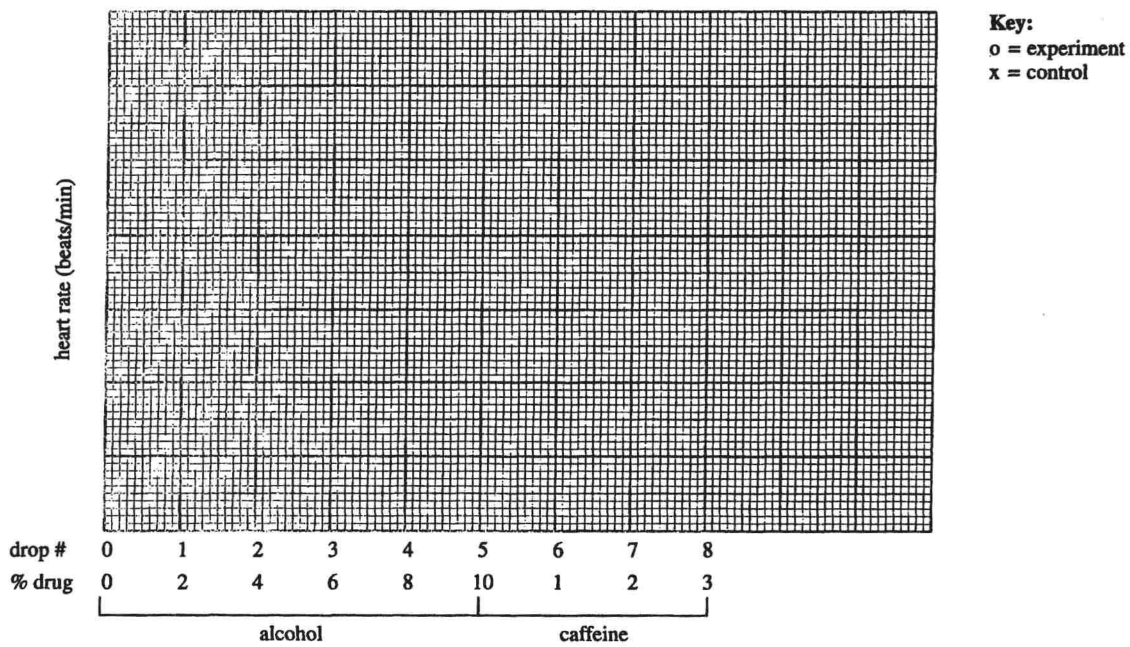
Experiment (beats/min)																							
% of Drug	Student Number																						Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
0% water																							
2% alcohol																							
4% alcohol																							
6% alcohol																							
8% alcohol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

Analyzing Data

Individual Results



Compiled Results



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 toconduct 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:

WEEK

2

Learning Objectives

Students should be able to

2.1 The Metric System

1. state and use metric units of measurement for length, temperature, weight, and volume.

2.2 Microscopy

2. describe three differences between the compound light microscope and the electron microscope.

2.3 Use of the Compound Light Microscope

3. name and give the function of the basic parts of the compound light microscope.
4. list, in proper order, the steps used for bringing an object into focus with the compound light microscope.
5. describe how the slide of the letter *e* provides information on the inversion of the image in the compound light microscope.
6. calculate the diameter of the field and the total magnification for both the low- and high-power lens systems.
7. explain how the slide of colored threads provides information on the depth of field.

2.4 Microscopic Observations

8. name the three kinds of cells studied in this exercise.
9. state two differences between onion epidermal cells and human epithelial cells.

2.5 Binocular Dissecting Microscope (Stereomicroscope)

10. identify the parts and tell how to focus the binocular dissecting microscope.