INTRODUCTORY BIOLOGY LABORATORY MANUALS

THE DEVELOPMENT OF LABORATORY COURSES

FOR

INTRODUCTORY BIOLOGY

By

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ABSTRACT

This project describes the development and evaluation of two laboratory courses in First Year Biology, each of which is part of a larger full-year course of instruction given by the Biology Department at McMaster University. Introductory Human Physiology is prepared for Physical Education students. Adaptation in the Biological World - a general Biology course - is prepared for Natural Sciences students.

Design of the laboratory exercises utilizes a variety of different educational models which are intended to stimulate the students' interest in Biology. The exercises give students first-hand experience with important principles and concepts related to the lecture material.

This project stresses the role of the Teaching Assistants who supervise activities in the laboratories and who demonstrate the basic skills we expect students to learn.

Conclusions drawn from this project are:

1. The majority of students consider the laboratory courses to be useful.
2. Educational goals established for the courses are being met.
3. Change and improvement are important ongoing components of the curriculum.
4. As funds become available, we must introduce more interesting techniques and methodologies to the curriculum.
5. It is essential to maintain a high level of efficiency and organization within the team of people associated with laboratories.

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CHAPTER I

GENERAL INTRODUCTION

The development of laboratory courses in Biology involves a great deal more than manuals of instruction. In essence, it means the development of a laboratory curriculum for the courses. Accordingly, the curriculum includes all those elements which contribute to the laboratory setting for purposes of education and which are involved in the interaction between students, demonstrators, instructors and the subject matter.

This project shows how the design of laboratory (lab) courses for Biology 1H6 and Biology 1A6 is related to learning theory. A critical ongoing assessment of the application of the laboratory exercises has prompted modifications and improvements which are now important aspects of the courses. These changes are explained with regard to their contribution to more enjoyable student learning experiences.

In 1980, the Biology Department was asked to give an introductory course in Human Physiology to 320 First Year Physical Education students. During the first trial year, instructions for eight labs were prepared by Dr. C. M. Wood, the instructor for the course. They were printed as individual handouts and given to students at the beginning of each lab. When it was decided to offer the course on a permanent basis, I was put in charge of the laboratory section as Instructional Assistant and given the task of reorganizing and expanding the original handouts into a permanent lab manual of ten units. This was completed between May and August of 1981.

In 1983, I was asked by the Departmental Chairman to take over

responsibility for the labs of the First Year Introductory Biology course with its enrollment of 500 students. This new assignment meant that I left the Physiology (Biology 1H6) course.

As Instructional Assistant in charge of labs for Biology 1A6, I developed a totally new lab manual over the summer and fall of 1983.

The educational goals which have guided the writing of these two manuals are based upon my perceptions of the students, their academic objectives, the nature of Biology as subject matter, and my practical philosophy of the best learning environments, developed after many years of experience. These educational goals are:

- to involve students actively in learning situations
- to help students achieve an excitement for learning
- to help develop laboratory skills and an understanding of basic laboratory procedures
- to foster the development of an inquiring mind
- to develop the students' ability to write clear, well-organized reports
- to assist students in reaching their potential for learning by expanding their intellectual capabilities

The laboratory environment is central to the achievement of these objectives. It is organized to be a friendly, non-threatening place where students can ask any question without fear of appearing foolish. In addition to discussion with the demonstrator or myself, students are encouraged to discuss experimental outcomes among themselves.

The technical aspects of laboratories and their administrative organization are of considerable importance. Unless experiments can

produce clear and visible results and unless the laboratory is set up in a workable manner, the essentials of the laboratory experience can be lost in a fog of confusion. It is therefore important to test in advance all procedures, equipment and materials to be used by the students.

It is equally important to establish a solid working team of demonstrators (Teaching Assistants or TAs) who are as dedicated to the principles of excellence in teaching as are the instructors. TAs are extremely busy graduate students themselves with tight schedules. They may have little inherent feeling for the freshman students who do not appear as concerned with proficiency as we think they should. The TAs must be coached to become experts on "the lesson-of-the-week". They must indicate enthusiasm for the lab itself and patience for those who are confronting its demands for the first time. Finally, they must spend many hours marking reports and making appropriate comments for improvement. As a matter of principle, it is important that marking be consistent throughout the team. Consistency in marking also helps to forestall dissatisfaction and grumbling by students over marking procedures. Since the demonstrators have the closest and most prolonged contact with individual students, they have by far the greatest influence on laboratory success. Consequently, their preparedness, attitude, enthusiasm and consistency are all of crucial importance.

Although the TAs must be careful not to give away specific answers to lab questions, they are available to discuss student reasoning or to direct this, if necessary. In addition to the lab manual questions, appropriate questions posed by the demonstrator while

moving among the students are an extremely important tool to ensure that learning is occurring.

Questions can uncover areas of confusion.

They can clarify issues.

Questions focus student concentration on a particular area.

They separate relevant from irrelevant information.

They help to assess information.

They help to create insight.

However, students should never leave the lab with all the questions still unanswered in their minds. This is frustrating for them and counter-productive for us, especially if they have picked up wrong information. Ten or fifteen minutes before the end of the lab time, demonstrators are expected to schedule a "debriefing session". All gather into an informal group to discuss their results and the significant points.

The TA sits down among the students and encourages them to explain points of difficulty to each other. However, the TA does not do the explaining. If some students do not understand or are reluctant to speak out, the TA prompts their understanding by asking sequences of open-ended questions, forcing students to verbalize their thinking. Thus, the TA can help direct their reasoning. Students are encouraged to formulate clear and logical statements, always backed up with evidence. The TA verifies the accuracy of their reasoning if it is correct, but asks others to make modifications or corrections when necessary. The TA may then paraphrase or summarize their ideas since repetition of this kind clearly contributes to student learning and to

reinforcement of what is already known. Instead of answering specific questions, TAs are directed to practise the art of asking alternative questions which lead students to answer their own queries. By way of caution, however, TAs are warned to avoid the type of question which has a yes or no answer because it limits the discussion; similarly, they avoid questions which are obscure or which might trap the student into a blunder of any kind.

Thus, the role of the demonstrator is an extremely important one. In most cases, it is the determining factor between student attainment of laboratory learning objectives and failure in this regard. In recognition of the importance of their role, the University sponsors a TA Day in early September, during which the TAs are invited to participate in seminars and workshops which prepare them for their teaching responsibilities. The Biology Department also hosts an informal gathering on the same day for this purpose. At the first TA meeting before the beginning of labs, I outline the perspective of our course regarding the responsibilities of TAs in facilitating the instructional goals. I also give them written guidelines to study (Appendix C, pp. 1H6 i-iv and 1A6 i-v). There is a one-hour TA meeting before the start of each unit during which we review all important points.

Ultimately, however, the laboratory manual of instruction is the most important factor in the development of a laboratory course. All other aspects depend upon and are shaped by the manual. Decker F. Walker (1980) calls the writing of curriculum documents a difficult craft requiring a high level of writing skill. He states that, "locally produced curriculum documents are more important than any

other sort of writing about the curriculum because they are more closely bound up with the actual people and events that constitute the curriculum in practice".

CHAPTER II

RELATIONSHIP OF THE MANUALS TO TEACHING

Whether consciously or not, all of our lives are ordered by some sort of theoretical basis. Accordingly, the orientation of an effective teacher is reflected in the focus of his or her personal theories of teaching, teaching strategies, and learning processes. Theory is equally important for good and effective curriculum planning. Its ultimate test, however, lies in the relationship of theory to practice. Unfortunately, it should be noted that instructors do not always follow through with espoused theory.

Educators may call upon a wide variety of theoretical models of teaching depending upon their personal preference and the nature of the subject matter involved. Some are oriented toward social relations, others toward personality development or to learning systems. I do not feel it is necessary to follow a single conceptual model, but it is advisable for people in education to have a well-defined primary focus which reflects priorities and which gives a rational consistency to the curriculum.

The ideological basis of my thinking on curriculum has come mainly from two sources - the Academic Model as outlined by Tyler (1949) and the Psychology-based Development of Cognitive Processes Models outlined by Eisner and Vallance (1974).

Tyler states that the process of curriculum development begins with the identification of objectives and continues through the careful

selection of content, learning activities, teaching techniques and evaluation procedures. I agree with Tyler. In fact, I have already outlined my educational objectives for the lab manual according to Tylerian guidelines and each lab exercise begins with a series of behavioral objectives. Tyler's Academic Model stresses student academic achievement in the form of acquisition of knowledge. Certainly, knowledge per se is of great importance. However, I feel that processes for acquiring knowledge are of even greater importance in laboratory education. Skills such as organizing, hypothesizing, analyzing and interpreting, when once mastered, are transferable to unfamiliar materials or problems. They are also of greater long-term value than the memorization of facts. Hence, I also espouse the Development of Cognitive Processes approach to curriculum (Eisner & Vallance, 1974). This orientation specifies that the goals of schooling provide a repertoire of content-independent cognitive skills that apply to a variety of situations. Thus, it emphasizes the processes of thinking and of learning over the acquisition of knowledge in the form of facts.

Joyce, Wald & Weil (1981) define "creative learning" as the process of learning something totally new or rediscovering what has already been known by others, or as the rearrangement of existing knowledge. It is based on Synectics - a system originated by Gordon (1961) for the development of creativity. I would also like to promote "creative learning" in our laboratories by the planning of exercises which involve the processes of inquiry, problem-solving, creative thinking, and discovery.

Having established the conceptual framework of curriculum development which has influenced me, I turned to Joyce and Weils' (1980)

Information Processing family of models for the design of individual labs. Some information processing models are concerned with productive thinking, i.e., the ability of the learner to solve problems; others are concerned with intellectual ability. Several emphasize concepts and information derived from the academic disciplines. Within this family are seven different models of teaching, all expanded and refined by Joyce and Weil, each with its own unique features. Of these seven I shall give a brief outline of six which have been considered for use.

1. Concept Attainment Model

This model was developed by Joyce and Weil (1980) from the work of Bruner, Goodnow & Austin (1967), to teach concept learning to children. The method uses inductive reasoning, meaning that it is a teaching strategy which uses data to teach concepts and generalizations.

Phase I is the presentation of a large amount of data. The student analyzes the data and formulates an hypothesis about the concept involved.

Phase II is the testing or evaluation by students of the hypothesis.

Phase III is the analysis by students of the thinking strategy.

The specific use of this model can direct the particular learning activities chosen. For instance, if the emphasis of a lesson is on a concept, the teacher emphasizes this in his or her questions. Similarly, the emphasis may be on the inductive process, so the teacher provides fewer clues, forcing students to do their own reasoning. In other situations, the emphasis could be on

analysis of the thinking phase. Thus, the model is adaptable to different learning situations.

2. Inductive Thinking Model

This model was developed by Taba (1967). It is process-oriented but may also be used to teach content. Her models explore some of the same territory as Bruner's Concept Attainment Model (1967). Although Taba's model uses the same processes, it teaches generalization rather than concepts. She believed that thinking skills should use specific questioning strategies designed for those thinking skills. The strategies should be used sequentially because one thinking skill builds upon the other. Taba has developed three teaching strategies to promote inductive thinking.

Stage I - Concept Formation

This is a basic teaching strategy.

Phase I involves the identification and enumeration of relevant data.

Phase II requires the grouping or organization of items into some logical order.

Phase III involves the labelling of items.

Stage II - Interpretation of Data

Taba calls this stage the interpreting, inferring and generalizing strategy. Each phase of the activity requires certain mental processes and the teacher guides these by appropriate questions from one phase into the next.

Phase I is the differentiation of characteristics.

Phase II involves relating points one to another. It also involves the determination of cause/effect relationships.

Phase III is finding implications and making inferences.

Stage III - Application of Principles

The third cognitive task Taba uses is the prediction of consequences from established conditions.

Phase I requires students to predict consequences that would result from modification of the data.

Phase II Students explain and support those predictions.

Phase III Students verify their predictions or outline conditions which might verify them.

3. Inquiry Training Model

This system was developed by Suchman (1962) to teach students a process for investigating and explaining unfamiliar phenomena. Origin of the model is a belief in the development of independent learning through active participation by students in inquiry.

Phase I involves confronting the students with a puzzling situation or problem.

Phase II involves data gathering by students and verification of conditions.

Phase III involves further data gathering or experimentation to

isolate relevant variables and test relationships.

Phase IV is the drafting of an explanation.

Phase V is an analysis of the inquiry procedure by students to determine whether it was successful, or efficient and to look for improvements of strategy.

4. Advance Organizer Model

This model, developed by Ausubel (1963), applies to situations where the teacher acts as lecturer or explainer. It is a deductive information processing model which means that it gives the broader, more general ideas first, then moves to more specific ideas afterwards. The main purpose of the model is to help students learn subject matter by the presentation of concepts or principles directly. Ausubel calls this an "intellectual scaffolding" upon which new ideas and facts may be applied. The design is based upon the theory that meaningful learning (that which will be remembered long-term) builds upon previously acquired knowledge, making new material easier to understand and therefore to remember.

Phase I involves the establishment of the aims and context of the lesson. It also involves presentation of the advance organizer and integration of the organizer with the students' existing knowledge.

Phase II is the presentation of new learning tasks or materials.

Phase III is the anchoring of the new material into the existing cognitive structure of the student.

N.B.: The advance organizer is not to be confused with simple introductory comments. It must be an idea distinct of itself and of a higher level of abstraction and generality than the learning material which follows, e.g., when planning a lesson on world geography, one teacher gave his students this advance organizer. "Geography is a science which tries to understand the relationships between mankind and the earth on which he lives." The lesson then went on to explore various related specifics.

5. Cognitive Growth Model

This model is an application of the learning philosophy of the Swiss psychologist Piaget (1952). It utilizes the theory that children develop increasingly more complex levels of thinking in distinct stages of maturation, thus moving through assimilation (the incorporation of new experience) to a higher level of thought by accommodation (changing thought structure to fit new experiences) (Joyce & Weil, 1980).

Phase I is the presentation of puzzling situations which the individual may be expected to handle.

Phase II is the posing of questions to determine the students' level of reasoning and degree of understanding. Justification may be expected.

Phase III is the presentation of a related task or set of questions to determine whether the student is able to transfer understanding to the new area.

6. Biological Science Inquiry Model

Schwab was one of the foremost members of the Academic Reform Movement in American education during the 1950s and 1960s. He developed the Biological Sciences Curriculum Study (BSCS) (1965) which teaches students to process information using techniques similar to those of research biologists. The Inquiry Model, which is based on Schwab's BSCS study, is designed for areas of inquiry where end results are still unknown and the texts do not give answers. Groups of students are assigned separate but related areas of investigation. Students are expected to design their own ways of handling any problems which may appear.

Phase I The area of investigation is outlined.

Phase II The students structure problems to be solved.

Phase III Students identify difficulties of the investigation.

Phase IV Students speculate on ways of clearing up the difficulties.

CHAPTER III

BIOLOGY 1H6: HUMAN PHYSIOLOGY

FOR FIRST YEAR PHYSICAL EDUCATION STUDENTS

Introduction

(i) Student Background and Laboratory Organization

Of the 320 students enrolled in Biology 1H6 in 1981, most of them intend to make Physical Education and working with young people their career path. A few have other purposes in mind but all of these appear to have some basis in health sciences. In general, their biological background has been meagre and for most of them it is their first experience in a scientific laboratory.

Students attend a 2 h lab period every other week, each lab emphasizing a different physiological system. Since students come from diverse non-science backgrounds, it is essential that instructions be simple, clear and precise. Each of the topics is relevant to human behaviour and to everyday life, but also sets standards for physical health, fitness and ability, areas of prime concern to these young athletes.

Twenty percent of the students' final course mark comes from their ten lab reports. Guidelines for the preparation of these standard scientific reports are given in the manual. TAs mark, make appropriate comments and return the reports during each following lab period. In addition, quizzes include a few questions on lab material, so, overall, the laboratories account for about 25% of the final grade in Human

Physiology 1H6.

In 1981, Instructors for this course were Dr. C. M. Wood and Dr. D.G. McDonald. The assigned textbook is Human Physiology, the Mechanisms of Body Function, Vander, Sherman and Luciano (1980).

(ii) Internal Laboratory Assessment

The effectiveness of the laboratory manual as a learning tool is weighed from my vantage point as Instructional Assistant coordinating these labs. Included is a personal assessment of student performance as well. This appraisal follows the discussion of each laboratory unit: a more complete evaluation is given on p. 103.

In May 1981, the TAs were called to a final course meeting to discuss what had gone well in the labs and what needed modification. This information is summarized in the evaluation (p. 103), but may also be referred to in discussions of individual units.

Many of the laboratory units were designed using the same or similar models of teaching. For that reason it would be tedious and repetitious to give a full accounting of the organizational details of all ten Biology 1H6 labs and all ten Biology 1A6 lab exercises. Accordingly, I have elected to choose five examples from each course which reflect different approaches and different models of design. Those discussed in full detail are:

Lab I - Diffusion and Osmosis - Inductive Thinking Model

Lab II - Neurophysiology I - Reaction Time - Concept Attainment Model - Technical Model

Lab VI - Respiratory Gas Exchange in the Mouse - Biological Science Inquiry Model

Lab VII - Fitness - Advance Organizer Model

Lab X - Nutrition - Inquiry Training Model

Those given in brief outline are:

Lab III - Sensory Function - Biological Science Inquiry Model

Lab IV - The Blood - Inductive Thinking Model

Lab V - Cardiovascular Function - Inductive Thinking Model

Lab VIII - Kidney Function - Inductive Thinking Model

Lab IX - Salivary Amylase - An Investigative Process Lab

LAB I - DIFFUSION AND OSMOSIS

- A Detailed Account

- Appendix A, p. 8

Diffusion is the most common form of molecular movement within all forms of life. Osmosis is a specific form of diffusion - the diffusion of water across semipermeable membranes.

Focus of the Unit (Orientation and Goals)

This lab unit is special because it is the students' first introduction to Biology labs. Accordingly, the primary purpose is to create a laboratory experience by which students will appreciate and respect the learning potential of future labs in this course.

The primary objective of the lab itself is to ensure that students clearly understand the process of diffusion and how osmosis differs from diffusion.

The model of teaching is Taba's Inductive Thinking Strategy (1967) for the processing and integrating of information. This model introduces the procedures of academic or research reasoning and of concept formation. These learning skills will be of benefit in tackling future scientific investigations. The students are actively involved in setting up the exercise. It requires them to identify cause/effect relationships and tests their observational abilities, since they must interpret observations in order to develop the concept which drives the process of diffusion.

Living cells must be able to take in nutrients from the extracellular environment and give off metabolic end products to it.

This requires the plasma membrane to be selectively permeable. Similarly, organelle (subcellular) membranes permit the isolation of various chemical reactions within organelles. The process of diffusion is central to the movement of nutrients and other substances through the cellular and subcellular membranes of living organisms. Nerve cells and muscle cells are able to function because of diffusion. The rate of diffusion is also important in determining the rate of metabolic processes.

Instructional Design (The Model in Action)

Stage I involves careful observation in order to identify important facts.

a) Working in pairs, students bore small holes in plates of agar, each to be filled with similar volumes of different chemical solutions. Ions from these solutions diffuse out into the agar at different rates as observed by measuring the distance per unit time that the diffusion front moves from its origin.

b) Osmosis is a specific type of diffusion; it is the diffusion of water across a semipermeable membrane. Different concentrations of sucrose solutions are placed inside semipermeable dialysis bags. Their volumes are measured by submerging them in graduated cylinders containing water, then measuring and recording the displacement volume. All are then placed in pure water. At specific time intervals, bags are removed and remeasured to calculate any change in volume.

c) Students add a small amount of whole blood to test tubes

containing salt solutions of varying concentrations. In this situation the red blood cells are, in fact, living semipermeable bags. Osmosis is occurring across their membranes. The contents of the tubes are mixed, then held up to a printed page. One of the tubes is clear enough to allow the print to be read through it. One is slightly clear. Two of the tubes are still opaque, such that one cannot read the print at all (Appendix C, p. 4).

Questions Related to Stage I

a) Record the relative positions of the precipitates in a drawing. Measure the diffusion distances to the precipitation fronts.

b) Plot the volume of each bag versus time.

c) In which tubes did haemolysis occur?

Stage II involves the interpretation of the facts observed in Stage I. The facts can be related to each other to determine cause/effect relationships. The students might find implications and/or conclusions in the data.

a) The students are asked to compare the rates of diffusion of the ions. They are asked to interpret any differences observed.

b) Students interpret the graph to compare osmotic flux rate with solute concentration.

c) The students must explain why it is possible to read the print through one of the tubes, and why the others exhibit different degrees of clarity; i.e., they must interpret the

results in terms of haemolysis (the swelling and bursting of cells).

Questions Related to Stage II

a) What can you conclude about the rates of diffusion of Cl^- and of $Fe(CN)_6^{\equiv}$ based on the relative positions of the precipitates?

Can you say anything about the rate of diffusion of Ag^{++}? Why?

b) Interpret the differences in rate of diffusion.

Can you calculate the rate of osmosis from the graph? Do you expect straight lines? Explain.

How does the rate of osmosis vary with solute concentration?

c) What happened in each of the tubes containing red blood cells?

Stage III asks students to predict the consequences of a related but different situation. This directs students to analyze the problem further.

a) Students are asked to predict the final outcome of leaving the bags indefinitely in the water.

b) Students must predict the outcome of placing red blood cells in hypotonic, isotonic and hypertonic solutions. Those unfamiliar with these terms would find definitions in their texts as well as lecture notes.

Questions Related to Stage III

a) What would happen if the bags (containing sucrose solutions) were left indefinitely?

b) What occurs when red blood cells are placed in hypotonic, isotonic and hypertonic solutions? Explain why.

The function of the demonstrator in this lab is to assist students technically with their equipment and observations. They may suggest coordinates for the graph and ask additional questions to guide understanding of the processes.

Preparation of the report develops the ability of students to communicate logically. They learn to defend their answers with facts and evidence. They learn to organize, draw and label a scientific graph. Most importantly, though, they learn a process for thinking stepwise through unfamiliar material.

Evaluations and Modifications

As chief demonstrator, I see this conceptually, as a very difficult lab. The processes and the equipment involved are simple. Diffusion is commonplace. Yet the explanations are deceptively complex. Again, this is the first lab of the year and students do not yet understand what is expected of them in reports. They showed a disappointing ability to reason out the "why" of their results and even less ability to write a good discussion of results.

I found from moving around among the lab rooms and from office conversations with individuals, the students themselves genuinely enjoyed the fun of this lab, but considered it to be elementary and therefore not demanding enough. In spite of this confidence, they were confused about the interpretation of their agar plates - in most cases because they had not consulted the text to explain or to help reason out their findings. Similarly, many students failed to read the guidelines for report writing in the lab manual and did not understand the standard format for scientific reports. Answers to the questions asked in the

manual are designed to guide student reasoning, but if these questions are ignored or not taken seriously, then the thinking process is "short circuited" and interpretations become inadequate.

The demonstrators found that in the lab itself, many students did not recognize or understand the use of basic laboratory equipment such as pipets, graduated cylinders or beakers. TAs were also critical of lab reports which they said were badly organized and lacked reasoning. Some students failed to include whole areas of discussion. From this experience, TAs realized that they themselves had been unprepared for their responsibility of getting the information across. Following this lab, I recorded many recommendations for following years:

1. Diffusion and osmosis should be divided into two separate labs.
2. Demonstrators should spend more time in the prelab talk explaining precisely what is expected in the report.
3. The prelab talk should include a demonstration of the technical equipment and its use - especially the pipetting technique.
4. Lab manual questions should be clarified and simplified.
5. At the end of the lab, students should be encouraged to discuss results among themselves. The demonstrator should guide but not lead this discussion.

LAB II - NEUROPHYSIOLOGY I - REACTION TIME

- A Detailed Account

- Appendix A, p. 14.

This laboratory unit is one of two designed to investigate the nervous system. Students study components of simple and of complex stimulus-response sequences by monitoring reaction time.

Focus of the Unit (Orientation and Goals)

The simplest reflex arcs in humans involve only two neurons - one sensory and one motor. Reflexes produced are usually rapid and relatively automatic. However, reflex arcs always interconnect with other neural pathways, so it is common for many interneurons to be involved. Reaction time is the latency between a stimulus and the response to that stimulus by an effector. It is largely determined by the number of synapses involved.

This unit lends itself to the Concept Attainment Model of Joyce and Weil (1980), developed from the work of Bruner et al. (1967). The students collect a large amount of data which must be organized into tables, then analyzed in order to formulate an hypothesis to explain the underlying concepts.

The primary purpose of this unit is to study the reflex arc in terms of reaction time and to compare reaction time with complex reaction time involving integration of several messages to the brain.

Because of the technical nature of the equipment, I have also

adapted segments of theory from Gay's Technical Model (1980). As in technological manuals, the language of instruction is terse and energetic. It uses the logic of "systems" analysis, and scientific objectivity.

Instructional Design (The Model in Action)

This unit utilizes a Discriminatory Reaction Time Apparatus which records response time to 0.01 s. It measures the time required for a stimulus to be processed by the central nervous system, where speed of response to visual and auditory stimuli is indicative of the number of synaptic connections involved and frequency of use.

The students work in groups of four, rotating through the functions of subject, presenter, recorder and observer. The presenter gives the stimulus. The subject responds, using his or her dominant hand and the recorder notes the response time. The average of the ten fastest times from fifteen trials is taken as that subject's reaction time. By using only the ten fastest of fifteen trials, we eliminate any trials for which the subject was not fully prepared, so the average is more representative of his or her true reaction time. The tests are repeated, using the non-dominant hand and then with an auditory stimulus, using both dominant and non-dominant hands to record the time.

To determine complex reaction time, stimuli are presented randomly as either a light or as a buzzer. The subject responds with the dominant hand to the light and the non-dominant hand to the buzzer.

All the data for each student are recorded on the blackboard. Students collect the class data for analysis in their reports.

Questions Related to Phase II and III require students to give the components of all reflex arcs. They are asked to interpret any differences in response time between individuals, and to compare their own data with the class data.

Evaluations and Modifications

Students gave a very favourable response to the content of this lab. The friendly rivalry and competition was a noticeable stimulus. Everyone was required to participate. The fact that students controlled the trials and were kept extremely busy contributed to their sense of accomplishment.

Demonstrators suggested that standardization of both equipment and instructions would be an improvement. The use of both white and red lights in Part A, the determination of response time, interfered with discrimination in Part B, the determination of complex response time, so the manual was changed to use only white light in Part A. By discussion with students, I recognized that it was desirable to eliminate all but one variable in each test. The manual was changed for the following year to ensure this and to clarify instructions. New reaction time boxes were ordered for consistency of data among groups. These changes eliminated any confusion during the laboratory session.

A table for the collection of class data was added to the manual where it acts as a visual framework of procedure or a flow chart of activities.

These changes smooth out the unit to better reflect the precise technical nature of the model.

LAB III - SENSORY FUNCTION

- A Brief Summary

- Appendix A, p. 17

This lab illustrates a homeostatic control system. It examines the conversion of stimulus energy into action potentials by the nervous system.

A. Tactile Sensitivity - Von Frey hairs explore the sense of touch in order to find the absolute threshold over various regions of the body.

B. Two-Point Threshold - Using the two points of an esthesiometer, the students determine whether there is any variation in the two-point threshold over different areas on the body surface.

C. Colour Vision - Test cards are used to determine if students have normal colour vision or some form of colour blindness.

D. Taste - Different areas of the tongue are tested with sweet, sour, salty and bitter solutions to determine if all areas are equally responsive to these tastes.

The main purpose of this unit is to teach students a method of inquiry. The model chosen was the Biological Science Inquiry Model developed by Schwab (1965).

In practice, this lab was found to be rather boring and excessively time-consuming due to the many repetitions required. Consequently, the colour vision and taste sections were incorporated into the Reaction Time lab for 1982-3. The sections on Tactile Sensitivity and Two-Point Threshold were discontinued.

LAB IV - THE BLOOD

- A Brief Summary

- Appendix A, p. 22

Blood is a special type of connective tissue consisting of red blood cells, white blood cells and platelets (in mammals) suspended in an extracellular fluid matrix called plasma.

A. Haematocrit - This exercise involves the separation of blood into its three compartments by centrifugation to allow observation of the relative volumes.

B. Haemoglobin Concentration - Students prepare a standard curve of haemoglobin concentration vs. optical density. This is a graph which shows established activity over a range of haemoglobin concentrations. By applying their own sample to the graph, students can judge its haemoglobin concentration (Appendix C, p. 18).

C. Determination of Blood Type - Students each perform this test to determine whether their blood type is A, B, AB or O. The test is based upon antigen/antibody reactions.

The primary purpose of this lab unit is the introduction of new information and new laboratory techniques. The unit is based upon the Inductive Thinking Model of Taba (1967) which uses academic reasoning to handle new material. The scientific approach of the manual helps the students to assess the results of their experiments and to organize their findings.

In spite of the complex techniques and graphing required, the

students performed well. Written reports were also well-prepared. At least in part this was because they were extracting and testing their own blood. The experimental nature of the tests was also a stimulus.

LAB V - CARDIOVASCULAR FUNCTION

- Brief Summary

- Appendix A, p. 30

This laboratory exercise examines a typical heart beat, listens to heart sounds, measures arterial and venous blood pressures and also demonstrates actual blood capillaries.

A. The Electrocardiograph - The TA sets up the ECG machine with a volunteer, to make original records of the normal heart beat. Students are able to compare these with examples of abnormal tracings.

B. Heart Sounds - Using stethoscopes, the students listen to and interpret each other's heart sounds. They also measure heart rate.

C. Measurement of Arterial Blood Pressure - Students measure each other's blood pressure using clinical sphygmomanometers and stethoscopes.

D. Venous Blood Pressure - Using the hydrostatic principle, students can measure the height of a column of blood which just opposes the venous pressure in the hand. This allows them to calculate the venous pressure.

E. The Venous Pump - Students examine the large veins and valves which are visible in the forearm.

F. Capillary Circulation - By scrubbing the fingers at the base of fingernails with a brush and soap and water, it is possible to observe the extensive capillary beds in this area when the hand is

examined under the dissecting scope.

This lab also makes use of Taba's Inductive Thinking Model (1967). Its primary purpose is the introduction of new information for the development of inductive mental processes and the building of academic theory. Seeing and performing these several clinical tests makes a lasting impression on students, partly because of the close relationship of tests to the field of medicine. The students enjoy and work hard on the techniques involved in the proper use of the technical equipment.

LAB VI - RESPIRATORY GAS EXCHANGE IN THE MOUSE

- A Detailed Account

- Appendix A, p. 35

Most of the energy which fuels human activity comes from the chemical breakdown of foodstuffs by aerobic respiration, an Oxygen-requiring reaction. Breathing carries oxygen to the lungs where it diffuses into the bloodstream and is carried to all the tissues of the body. There, it is exchanged for CO_2 and the process is reversed. In this lab, students measure the respiration of a mouse and calculate the Respiratory Quotient (RQ) of the mouse where:

$$RQ = \frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}}$$

Focus of the Unit (Orientation and Goals)

The parameters of this experimental physiology lab, as originally set out, resemble Schwab's Biological Science Inquiry Model (1965) because the lab requires students to identify and to solve problems related to an investigation. Both the content and the process are emphasized. Therefore, the unit is designed to help students better understand the nature of science and its methods of inquiry.

A short introductory section uses Suchman's Inquiry Training Model (1962), to teach students another process for investigating and explaining an unusual situation by inquiry.

The use of both of these models plays a large part in the realization of many of the course objectives:

- developing an inquiring mind
- learning to tell relevant from irrelevant information
- assessing the results of investigation
- bringing about an excitement for learning
- learning to defend answers with fact or evidence
- involving students actively in the procedures

Instructional Design (The Model in Action)

There are three lessons or "invitations to inquiry" as Schwab calls them. Each invitation poses examples of the process itself and engages the participation of the students. The invitations build sequentially in difficulty. Each is a real-life scientific study requiring the students to resolve some perplexity by their own reasoning and judgment.

A. A Problem to Be Answered by Inquiry

Phase I - Immediately prior to the lab, each TA sets up a mystery apparatus of three flasks. The two outside flasks each contain a red fluid. The centre flask contains a mouse. Air is pumped gently into one of the flasks, through the flask with the mouse and into the third flask, all in a continuous series (Appendix C, p. 29). The students collect in a group around the demonstration, but they are not given any explanatory information. Within a few minutes, the fluid in the third flask gradually begins to turn yellow.

Phase II - The TA explains that the students are collectively to solve the riddle of what is happening in this system. They must ask questions in such a way that the TA can answer either yes or no. The questions must establish what the fluids are, why only one is changing colour, the function of the mouse, and other factors.

Phase III - When several of the students feel they have solved the riddle, they answer the questions of the other students until everyone understands the details. Volunteers are invited to work out the equation of the reaction on the blackboard.

B. The Second Invitation to Inquiry

Measurement of O_2 Consumption and CO_2 Production

Phase I - Pairs of students set up their apparatus for measuring O_2 consumption by the mouse, then proceed to carry out the measurements required for calculations. Many small technical problems arise in setting up the apparatus, each requiring students to make a judgment before proceeding.

Lab manual questions (Q) are designed to ensure that students understand all sequential steps of the experiment.

Q - Calculate the change in volume (ΔV) and record cumulatively.

ΔV must now be corrected to take into account the

volume of the tube occupying part of the space in the cylinder.

Q - Plot the corrected ΔV vs. time on a graph.

Q - Draw the straight line which best represents the data and calculate the slope of this line in ml/s.

Phase II - The third invitation to inquiry occurs when students are told to modify the apparatus so they can calculate the CO_2 production of the mouse. Having modified the apparatus appropriately, they then take the measurements.

Q - Give your reasoning for the changes made to the apparatus.

Phase III - The students plot these new data on the same graph as that in Phase I. The slope of this line will also give them ml/s. By subtracting Slope 2 from Slope 1, they determine the rate of CO_2 production.

Once all these data are collected, the students calculate the Respiratory Quotient (RQ) of their mouse.

The TA is available throughout the lab period to help set up the apparatuses and to answer specific questions, but not to give away answers until students have worked through their own hypotheses.

Because some students are always much slower than others, it is essential to have a "debriefing session" following this lab to verify the accuracy of the information collected and to help formulate concepts. The TA acts only as questioner and verifier; the students themselves manage the explaining and answering.

Evaluations and Modifications

This lab was very demanding of students. It required them to work hard and to give real thought to its intellectual challenges. During the first year of operation, before the lab manual was available, many students were totally confused about how to make calculations, how to adjust readings and how to organize their data. In writing the manual, I clarified instructions and added the diagram of the one-way valve to assist in setting up the system. I also added the table for data collection. Instructions to adjust readings to standard temperature and pressure were eliminated because they seemed to be a procedural refinement of data treatment which could better be introduced in more advanced courses. To retain this step would have required special instructions and therefore would take time away from the deductive reasoning required by the experiment.

Further clarification was needed again, however, at the end of 1982, both for comprehension of procedure and for calculations. An important modification was made to the apparatus, allowing students to take readings of O_2 consumption directly without having to correct for the volume of enclosed tubing or to sum up individual volume changes.

As a consequence, this has become the preferred lab of the year by many students. Of course, they particularly enjoy working with a cute, live animal. They rise to the challenge of the puzzling situations well. Also, their calculations and discussions show marked improvement over the beginning of the term. Equally

important, though, the students feel they have learned a great deal. In part, this may be due to the fact that the unit draws upon concepts learned in the labs on Diffusion, Blood, and Cardiovascular Function.

LAB VII - FITNESS

- A Detailed Account

- Appendix A, p. 39

In this exercise, students assess their own cardiovascular and pulmonary fitness by the performance of several applicable clinical tests.

Focus of the Unit (Orientation and Goals)

The goal of this lab unit is for students to explore the role of the cardiovascular system in the maintenance of physical fitness. A secondary goal is to determine whether there is a correlation between physical ability and fitness, between gender and fitness, and between smoking and fitness. Fitness is defined as a good state of health and physical ability.

The model of instruction chosen is Ausubel's Advance Organizer (Ausubel et al., 1963). The presentation of material in this unit does not relate well to discovery learning or to inductive or deductive teaching. The Advance Organizer Method is more suitable because it provides new concepts or principles to the students directly and because the general concepts of fitness, gender, smoking and even cardiovascular fitness are already familiar to most people. The students' role is to master the ideas and information. Learning material is presented by the method of progressive differentiation which means that the most general ideas are given first, followed by a gradual increase in detail or specificity. The new material becomes integrated into a student's

existing cognitive structure by relating it back to the "advance organizer".

Besides actively involving students in the learning process, this unit develops their ability to organize findings and to interpret them in a manner which will verify or refute the concepts of the organizer. Preparation of a report helps students learn how to communicate logically and obliges them to defend arguments with fact and evidence. Preparation of data tables helps students to identify cause/effect relationships.

Instructional Design (The Model in Action)

Phase I involves the presentation of the "advance organizer". In this case we use an expository organizer because it provides a general outline of the relationships which follow. (A second type, called a comparative organizer, is used when the new material is relatively familiar.)

Aims of the lesson are clearly outlined in the objectives. The "advance organizer" itself consists of three basic principles which are distinct ideas in themselves and are also more inclusive than the specifics of the lab exercise.

1. Fitness is positively correlated with low resting heart rate.
2. Fitness is negatively correlated with the degree of elevation of heart rate after exercise.
3. Fitness is positively correlated with rate of return of the heart rate to "rest" after exercise.

These statements are strengthened by the equation:

$$C = R \times V \times D$$

where $C = O_2$ consumption

R = heart rate

V = stroke volume

D = arterial/venous O_2 difference

The interpretation of this equation is well documented in human physiology as meaning: the more fit the individual, the heavier is his or her reliance on cardiac stroke volume as well as arterial/venous O_2 difference and the less on heart rate (Vander, Sherman & Luciano, 1986).

Phase II involves the collection of data from the various tests.

A. The resting pulse rate is central to fitness tests and is therefore determined first with the subject in a sitting position. Then the reclining heart rate (pulse rate) is taken.

The effect of standing or motion on heart rate is next determined, followed by the effect of exercise.

B. The Schneider Test to assess cardiovascular fitness has a broad general application for people in a wide range of physical condition. It is a simple, straightforward test, easily performed under laboratory conditions.

C. Spirometers are used for the Pulmonary Fitness Test to determine maximum breath-holding ability and lung vital capacity. There is a logical progression among these tests, each step being related to the previous step. Results of the trials earn for the individual a number of points. Each student is then able to total up a score

which may be compared to a standard chart in order to determine his or her degree of fitness.

Phase III is designed to anchor the new learning material into the student's existing body of knowledge. Students analyze the class data to see if there are correlations among the various results. By further analysis, they can determine whether or not smoking has an influence on results and whether or not results vary between males and females.

The TA gives an introductory prelab talk to the whole group. It is essential that TAs themselves have a thorough understanding of the factors at work in this exercise. To ensure that all are knowledgeable, I present a summary of the essence of the unit, but each TA is expected to read the pertinent chapter in the text. At the TA meeting, the instructor explains each factor to the demonstrators and outlines what students have been told in lectures.

Evaluations and Modifications

Of all the exercises from the lab manual, this is unquestionably the most relevant for these budding young athletes who are greatly concerned with endurance training and in physical performance.

In 1982, after consultation with instructors and demonstrators, I decided to introduce more background and more content to this unit. The name was changed from Fitness to Exercise and Physical Fitness. A much more complete introduction to the topic was added. Explanations and definitions of the various factors involved were added to the manual. Also, a new description of differences in body response to mild vs. extreme exercise was inserted.

Demonstrators and students both found Part A, The Influence of Position on Heart Rate, to be monotonous. The essentials of it were therefore retained, but the rest was dropped for 1982. To compensate, a new section was added to demonstrate the effect of strenuous activity (via an exercycle) on the normal ECG record and on tidal volume.

This unit was always popular with the students. Modifications have now made it a polished and effective procedure for students to predict the consequences of exercise-related activities.

LAB VIII - KIDNEY FUNCTION

- A Brief Summary

- Appendix A, p. 47

All vertebrate kidneys contain units called nephrons which receive filtrate from the blood. This fluid is filtered through the kidney at a rate of 180 ℓ per day. Ninety-nine percent of it, however, is reabsorbed by the body, along with nutrients such as glucose and amino acids. Only about one ℓ remains to be excreted daily, along with nitrogenous wastes and excess salts.

The following laboratory tests on urine are performed:

A. Clinical tests of a normal sample
   - for pH, using litmus paper
   - for glucose, using clinistix
   - for protein, using albustix reagent strips

B. Clinical tests of physiological and pathological samples
   - test for pregnancy - samples are both positive and negative
   - glucose - positive and negative samples
   - for protein - positive and negative samples

Two puzzling questions are put to the students regarding abnormal urine tests. In their reports, students are asked to answer these questions in essay form.

The primary purpose of this lab is to create a setting wherein students come to understand the role of the kidney in the maintenance of

homeostasis. This is accomplished by the performance of a number of clinical tests which verify the introductory statement above.

The model for this unit is Hilda Taba's (1967) second and third teaching strategies. The Interpretation of Data Strategy requires the students to differentiate among the various urine test results to relate factors one to another. In this way students are able to determine cause-and-effect relationships and explain the meaning of their results in terms of normal kidney function.

According to the Application of Principles strategy (Stage II of the model), the students formulate an hypothesis to explain abnormal test results. They must be able to explain and support their hypotheses.

In 1981, student performance in this lab was average but lacked enthusiasm. A more challenging section was added in 1982, calling for three volunteers from each room to consume an excess of salt, sugar or alcohol. Their urine was monitored during the afternoon to observe the effects of the excess. Results obtained were not as clear-cut as desired, but did demonstrate the expected pattern and certainly created a great deal of enthusiasm for the kidney lab.

LAB IX - SALIVARY AMYLASE

- A Brief Summary

- Appendix A, p. 52

Salivary amylase is an enzyme found in saliva, which contributes to the breakdown of starch in the stomach during digestion. Laboratory tests are used to simulate this process. Factors are explored which speed up or slow down the process.

The main purpose of this lab is to observe the activity of an enzyme, but also to examine the effects of temperature and of changing pH on enzymatic activity. This unit stresses the process of investigation. It also gives students practice in the use of basic laboratory skills such as pipetting. It requires them to reflect on their results and to interpret the significance of the findings.

For variety, and because this exercise was relatively uncomplicated, the formal report for this lab was set aside in favour of students completing a hand-in sheet during the lab period itself (Appendix A, p. 55). This procedure was not satisfactory. The students were uncomfortable, preferring to take the extra time and hand in a complete report. The answer sheets clearly reflected their lack of thought and preparation time.

Following this lab, I discussed its shortcomings with Dr. C. M. Wood to draw up a new set of questions. These modified questions resulted in more thoughtful responses and more reasoned interpretations of the data. The unit was also moved to the earlier

part of the first term since it is more appropriate as an introductory lab than as a concluding one. It has been quite satisfactory since these changes.

LAB X - NUTRITION

- A Detailed Account

- Appendix A, p. 57

The production of energy to maintain living systems and to build new body structure requires a continuous supply of organic nutrients. The processes and problems related to these activities are termed nutrition.

Focus of the Unit (Orientation and Goals)

The primary purpose of this lab is to evaluate the adequacy or inadequacy of the student's diet by monitoring daily food consumption and exercise. A secondary purpose is to determine whether or not the student is in energy balance.

The unit is designed from a simplification of the Inquiry Training Model developed by Suchman (1962) to teach students a process for investigating and explaining unusual phenomena. Suchman explains the model as a miniature version of the kind of procedure scholars use to organize knowledge and to generate principles. The general goal of inquiry training is to help students inquire independently but in a disciplined way. Since this lab unit requires them to conduct the investigation independently, it was logical to follow Suchman's guidelines. The variation in our application lies in the fact that there is no opportunity for students to ask questions of the teacher.

The students themselves are vitally concerned with maximizing physical performance. Consequently, they are very keen about performing a complete and accurate survey of their eating and exercising habits.

This unit focusses on four educational goals:

- to help students assess the results of an investigation
- to help students recognize relationships among data
- practice in determining cause/effect relationships
- practice in the organization of data tables and of graphs

Instructional Design (The Unit in Action)

Phase I The demonstrator presents the guidelines of the investigation to the students in a twenty-minute discussion of the parameters and the format for the final report. He or she makes suggestions for the organization of the data and the graph. For assistance there is an abundance of reference information discussing relationships between foods and calories or activities and calories. Actual examples of average portions of meat and beverage are exhibited to enable students to estimate more accurately the portions for consumption.

Phase II is performed by the students independently. They gather data in the form of a journal which includes a record of all food and drink consumed, and all activities performed. These data are put into a table from which each student constructs a standard graph that charts recommended caloric intake for corresponding weights. By applying the individual's weight to the chart it gives a read-out for recommended daily caloric intake and protein consumption (Appendix C, p. C50).

