Biological Investigations

Sixth Edition

Form,

Function,

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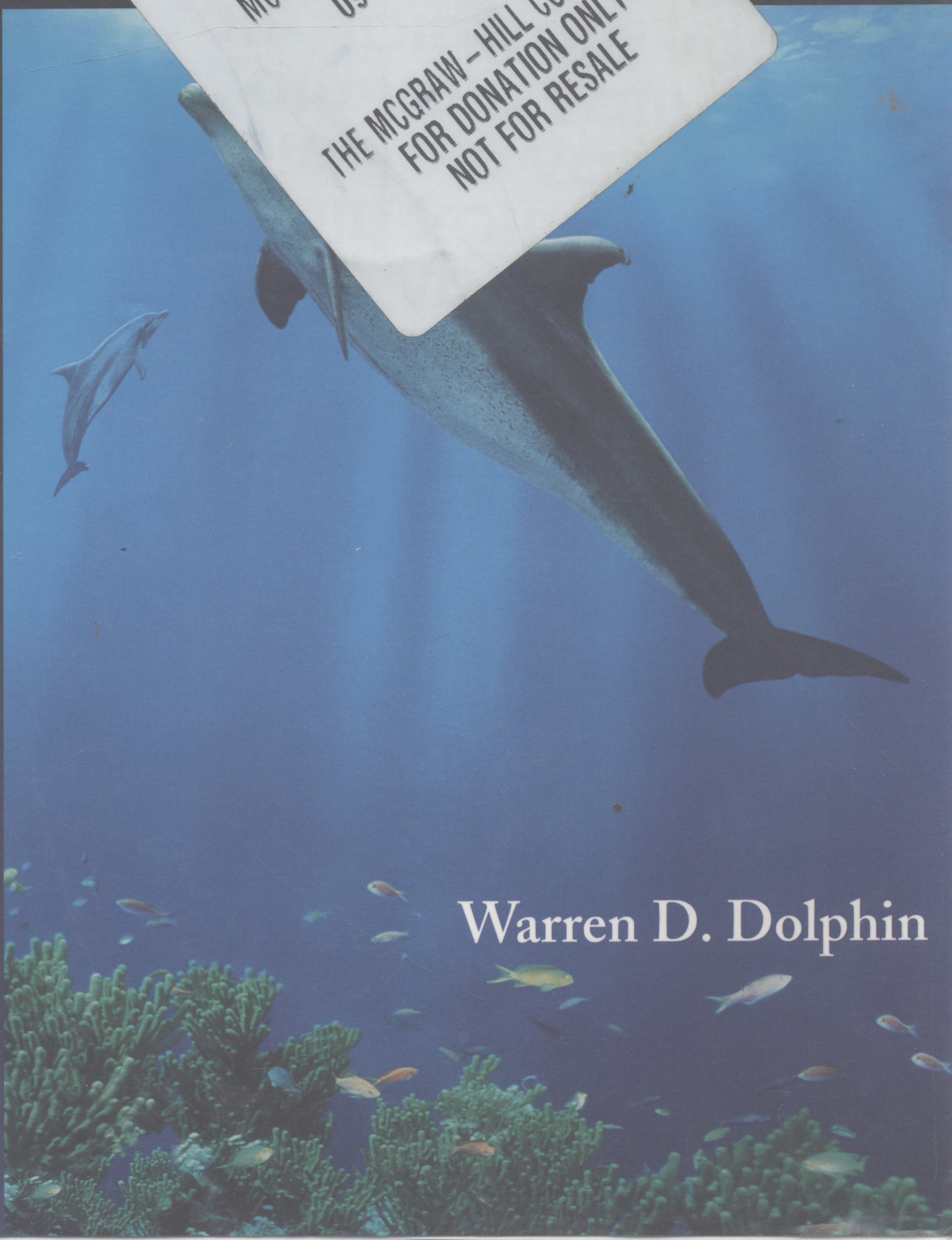
and

Process



Warren D. Dolphin

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Sixth Edition

Form, Function, Diversity, and Process

Warren D. Dolphin

Iowa State University

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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PREFACE

This lab manual is dedicated to the many students and colleagues who have been my patient teachers. I hope that it returns some of what has been learned so that a new generation of biologists may soon add to our wonder of nature's ways while advancing our understanding of life's diverse forms and processes.