Phase III is the analysis of the data to generate an hypothesis in the

form of a conclusion to the original purpose regarding energy balance. It includes an analysis of the effectiveness of students' inquiry and a criticism of methods or procedures.

Phase IV Students are asked to discuss other factors which might contribute to energy balance.

The demonstrators are directed to prepare a comprehensive twenty-minute talk outlining the method of procedure for the investigation. They draw an example of the recommended chart on the blackboard and also a rough estimation of the standard graph for caloric intake determination.

Evaluation and Modifications

This was the final lab of the year for students. Consequently, they were pleased that the classroom component of it was extremely short.

This lab was very well-executed and well-reported, a surprising result considering the independent nature of the exercise. One area which needed clarification was the standard graph. Students misunderstood its significance; i.e., that they were to apply their own weight to the graph in order to calculate what their personal caloric intake should be.

After consultation with Dr. G. McDonald who was in charge of the course for the following year (1982), I decided to broaden the introduction for further clarification and to introduce a new component which could be used to calculate the metabolic rate of the individual. Another question was added to the manual asking students to comment upon

the differences in expectations within the given data tables for males and females. This was a very relevant topic in 1982 when many women were quite sensitive to the suggestion that they were less capable than their male counterparts in any sense. Such an attitude was considered "sexist".

Concepts of this lab are extremely relevant to general student interests. The calculation of "energy balance " constitutes a technique which may be referenced by students in the future either for themselves or for others whom they may be coaching or supervising.

Addition of these changes has resulted in a particularly popular and effective laboratory unit, quite appropriate as a conclusion to the course.

CHAPTER IV

BIOLOGY 1A6: ADAPTATION IN THE BIOLOGICAL WORLD

FOR FIRST YEAR NATURAL SCIENCES STUDENTS

Introduction

(i) Student Background and Laboratory Organization

Ninety percent of the 500 Biology 1A6 students are First Year Natural Sciences students who expect to continue within the Faculty of Science or to be accepted into the Faculty of Medicine. They are serious students, sincerely interested in their subjects and anxious to achieve high grades. Only a small proportion of them do not have a Grade XIII Biology background. Like 1H6 students, however, their experience in Biology laboratories has been limited. Students attend a three-hour lab period every other week, ten in all for the year. Efforts have been made to synchronize laboratory material with lecture outlines. Subject matter has been chosen to introduce the different areas of Biology such as Genetics, Physiology, Microbiology, and others. A real effort is made to relate concepts and techniques to the more complex methodology practiced in working labs by career biologists. For realism and interest, fresh or living material is used whenever possible. In this way, the laboratory program seeks to provide a sound biological foundation. It also gives students insight into more advanced courses available to them in senior years. Thus, I am hopeful that many of the brightest students will choose Biology as their career path.

Twenty percent of the students' final course mark comes from

lab reports. Guidelines for report writing are given in the manual and by the TAs in pre-lab talks. The demonstrators grade, make appropriate comments and return reports during each following lab period. Several lab questions are included in bi-weekly quizzes and in the final exams. Overall, then, laboratories account for about thirty percent of the final grade in Biology 1A6. The instructor for Term I is Dr. S.F.H. Threlkeld of the McMaster University Department of Biology. In Term II the instructor is Dr. C. M. Wood. The assigned textbook is Biological Science, Keeton & Gould, 3rd ed. (1980).

(ii) Internal Laboratory Assessment

Evaluation of student performance and lab manual effectiveness is discussed following each laboratory unit. Data to substantiate the evaluations are included in the Critical Evaluation, p. 103.

After the end of classes in May, it has become customary to have an informal Biology 1A6 "wrap-up meeting" to which all TAs and instructors are invited; the successes and failures of the year's work are discussed and recommendations for subsequent years are recorded. References to feedback from these meetings are included in evaluations of individual units.

The five labs which will be dealt with in detail together with the design model employed are:

Lab I - Evolution, Classification and Identification of Unknown Specimens - Biological Science Inquiry Model

Lab II - The Chemical Basis of Biological Systems - Creative Learning Model

Lab IV - Genetics and Heredity - Inductive Thinking Model

Lab VII - Gas Exchange and Internal Transport - Inquiry Training Model

Lab VIII - Regulation of Body Fluids - no precise model

The other five labs which are outlined briefly are:

Lab III - Cell Structure - Concept Attainment Model

Lab V - Photosynthesis - Creative Learning

Lab VI - Unicellular and Colonial Organisms - Creative Learning

Lab IX - Plant Hormones - Academic Model

Lab X - Animal Behaviour - Inductive Thinking

LAB I - EVOLUTION, CLASSIFICATION AND IDENTIFICATION OF UNKNOWN SPECIMENS

- A Detailed Account

- Appendix B, p. 4

As human beings, our lives are sustained by natural processes and ordered by natural laws. A study of nature, then, lies at the foundation of education. This first laboratory unit for Biology 1A6 is designed as an introduction to the study of Biology in general and Field Biology in particular.

Focus of the Unit (Orientation and Goals)

It seems appropriate that an introductory laboratory unit be concerned with the beginning of the universe and of the world in which we live. Although it is not possible for laboratory exercises to study events which occurred on the primordial earth, the introductory film presents current theories about the origin of the earth and planets. T. H. Huxley (as cited in Keeton & Gould, 1980) wrote that, "Science is nothing but trained and organized common sense." The main purpose of this lab unit is to examine this training and organization to which Huxley refers. Its secondary purpose is to promote an appreciation of, and a curiosity about, the plants and organisms which are so abundant on our earth but about which most students have only a superficial understanding.

The Biological Science Inquiry Model (Joyce & Weil, 1980) is chosen as the model for this unit because its format includes a

procedure for the investigation of a genuine biological inquiry problem. The procedure uses techniques similar to those of research biologists. It involves students actively and challenges their powers of observation as well as deduction. Both the context of the lesson and the process of investigation are stressed, so that learning occurs by discovery rather than by instruction.

Instructional Design (the Model in Action)

As an introduction to this lab, students watch an excellent colour film entitled "Evolution and the Origin of Life" which describes the probable conditions of the earth's atmosphere at the time of the origin of life. The film also tells the story of Charles Darwin's life and history leading up to his revolutionary book The Origin of the Species. Darwin's work is described as a true example of the Scientific Method - the universally accepted procedure for scientific investigation. The method is explained in the manual for our students to follow. The film also gives a simulation of Miller and Urey's experiments (Keeton & Gould, 1986) in which complex organic compounds are synthesized from mixtures of ammonia, methane, water and hydrogen. These experiments explain how the purine and pyrimidine bases necessary for the beginning of life, might have been formed on the primitive earth.

The lab manual outlines the Binomial System of Nomenclature which utilizes evolutionary characteristics to systematize the tremendous diversity of life. It explains how the system is able to identify each individual, precisely and logically according to observable characteristics.

An aquarium filled with pond water and containing abundant aquatic life is set up in each lab room. Students capture one of these organisms in order to identify it according to a dichotomous key. This key offers students a series of two contrasting choices. They must determine which is correct for that organism. Choices are made in continuous steps by comparing structural features until all possibilities of identification but one are eliminated.

A second question to be solved by students is the identification of any one of seven different species of local evergreen tree. The manual also provides a dichotomous key to the evergreens which compares the size, shape and positioning of needles as well as features of the wood and cones.

Dissecting microscopes are used for recognition of the correct characteristics of both plants and animals. Reference books on the classification of both insects and trees are available to students in the lab. Thus, students learn to use a variety of tools from which to develop theories or to draw conclusions.

Phase I of the inquiry procedure is the outline of the two areas of investigation together with the two dichotomous keys.

Phase II, in this case, is the collection of data regarding observable features. Following the lab, a full report is required to describe and discuss these observations. Laboratory drawings of the unknown specimens showing the features used as evidence are included with the report.

Phase III requires students to speculate upon the data in order to solve the problem of identity.

The special function of the demonstrator throughout this lab is to encourage the inquiry nature of the exercise, emphasizing the process itself over final conclusions.

Evaluations and Modifications

From my observations as Coordinator of Labs, the general student response to this lab, unfortunately, was negative. Although many students were stimulated and excited by the experience, too many were unenthusiastic and a few actually detested working with "bugs". Due to lack of experience, many students had difficulty in the use of the dissecting microscopes. Also, one of the evergreen specimens did not fit the criteria of the key. Consequently, this was an area of frustration for some.

More importantly, however, neither the students nor the demonstrators were able to function with ease in this inquiry environment. Previous experience had not taught them to be comfortable working in areas where the answers were unknown. Experienced TAs were able to emphasize the process of inquiry and to minimize identification as the central aim. However, several of the TAs were inexperienced and therefore unable to handle the exercise with confidence.

Theoretically, this lab offers excellent potential as a learning experience. As an introductory unit, however, it was too difficult to handle. Neither did it generate enough excitement nor enthusiasm to justify its retention as an introduction to the laboratory component of 1A6.

Therefore, in a general rearrangement for the following year, I decided that this unit should be discontinued. The greatest strength of a process model of curriculum design rests upon the quality of the teacher (Stenhouse, 1975). Unfortunately, this is also its major weakness.

LAB II - THE CHEMICAL BASIS OF BIOLOGICAL SYSTEMS

- A Detailed Account

- Appendix B, p. 19

The underlying principles of this lab exercise come from Molecular Biology, an area at the forefront of industrial and research Biology today. Moreover, this unit reflects the primary focus of the McMaster Biology Department and of related departments in Health Sciences. Therefore, it is an exciting and stimulating area of study for students.

Focus of the Unit (Orientation and Goals)

The primary purpose of this unit is to emphasize how chemical analysis can be used to isolate and study the structure and properties of subcellular components. It demonstrates that chemical bond energy is the key to understanding the nature of chemical reactions and consequently reactions which occur within living cells. Students learn about the different classes of organic molecules and about their functional groups.

The method chosen to convey this information as meaningfully and efficiently as possible is "creative learning", or rediscovering what has already been known by others. This laboratory unit departs from Gordon's Synectics model insofar as students are not required to make direct analogies of cellular components. Rather, they are encouraged to experience creative achievement by reinvestigation of the processes which demonstrate

the existence and behaviour of molecules. In this unit, students learn laboratory techniques such as pipetting, centrifugation and chromatography. They also learn the concepts behind very complex and sophisticated procedures such as electrophoresis and ultracentrifugation.

Instructional Design (the Model in Action)

Phase I Because of the unfamiliar nature of the subject matter for students, the topic is introduced by way of a summary of chemical bonds, an outline of the various classes of organic molecules, and a description of functional groups. This establishes the framework of the lab exercise and provides an organizing concept for the tests and exercises which follow (Appendix B, p. 19).

A short, animated colour film entitled "DNA, the Blueprint of Life" shows important molecules and subunits (functional groups) as humorous little characters each with a unique type of behaviour. Imaging, or pictorial repetition of this nature contributes to mastery of the material.

Phase II involves the presentation of tests which explore and verify existing knowledge. The first tests are for the presence of carbohydrates. Benedict's reagent is used to identify reducing sugars (simple carbohydrates) and iodine identifies starch (a complex carbohydrate).

The Sudan III test is used to identify lipids or

fats and the Biuret test identifies proteins. Each trial is simple and straightforward, giving a clear positive or negative indication of the presence of specific molecules.

There are two tests for nucleic acids. In the presence of DNA, diphenylamine (Dische diphenylamine reagent) exhibits a strong blue colour. Similarly, in the presence of RNA, orcinol (Bial's orcinol reagent) gives a blue green colour. These tests are both quite specific for the target molecules.

In the last exercise of this unit, students perform paper chromatography. This important technique is widely used in research and industry for the separation of individual substances from mixtures.

The mixture is first dissolved in a suitable solvent, then exposed simultaneously to two different phases. Each of the components becomes partitioned between the two phases according to its affinity for each. In this way, substances with only very slight molecular variations may be clearly differentiated, one from another.

In this laboratory, the students are given four different amino acids to be spotted on chromatography paper in order to determine the R_f of each (ratio of the fronts) (Appendix B, p. 31).

$$R_f = \frac{\text{distance travelled up the paper by the solute}}{\text{distance travelled by the solvent}}$$

By analyzing and comparing the behaviour of these standard solutions, students are able to determine the identity of two unknown amino acid solutions. Each day the composition of these unknowns is changed (Appendix C, p. 60).

Phase III The cognitive nature of the concepts learned in the laboratory is strengthened and reexamined by the preparation of a report describing test results and including a discussion of their relationships and significance. This should result in higher level information processing by students.

The demonstrator's function is to promote an environment of freedom and support, so as to facilitate the work of the students. He or she must see that tests are properly performed so that appropriate conclusions can be made (Joyce et al., 1981).

Evaluation and Modifications

It was clear to me as I moved among the lab rooms, that in spite of their intense industry, students enjoyed this lab. In most cases, procedures were straightforward and results were clear-cut. Some students, however, had difficulty differentiating between the colour reactions of DNA and RNA because both produced a blue colour.

At the wrap-up meeting in May, the statement was made that it was important for students to see the difference between DNA and RNA. This, and the fact that most of the tests are relatively

simple, convinced me to modify the nucleic acid section and also to eliminate the starch test which was both uninteresting and unnecessary. Since orcinol must be prepared immediately prior to use, dropping the RNA test was an obvious and justifiable simplification.

It was replaced in 1985 with a DNA extraction and identification, the results of which are impressive even to seasoned biologists. Calf thymus tissue was chosen as the source of DNA because of its high proportion of nuclear material. Because this process involves several steps, the TAs draw up a procedural flow chart on the blackboard as an example of good scientific organization for the students. TAs also recommend that in the future students make their own experimental flow charts to simplify procedures.

When the extraction is well-executed, students obtain an ample amount of semi-purified DNA from this experiment. Molecules of DNA are several cm long. They may clearly be seen coiling around a glass rod if it is moved slowly through the dish containing the final mixture. The depth of blue colour achieved with the diphenylamine test, when compared with commercial DNA is indicative of the purity of both products.

This new lab section is an extremely worthwhile addition to the lab. It creates an exciting learning experience in addition to providing in-depth experimentation. The whole unit gives students an opportunity to try out first-hand, some of the sophisticated procedures described in the introduction.

LAB III - CELL STRUCTURE

- A Brief Outline

- Appendix B, p. 34

The students have now looked at the origin of life, evolution and at subcellular structures. As a next step, this lab is designed to examine different cell types and their associations into tissues, organs and organ systems. Students compare cellular structure visible by light microscopy with that visible by electron microscopy. They are also introduced to the study of Developmental Biology through the examination of live Medaka fish eggs. Some of these eggs are only a few hours old; others show stages of development up to and including hatching.

Since this is the first time students use microscopes, this unit begins with identification of microscope parts and a guide to microscope procedure.

A. Representative Plant Cells

I Elodea is chosen as an example of simple plant cells with clearly observable chloroplasts and cell vacuole.

II Tradescantia stamen hairs show cytoplasmic streaming.

III Strategies for Water Retention - Examination of the upper and lower epidermal surfaces of Tradescantia leaves permits students to discover that stomata, through which air and moisture enter into leaves, are more abundant on the lower surfaces; i.e., the side away from the heat of the sun.

B. Representative Animal Cells

I Cheek epithelium cells are representative of morphologically simple animal cells.

II Skeletal muscle fibres, by their contractile ability, show modification of structure to function.

III Nerve cells also show modification for their unique function of irritability.

C. Cell Ultrastructure

Students examine micrographs of a "typical" plant and a "typical" animal cell to observe the subcellular detail.

D. Multicellular Organization

Using a microscope, the students observe how combinations of cells work together as a unit to perform specialized functions. They identify five different layers of cells from slides of frog intestine in cross-section.

E. Animal Development

This section is a brief introduction to Embryology and Developmental Biology. Individual Medaka eggs are about 2.0 mm in diameter. Those collected on the day of the laboratory are usually at the 64-cell stage of cleavage by lab time. (Appendix B, p. 50).

The relationship of form to function is a basic tenet of Zoology. The main purpose of this lab unit then is to observe,

first hand, how individual cells became adapted over time to perform specific functions. Muscle cells are contractile for motility. Nerve cells are adapted to receive and transmit nerve impulses.

The design of this unit is a simplification of the Concept Attainment Model developed by Bruner et al. (1967). The lab follows a logical sequence from the simplest of cells to more specialized types, then to their organization into tissues and organs.

Slides are prepared by the students themselves from living materials on the assumption that live material is more dramatic and relevant. Students submit drawings of most of the examples with their reports.

As with any observational and open-ended lab, the students were uncomfortable with the dependence for success upon their own inexperienced technique.

Consequently, they were frustrated by their inability to see the features as described in the lab manual. I recognized that this was an area where the TAs could manage the mood of the lab room better by interpreting the spirit of the lab more as an investigation and less for specific results. Demonstrators agreed among themselves that they had not given enough attention to discussion of the perspective or internal view of the frog's intestine for students to be able to relate structure to function. This will be given more attention in future years.

Although unwilling to revert to commercially prepared

slides, I decided to obtain some for students who were unable to make a good preparation by themselves and also for comparison purposes. The section on cytoplasmic streaming in the stamen hairs was dropped because it was too dependent upon the availability of flowers and also because streaming had already been observed in Elodea.

Students enjoy viewing the live Medaka fish embryos under the dissecting microscope. The first visible structure after cell cleavage is the neural tube, soon to differentiate into the brain. Within ten more hours, large eyes and a beating heart become visible; blood cells may even be seen moving through the vessels. It is relatively easy to determine the age of each embryo by identification of the developmental stage.

This lab unit is inspiring for those students who have mastered microscopic technique. Those who have not are unable to fully appreciate its interest.

LAB IV - GENETICS AND HEREDITY

- A Detailed Account

- Appendix B, p. 54

It has been recognized for centuries that offspring tend to resemble their parents, whether one is observing plants or animals (including human beings). Genetics is a study of the mechanisms which control the patterns of inheritance and of variation among individuals. These mechanisms are passed on from one generation to another through the information molecules of DNA that compose the genes and chromosomes located in cell nuclei.

Focus of the Unit (Orientation and Goals)

The primary purpose of this lab exercise is to introduce students to some of the most important concepts which form the basis of Genetics. The technology associated with this subject has developed explosively in the last few years and its future promises to be even more dynamic. Therefore, it is critical that this area of Biology be highlighted to our First Year classes.

In so doing, I expect to help develop the ability of students to interpret observations leading to concepts and also to help develop their potential for learning. Of all the laboratory exercises, this is the most demanding of the students' ability to comprehend. It stretches their intellectual capabilities to the utmost and in the section on crossing over, even beyond the capability of many students. In spite of this, most of the sections

may be understood by everyone and all students should be able to grasp the more fundamental points.

The model chosen is Taba's (1967) Process for Inductive Thinking which is designed to increase a student's capacity to think. Like Piaget, Taba believes that teaching strategies must be used in a logical and progressive sequence because one thinking skill is built upon another and the earlier ones must be mastered first.

Instructional Design (The Model in Action)

The language of Genetics is very precise. In order for students to discuss the mechanisms of inheritance, they must be able to understand and use established terminology. Therefore, the exercise begins with brief explanations or definitions of the processes and terms referred to in the text of the manual (Appendix B, p. 55).

Taba outlines three inductive thinking stages, each of which builds upon the previous stage. Each of the four sections of this unit does not necessarily utilize all of Taba's three stages, but I shall explain them according to Taba's criteria.

A. Fruit Fly Genetics

Stage I - Concept Formation

The students look at vials of living fruit flies to learn about the life cycle stages of egg, larva, pupa and adult fly. They practice separating the males and females from vials containing recently-killed flies. This is necessary to achieve competence for counting the numbers of male and female flies in the F_2 vials (Appendix B, p. 60). The students work in groups of

four to collect these data, then combine their counts in a blackboard chart of class data. Totals are accumulated from each of the twenty-eight lab sections, making data from thousands of flies available for analysis.

All work is performed under the dissecting microscope. Accuracy requires a sharp eye for the identification of characteristics and good eye/hand coordination. This stage involves grouping the flies by sex according to their common properties; i.e., vestigial-wings vs. wild-type wings and white-eyes vs. red-eyes.

Stage II - Interpretation of Data

This stage begins with the establishment of statements of fact. Questions are designed to generate important information.

Q: From the two crosses, which allele is dominant? Which is recessive? Give your reasoning.

The students must explain their answers by relating the information generated to established ratios and thereby determine cause/effect relationships.

Q: Do the data from the crosses support the expected ratio of a monohybrid cross?

The next step requires students to go beyond the data and make inferences or find implications within it.

Q: Is this locus autosomal or is it sex-linked? Give your reasoning.

No Stage III is involved in this section.

B. Hybrid Corn

Students examine five glass cases containing various monohybrid and dihybrid corn crosses. They count the numbers of kernels (F_2 generation) which represent traits inherited from the parents.

Stage I - Data Collection

Stage I involves the collection of data, i.e., the number of kernels of each type, on each of the F_2 cobs.

Stage II - Interpretation of Data

This includes the identification of distinct points or statements of fact.

Q: For each monohybrid cross would you say that one of the alleles was dominant?

This stage also explores the relationship between statements.

Q: Was the dominant allele the one most frequently expressed?

Stage III - Application of Principles

By analysis of the situation, one may predict consequences.

Q: Would you be able to say that the more common allele is always dominant?

This question is designed to make students think about and explain an unfamiliar situation. They must also support their hypothesis with reasoning. The most common allele is not always dominant; e.g., black corn kernels are dominant to yellow, but yellow is more common.

C. A Dihybrid Cross in Tomatoes

Each lab room contains two flats of young tomato plants - the F_2 generation of a cross between green stemmed plants with smooth edged leaves and purple stemmed plants with serrated leaves.

Stage I - Concept Formation

Students examine the plants to differentiate among observable types and to group them according to the criteria of leaf edge and stem colour. The students are asked to assign genotypes of their own choice to the parent plants. This allows them to draw up a Punnett Square and to determine what the expected F_2 generation should produce.

Stage II - Interpretation of Data

Students compare actual numbers of plants to the numbers predicted by the Punnett Square in order to verify or refute the expected ratios of a dihybrid cross.

Stage III - Application of Principles

In order to analyze the true nature of the problem, students are asked to write a paragraph explaining how one would

obtain the F_2 plants had they been given this assignment. I intended the students to think about the length of time required for a tomato to ripen to the stage where the seeds would be mature enough for planting. I hoped they might also consider how pollen from the one type of tomato plant must be dusted on flowers of the other for fertilization, and that flowers must be prevented from self-pollination.

D. Sexual Reproduction in a Fungus

When the mycelia (vegetative cells) of a strain of the fungus Sordaria fimicola, which produces grey spores, are crossed with another strain which produces black spores, the resulting fruiting bodies produce asci that contain both grey and black spores (Appendix B, p. 64). Examination of these asci reveals six different combinations of the grey and black spores. Two of the combinations indicate that no crossing over occurs between the centromere and the gene locus for spore colour. Four other combinations are proof that a crossover does occur. Scoring the numbers of different combinations and applying these to a formula allows students to calculate the number of map units between the gene locus for spore colour and the centromere of the chromosome.

Stage I - Concept Formation

Students search for perithecia (fruiting bodies) that contain both grey and black spores. When found, they examine at least thirty asci, counting the number which show the different possible combinations. This requires the use of high power

microscopes and considerable skill. Several perithecia are mounted on a microscope slide in a drop of water. A coverglass is applied, using just enough force to burst each, thereby producing a sunburst of asci, but not enough force to break the walls and release the spores.

Stage II - Interpretation of Data

Students explain the significance of different combinations of spores by filling out a chart that follows the pattern of spore development from the original spore mother cell to production of a single ascus. They also explain what the chart means in terms of crossing over (Appendix B, p. 69). To make this easier, the lab manual explains what happens during a similar event when there is no crossing over between centromere and gene locus.

Stage III - Application of Principles

Predicting the distance of the gene locus from the centromere requires the ability to visualize crossing over as a three-dimensional occurrence - one which happens frequently during meiosis (gamete production). The farther away the locus is from the centromere, the more frequently crossing over occurs. Conversely, if the locus is close to the centromere, crossing over will be less frequent. This principle forms the basis of the calculation of map units which is the substance of Stage III.

Evaluation and Modifications

During the performance of this lab the students obviously

enjoyed what they were doing. They appeared to be very confident and proficient in the activities of the four components. Yet, upon checking their work, many were making too many unwitting errors. Sexing of the flies was frequently incorrect. Many microscopes were poorly focussed so that accurate spore counts were in serious doubt. More attention in this area was obviously required by the demonstrators.

The section on hybrid corn was not only boring for the students but also excessively time-consuming. In 1984, therefore, we retained one set of corn cases in each room as a demonstration, but eliminated the need for students to count individual kernels.

The dihybrid cross of tomatoes was very graphic and produced good results. However, the plants became badly mauled by the students. At the end of the week, results were difficult to observe and no fresh flats were available. Production had required more time and effort from the greenhouse staff than the results merited. Consequently, I decided to replace this section with a cross which demonstrated albinism in corn; 25% of the offspring from this cross receive a double recessive of the gene for colour and are therefore white. Because of their inability to photosynthesize, the fate of these plants is death. The students found this new section equally interesting in comparison with the tomato cross and the plants were much more easily produced.

The section on Sordaria fimicola has several inherent problems. Since very few students were able to score thirty

asci, they were told to score as many as possible. Reports showed a general lack of understanding of the concept of crossing over, and of the gene locus calculation. For many of the students, the concept of crossing over, combined with meiosis and the gene locus calculation, were beyond their capabilities. Nevertheless, I have resisted any change to this part of the lab. Instead, the technical preparation has been perfected. Students may now expect to find more than an adequate number of perithecia containing both colours of spore at the correct stage of development. Demonstrators are also better coached to assist with data collection and interpretation.

Lab manual instructions have been clarified and more explanatory labels were included in the charts (Appendix B, p. 68). A section explaining how the Chi Square Test may be used for the evaluation of data has been added. This test allows students to determine whether their data reflect an acceptable level of probability for an hypothesis or whether the hypothesis should be rejected. This test can be aptly applied to the fruit fly and to the Sordaria sections.

Although this lab unit is conceptually difficult, an encouraging number of the students rise to the intellectual challenge very successfully and all may learn a great deal about introductory Genetics.

LAB V - PHOTOSYNTHESIS

- A Brief Summary

- Appendix B, p. 71

In biological terms, photosynthesis uses energy and water to convert CO_2 into carbohydrate which is polymerized by the plant into starch or cellulose for storage.

In chemical terms, photosynthesis is the process that converts light energy into chemical energy which is stored by the reduction of carbon dioxide within green plants. In essence, this is the synthesis of energy-rich sugar from energy-poor carbon dioxide.

The introduction to this lab unit includes explanations of oxidation and reduction reactions and summaries of Photosystem I, Photosystem II, and the Calvin Cycle - the stages of photosynthesis.

A. Role of Light in Photosynthesis

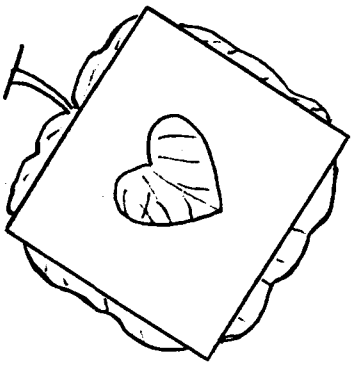
I. Necessity of Light

This is a demonstration experiment in which various different types of leaf are boiled in H_2O to break down the cell walls. Then they are boiled in alcohol to extract the pigments. The areas of starch storage show up clearly when the leaves are soaked in Lugol's iodine. Interestingly, these do not necessarily coincide with intensely pigmented areas.

Other leaves are also tested for starch. Some have been kept in the dark for 48 h and show very little starch. Another

has been shielded from the sunlight except for a cut-out patterned area.

More starch is observed in the cut-out area which has received sunlight than in the darkened area.



II. Effect of Light Intensity

Students perform this experiment to compare the rate of photosynthesis that occurs when the light source is at various distances from the plant (Appendix B, p. 79).

B. The Hill Reaction

This experiment simulates the photolytic cleavage of water which occurs in Photosystem I. By substituting the artificial dye DCIP for the electron acceptor NADP, it is possible to monitor the reduction of DCIP during photosynthesis by taking readings on the spectrophotometer.

The main purpose of this lab is that students learn about the process of photosynthesis. The model chosen is Creative Learning (Joyce et al., 1981). The students are repeating experiments already known. This not only verifies experimental outcomes, it gives students experience in working through a scientific problem and in handling scientific equipment. For example, the spectrophotometer is a very useful and delicate instrument used to measure the absorbance or the transmittance of light (Appendix B, p. 85).

The demonstration experiment on the necessity of light for photosynthesis was very successful. It has now been modified for

students to perform themselves. The experiment on the effect of light intensity, however, was found to be boring. It was modified to work faster, but even so it still lacks interest. Therefore, it will be replaced in 1986 with a thin layer chromatography experiment which separates pigments extracted from leaves.

In their reports, the students identify and explain the Hill Reaction, likening it to comparable reactions occurring in Photosystem I. This is a graphic illustration of the process of Photosynthesis which contributes to student understanding.

The Hill Reaction experiment always has difficulties inherent with using a mixture of chloroplasts. The flocculent nature of the mixture causes fluctuations in spectrophotometer readings. Nevertheless, when well-organized, it is a valuable learning experience, well worth retaining.

LAB VI- UNICELLULAR AND COLONIAL ORGANISMS

- A Brief Summary

- Appendix B, p. 86

This laboratory unit compares procaryotic and eucaryotic cells. It also examines the different groups of unicellular organisms.

A. Kingdom Monera

I. Cyanophyta - the blue-green algae

Students observe and draw living Anabaena sp. and Gloeocapsa sp. cells.

II. Schizomycetes

Students observe and draw examples of three different bacterial types. An experiment is performed applying antibiotic discs to gram-negative and gram-positive bacterial plates. After 48 h, students measure the diameters of the clear area visible around each disc.

B. Kingdom Protista

I. Sarcodina

Students observe and draw from living cultures of Amoeba proteus.

II. Sporozoa

These parasitic organisms are discussed briefly but are not studied.

III. Ciliata

Living cultures of Paramecium caudatum are observed and organelles are identified.

Contractile vacuoles take in water from the surrounding cytoplasm, then release it to the outside. Students count the number of seconds which elapse between expulsions. The contractile vacuole located near the area where food and water enters is expected to burst more frequently than the vacuole located anteriorly in a region of less water (Appendix C, p. 77).

IV. Mastigophora

These flagellates are considered to be the most primitive of the protozoa. Students examine blood smears taken from humans suffering from African sleeping sickness. The causative organism is the flagellate Trypanosoma sp. which is seen among the blood cells.

C. Plant-Like Protista

I. Diatoms

Prepared slides of this aquatic organism are observed and drawn (Appendix B, p. 97).

II. Dinoflagellates

Living cultures of this marine organism are observed and drawn.

D. Fungus-Like Protista

Physarum polycephalum is an example of the true slime molds. Students examine plates of this organism in order to

describe its colour, growth pattern and cytoplasmic streaming.

The main purpose of this lab is to give students experience looking at living unicellular organisms under the microscope in order to learn about the true nature of their structure. They also learn how to make biological drawings, a skill which will be utilized in future courses. The model chosen is Creative Learning (Joyce et al., 1981) or rediscovering what has already been known by others.

This lab was very successful. During the restructuring of the lab course in 1984, it was moved to Term I where it became the introductory lab for Biology 1A6. The bacterial experiment was removed, however, and expanded into a new Microbiology unit to introduce labs in Term II.

LAB VII - GAS EXCHANGE AND INTERNAL TRANSPORT

- A Detailed Account

- Appendix B, p. 100

This laboratory section explores the nature of respiration among different forms of life, both plant and animal. Of course, respiration always requires a ready supply of oxygen. Students measure and compare oxygen uptake with carbon dioxide output by a laboratory mouse. The section of the lab using the mouse is very similar to Lab VI of the Physiology 1H6 course, but the context has been given a different treatment.

Focus of the Unit (Orientation and Goals)

The primary purpose of this laboratory unit is to stimulate the development of investigative skills necessary for the solution of scientific problems. The secondary purpose is to promote progressive intellectual discipline and scientific competency among students.

The model chosen for this unit is Suchman's Inquiry Training method (1962) which is based upon the development of independent learning. Suchman's theory is that:

1. People inquire naturally when they are puzzled.
2. They can be made more conscious of and learn to analyze their own thinking strategies.
3. New strategies of thought can be taught directly and added to students' existing capabilities.

In part, this unit also utilizes the Cognitive Growth Model of Piaget (1952) which is inherently similar to Inquiry Training.

The lab exercise focuses on many of the general course objectives. For example:

1. The recognition of major relationships within the information;
2. Learning to assess the results of investigation;
3. Active involvement of students in the procedures;
4. Development of student potential for learning;
5. Development of student ability to communicate logically and critically;
6. Development of an ability to draw clearly labelled graphs.

Instructional Design (The Model in Action)

Phase I The introduction to this lab exercise outlines the problem which confronts all larger plants and animals. Diffusion alone cannot carry the oxygen necessary for respiration, to inner tissues, nor can it remove the carbon dioxide.

Every gas exchange surface, then, must answer the four basic requirements for respiratory surfaces, namely, sufficient size, system of transport, protection and moisture. Different forms of life solve the problems in different ways. This lab examines four fundamentally different adaptive strategies.

Phase II a) requires students to observe how insects move oxygen to their innermost tissues through tracheae and tracheoles. The system is very extensive. Seen in

living organisms, it is silvery-white in colour with numerous reservoir-like sacs for retaining air. Many of the larger tubes are reinforced with rings similar in appearance to the cartilaginous rings of the mammalian trachea. Although the lab manual gives a description of the tracheal system, students must verify its appearance under the dissecting microscope.

b) Necturus maculosus, the mud puppy, is an amphibian with a curious method of respiration. Several of these salamanders are in an aquarium in the laboratory. Students watch as nitrogen is bubbled into the aquarium for about five minutes. During this time, the nitrogen almost totally displaces oxygen in the water. This procedure should cause the mud puppies first to actively extend and move their gills and later to come up to the surface and start gulping air into their primitive and little-used lungs. The students are not given any information either about the animals or about the surprising results.

c) The third strategy for investigation is mammalian respiration. The laboratory mouse is representative of mammals. Students perform an experiment to measure the O_2 consumption of the live mouse according to detailed instructions given in the manual (Appendix B, p. 103).

d) Lastly, according to instructions in the manual, students perform an experiment to determine the rate of transpiration by a geranium plant and to examine the

Phase IV In their written reports, students must tie the data together and explain the nature of the various inquiries.

c) Mouse - When all the data have been collected, students calculate the O_2 consumption by the mouse as $ml/O_2/g$ of mouse body weight/h. They also calculate the CO_2 production and the Respiratory Quotient where:

$$RQ = \frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}}$$

In turn, the RQ is an indicator of the metabolic substrate utilized by the mouse during the respiratory period of the experiment. The students identify this substrate.

In the report, students must explain the reasoning behind their decision regarding experimental modifications to determine CO_2 output. They must plot a graph of O_2 consumption and of O_2 consumption + CO_2 production, calculate the slopes of these graphs, and interpret the information obtained.

d) Plants - Plots of the rates of transpiration during the three trials are all applied to one graph for direct comparison and interpretation. Discussion in the report requires students to explain how plants have adapted to meet the four basic needs of respiratory surfaces. (Appendix C, p. C81).

In addition to checking the technical aspects of the experimental setups, the function of the demonstrators is to

continually ask questions about conditions and events in order to expand the basis of students' understanding of the experiments.

Evaluations and Modifications

Although they were busy every minute of the period in some aspect of these procedures, the students had an exciting time in this lab. Many did not care to handle the mice, but there was always someone available who was willing to help. I cannot say, however, that the lab was an unqualified success. There was a general lack of understanding and of interest regarding the insect tracheal system; its value is therefore being reassessed. As reported by demonstrators, the reports were disappointing. Many students had difficulty making the intellectual leap between data accumulated and a clear explanation. The reasoning for Necturus' activity in most cases was inadequate. Many omitted essential areas of discussion in their reports, and graphs were not well drawn or labelled.

At the year-end demonstrators' meeting, there were complaints about the aggressiveness of the mice; this problem was easily solved, however, by ordering only females for the following year. Demonstrators also reported that the size and number of shoots available on geranium plants was insufficient. Greenhouse personnel were asked in future to increase the plant growth for this time of year. Instructions in the lab manual were simplified and clarified to avoid undue student confusion.

This lab exercise appeared to require additional effort to assist student understanding. In the following year, I insisted

that TAs conduct a more discerning post-lab discussion in order to explore the several fundamental issues involved, and to give students a chance to analyze their reasoning while the experiment was still fresh in their minds. As a consequence, students have indicated that, in spite of its demanding nature, this lab unit is not only a favourite lab, but is also a superior learning experience. These improved results have proved the importance of good organization and of good team work to the success of the labs.

LAB VIII - REGULATION OF BODY FLUIDS

- A Detailed Account

- Appendix B, p. 110

All life must maintain within itself a fluid environment suitable for its cellular make-up. Thus, living organisms have evolved many different mechanisms to maintain homeostasis of body fluids.

Focus of the Unit (Orientation and Goals)

The primary purpose of this unit is for students to understand why organisms must control their water balance. A secondary purpose is to relate the structure of the nephron to the internal structure of the vertebrate kidney. No single teaching model is used as a guide.

The educational objectives are:

- that students learn to assess the results of investigation;
- that students develop laboratory skills;
- that students develop the ability to organize experimental results.

Young people are naturally curious; they are eager to learn and to see interesting, new things. This lab gives them a specific direction to explore - one which challenges their intellectual ability and laboratory skills.

Instructional Design (The Model in Action)

As an introduction to this lab, we show an excellent colour

film which examines the structure of the mammalian kidney. The film was produced for medical students at the McMaster University Medical Centre. It is entitled "Functional Anatomy of the Human Kidney."

There are three distinct sections to this lab, but one does not necessarily build upon any other. Rather, each is a study of the evolutionary adaptations made by a particular group of animals, to control water balance.

I. Invertebrates - Earthworms

Working in pairs, the students weigh three earthworms. This requires the use of a micro-balance which is capable of giving accurate readings to 0.001 g. One worm is put into distilled water. A second is put into a weak salt solution of known concentration and the third is put into a strong salt solution. After one hour, each worm is reweighed and the change in body weight is recorded. The class data on all worms are collected, from which students calculate the average increase or decrease in body weight, due to water fluxes, for each treatment.

With these data, students construct a standard graph from which they may deduce the salt concentration at which there would be no increase or decrease in worm body weight, and thus one which is isotonic to the worm's normal body fluids (Appendix C, p. 84).

Students are asked to assess the effectiveness of the nephridial system of earthworms in controlling water balance.

II. Insects - Grasshopper

To illustrate divergent evolution, students observe and draw the Malpighian tubules found in the body cavity of a freshly-killed grasshopper. They are a mass of long thin tubes which lie free in the hemocoel (body cavity) where they pick up waste material from the body fluids, process it and dump dry wastes into the large intestine for removal through the rectum. This system is a totally different concept from the nephridia of earthworms.

III. Vertebrate Kidney

Each pair of students is given a whole pork kidney, cut in half to show the major processing areas for the formation of urine. By taking a very thin slice from the cut surface, and examining it under the dissecting microscope, the students are able to distinguish parts of individual nephrons and structural areas of the kidney. Students are asked to make diagrams because visual observation of processing areas and the recording of them in diagrams will reinforce understanding of the vertebrate kidney structure given in lectures, thus anchoring it in the students' minds.

In addition to instructing the students in the use of the micro-balance, the TAs introduce the lab objectives and make sure that they are accomplished. Each lab room has on demonstration, a preserved pork kidney in which the arteries, veins and collecting ducts are injected with different colours of latex.

Evaluations and Modifications

The Malpighian tubules did not show up well in the dissection of the grasshopper. It was suggested that cockroaches are better organisms for this purpose, so a change was made for the following year. However, there was still difficulty in interpretation of the system.

The experiment on water balance by earthworms was very successful. The students enjoyed the experiment, results were very close to expected values and the report write-ups including the standard graph were well executed.

Many students were unable to see the structure of the nephron in the pork kidney, usually because their cut sections were not thin enough. To clarify understanding of kidney structure, slides of the rat kidney were borrowed for use as a reference. Students seemed better able to interpret from these prepared slides than from the fresh material so a complete set of rat kidney slides has been obtained.

One demonstrator suggested that this lab unit was not busy enough. Some of his students had left early without participating in all the activities of the lab. With this in mind, I discussed various alternatives to the pork kidney with Dr. C. M. Wood, and have decided to change the mammalian kidney section of the lab to a dissection of the foetal pig. This will allow students to explore relationships among all the various organ systems with particular emphasis on the kidney and its related vessels. It will also provide an opportunity to learn or to practice skills of dissection, an area

which has been lacking in this course. As many of our students wish to enter medicine, this new part on dissection should generate a good deal of enthusiasm.

The introductory film on kidney function was dropped. Although it was excellent, the subject matter and medical terminology were at too great a depth for our students to comprehend.

This lab unit on the kidney is therefore still in a state of alteration, but the planned changes should pose a greater challenge for the students, and should also generate more enthusiasm.

LAB IX - PLANT HORMONES

- A Brief Summary

- Appendix B, p. 116

Plant hormones are often called growth substances. Their main function is the regulation of plant growth and development. This lab unit examines the activity of two types of hormone - auxins and gibberellins.

A. Auxins

I. Phototropism

That plants bend towards a source of light is a very well-known phenomenon. The first section of this lab requires students to search out and explain why plants turn towards the light. In the lab, the students place two young bean seedlings under direct overhead light. Another two are placed in front of a side-directed light. Within a few hours the plants are observed to be turning towards the illumination.

II. Geotropism

Students plant four corn seeds in small pots. Two are planted horizontally, one with the embryo facing down, the other facing up. Two others are planted vertically, the embryo of one pointed down and the other pointed up. After two weeks the seeds are removed to examine the growth patterns of emerging shoots and roots. Regardless of the original orientation of the corn seeds, all shoots are observed to be growing upwards and the roots to be growing downwards.

Geotropism in older plants is examined by placing a growing bean plant on its side under an overhead light for two weeks. When removed from the pot, roots are observed to have turned downwards and the shoot has turned upwards.

Because two weeks were required for these experiments, the students were given an extension of one week for their reports. It was found, however, that the desired results for all parts of this lab were observable after only one week. Therefore, in 1984, the manual was changed to allow only one week growth period for observations.

Instructions for keeping the bean plant on its side were modified to direct students to take the plant home and to put it into a dark cupboard on its side for one week. This eliminated light as a factor in the bending of stems to the upright from the horizontal position.

III. Apical Dominance

Plant growth is controlled by auxins produced in meristematic cells of the growing tip and carried downwards in the vascular system of the plant. A concentration of auxin which stimulates growth of stems will inhibit the development of adjacent lateral buds. This is the hypothesis behind apical dominance. The students try to verify the truth of this hypothesis by growing four bean plants under various treatments. A control plant (A) is left to grow naturally. The growing tips of the other three are removed. One of these is left without further treatment (B). A mixture of lanolin (a vaseline-like substance) and indoleacetic acid (a naturally

occurring auxin) is applied to the cut stem of a third (C), and lanolin alone is applied to a fourth (D). After two weeks, the two topmost lateral buds of B are observed to have grown into new shoots. Plant C appears to be unchanged following removal of its growing tip. The two topmost lateral buds of Plant D have also grown into new shoots, proof that the indoleacetic acid applied to the cut stem of Plant C has prevented the growth of lateral buds in a manner similar to its prevention naturally, i.e., by auxin production in growing meristematic cells.

This experiment proved to be very successful. Its only problems were technical - the movement of so many plants back and forth to the greenhouse. In 1984 the students did their preparations at the greenhouse itself. This gives students an opportunity to view the greenhouse facilities and prevents transportation damage to the delicate plants.

B. Gibberellins

Gibberellic acid is a plant hormone which causes growth of dwarf plants but has little or no effect on plants of normal size.

Each student pair is given two normal pea plants and two dwarf plants. One of each type is put into a tray to be watered every day. The second of each type is also watered each day but in addition is sprayed with gibberellic acid every other day. After two weeks, it is observed that the dwarf plants treated with gibberellic acid are as tall, if not taller, than the normal plants, whereas the untreated dwarf plants are still much smaller

than the normal plants.

The only change made to this experiment in 1984 was to have the students measure and record the length of each plant both before and after the treatment period.

The main purpose of this unit is for students to see first-hand how plants are able to control growth and development. This is one of the most active areas of modern botanical research and is therefore an introduction to another direction for future biological study. The laboratory material is closely related to lectures in this area.

Design of the unit is Tyler's Academic Model which is a subject-centred method of teaching based upon specific objectives.

Although the background of this lab unit is easily understood, and although the lab sections give clear results, a surprising number of students indicated a distaste for any sort of laboratory unit involving plants. Nevertheless, the unit is being retained because it not only closely parallels lecture material but also because Botany is an important part of any general biological course.

LAB X - ANIMAL BEHAVIOUR

- A Brief Summary

- Appendix B, p. 123

The science of animal behaviour is called Ethology. It requires careful and detailed observation, descriptions and interpretations of behavioral events under study. Since this is the last laboratory exercise of the year, it is planned to be enjoyable as well as informative.

A. Schooling Behaviour of Fish

I. Effect of Visual Markings on Behaviour

This experiment is designed to determine whether a fish will choose to remain with its own species or will congregate with other species of similar body size.

During observations, students are expected to report any behaviour on the part of the test fish which may be relevant.

Q. Does the activity in the jar appear to stimulate the test fish?

Results of this experiment will probably indicate that goldfish are not greatly attracted to other goldfish and might therefore be considered as non-schoolers. On the other hand, the zebra danios appear plainly to be more attracted to other zebras than to the goldfish. The students hypothesize, therefore, that danios are schooling fish and that their striped

body pattern may in part be responsible for their ability to maintain a close relative position, one to another while swimming.

II. Effect of Group Size on Behaviour

This experiment is designed to determine whether goldfish and zebra danios prefer to remain close to a large group of their own species or whether they prefer to swim randomly in the aquarium.

The expectation is that students will observe that goldfish swim randomly, not preferring any particular area. The zebra danio, however, will prefer to remain near its own kind, another indication that the zebra is a schooling fish and the goldfish is a non-schooler.

B. Agonistic Behaviour in Siamese Fighting Fish

In the laboratory, students observe male Siamese fighting fish in order to establish the pattern of normal behaviour. Upon the sight of each other, but separated by a glass barrier, two male Siamese will show an agonistic display (having to do with attack, escape or fear). Colour is heightened, and fins become erect. One or both exhibits a broadside display to the other (Appendix B, p. 128). The dominant fish may adopt a frontal attack position. If the glass barrier is removed, the dominant fish may pursue and attack the more submissive fish. Behaviour of the submissive fish is also noteworthy. Its fins are lowered, colour decreases and it may flee to a corner. This "appeasement" behaviour tends to reduce the aggressiveness of the dominant fish, thus establishing dominance

without the necessity of a fight. If the dominant fish sees its own image in a mirror, it will maintain the agonistic display for a considerable length of time.

C. Grasshopper Behaviour

Grasshoppers have an extremely interesting life cycle. When ready to lay eggs, the female uses her ovipositors (appendages used for egg laying) to dig a hole. About twenty-four eggs are laid. After ten days, the young nymphs emerge. During the next four weeks, they pass through five successive stages of development called instars, during each of which the old exoskeleton is shed and a new one is formed.

Cages are set up in our lab rooms in February for students to watch the stages of development. An experiment is performed during which one set of nymphs reared in an uncrowded cage develop a green or tan coloration similar to the green of the grass upon which they feed. Nymphs reared in a crowded cage remain smaller in size and darker in colour, often with a yellow and black pattern but never green. They also exhibit a more aggressive nature. Such experiments form the basis of studies which determine the cause of locust outbreaks in hot, dry weather. The students are asked to observe the grasshoppers for a few minutes twice a week over a period of four weeks. During this time, volunteers are allowed to care for the grasshoppers. Observations are recorded with respect to colour, growth and development, molting, sex differentiation, eating behaviour, stridulation (a "song" produced by rubbing the hind femur against the forewing), copulation, and oviposition. Discussion and

interpretation of observations are to be included in the report.

The main purpose of this lab exercise is to give students first-hand experience in making behavioral observations. A secondary purpose is to provide insight into the natural activities of fish and of insects.

The design of this unit is modelled after Tabas's Inductive Thinking Model (1967).

Feedback from the students regarding this behavioral lab was generally very positive as regards the fish section, but negative for the grasshoppers. The students were turned off by anything to do with insects, especially grasshoppers. Consequently, this section was replaced in 1984.

This is a very social lab unit. The students work in groups of four, but if one group has something interesting going on, others will also come over to watch. Even though the subject matter parallels the lecture material closely, this lab unit is not very demanding academically. On the last day of the lab we give the fish away to the students. It is therefore a very popular lab, one which finishes off the academic year in high style.

CHAPTER V

CRITICAL EVALUATION

Scriven (1967) explains formative evaluation as the ongoing process of examining feedback from within the circle of a project, by those who are involved with it. In the context of this project, evaluation is regarded similarly; it is the procedure of examining information from different sources:

I to make a judgment regarding the worth of the laboratory curriculum;

II to determine whether the educational goals have been attained - p. 2;

III to assess the individual exercises of the lab manual for purposes of modification.

I. Evaluation to Determine the Worth of the Curriculum

An important measure of the success of a laboratory curriculum lies in its acceptance by the students as a worthwhile part of their education. Accordingly, at the end of each academic year, students anonymously fill out an evaluation form rating their lab demonstrator, the professors and the laboratories on a scale of 1 to 5. These forms are summarized, then given to the demonstrators and to the professors, respectively.

Summaries of original TA evaluations from 1982 were recorded for Biology 1H6, representing a 90% response. Unfortunately, laboratory evaluations were not retained. Although we do not

have actual figures, the consensus of opinion each year I was involved with Biology 1H6 was that the laboratory component was of considerable value. Because the ongoing evaluative emphasis has been directed toward curriculum improvement, it may appear that feedback on the laboratory program was negative. In fact, however, student and TA opinion has always been very positive in nature.

Student evaluations of TAs are based upon five criteria: preparation, knowledge, interest and involvement, availability, and grading. In 1982, the average individual scores out of a possible five for the ten TAs were: 3.62, 4.64, 2.66, 3.96, 4.40, 4.02, 4.12, 4.56, 4.28, and 3.56. The overall average was 3.98 (79.6%).

In 1984, 90% of Biology 1A6 students anonymously filled out forms evaluating their TAs, the professors, and the labs. The TA evaluations were based on the same five criteria. Average individual scores out of a possible five for the twenty TAs were: 4.42, 4.03, 4.41, 3.24, 4.13, 4.02, 3.44, 3.62, 4.23, 4.14, 3.84, 4.50, 4.51, 3.13, 3.94, 4.01, 4.62, 3.41, 3.30 and 4.31. The overall average was 3.96 (79.2%).

Laboratories are evaluated on a scale of one to five using the following criteria:

very helpful - 5

fairly helpful - 4

satisfactory - 3

not very helpful - 2

poor - a waste of time - 1

In 1984, Biology 1A6 laboratories scored 2.8 out of 5. 58.0% of students indicated labs were satisfactory or better.

In 1985, they scored 2.9 out of 5. 62.3% of students indicated labs were satisfactory or better.

In 1986, they scored 3.1 out of 5. 71.4% of students indicated labs were satisfactory or better.

At the year-end wrap-up meeting in 1982, Biology 1H6 TAs were unanimous in recommending that the lab manual remain unchanged except for planned alterations and small revisions to improve comprehension.

There was no wrap-up meeting of TAs and instructors in 1984. Several major changes to the lab manual were planned and completed. In 1985 and again in 1986, TAs indicated satisfaction with the laboratory curriculum in its revised form, with only minor changes for improved comprehension.

From this information, I conclude that laboratories for both Biology 1H6 and Biology 1A6 make a worthwhile contribution to the courses which they complement.

II. Evaluation to Determine Attainment of Educational Goals

In the fall of 1986, questionnaires were distributed to former Biology 1H6 students still within the Department of Physical Education and to former Biology 1A6 students still within the Department of Biology. All were requested to respond anonymously to a series of twelve questions and to rate the individual lab exercises according to merit. A very low response rate of only 10% was obtained. Results, therefore, are only indicative of the

opinions of those who took the time and trouble to complete the questionnaires.

For each statement in the questionnaire (Appendix D), students were asked to respond:

strongly agree, agree, neutral, disagree, strongly disagree

1. Instructions in the lab manual were clear and understandable.
   Biology 1H6 - 83% of students agree
   Biology 1A6 - 56% agree, 33% neutral

2. The objectives and introductions at the beginning of each exercise were helpful.
   Biology 1H6 - 58% agree, 33% neutral
   Biology 1A6 - 72% agree, 16% neutral

3. I was able to reason out the concepts behind the lab exercises by myself.
   Biology 1H6 - 58% were neutral, 25% disagree
   Biology 1A6 - 61% agree, 28% neutral

4. I enjoyed working through the lab experiments.
   Biology 1H6 - 42% agree, 25% neutral, 25% disagree
   Biology 1A6 - 22% agree, 50% neutral, 16% disagree

5. My demonstrator was accessible for questions and for discussion of theory during the labs.
   Biology 1H6 - 66% agree, 33% neutral
   Biology 1A6 - 39% strongly agree, 33% agree, 17% neutral

6. Laboratory skills learned in these labs have been useful to me since finishing this course.

   Biology 1H6 - 33% neutral, 42% disagree

   Biology 1A6 - 17% agree, 39% neutral, 22% disagree

7. Preparation of lab reports helped me to learn the concepts.

   Biology 1H6 - 66% agree, 25% neutral

   Biology 1A6 - 50% agree, 28% neutral, 22% disagree

8. Preparation of lab reports has improved my ability to write or communicate logically and precisely.

   Biology 1H6 - 25% agree, 42% neutral, 33% disagree

   Biology 1A6 - 33% agree, 56% neutral

9. Lab exercises aroused my interest in Biology and/or Science.

   Biology 1H6 - 17% strongly agree, 25% agree, 25% neutral, 25% disagree

   Biology 1A6 - 33% agree, 39% neutral, 22% disagree

10. Lab manual questions were helpful in interpretation of data.

    Biology 1H6 - 43% agree, 33% neutral

    Biology 1A6 - 56% agree, 17% neutral, 17% disagree

11. Lab manual questions were frequently an intellectual challenge.

    Biology 1H6 - 25% strongly agree, 43% agree, 25% neutral

    Biology 1A6 - 17% strongly agree, 33% agree, 17% neutral, 22% disagree

12. My impressions of the usefulness of these labs have improved since taking the course.

    Biology 1H6 - 25% strongly agree, 50% neutral, 25% disagree

    Biology 1A6 - 33% agree, 39% neutral, 17% disagree

To summarize the results of this questionnaire:

1. The instructions in both manuals are clear and understandable.
2. The objectives and introductions at the beginning of each exercise are extremely helpful.
3. Biology 1H6 students had difficulty reasoning out the concepts behind the lab exercises on their own. Biology 1A6 students had no such difficulty.
4. Most students enjoyed working through the lab exercises.
5. Most students found their demonstrator to be accessible and helpful in discussions about lab exercises.
6. Laboratory skills learned in the labs have not been useful to the students since finishing the course.
7. The preparation of lab reports was very helpful to students in learning the lab concepts.
8. Most students believe that the preparation of laboratory reports has improved their ability to write and to communicate logically and precisely.
9. Most Biology 1H6 students believe that the lab exercises increased their interest in Biology or in Science. Biology 1A6 students were more neutral regarding increased interest in Biology or Science.

10. Lab manual questions were definitely helpful in the interpretation of lab data.
11. Most Biology 1H6 students found the questions to be an intellectual challenge. Similarly, most Biology 1A6 students found the questions challenging, but a significant number did not.
12. Student opinion regarding the usefulness of lab exercises improved since finishing the course for one-fourth of the Biology 1H6 students. The opinions of another fourth had not improved. A majority of Biology 1A6 students have a better opinion of the usefulness of labs since finishing the course.

The top three labs in Biology 1H6 according to the questionnaire were:

1. Cardiovascular Function
2. The Blood
3. Nutrition

The least preferred lab was Diffusion; however, only half of the respondents answered this question.

The top three labs in Biology 1A6 according to the questionnaire were:

1. Gas Exchange
2. Photosynthesis
3. Genetics

The least preferred was Behaviour. Again, however, only 50% of respondents answered this question.

As a means of interpreting the information from the questionnaires further, results can be related to the educational goals:

1. to involve students actively in learning situations

   Although no questions relate directly to this goal, involvement is an essential part of each lab exercise. Unquestionably, I believe, this goal is met.

2. to help students achieve an excitement for learning

   Questions 4 and 9 relate to this objective. More than 66% of students from both classes signified neutral or better for enjoyment of labs. More than a third of each group agreed that their interest in Biology had been aroused, and two-thirds were neutral or better. A moderate degree of success has therefore been achieved.

3. to help develop laboratory skills and an understanding of basic laboratory procedures

   According to the response to Question 6, 42% of Biology 1H6 students disagreed that they learned laboratory skills which were subsequently useful. Similarly, more Biology 1A6 students gave a negative response than a positive response to this question.

4. to foster the development of an enquiring mind

   From the data available, it is not possible to make an assessment regarding this goal. Neither the questions nor personal contact indicate whether labs were successful in

its achievement.

5. to develop the students' ability to write clear and well-organized reports

Questions 7, 8, 10, and 11 all assess this goal. It appears that the goal was better achieved with Biology 1A6 students than with Biology 1H6 students. Perhaps this reflects less interest in the value of scientific report-writing by Physical Education students, whereas Natural Sciences students expect to be required to prepare scientific reports throughout their careers. TAs work very hard at encouraging improvement in the quality of reports.

They have agreed in wrap-up meetings that approximately two-thirds of the students demonstrate a marked improvement in report-writing over the course of the year.

6. to assist students in reaching their potential for learning by expanding their intellectual capabilities

The success of this goal is also difficult to assess. The fact that many Biology 1A6 students chose the three most difficult labs as being the most preferred indicates that they have been stimulated by the intellectual challenge of these units. The least useful Biology 1A6 lab was Behaviour - the one which is the least demanding. Therefore, I believe this goal is being met in Biology 1A6.

In general, most of the educational goals are being met. It seems, however, that from the students' point of view, the goal of

developing laboratory skills has not met their expectations. This response suggests a need for more challenging or sophisticated techniques and methodologies in lab exercises. In the future, as funds become more available, it may be possible to move in this direction. However, students frequently have a misconception regarding their own level of proficiency. Having practiced a technique once or twice does not ensure an understanding of it; repetition of the procedure at greater and greater depth is necessary before full understanding or competence may be achieved. Simple skills and disciplines must be mastered before students are ready to move up to more demanding, highly technical skills and to more delicate and expensive equipment. Such opportunities will come in future courses where enrollment is lower and the focus is more narrow.

The Biology 1H6 and 1A6 courses are broad and basic introductions to biology, purposely designed to cover as many areas as possible. They are intended to point students in diverse directions. Consequently, the skills taught in these courses are also basic, for example:

1. the use of microscopes and dissecting microscopes
2. pipetting
3. use of the centrifuges
4. operation of the spectrophotometer
5. writing of standard scientific reports
6. construction of graphs and tables
7. use of the electrocardiograph machine (1H6)

8. use of stethoscopes and sphygmomanometers (1H6)
9. use of the spirometer (1H6)
10. use of microbalances (1A6)
11. thin layer and paper chromatography (1A6)
12. translation of laboratory observations to the permanent record of laboratory drawings (1A6)
13. aseptic technique (1A6)

These are simple and basic skills which must be mastered before students are ready to tackle other projects. Unfortunately, this is an area of frustration to students whose aspirations surpass their level of competence.

III. Evaluation of Individual Exercises

As stated earlier, the TAs have the closest and most prolonged association with the students. Each of them is responsible for his or her own lab room and for marking the students' reports. Therefore, input from the TAs carries much weight in assessment of lab manual exercises.

General recommendations which arose from the May 1982 and 1983 wrap-up meetings of Biology 1H6 are:

Reports

1. Guidelines for preparation of written reports should include a mandatory paragraph combining purpose and introduction.
2. In order to make the students read the lab manual in advance,

a pre-lab quiz or a random quiz should be considered for the beginning of each lab.

3. Consider the use of a letter grade for lab reports instead of a number grade.
4. Guidelines should require students to number all tables, diagrams and graphs for quick and handy reference in the body of their report.
5. After each lab, a copy of the best lab report should be posted where others may see it.

Lab Manual

6. Each new lab unit should start on a right-hand page.
7. Each unit should end with a blank page (or two) for work notes and calculations.
8. Replace graph paper in the manual with a colour which copies better.
9. Give textbook page references in the manual.

Specific suggestions made for individual labs were:

Lab I - Diffusion and Osmosis

1. The lab unit should be split into two sections:
   (i) Diffusion; and (ii) Osmosis.
2. Because NaCl is colourless, consider other chemicals which would show diffusion rate and pattern better.
3. Clarify and simplify the questions.
4. TAs should spend more time explaining how to write reports.
5. A lengthy pre-lab talk should be organized to demonstrate the

pipetting technique and to explain procedures as well as the use of equipment.

6. The section on the osmotic fragility of the cell membrane of blood cells could be transferred to the lab unit on Blood.

Lab II - Reaction Time

1. Try to standardize equipment and instructions.
2. Add a table for collection of data to the manual.

Lab III - Sensory Function

1. Eliminate tactile sensitivity and the section on 2-point threshold. They are boring and time-consuming.
2. Incorporate the sections on taste and on colour vision into the section on Reaction Time.
3. Increase the concentration of solutions typifying the four basic taste groups.
4. Use quinine for the bitter solution - not phenylthiocarbamide (PTC)

Lab IV - The Blood

1. Try "Sealize" to replace the plasticene plugs in the capillary tubes in order to prevent leakage during centrifugation.
2. Explain the relationship between Sickle Cell Anemia and Malaria.

Lab V - Cardiovascular Function

1. TAs should plan a longer pre-lab talk to explain the use of the ECG machine. Consider linking up an oscilloscope to the

ECG to give a more graphic display.

2. The venous pump section did not work well because the veins of most students were not prominent enough. However, do not cancel the section altogether. Consider making it a demonstration instead.
3. The capillary beds in the fingernails were not well seen. Neither did the hot and cold treatments add to the results.
4. Get fresh beef hearts to show the valves and chambers of the heart.

Lab VI - Respiratory Gas Exchange

1. Stress to students that the R.Q. is based on the substrate being metabolized by the mouse rather than its diet. For better comprehension, clarify instructions for the calculation of CO_2 production.
2. Experiment with other equipment which might be more successful, e.g.,
   - narrower tubing;
   - try dye in water to improve ease of reading volumes;
   - use a pipet in place of the inverted cylinder to suck up water from the system, thus eliminating the need to compensate for the volume of the tubing in calculations.

Lab VII - Fitness

1. Add a section to the manual explaining how various factors affect the equation for O_2 consumption.
2. Delete Part A - the influence of position on heart rate.

3. Delete the tedious correlations between Parts A, B, and the Schneider's Test.
4. Substitute the ECG machines used in Lab V to illustrate various degrees of exercise on heart rate.

Lab VIII - Kidney Function

1. Prepare a "hand-in" sheet in place of a "report" for this lab.
2. Use fresh reagents each day for the pregnancy tests.
3. Use all-purpose labstix instead of separate test strips for each substance.
4. Consider adding an immunological test to utilize antibodies.

Lab IX - Salivary Amylase

1. Stress the different sizes of pipets used and the significance of the colour changes.

Lab X - Nutrition

1. Clarify the parameters to be used for the graph that students draw.
2. Clarify how the curve is to be utilized in the report.

All of these suggestions were given full consideration. Most of them have been incorporated into the lab manual as improvements. The rest were tested and rejected as being unsuitable.

General recommendations which arose from the May 1985 and 1986 wrap-up meetings of Biology 1A6 are:

1. It would be helpful for TAs to know what students have been given in lectures regarding the lab material.

Reports

2. Students should be cautioned against plagiarism with regard to report-writing.
3. References should be regularly included in reports.

Lab Manual

4. It is important for TAs to interpret the difficulties of the lab to students in advance.
5. Encourage students to read the lab manual before coming to the lab.

Specific suggestions made for individual labs are:

Lab I - Unicellular and Colonial Organisms

1. Use fewer organisms and more prepared slides.
2. Order large specimens, as they are easier to find under the microscope.

Lab II - Chemical Basis of Biological Systems

1. This lab was fun; the students were kept very busy.
2. It is important to see the difference between RNA and DNA.

Lab III - Cell Structure

1. Lectures do not follow the sequence of labs.
2. Retain both fresh and prepared muscle slides - consider trypsin treatment for fresh muscle.
3. The Department has an excellent film we should consider using, called "The Development of Zebra Fish".
4. Interpretation of the frog intestine needs more attention by demonstrators.
5. Get a better micrograph which shows good chloroplasts.
6. Consider slides of blood cells showing Barr bodies.

Lab IV - Genetics

1. Students find the section on Sordaria difficult.
2. The lab is too long - put corn in as a demonstration.
3. The students enjoyed working with fruit flies.
4. Have Dr. Threlkeld interpret the lab in lectures.

Lab V - Photosynthesis

1. Retain the section on testing for starch storage in leaves.
2. Delete the section on light intensity.

Lab VI - Microbiology

1. The lab is not too long. There are a lot of problems. Therefore, technical set-up should be reviewed.
2. Organize the lab so that students do not have to come in over the weekend to read plates.
3. Clarify the protocol, e.g., show how to make the calculation for titre (bacterial concentration).

Lab VII - Gas Exchange and Internal Transport

1. Get a more docile strain of mouse.
2. This was one of the best labs - a favourite.
3. Consider dropping the insect tracheal system.
4. Ensure that large leafy plants are available.

Lab VIII - The Kidney

1. Stress that the kidney slice must be very thin.
2. Do not use the film again - too long and the terminology is unfamiliar.
3. Some students only came in and observed, then left because there was not enough to do in the lab itself.
4. Replace the kidney with a dissection, e.g., fresh rat.

Lab IX - Plant Hormones

1. Make observations after one week rather than two.
2. The phototropism section should be retained because the rationale for it is important. However, it should be set up in advance to show better results.
3. Synchronize the unit with lectures.

Lab X - Animal Behaviour

1. The fish section was excellent.
2. Consider setting up the tanks in advance rather than students handling the fish.
3. Replace the section on woodlice with something else.
4. Consider introducing some aspect of human behaviour for more

interest.

Most of these suggestions have already become a part of the laboratory curriculum of Biology 1A6. Others are still being considered for the future.

At the end of the introductory year of Biology 1A6, students were asked to complete an evaluation questionnaire asking:

- which lab they liked the most
- which lab they liked the least
- which lab(s) was/were of the most benefit
- which lab(s) should be dropped

The following table indicates the range of feedback received from the questionnaires:

LABORATORY EVALUATIONS - BIOLOGY 1A6 (1982)

Most-Liked Lab	Least-Liked Lab	Lab of Most Benefit	Recommend Drop Lab(s)
Microscopic Labs Cell Structure and Unicellular Organisms	Behaviour (Insects)	Gas Exchange	Observational only
Unicellular Organisms	Photosynthesis	Genetics & Behaviour	Chemical Basis
Plant Hormones & Gas Exchange	Unknown Specimens (Classification)	Plant Hormones	Unknown Specimens (Classification)
Chemical Basis	Behaviour (trivial)	"	"
Behaviour	Unknown Specimens (Classification)	Kidney	"
"	"	"	"
Genetics (Flies)	Behaviour	-	Behaviour (too abstract)
Gas Exchange	"	Gas Exchange	Behaviour & Plant Hormones
Plant Hormones	"	Kidney	Behaviour
Gas Exchange	"	"	Behaviour & Plant Hormones
Gas Exchange	"	"	"
Behaviour (Grasshoppers)	Plant Hormones	"	Plant Hormones
2nd Term	Genetics	Kidney & Plant Hormones	None
Behaviour	Genetics	Behaviour	Genetics
Gas Exchange	Plant Hormones	Gas Exchange	None
Gas Exchange	Behaviour & Plant Hormones	Gas Exchange	Behaviour (Grass-hoppers & Fish)
Gas Exchange	Plant Hormones	"	Behaviour

(Cont.)

Most-Liked Lab	Least-Liked Lab	Lab of Most Benefit	Recommend Drop Lab(s)
Gas Exchange	Plant Hormones	"	Behaviour & Plant Hormones
Unicellular Organisms	Genetics	Chemical Basis	Gas Exchange & Plant Hormones
Gas Exchange	Behaviour (Grasshoppers)	Genetics	Behaviour
Behaviour	Kidney	Gas Exchange	Plant Hormones
Kidney	Plant Hormones	Kidney Dissection	" " & Behaviour
Behaviour	Plant Hormones	Genetics	None
Kidney	Genetics	Kidney	Plant Hormones
Unicellular Organisms	Plant Hormones	Behaviour	Gas Exchange & Genetics
Behaviour	Genetics	None	Unknown Specimens
Gas Exchange	Chemical Basis	Gas Exchange	Photosynthesis (Didn't work)
Behaviour & Plant Hormones	Kidney	Chemical Basis	Unicellular Organisms (Modify it)
Chemical Basis	Unknown Specimens	-	-
"	Behaviour	Genetics	Kidney
Photosynthesis	Behaviour & Plant Hormones	Chemical Basis	Behaviour & Plant Hormones
Kidney	Unknown Specimens	Kidney	Unknown Specimens
-	Photosynthesis & Behaviour (Grasshoppers)	All the same	
Gas Exchange	Behaviour (Grasshoppers)	Behaviour (Grasshoppers) (Research)	Plant Hormones & Photosynthesis (Didn't work)
Kidney	Genetics	Gas Exchange	None
Chemical Basis	Plant Hormones	Cell Structure	Plant Hormones & Unknown Specimens

(Cont.)

Most-Liked Lab	Least-Liked Lab	Lab of Most Benefit	Recommend Drop Lab(s)
Kidney	Behaviour	Kidney	Unknown Specimens
Cell Structure	Genetics	Kidney	Films
Chemical Basis	Kidney	Genetics	None
-	Behaviour	-	-
Behaviour	Plant Hormones	Genetics	None
Chemical Basis	Genetics	Cell Structure	Plant Hormones
Behaviour	Chemical Basis	Genetics	None
Gas Exchange	Cell Structure	Gas Exchange	Genetics
Cell Structure	Genetics	Unicellular Organisms	Behaviour
Gas Exchange	Genetics	Cell Structure	Behaviour
Gas Exchange	Unicellular Organisms	Genetics	Unknown Specimens
Gas Exchange	Chemical Basis	Kidney	Plant Hormones
Plant Hormones	Behaviour	-	None
All	None	All-by researching	None
Chemical Basis	Genetics	All-by researching	None
-	Plant Hormones	-	Plant Hormones
Behaviour	Behaviour (Grasshoppers)	-	Behaviour (Grasshoppers) & All 1st Term
Gas Exchange	Genetics	2nd Term Labs	Genetics
Gas Exchange	Genetics	All except Plant Hormones	Genetics & Behaviour

SUMMARY

	Most-Liked Lab		Least-Liked Lab		Lab of Most Benefit		Recommend Drop Lab(s)
1.	Gas Exchange	1.	Behaviour	1.	Kidney	1.	Plant Hormones
2.	Behaviour	2.	Genetics	2.	Gas Exchange	2.	Behaviour
3.	Chemical Basis } Kidney	3.	Plant Hormones	3.	Genetics	3.	Unknown Organisms

More information can be obtained from the questionnaire than is indicated by the summary alone. The following observations were helpful in planning modifications for the following years:

1. Modify the Unicellular Organisms lab but do not drop it.
2. The Behaviour lab was trivial.
3. The Behaviour lab was too abstract.
4. The use of films should be discontinued.
5. All labs are useful because of the research required for the report.

These observations were helpful in interpreting or clarifying the results of the questionnaire:

6. The Photosynthesis lab did not work. Failure such as this can be fixed whether it is equipment, instructional or technical.
7. One student did not enjoy handling the fruit flies.
8. In seven cases, students rejected only the grasshopper component of the Behaviour lab.
9. All of the labs which employed the microscope were enjoyable.
10. Nine students wished to retain all the units. None should be dropped.
11. There was a wide range of units mentioned as the "most useful lab". This reflects the variation of individual preference rather than a consensus.
12. One student did not enjoy working with live animals. Responses of this type indicate a personal bias which is not significant

for our purposes. However, it indicates that the choice of "least-liked" lab was not based on the quality of the instructional unit itself. Similar thinking was probably the basis of other responses as well.

CHAPTER VI

FINAL SUMMATION

The development of a laboratory course in Biology is essentially the development of a laboratory curriculum to accompany and to complement a larger course of Biology. As such it is not expected to remain static; rather, it is a viable and dynamic structure within which change and improvement are ongoing.

Of special importance in the laboratory environment is the relationship between students and demonstrators. Because the instruction and guidance of demonstrators is of paramount concern, the project stresses this area. However, the technical and administrative functions are also important and are also given consideration.

The primary purpose of the Biology 1H6 laboratory curriculum is to relate the theory of human physiology to the performance of physical activities including exercise. The assumption is made that understanding human physiology gives the students a better context for understanding everything they learn in the entire Physical Education program than would otherwise be possible.

The Biology 1A6 laboratory curriculum is designed to scan the whole spectrum of Biology in order to provide Natural Sciences students with a broad fundamental background in Biology. They can then make informed choices for future study. The curriculum seeks to provide students with a scientific perspective and a repertoire of basic laboratory skills.

Central to laboratory courses is the writing of the manuals of

instruction. For these courses, the laboratory manuals are designed to reflect specific educational goals. Their theoretical basis is founded upon the specification of objectives and an emphasis on the development of scientific competence through the development of the students' ability to hypothesize, analyze or theorize when confronted with a variety of different circumstances. The rationale for this approach is that people tend to forget the precise details of what they learn. However, when the processes of learning are stressed by the curriculum, these abilities will persist and may be adapted to a wide range of other intellectual problems (Eisner & Vallance, 1974). This is not to say that knowledge and facts per se are set aside by the curriculum. Rather, it indicates an emphasis on the process approach to teaching which requires students to think.

Individual units of the manuals use specific models of teaching. Each one incorporates the steps of procedure specified by the model itself. However, the use of models is not always intended to be a pure interpretation of theory. Adaptations are occasionally made to fit the model to specific teaching situations.

Because the definition of curriculum includes change and improvement, its evaluation becomes an important part of the process of curriculum development. In this project, evaluation examines the overall worth of the laboratory curriculum. The conclusion is drawn that the laboratory curriculum is a worthwhile reinforcement to lecture material but, more importantly, it provides valuable practical experience for students. Evaluation also questions whether or not educational goals are achieved. Examination of feedback indicates that

INTRODUCTION

Science seeks to discover facts about the universe, and to fit these facts into conceptual schemes which give order and meaning to them. Science can only deal with that which may be observed and tested. Whenever a scientific experiment is being conducted, it is important to run a "control" at the same time in order to compare the experimental with normal results.

The laboratory exercises in this manual will stress physiological and experimental approaches to the study of the systems of the human body. The material is oriented to practical applications of principles learned in lectures. i.e. to study living processes at work in the normal environment.

In order to make the most productive use of time spent in the laboratory, it is essential that students read over each exercise in advance. Textbook references to specific topics should be read, and lecture notes relevant to each lab should also be reviewed before coming each week.

Most of the labs require that students write a "report" which must be handed in to the demonstrator for marking by noon of the day one week from the time of the lab itself. LABS WHICH ARE HANDED IN LATE WILL NOT BE MARKED. Boxes are set up to receive these reports on the first floor of the Life Sciences Building a few yards to the south of the elevators. You must put your report in the correct box for your room and day and demonstrator. Otherwise it will not be marked and you will not receive credit for it. Be sure to put your name, your student I.D. number, your demonstrator's name and the day of your lab on the front page along with the title.

GUIDELINES FOR PREPARING WRITTEN REPORTS

There is a standard format for the writing of scientific reports which ensures a logical presentation of the information and a generally accepted style which avoids confusion in relating the information to others.

A report on a scientific experiment should be divided into 6 sections:

Purpose - A brief statement indicating what the experiment set out to achieve.

Introduction - The introduction is optional - when used, it orients the reader by giving background material which shows how the subject of the report fits into the general scheme of Biology. It supplies a framework for the outcomes you will be obtaining and defines the scope of the study.

Materials and Methods - This section of the report should briefly outline the way you carried out the experiment including a diagram (when possible) of the apparatus used. Usually the lab manual presents a complete description of the materials and methods used. If no changes have been made in the procedure, it will suffice to refer to the lab manual, but if changes have been made they should be described.

Results - Describe, using written sentences, precisely and concisely the results of the experiment. Be sure to include both quantitative (i.e., data) and qualitative results (i.e., observations).
Calculations, if required, should be made here and should be complete enough to show all the steps which were involved. The quality of scientific work depends greatly on the accuracy of the observations. Accurate observation is an extremely difficult art, requiring considerable practice. Data should be drawn up into tables and/or graphs so that the maximum information can be seen at a glance. Diagrams may also be very useful here, as they may be able to replace paragraphs of written description. There should be no discussion or interpretation of results at this time.

Discussion - This is the most important part of the report. The "discussion" is where students explain and justify their reasoning. It is where you will interpret the results obtained, and draw inferences from them if this is possible. Discuss the results in the light of their implications to living systems, based on background material such as readings from the text, from lecture notes, or from other references. This is where you answer specific questions asked during the course of the experiment, either by your lab manual or your demonstrator. Also, discuss briefly the reliability of your results - was the experiment soundly designed and executed? Suggest what sources of error may have existed and propose your ideas for improvement of the experimental design. (Remember to cite your references). Usually, experiments are separated into sections A, B, C, D, etc. For clarity, you should refer to the specific section being discussed. Use, and underline headings, rather than writing long and rambling general discussions.

Conclusions - Briefly formulate a statement of what the results of the experiment have told you in the light of your original purpose(s) and your understanding of background material. A conclusion should be a concise statement which emphasizes the most important results or generalizations.

General Recommendations

Length - Generally, lab reports should be no more than 3 pages in length, single spaced, excluding graphs and tables. Marks will be deducted for excessively long reports.

Calculations - All scientific measurements are in metric. Always include the units of measurement used eg.

mm	millimeters
ml	milliliters
kg	kilograms

When the outline asks for averages, this will refer to the mean average unless otherwise specified. "Variation" refers to the numerical variation or variance among data.
Remember that mathematical calculations have no more reliability than the initial values had eg. 7.2 × 9.8 is 70.6 rather that 70.56.

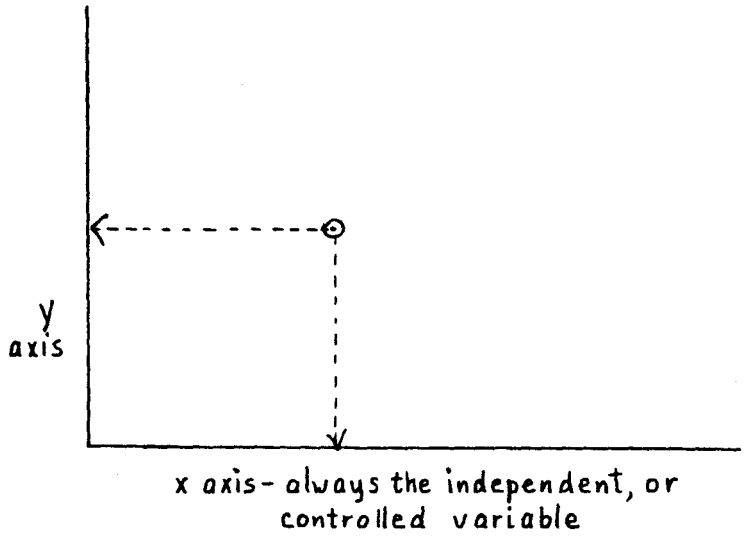
Organization of Data

There are 2 common methods of presenting quantitative data. One method is in the form of a table which contains an organized presentation of the numbers themselves, while the other is a pictorial form consisting of graphs or diagrams.

Tables are used when you have data that does not lend itself to graphic interpretation. When drawing up tables, use precise headings to explain the sources of your data. Each table must have an explanatory title.

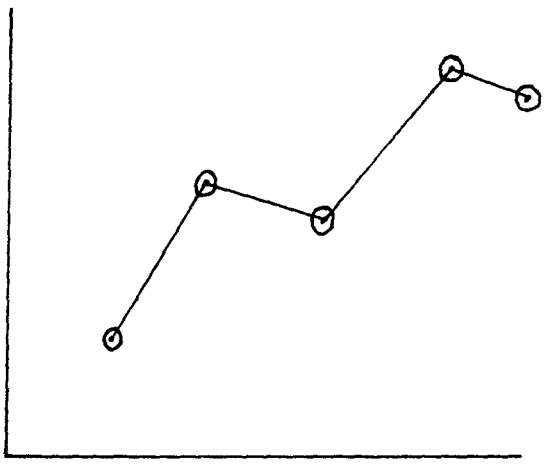
Graphs frequently show relationships more quickly than tables. Each graph must have an explanatory title and clearly labelled coordinates, with reference to the specific units used.

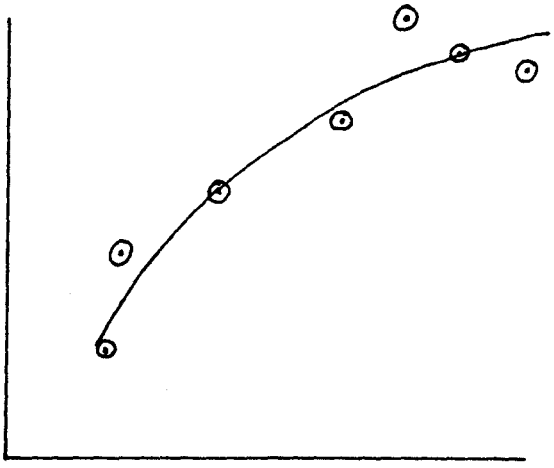
The dependent or experimental variable - changes are due to changes in the independent variable



Units may be chosen which best reflect the data which you wish to display. After the points have been located on the graph, it is customary to plot them as dots with a circle around each ⊙ . A straight line from point to point may then be made.

Frequently for our purposes, it is more graphic to draw the straight or curved "line of best fit". This line will fall uniformly through the midst of the points, not necessarily touching any of them.

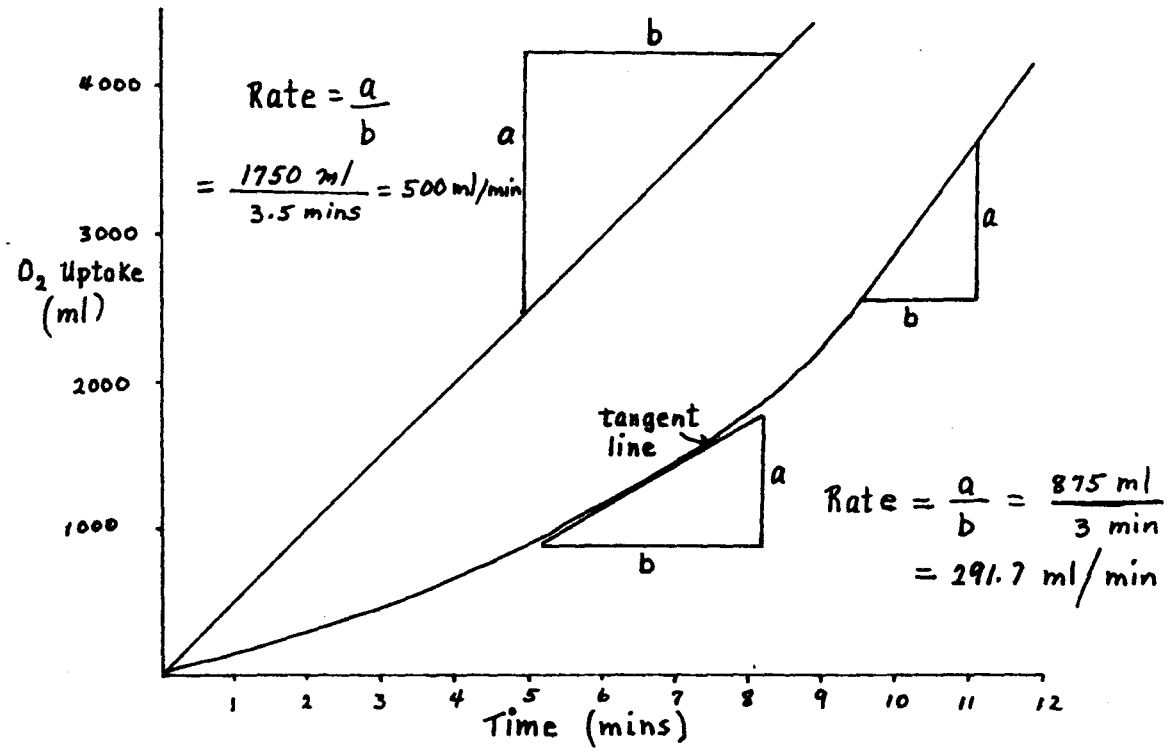




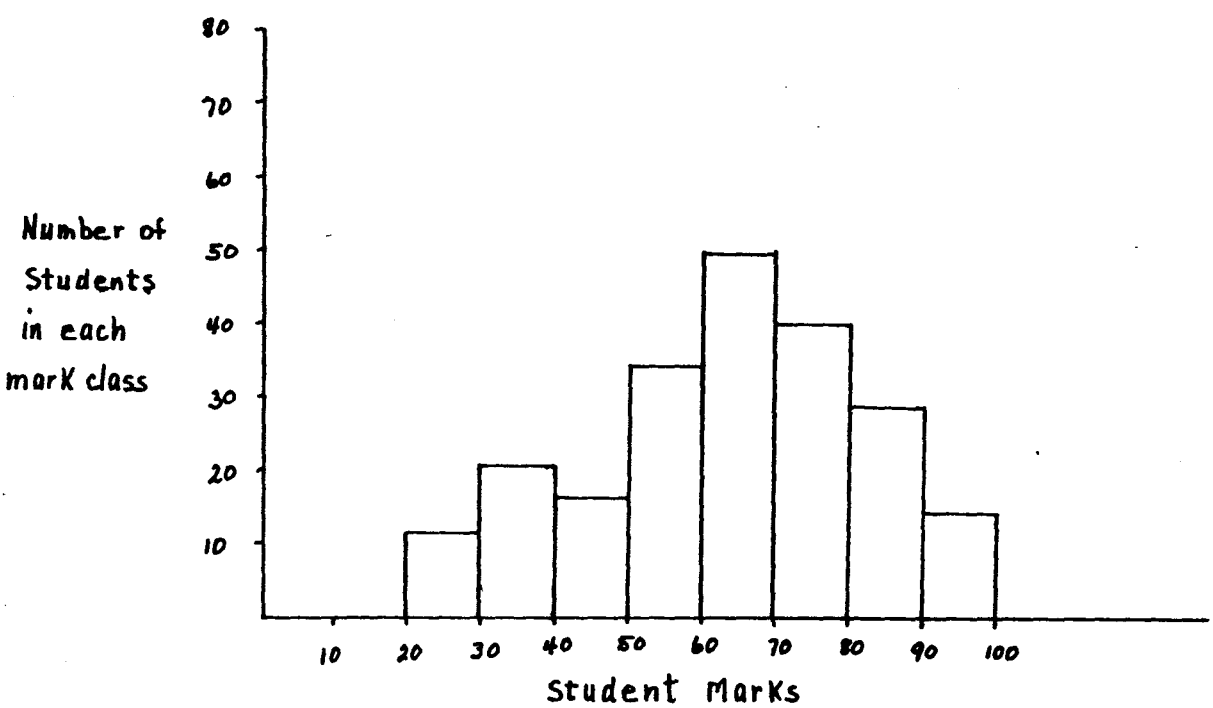
Determination of Rates from Graphs

You will frequently be asked to calculate the "rate of change" for a particular situation. This is easily accomplished by taking the slope of a line on a graph, and should be done on the graph itself. Intercepts are drawn to form a triangle and labeled as 'a' and 'b' respectively. The values of the intercepts may be used to calculate the rate

directly, as in this example. If the data does not give a straight line, one may draw a tangent to the curve at the specific area of interest, drawing intercepts from the tangent.



Histograms are useful to show the breakdown of data into groups or classes.



The Spectrophotometer

A test tube containing a red substance e.g., cyanmethemoglobin appears as it does because the light which enters the tube is absorbed by the contents, except for the red part of the spectrum which is reflected. A clear solution allows almost all light to pass through. A very dark solution absorbs almost all of the light.

The spectrophotometer is an instrument which measures quantitatively how much of the light is absorbed (readings on the absorbance scale) in comparison to a clear solution, and how much of the light is able to pass through (readings on the transmission scale).

The light inside the spec 20 shines through a filter which can be adjusted to control the colour (wavelength) of the light before it passes through the sample. Upon emergence from the sample, the light falls on a light-sensitive phototube, producing an electric current proportional to the amount of light striking it. Readings are always taken by comparison with a tube of clear water or the appropriate solvent of the test material. This tube is called the blank.

When reading a sample, follow these steps:

1. Turn the power switch knob to "on" and allow 10 minutes warm-up time. See #1 on diagram, next page.

2. Adjust to the desired wavelength in nanometers (nm), using knob #2, on diagram.

3. With the sample chamber #6 empty and its cover closed, use the power switch knob to zero the machine to infinity absorbance (∞) i.e., a shutter blocks all light from the phototube. See #3.