As reflected in the subtitle, this lab manual reflects fundamental biological principles based on the common thread of evolution: form reflects function; unity despite diversity; and the adaptive processes of life. The manual was written for use in a two-semester introductory biology course serving life science majors. I have emphasized investigatory, quantitative, and comparative approaches to studying the life sciences and have integrated physical sciences principles where appropriate. In choosing topics for inclusion, I sought to achieve a balance between experimental, observational, and comparative activities. The comments of several expert reviewers were incorporated into this revision, clarifying many points from previous editions. The activities included in each lab topic have been tested in multisection lab courses and are known to work well in the hands of students.

Throughout the manual, the concept of hypothesis testing as the basic method of inquiry has been emphasized. Starting with lab topic 1 on the scientific method, and reiterated in experimental topics throughout the manual, students are asked to form hypotheses to be tested during their lab work and then are asked to reach a conclusion to accept or reject their hypotheses. Hypothesis testing and a comparative trend analysis also have been added into the more traditional labs dealing with diversity so that students are guided to look across several labs in reaching conclusions. Labs investigating physiological systems and morphology emphasize the concept of form reflects function. Comparative activities are included to demonstrate the adaptations found in several organisms.

Nature of the Revisions

Several major changes were made in this edition. The plant section was thoroughly revised. The old plant phylogeny lab topic is now divided into two topics, the seedless and seed plants, to better reflect the time needed to study plant phylogeny, and alternation of generations is given greater emphasis. The section on the functional biology of angiosperms was also extensively revised. The old transport lab topic was divided into two lab topics, one emphasizing plant tissue systems and primary root structure, and the other emphasizing primary and secondary growth in stems. In addition some experiments were changed in other labs. In Lab Topic 1 about the scientific method, the experiment was changed from one testing physical fitness to one that emphasizes reaction time so that less athletic students will feel included and the results are not as predictable before the experiment. A new fruit fly experiment has been added which has more of an investigative theme requiring students to determine the genotypes of unknowns they are given. It can be completed in two weeks rather than the four required for the old experiments. The microevolution lab topic was rewritten and now includes student activities and computer simulations to teach the Hardy-Weinberg Principle instead of drawing beads from a container to illustrate statistical sampling. The taxonomic classifications for bacteria and protists were updated to reflect current thinking and the information in textbooks. In several of the exercises, the student activities were streamlined deleting experiments that usually were not performed for lack of time. All exercises were edited to improve clarity based on experience with students at Iowa State University.

New teaching elements were added as well. Each lab topic now starts with a Pre-lab Preparation section. In this section key vocabulary terms are listed and key concepts are named. The expectation is that students will realize that they must study vocabulary and concepts before coming to lab. Lab instructors can reinforce this realization by giving short quizzes before starting lab work. At the end of each lab topic, there is a section entitled "Learning Biology by Writing." For those departments that have strong writing-across-the-curriculum emphases, the suggested assignments will complement their goals. Several new Critical Thinking and Lab Summary Questions have also been added at the end of each lab topic.

Organization of Lab Topics

The lab topics have a standard format. All start with the Pre-lab Preparation section. This is followed by a list of equipment, organisms, and solutions to be used during the lab, informing students about what they will encounter in the lab. A brief introduction explains the biological principles to be investigated. These introductions are not meant to replace a textbook. They are included to summarize ideas that students will have had in lecture and to discuss how they apply to the lab. The lab instructions are detailed and allow students to proceed at their own pace through either experimental or observational lab work. Dangers are noted and explained. Data tables help students organize their lab observations. Questions are interspersed to avoid a cookbook approach to science and spaces are provided for answers and sketches. New terms are in boldface the first time used and are followed by a definition. At the end of each lab topic, several alternative suggestions are given for summarizing the lab work. A Learning Biology by writing section usually describes a writing assignment or lab report. Critical thinking questions emphasize applications. A lab summary based on several questions organizes the reporting of lab activities in a more stepwise approach. An Internet sources section points the students toward information sources on the WWW. Appendices include discussions of the use of significant figures, directions on making graphs, a description of elementary statistics, and instructions of how to write a lab report.