4. Fill a spectrophotometer tube halfway with water, or the appropriate solvent if other than water. Wipe this "blank" free of moisture and fingerprints with a tissue. Insert it into the sample holder #4 and close the cover.

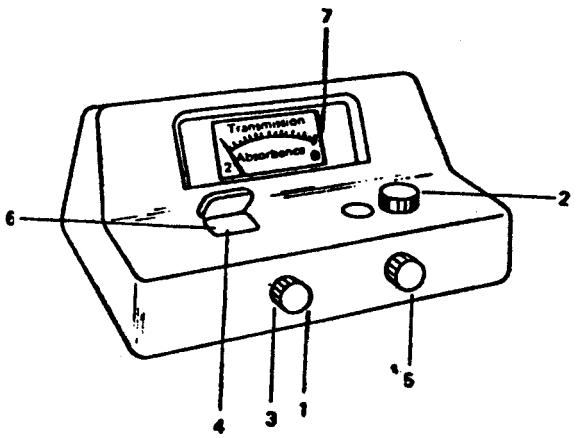
5. Use the lower right-hand knob, #5, to adjust the meter to read zero absorbance. Remove the blank, but keep it available to check or readjust the zero setting.

6. Fill, wipe clean and insert the sample tube, closing the cover. See #6.

7. Read the absorbance directly from the meter. See #7.

8. Over time, the settings tend to drift slightly. One should reset the infinity absorbance and zero absorbance for each new set of readings, or whenever the wavelength is changed.

OPERATION OF THE SPECTRONIC 20



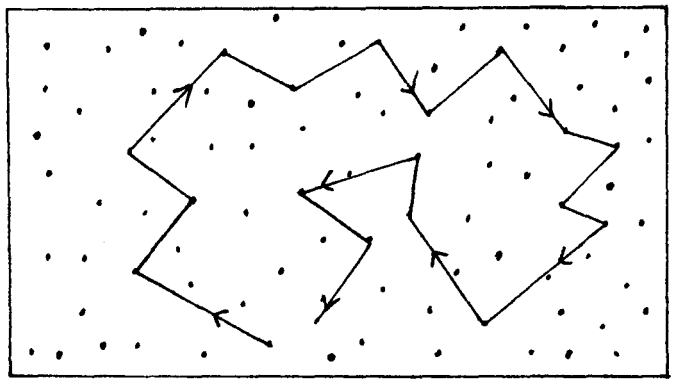
Lab 1 Diffusion and Osmosis

Objectives:
- to understand the processes of diffusion and osmosis.
- To observe the processes of diffusion and osmosis in order to apply them to living systems.
- To observe the osmotic fragility of cells and to explain the results of placing cells into salt solutions of varying concentrations.

A) DIFFUSION

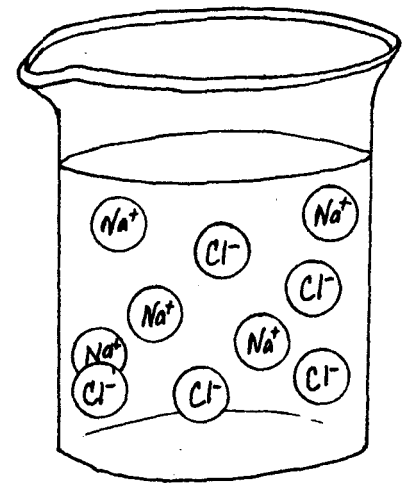
DIFFUSION IS THE SPONTANEOUS PROCESS BY WHICH MOLECULES OR IONS MOVE FROM A REGION OF HIGH CONCENTRATION TO A REGION OF LOW CONCENTRATION AS A RESULT OF RANDOM MOVEMENTS DUE TO THEIR INTERNAL KINETIC ENERGY.

Diffusion is the most common form of molecular movement within a living organism. During this random motion, molecules or ions bump into other molecules or ions millions of times each second, bouncing off each other in all directions. Different molecules may move at different rates depending upon temperature, concentration difference, molecular size and molecular weight.



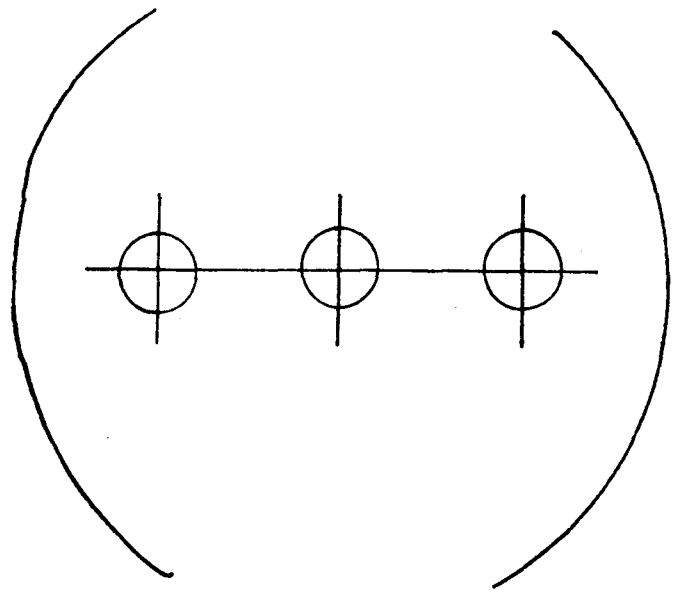
DIFFUSION OF A FLUID MOLECULE DURING A FRACTION OF A SECOND

When in solution, some molecules dissociate into separate ions, and both ions move according to the laws of diffusion, eg $NaCl \rightarrow Na^+$ and Cl^-



SOLUTION OF NaCl IN WATER

1. Using a small test tube, practice boring wells in the agar of the Petri dish. Use the boring guide below (yes, we know, the whole lab guide is boring). Having satisfied yourself that you can do it competently, bore out a second Petri dish exactly as per the boring guide.



2. Put 8 drops of 1N silver nitrate ($AgNO_3$) in the centre well.

   Put 8 drops of 1N potassium ferricvanide ($K_3Fe(CN)_6$) in the left hand well.

   Put 8 drops of 1N common salt solution (NaCl) in the right hand well. Cover the Petri disn and put it aside carefully, so the solutions do not spill over, while you continue with parts B and C. Periodically, inspect the Petri dish.

3. Ions will diffuse out into the agar from each of the 3 wells. Visible precipitates will form where silver ions (Ag^{++}) from the central well meet with $Fe(CN)_6^{\equiv}$ ions from the left hand well and Cl^- ions from the right hand well. Carefully inspect the interface areas. Colour is of no importance here. Record the <u>relative</u> position of the precipitates in a drawing. For our purposes we will compare the diffusion rates of the $Fe(CN)_6^{\equiv}$ and Cl^- anions. Measure the distance from the $K_3Fe(CN)_6$ well to the precipitation front and from the NaCl well to the precipitation front.

   What can you conclude about the rates of diffusion of these two ions based on the relative positions of the precipitates?

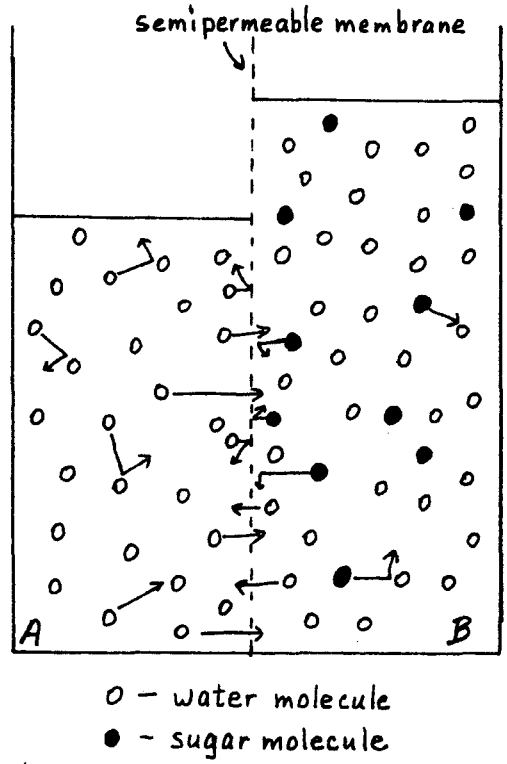
Can you say anything about the rate of diffusion of the Ag^{++} ions? Why? Interpret the difference in rate of diffusion.

B) OSMOSIS

OSMOSIS IS A SPECIFIC TYPE OF DIFFUSION--THE DIFFUSION OF WATER ACROSS A DIFFERENTIALLY PERMEABLE (SEMIPERMEABLE) MEMBRANE.

Water is normally the solvent through which this diffusion of water occurs. It diffuses from an area of high concentration of water to an area of low concentration of water.

Let us consider a system where pure water is on one side of a differentially permeable membrane, and a sugar and water solution is on the other side. The sugar solution B contains water molecules and also sugar molecules which are too large to pass through the pores of the membrane. Consequently there are more water molecules per unit volume in A than there are in B - i.e. water is more concentrated on side A. Therefore, more water molecules per unit time will bump into the membrane on side A than on side B. As a result, more water molecules will be able to pass through the membrane from A into B than will be able to pass from B into A.



This agrees with our earlier generalization that water diffuses from an area of high concentration of water (A) to an area of lower concentration (B). As a result of this net movement of water from A to B the volume of fluid on side B will increase and on A will decrease. By convention, however, we usually discuss osmosis in terms of the effects that the dissolved solute (in this case sugar) has on the kinetic activity of water molecules. The greater the concentration of solute in the solution, the less the kinetic activity of the water molecules in that solution. Thus, water will tend to diffuse by osmosis from a region of low solute concentration (high water) to a region of high solute concentration (low water).

1. Dialysis bags are artificial semi-permeable membranes, freely permeable to water, less so to many other substances. They are difficult to work with when they dry out, so keep them moist while setting up this experiment. Tie a tight knot in the end of four dialysis bags.

2. Using a 10 ml pipette, work quickly to fill the bags as follows:

   Bag 1 - 15 ml H_2O

   Bag 2 - 15 ml 20% sucrose (sugar solution)

   Bag 3 - 15 ml 40% sucrose

   Bag 4 - 15 ml 60% sucrose

After each bag has been filled, expel all trapped air by working it out with your fingers, and draping the unfilled end over the index finger to prevent reentry of air. Fold the top of the bag over one inch and tie securely with a piece of string. Use a knot code on the strings to identify the bags - 1 knot for bag 1, 2 knots for bag 2 etc.

NOTE: IT IS VERY IMPORTANT THAT ALL AIR BE REMOVED YET A MAXIMUM OF SPACE BE LEFT FOR CHANGE OF INTRA-BAG VOLUME. FOLLOW THE DEMONSTRATORS INSTRUCTIONS CAREFULLY.

3. Measure the volume of each bag by submerging it in water in a graduated cylinder and accurately recording the displacement volume.

4. Place all four bags in a water-filled sink simultaneously.

5. At 15 minute intervals, briefly remove each bag in turn, gently wipe off external water, and measure the volume in the graduated cylinder as before. Record this value and re-submerge it in the sink. Continue for 4-5 intervals (Part C can be performed during these intervals).

6. On a single graph, plot the volume of each bag versus time. Refer to page #4 concerning graphs.

   Do you expect straight lines? Explain.

   Can you calculate the rate of osmosis from these graphs?

How does rate of osmosis vary with solute concentration?

What would happen if the bags were left indefinitely?

Is hydrostatic pressure of any importance here?

C) OSMOTIC FRAGILITY OF CELL MEMBRANE

The membrane of the red blood cell is a natural differentially permeable membrane rather similar to the dialysis bag. As water enters from the outside, the erythrocyte swells until it bursts. The haemoglobin spills out, and the empty membrane (ghost) sinks to the bottom. This phenomenon is known as haemolysis. Alternatively, if water leaves, the red cell shrinks. Haemolysis can be easily detected because dilute suspensions of intact red blood cells are turbid. After haemolysis has occurred the suspension becomes transparent.

1. Fill four test tubes as follows:

   Tube 1 - 2 pasteur pipettes full of 1/18 M NaCl

   Tube 2 - 2 pipettes full of 1/13 M NaCl

   Tube 3 - 2 pipettes full of 1/6 M NaCl

   Tube 4 - 2 pipettes full of 1/2 M NaCl

2. Add 2 drops of blood to each tube and mix.

3. Immediately, and again after 15 minutes, try to read the print on white paper held behind the tubes.

4. In which tubes did haemolysis occur?

   What happened in the other tube(s)? Explain.

   Which solution is closest to the normal osmotic pressure of blood plasma?

   What occurs when red blood cells are placed in:
   a hypotonic solution? Why?
   a hypertonic solution? Why?
   an isotonic solution? Why?

Lab II Neurophysiology I - Reaction Time

Objectives:
- to determine simple reaction time for a single stimulus using the dominant hand, or the non-dominant hand.
- to determine complex reaction time for two different stimuli, each calling for a different response.

The capacity to respond to stimuli is a universal characteristic of all life. A stimulus-response sequence is known as a reflex, and the pathway mediating this reflex is known as the reflex arc. The components of a typical reflex arc are:

receptor
afferent pathway
integrating center
efferent pathway
effector

Receptors may be specialized cells in intimate contact with nerve cells, or they may be portions of nerve cells themselves. In general, each type of receptor is responsive to a particular kind of stimulus and will not respond to other types of stimuli. A stimulus is some sort of a detectable change in the environment, which acts upon the receptor to alter the signal being emitted. This new signal is the information which is sent by the afferent pathway to the integrating center for processing. The integrating center is continuously receiving input from many receptors with all manner of messages. It must process, filter and integrate these messages simultaneously. When it sends out a response by efferent pathway, this response represents a summation of all the diverse bits of information which had been received. The effectors which make the eventual change are generally either muscles or glands.

The reflex arc may be a simple pathway, as for example the knee jerk reflex where the stimulus travels only to the spinal cord and back, or it may be much more complex, involving the spinal cord, the brain and/or hormonal glands and secretions, depending upon the nature of the reflex.

Reaction time is the latency between a stimulus and the response to that stimulus by an effector. Simple reaction time is the latency between a fixed response and a fixed stimulus Complex reaction time is a measure of the response latency when two or more stimuli must be discriminated and responded to differently.

Students will be working in teams of four, and should perform each of the following functions in rotation:

a) Subject - will be seated in front of the reaction time board (the smaller box).

b) Presenter - will be seated opposite the subject, and will choose and present the stimulus by pressing the contact key on the larger board, simultaneously activating the timer.

c) Recorder - records the latent response time for each trial to 0.01 seconds, and resets the clock for the next trial.

d) Observer - watches the others to ensure the procedure is correct, and assists when needed.

A) SIMPLE REACTION TIME

1.) Begin by using the white light as the stimulus. The Presenter should say "Ready" and present the stimulus between 1 and 4 seconds later at random. Each subject should be given 5 "training" presentations and 15 "test" presentations.

The subject will place the index finger (the Peter Pointer next to Tom Thumb) of his dominant hand (right hand of right-handed people, left if left-handed) on the contact key which is to be pressed as rapidly as possible after the stimulus is presented, thereby breaking the circuit and stopping the timer.

The recorder should record all the response times. The average of the 10 fastest responses should then be determined and taken as that subject's reaction time.

Allow 2 minutes rest for everyone.

2.) Repeat Trial 1 using the red light as stimulus. The subject should use the index finger of his or her non-dominant hand.

Take 2 minutes rest.

3.) Repeat Trial 1. using an auditory stimulus. The subject should either use a blindfold or close his or her eyes and should use the non-dominant hand to respond to the buzzer.

Take 2 minutes rest.

B) COMPLEX REACTION TIME

1.) Repeat Trial 1. using two visual stimuli, calling for two different responses:

White light - use dominant hand.
Red light - use non-dominant hand.

In determining complex reaction time, the stimuli should be presented randomly at the discretion of the presenter. Again, each subject should be given 5 "training" and 15 "test" presentations.

Take 2 minutes rest.

2.) Repeat Trial 1. using one visual and one auditory stimulus calling for two different responses:

White light - use dominant hand.
Buzzer - use non-dominant hand.

Repeat this procedure for each member of the team.

Calculate the mean averages for each of the five trials.

Record your data on the blackboard. Take down the class data for comparison.

What are the components of the reflex arc for a simple visual reflex? For a complex reflex requiring discrimination?

What are the components of the reflex arc for the auditory stimulus?

Was there a difference in latent response time between individual trials? If so, interpret these differences.

Did your data concur with the class data?
If it did not, explain why.

Lab III Neurophysiology II - Sensory Function

Objectives:

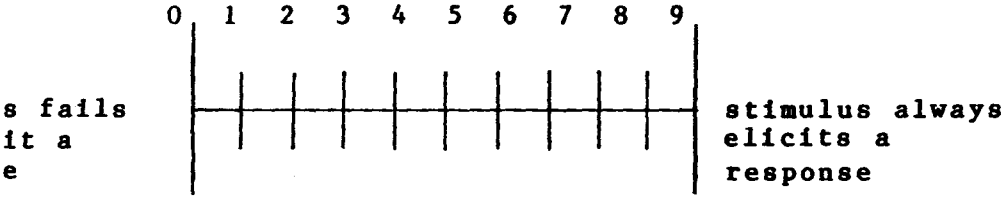
- to compare and quantify the tactile sensitivity of different regions of the body.
- to explore the ability of individuals to detect separation between two points on various areas of the body.
- to test colour perception.
- to map the areas of the tongue which are sensitive to sweet, sour, salty and bitter substances.

DO NOT HURRY ANY OF THESE PROCEDURES. ALTHOUGH THEY ARE SIMPLE, CONCENTRATION IS IMPORTANT; SO ARE ADAPTATION AND FATIGUE.

A) TACTILE SENSITIVITY - Work in pairs.

The sense of touch is a complicated system involving activation of receptors in the joints as well as receptors under the surface of the skin. Although very little is known for certain about the mechanism, we do know that there are a great many different kinds of receptors in the skin, and that sensitivity varies considerably from one point to another on the body surface.

We will be using von Frey hairs to determine tactile sensitivity. These are bristles of graded diameters and hence of graded tensile strength which are mounted on a moveable wheel. The force required to bend any one of these hairs is thus proportional to its diameter. Individual ability to detect a touch stimulus ranges over a continuum between feeling nothing, and feeling everything.



The absolute threshold of a stimulus is considered to be that value at which a stimulus is perceived 50% of the time. Each pair of students will act in turn as Subject and as Presenter/Recorder. The subject should either keep his or her eyes closed or be blindfolded throughout. This is standard procedure whenever a subjective assessment is required.

Using a felt-tipped pen, make a dot on each of these areas:

a) tip of index finger
b) heel of the hand
c) back of the hand
d) the inside surface of the forearm
e) forehead
f) small of back (if the subject agrees)

Stimulate the touch receptors of each area by gently pressing Hair #9 against the skin with just enough force to bend it. When the stimulus is felt, the subject should say "yes" and identify the area stimulated. Randomly, test each area ten times with each of the 9 hairs, recording whether the response was "yes" or "no", i.e., when the subject failed to detect the stimulus. Vary the interval between presentations over a range of 4 seconds. Find the hair which produces a 50% "yes" response and record the diameter of the hair as a measure of the threshold for that area. Find the threshold for each of the areas marked.

Does the absolute threshold for tactile stimulation vary over the regions of the body which were tested?

In physiological terms, explain why this is so. Record your results on the blackboard.

Does your data compare with that of the rest of the class?

Why or why not?

B) TWO POINT THRESHOLD - work in pairs.

The two-point threshold, is the distance two points of pressure on the skin must be apart, in order to be felt as two distinct sensations. The instrument we use to determine two-point threshold is the esthesiometer. It has 2 identical pressure points, the distance between which can be varied and read on a vernier scale.

Both members of each pair will act in turn as Subject and as Presenter/Recorder. The subject should keep his or her eyes closed or be blindfolded throughout.

Sterilize the pressure points of the esthesiometer and dry them. Use the same areas for testing as were used in Part A for tactile sensitivity, but also include the tip of the tongue.

a) tip of index finger
b) heel of hand
c) back of hand
d) inner surface of forearm
e) forehead

f) small of back (if the subject agrees)
g) tip of tongue.

If not already done, mark a dot with a pen for each of the areas to be tested (except the tongue). The presenter should take care to have both points touch at exactly the same time. Test each area with 4 different separations of the pressure points; each area should be tested 10 times. The distance between the points, and the areas to be tested should be randomly varied. At each test, the subject reports whether 1 point or 2 points are felt. The procedure should not be hurried if good results are to be achieved. A "one" response means that the separation is below threshld; a "two" response means that it is above. Find the separation for each area which produces a 50% "two" response, and record this distance as the threshold separation for that point. Record your results on the blackboard and copy down the other class data.

Is there variation in the two-point threshold at different areas of the body surface? Discuss this.

Is the direction of stimulation important (e.g, "along" the arm as compared with "across" the arm)?

Are practice, fatigue and concentration important? Explain.

Are your results consistent with the class results?

If they are not, explain why.

C COLOUR VISION - Work in pairs.

Not all people are able to detect all the frequencies of light in the visible spectrum which are perceived as colours. Many colourblind people are unaware of their defect because they are able to make such skillful use of their remaining colour discrimination, combining it with the learned colours and colour names of familiar objects.

The test cards for colour blindness should be left face down on the bench after use. Each card has a number (1 to 15) on the back, which has no relationship to the patterned number on the face of the card. Both members of each pair will act in turn as Subject/Recorder and as Presenter.

The presenter will shuffle the cards into random order without looking at their faces. He or she will present card #1 first as an orientation card. Everyone should be able to see #1 clearly. The subject should record the face or pattern number which he sees, opposite #1 on the record sheet. The presenter should hold each card about 30 inches from the subject and should hold it steady - for not more than 5

seconds. As the presenter calls out the number of the card, the subject silently records the pattern number opposite the number of the card. Do not record them sequentially or you will be unable to compare numbers with the master card. Check your results. Incorrect responses to 3 or more cards indicate defective red/green vision.

Record your results with the class data i.e whether your vision was N-normal or C.B. colourblind.

What is the physiological basis of normal colour vision? Of colourblindness? What is the likelihood of your being colourblind?

D) TASTE - Work in pairs.

Taste sensations are traditionally divided into four basic groups: sweet, sour, salt, and bitter.

There is some evidence that taste receptors are tuned to respond more strongly to some chemicals than to others, but most receptors will respond to all of the substances.

Four beakers containing the test solutions have been set up on each bench. They contain very small pieces of filter paper which are to be applied to various regions of the tongue - tip, sides, middle and back - with the forceps. Sterilize the forceps in alcohol berore use, and dry them.

Both members of each pair will act as Subject and Presenter/Recorder in turn. Because a subjective response is called for, the subject should close his or her eyes or be blindfolded. DO NOT DRINK THE SOLUTIONS.

The subject protrudes his or her tongue. It is essential that the tongue be dry in order that the stimulus not be diluted with saliva. Before each application, the presenter should blot the tongue dry with a piece of filter paper. A square of filter paper from each solution should be applied to the various regions of the tongue by separate trials and in random order.

Record the response for each

a. The sour solution is a very dilute sulphuric acid
b. The sweet solution is sucrose
c. The salt solution is sodium chloride
d. The bitter solution is phenylthiocarbamide (PTC) phenylthiourea.

About 30% of people are unable to taste (PTC) regardless of its strength. The other 70% perceive a strong bitter taste

which may last for hours. This "trait" of taste-blindness has a genetic basis; it is due to a single pair of alleles which are inherited according to Mendelian laws. There is no advantage to a person either to have this trait or to lack it.

Which area of the tongue is best able to detect the presence of the solution ?

Since there is no obvious difference in the specificity of the taste receptors, how do you think the different qualities are perceived?

Report your findings of "tongue-mapping" on the board along with your PTC response.

Compare your findings with those of the class.

What percentage of the class is taste-blind?

Sub-ject	Tactile Sensitivity						Two Point Threshold							Colour Vision	Taste				PTC
	a	b	c	d	e	f	a	b	c	d	e	f	g		a	b	c	d	

Lab IV The Blood

Objectives: - To observe the various compartments of blood.

- To perform some simple clinical tests of blood status.

- To demonstrate the degree of variability inherent in blood within a normal population.

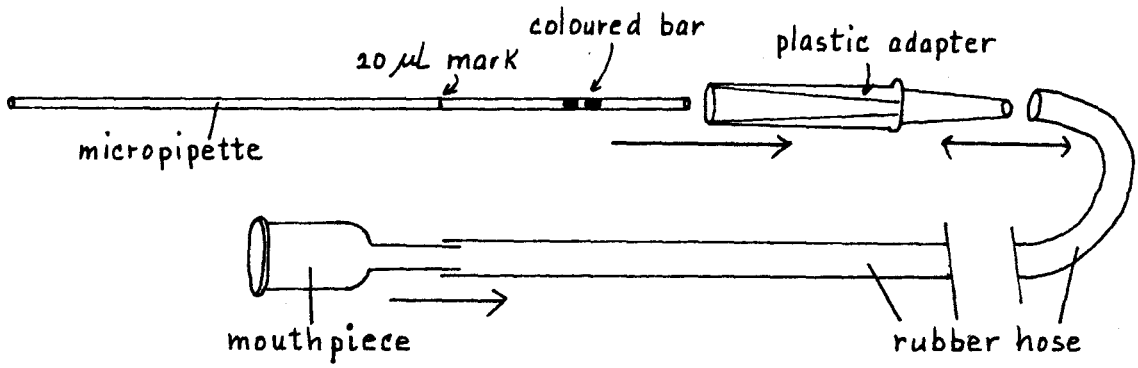
A SEPARATION OF BLOOD INTO PHASES BY CENTRIFUGATION AND DETERMINATION OF HAEMATOCRIT

1. Make a finger puncture on your partner with the lancet, as shown by your lab demonstrator.

2. Wipe off the first drop of blood with a piece of sterile gauze and let several more drops accumulate.

3. Take a heparinized "micro-hematocrit" capillary tube - it is the small glass tube without any markings. Holding the tube at a 45° angle, touch it to the drop, allowing the tube to fill three-quarters full by capillarity. (NOTE: Heparin is an anticoagulant.) Cover the upper end of the tube with your index finger to prevent the blood from leaking out. Jab the lower end into the piece of plasticene at your bench several times in order to plug the tube. It is important that you have a good solid plug of plasticene in the end of the tube to prevent loss of the sample during subsequent centrifugation.

4. Assemble the 20 µL micropipette according to this diagram.



Insert one end of the rubber hose over the end of the mouthpiece. Fit the other end over the smaller end of the plastic adapter. Insert the micropipette (the end with the coloured bar) firmly into the shaft of the plastic adapter.

5. Allow several more drops of blood to accumulate on your finger. Touch the micropipette to the drops and using the mouthpiece, draw up exactly 20 µL (to the black line). Transfer the blood into a test tube containing 5 ml of Drabkin's solution. Wash the contents out by drawing fluid up into the tube and blowing it back into the Drabkin's several times. Mix thoroughly. Set this tube aside for haemoglobin determination in B below.

6. Place your micro-hematocrit capillary tube in the centrifuge, plasticene plug to the outside. *(Note the position number of your own tube.) When the centrifuge is full, spin them at 10,000 rpm for 5 minutes.

7. Examine the contents of your tube carefully, as one of the compartments is extremely small.
How many phases are there?
What is the major component of each layer?
Draw a diagram to show the relative volume of each.
Explain why centrifugation was able to separate the phases.
Calculate the relative volume of each phase using a ruler or graph paper (1 division = 1 mm).
For example, the relative volume of erythrocytes (red blood cells) is the haematocrit:

$$\text{haematocrit (\%)} = \frac{\text{height of packed erythrocytes}}{\text{total height of blood column.}} \times 100$$

B) DETERMINATION OF HAEMOGLOBIN CONCENTRATION IN BLOOD

NOTE: DRABKIN'S REAGENT IS POISONOUS. DO NOT UNDER ANY CIRCUMSTANCES GET IT IN YOUR MOUTH. WASH YOUR HANDS IF IT CONTACTS YOUR SKIN.

CYANMETHEMOGLOBIN TEST

The cyanmethemoglobin method has gained international acceptance as a clinical test for measuring blood haemoglobin concentration. In brief, the principle involved is that all normal forms of haemoglobin react with cyanide at alkaline pH (Drabkin's Reagent) to produce cyanmethemoglobin, a compound which absorbs light at a wavelength of 540 nm. Absorbance readings of the sample at 540 nm are therefore directly proportional to the concentration of haemoglobin.

1. Refer to the use of the "Spectronic 20" spectrophotometer on Pg. 6. Consult your demonstrator if you have any problem. To break a "spec 20" is to invite the death penalty.

2. A set of four standard solutions are beside each spec 20. Each corresponds to 20 μL of blood in 5 ml Drabkin's solution.

   Tube 1 contains blood with 0.0 gm haemoglobin/100 ml
   Tube 2 contains blood with 6.0 gm haemoglobin/100 ml
   Tube 3 contains blood with 12.0 gm haemoglobin/100 ml
   Tube 4 contains blood with 18.0 gm haemoglobin/100 ml

   Read the "absorbance" of these standard solutions on the machine using Tube 1 as the blank. On graph paper, plot a calibration curve of these absorbance readings (optical density) versus haemoglobin concentration.

3. Read the absorbance of your own blood sample which you prepared in A. From the graph, determine the haemoglobin concentration of your sample.

4. Demonstrators will draw up a chart on the black board to collect class data. Add your values as quickly as possible.

Student	Sex	Haematocrit	Haemoglobin Conc.	Blood Type
initials	M or F	%	[H] gm/100ml	A, B, AB, or O

5. Copy down the class data. Using this data, answer these questions:

   Is there a correlation between haematocrit and haemoglobin?
   If so, why might this be?
   Calculate the mean values for males and females. Is there any difference between the two?
   Normal male haematocrits are approximately 42%
   Normal female haematocrits are approximately 38%
   If a person is anemic the haematocrit may drop as low as 10% or during polycythemia it may reach 65% or higher.
   Normal male haemoglobin concentrations are approximately 15 gm/100 ml.
   Normal female haemoglobin concentrations are approximately 14 gm/100 ml.
   Is there much variability in the class data?
   Illustrate this variability in a graph or table.
   What are the main causes of this variability?

C) DETERMINATION OF BLOOD TYPE

Human beings may be divided into four categories on the basis of their blood types - A, B, AB, and O. Red blood cells may contain antigens on their surfaces. When transfused into another person, these antigens may react with antibodies produced by the recipient's immune system.

People with blood type A have A antigens and produce anti-B antibodies.

People with blood type B have B antigens and produce anti-A antibodies.

People with blood type AB have both A and B antigens and therefore produce no anti-A or anti-B antibodies.

People with blood type O have no antigens but produce both anti-A and anti-B antibodies.

If the blood from an A person is transferred into a B person the recipient's anti-A antibodies would react with the A antigen of the donor's blood causing agglutination (clumping together). This clumping might block the blood vessels and could cause death. Similarly, if blood from a B person is transferred into an A person, the recipient's anti-B antibodies would react with the B antigen of the donor's blood causing agglutination.

1. Take a clean slide.

2. Lance your finger as before, and place a small fresh drop of blood at each end of the slide.

3. Immediately add <u>one</u> drop of anti-A serum (from group B donors) to one of the drops.

4. With a <u>clean</u> toothpick, mix the blood and serum together for several seconds. Observe closely to see if agglutination occurs.

5. Add <u>one</u> drop of anti-B serum (from group A donors) to the other drop, and using a <u>clean</u> toothpick mix the blood and serum together.

6. Did you get agglutination with the anti-A serum and/or the anti-B serum, or neither?

7. What is your blood type?

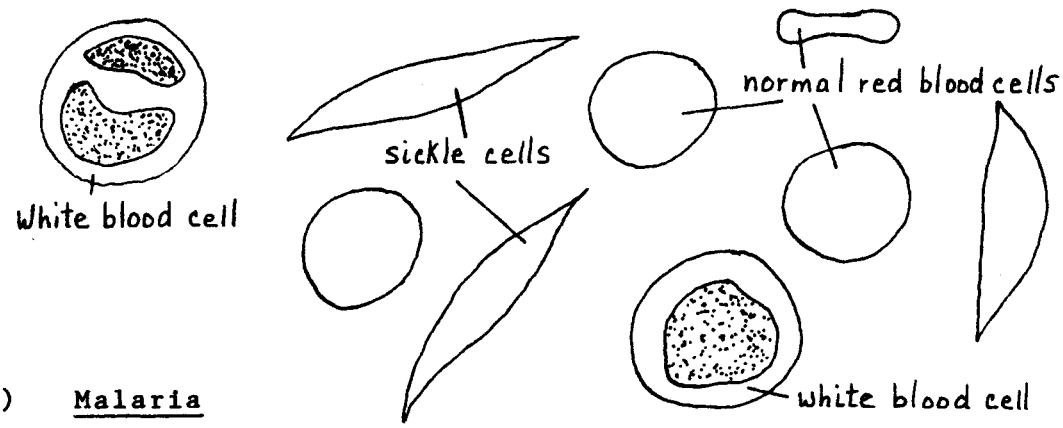
8. Is there a person of one blood type who could safely <u>donate</u> blood to all of the other three?

9. Is there a person of one blood type who could safely <u>receive</u> blood from all of the others?

D) <u>DEMONSTRATIONS</u>

1) <u>Sickle Cell Anemia</u>

This is a <u>genetic</u> condition very common among black people of African origin. Red blood cells contain an abnormal form of haemoglobin called Haemoglobin S. On exposure to oxygen it precipitates into long crystals, causing the cells to change shape from biconcave to sickle shaped. Such cells become very fragile and are easily ruptured causing severe anemia, impairment of circulation, and low oxygen tension in tissues.

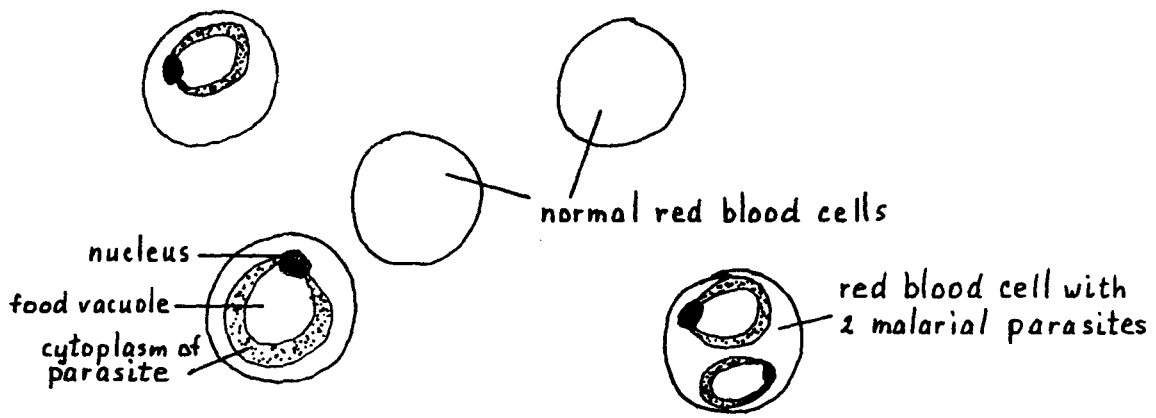


2) <u>Malaria</u>

Malaria is a tropical <u>parasitic</u> disease. Its most virulent form is "malignant tertian malaria" which is caused by the sporozoan <u>Plasmodium falciparum</u>. The parasite is transmitted to humans from <u>Anopheles</u> mosquitoes during a bite. In the human bloodstream, young parasites enter

red blood cells where they feed, taking on the appearance of a signet ring.

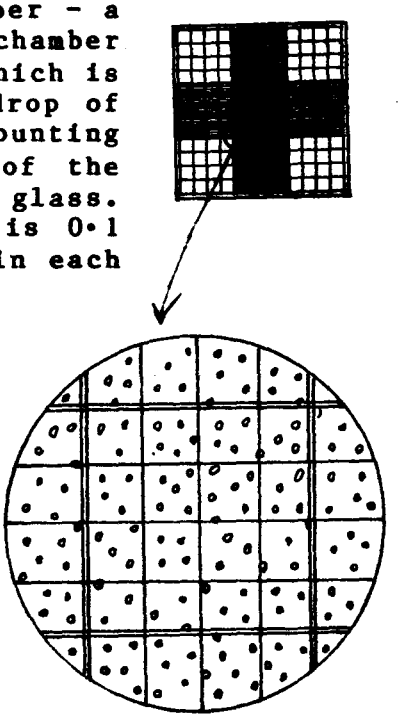
When stained, the parasite cytoplasm making up the "ring" appears blue. The nucleus which stains red, has the appearance of the "jewel" of the ring. While in the red blood cell the parasite feeds on the haemoglobin and when the host blood cell ruptures, hemozoin (a breakdown product from the metabolism of haemoglobin) and metabolic wastes are released into the blood stream, causing the characteristic fever symptoms of malaria.



3) Erythrocyte Counting Chamber - Haemocytometer

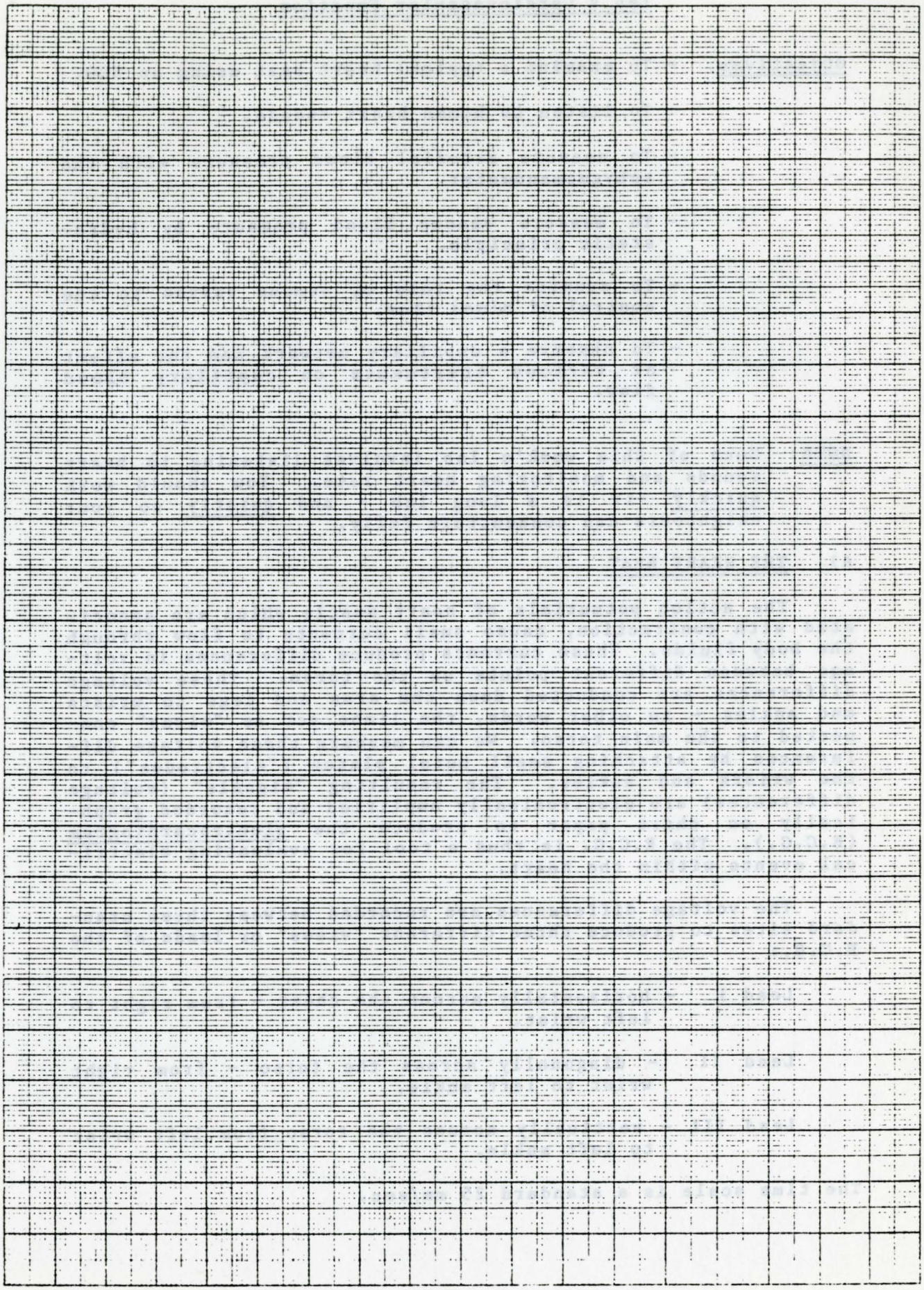
There are approximately 5 billion red blood cells in each ml of your blood. Each is a biconcave disc, thicker at the outside and thinner in the middle, and only 7 micrometers in diameter.

Red blood cell counts are determined by counting cells in a special counting chamber - a haemocytometer-under the microscope. The chamber contains a central area of one square mm which is subdivided into 400 smaller squares. A drop of blood was diluted 1 to 200 parts with counting fluid, then expressed onto the surface of the counting chamber, and covered with a cover glass. The depth of this film of diluted blood is 0·1 mm. Therefore the total volume of fluid in each of the smaller squares is 1/4000 cu mm.



The most established procedure is to count 5 sets of 16 squares within the central area of 1 square mm, preferably in each of the four corners and one in the centre. If we were to count 480 cells total in the 5 groups of 16 squares (5 × 16 = 80) and the dilution factor was 1:200 and the volume of fluid was 1/4000 cu mm. The cell count would be:

$$\frac{480 \times 200 \times 4000}{80} = 4{,}800{,}000 \text{ erythrocytes/cu mm}$$



Lab V Cardiovascular Function

Objectives:
- To examine a typical heart beat using an ECG.
- To listen to human heart sounds.
- To measure arterial blood pressure with the sphygmomanometer.
- To measure venous blood pressure by hydrostatic principle.
- To examine the role of venous valves in the control of blood flow.
- To observe a capillary network and the effect of various treatments on capillary blood flow.

NOTE: Much of this week's lab involves listening to heart sounds and monitoring heart rate. You should work quickly -it is a long lab - and quietly so your neighbours can concentrate fully.

A) THE HEART BEAT

The action potentials of heart muscle which are associated with contraction, cause small currents to flow through the body fluids. These currents produce differences in voltage between different points on the torso. These voltage differences are conducted down the arms and legs to wrists and ankles - in other words, the limbs act as "wires" connected to the main torso. We can measure these voltage differences by attaching small metal plates ("electrodes") to the wrists and ankles. The resulting "signals" (voltage differences) are electronically amplified and recorded graphically on chart paper to produce the electrocardiogram (E.C.G.). The E.C.G. is thus a tool for evaluating electrical events within the heart.

The voltage differences are recorded between three standard sites to produce three different "views" or leads of the E.C.G.:

Lead I - horizontally across the chest - from right to left wrist.

Lead II - diagonally across the torso - from right wrist to left ankle.

Lead III - vertically across the torso from left wrist to left ankle.

The time scale is a standard 25 mm/sec.

A sample E.C.G. recording will be made on a single subject and distributed to each student in the lab. On your tracing, label the P-wave QRS complex, and the T-wave. Explain the origin of each.

Calculate the heart rate in beats per minute from the inter-beat interval.

Can you detect any abnormalities in the E.C.G.?

B) HEART SOUNDS

Have your partner sit quietly for one minute. Using the stethoscope (some of them must be turned on) listen to his or her heart sounds.
Describe the sounds you hear.
What is their origin?
What is the heart rate in beats per minute?

C) MEASUREMENT OF ARTERIAL BLOOD PRESSURE

The arterial blood pressure is the lateral pressure (a force per unit area) exerted by the arterial blood flow on the vessel wall. You will measure this pressure by finding the appropriate opposing lateral pressure which just prevents the blood flow. Arterial blood pressure is constantly changing during the course of the cardiac cycle, the highest pressure being the systolic, and the lowest the diastolic pressure. The numerical difference between systolic and diastolic is the pulse pressure. A typical blood pressure is expressed as:

$$\frac{\text{systolic pressure}}{\text{diastolic pressure}} \qquad \text{eg. } 120/80 \text{ mm Hg}$$

In this case the pulse pressure would be 40 mm Hg.

1. Have your partner sit quietly. Place the compression cuff of the sphygmomanometer snugly on the upper arm such that the cuff is at the same level as the heart. Why?

2. Place the bell of the stethoscope tightly over the radial artery just below the cuff in line with the crook of the arm.

3. Inflate the cuff to a pressure of over 150 mm Hg and then deflate it slowly and evenly - a rate of 3 mm Hg per heart beat is about right. Note the pressure at which the "thud" sound is first heard. This is the systolic pressure and represents the vibration of the first blood flow to get through the almost totally occluded vessel.