WWW Site

Under the sponsorship of McGraw-Hill, a WWW site has been established for this manual at http//www.mhhe.com/dolphin/

There you will find a preparator's manual giving recipes of chemical solutions and sources of supplies for each of the exercises. Also included is a list of links to other WWW sites which have materials relevant to the topics that students are investigating in the labs. If you know of links that should be included, please send them to me by E-mail (wdolphin@iastate.edu).

Acknowledgments

I would especially like to thank James Colbert, Associate Professor of Botany at Iowa State University, for his helpful comments and his patience in explaining plant biology. I also wish to thank the critical reviewers who made constructive suggestions throughout the writing of this manual: William Barstow, University of Georgia; Daryl Sweeney, University of Illinois; Gerald Gates, University of Redlands; Marvin Druger, Syracuse University; Thomas Mertens, Ball State University; Cynthia M. Handler, University of Delaware; Stan Eisen, Christian Brothers College; Paul Biebel, Dickinson College; Stephen G. Saupe, St. Johns University (Minnesota); Sidney S. Herman, Lehigh University; Margaret Krawiec, Lehigh University; Charles Lycan, Tarrant County Junior College; Olukemi Adewusi, Ferris State University; Karel Rogers, Adams State College; Peter A. Lauzetta, Kingsborough Community College (CUNY); Maria Begonia, Jackson State University; Thomas Clark Bowman, Citadel Military College; Gary A. Smith, Tarrant County Junior College; Timothy A. Stabler, Indiana University Northwest; William J. Zimmerman, University of Michigan-Dearborn; and Nancy Segsworth, Capilano College (British Columbia).

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If you have questions or comments, please contact me by E-mail (wdolphin@iastate.edu.).

CORRELATION TABLE How lab topics correlate with chapters in major textbooks

Lab Topic	Audesirk & Audesirk & Biology, 5th ed.	Campbell, Reece & Mitchell Biology, 5th ed.	Lewis et al. Life, 4th ed.	Mader Biology, 7th ed.	Purves, Sadava, Orianes & Heller Life, 6th ed.	Raven & Johnson Biology, 6th ed.	Solomon, Berg, & Martin Biology, 5th ed.
1. Science: A Way of Gathering Knowledge	1	1	1	1	1	1	1
2. Techniques in Microscopy	6	7	3	4	4	5	4
3. Cellular Structure Reflects Function	6	7	3	4	4	5	4
4. Determining How Materials Enter Cells	5	8	4	5	5	6	5
5. Quantitative NA Techniques and Statistics		NA	NA	NA	NA	NA	NA
6. Determining the Properties of an Enzyme	4	6	5	6	6	8	6
7. Measuring Cellular Respiration	8	9	6	6	7	9	7
8. Determining Chromosome Number in Mitotic Cells	11	12	8	9	9	11	9
9. Observing Meiosis and Determining Crossover Frequency	11	13	9	10	9	13	10
10. Using Mendelian Principles to Determine the Genotypes of Fruit Flies	12	14, 15	10, 11	11, 12	10	13	10
11. Isolating DNA and Working with Plasmids	9, 13	16, 20	12	14, 17	11, 17	14, 19	11, 14
12. Testing Assumptions in Microevolution and Inducing Mutations	15	23	13, 15	16, 19	21	20, 21	18
13. Using Bacteria as Experimental Organisms	19	27	20	29	26	34	23
14. Diversity Among Protists	19	28	21	30	27	35	24
15. Plant Phylogeny: Seedless Plants	21	29	22	32	28	37	26
16. Plant Phylogeny: Seed Plants	21	30	22	32	29	37	27
17. Fungal Diversity and Symbiotic Relationships	20	31	23	31	30	36	25
18. Early Events in Animal Development	36	32, 47		51	16, 43	60	49

CORRELATION TABLE How lab topics correlate with chapters in major textbooks (continued)

19. Animal Phylogeny: Evolution of Body Plan	22	33	24	33	31	44	28
20. Protostomes I: Evolutionary Development of Complexity	22	33	24	34	31	45	29
21. Protostomes II: A Body Plan Allowing Great Diversity	22	33	24	34	32	46	29
22. Deuterstomes: Origins of the Vertebrates	22	37	25	35	33	47, 48	30
23. Investigating Plant Tissues and Root Structure	23	35	26, 27	36	34	38, 39	31
24. Investigating Stem Structure, Growth, and Function	23	36	27	36, 37	35	39	33
25. Investigating Leaf Structure and Photosynthesis	7	10	6	7	8	10	8, 32
26. Investigating Angiosperm Reproduction and Development	24	38, 39	28, 29	39	37, 38	40, 42, 43	35, 36
27. Investigating Digestive and Gas Exchange Systems	28, 29	41, 42	36, 37	43, 44	48, 50	51, 53	44, 45
28. Investigating Circulatory Systems	27	42	35	41	49	52	42
29. Investigating the Excretory and Reproductive Systems	30, 35	44, 46	38	50	40, 42, 51	58, 59	46, 48
30. Investigating Form and Function in Muscle and Skeletal Systems	34	49	34	48	47	50	38
31. Investigating the Nervous and Sensory Systems	33	48, 49	31	46, 47	44, 45, 46	54, 55	39, 40, 41
32. Statistically Analyzing Simple Behaviors	37	51	41	22	52	27	50
33. Estimating Population Size and Growth	38	52	43	23	54	24	51
34. Standard Assays of Water Quality	40	54	44	25	56	29, 30	54, 55

BRIEF CONTENTS

CONTENTS

LAB TOPIC 1

Science: A Way of Gathering Knowledge

Supplies

Preparator's guide available at
http://www.mhhe.com/dolphin

Materials

Meter sticks
Photo copies of newspaper, magazine, and journal articles about biology (AIDS, rainforests, or cloning would be good examples, especially if articles were coordinated so students see same material intended for different audiences.)

Prelab Preparation

Before doing this lab, you should read the introduction and sections of the lab topic that have been scheduled by the instructor.

You should use your textbook to review the definitions of the following terms:

Dependent variable
Hypothesis
Independent variable
Scientific literature

You should be able to describe in your own words the following concepts:

Critical reading
Experimental design
Reaction time
Scientific method

As a result of this review, you most likely have questions about terms, concepts, or how you will do the experiments included in this lab. Write these questions in the space below or in the margins of the pages of this lab topic. The lab experiments should help you answer these questions, or you can ask your instructor for help during the lab.

Objectives

1. To understand the central role of hypothesis testing in the modern scientific method
2. To design and conduct an experiment using the scientific method
3. To summarize sample data as charts and graphs; to learn to draw conclusions from data
4. To evaluate writing for its science content and style

Background

Many dictionaries define science as a body of knowledge dealing with facts or truths concerning nature. The emphasis is on facts, and there is an implication that absolute truth is involved. Ask scientists whether this is a reasonable definition and few will agree. To them, science is a process. It involves gathering information in a certain way to increase humankind's understanding of the facts, relationships, and laws of nature. At the same time, they would add that this understanding is always considered tentative and subject to revision in light of new discoveries.

Science is based on three fundamental principles:

The *principle of unification* indicates that any explanation of complex observations should invoke a simplicity of causes such that the simplest explanation with the least modifying statements is considered the best; also known as the law of parsimony.

The second principle is that *causality is universal;* when experimental conditions are replicated, identical results will be obtained regardless of when or where the work is repeated. This principle allows science to be self-analytical and self-correcting, but it requires a standard of measurement and calibration to make results comparable.

The third principle is that of the *uniformity of nature;* it states that the future will resemble the past so that what we learned yesterday applies tomorrow.

For many, science is just a refined way of using common sense in finding answers to questions. During our everyday lives, we try to determine cause and effect relationships and presume that what happened in the past has a high probability of happening in the future. We look for relationships in the activities that we engage in, and in the phenomena that we observe. We ask ourselves questions about these daily experiences and often propose tentative explanations that we seek to confirm through additional observations. We interpret new information in light of previous proposals and are always making decisions about whether our hunches are right or wrong. In this way, we build experience from the past and apply it to the future. The process of science is similar.