4. Continue deflating the cuff at the same rate until the sounds may be clearly heard. They should be getting

louder. Measure the heart rate again in beats per min. Is it the same as in B? If different, explain.

5. Continue deflating the cuff until all sound has just disappeared. Record the exact point. It is the diastolic pressure.

DO NOT KEEP THE CUFF ON FOR MORE THAN 3 MINUTES AT A TIME. ALLOW THE ARM AT LEAST 2 MINUTES "REST" BETWEEN MEASURMENTS.

The mean arterial blood pressure may be calculated as:

$$\frac{2 \text{ diastolic} + 1 \text{ systolic}}{3}$$

Calculate the pulse pressure and the mean arterial blood pressure for your subject.

6. Repeat the above measurements immediately after the subject has performed 2 minutes of brisk running on the spot.
Is there a difference? Explain.

Note: DO NOT PERFORM THE RUNNING ON THE SPOT IF YOU ARE ILL, HAVE HIGH BLOOD PRESSURE, OR ARE TAKING MEDICATION.

D) MEASUREMENT OF VENOUS BLOOD PRESSURE

Venous pressure is much lower than arterial pressure and almost non-pulsatile. A crude measurement of venous pressure, which is often used by doctors, can be made by hydrostatic principle: i.e. by finding the depth (in our case height) of a column of blood which gives a negative pressure just equal and opposite to the venous pressure, thereby causing the veins to collapse.

1. Have your partner stand at the blackboard. Mark the point on the blackboard which is at the level of his or her right auricle - the zero pressure level of the circulation. If it will be of any help, this is the sternal junction of the 5th right costal cartilage.

2. Have your partner allow one arm to hang loose, muscles and hand relaxed until the veins are well distended.

3. Now you slowly raise his or her hand until a height is reached at which the veins just disappear, i.e. collapse. Mark this point on the blackboard and measure the vertical distance in mm between the points. This distance represents the depth of a column of blood which just opposes (is equal and opposite to) the venous pressure in the hand.

The specific gravity of blood is 1.055 and the specific gravity of mercury (Hg) is 13.55.

Calculate the venus pressure of the blood in the veins of your hand.

$$V.P. = \text{distance} \times \frac{1.055}{13.55} \text{ mm Hg}$$

How would you expect this figure to compare with the venous pressure in the right auricle? Why?

What happens to blood flow in the vein when it collapses? Draw a diagram.

E) THE VENOUS PUMP

Consider that 60% of the total blood volume is present in the systemic veins and that there is only a small non-pulsatile venous pressure tending to force it back to the heart. Also consider the hydrostatic pressure (weight of the blood) in the veins of a person who is standing still. This pressure may reach 90 mm Hg because of pooling and because of the distance between the feet and the heart. The body compensates for these factors by a pumping mechanism known as the venous pump, which works in 2 ways.

a) Every time a person moves his legs, the muscles tighten, compressing the veins, squeezing the blood out, and breaking up the column of blood into small segments.

b) One way valves located in the veins prevent backflow and propel the blood in the direction of the heart.

1. Have one partner relax and lower his or her arm. Then apply the rubber hose tubing tightly around the arm above the elbow (N.B.: REMOVE IMMEDIATELY IF ANY DISCOMFORT OR NUMBNESS IS FELT). Note the great filling and distention of the veins of the hand and forearm. Also note the localized swellings seen at intervals on the veins. These are the valves.

2. With one finger, press down on a vein in the lower part of the arm. With a finger of the other hand press the blood upwards beyond the next valve.
Are you able to mechanically empty the vein in this way?
Remove the second finger - the upper one. Does the vein refill from above?

Can you force the blood back downward from above with your second finger?

Watching closely, remove the first finger. From which end did the vein start to refill? Diagram and explain your observations.

3. Take off your shoes and stand still for 2 minutes. Relax the leg muscles. Notice how prominent the veins become. What kind of pressure causes this?
Now run on the spot 5 or 6 steps and re-examine the veins. Has muscle action had any effect?

F) THE CAPILLARY CIRCULATION

It is in the capillaries where the exchange processes between blood and interstitial fluid occur. Capillaries permeate every tissue of the body. The best area for viewing capillaries in humans is in the fingernail capillary bed. Indeed the first measurements of capillary blood pressure were made from the capillaries in the fingernail.

1. Scrub the skin covering the root of the fingernail thoroughly with soap, water and a stiff brush. (The idea here is to remove the outer dead keratin layer of the skin so as to increase the skin's transparency.) Dry thoroughly. Moisten the area with mineral oil. Examine the nail bed under the dissecting microscope. The demonstrator will help if necessary. Use brilliant illumination and take the magnification up to the highest power.

   Draw a diagram of the capillary loops as you see them.

2. Examine the capillary loops after plunging the hand into ice cold water, and again after plunging it into hot water. What did you observe?

Lab VI Respiratory Gas Exchange in the Mouse

Objectives:
- To solve a scientific problem by inquiry methods.
- To measure and compare the rates of oxygen consumption and carbon dioxide production of an animal under laboratory conditions.
- To calculate the Respiratory Quotient (R.Q.) of a mouse.

In a healthy animal, respiratory gas exchange through the lungs reflects the summated respiratory activity of all the cells of the body. It is the result of the coupled processes of aerobic glycolysis, the Krebs cycle, and oxidative phosphorylation at the cellular level which together comprise aerobic respiration. The gases (O_2 and CO_2) which move in and out through the lungs are transported between the cells of the tissues and the lungs by the bloodstream.

Aerobic respiration is the process whereby humans obtain the vast majority of their energy, at least when at rest, through the chemical breakdown of foodstuffs.

Respiratory quotient (R.Q.) is the $\frac{\text{volume of } CO_2 \text{ produced}}{\text{volume of } O_2 \text{ consumed}}$

The value of the R.Q. varies and is dependent upon the oxidation level of the food which is being metabolized. For example, the complete oxidation of glucose by respiration follows this equation:

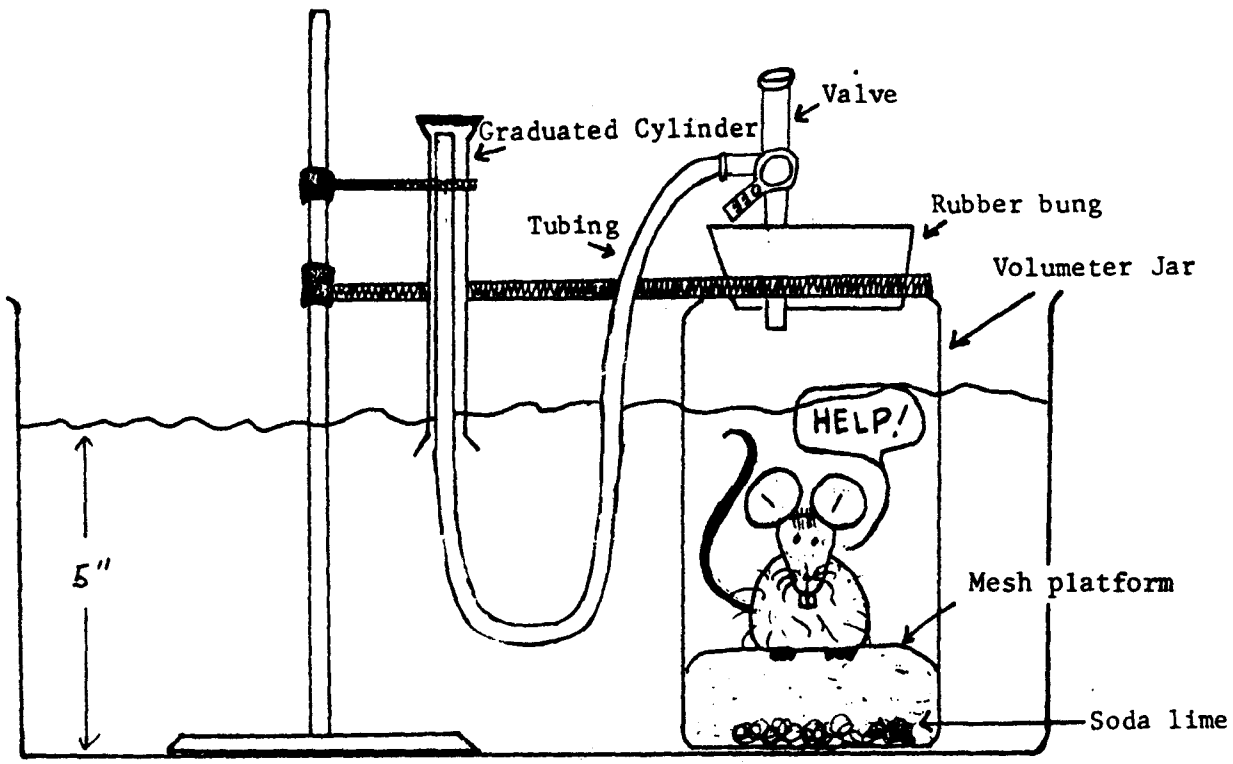
$$(CH_2O)_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

Since 6 moles of O_2 are consumed and 6 moles of CO_2 are produced for each mole of glucose respired, the R.Q. of glucose is 1.0.
Average R.Q. values for typical proteins are lower at 0.8.
Average R.Q. values for fats are lower still at 0.72.
The rate of respiration and the R.Q. are largely dependent upon the environment imposed on the cells by the organism as a whole. Thus they are affected by such factors as nutritional levels, activity level, body temperature, circulatory efficiency, and the production of endogenous agents such as the hormones thyroxine and epinephrine.

A. A PROBLEM TO BE ANSWERED BY INQUIRY

B. MEASUREMENT OF O_2 CONSUMPTION, CO_2 PRODUCTION AND RESPIRATORY QUOTIENT

Respiratory gas exchange will be measured with the volumeter system shown below.

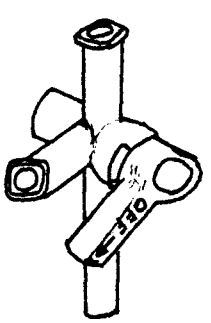


1. Fill the basin with water at approximately room temperature to a depth of about 5 inches.
Set up the inverted 10 ml graduated cylinder in the basin so that the water level is at about the 9 ml mark.

2. Place a packet of soda lime in the jar and then insert the mesh platform which will support the animal and protect it from the caustic effect of the soda lime.
N.B. THE SODA LIME MUST BE KEPT DRY.

3. Weigh the mouse by placing it in the container on the balance. CAUTION: MICE CAN BITE, SO HANDLE WITH CARE. Put the mouse into the jar.

4. Examine the 1-way valve in the rubber bung in order to understand how it works. Then <u>open it to the outside atmosphere</u>.



Close the jar by putting the rubber bung in place. It is important that the seal be airtight - wetting the edge of the rubber bung will ensure this.

5. Place the jar in the water-filled basin, holding it in place with the iron ring, and connect it via the tubing to the air trapped in the inverted cylinder. You will have to raise the cylinder to do this because water must not get into the tubing. If water should get in, blow it out with a jet of air. Be sure the tubing goes to the very top of the graduated cylinder.

6. Equilibrate the pressure within the chamber to the atmospheric pressure by moving the 1-way valve back and forth several times, so as to alternately connect the volumeter to the air pocket in the graduated cylinder and to the outside atmosphere. This procedure should bring the water level in the graduated cylinder approximately level with that of the outside basin.
Set the 1-way valve so that the volumeter jar is connected to the outside atmosphere and allow the system to stand for 5 minutes in order to:

   a) allow the animal to settle down
   b) let the temperature equilibrate throughout the system.
   c) allow the absorption of any excess CO_2 initially in the system.

7. Read and record the temperature of the water.

8. After the 5 minute period, repeat step 6 to ensure complete pressure equilibration. Now set the 1-way valve so that the volumeter jar is connected to the air pocket in the graduated cylinder and start timing.

   Take readings of the water level in the graduated cylinder every 15 seconds in order to determine the rate of O_2 consumption. Once the level begins to move, the whole process will probably take less than 3 minutes. At completion, open the valve to the atmosphere to prevent the tube and jar from filling with water, and the mouse from being asphyxiated. Record the readings you obtain in the table provided. From these values, calculate the change in volume (ΔV) and record cumulatively in the column labeled ΔV. ΔV must now be corrected to take into account the volume of the tube which occupied a portion of the space in the cylinder. For each ml of calculated air removed from the cylinder, the true volume of air removed was 0.7 ml because the tube occupied 0.3 ml. Apply the factor of 0.7 to the values in the ΔV column and record the new values as the "corrected ΔV" in the next column. Plot these data on the graph. Draw the straight line which best represents the data.

and calculate the slope of this line in ml/sec. Refer to page 5 at the beginning of the manual on "Determination of Rates From Graphs." This represents the O_2 consumption rate of the mouse. Finally, express it as ml O_2/gm of mouse body weight/hr.

9. What changes in the arrangement of the apparatus would allow you to measure the rate of CO_2 production? Give your reasoning.
Note: You may assume that the rate of O_2 consumption will remain at the level which you have measured.
Make the change and repeat the experiment to obtain a new series of readings which will allow you to calculate the CO_2 production rate. Record the readings and the cumulative ΔV and corrected ΔV values as before in the table. Plot these data on the same graph and determine the slope as before. From your data on the graph calculate the CO_2 production rate.

i.e. ml CO_2/gm of mouse body weight/hr.
Also calculate the respiratory quotient (R.Q.) for your mouse.

The soda lime packets may be reused if they are not wet. Please return them to the closed jar.

O_2 Consumption				CO_2 Production			
Time in seconds	Level of Water in cylinder	Cumulative Change in Vol (ΔV) in ml	Corrected ΔV in ml	Time in seconds	Level of Water in cylinder	Cumulative Change in Vol (ΔV) in ml	Corrected ΔV in ml
			0			0	0

Lab VII Fitness

Objectives:

- To determine whether changes in body position and muscle activity have a measurable effect on heart rate.
- To assess cardiovascular fitness by performance of the Schneider Test.
- To assess pulmonary fitness by performance of the Pulmonary Fitness Test.
- To determine by analysis of class data whether there are relationships between physical ability and physical fitness.

There are a variety of cardiovascular fitness tests available, the various merits of which are subject to considerable dispute amongst their respective proponents. Most are based on the following principles:

i) Fitness is positively correlated with low resting heart rate.

ii) Fitness is negatively correlated with the degree of elevation of heart rate after exercise or other disturbance, such as standing up.

iii) Fitness is positively correlated with rate of return of the heart rate to "rest" after exercise or disturbance

OXYGEN CONSUMPTION = HEART RATE × STROKE VOLUME × ART-VEN O_2 DIFFERENCE.

The interpretation of this equation is that, "the more fit the individual, the more heavily he or she relies on cardiac stroke volume and the art-ven O^2 difference to achieve or sustain any given level of oxygen consumption and the less he or she relies on heart rate."

In this laboratory we will be attempting to verify this statement. Since the tests rely heavily on the accurate measurement of true resting heart rate we would ask students to:

i) Not eat, smoke, drink coffee, tea or coke, or exercise unduly during the 1 hour (ideally 2 hour) period prior to the tests.

ii) Recline for 5 minutes before the test is begun.

Tests are to be performed in turn by both partners. The pulse rate may be taken from the wrist or by stethoscope.

A) INFLUENCE OF POSITION ON HEART RATE

Rapid changes in the position of the body can alter arterial blood pressure due to the effects of gravity on the rate of venous return. Such changes in arterial blood pressure will be detected by baroreceptors which can initiate reflex changes in heart rate.

1) One partner will lie on his or her back, relaxing, for 5 minutes. The other partner will then take a control pulse for 4 successive 20 second intervals recording each interval.

2) The subject then begins to stand up very slowly taking about 20 seconds in all, and moving the legs as little as possible. During the standing up period and for at least 6 successive 20 second periods, the partner should monitor and record the pulse rate. If the rates for the 5th. & 6th 20 second periods are not the same, continue counting until 2 consecutive 20 second counts are the same.

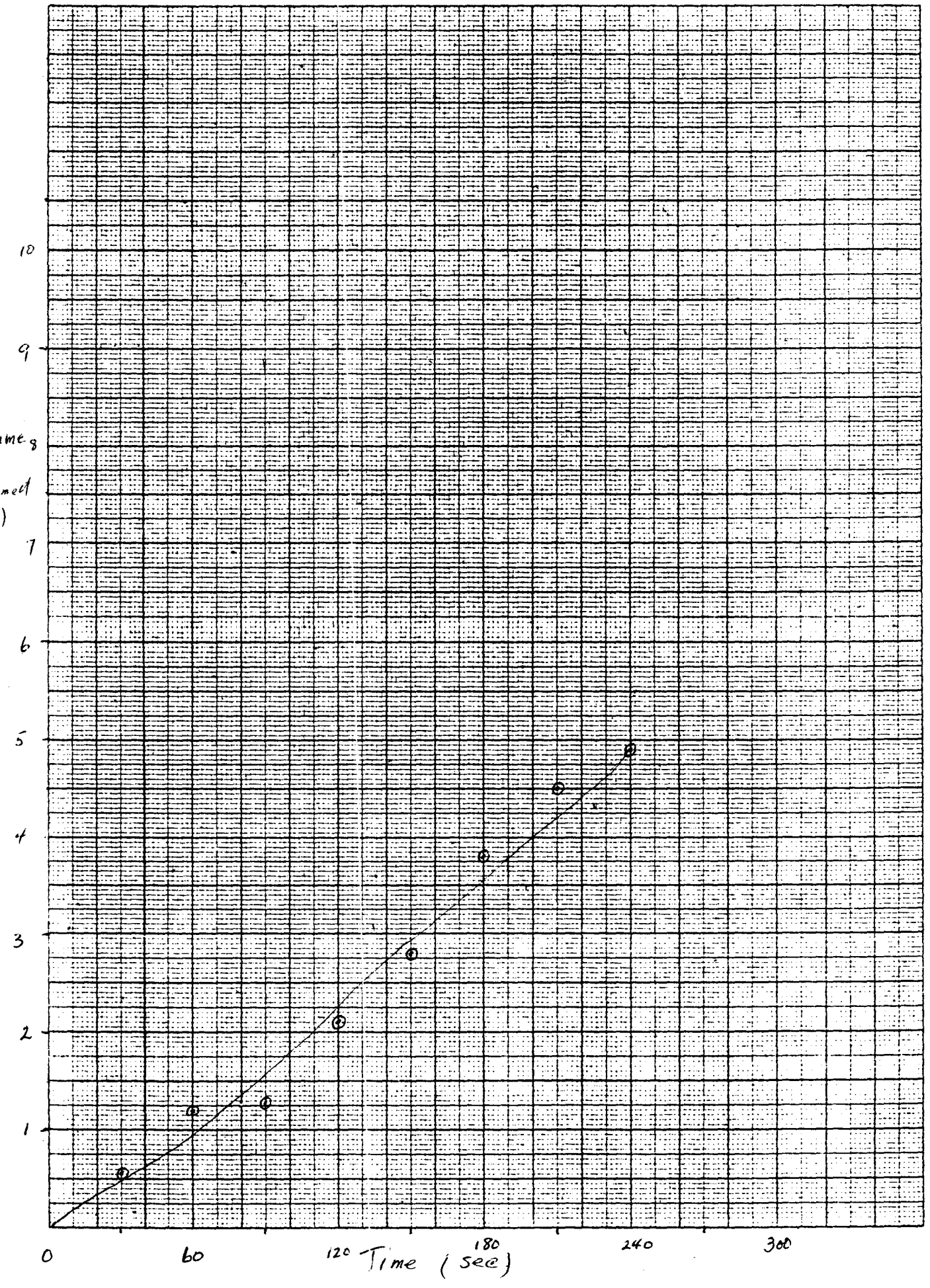
3) On graph paper, plot all the changes in pulse rate versus time.

4) Repeat the experiment after a further 5 min. of reclining, but this time the subject should actively move the legs during and after standing up.

5) Plot the changes in pulse rate on the same graph.

Is there a difference?

What is your interpretation of the findings?


10
9
8
7
6
5
4
3
2
1
0
60
120
180
240
300
Time (sec)

B) THE SCHNEIDER CARDIO-VASCULAR FITNESS TEST

The Schneider Test allows us to assess cardio-vascular fitness. It is a very straightforward and simple test which may be used with people in a wide range of physical condition, for it involves only a small degree of exertion on the part of the subject, and is therefore very safe.

NEVERTHELESS YOU SHOULD NOT PARTICIPATE IN THIS TEST IF YOU ARE ILL, IF YOU HAVE HIGH BLOOD PRESSURE, OR IF YOU ARE TAKING ANY MEDICATION.

1) From the data collected on pulse rate in section A TRIAL 1 select the most representative rates for

a) the reclining pulse rate in beats / minute

b) the standing pulse rate in beats / minute (i.e. when 2 consecutive counts were the same)

Apply these values to Tables A and C Page 43 in order to determine your scores.

2) Calculate the difference between the reclining and the standing pulse rates and determine your score from Table B.

3) Timed by a watch, the subject next steps up upon a bench at a regular rate of 5 times in 15 seconds (i.e., 1 step every 3 seconds) for one 15 second period only.

4) Immediately after exercise, start counting the pulse immediately in 15 second counts until the rate has returned to the normal standing rate (values to be multiplied by 4). Discontinue after 2 min if the heart rate has still not returned to normal, but record the number of beats/min above normal at that time. Record:

   (i) The increase in heart rate in the 15 second period immediately after exercise (see Table D).

   (ii) The return to normal (see Table E). In computing this return, count from the end of the 15 seconds of exercise to the beginning of the first normal 15 second pulse count.

5) Add up the total score (A,B,C,D, and E) and enter it on the blackboard together with sex, and smoking history.

POINTS FOR GRADING CARDIO-VASCULAR CHANGES

A. Reclining pulse rate	
Rate	Points
50-60	3
61-70	3
71-80	2
81-90	1
91-100	0
100-110	-1

B. Pulse rate increase on standing				
0-10 beats, points	11-18 beats, points	19-26 beats, points	27-34 beats, points	35-42 beats, points
3	3	2	1	0
3	2	1	0	-1
3	2	0	-1	-2
2	1	0	-1	-2
1	0	-2	-3	-3
0	-1	-3	-3	-3

C. Standing pulse rate	
Rate	Points
60-70	3
71-80	3
81-90	2
91-100	1
101-110	1
111-120	0
121-130	0
131-140	-1

D. Pulse rate increase immediately after exercise				
0-10 beats, points	11-20 beats, points	21-30 beats, points	31-40 beats, points	41-50 beats, points
3	3	2	1	0
3	2	1	0	0
3	2	1	0	-1
2	1	0	-1	-2
1	0	-1	-2	-3
1	-1	-2	-3	-3
0	-2	-3	-3	-3
0	-3	-3	-3	-3

E. Return of pulse rate to standingnormal after exercise	
Seconds	Points
0-30	3
31-60	2
61-90	1
91-120	0
After 120; 2-10 beats above normal	-1
After 120; 11-30 beats above normal	-2

Interpretation of Scores

Very good	14-15
Good	11-13
Reasonable	8-10
Fair	5-7
Poor	<4

Name Initials	Smoker yes or No	Schneider's Score	Breath Holding Time in sec	A Percentage of Mean Breath Holding Time	Vital Capacity in cc	B % Normal Vital Capacity	A + B
	Mean						
	Mean						

♂

♀

C) PULMONARY FITNESS

As before, this test is to be performed in turn by both partners. You will be determining maximum breath holding time and lung vital capacity.

1. To determine breath holding time the subject should take 3 forced inspirations followed by 3 complete expirations, then a maximum inspiration. At the end of this inspiration, measure the breath holding time in seconds and record the value on the blackboard. When all the class data are entered, determine the mean breath holding time and express each individual value as a percentage of the mean.

2. Determine lung vital capacity (Maximum lung volume available for breathing) using the spirometer. This should be performed in the same manner as for breath-holding (i.e., 3 forced inspirations + exhalations, a maximum inspiration, and then a maximum expiration on which the vital capacity is measured). Using the Table supplied, with reference to your height and sex, determine the percentage of normal vital capacity. Enter the value on the blackboard.

3. Analyse the class data to see if there is a correlation between percentage of the mean breathholding time and percentage of normal vital capacity, and between either of these pulmonary indices (or the two added together) and the cardiovascular fitness score.

Are sex and smoking history of any influence?

Try to explain the relationships observed.

PERCENTAGE OF VITAL CAPACITY **MEN** (CALCULATED FROM STANDING HEIGHT) IN CUBIC CENTIMETERS

Standing height in cms	ins	200	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	2400	2500	2600	2700	2800	2900	3000	3100	3200	3300	3400	3500	3600	3700	3800	3900	4000	41	10	4200	4300	4400	4500	4600	4700	4800	4900	5000	5100	5200	5300	5400	5500	5600	5700	5800
144 8	57	19	22	25	28	30	33	36	39	41	44	47	50	52	55	58	61	64	66	69	72	75	77	80	83	85	88	91	94	97	99	102	105	108	110	1	12	116	119	121																
147 3	58	19	22	24	27	30	33	35	38	41	43	46	49	52	54	57	60	63	65	68	71	73	76	79	82	84	87	90	92	95	98	100	103	106	109	1	11	114	117	120	122																
149 8	59		21	24	27	30	32	35	37	40	43	45	48	51	53	56	59	61	64	67	69	72	75	77	80	83	85	88	91	93	96	99	101	104	107	1	10	112	115	117	120	123															
152 4	60		21	24	25	29	32	34	37	39	42	45	47	50	53	55	58	60	63	66	68	71	74	76	79	81	84	87	89	92	95	97	100	102	105	1	08	110	113	115	118	121	123														
154 9	61		21	23	26	28	31	34	36	39	41	44	47	49	52	54	57	59	62	65	67	70	72	75	78	80	83	85	88	90	93	96	98	101	103	1	06	108	111	114	116	119	121	124												
157 4	62		20	23	25	28	30	33	36	38	41	43	46	48	51	53	56	58	61	64	66	69	71	74	76	79	81	84	86	89	91	94	97	99	102	1	04	107	109	112	114	117	119	122												
160 0	63		20	23	25	28	30	33	35	38	40	43	45	48	50	53	55	58	60	63	65	68	70	73	75	78	80	83	85	88	90	93	95	98	100	1	03	105	108	110	113	115	118	120	123											
162 5	64		20	22	25	27	30	32	35	37	39	42	44	47	49	52	54	57	59	62	64	67	69	71	74	76	79	81	84	86	89	91	94	96	99	1	01	103	106	108	111	113	116	118	120	123										
165 1	65			22	24	27	29	32	34	36	39	41	44	46	48	51	53	56	58	61	63	66	68	70	73	75	78	80	82	85	87	90	92	95	97		99	102	104	107	109	111	114	116	118	121	123									
167 5	66			21	24	26	29	31	33	36	38	41	43	45	48	50	52	55	57	60	62	64	67	69	71	74	76	79	81	83	86	88	91	93	95		98	100	102	105	107	109	112	114	117	119	121	124								
170 2	67			21	24	26	28	31	33	35	38	40	42	45	47	49	52	54	56	59	61	63	66	68	71	73	75	78	80	82	85	87	89	92	94		97	99	101	104	106	109	111	113	115	118	120	122								
172 7	68			21	23	26	28	30	32	35	37	39	42	44	46	49	51	53	56	58	60	63	65	67	70	72	74	76	79	81	83	86	88	90	93		95	97	100	102	104	107	109	111	114	116	118	120	123							
175 5	69			21	23	25	27	30	32	34	37	39	41	43	46	48	50	52	55	57	59	62	64	66	68	71	73	75	78	80	82	84	87	89	91		94	96	98	100	103	105	107	109	112	114	116	119	121	123						
177 8	70			20	22	25	27	29	31	34	36	38	40	43	45	47	49	52	54	56	58	61	63	65	67	70	72	74	76	79	81	83	86	88	90		93	95	97	100	102	104	106	109	111	113	115	117	119	121	124					
180 4	71			20	22	24	27	29	31	33	35	38	40	42	44	47	49	51	53	55	58	60	62	64	67	69	71	73	75	78	80	82	84	86	89		91	93	95	98	100	102	104	106	109	111	113	115	117	120	122	124				
182 9	72			20	22	24	26	28	31	33	35	37	39	42	44	46	48	50	53	55	57	59	61	63	66	68	70	72	74	77	79	81	83	85	88		90	92	94	96	98	101	103	105	107	109	111	113	116	118	120	122	125			
185 4	73			19	22	24	26	28	30	32	35	37	39	41	43	45	47	50	52	54	56	58	60	63	65	67	69	71	73	76	78	80	82	84	86		89	91	93	95	97	99	101	103	106	108	110	112	114	117	119	121	123			
188 0	74			19	21	23	26	28	30	32	34	36	38	40	43	45	47	49	51	53	55	57	60	62	64	66	68	70	72	75	77	79	81	83	85		87	89	92	94	96	98	100	102	104	106	108	111	113	115	117	119	121	123		
190 5	75				21	23	25	27	29	32	34	36	38	40	42	44	46	48	50	53	55	57	59	61	63	65	67	69	71	73	76	78	80	82	84		86	88	90	92	94	96	98	100	102	104	106	108	110	113	115	117	119	121	123	
193 0	76				21	23	25	27	29	31	33	35	37	39	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	75	77	79	81	83		85	87	89	91	93	95	97	99	101	103	105	107	109	112	114	116	118	120	122	
		42 7	46 8	54 9	61 0	67 1	73 2	79 3	85 4	91 5	97 6	103 7	109 8	115 9	122 0	128 1	134 2	140 3	146 4	152 5	158 6	164 7	170 8	176 9	183 0	189 1	195 2	201 3	207 4	213 5	219 6	225 7	231 8	237 9	244 0	250 1	256 2	262 3	268 4	274 5	280 6	286 7	292 8	298 9	305 0	311 1	317 2	323 3	329 4	335 5	341 6	347 7	353 8

PERCENTAGE OF VITAL CAPACITY IN CUBIC INCHES

PERCENTAGE OF VITAL CAPACITY **WOMEN** (CALCULATED FROM STANDING HEIGHT) IN CUBIC CENTIMETERS

Standing Height in cm	Standing Height in inches	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	2400	2500	2600	2700	2800	2900	3000	3100	3200	3300	3400	3500	3600	3700	3800	3900	4000	4100	4200	4300	4400	4500
139 7	55	21	23	29	32	36	39	43	47	50	54	57	61	64	68	71	75	79	82	86	89	93	96	100	103	107	111	114	118	121											
142 2	56	21	23	28	32	33	39	42	46	49	53	56	60	63	67	70	74	77	81	84	88	91	95	98	102	105	109	112	116	119	123										
144 8	57	21	24	28	31	35	38	41	45	48	52	55	59	62	66	69	72	76	79	83	86	90	93	97	100	103	107	110	114	117	121										
147 3	58	20	24	27	31	34	38	41	44	47	51	54	58	61	64	68	71	75	78	81	85	88	91	95	98	102	105	108	112	115	118	122									
149 8	59	20	23	27	30	33	37	40	43	47	50	53	57	60	63	67	70	73	77	80	83	87	90	93	97	100	103	107	110	113	117	120	120								
152 4	60	20	23	26	30	33	36	39	43	46	49	52	56	59	62	66	69	72	75	79	82	85	88	92	95	98	102	105	108	112	115	118	121								
154 9	61		23	26	29	32	36	39	42	45	48	52	55	58	61	65	68	71	74	77	81	84	87	90	94	97	100	103	106	110	113	116	119	122								
157 4	62		22	25	29	32	35	38	41	44	48	51	54	57	60	63	67	70	73	76	79	83	86	89	92	95	98	102	105	108	111	114	118	121								
160 0	63		22	25	28	31	35	38	41	44	47	50	53	56	59	62	66	69	72	75	78	81	84	88	91	94	97	100	103	106	109	112	116	119	122							
162 5	64		22	25	28	31	34	37	40	43	46	49	52	55	58	62	65	68	71	74	77	80	83	86	89	92	95	98	101	105	108	111	114	117	120							
165 1	65		21	24	27	30	33	36	39	42	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	103	106	109	112	115	118	121						
167 6	66		21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	98	101	104	107	110	113	116	119	122					
170 2	67		21	24	27	29	32	35	38	41	44	47	50	53	56	59	62	65	68	71	74	77	79	82	85	88	91	94	97	100	103	106	109	112	115	118	120	123				
172 7	68		20	23	26	29	32	35	38	41	44	46	49	52	55	58	61	64	67	69	72	75	78	81	84	87	90	93	96	98	101	104	107	110	113	116	119	121				
175 3	69		20	23	26	29	31	34	37	40	43	46	49	51	54	57	60	63	66	68	71	74	77	80	83	85	88	91	94	97	100	102	105	108	111	114	117	120	123			
177 8	70		20	22	25	28	31	34	37	39	42	45	48	51	53	56	59	62	65	67	70	73	76	79	82	84	87	90	93	96	98	101	104	107	110	112	115	118	121			
180 4	71			22	25	28	31	33	36	39	42	44	47	50	53	55	58	61	64	66	69	72	75	78	80	83	86	89	91	94	97	100	102	105	108	111	114	116	119	122		
182 9	72			22	25	27	30	33	36	38	41	44	47	49	52	55	57	60	63	66	68	71	74	77	79	82	85	88	90	93	96	98	101	104	107	109	112	115	118	120		
185 4	73			22	24	27	30	32	35	38	40	43	46	49	51	54	57	59	62	65	67	70	73	76	78	81	84	86	89	92	94	97	100	102	105	108	111	113	116	119	121	
188 0	74			21	24	27	29	32	35	37	40	43	45	48	51	53	56	59	61	64	67	69	72	75	77	80	83	85	88	90	93	96	99	101	104	106	109	112	114	117	120	
		36 6	42 7	48 8	54 9	61 0	67 1	73 2	79 3	85 4	91 5	97 6	103 7	109 8	115 9	122 0	128 1	134 2	140 3	146 4	152 5	158 6	164 7	170 8	176 9	183 0	189 1	195 2	201 3	207 4	213 5	219 6	225 7	231 8	237 9	244 0	250 1	256 2	262 3	268 4	274 5

PERCENTAGE OF VITAL CAPACITY IN CUBIC INCHES

Lab VIII Kidney Function

Objectives:
- to understand the role of the kidney in the maintenance of homeostasis.
- to understand why kidney malfunction sometimes occurs.
- to compare samples of urine by standard clinical tests.

All body cells are bathed in extracellular fluid (ECF) - a fluid volume which consists of a blood plasma compartment and an interstitial fluid compartment. In order for cells to function at maximum efficiency, ECF homeostasis must be maintained. That is, the volume, the composition and the degree of acidity of the extracellular environment must be regulated within very close tolerances. During the course of a normal day, the intake of fluids, salts and nutrients, and the metabolic demands of the cells fluctuate markedly. Therefore, ECF homeostasis largely depends on the kidneys' ability to excrete those substances which are in excess or are toxic by-products of metabolism, and to selectively reabsorb those substances which are needed by the body.

The 2 million nephron units of the kidneys accomplish this task by filtering about 180 liters of fluid through their glomeruli every day. This fluid has a composition very similar to ECF; as it is being formed, 99% of this fluid is reabsorbed along with all essential nutrients which had been filtered, such as glucose, amino acids etc. The 1 liter of urine remaining, contains the nitrogenous wastes of metabolism as well as any excess salts.

The kidneys also have the ability to continuously adjust the pH of the urine between the rather wide range of 4.5 and 8.5. This allows excretion of either basic or acid ions in order to maintain an amazingly constant plasma pH in the vicinity of 7.4.

Analysis of urine can yield valuable information about the normal or abnormal condition of the body. Patients with kidney disease frequently develop a so called "nephrotic syndrome" which is characterized by large quantities of plasma proteins in the urine. Another well-known disease is characterized by the appearance of glucose in the urine. Women in the initial stages of pregnancy exhibit detectable levels of the hormone chorionic gonadotropin (CG) in the urine. This hormone, which is responsible for the mainten

ance of a healthy environment for early fetal development, rises to high levels in maternal blood in the first months of pregnancy. The volume and specific gravity of urine are important indicators of the body's state of hydration.

CLINICAL EXAMINATION OF URINE

A) CLINICAL TESTS OF A NORMAL SAMPLE

Because these tests are simple and fast, each student should collect his or her own "ample sample" in the vial provided. Perform each of the following tests on your fresh specimen and add your data (anonymously if you wish) to the class results on the board.

1. Test for pH

Using the forceps provided, dip a strip of litmus paper into the fresh sample; Remove immediately, and drain. Match the litmus to the colour blocks on the chart. Record your results.

2. Qualitative Test for Glucose in Urine

Clinistix used in this test must be kept in the bottle with the cap tightly closed to protect the reagents from deterioration.

i) Completely immerse the reagent area of the strip in fresh, well mixed urine, and remove immediately.

ii) Tap edge of strip against side of container to remove excess.

iii) Exactly 10 seconds after dipping, compare the test area closely with the colour chart.

iv) Results with Clinistix are qualitative; they are read from the chart as either negative (-) or varying degrees of positive (+, ++ and +++) which indicate relative amounts of glucose present. Concentrations of 0.1% glucose are considered significantly abnormal if found consistentlv.

3. Quantitative Test for Protein in the Urine

Reagent strips must be kept in the bottle with the lid tightly closed to maintain reagent reactivity.

i) Completely immerse the reagent area of the stip in fresh urine, and remove immediately.

ii) Tap edge of strip against side of container to remove excess.

iii) Compare the test area closely with the colour chart on the bottle label. The reading should be made within 60 seconds after dipping, and any colour development is considered to indicate significant proteinurea.

iv) Results are to be recorded as negative (-) or varying degrees of positive which indicate the amount of protein present:

30 mg/100 ml	(+)
100 mg/100 ml	(++)
300 mg/100 ml	(+++)
1000 mg/100 ml	(++++)

Collect the class data on normal samples.

Calculate the mean classroom pH. What is the variance? Make a general statement about normal pH levels from the class data.

Would you expect to find glucose in normal urine? Why or why not?

Would you expect to find protein in normal urine? Why or why not?

B) CLINICAL TESTS OF PATHOLOGICAL SAMPLES

You will work in pairs for this section. Each pair of students has been given an "unknown" pathological sample for analysis - either A, B, C or D, but since each group is testing only one unknown, be prepared to share your findings with others. The demonstrator will tell you whether to test for glucose and protein or whether you should do the pregnancy test.

1. Pregnosis - Test for Pregnancy

The reagents for this test are very costly, so read the whole procedure through beforehand and follow the instructions carefully. The test components for pregnosis consist of 2 reagents.

a) An antigen in the form of a suspension of HCG (human chorionic gonadotropin) chemically bonded to latex polymer particles.

b) An antiserum (from rabbits) containing antibodies to HCG. When the antiserum is mixed with the urine from a pregnant female, it is neutralized. Then, when the antigen is added, no agglutination (clumping of the particles) occurs. This is a positive test for pregnancy. Urine containing no HCG cannot neutralize the antiserum, and agglutination of the antigen results. This is a negative test for pregnancy.

PROCEDURE

i) Shake the antigen reagent to assure a uniform suspension.

ii) With a glass marking pencil, draw a circle approximately 2 cm in diameter on each of 2 glass microscope slides. Label the slides as 1. and 2.

iii) Place 1 drop of the urine from one sample in the circle on one slide, and 1 drop from the second sample in the circle on the second slide. Record which sample corresponds to slide 1. And which sample corresponds to slide 2. They will vary from day to day.

iv) Holding the dropper perpendicular to, and well above the slide, add 1 drop of Antiserum Reagent (BLACK CAP) to each circle. Replace the bottle cap and set aside,

IT IS EXTREMELY IMPORTANT THAT DROPPER CAPS BE REPLACED ON THE PROPER BOTTLES TO PREVENT CROSS-CONTAMINATION OF INGREDIENTS.

v) Using a separate clean toothpick for each, mix the drops and rotate each slide gently for 30 seconds.

vi) Holding the dropper perpendicular to and above the slide, add 1 drop of Antigen Reagent (WHITE CAP) to each of the slides 1 and 2. Mix thoroughly with the same toothpick, spreading the mixture out over the full area of the circle.

vii) Rotate each slide for about 2 minutes, observing as you rotate for agglutination.

When used according to instructions, results of this test should be 97.3% accurate.

If you did the Pregnosis test, make a statement in your report about the examples which you tested.

If you did the glucose and protein tests, report your results.

Everyone should answer these questions.

Proteins are normally too large (or have too high a molecular weight) to pass through the glomerular membrane filter. Suggest a reasonable explanation of why protein is found in the urine in cases of "nephrotic syndrome".

Normally glucose is almost completely reabsorbed by active processes in the proximal tubules of the kidneys. Suggest a possible rationale to explain why a test for glucose may come out positive.

Why does chorionic gonadotropin appear in the urine of women during early pregnancy. Include your reasoning.

Lab IX Salivary Amylase

Objectives:
- To demonstrate the digestive action of an enzyme found in human saliva.
- To illustrate the sensitivity of an enzyme to pH.
- To determine whether ionic activation is necessary for enzymatic activity.
- To examine the effect of temperature on enzymatic activity.

Human saliva contains salts, mucus and the enzyme "salivary amylase" which is also known as ptyalin. Ptyalin is a catalyst which speeds up the breakdown of starch during the process of digestion. This breakdown of starch occurs in two stages:

starch - a complex branched chain, polysaccharide
↓
dextrins - short chain polysaccharides
↓
maltose - a disaccharide of 2 glucose subunits

Most starch digestion in humans occurs in the duodenum, as a result of "pancreatic amylase" but a significant fraction is due to salivary amylase -- especially the digestion of soft starchy foods such as bread.

In this experiment you will use an iodine test to determine the extent to which starch has been hydrolyzed (broken down) by salivary amylase.

Iodine plus starch produces a very dark blue colour.

Iodine plus dextrins produces a red brown colour

Iodine plus maltose produces the amber colour of dilute iodine alone.

The point in this sequence of reactions at which the hydrolysis mixture just fails to give any colour with iodine is called the achromic point. The time required to cause a given colour change (ideally to reach the achromic point) can be used as a measure of the activity of the enzyme. We will be using NaCl to determine if ionic activation is required for the reaction. As mentioned earlier however, saliva itself contains some salts.
Buffers of pH 4.0, 6.0, 7.0, and 9.0 have been prepared for the test of pH sensitivity.

Water baths at 0°C, 37°C, 57°C and 70°C have been prepared and labelled for testing the effects of temperature on enzyme activity.

A) PREPARATION OF THE ENZYME

1. One partner should rinse his or her mouth with warm water and then chew a piece of paraffin wax to stimulate salivary flow. Chew it for a few minutes until the wax comes together into one piece, then discard.
Collect a few ml of saliva in a 50 ml beaker.

2. Measure and record the pH of the saliva using a small sliver of pH paper.

3. Fill a 100 ml beaker with distilled water.

4. Dilute exactly 1 ml of saliva with 19 ml of the distilled water in a 100 ml Erlenmyer flask.

5. Pipette 3 ml of the diluted saliva preparation into a test tube. Mark it with your initials and place in the boiling water bath (under the supervision of the demonstrator) for 5 minutes. You will use this boiled saliva in part C.

B) SENSITIVITY OF ENZYME TO pH AND IONIC ACTIVATION

1. With a single 5 ml pipette, prepare the following test tubes. Initial and number each. NOTE: RINSE THE PIPETTE THOROUGHLY WITH DISTILLED WATER WHENEVER CHANGING SOLUTIONS.

Tube 1 - 5 ml of 1% starch solution + 2 ml of 1% Na Cl + 2 ml of phosphate buffer pH = 4.0

Tube 2 - 5 ml of 1% starch solution + 2 ml of 1% Na Cl + 2 ml of phosphate buffer pH = 6.0

Tube 3 - 5 ml of 1% starch solution + 2 ml of 1% Na Cl + 2 ml of phosphate buffer pH = 7.0

Tube 4 - 5 ml of 1% starch solution + 2 ml of DISTILLED WATER + 2 ml of phosphate buffer pH = 7.0

Tube 5 - 5 ml of 1% starch solution + 2 ml of 1% Na Cl + 2 ml of phosphate buffer pH = 9.0

2. Using a small sliver of pH paper, verifv and record the pH of each tube. Do not leave the paper longer than 5 seconds, as it will "bleed".

3. Place all the tubes in the 37°C water bath and leave there for the rest of the experiment. NOTE: ALLOW 10 MINUTES FOR THE TUBES TO WARM UP TO WATER BATH TEMPERATURE BEFORE PROCEEDING WITH THE REST OF THIS PART OF THE EXPERIMENT. Meanwhile, put one drop of iodine in each depression of a spot plate, ready for step 5.

4. At intervals staggered by 30 seconds, add to each of the tubes 1 to 5, 1 ml of the diluted saliva prepared in section A. Leave the 1 ml pipette in the flask for future use. MIX EACH TUBE THOROUGHLY BY INVERSION, PLUGGING THE TOP WITH YOUR THUMB. RECORD THE TIME OF EACH ADDITION.