The origin of today's scientific method can be found in the logical methods of Aristotle. He advocated that three principles should be applied to any study of nature:

1. One should carefully collect observations about the natural phenomenon.
2. These observations should be studied to determine the similarities and differences; i.e., a compare and contrast approach should be used to summarize the observations.
3. A summarizing principle should be developed.

While scientists do not always follow the strict order of steps to be outlined, the modern scientific method starts, as did Aristotle, with careful observations of nature or with a reading of the works of others who have reported their observations of nature. A scientist then asks questions based on this preliminary information-gathering phase. The questions may deal with how something is similar to or different from something else or how two or more observations relate to each other. The quality of the questions relates to the quality of the preliminary observations because it is difficult to ask good questions without first having an understanding of the subject.

After spending some time in considering the questions, a scientist will state a research **hypothesis,** a general answer to a key question. This process consists of studying events until one feels safe in deciding that future events will follow a certain pattern so that a prediction can be made. In forming a hypothesis, the assumptions are stated and a tentative explanation proposed that links possible cause and effect. A key aspect of a hypothesis, and indeed of the modern scientific method, is that the hypothesis must be falsifiable; i.e., if a critical experiment were performed and yielded certain information, the hypothesis would be declared false and would be discarded, because it was not useful in predicting any natural phenomenon. If a hypothesis cannot be proven false by additional experiments, it is considered to be tentatively true and useful, but it is not considered absolute truth. Possibly another experiment could prove it false, even though scientists cannot think of one at the moment. Thus, recognize that science does not deal with absolute truths but with a sequence of probabilistic explanations that when added together give a tentative understanding of nature. Science advances as a result of the rejection of false ideas expressed as hypotheses and tested through experiments. Hypotheses that over the years are not falsified and which are useful in predicting natural phenomena are called theories or principles—for example, the principles of Mendelian genetics.

Hypotheses are made in mutually exclusive couplets called the **null hypothesis (H_o)** and the **alternative hypothesis (H_a)**. The null hypothesis is stated as a negative and the alternative as a positive. For example, when crossing fruit flies a null hypothesis might be that the principles of Mendelian genetics do not predict the outcomes of the experiment. The alternative hypothesis would be that Mendelian principles do predict the outcome of the experiment. As you can see, rival hypotheses constitute alternative, mutually exclusive statements: both cannot be true.

The purpose in proposing a null hypothesis is to make a statement that could be proven false if data were available. Experiments or reviews of previously conducted experiments provide the data and are therefore the means for testing hypotheses. In designing experiments to test a hypothesis, predictions are made. If the hypothesis is accurate, predictions based on it should be true. In converting a research hypothesis into a prediction, a deductive reasoning approach is employed using if-then statements: if the hypothesis is true, then this will happen when an experimental variable is changed. The experiment is then conducted and as certain variables are changed, the response is observed. If the response corresponds to the prediction, the hypothesis is supported and accepted; if not, the hypothesis is falsified and rejected.

The design of experiments to test hypotheses requires considerable thought! The variables must be identified, appropriate measures developed, and extraneous influences must be controlled. The **independent variable** is that which will be varied during the experiment; it is the cause. The **dependent variable** is the effect; it should change as a result of varying the independent variable. **Control variables** are also identified and are kept constant throughout the experiment. Their influence on the dependent variable is not known, but it is reasoned that if kept constant they cannot cause changes in the dependent variable and confuse the interpretation of the experiment.

Once the variables are defined, decisions must be made regarding how to measure the effect of the variables. Measures may be quantitative (numerical) or qualitative (categorical) and imply the use of a standard. The metric system has been adopted as the international standard for science. If the independent variables are to be varied, a decision must be made concerning the scale or level of the treatments. For example, if something is to be warmed, what will be the range of temperatures used? Most biological material stops functioning (dies) at temperatures above 40°C and it would not be productive to test at temperatures every 10°C throughout the range 0° to 100°C. Another aspect of experimental design is the idea of replication: how many times should the experiment be repeated in order to have confidence in the results and to develop an appreciation in the variability of the response.