5. Place a separate pasteur pipette in each tube, and leave it there. Beginning 4 minutes after the first addition of saliva, place 1 drop from tube 1 in a depression of the spot plate containing iodine. Note the colour. Continue testing each tube in rotation so that each tube is tested at 4-minute intervals for 28 minutes or until the achromic point is reached. (You should carry the spot plate around with you from bath to bath). NOTE: MIX EACH TUBE BEFORE TAKING THE SAMPLE-BY BLOWING OUT THE CONTENTS OF THE PIPETTE TWICE.

C) SENSITIVITY OF ENZYME ACTIVITY TO TEMPERATURE

1. Prepare 5 more test tubes identical to tube 3. Label them 6, 7, 8, 9 and 10. Again, be sure to rinse the pipette thoroughly with distilled water whenever changing solutions.

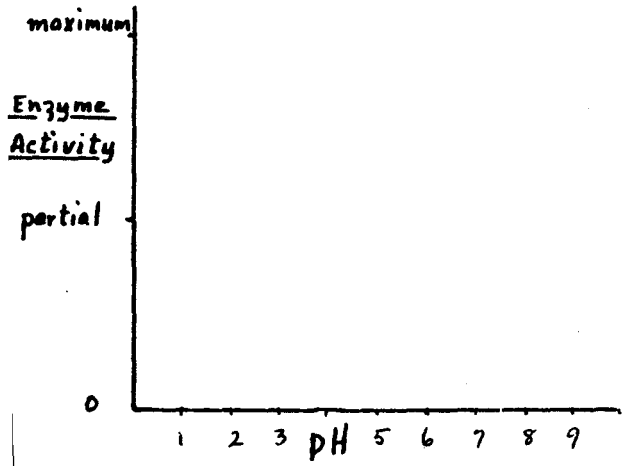
2. Place tube 6 in the ice-water bath, tubes 7 and 8 in the 37°C waterbath, tube 9 in the 57°C bath, and tube 10 in the 70°C water bath. Again allow 10 minutes for the tubes to equilibrate to temperature before proceeding. While waiting, prepare the other spot plate with iodine.

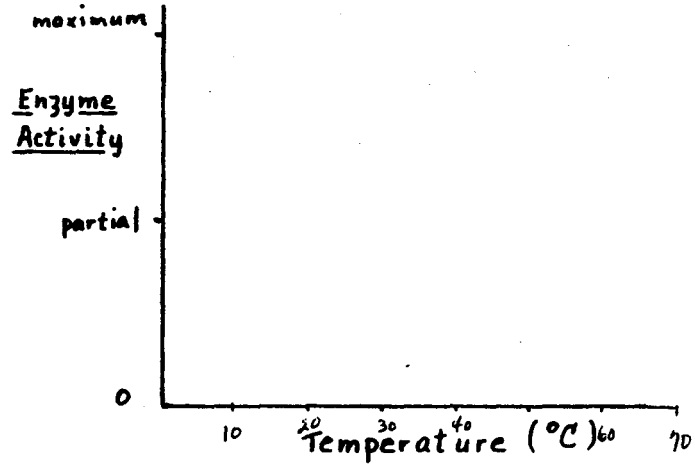
3. At intervals staggered by 30 seconds, add to tubes 6, 7, 9 and 10, 1 ml of the diluted saliva prepared in section A, recording the time of each. Lastly, add 1 ml of the BOILED diluted saliva from section A to tube 8.

4. As in section B, iodine test the 5 tubes in rotation at 4 minute intervals for 28 minutes or until the achromic point is reached.

Organize all your data into a chart or Table to <u>best</u> show the results of all the tests.

From your data, draw the graph to show the activity of the enzyme according to these parameters. The Y axis can only be a semi-quantitative scale.





What was the original pH of your saliva? _____

Define a pH optimum for salivary amylase activity. _____

How does this optimum pH compare with the pH of your saliva?

Would "pancreatic amylase" have the same pH optimum? Explain.

Does ionic activation appear to be important?

Describe the effect of increasing temperature on salivary amylase activity. Explain the effects.

Explain why the results with tube 7 and tube 8 were different.

Lab X Nutrition

Objectives: - To evaluate the adequacy (or inadequacy!) of your diet by careful monitoring of daily food consumption.

Most of us actually eat a reasonably healthy diet by a combination of conscious and subconscious motivation. A few of us eat a nutritionally poor diet and eventually pay the consequences. In this lab you will survey your own diet and compare it to both recommended standards and your own estimated energy expenditure in order to determine its adequacy. The results should be treated with some caution as they are subject to many inaccuracies due to the need for estimating or "guesstimating". Furthermore, you will only monitor total Calorie and protein intake. It is possible that a diet adequate in proteins and Calories may be inadequate in minerals or vitamins; however, this is unlikely unless your diet is unusual (e.g., all your protein comes from fish, or all your calories come from beer!).

(1) For a period of 3 days, keep a record of all the foods you eat, both types and amounts. Try to eat a normal diet (i.e., whatever you would normally eat; don't modify your habits because of this survey). In the accompanying tables, the food energy (Calories) and protein content of most common food is listed. The measures or portions are largely listed in the tables, in units of "cups" or "oz". These will be demonstrated in the lab for those unfamiliar with these units. Using these units, try to "guesstimate" your portions as accurately as possible. Of course, not all foods will be listed in the tables, and for these you will just have to use your common sense to choose the best "equivalents" for purposes of calculation.

(2) From the record in section 1 above, calculate your average daily intake of total Calories and protein. Compare with the following figures recommended by the National Research Council for healthy 25 year old men and women (why the difference between men and women?). How much of your daily calorific intake comes from protein? (Protein has calorific value of about 4.2 Calories/gm.).

	Men	Women
Calories	3200	2300
Protein	1 g/kg body weight	1 g/kg body weight

The Calorie figures assume a body weight of 65 kg for men and 55 kg for women. They may be corrected to your own body weight by interpolation from the following table (1 lb. = .4536 kg). This is most easily done by constructing a graph. Explain the nature of the relationship.

Weight				Percentage of Recommended Figure
Men		Women		
kg	lb	kg	lb	
47.3	104	40.5	89	80
55.8	123	46.8	103	90
64.4	142	54.5	120	100
73.4	162	61.2	135	110
82.4	181	70.2	154	120
97.0	213	84.5	186	130

All these figures are for a "fairly active" person and would have to be adjusted upwards or downwards for very active and very inactive people. Section 3 (below) will relate your activity level to calorific intake.

(3) Keep a record of your activities for 1 of the 3 days (we only ask for 1 day here as it can be very tedious). Was this typical of the three? If not, adjust the figures to be representative of a "typical" day. Produce a summated record - e.g.,

Sleeping	8.50 h
Awake, lying still	.75 h
Dressing	.25 h
Driving	1.00 h
Taking notes	4.25 h
Walking slowly	1.75 h
Eating	1.50 h
Lab Work	2.00 h
Swimming	1.00 h
Standing	1.50 h
Typing	1.25 h
Running	0.25 h
Total	24 h

Calculate from the table on the following page (use common sense and "guesstimate" for unlisted items) what the energy expenditure is in Calories/kg for each of the activity periods. Sum the figures and multiply by your body weight in kg to find the average daily energy expenditure. How does this compare with your average daily energy intake calculated in Section "2"? Are you in energy balance?

± 250 Calories/day is probably an insignificant deviation from balance given the inaccuracies in the methods. If you are further than 250 Calories/day from balance, translate the imbalance into a rate of weight gain or weight loss. If we assume that the weight gain or loss is in the form of fat (adipose tissue), a reasonable conversion factor is 8 Calories/g tissue (the calorific value of pure fat is about 9.3 Calories/g, but adipose tissue contains some water).

What other factors must be taken into account to construct a more accurate energy balance sheet?

Form of Activity	Calories/kg/hour
Awake, lying still	1.10
Bicycling (moderate speed)	3.46
Boxing	54.20
Cleaning windows	12.30
Dancing	4.75
Dishwashing, ironing	2.02
Dressing and undressing	1.68
Driving automobile	1.87
Fencing	34.80
Football	40.80
Gardening (weeding)	18.50
Horseback, riding, walk	6.67
Horseback, riding, trot	20.40
Horseback, riding, gallop	31.80
Laboratory work	9.98
Playing cards	2.40
Playing ping pong	20.90
Reading	1.92
Running	33.30
Sitting, at rest	1.39
Sitting, and eating	1.58
Sleeping	0.91
Skating	16.70
Skiing (moderate speed)	49.00
Standing, relaxed	1.49
Sweeping with broom	6.67
Swimming	7.01
Taking notes in class (sitting)	1.54
Typing, rapidly	1.97
Walking, slowly (2.6 mi/hr)	2.83
Walking, fast (3.5 mi/hr)	4.27
Walking down stairs	5.09
Walking up stairs	15.50
Writing, sitting	1.92
Sex (participatory, not imaginary)	4.00
Sawing wood	6.86
Jogging	8.00
Rowing	11.80
Shovelling snow	8.00

These figures represent a recreational type of activity, not what energy expenditure would be while training. If you play really hard you can claim more Calories/hr.

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
MILK, CHEESE, CREAM; RELATED PRODUCTS					
Milk, fluid:					
Whole, 3.5% fat	1 cup	244	87	160	9
Non fat (skim)	1 cup	245	90	90	9
Partially skimmed, 2%	1 cup	245	87	123	9
Buttermilk (from skim milk)	1 cup	245	90	90	9
Milk, processed:					
Evaporated, canned, unsweetened, whole	1 cup	252	74	345	18
Condensed, canned, sweetened	1 cup	306	27	980	25
Dry, skim, instant (1½ c. needed for reconstitution to 32 ounces)	1 cup	68	4	245	24
Dry, whole	1 Tbsp	6	2	32	2
Milk Beverages:					
Cocoa (made with whole milk)	1 cup	250	79	245	10
Chocolate drink (partially skimmed milk)	1 cup	250	83	190	8
Instant Breakfast (made with whole milk)	1 cup	298	–	304	18
Instant Breakfast, powder	1 envelope	37	–	146	9
Malted milk – beverage	1 cup	270	83	280	13
Ovaltine (with whole milk)	1 cup	259	83	221	11
Milk Desserts:					
Cornstarch Pudding – cooked	½ cup	124	38	137	5
Cornstarch Pudding – instant	½ cup	157	–	182	6
Custard – baked	½ cup	124	38	142	7
Rice pudding	½ cup	97	–	141	3
Tapioca pudding (minute)	½ cup	105	–	133	5
Ice Cream:					
Regular (approx. 10% fat)	1 cup	133	63	255	6
Rich (approx. 16% fat) ¼ brick = ½ cup	1 cup	148	63	330	4
Ice Milk, soft serve	1 cup	175	67	265	8
Ice cream bar	1 bar	60	–	144	3
Sherbet, lemon	1 cup	130	67	223	5
Yoghurt:					
Made from partially skimmed milk, plain	6 oz	185	89	112	9
Made from partially skimmed milk, fruit flavoured (average)[7]	6 oz	185	89	170	8
Cheese:					
Blue	1 oz	28	40	105	6
Camembert	1 oz	28	52	84	5
Cheddar (1" cube = 17 gms.)	1 oz	28	37	116	7
Cheddar grated	1 Tbsp.	7	37	30	2
Cheddar processed	1 oz	28	40	105	7
MILK, CHEESE, CREAM; RELATED PRODUCTS (continued)					
Cottage creamed – (4% fat)	1 cup	225	79	240	31
Cottage not creamed	1 cup	200	79	170	34
Cream	1 oz 2 Tbsp.	28	51	105	2
Parmesan – grated	1 Tbsp.	7	–	28	2
Pasteurized, processed, cheese spread	1 oz , 2 Tbsp.	28	43	90	6
Swiss – natural	1 oz	28	39	105	8
Swiss – processed – domestic	1 oz	28	40	100	8
Swiss – processed – gruyere	1 oz	28	40	115	8
Cream:					
Coffee Cream (10% fat)	1 cup	242	80	293	7
Coffee Cream (10% fat)	1 Tbsp.	15	80	18	tr.
Table cream (18% fat)	1 cup	240	72	505	7
Table cream (18% fat)	1 Tbsp.	15	72	30	1
Whipping cream (35% fat)(unwhipped)	1 cup	239	57	691	5
Whipping cream (35% fat)(unwhipped)	1 Tbsp.	15	57	43	tr.
Sour cream (10-18% fat)	1 cup	230	72	485	7
Sour cream (10-18% fat)	1 Tbsp.	12	72	25	tr.
Whipped cream (pressurized)(18-26% fat)	1 Tbsp.	3	62	10	tr.
Imitation Cream Products (vegetable fat)					
Creamers:					
Powdered	1 Tsp.	2	2	10	tr.
Liquid (frozen)	1 Tbsp.	15	77	20	tr.
Toppings:					
Whipped – pressurized	1 Tbsp.	4	61	10	tr.
Powdered – made with whole milk	1 Tbsp.	6	58	14	0
EGGS					
Eggs, large, (24 ounces per dozen):					
Raw or cooked in shell:					
Whole, without shell	1 egg	50	74	80	6
White of egg	1 white	33	88	15	4
Yolk of egg	1 yolk	17	51	60	3
Scrambled with milk and fat	1 egg	64	72	110	7
Fried	1 egg	55	–	113	6
MEAT, POULTRY, FISH, SHELLFISH; RELATED PRODUCTS					
Beef cooked:					
Ground beef, broiled:					
Lean	3 oz	85	60	185	23
Regular	3 oz	85	54	245	21
Roast, oven, no liquid:					
Relatively fat, such as rib:					
Lean and fat	3 oz	85	40	375	17
Lean only	1.8 oz	51	57	125	14
Relatively lean, such as round:					
Lean and fat	3 oz	85	62	165	25
Lean only	2.7 oz	78	65	125	24
Steak, broiled:					
Relatively fat, such as sirloin:					
Lean and fat	3 oz	85	44	330	20
Lean only	2.0 oz	56	59	115	18
Relatively lean, such as round:					
Lean and fat	3 oz	85	55	220	24
Lean only	2.4 oz	68	61	130	21
Stewing beef or pot roast, braised:					
Lean and fat	3 oz	85	53	245	23
Lean only	2.5 oz	72	62	140	22
Beef, canned:					
Corned beef	3 oz	85	59	185	22
Corned beef hash	3 oz	85	67	155	7
Pork, cured, cooked:					

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
MEAT, POULTRY, FISH, SHELLFISH; RELATED PRODUCTS (continued)					
Bacon, back, sliced	1 slice	21	54	65	6
Bacon, side(20 slices per lb, raw)fried crisp	2 slices	15	8	90	5
Ham, lean and fat, roasted	3 oz	85	54	245	18
Ham, boiled, sliced	2 oz	57	59	135	11
Pork,[2] fresh, cooked:					
Chop, thick, with bone (4 per lb)	1 chop	98	42	260	16
Chop, lean and fat	2.3 oz	66	42	260	16
Chop, lean only	1.7 oz	48	53	130	15
Cuts, simmered:					
Cuts, lean and fat	3 oz	85	46	320	20
Cuts, lean only	2.2 oz	63	60	135	18
Roast, oven cooked, no liquid:					
Lean and fat	3 oz	85	46	310	21
Lean only	2.4 oz	68	55	175	20
Salt pork (3" x 1½" X ¼")	2 pieces	50	–	341	6
Spareribs – 1¾" pieces	6 pieces	45	0	123	8
Sausage:					
Bologna, slice 3" diam. x ⅛"	2 slices	26	56	80	3
Braunschweiger, slice 2" diam. x ¼"	2 slices	20	53	65	3
Pork links, cooked (16 links per pound, raw)	2 links	26	35	125	5
Salami, dry type	1 oz	28	30	130	7
Salami, cooked	1 oz	28	51	90	5
Wieners, heated (8 per pound)	1 wiener	56	57	170	7
Veal and Lamb:					
Lamb,[2] cooked:					
Chop, thick with bone, broiled, 1 chop:	4.8 oz	137			
Lean and fat	4 0 oz	112	47	400	25
Lean only	2.6 oz	74	62	140	21
Leg, roasted:					
Lean and fat	3 oz	85	54	235	22
Lean only	2.5 oz	71	62	130	20
Shoulder, roasted:					
Lean and fat	3 oz	85	50	285	18
Lean only	2.3 oz	64	61	130	17
Veal, cooked, bone removed:					
Cutlet or chop without bone	3 oz	85	60	185	23
Roast	3 oz	85	55	230	23
Liver:					
Beef liver, fried	2 oz	57	57	130	15
Calves liver, fried (3" X 2¼" X ⅜")	2 slices	72	52	147	16
Chicken liver, fried	2 livers	60	65	148	18
Pork liver, fried (3" X 2¼" X ⅜")	2 slices	74	54	170	18
Poultry:					
Chicken, cooked:					
Breast, fried (½ breast):					
with bone	3.3 oz	94	58	155	25
flesh and skin only	2.7 oz	76	58	155	25
Drumstick, fried:					
with bone	2.1 oz	59	55	90	12
flesh and skin only	1.3 oz	38	55	90	12
Meat only (1 c. diced = 5⅓ oz)	3 oz	85	71	115	20
Chicken, canned, no bone	3 oz	85	65	170	18
Turkey, roast, meat only	3 oz	85	56	160	25
Variety Meats:					
Beef Heart (braised)	3 oz	85	61	160	27
Beef Kidney, cooked(3¼" X 2½" X ¼")	3 pieces	93	53	169	18
Beef Tongue, braised	3 oz	85	61	210	18
Pork Kidney, cooked	3½ oz	100	55	114	16
Rabbit	3½ oz	100	60	177	31
Sweetbreads	3½ oz	100	63	184	15
Luncheon meat	2 oz	57	55	165	8
Fish and Shellfish:					
Anchovy (fillet)	3 fillets	12	59	21	2
Clams, canned, solid and liquid	3 oz	85	86	45	7
Cod, fried in butter	4 oz	95	65	162	26
Crab, canned	3 oz	85	77	85	15
Fish sticks, breaded, frozen (3.8" X 1" X ½")	8 oz pkg	227	66	400	38
Haddock, fried, breaded	3 oz	85	66	140	17
Halibut, grilled with butter	3 oz	85	67	146	21
Herring, grilled	1 herring	85	63	217	21
Lobster, boiled + 2 Tbsp butter	1 lobster	334	77	308	20
Lobster, canned	3 oz	85	77	75	15
Mackerel, cooked	3 oz	85	62	200	19
Ocean perch, breaded, fried	3 oz	85	59	195	16
Oysters, raw, meat only, medium size (13-14)	1 cup	240	85	160	20
Pike	3½ oz	100	80	84	19
Salmon, canned	3 oz	85	71	120	17
Salmon, fresh, fried in butter	3 oz	85	71	155	23
Sardines, canned in oil, solids only	3 oz	85	62	175	20
Scallops, cooked	6 scallops	100	73	112	23
Shrimp–raw–20 small	⅔ cup	100	70	91	19
–fried–1 large 15 g	6(approx.)	100	70	225	20
–canned, meat only	⅔ cup	85	70	100	21
Sole–fillet, in butter	3 oz	85	58	172	26
Trout–raw	3½ oz	100	78	167	18
Tuna–canned, solids only	3 oz	85	61	170	24
Meat, Poultry and Fish Combination Dishes:					
Beef and Vegetable Stew	1 cup	235	82	210	15
Beef pot pie baked, 4¼" diam., weight before baking 8 ozs	1 pie	227	63	560	23
Cabbage rolls with meat	2 rolls	206	–	261	9
Chili con carne, canned					
with beans	1 cup	250	72	335	19
without beans	1 cup	255	67	510	26
Fish cake	¾ cup	134	53	166	18
Fish stew	1 cup	225	82	157	19
Irish stew	1 cup	200	82	280	23
Meat Loaf (4" X 3" X ⅜")	1 slice	70	64	264	10
Poultry Pot pie, baked, 4¼" diam., weight before baking 8 ozs.	1 pie	227	63	535	23
Spaghetti sauce, meat and tomato	¼ cup	66	–	64	3
Tourtière (Pork pie)(1/6 of 9" pie)	1 piece	139	–	451	15
MATURE DRY BEANS AND PEAS, NUTS; RELATED PRODUCTS					
Almonds, shelled, whole	1 cup	142	5	850	26
Beans, dry:					
Cooked, drained, common white	1 cup	180	69	210	14
Canned, solids and liquid					
White with					
Wieners (sliced)	1 cup	255	71	365	19
Pork and Tomato Sauce	1 cup	255	71	310	16
Red Kidney, canned	1 cup	255	76	230	15
Cashew Nuts, roasted	1 cup	140	5	785	24
Coconut, shredded, dried, packed	1 cup	62	4	344	2
Lentils, cooked	1 cup	150	69	159	12
Lima Beans, cooked, drained	1 cup	190	64	260	16
Peanuts, roasted, salted, halves	1 cup	144	2	840	37
Peanut butter	1 Tbsp.	16	2	95	4
Peas, split, dry cooked	1 cup	250	70	290	20
Pecans, halves	1 cup	108	3	740	10
Walnuts, English, halves	1 cup	100	4	650	15
Walnuts, English, chopped	1 Tbsp.	8	4	50	1

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
VEGETABLE AND VEGETABLE PRODUCTS					
Asparagus, green:					
Cooked, drained:					
Spears, ½" diam. at base	4 spears	60	94	10	1
Pieces, 1½-2" lengths	1 cup	145	94	30	3
Canned, drained	1 cup	145	94	30	3
Beans:					
Lima, immature seeds, cooked, drained	1 cup	170	71	190	13
Green:					
Cooked, drained	1 cup	125	92	30	2
Canned, drained	1 cup	125	92	30	2
Yellow or Wax:					
Cooked, drained	1 cup	125	93	30	2
Canned, drained	1 cup	125	92	30	2
Sprouted, mung beans, cooked	1 cup	125	91	35	4
Beets:					
Cooked, diced or sliced	1 cup	170	91	55	2
Canned, drained	1 cup	166	91	62	2
Beet Greens, cooked, drained	1 cup	145	94	25	3
Broccoli, cooked, drained:					
Whole stalks, med. size	1 stalk	180	91	45	6
Stalks, cut in ½" pieces	1 cup	155	91	40	5
Brussels Sprouts, cooked, 7-8 sprouts per cup	1 cup	155	88	55	7
Cabbage, common varieties:					
Raw, finely shredded	1 cup	90	92	20	1
Cooked, finely shredded, small amount water	1 cup	170	94	35	2
Cabbage, red, raw, shredded	1 cup	70	90	20	1
Carrots:					
Raw:					
Whole, 5½" x 1"	1 carrot	50	88	20	1
Grated	1 cup	110	88	45	1
Cooked, diced	1 cup	145	91	45	1
Canned, strained or chopped (baby food)	1 oz	28	92	10	tr.
Cauliflower, cooked	1 cup	120	93	25	3
Celery, raw:					
Stalk, large, outer, 8" x 1½"	1 stalk	40	94	5	tr.
Pieces, diced	1 cup	100	94	17	1
Celery, cooked, pieces	4/5 cup	100	94	14	1
Corn, sweet:					
Cooked, ear 5" x 1¾"	1 ear	140	74	70	3
Canned, kernels, drained	1 cup	166	79	140	4
Canned, cream style	1 cup	230	82	187	5
Cucumbers, 10 oz, 7½" x 2"					
Raw, pared, center slice, ⅛" thick	6 slices	50	96	5	tr.
Eggplant, cooked, slices ½" thick, 4 slices	1 cup	200	19	38	2
Lettuce, raw:					
Crisphead, as Iceberg, head 4¾" diam.	1 head	454	96	60	4
Looseleaf, leaves	2 large	50	94	10	1
Mushrooms, canned, solids and liquid	1 cup	244	93	40	5
Fresh, sautéed	4 avg	70	93	78	2
Onions:					
Mature:					
Raw, onion 2½" diam.	1 onion	110	89	40	2
Cooked	1 cup	210	92	60	3
Fried	½ cup	214	92	176	3
Young green, small without tops	6 onions	50	88	20	1
Parsley, raw, chopped	1 Tbsp.	4	85	tr.	tr.
Parsnips, cooked	1 cup	155	82	100	2
Peas, green:					
Cooked	1 cup	160	82	115	9
Canned, drained	1 cup	140	79	112	9
Canned, strained (baby food)	1 oz	28	86	15	1
Peppers, hot, red, dried, powder	1 Tbsp.	15	8	50	2
Peppers, sweet:					
Raw, about 5 per pound					
Green, without seeds	1 pod	74	93	15	1
Cooked, boiled, drained	1 pod	73	95	15	1
Potatoes, medium (about 3 per pound, raw):					
Baked, peeled after baking	1 potato	99	75	90	3
Boiled,					
Peeled after boiling	1 potato	136	80	105	3
Peeled before boiling	1 potato	122	83	80	2
French Fried, piece 2" X ½" X ½"					
Cooked in deep fat	10 pieces	57	45	155	2
Frozen, heated	10 pieces	57	53	125	2
Mashed:					
Milk added	1 cup	195	83	125	4
Milk and butter added	1 cup	195	80	185	4
Potato chips medium, 2" diameter.	10 chips	20	2	115	1
Pumpkin, canned	1 cup	228	90	75	2
Radishes, raw, small, without tops	4	40	94	5	tr.
Sauerkraut, canned, solids and liquid	1 cup	235	93	45	2
Spinach:					
Cooked	1 cup	180	92	40	5
Canned, drained solids	1 cup	180	91	45	5
Squash:					
Cooked:					
Summer, diced	1 cup	210	96	30	2
Winter, mashed	1 cup	205	81	130	4
Sweet potatoes:					
Cooked, medium, 5" x 2"					
weight raw about 6 ounces:					
Baked, peeled after baking	1 potato	110	64	155	2
Boiled, peeled after boiling	1 potato	147	71	170	2
Candied, 3½" X 2¼"	1 potato	175	60	295	2
Canned, vacuum or solid pack	1 cup	218	72	235	4
Tomatoes:					
Raw, 2" X 2½", 3/lb or 7-8 miniatures	1 tomato	150	94	35	2
Canned, solids and liquid	1 cup	241	94	50	2
Tomato aspic	½ cup	120	94	32	3
Tomato catsup	1 Tbsp.	15	69	15	tr.
Tomato juice, canned	1 cup	243	94	45	2
Tomato sauce, canned	¼ cup	68	82	79	1
Turnips (Yellow Rutabagas)					
Raw, sliced (3½" diam., ½" thick)	¾ cup	100	87	46	1
Cooked, cubes	½ cup	100	91	35	1
Vegetable juice, canned	1 cup	243	94	43	2
PICKLES					
Assorted, sweet	2 pieces	20	–	6	tr
Dill pickles (4" X 1¾")	1 pickle	135	93	15	1
Gherkins (2¾" X ¾")	1 pickle	20	61	30	tr
Olives – Black – large	2 large	10	73	15	tr
Green – medium	4 medium	16	78	15	tr
Relish	1 Tbsp.	13	–	18	tr
FRUIT AND FRUIT PRODUCTS					
Apples, raw (3 per pound) 2½" diam.[5]	1 apple	150	85	70	tr
Apple juice, canned, vitaminized	1 cup	248	88	120	tr
Applesauce, canned:					
Sweetened	1 cup	255	76	230	1
Unsweetened	1 cup	244	88	100	1
Apricots:					
Raw (about 12 per pound)[5]	3 apricots	114	85	55	1
Canned in heavy syrup	1 cup	259	77	220	2
Dried, uncooked (40 halves per cup)	1 cup	150	25	390	8
Cooked, unsweetened, fruit & liquid	1 cup	285	76	240	5
Apricot nectar, canned	1 cup	251	85	140	1
Avocados, whole fruit, raw[5]					
California (3⅛" diam.)	1 avocado	284	74	370	5
Florida (3⅝" diam.)	1 avocado	454	78	390	4
Bananas, raw, medium size[5], 7" - 8" long	1 banana	175	76	100	1
Blackberries, raw	1 cup	144	84	85	2
Blueberries, raw	1 cup	140	83	85	1

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
FRUIT AND FRUIT PRODUCTS (continued)					
Cantaloup, raw, medium 5" diam.[5]	½ melon	385	91	60	1
Cherries, canned, red, sour					
Pitted, water pack	1 cup	244	88	105	2
Cranberry juice cocktail	1 cup	250	83	165	tr.
Cranberry sauce, sweetened, canned, strained	1 cup	277	62	330	tr.
Dates, pitted, cut	1 cup	178	22	490	4
Figs, dried, large, 2" x 1"	1 fig	21	23	60	1
Fruit cocktail, canned, heavy syrup	1 cup	256	80	195	1
Grapefruit:					
Raw, medium 3¾" diam.[5]					
White	½ grapefruit	241	89	45	1
Pink	½ grapefruit	241	89	50	1
Canned, syrup pack	1 cup	250	81	180	2
Grapefruit juice:					
Fresh, white	1 cup	246	90	95	1
Canned, white:					
Unsweetened	1 cup	247	89	100	1
Sweetened	1 cup	250	86	130	1
Frozen, concentrate, unsweetened:					
Undiluted, can, 6 fluid ounces	1 can	207	62	300	4
Diluted with 3 parts water	1 cup	247	89	100	1
Grapes, raw:					
Canadian type (slip skin)	30 grapes	153	82	106	2
European type (adherent skin)	1 cup	160	81	95	1
Grape juice:					
Canned or bottled	1 cup	253	83	165	1
Frozen concentrate, sweetened					
Undiluted can, 6 fluid ounces	1 can	216	53	395	1
Diluted with 3 parts water	1 cup	250	86	135	tr.
Lemons, raw, 2½" diam.[5]	1 lemon	110	90	20	1
Lemon juice, raw	1 cup	244	91	60	1
Lemon juice, canned, unsweetened	1 cup	245	92	55	1
Lemonade concentrate:					
Frozen, 6 fl. oz per can	1 can	219	48	430	tr.
Diluted with 4½ parts water	1 cup	248	88	110	tr.
Lime juice:					
Fresh	1 cup	246	90	65	1
Canned, unsweetened	1 cup	246	90	65	1
Oranges, raw, 2⅝" diam.[5]	1 orange	180	86	65	1
Orange juice, fresh, all varieties	1 cup	248	88	110	2
Canned, unsweetened	1 cup	249	87	120	2
Frozen concentrate:					
Undiluted, can, 6 fl. oz	1 can	213	55	360	5
Diluted with 3 parts water	1 cup	249	87	120	2
Orange and grapefruit juice:					
Canned, sweetened	1 cup	251	–	130	1
Frozen concentrate:					
Undiluted, can, 6 fl. oz	1 can	210	59	330	4
Diluted with 3 parts water	1 cup	248	88	110	1
Peaches:					
Raw:					
Whole, medium, 2" diam. 4 per lb[5]	1 peach	114	89	35	1
Sliced	1 cup	168	89	65	1
Canned, solids and liquid:					
Syrup pack, heavy:					
Halves or slices	1 cup	257	79	200	1
Water pack	1 cup	245	91	75	1
Dried, uncooked	1 cup	160	25	420	5
Cooked, unsweetened	1 cup	270	77	220	3
Frozen, carton, 12 oz	1 carton	340	76	300	1
Pears:					
Raw, 3" X 2½" diam.[5]	1 pear	182	83	100	1
Canned, solids and liquid:					
Syrup pack, heavy:					
Halves or slices	1 cup	255	80	195	1
Pineapple:					
Raw, diced	1 cup	140	85	75	1
Canned, heavy syrup, solids and liquid:					
Crushed	1 cup	260	80	195	1
Sliced – large, with juice	1 slice	122	80	90	tr.
Pineapple juice, canned	1 cup	249	86	135	1
Plums:[5]					
Raw, 2" diam., about 2 oz	1 plum	60	87	25	tr.
Canned, syrup pack (with pits) and juice	1 cup	256	77	205	1
Prunes, dried, "softenized", medium:					
Uncooked[5]	4 prunes	32	28	70	1
Cooked, unsweetened	1 cup	270	66	295	2
Prune juice, canned or bottled	1 cup	256	80	200	1
Raisins, seedless:					
Packaged, ½ oz or 1½ tbsp. per pkg	1 pkg	14	18	40	tr.
Cup, pressed down	1 cup	165	18	480	4
Raspberries:					
Raw	1 cup	123	84	70	1
Frozen – 10 oz carton	1 carton	284	74	275	2
Rhubarb, cooked, sugar added	1 cup	272	63	385	1
Strawberries:					
Raw, capped	1 cup	149	90	55	1
Frozen, 10 oz carton	1 carton	284	71	310	1
Tangerines, raw, medium, 2⅜" diam.[5]	1 tangerine	116	87	40	1
Tangerine juice, frozen, concentrated:					
6 oz can	1 can	210	58	340	4
Diluted with 3 parts water	1 cup	248	88	115	1
Canned, unsweetened	1 cup	248	89	105	1
Watermelon, raw, wedge 4" X 8"[5]					
(1/16 of 10" X 16" watermelon) or					
slice (8¼" diam. X 1" thick)	1 wedge	925	93	115	2
Fruit and Fruit Flavoured Breakfast Drinks					
Canned or bottled with added Vitamin C					
Individual fruit flavors or combinations of two or more	1 cup	250	90	135	tr.
Made with crystals with added Vitamin C					
Orange, grapefruit, apple etc. (Start, Tang, etc.)	1 cup	248	–	135	tr.
CEREALS AND CEREAL PRODUCTS					
Barley, pearled, light, uncooked	1 cup	200	11	700	16
Biscuits, baking powder, 2" diam., enriched flour	1 biscuit	28	27	105	2
Bran flakes, with added nutrients	¾ cup	28	3	104	3
Branflakes, with raisins & nutrients	⅔ cup	28	3	100	2
Breads:					
Cracked Wheat	1 slice	30	35	77	3
Melba toast	1 piece	4	4	15	tr.
Raisin – 18 slices per loaf	1 slice	25	35	65	2
Rye, light	1 slice	30	35	73	3
Rye, dark, pumpernickel	1 slice	32	34	79	3
White, enriched, 24 slices per loaf	1 slice	30	35	82	2
White, enriched (Newfoundland)[3]	1 slice	30	35	82	2
Whole wheat (60% whole wheat)	1 slice	30	35	72	3
Breadcrumbs, dry, grated	1 cup	100	6	390	6
Cakes made from cake mixes:					
Angelfood:					
Piece, 1/12 of 10" diam. cake	1 piece	53	34	135	3
Devil's food, 2-layer, with chocolate icing					
Piece, 1/16 of 9" diam. cake	1 piece	69	24	235	3
Cupcake, small, 2½" diam.	1 cupcake	35	24	120	2
Gingerbread					
Piece, 1/9 of 8" square cake	1 piece	63	37	175	2
White, 2-layer, with chocolate icing:					
Piece, 1/16 of 9" diam. cake	1 piece	71	21	250	3
Cakes made from home recipes:					
Boston cream pie; piece 1/12 of 8" diam.	1 piece	69	35	210	4

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
CEREALS AND CEREAL PRODUCTS (continued)					
Fruitcake, dark, made with enriched flour:					
Slice, 1/30 of 8" loaf	1 slice	15	18	55	1
Plain sheet cake:					
Without icing:					
Piece, 1/9 of 9" square cake	1 piece	86	25	315	4
With boiled white icing:					
Piece 1/9 of 9" square cake	1 piece	114	23	400	4
Pound:					
Slice, ½" thick	1 slice	30	17	140	2
Sponge:					
Piece, 1/12 of 10" diam. cake	1 piece	66	32	195	5
Yellow, 2-layer, without icing:					
Piece, 1/16 of 9" diam. cake	1 piece	54	24	200	2
Yellow, 2-layer, with chocolate icing:					
Piece, 1/16 of 9" diam. cake	1 piece	75	21	275	3
Cookies:					
Brownies with nuts:					
Made from home recipe with enriched flour	1 brownie	20	10	95	1
Chocolate chip:					
Made from home recipe with enriched flour	1 cookie	10	3	50	1
Commercial	1 cookie	10	3	50	1
Chocolate Marshmallow	1 biscuit	19	–	75	1
Fig bars, commercial	1 cookie	14	14	50	1
Sandwich, chocolate or vanilla, commercial	1 cookie	10	2	50	1
Social Tea or Arrowroot	1 biscuit	5	2	20	tr.
Oatmeal, Dad's	1 biscuit	19	–	86	1
Cornflakes:					
Plain	1 cup	21	4	80	2
Sugar coated	1 cup	28	2	107	1
Cornflakes, added nutrients	1 cup	21	4	80	2
Cornflakes, presweetened, added nutrients	1 cup	28	2	107	1
Cornmeal, degermed, dry form	1 cup	138	12	500	11
Corn muffins, made from mix	1 muffin	40	33	125	3
Corn, puffed, presweetened[1]	1 cup	28	2	108	1
Corn, puffed, presweetened, added nutrients	1 cup	28	2	110	1
Corn, shredded, added iron	1 cup	28	4	104	2
Crackers:					
Graham – 2½" square	4 crackers	28	6	110	2
Saltines 2" square	4 crackers	11	4	50	1
Cream of Wheat (see Farina 379)					
Danish pastry, plain, round 4½" diam. x 1"	1 pastry	65	22	275	5
Date Squares	1 square	90	–	226	2
Doughnuts, cake type, 3" diam.	1	32	24	125	1
Eclairs, chocolate, custard filling	1	110	–	315	8
Farina, quick cooking, cooked:					
Enriched (e.g. Cream of Wheat)	1 cup	245	89	105	3
Unenriched	1 cup	245	89	105	3
Macaroni, cooked	1 cup	140	72	155	5
Macaroni and cheese, baked	1 cup	220	58	430	18
Muffins, with enriched white flour:					
Muffin 3" diam.	1 muffin	40	38	120	3
Muffins, bran	1 muffin	35	–	86	3
Noodles (egg noodles), cooked, unenriched	1 cup	160	70	200	7
Oats, puffed, with or without corn	1 cup	25	3	100	3
Oats, puffed, presweetened	¾ cup	25	2	107	2
Oatmeal or rolled oats, cooked	1 cup	240	87	130	5
Oatmeal, dry, regular or quick cooking	1 cup	80	–	312	11
Oatmeal, dry, instant (¾ c. cooked)	1 envelope	28	–	107	5
Pancakes, 4" diam.:					
Plain, made from mix with egg and milk	1	27	50	60	2
Buckwheat, made from mix with egg and milk	1	27	58	55	2
Pie (piecrust made with enriched flour):					
Sector, 4", 1/6 of 9" pie					
Apple (2-crust)	1 sector	160	–	410	3
Blueberry (2-crust)	1 sector	160	–	387	4
Cherry (2-crust)	1 sector	160	–	387	4
Custard (1-crust)	1 sector	150	–	327	9
Lemon meringue	1 sector	140	–	357	5
Mince (2-crust)	1 sector	160	43	434	4
Peach (2-crust)	1 sector	165	–	421	4
Pumpkin (1-crust)	1 sector	150	–	317	6
Raisin	1 sector	120	–	325	3
Piecrust, baked shell for pie, enriched flour	1 shell	135	15	675	8
Pizza (cheese) ⅛ of 14" diam. pie	1 sector 5½"	75	45	185	7
Pizza (sausage) ⅛ of 14" diam. pie	1 sector 5½"	105	45	315	14
Popcorn, popped:					
With oil and salt	1 cup	9	3	40	1
Sugar coated	1 cup	35	4	135	2
Pretzels:					
Dutch, twisted	1 pretzel	16	5	60	2
Stick, regular, 3⅛"	5 sticks	3	5	10	tr.
Puff Pastry – 10 shells – 4" diam.	10 shells	284	–	1198	14
Rice, brown, cooked (¼ c. raw)	1 cup	160	71	198	4
Rice, white:					
Unenriched, cooked, short grain (¼ c. raw)	1 cup	170	73	215	4
Parboiled, cooked (converted)(¼ c. raw)	1 cup	160	73	185	4
Fried, unenriched, chicken	1 cup	185	73	286	3
Rice, puffed or oven popped	1 cup	20	4	78	1
Puffed or oven popped with added nutrients	1 cup	28	4	106	2
Puffed or oven popped, presweetened	1 cup	28	2	120	1
Puffed or oven popped, presweetened with added nutrients	1 cup	28	4	120	1
Shredded, with added nutrients	1 cup	28	4	108	2
Rolls, enriched:					
Cinnamon	1 roll	50	31	158	3
Hamburger	1 roll	60	31	164	5
Hot dog	1 roll	50	31	137	5
Spaghetti, cooked	1 cup	140	72	155	5
Spaghetti, with meat balls and tomato sauce	1 cup	248	70	330	19
Spaghetti, with tomato sauce and cheese, canned	1 cup	250	80	190	6
Spaghetti, Italian, with meat sauce	1 cup	292	–	396	13
Waffles, made from mix with egg and milk	7" diam. waffle	75	41	210	7
Wheat, flakes	1 cup	28	4	105	3
Wheat flakes, with added nutrients	1 cup	28	4	105	3
Wheat puffed	1 cup	15	3	55	2
Wheat puffed, presweetened	1 cup	21	3	80	1
Wheat puffed, presweetened with added nutrients	1 cup	21	3	80	1
Wheat flours					
Whole wheat	1 cup	120	12	400	17
All purpose, enriched	1 cup	110	12	386	13
Cake	1 cup	100	12	350	9
Wheat, shredded	1 biscuit	25	7	80	1
1 biscuit = 12 spoon size					
Wheat, shredded, with added nutrients	2/3 cup	30	35	138	3
FATS AND OILS					
Butter:	½ cup	113	16	812	1
Tablespoon	1 Tbsp.	14	16	100	tr.
Pat (90 per lb)	1 pat	5	16	35	tr.
Fats, cooking:					
Lard	1 cup	205	0	1850	0
Lard	1 Tbsp.	13	0	115	0
Vegetable fats (shortening)	1 cup	200	0	1770	0
Vegetable fats (shortening)	1 Tbsp.	13	0	110	0
Margarine:					
Regular	½ cup	113	16	815	1
Regular	1 Tbsp.	14	16	100	tr
Soft, 2-8 oz tubs per pound	1 tub	227	16	1635	1
Soft, 2-8 oz tubs per pound	1 Tbsp.	14	16	100	tr.
Oils, salad or cooking, corn	1 cup	220	0	1945	0
Oils, salad or cooking, corn	1 Tbsp.	14	0	125	0

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
FATS AND OILS (continued)					
Salad dressings:					
Blue cheese	1 Tbsp.	15	32	75	1
Commercial, mayonnaise type	1 Tbsp.	15	41	65	tr.
French:					
Regular	1 Tbsp.	15	16	59	tr.
Low calorie	1 Tbsp.	16	95	13	tr.
Home cooked, boiled	1 Tbsp.	16	68	25	1
Mayonnaise	1 Tbsp.	14	15	100	tr.
Thousand Island	1 Tbsp.	16	32	50	tr.
SUGARS AND SWEETS					
Cake icings:					
Chocolate made with milk and fat	1 cup	275	14	1035	9
Creamy fudge from mix with water only	1 cup	245	15	830	7
White, boiled	1 cup	94	18	300	1
Candy:					
Caramels, plain or chocolate 1 = 10 g	1 oz	28	8	115	1
Chocolate, bitter or baking	1 oz	30	2	145	3
Chocolate, milk, plain	1 oz	28	1	145	2
Chocolate coated peanuts	1 oz	28	1	160	5
Chocolate fudge	1 oz	28	8	112	1
Chocolate, baking, sweet	1 oz	28	1	150	1
Gum drops	1 oz	28	12	100	tr.
Hard 1 = 5 g	1 oz	28	1	110	0
Marshmallows 1 = 8 g	1 oz	28	17	90	1
Milk chocolate bars:					
All varieties	1 oz	28	–	134	3
"Caravaa", "Caramilk" type	1 oz	28	8	116	1
"Oh Henry" type	1 oz	28	7	129	3
Chocolate flavoured syrup:					
Thin type	1 fl. oz	38	32	90	1
Fudge type	1 fl. oz	38	25	125	2
Chocolate-flavoured beverage powder (4 heaping teaspoons per ounce):					
With skim milk powder	1 oz	28	2	100	5
Without skim milk powder	1 oz	28	1	100	1
Honey strained	1 Tbsp.	21	17	65	tr.
Jams and preserves	1 Tbsp.	20	29	55	tr.
Jellies	1 Tbsp.	18	29	50	tr.
Molasses, cane:					
Light (first extraction)	1 Tbsp.	20	24	50	–
Blackstrap	1 Tbsp.	20	24	45	–
Syrups:					
Table blends	1 Tbsp.	21	24	60	0
Maple	1 Tbsp.	21	–	50	0
Sugars:					
Brown – firm packed	1 cup	220	2	820	0
White:					
Granulated	1 cup	200	tr.	770	0
Granulated	1 Tbsp.	11	tr.	40	0
Powdered, stirred before measuring	1 cup	120	tr.	460	0
MISCELLANEOUS ITEMS					
Barbecue Sauce	1 cup	250	81	230	4
Beverages, alcoholic:					
Beer	12 fl. oz	360	92	150	1
Gin, rum vodka, whiskey	1½ fl. oz	42	–	105	–
Wines:					
Dessert	3½ fl. oz	100	–	137	–
Table	3½ fl. oz	100	–	85	–
Beverages, carbonated, sweetened:					
Carbonated water (e.g. Tonic)	12 oz	366	92	115	0
Cola type	12 oz	369	90	145	0
Ginger ale	12 oz	366	92	115	0
Bouillon cubes, approx. ½"	1 cube	4	4	5	1
Chop suey, with meat or chicken	1 cup	155	–	180	16

Food	Measure	Weight	Moisture	Food Energy	Protein
		g	%	Cal	g
MISCELLANEOUS ITEMS (continued)					
Egg roll (pork)	2	146	–	479	7
Gelatin:					
Plain, dry powder in envelope	1 envelope	7	13	25	6
Dessert powder, 3 oz pkg	1 pkg	85	2	315	8
Gelatin dessert, prepared with water	½ cup	120	84	80	2
Popsicle, 3 fl. oz size	1 popsicle	95	80	70	1
Soups:					
Canned, condensed, ready-to-serve:					
Prepared with an equal volume of milk:					
Cream of chicken	1 cup	245	85	180	7
Cream of mushroom	1 cup	245	83	215	7
Tomato	1 cup	250	84	175	7
Prepared with an equal volume of water:					
Bean with pork	1 cup	250	84	170	8
Beef broth, bouillon consomme	1 cup	240	96	30	5
Beef noodle	1 cup	240	93	70	4
Clam chowder, Manhattan type (with tomatoes, without milk)	1 cup	245	92	80	2
Cream of chicken	1 cup	240	92	95	3
Cream of mushroom	1 cup	240	90	135	2
Minestrone	1 cup	245	90	105	5
Split pea	1 cup	245	85	145	9
Tomato	1 cup	245	90	90	2
Vegetable beef	1 cup	245	92	80	5
Vegetarian	1 cup	245	92	80	2
Dehydrated, dry form:					
Chicken noodle (2-oz package)	1 pkg	57	6	220	8
Onion mix (1½ oz package)	1 pkg	43	3	150	6
Tomato vegetable with noodles (2½-oz pkg)	1 pkg	71	4	245	6
Snack Foods:					
Puffs, cheese flavoured, etc.	1 cup	28	–	160	2
Chips, shapes, etc. (corn)	1–1½ cup	28	–	160	2
White Sauce, medium	1 cup	250	73	405	10
Yeast:					
Baker's, dry, active	1 pkg	7	5	20	3
Brewer's, dry	1 Tbsp.	8	5	25	3

APPENDIX B

BIOLOGY 1A6/1B7

LABORATORY MANUAL

McMASTER UNIVERSITY

DEPARTMENT OF BIOLOGY

1983-1984

1A6/1B7 LAB SCHEDULE

BIOLOGY 1A6/1B7 1983-84

Professors: Term I - Dr. S.F.H. Threlkeld - Room 541
Term II - Dr. C.M. Wood - Room 528

Lab Coordinator: Mrs. M.A. Service - Room 330

Text: Biological Science 3rd edition Keeton

Laboratory Requirements:

Drawing pencil - 3H Berol turquoise	.79
Biology drawing paper - pkg.	.30
Dissecting Kit	4.25
- better quality (optional)	19.85

Coloured pencils (2 or 3 for graphs)
Metric ruler (included in 4.25 dissecting kit)
All available at McMaster bookstore

Grading System: Suggested weightings of course components are listed below. However, final marks for this course are based on a total assessment of each student's record.

Weekly lab reports	- 20%	
Multiple choice quizzes	- 30%	(5 each term)
Christmas exam	- 25%	(Final, term I)
Final exam (Term II)	- 25%	

INTRODUCTION

Science is concerned with the material universe, seeking to discover facts about it and to fit these into theories or laws to clarify our understanding of the universe. Science deals only with things which may be observed or tested.

The scientific method has been developed over many years as the universally accepted procedure for scientific investigation. Its steps are as follows:

1. Formulate the question for which you want the answer.
2. Make detailed, honest observations in order to collect relevant data or information.
3. Analyze these data by fitting it into some coherent pattern or generalization i.e. a hypothesis.
4. Test this hypothesis by reliable methodology.
5. Analyze the test results and modify or change the hypothesis according to observable facts or evidence.

A good scientist should keep an open mind, and be prepared to alter or abandon any hypothesis if new facts are found which are contradictory.

A hypothesis which is able to stand the test of time and continuously be proven correct, may eventually be given the label of Theory or Law, but must always be dependent upon observable fact and not vice versa.

Limitations of the scientific method are that it cannot make value or moral judgments as such, but it can supply the information or evidence by which intelligent judgments may be made.

"People developed from monkeys who fell off the tree and couldn't climb back."

1A6/1B7 PERSPECTIVE

The laboratory exercises in this manual are designed to introduce students to the discipline of Biology as it is being practised in this department and in other research-oriented universities or industry.

Attempts will be made to relate the basic concepts and techniques of laboratory exercises to the more complex methodology being practised in working labs by career biologists. Whenever possible, we will acquaint you with the actual equipment being used and if available, examples will be of fresh or living material.

The labs are held simultaneously in Rooms 107, 108, 109, and 110 of the Life Sciences Building according to the schedule on page i. Each will be preceded by a pre-lab briefing in Room 1A6 of the Health Sciences Building (attached to Life Sciences by a skywalk at the 2nd floor level). These are all 3 hour labs from 2:30 to 5:30 p.m. Attendance at labs and pre-lab briefings is mandatory. In order to derive the most benefit from them, always read the lab outline in advance as well as textbook references and relevant lecture notes.

LAB REPORTS

A formal lab report is to be handed in by noon, one week following each lab. Your demonstrator will evaluate it out of 10, make suitable comments for improvement and return it to you during the next lab period. Reports which are handed in late will not be marked. Boxes for these reports, specifically marked for 1A6/1B7, are in the hall outside the lab rooms.

Guidelines

Every report should have your name, your demonstrator's name, the day you regularly come for labs and the title on the outside page. Do them on notepaper to be kept in the binder with lab instructions and/or notes.

Reports should not exceed 4 pages (preferably 3) of written material, single spaced, plus extra pages for graphs, tables or drawings. Measurements are always in metric. e.g. mm - millimeters

ml - milliliters

kg - kilograms

Always include the unit of measurement used. When making graphs or tables, give each

a reference number for referral in the body of the report, as well as a complete and descriptive title.

Formal lab reports should follow the standard format used in scientific journals, which reflect the steps of the scientific method outlined on page iii. The report should have 5 dis-tinctly marked sections:

Purpose and Introduction

The purpose is a brief statement in your own words which outlines what the exercise set out to achieve. It should not be a repetition of the objectives.

The introduction gives relevant background material which shows how the subject of the report fits into the general scheme of Biology. It supplies the framework of your results and defines the scope of the study.

Materials and Methods

This section of the report should briefly outline the way you carried out the experiment. Usually, however, the lab manual has presented a complete description of the materials and methods used, so if no changes were made in the procedure, simply refer to the lab manual under this heading. If any changes were made, they should be described. Whenever appropriate, you should include a diagram of the apparatus used.

Results

In paragraph form, and using headings as well as subheadings to clarify references for the reader, report precisely and concisely the results which you obtained, whether in the form of observations or of data. Take time to organize these results into a form which is clear and understandable to the reader. Charts or graphs are useful, but should be summarized to point out trends. Point form is acceptable for details or series of features, and frequently saves space.

Any calculations of numerical data should be done here and should include all of the steps involved in the production of results.

Drawings or diagrams are part of your observations. Include them on drawing paper at the end of the report, but refer to each in the body of this section.

The quality of scientific work depends to a large extent, on the accuracy and insight of the observations. Do not include any discussion or interpretation of the

results under this heading --just the facts.

Discussion

The discussion is the most important part of the report. This is where you interpret the results obtained and draw inferences or deductions from the findings or observations. The logic and quality of your reasoning are the principal ingredients of the discussion.

If any specific questions were asked in the manual or by your demonstrator, work the anwers into your discussion.

- What is the significance of the results?
- Did they support or disprove your original hypothesis?
- Relate the results to the background material of section 1 or to any other references.
- Do they have any implication beyond the original purpose?

Briefly discuss the reliability of the results. If something went wrong, attempt to explain why, and if there were any ambiguities, talk about them. Keep your discussion as brief and as logical as possible, always including the reasoning behind each point.

Summary and Conclusions

Conclusions should be brief statements which summarize the most important implications from the discussion and should reflect the original purpose or introduction. Sometimes the conclusion may be a generalization.

At the end of your report you must refer to all the sources of information which you have used. For this course, refer only to those sources other than the assigned textbook.

LAB I EVOLUTION, CLASSIFICATION, AND IDENTIFICATION OF UNKNOWN SPECIMENS

Objectives: Upon completion of this lab you should be able to:

1. Describe the probable conditions of the earth's atmosphere which existed at the time of the origin of life according to current hypotheses.

2. Outline Darwin's Theory of Evolution.

3. Describe the 1953 experiments of Miller and Urey and explain their significance.

4. Understand the binomial system of classification.

5. Use a dichotomous key to identify an unknown invertebrate and an evergreen.

6. Understand the scientific method in order to apply it to future investigation.

7. Write a scientific report by the accepted format.

A THEORY OF EVOLUTION

In 1859 Charles Darwin published his famous book "The Origin of the Species", the result of a meticulously detailed investigation conducted according to the tenets of the scientific method outlined on page iii. Darwin's theory consists of two major parts:

1. Organisms or creatures which are alive today have descended by gradual changes from ancient ancestors, frequently very unlike themselves.
2. The guiding factor to evolutionary change is natural selection which occurs continuously without purpose or design, and is based upon the following basic assumptions:

   - more offspring are born than will survive and reproduce
   - because of genetic variation, offspring exhibit a range of inherited characteristics, some being advantageous for the specific environment and some being

disadvantageous.

- within nature, well-adapted individuals survive and reproduce, often passing advantageous characteristics to their progeny.
- poorly adapted individuals are eliminated.

B CLASSIFICATION (TAXONOMY)

Since the simplest forms of life arose, innumerable different kinds of increasingly complex organisms have evolved and have become adapted to the wide variety of different environments to be found on the earth.

To deal with all this organic diversity, some sort of organization was needed by which individuals could be classified in a logical and meaningful way. There are many different systems of classification which could be used, but the system generally employed in Biology today is called the Binomial System of nomenclature which utilizes evolutionary characteristics or phylogenetic relationships among the various groups as its basis, and includes a hierarchy of categories (taxa), each of which is more precise than the category (taxon) above it. The last two taxa are the most precise and give to the organism its specific scientific name which, by convention, is always underlined. The hierarchy of taxa used should be memorized. They are as follows:

Kingdom
Phylum
Class
Order
Family
Genus
Species

The classification system used by our textbook recognizes five broad categories of kingdoms -- the Monera, Protista, Plantae, Fungi and Animalia. Each of these kingdoms may be separated into several phyla, differing one from another in some important aspect. Each phylum may be separated into several different classes on the basis of other characteristics, and so on until the genus and species (scientific name) is reached which identifies that particular individual as precisely as possible. Thus a genus is a group of closely related species; a family is a group of

related genera; an order is a group of related families etc.

A species is a genetically distinctive population within which interbreeding occurs i.e. there is a free exchange of genetic material. Interbreeding between members of closely related species is rare and if offspring are produced, they are usually sterile e.g. horse and donkey to produce a mule.

Anatomical, physiological and behavioural characteristics may only be considered as "clues" in the determination of a species because the final criterion is always reproduction.

Species do not remain static however -- they are dynamic, every generation leading to divergence in characteristics. If interbreeding of a single population is prevented by some geographic barrier, over time it may become two or more distinct species. Factors contributing to this speciation could be the occurrence of a mutation in one group, or exposure to different environmental selection pressures.

The evolutionary splitting of populations into many separate descendent species is called divergent evolution. The bewildering number of species of insects now seen, or differences among the finches on the Galapagos Islands are the direct result of divergent evolution.

The evolutionary history of any group of organisms is called its phylogeny.

C DICHOTOMOUS TAXONOMIC KEYS

Anyone working in field biology must learn how to use taxonomic keys to identify different plants and animals. The use of a key also points up the major differences or similarities that exist within groups of plants or animals.

A dichotomous key offers two brief contrasting choices at each step which compare structural features that offer apparent differences between taxa. By moving down through the hierarchy of alternatives until all possibilities but one are eliminated, you should reach the name of the Order to which the invertebrate specimen belongs, or the common name of the evergreen.

Procedure - work in pairs

1. The Invertebrate - Many of these organisms have burrowed into the substrate (the gravel on the bottom) or are hiding under rocks etc. Gently move the rocks to find an organism, but disturb them as little as possible.

With forceps, (or hands) take one specimen from the aquarium and place it in a watch glass. If it will not remain still long enough for careful observation, place it in a vial of alcohol for a few seconds and return it to the watch glass.

Use the dissecting microscope to observe details of the specimen's structure. At step 1 of the key, choose the most appropriate of the two choices 1a or 1b. When you have made your decision, move next to the number indicated at the end of the line of your choice. You will again find two alternative choices a or b. Choose whichever best fits your organism and move next to the number indicated at the end of the line of that choice. Continue in this manner until your choice gives you the name of the order to which your animal belongs.
If you get stuck, or do not understand the wording of the key, ask your demonstrator for help. While observing your specimen, remember that its features represent an adaptation to its habitat (where it lives). Think about how these features improve its chances of survival in its niche (position in the ecosystem), and record as many features as possible along with the rationale for each, to be included in your report. The inter-relationship of form and function is a basic concept of Biology and an understanding of one is always helpful in predicting or understanding the other. Record the identification you have made and make a simple sketch on drawing paper for your report and for future reference. If the specimen is still alive, return it to the aquarium, otherwise into the garbage pail.

2. The Evergreen

Choose an evergreen branch and using the same procedure as before, key it out to its common name.

Record the common name for your report along with a descriptive paragraph outlining the important identifying features.

General Key to Orders of Aquatic Insects

1.a) Wings or rudimentary wings present; legs present 2
b) Wings or rudimentary wings absent; legs either present or absent 9

2.a) Wings fully developed ..... ADULTS 3
b) Rudimentary wings present ..... NAIADS or PUPAE 5

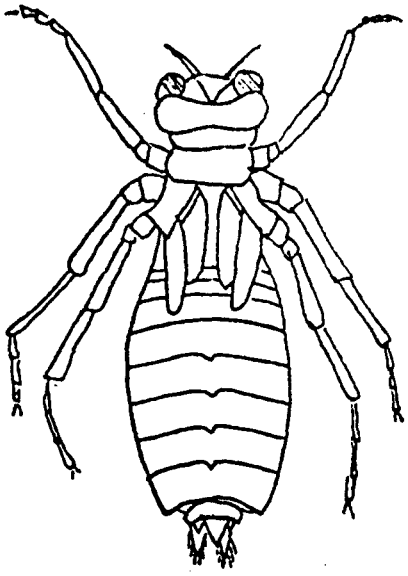
3.a) Anterior wings all or partly hard and leathery; posterior wings membranous 4
b) All wings membranous ..... NON AQUATIC INSECTS (present by accident)

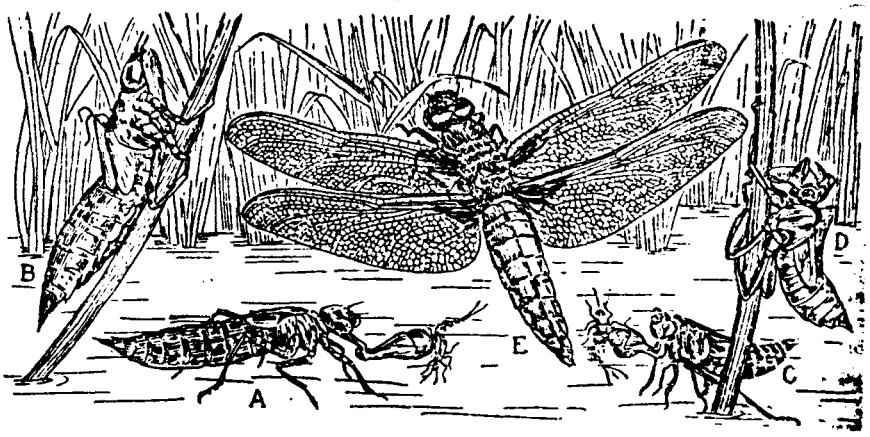
4.a) Anterior wings all leathery or hard, meet along middorsal line when folded; mouthparts are of the chewing type with a distinct mandible; antennae of 11 segments ..... COLEOPTERA (beetles)
b) Only basal part of anterior wing is leathery or hard; mouthparts jointed and elongated to form a tube for piercing or sucking; wings of adults are crossed when at rest leaving a triangular area between wings at anterior .. HEMIPTERA (true bugs)

5.a) Active with freely movable legs ..... NAIADS (larval stages of aquatic insects 6
b) Inactive; limbs drawn up or fused to body; sometimes in capsule-like cases ..... PUPAE (not keyed out)

6.a) Mouthparts elongated for piercing and sucking ..... HEMIPTERA (true bugs)
b) Chewing mouthparts with distinct mandibles 7

7.a) Anterior mouthparts (labium) mask-like, or scoop-like, covering lower part of head; eyes large; no cerci ..... ODONATA (dragon flies)



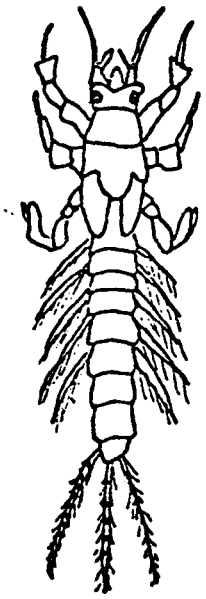


—Order ODONATA. Dragon-flies. *Aeschna: A.* Nymph capturing prey with labium extended. *B.* Nymphal skin. *Libellula: C.* Nymph. *D.* Nymphal skin. *E.* Adult at rest with wings extended. (*After Brehm.*)

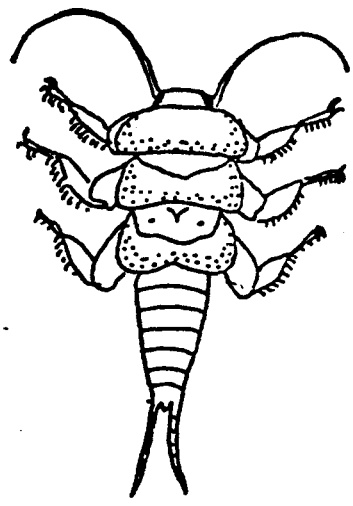
b) Mouthparts (labium) not larger than head 8

8.a) Tarsus (terminal part of leg) with one claw; abdomen with 2 or 3 terminal jointed spines; feather-like gills may extend from sides of abdomen .....
EPHEMEROPTERA (may flies)

b) Tarsus has 2 claws; abdomen has 2 terminal jointed spines; finger-like gills extend from the base of the mouthparts or from the base of each leg ....
PLECOPTERA (stone flies)



Order Ephemeroptera



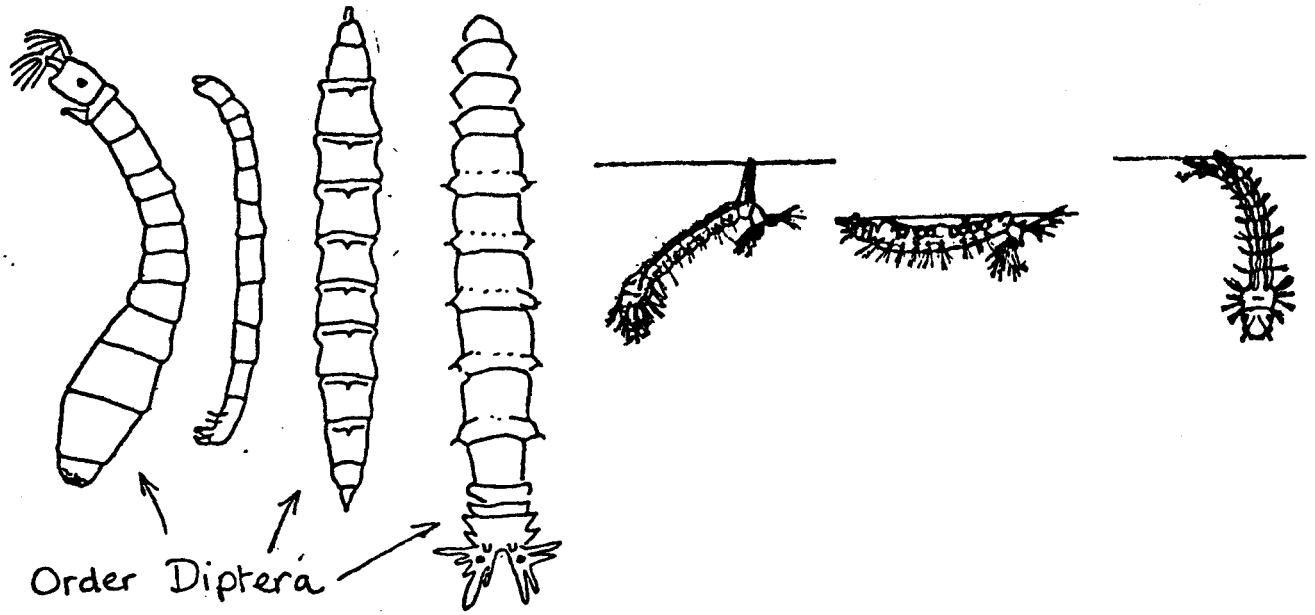
Order Plecoptera

9.a) Abdomen with 6 or fewer segments; ventral springing apparatus on 4th abdominal segment, held by hook on 3rd segment ..... COLLEMBOLA (Springtails)

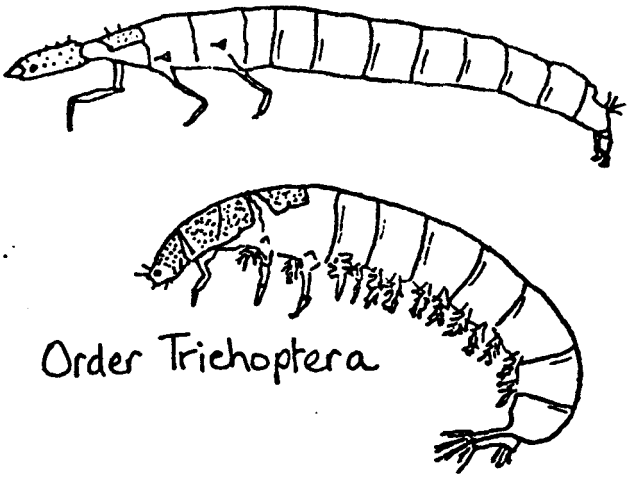
b) Abdomen with more than 6 segments 10

10.a) With 3 pairs of jointed legs on thorax 11

b) Without jointed legs on thorax; leg-like, non-jointed protuberances (prolegs) may be visible on thorax .....DIPTERA



11.a) Last abdominal segment with a pair of lateral appendages bearing anal hooks; antennae are 1-segmented and inconspicuous ..... TRICHOPTERA (caddis flies)

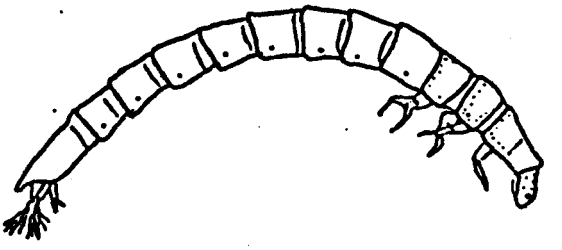


Order Trichoptera

b) Last abdominal segment without anal hooks (or, if present, antennae have 2 or more segments) 12

12.a) Abdominal segments each with a pair of lateral filaments; posterior segment has long single filament ..... MEGALOPTERA (dobson fly)

b) Abdomen usually without lateral filaments; caudal appendage is paired or absent ..... COLEOPTERA (beetles)

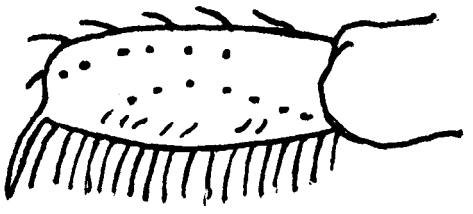


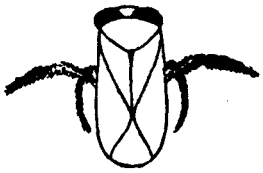
Order Coleoptera

Order Coleoptera

Classes of Order Hemiptera (true bugs)

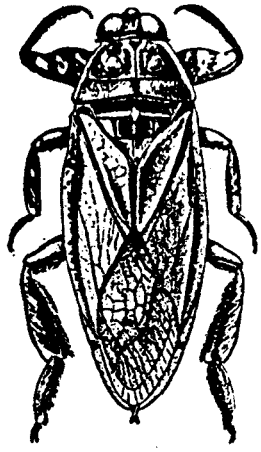
Corixidae - (Water boatman) - beak triangular and very short
-foreleg tarsus a single scoop-like segment fringed with stiff setae





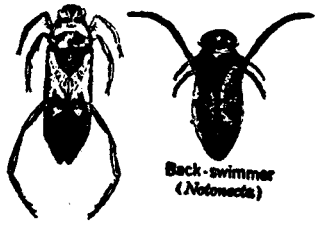
- swim upright by fringed oar-like hind legs.

Nepidae (water scorpion) slender and stick-like
- long anal breathing tubes.
- crawl in shallow waters

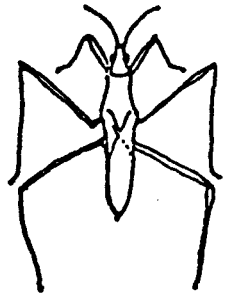


Belostomatidae (giant water bug)
- large size, heavy body
- up to 35 mm.

Notonectidae (back swimmers)
- swim by long oarlike hind legs
- antennae 3 or 4 segmented
- body elongate > 5 mm

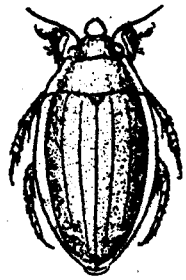


Gerridae (water striders)
- delicate, long legged
- walk on surface film of water



Classes of Order Coleoptera

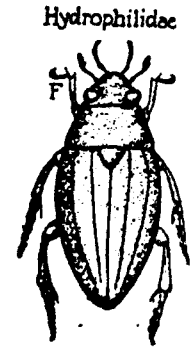
Dytiscidae - (predaceous diving beetle)
- to 37 mm long
- oval, smooth, shiny
- hind legs flat, fringed with hairs which serve as oars in swimming
- air stored under wings when diving



Gyrinidae (whirligig beetles)
- small, oval, lustrous
- skate rapidly on water surface

Hydrophilidae (water scavengers) < 27 mm

- resemble Dytiscidae but are black
- lengthwise keel under body



A Key to the Conifers

1.a) Leaves are in clusters 2
b) Leaves are borne singly along the stem 13

2.a) More than 5 leaves in a cluster 3
b) Leaves occur in clusters of 2 to 5 4

3.a) Leaves are deciduous (fall off in winter)
- are soft anf flattened ..... Tamarack (larch)
b) Leaves are persistent, stiff, and 4 sided..... True cedar

4.a) Leaves mostly 5 in a cluster ..... White Pine
b) Leaves 2-3 in a cluster 5

5.a) Leaves mostly 3 in a cluster 6
b) Leaves mostly 2 in a cluster 8

6.a) Twisted needles, less than 5 in long ..... Pitch Pine
b) Straight needles, more than 5 in long 7

7.a) Cones very thorny ..... Loblolly Pine
b) Cones not thorny .....Longleaf Pine

8.a) Limbs orange in colour ..... Scotch Pine
b) Limbs not conspicuously orange 9

9.a) Needles more than 3 in long 10
b) Needles less than 3 in long 11

10.a) Bark a distinctive grey in colour ..... Austrian Pine
b) Bark red-brown or brown ..... Red Pine

11.a) Cones have very long thorns ..... Mountain Pine
b) Cones have tiny thorns or no thorns 12

12.a) Twigs not whitened ..... Jack Pine
b) Twigs are whitened ..... Scrub Pine

13.a) Leaves are scale-like or triangular and opposite or whorled 14
b) Leaves needle-like or flattened 17

14.a) Branchlets flattened; all leaves scale-like and opposite ..... White Cedar
b) Branchlets not flattened 15

15.a) Leaves needle-like to triangular in tight spirals ..... Monkey Puzzle Tree
b) Leaves needle-like in whorls or pairs 16

16.a) Leaves 3-sided in whorls of 3 on 3-sided twigs ..... Dwarf Juniper
b) Leaves 3-sided in pairs in 4 rows on 4-sided twigs ..... Red Cedar

17.a) Leaves soft on green branchlets (like compound) 18
b) Leaves rigid on brown branchlets (solitary) 20

18.a) Leaves dark green and persistent ..... Redwood
b) Leaves light yellow-green, deciduous 19

19.a) Leaves to 1 inch, subopposite ..... Dawn Redwood
b) Leaves less than 1 inch, alternate ..... Bald Cypress

20.a) Leaves 4-sided with persistent woody petiole 21
b) Leaves flattened in cross section 25

21.a) Young twigs are hairy 22
b) Young twigs are not hairy 23

22.a) Leaves are dark or yellow green ..... Red Spruce
b) Leaves may be blue green with white powder ..... Black Spruce

23.a) Leaves are sharp and at right angles to the stem ..... Blue Spruce
b) Leaves point forward 24

24.a) Leaves dark green and branchlets droop ..... Norway Spruce
b) Leaves blue green and branchlets do not droop ..... White Spruce

25.a) Leaves do not have stomata underneath; seed in red berry (shrub) 26
b) Leaves have 2 lines of stomata (tree) 27

26.a) Leaves 3/4 inch or less with inconspicuous midrib ..... American Yew
b) Leaves 1 inch or longer with prominent midrib ..... Japanese Yew

27.a) Leaves round tipped, white beneath, attached by slender stalks;
twigs rough ..... Eastern Hemlock
b) Leaves pointed, green beneath, not attached by stalks;
twigs smooth ..... Balsam

The Dissecting Microscope

The dissecting (or stereoscopic) microscope magnifies specimens to approximately 50 times their actual size with clarity. It is used to examine large specimens or when doing precise dissections. The image is seen to be upright.

Objects may be viewed by reflected light when the bulb is mounted above the stage, or by transmitted light when the bulb is placed under the stage.

Dissecting scopes are binocular i.e. they have two oculars which may be moved closer together or further apart until you feel comfortable looking into the microscope. The two fields of view should overlap completely so that you see a single circle of light. In addition, one of the oculars is adjustable by rotation of its focussing sleeve -- a useful feature for students requiring more correction for one eye than the other. Proper adjustment of the oculars will give a single field of view and a 3-dimensional image.

The nosepiece of these scopes is also adjustable by rotation of the control knob from 0.63, to 1.0, 1.6, 2.5 or 4X. With some instruments, the whole nosepiece slides either forward or back to give the low or high powered objective.

Most dissecting scopes have a removable plate on the stage, either frosted to transmit light from below or reversible black and white for maximum background contrast.

Guide for Laboratory Drawings and Diagrams

1. Use good quality unlined white paper, from the bookstore.
2. Use a sharp 3 H pencil and a rule to keep labels and label lines horizontal and straight.
3. Leave an area 1.5 cm wide around the edge of the paper free from printing or drawing.
4. Place the classification in the upper left hand corner when it is known. This should include the phylum, class, order, family, and scientific name (genus and species). Capitalize each of these taxa except the species. Always underline the scientific name e.g. Homo sapiens. If the species name is not known, substitute sp. in which case it is not underlined. e.g. Amoeba sp.
5. Put your name, course and date in the upper right hand corner. Each goes on a separate line in vertical order.
6. Labels go in a vertical line down the right hand side of the page under your name. Use lower case letters for labels unless using a proper name e.g. Golgi body.
7. The drawing itself is centered in the remaining area of the page.
8. Use solid, precise lines to outline the specimen. Avoid shaggy, imprecise outlines which do not link up with each other.
9. Proper laboratory drawings use stippling to give depth of colour. We do not require stippling in 1A6/1B7 but any shading must be carefully done.
10. All label lines should be horizontal. They should never cross one another. If congestion is inevitable, it is permissible to slant the inner end of a line.
11. Under each drawing, print a legend to explain what the drawing shows, whether the specimen was alive or preserved, the view, where it was found, and the magnification. The form of the legend is one or two sentences.
12. Neatness is important; it will be considered when drawings are marked.

LAB II CHEMICAL BASIS OF BIOLOGICAL SYSTEMS

Objectives: Upon completion of this lab you should be able to:

1. Understand how molecular biologists approach their particular field of interest.

2. Explain the theory behind the process of chromatography and electrophoresis.

3. Define the term functional group and be able to identify molecules on the basis of their functional groups.

4. Recognize the four main classes of organic molecules.

5. Describe how to perform simple laboratory tests to identify specific biological molecules.

6. Show how one is able to use paper chromatography to separate individual amino acids from a mixture.

Cells are the microscopic units of structure of all living things. Each one is an independent functional unit and the processes of the body are the sum of the coordinated functions of all its individual cells.

Much has been learned in the last two decades about the various parts of cells by a variety of different techniques. Cytologists use sophisticated light microscopes or electron microscopes to look at cells and their components. Biochemists, however, use chemical analysis to study the structure and properties of subcellular components. To accomplish this, the cells must first be broken open to release their contents. The contents are then separated and purified before analysis can begin.

1. Cellular Homogenization is the process used to disintegrate individual cells, either for example mechanically with a Potter homogenizer or by ultrasonic waves which generate vibrations to rupture cell membranes or the cell walls of plants.
2. Separation is the process which separates the suspension into its various fractions.

a.) The Ultracentrifuge utilizes the difference in mass or density to spin out the components. Heavier particles will be deposited first on the outer wall of centrifuge tubes and may then be removed. Increased speed will collect the next heaviest particles and so on.

b.) Density Gradient Columns have been established which are very stable. The suspension is added to a column which is then rotated. Particles will separate out according to their density and lie at specific heights in the column. These may be drawn off and collected as separate fractions, e.g. mitochondria, ER, lipids, etc.

Proteins, however, usually remain in solution and are therefore in the supernatant. Purification of macromolecules such as proteins uses different techniques.

1. Dialysis - Dialysis bags have very small pores through which small molecules may flow, but large macromolecules like proteins may not. When the supernatant is poured into dialysis bags which are placed in water the small molecules diffuse out of the bag into the surrounding water. By changing the water several times, one can achieve more complete removal.
2. Electrophoresis - this is a salting out method making use of the fact that ions in solution carry a net electric charge. When an electric field is set up in a conducting medium, charged particles such as proteins migrate toward the electrode having an opposite charge. Some with heavier charges will migrate more rapidly than others and are thus separated more quickly. The supporting medium can be filter paper, a starch gel or agar, and some equipment will measure relative concentrations as well.
3. Chromatography is an extremely important techique used to separate all kinds of mixtures into their individual components. Analysis of proteins or other substances in present-day research or industry has access to expensive and sophisticated chromatographic techniques making possible very precise and very rapid separation and analysis of materials, but the underlying principle remains the same in all cases. The mixture is first dissolved in an appropriate solvent such as alcohol. It is then exposed simultaneously to two different substances (phases). Each component of the mixture will be partitioned between the two phases in concentrations proportional to its relative affinity for that substance.

   Column Chromatography uses a glass column packed with some hydrated adsorbent

material such as starch. The test mixture is poured into the top of the column and allowed to filter downward. The separated components of the original mixture will emerge from the bottom of the column at different times and can be collected separately for analysis.

Amino Acid Chromatography - The entire process of analysis has been automated. The test mixture is forced, under pressure, through ion-exchange columns, each component being caught by an automatic fraction collecter as it emerges. Ninhydrin (a colour indicator for amino acids) is added automatically to each container and the intensity of the blue colour in each is measured by a photometer attached to a recorder which prints out an activity graph. The position of the peaks indicate which amino acids were present and the height of the peak, determined by the intensity of the blue colour, provides an estimate of the concentration of each.

Since all life processes obey the laws of chemistry and physics, it is necessary to understand the behavior of the atoms and molecules which make up the compounds which form living material.

All matter is composed of naturally occurring or synthetic elements, the smallest unit of which is an atom. Six of these elements, hydrogen, carbon, oxygen, nitrogen, phosphorus and sulfur are present in all living things and are essential for life. Calcium, potassium, magnesium, sodium and iron are also very important and are frequently found, but are not essential. In addition, a few trace elements are present in minute amounts.

Most atoms bind together with other atoms to form molecules. The forces of attraction holding these atoms together, called chemical bonds, contain potential energy. The concept of "energy" is the key to understanding the properties of chemical reactions and ultimately the properties of living cells. Any physical or chemical change involves a redistribution of energy from one part of the system to another as these bonds break down and release energy or are reformed, and absorb energy. Normal functioning of cells depends upon their ability to extract and use the chemical energy locked within the structure of their organic molecules. The major energy-carrying molecule of all cells is adenosine triphosphate (ATP), to which, energy is transferred during the catabolism (breakdown) of carbohydrates, fats and proteins. Subsequent breakdown of ATP releases this energy in the form of calories to perform work for the cell when necessary, such as muscle contraction, synthesis of new organic molecules, active transport of molecules across membranes etc.

SUMMARY OF CHEMICAL BONDS

Ionic Bonds are the result of the mutual attraction between two atoms resulting from the complete transfer of one or more electrons from one to the other. Substances with ionic bonds tend to dissociate into separate ions when in solution e.g. $NaCl \rightarrow Na^{+} + Cl^{-}$. Ionic bonds in aqueous solution are usually weak and easily broken.

Covalent Bonds are the result of the mutual attraction between atoms resulting from the sharing of electrons.

a.) Non-polar covalent bonds share the electrons equally between the atoms e.g. H_2.

b.) Polar covalent bonds are formed when electrons are pulled more closely to one atom than the other e.g. H_2O.

Covalent bonds are strong and very stable.

Different atoms have different bonding capacities, depending on the valence i.e. number of electrons in the outer shell e.g. $O_2 \rightarrow O = O$ double bond, or $N_3 \rightarrow N \equiv N$ triple bond.

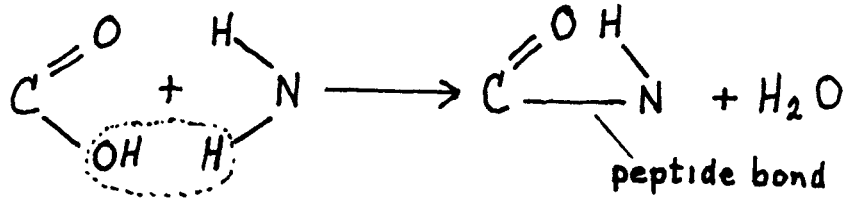
Hydrogen Bonds are weak bonds which arise when a single hydrogen is shared between two electronegative atoms, usually oxygen or nitrogen. Water molecules frequently form hydrogen bonds. Because of the polar nature of the bonds between hydrogen and oxygen in water, the oxygen is left with a slightly negative charge and the hydrogens with a slightly positive charge, permitting other nearby molecules to be attracted. Each water molecule has the potential of linking with four other water molecules. This property of water to form hydrogen bonds makes it very important biologically since most life reactions take place in water.

There is a general rule of solubility which states that "like dissolves like". Polar molecules dissolve in polar solvents and nonpolar molecules dissolve in nonpolar solvents.

Hydrophobic Interactions are very weak linkages which form between nonpolar groups when in the presence of polar water molecules. Such groups clump together to minimize the surface area in direct contact with the water.

Peptide Bonds form as a result of condensation reactions between the carboxyl

group (COOH) of one amino acid and the amino group (NH_2) of an adjacent amino acid

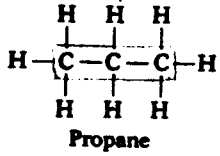


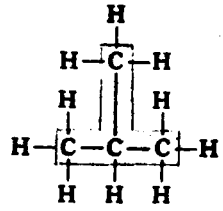
A series of amino acids connected by peptide bonds form a polypeptide chain. See pg. 55.

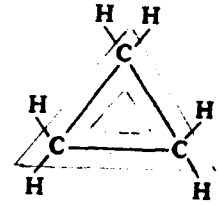
Disulphide Bonds may form between the two sulfur atoms of two units of the amino acid cysteine, producing folds in the polypeptide chain. See pg. 56.

CLASSES OF ORGANIC MOLECULES

Organic compounds are based upon the element carbon (valence 4) which is usually found bonded to hydrogen, oxygen, nitrogen or more carbon. Compounds containing carbon and hydrogen are called hydrocarbons


Propane


Isobutane

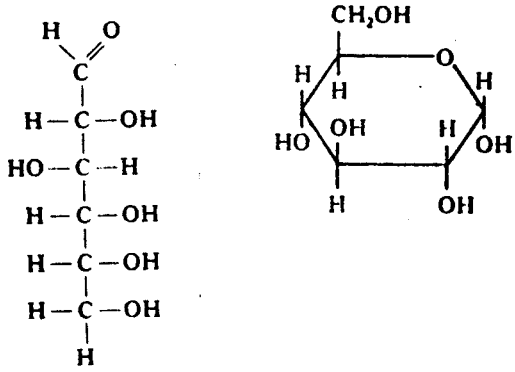

Cyclopropane

These carbon to hydrogen bonds are covalent nonpolar. Adjacent carbon atoms may form single, double or triple bonds, making such compounds very stable. There are four main classes of organic molecules

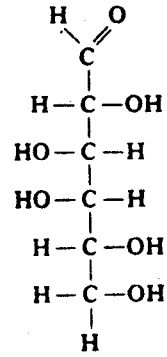
1. Carbohydrates are compounds composed of carbon hydrogen and oxygen, usually characterized as $(CH_2O)_n$ or H-C-OH which constitute 3% of the organic matter in the body. The sugars making up carbohydrates form hydrogen bonds with water and are therefore soluble.

   a.) Monosaccharides are simple sugars containing 6 or fewer carbon atoms. The

6 carbon sugars are the most important as building block compounds for more complex carbohydrates.

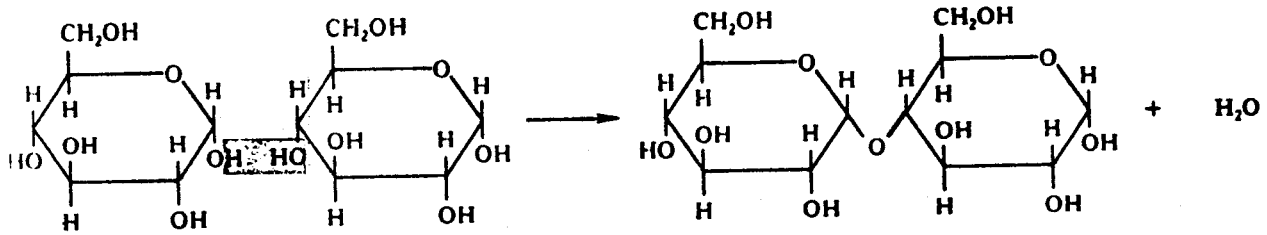


Two forms of glucose
Glucose may exist in the straight-chain aldehyde form shown at left or as a ring structure, as shown at right.



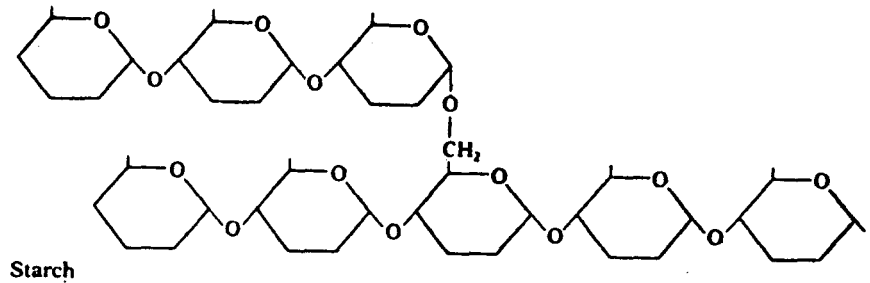
Galactose

b.) Disaccharides are composed of 2 simple sugars bonded together by a condensation reaction (removal of a water molecule) and may be broken down by the reverse process, hydrolysis (addition of a water molecule.)

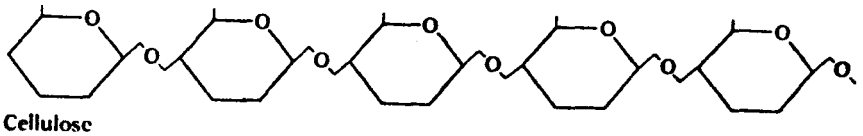


Glucose + Glucose = Maltose (a disaccharide) + Water

c.) Polysaccharides are complex carbohydrates composed of many simple sugars bonded together in long chains which may be synthesized or broken down like the disaccharides. The principal polysaccharides in living material are starch, glycogen and cellulose.