Once collected, experimental data are reviewed and summarized to answer the question: does the data falsify or support the null hypothesis? The research conclusions then state the decision regarding the acceptability of the null hypothesis and discuss the implications of the decision.

If the experimental data are consistent with the predictions from the null hypothesis, the hypothesis is supported, but not proven absolutely true. It is considered true only on a trial basis. If the hypothesis is in a popular area of research, others may independently devise experiments to test the same hypothesis. A hypothesis that cannot be falsified, despite repeated attempts, will gradually be accepted by others as a description that is probably true and worthy of being considered as suitable background material when making new hypotheses. If, on the other hand, the data do not conform to the prediction based on the null hypothesis, the hypothesis is rejected and the alternative hypothesis is supported.

Modern science is a collaborative activity with people working together in a number of ways. When a scientist reviews the work of others in journals or when scientists work in lab teams, they help one another with interpretation of data and in the design of experiments. When a hypothesis has been tested in a lab and the results are judged to be significant, she or he then prepares to share this information with others. This is done by preparing a presentation for a scientific meeting or a written article for a journal. In both forms of communication, the author shares the preliminary observations that led to the forming of the hypothesis, the data from the experiments that tested the hypothesis, and the conclusions based on the data. Thus, the information becomes public and is carefully scrutinized by peers who may find a flaw in the logic or who may accept it as a valuable contribution to the field. Thus, the scientific discussion fostered by presentation and publication creates an evaluation function that makes science self-correcting. Only robust hypotheses survive this careful scrutiny and become the common knowledge of science.

LAB INSTRUCTIONS

You will create a research hypothesis, design an experiment to test it, conduct the experiment, summarize the data, and come to a conclusion about the acceptability of the hypothesis. You will also practice evaluating scientific information from various published sources.

Using the Scientific Method

Description of the Problem

Working in groups of four, you are to develop a scientific hypothesis and test it. The topic will be neuromuscular reaction time. This can be easily measured in the lab by measuring how quickly a person can grasp a falling meter stick. The person whose reaction time is being measured sits at a table with her or his forearm on the top and the hand extended over the edge, palm to the side and the thumb and forefinger partially extended. A second person holds a meter stick just above the extended fingers and drops it. The subject tries to catch it. The distance the meter stick drops is a measure of reaction time.

Your assignment is to create a scientifically answerable question regarding reaction time in individuals with different characteristics and to express this as testable hypotheses. You will then design an experiment to test the hypotheses, collect the data, analyze, and come to a decision to reject or accept your hypothesis. For example, you might investigate the differences between those who play musical instruments and those who do not or try a more complex design that investigates gender differences in reaction time for students who are in some type of athletic training versus those who are not. The design will depend on the hypotheses that you decide to test as a group in your lab section. Continuing the example, you might propose a null hypothesis that there will be no significant differences in reaction time between musicians and nonmusicians. An alternative hypothesis would be that there is a significant difference in the reaction times between the two types.

Summarizing Observations

Start your discussion of this assignment by summarizing the collective knowledge of your group about neuromuscular response time. Are these responses the same for all people or might they vary by athletic history, gender, body size, age, hobbies requiring manual dexterity, left versus right hand, or other factors? Be sure to consider these factors in both a qualitative and quantitative light. You might expect differences in the physiological responses of those who exercise. What other factors might influence the response time? As your group discussion proceeds, make notes below that summarize the group's knowledge and observations about what characteristics influence reaction time.

Asking Questions

Research starts by asking questions which are then refined into hypotheses. Review the group observations that you listed and write down scientifically answerable questions that your group has about reaction time in people with different characteristics. Be prepared to present your group's best questions to the class and to record the best questions from the class on a piece of paper.

Forming Hypotheses

With your group, review the questions posed in the class discussion. Examine the questions for their answerability. Do some lack focus? Are they too broad? Are others too simple, with obvious answers? By what criteria would you judge a good question?