Starch



Cellulose

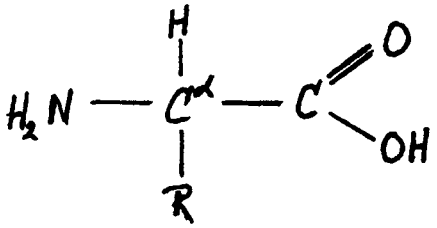
2. Lipids are also compounds composed of carbon, hydrogen and oxygen but may also contain such elements as phosphorus and nitrogen. Lipids account for about 40% of the organic matter in the body. Lipid molecules, because their atoms are linked by nonpolar covalent bonds are insoluble in water, but soluble in nonpolar solvents.

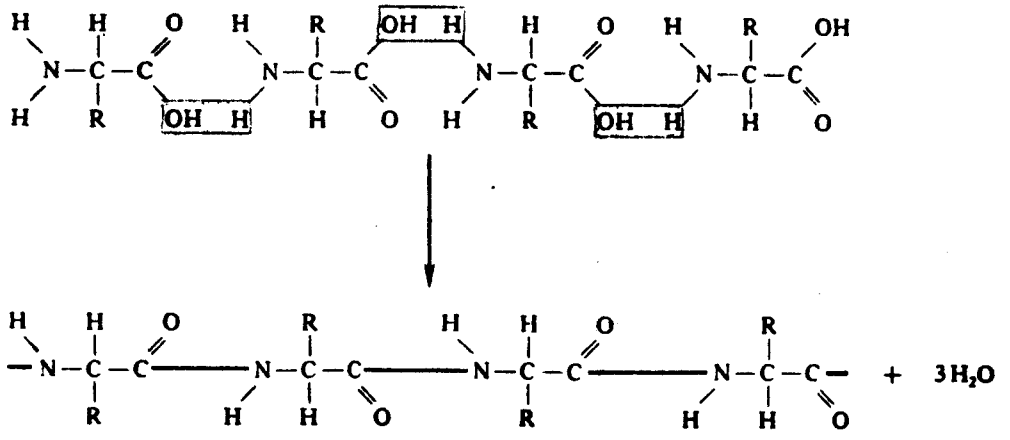
   a.) Neutral Lipids (fats and oils) are energy storage molecules of living organisms. They are composed of 2 types of building block molecules linked together -- glycerol and fatty acids.

   b.) Phospholipids are molecules having one end water-soluble (polar) but the other not (nonpolar), a feature which makes them very suitable for membranes.

   c.) Steroids are complex molecules of 4 interlocking rings of carbon with various side groups. Many vitamins and hormones are steroids.

3. Proteins are more complex than either carbohydrates or lipids. They account for about 50% of the organic material in the human body being components of most of the body's structures, and are therefore involved in almost all chemical interactions. Most proteins are very large molecules formed from linkages of many small subunits called amino acids. Proteins are polymers of the 22 amino acids, and contain carbon, hydrogen, oxygen, nitrogen and usually sulfur. Amino acids are characterized by this formula





Synthesis of a polypeptide chain
Condensation reactions between the COOH and NH_2 groups of adjacent amino acids result in peptide bonds (color) between the acids.

The α carbon atom has both a carboxyl group and an amino group attached to it, in addition to the side chain R which gives it its unique composition.

Primary structure (1°) Amino acids bond together by condensation reactions between the COOH group of one and the NH_2 group of another to form peptide bonds. This sequence of amino acids along a polypeptide chain constitutes the primary structure of the protein molecule.

Secondary structure (2°) of proteins is the α-helical and random-coil configuration which results from the formation of hydrogen bonds between the regularly-spaced and polarized peptide bonds.

Tertiary structure (3°) is the achievement of 3-dimensional shape by the electric interaction of additional polar or ionized sites on side chains of the amino acids. e.g. Disulphide bonds may form between the sulfur atoms of adjacent amino acids, forming strong and important covalent disulfide bonds.

Quanternary structure (4°) of proteins exists when two or more polypeptide chains are loosely held together by weak bonds.

The potential number of proteins formed from the chains of amino acids and side groups is nearly infinite, thus explaining why every individual human being is different from every other individual but is still able to maintain the integrity of the species.

4. Nucleic Acids are long polymers of building-block units called nucleotides. Each nucleotide is composed of a 5- carbon sugar with a phosphate group and a

nitrogenous base covalently bonded to it.

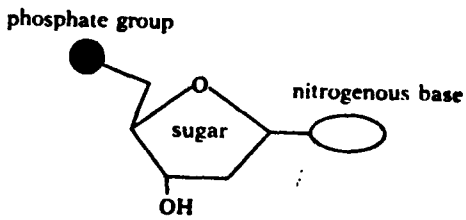
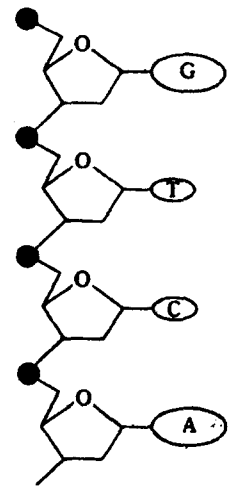


Diagram of a nucleotide
A phosphate group and a nitrogenous base are attached to a five-carbon sugar.

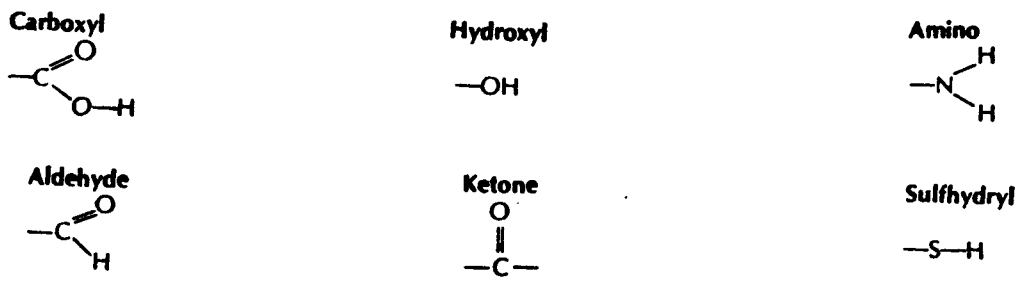


Portion of a single chain of DNA
Nucleotides are hooked together by bonds between their sugar and phosphate groups. The nitrogenous bases (G, guanine; T, thymine; C, cytosine; A, adenine) are side groups.

Nucleic acids are the largest of the organic compounds. They make up all of the genetic material of cells which is stored, replicated, and passed on from parent to offspring and from cell to cell during growth. There are two main types of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

A - TESTING FOR BIOLOGICAL MOLECULES

Each class of organic compound is composed of many similar subunits. The subunits have recurring groups of atoms which are also characteristic. We call these recurring parts functional groups because they determine the chemical behavior of the molecule. Some molecules such as the amino acids may have several functional groups but they all share those functional groups which are characteristic of amino acids.

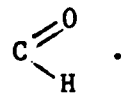


COMMON FUNCTIONAL GROUPS

Chemical tests sensitive to specific groups of molecules have been devised and may be used to identify molecules of that class.

I. Carboydrates

a.) Benedict's Test for Reducing Sugars Pages 47, 49.

Benedict's reagent tests for the presence of a free aldehyde group  .

Monosaccharides usually have this free aldehyde group as do some disaccharides such as maltose and lactose. These sugars, called reducing sugars, all give a positive test with Benedict's reagent.

If there are no reducing sugars in a test solution the blue colour of Benedict's reagent will remain blue.

A small amount of reducing sugar will change the colour to yellow green or green.

A large amount of reducing sugar will change the colour to orange or bright red.

Heat is required for this reaction to take place.

Procedure

1. Label 5 small test tubes 1, 2, 3, 4 and 5.
2. With glass marking pencil, mark each tube 1 cm and 3 cm from the bottom.
3. Fill #1 to the first mark (1 cm) with distilled water.
   Fill #2 to the first mark (1 cm) with albumen solution.
   Fill #3 to the first mark (1 cm) with honey solution.
   Fill #4 to the first mark (1 cm) with 1% glucose.
   Fill #5 to the first mark (1 cm) with starch solution.
4. Fill each tube to the second mark (3 cm) with Benedict's solution and mix well.
5. Place all 5 tubes in the boiling water bath for 3 min.
6. Remove tubes and record the colour of each.
7. Rinse out the tubes for use in other tests.

b.) Iodine Test for Starch

Starches are the principal carbohydrate storage products of higher plants. They are polymers, composed of hundreds of glucose units bonded together. One of the advantages of storing carbohydrate as starch rather than sugar is that starch,

which is insoluble, has much less osmotic activity than sugar which is highly soluble.

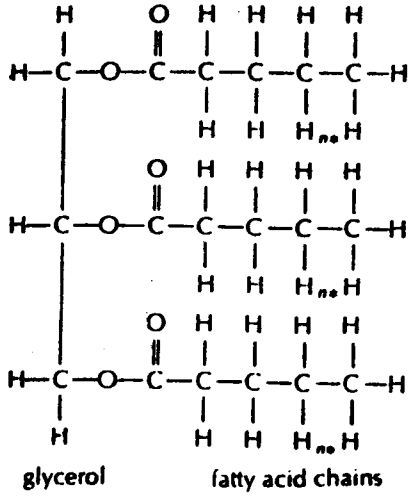
Similarly, glycogen is the principal storage product of animals.

Cellulose, important supporting tissue of plant cell walls, is also a polysaccharide, and also composed of glucose subunits.

Procedure

1. Add a drop of Lugol's Iodine to the square of filter paper (cellulose) at your bench.
2. Add a drop of Lugol's Iodine to a thin slice of potato from the demonstrator's bench.
3. Add a drop of Lugol's Iodine to a thin slice of apple from the demonstrator's bench.
4. Add 4 drops of Lugol's Iodine to each of the 3 test tubes containing a) water b) starch and c) glycogen already at the bench.
5. Record all results on the chart and rinse out the tubes for future use.

II Lipids - Pg. 51

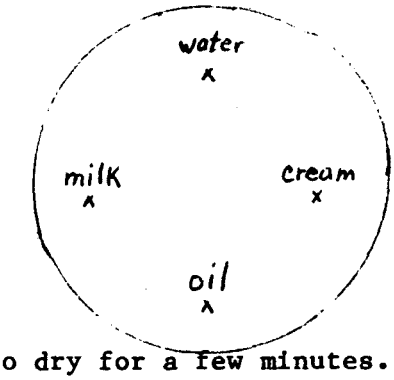


Sudan III Test for Fats

Sudan III is a red dye that stains the long H-C-H chains of the fatty acid part of fat molecules a red-orange colour

Procedure

1. Take 4 test tubes and label them 1, 2, 3 and 4.
2. Use the wax pencil to mark each 1 cm and 3 cm from the bottom.
3. Fill tube #1 to the first mark with distilled water.
   Fill tube #2 to the first mark with milk.
   Fill tube #3 to the first mark with cream.
   Fill tube #4 to the first mark with vegetable oil.
4. Fill all 4 tubes to the second mark with 95% alcohol (ETOH) and shake each until well mixed.
   What is the function of the alcohol?



5. Using lead pencil, mark a piece of filter paper according to this diagram.
6. Use a separate toothpick to transfer a drop from each tube to the appropriate spot on the filter paper, and allow the spots to dry for a few minutes.
7. Place the paper into the communal dish of Sudan III solution for exactly 60 sec.
8. Remove it with forceps and rinse in distilled water to remove the excess dye.
9. Record the colour of each spot.

III Biuret Test for Proteins

When amino groups ($\overset{H}{\diagdown}N\overset{H}{\diagup}$) are joined in the peptide linkages of a protein, they react with copper ions to give a purple colour.

Procedure

1. Label 5 small test tubes #1, 2, 3, 4, and 5.
2. Use the wax pencil to mark each 3 cm and 5 cm from the bottom.
3. Fill #1 to the first mark with distilled water.
   Fill #2 to the first mark with albumen solution.
   Fill #3 to the first mark with honey solution.
   Fill #4 to the first mark with milk.
   Fill #5 to the first mark with amino acid solution.
4. Fill all tubes to the second mark with 10% NaOH.

N.B.: SODIUM HYDROXIDE IS VERY CAUSTIC. IF ANY IS SPILLED ON YOU, FLUSH WITH WATER IMMEDIATELY. CALL THE DEMONSTRATOR FOR HELP.

5. Cover each tube with parafilm and mix well.
6. Add 5 drops of 1% $CuSO_4$ to each tube (gives a light blue colour).
7. Shake again and record the colour of each.

IV. Test for Nucleic Acids

The diphenylamine test exhibits a blue colour in the presence of DNA.
The orcinol test exhibits a blue colour in the presence of RNA.

Procedure

1. Label 3 small test tubes 1, 2 and 3.
2. Use the wax pencil to mark each 2 cm and 4 cm from the bottom, and identify with your initials.
3. Fill #1 to the first mark with distilled water.
   Fill #2 to the first mark with DNA suspension.
   Fill #3 to the first mark with RNA suspension.
   Both the DNA and the RNA suspensions have been made up in trichloracetic acid (TCA) to promote solution of the nucleic acids and to hasten their hydrolysis.
4. Add diphenylamine to the second mark. Mix again.
5. Place a marble on each tube and place tubes in a hot water bath for 10 min.
6. Remove, allow tubes to cool and record your observations.
7. Repeat the test as before, substituting orcinol for the diphenylamine.

In both tests the strong acids in the test solutions hydrolyze the purine nucleotides to sugars, bases and phosphoric acid. Sugars react with the dye to yield the characteristic colour.

B – PAPER CHROMATOGRAPHY

Overview

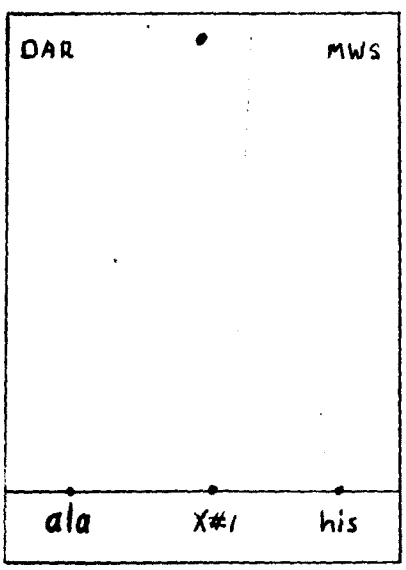
The mixture of amino acids to be analyzed has been dissolved in propanol. Several drops are placed near the bottom edge of the filter paper. The filter paper is inserted into a jar containing a small amount of a solvent (the spots must be above the solvent level). The solvent, (the flowing phase), migrates up the filter paper, (the stationary phase) by capillary action, carrying the mixture with it. Those parts of the mixture having a higher affinity for the solvent, travel freely up the paper with the solvent. Those parts of the mixture having a higher affinity for the filter paper will quickly transfer from the solvent to the paper. At the end of a measured time interval, the amino acids of the original mixture will be at different places along the paper. Thus, each will have its own characteristic rate of migration which is always the same for any given substance in a particular solvent system. We call this distance R_f - ratio of the fronts.

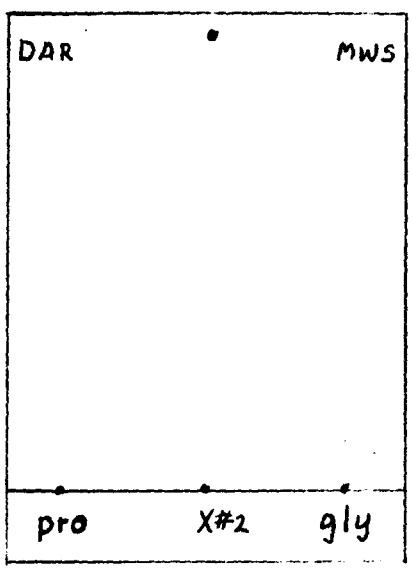
$$R_f = \frac{\text{distance travelled by solute (cm)}}{\text{distance travelled by solvent (cm)}}$$

N.B.: THE FILTER PAPER MUST BE HANDLED ONLY WITH FORCEPS TO PREVENT CONTAMINATION.

Procedure - Work in pairs.

1. Using forceps, partners should take two pieces of chromatography paper. When the exercise is completed, you may call them chromatograms.
2. Using a pencil, draw a line one centimeter from the bottom (opposite the side with the hole), and put your initials in each upper corner.
3. Also with pencil, mark 3 spots along each line which should be labeled as illustrated





Alanine (ala) histidine (his) proline (pro) and glycine (gly) are amino acids whose activity is already known. Hence they are being used as "standards". X1 and X2 are unknown amino acids which must be identified by comparing their activity with the standards.

4. Using a separate capillary tube for each, put one drop of solution on the paper at the appropriate spot.
5. Pour 15 ml of solvent into the glass jar. Hang the two chromatograms from the wire hooks in the rubber stopper and carefully lower them into the jar so that they do not touch the glass sides or each other. Seal the jar with the stopper, being careful to ensure that the bottom edges of paper are only 2-3 mm below the solvent surface.
6. Watch as the solvent moves up the paper by capillary action carrying the amino acids along with it.

7. When the solvent gets to within a few mm of the top of the paper (~ 30 min) remove the chromatograms from the jar. With pencil, mark the position of the solvent front and take the chromatograms to the fumehood to dry.
8. Spray the chromatograms with ninhydrin solution according to the instructions of the demonstrator. Allow a few minutes for them to dry.
9. Hang the chromatograms in the 85°C oven for 5 min for colour development to take place.
10. With pencil, carefully outline each spot and calculate the R_f value for each sample.
11. Record all observations and measurements for your report. Partners should each take one of the chromatograms to include with the report.
    What amino acid(s) did mixture #1 contain?
    What amino acid(s) did mixture #2 contain?

Table 2-I a)

I a)

Tube #	Test Substance	Colour	Reducing Sugar Present
1			
2			
3			
4			
5			

Table 2-I b)

b)

Sample	Colour	Starch Present

Table 2-II

II

Tube #	Test Substance	Colour	Fat Present
1			
2			
3			
4			

Table 2-III

III

Tube #	Test Substance	Colour	Protein Present
1			
2			
3			
4			
5			

Table 2-IV a) Diphenylamine Test

V a)

Tube #	Test Substance	Colour	DNA Present

Table 2-IV b) Orcinol Test

b)

Tube #	Test Substance	Colour	RNA Present

Table 2-V

B.

Standard	Colour	Distance moved by Amino Acid	Distance moved by Solvent	R_f
Unknown Mixture #1				
Unknown Mixture #2				

LAB III CELL STRUCTURE

Objectives: When you have completed this lab you should be able to:

1. Describe the structures you can expect to see in a typical plant cell using the light microscope.

2. Describe the structures you can expect to see in a typical animal cell using the light microscope.

3. Describe the early stages of cleavage in Medaka fish eggs.

4. Recognize different cell types according to their function in cross sections of frog intestine.

5. Handle a light microscope in order to obtain the best possible image of a specimen.

The Cell Theory, as we think of it today, states that all living things are composed of cells, the fundamental units of life.

The theory of Biogenesis arose from the cell theory: "omnis cellula e cellula," i.e. all living cells arise from pre-existing living cells.

These two theories are basic to our understanding of the subject of Biology today.

A group of cells similar in form and specialized to perform one or more particular functions is called a tissue. A group of tissues may be associated together as an organ and organs into organ systems. The digestive system is an example of an organ system.

Much of our knowledge of subcellular organization has been made possible by the development of better and more powerful microscopes in order to improve magnification, resolution (the capacity to separate adjacent areas) and contrast, the three most important factors in microscopy.

Today you will be using the compound microscope to examine a variety of different cells and tissues. Different models of microscopes will vary in some details but the principles remain the same for all. Microscopes are delicate and expensive pieces of equipment which should be treated as you would a fine watch or camera. It is your responsibility to handle it with care, use it correctly and to store it properly before you leave.

Identify these microscope parts on the diagram:

1. Ocular or eyepiece through which you look. Learn to leave both eyes open while working, to avoid eye fatigue. Soon you will be able to ignore anything seen with the unused eye.

2. Body tube.

3. Ocular turret which may be rotated through a full circle to allow infinite positions of the ocular.

4. Nosepiece with 3 or 4 different objectives, all of which are parfocal, i.e. the specimen should remain in focus as objectives are changed one to another, and require only minor adjustment of the fine focus at each change.

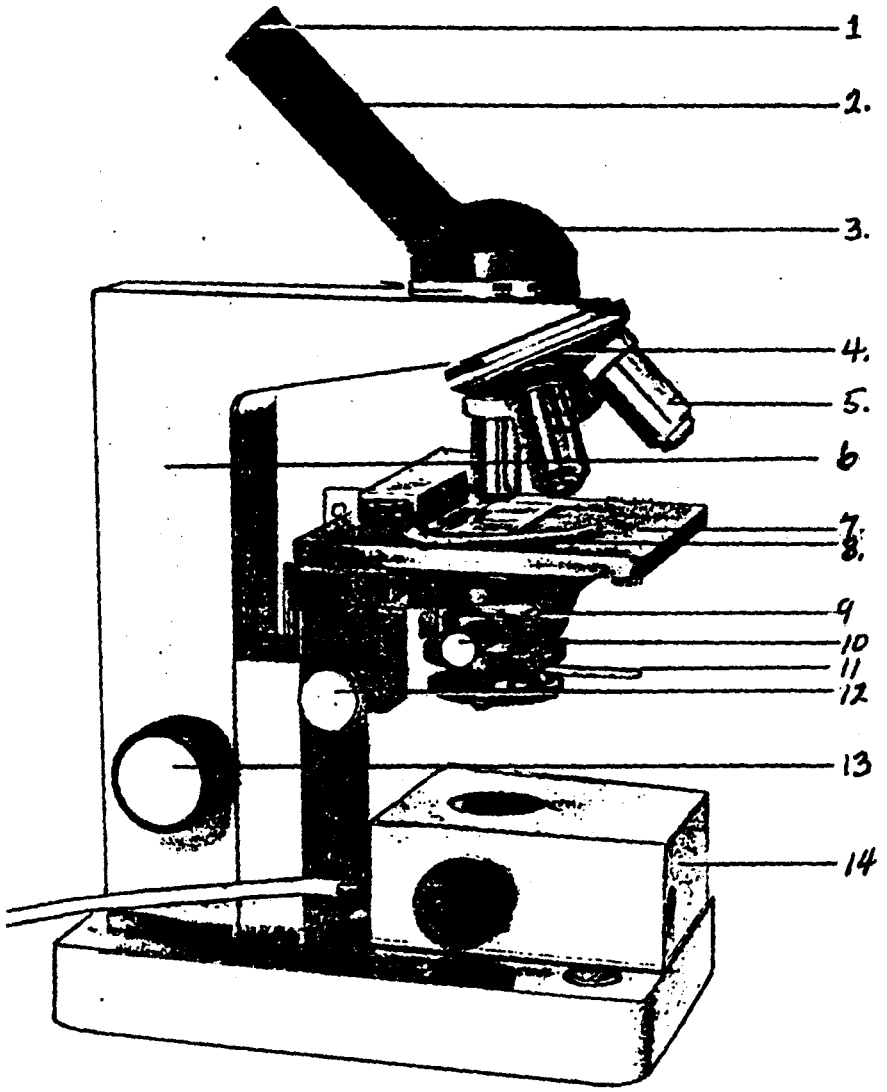
5. Objectives are the main working parts of any microscope - the compound lens system which produces the initial real specimen image. There are 4 interchangeable objectives - low power (4x), medium power (10x), high power (40x), and oil immersion (100x).

6. Arm - used to carry the microscope.

7. Stage - upon which the slide with its specimen are placed. Adjustment of the stage height is the method of focusing.

8. Spring clip - used to hold the slide firmly in position.

Fig. III-1



9. Condenser - the lens mounted below the stage, which catches the light rays from their source and focuses them on the specimen. The condenser should not need adjustment, it should remain in its position just below its uppermost level. The condenser control knob is #12.

10. Condenser front lens knob - used only with high power or oil immersion. Twist it towards you to remove this lens and away from you to position it for use.

11. Iris diaphragm lever controls the diameter of the beam of light coming up from below, and must be adjusted for each objective change. This adjustment is essential for clarity of image.

12. Control knob for condenser raises or lowers the condenser and is a real hazard for students who mistake it for the fine focus knob. The condenser should remain in a fixed position. See #9.

13 Coarse and fine adjustment knobs combined - initial turning gives fine adjustment - any continued turning switches automatically into coarse adjustment.

14. Light source - the rheostat which controls the intensity of light is on the right side of the housing containing the light bulb.

15. Vertical and horizontal control knobs are on the right hand side of the stage. They adjust the position of the slide in the clamp.

MICROSCOPIC PROCEDURE

1. Place the microscope on the bench 3 or 4 inches back from the edge. Most people are more comfortable with the arm away from them.

2. Plug in the cord and turn on the light to a low intensity.

3. Rotate the turret to put the ocular in position for your eye.

4. Rotate the nosepiece to bring the longest objective (4x low power objective) into the vertical position above the specimen.

5. Place the stage micrometer slide firmly in the clamps as far back as it will go. Check to be certain the slide is not upside down.

6. Using the vertical and horizontal control knobs, center the slide so the specimen is in the beam of light.

7. Watch from the side and turn the coarse adjustment knob to bring the stage to its highest position i.e. closest to the objective. The objective will still clear the slide nicely when using either the low or medium power objectives.

8. Look through the ocular and slowly turn the coarse adjustment knob in the opposite direction from before, lowering the stage, until a clear image of the specimen comes into focus. Note that the image is inverted and the left and right sides are reversed. Center the micrometer in the field of view.

9. Adjust the light source to optimum and the iris diaphragm lever to regulate the diameter of the circle of light coming through the condenser. Study the diagram, next page, to understand why you close the diaphragm for low power and open it for high power.

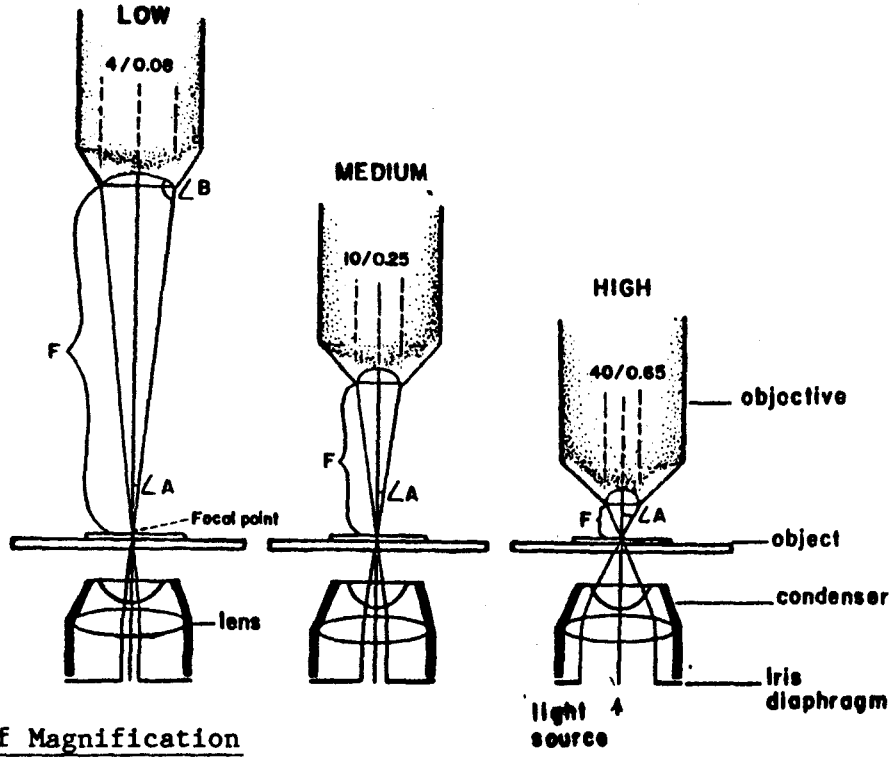
10. Once the image is clear, if greater magnification is required, rotate the nosepiece carefully to bring the 10x or 40x objective into vertical alignment above the slide. If the field of view is blank, the specimen was not centered at low power and is now outside the new field of view. Go back to low power and center it.

11. Use the fine focus knob to bring the image into best focus, being very careful not to hit the slide with the objective. NEVER USE COARSE ADJUSTMENT WITH HIGH POWER.

12. Swing the condenser front lens into position when using the high power objectives only. If it does not improve the image, remove it.

13. Practice focusing with your left hand only, in order to leave the right hand free for drawing or slide adjustment. With experience you will find that the most accurate observation is achieved by working the fine focus knob back and forth continuously over the surface of the specimen.

Fig. III-II



Calculation of Magnification

Measure the diameter of the field of view in mm for each of the objectives and record them in this table for future use. Use your metric ruler for low power, but the stage micrometer would be more accurate for medium and high power. Each small division of the micrometer scale is 0.05 mm (1/20 mm).

Table III-I

Objective	Magnifying Power	Diameter of Field
low	4x objective × 10x ocular = 40x	_____ mm
medium	10x " × " = 100x	_____ mm
high	40x " × " = 400 x	_____ mm

Every drawing or diagram must include an estimate of the magnification of the drawing.To determine the magnification (or scale) of your drawing, use this formula

$$\text{scale} = \frac{\text{size of drawing in mm}}{\text{actual size of the object in mm}}$$

The scale is always a ratio of the size of the drawing to the actual size of the object, and as such it does not have any dimensional unit.

e.g. Scale = 350:1 means that your drawing is 350 times larger than the actual size of whatever you drew.

Size of drawing should be measured directly from your drawing paper, using the largest dimension of the object.

Actual size of the object is found by comparing the size of the image you see in the microscope with the known diameter of the field. If the object fills 3/10 or 7/10 of the diameter of the field by its longest dimension, then the actual size is 3/10 or 7/10 of whatever value you have in your table as the diameter of the field for the

objective being used.

N.B.: Always record the proportion of the diameter of the field of view while the object is still seen in the microscope. You will not be able to remember it later when calculating the scale.

Although all cells have certain basic structural similarities, there is considerable diversity from one cell to another. Nevertheless, cell forms are closely related to cell function. This same form-function relationship holds true at higher levels of organization as well such as organs and organ systems.

A-REPRESENTATIVE PLANT CELLS (Pgs. 112, 122, 123.

I Elodea is a water plant found abundantly in fresh water ponds. Its leaves are thin so the cells are easily seen under the microscope. Take one of the small leaves growing at the tip of the plant and mount it on a microscope slide in a drop of water. Place a coverglass over it, taking care to eliminate air bubbles.

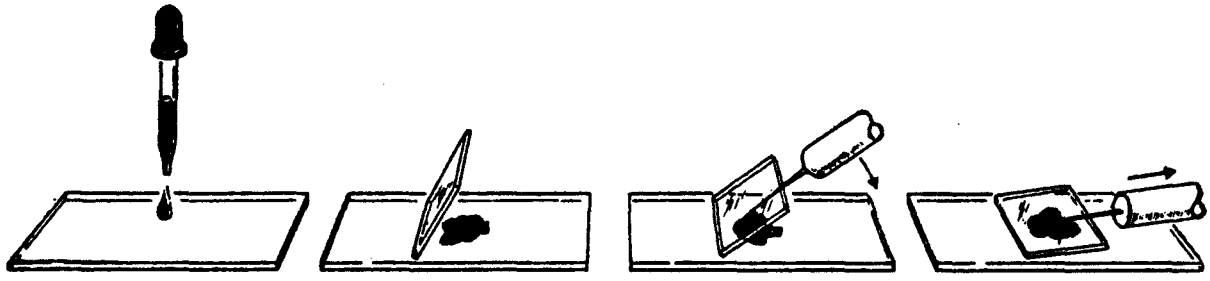


Fig. III-III

Add water to the edge of the coverglass as needed to prevent drying. Examine first under the low power of the microscope. Note the shape and arrangement of the cells. Observe and compare the cells at the leaf tip and along the edge.

Now change to high power. As you focus up and down you will notice that more

than one layer of cells comes into view at different levels, indicating that the leaf is more than one layer thick, but the depth of focus of the microscope is only able to keep one layer in sharp focus at any given time.

How many layers can you count in your leaf?
Are all the cells of the same size and form?
Adjust the focus so that one of the layers is in sharp outline and study one of the cells in detail. This will probably require adjustment of the iris diaphragm, condenser front lens and the illumination.
Locate the clear vacuolar area in the middle of the cell.
Are there several small vacuoles or one large vacuole?
What is the function of vacuoles?
This plant is green because the cells contain the pigment chlorophyll within the chloroplasts of the cytoplasm. You may be able to see the chloroplasts of some cells moving.
Can you account for this?
Look for the nucleus. It is oval, colourless, mobile and larger than a chloroplast. To find the nucleus, it helps to adjust the light intensity and the iris diaphragm for greatest contrast. If you can't find one, add a drop of Lugol's iodine and draw it through. This should stain the nucleus amber.
Each plant cell is enclosed by a nonliving cell wall just inside of which, is the cell membrane. Like most cell membranes, it is too small to be seen with the light microscope. The area between the cell membrane and the vacuole is filled with cytoplasm which is continuously moving around the periphery of the cell carrying the nucleus and chloroplasts with it.

Make a diagram of two adjacent Elodea cells showing the detail of one cell. Label it and include the scale.

II Cytoplasmic Streaming in Tradescantia

With forceps, remove a few filament hairs from a flower of the Tradescantia plant and put them in a drop of water on a microscope slide. Add a coverglass and examine under low power. The hairs will look like chains of beads. Select a good area and switch to high power. Look for the vacuole, the nucleus and for movement of the cytoplasm. Some vacuoles may appear purple due to the presence of anthocyanin, a pigment which is water soluble.

Compare this with the position of pigment in Elodea.

Make a diagram of two adjacent hair cells and label it. Show the detail of one of the cells.

III Plant Strategies for Water Retention (Text pg. 241)

Have ready a drop of water on a clean slide. Take a leaf from the Tradescantia plant and holding it with both hands, twist the leaf obliquely to slowly rip it apart. This procedure, called an epidermal "peel" should give you one very thin transparent sheet of cells from the bottom of the leaf and one from the top of the leaf. Put a very small sample of each of these peels in the drop of water and examine on low power. Then switch to high power.

Are the cells similar to those seen in Elodea? Differences?

Look for unusual cell clusters or pores, called stomata (singular stoma). What is their function?

In which of the peels were they found more abundantly? What is the rationale for this discrepancy?

Make a diagram of a stoma with surrounding cells.

B—REPRESENTATIVE ANIMAL CELLS (Text Pg. 127, 128)

All animal tissues belong to one of four types, epithelium, connective tissue, muscle, or nerve, but within each of these groups you will find considerable variety.

I Cheek Epithelium

Place a drop of methylene blue stain on a microscope slide. Gently scrape the inside of the cheek of your mouth with the flat end of a toothpick and stir the scrapings well in the stain. Apply a coverglass and examine under low power to find isolated cells. These cells are from the top layer of squamous epithelium and are continuously being sloughed off as they are replaced by new growth from below. Folds on the surface of some cells will appear as darker areas. They are caused by the flattening of the cell by the coverglass.

Identify the following structures to be included on your labeled drawing:

nucleus - spherical, darkly stained body located centrally.

nucleolus - smaller body located within the nucleus.

cytoplasm - the light blue viscid material located outside of the nucleus but within the cell membrane.

organelles - tiny granular and filamentous inclusions which are frequently seen in the cytoplasm.

plasma membrane - very thin outer boundary of the cell which cannot be clearly seen by light microscope.

Look for small rod-shaped  structures on the cell surface. These are bacteria from your mouth, and may or may not be present in all preparations.

What is the shape of the cells?

What important structures were visible in the plant cell but were lacking in the cheek epithelium?
Which of the two types of cell was the larger, plant or animal?
Did you remember to note the proportion of the field for your magnification?

Draw and label one epithelial cell from your preparation.

II Muscle Cells (Text Pgs. 471, 477)

Muscles are responsible for movement in higher animals, and as such they have good capacity for contraction. Individual muscle cells, called fibers are elongate in shape. They are bound together into sheets or bundles by connective tissue. There are three types of muscle fiber, each having a different structure and function - skeletal muscle, smooth muscle and cardiac muscle.

Skeletal muscle produces most conscious body movement. It is the most abundant

tissue in the vertebrate body and constitutes most of the meat that we eat. Today you will look at muscle fibers from the shank of a cow.

Each skeletal muscle fiber is cylindrical in shape, is covered with a sheath which contains many nuclei, and is crossed by alternating light and dark bands called striations. These striations are formed by the pattern of the individual contractile myofibrils which are packed into each fiber.

Take a very small piece of the red muscle tissue from the ice on the demonstrator's bench. Mount it in a drop of methylene blue dye and tease it extensively with dissecting needles to separate the individual fibers one from another.

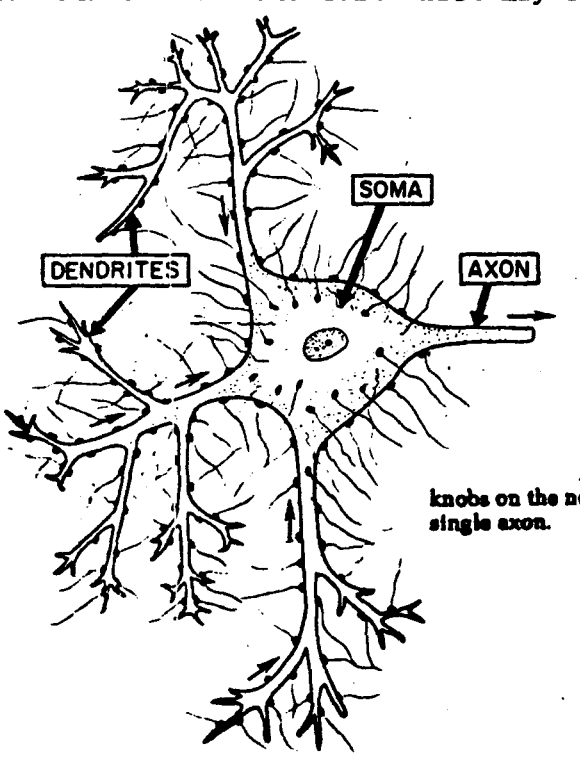
Examine it under low power to find an area which shows a single muscle fiber. Adjust the magnification to high power to see fine detail.

Draw a section of one striated muscle fiber to show its structure.

III Nerve Cells (Pgs. 133, 405)

Cells specialized for the reception of stimuli and the transmission of impulses are called neurons. A neuron typically has an enlarged cell body (soma) containing the nucleus, and two or more cytoplasmic processes, or fibers along which the impulse travels. The fibers which receive the stimulus and carry it to the cell body for processing are called dendrites. Usually only one fiber, the axon, transmits the impulse away from the soma to the next cell which may be another neuron, a secretory cell or a muscle.

Fig. III-IV



A typical motoneuron, showing synaptic knobs on the neuronal soma and dendrites. Note also the single axon.

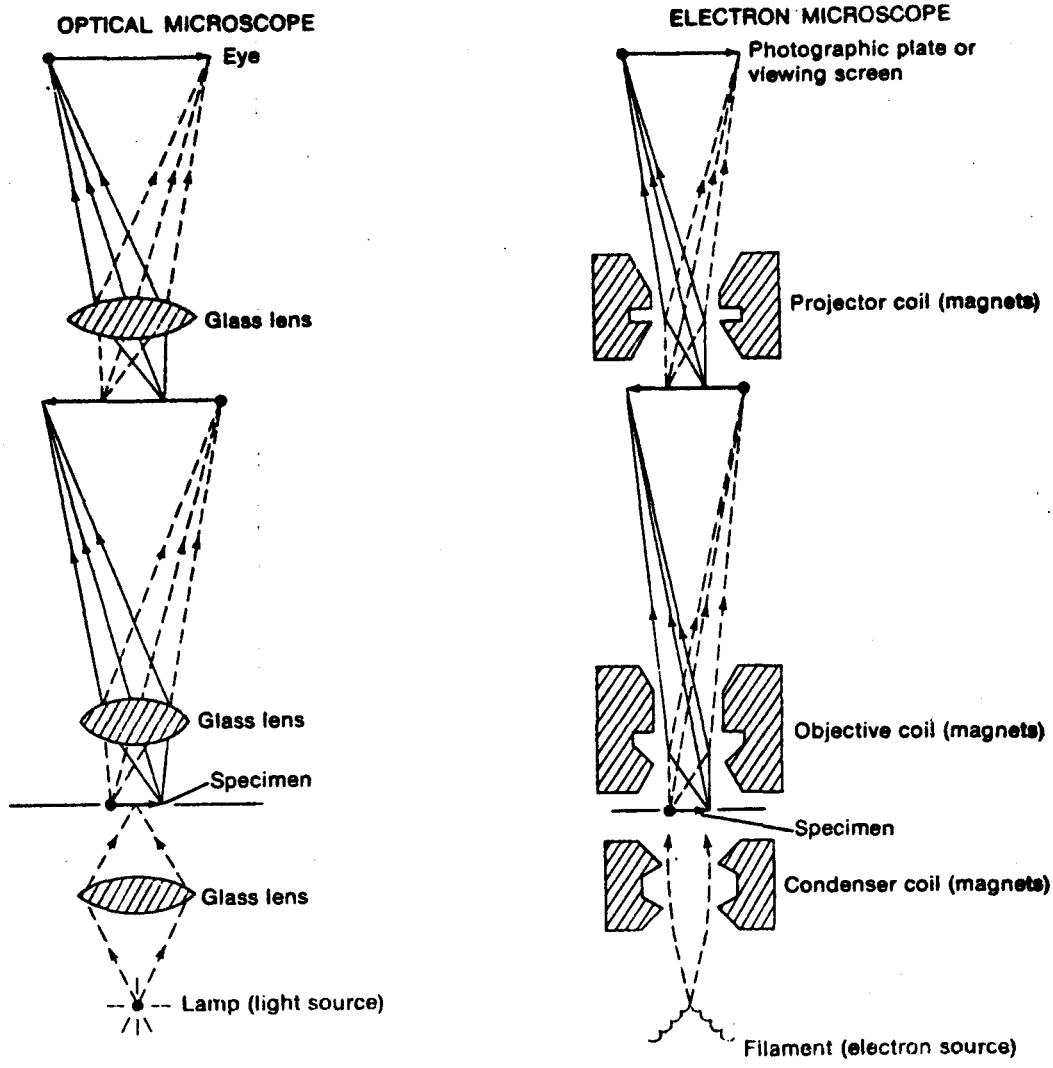
Put a drop of aceto carmine on a microscope slide; add a very small amount of material from the central nerve cord of a cow and mix together thoroughly. Add a coverglass and examine for cell bodies. Since most axons are proportionally long, it is unlikely that you will find a whole intact neuron.

Draw and label one neuron from your preparation.

C-CELL ULTRASTRUCTURE (Pgs. 98, 100, 105-110, 118, 119)

Adequate magnification has been a barrier for cytology - the study of cells - in spite of the best lens systems available. This problem was really one of resolving power or the ability of a microscope to differentiate clearly between two separate lines or objects which are lying close together. The resolving power of optical microscopes is related to the wavelength of light, and white light cannot resolve two discreet points less than 0.27 micrometers (μm) apart. Membranes, for example, are ~ 0.01 μm across.

The electron microscope has jumped this barrier by using high speed electrons, the negatively charged particles of atoms, as a source of illumination instead of light. Electrons have much shorter wavelengths than light, producing clear images at considerably higher magnifications. As electrons hit or pass through the specimen, depending upon the type of scope, parts of the cell differentially absorb or scatter the electrons, thus forming an image of the specimen on an electron - sensitive fluorescent screen or photographic plate. The optical system of the TEM (transmission electron microscope) is similar to that of a light microscope except that the beam of electrons is focused by means of magnetic coils instead of glass lenses and the system must be enclosed in a vacuum because electron movement is affected by the presence of air molecules. The electron microscope can resolve down to 0.0003 μm.



Comparison between optical (light) microscope and electron microscope.

Fig. III-V

1 meter = 1000 millimeters (10^3 mm)
1 mm = 1000 micrometers (10^3 μm)
1 μm = 1000 nanometers (10^3 nm)
1 nm = 10 Angstrom units (10 Å)

The electron microscope therefore, has enhanced our understanding of the structure and function of parts of the cell that were not previously visible to the cytologist.

Examine the micrographs at your bench to become familiar with the basic ultrastructure of plant and animal cells. Look particularly for these structures:

cell membrane - composed of two dark protein layers and a middle, light coloured, lipid region.

nuclear membrane - composed of a double set of similar membranes.

endoplasmic reticulum (ER) - the complex canalicular system of membranes extending throughout most of the cytoplasm frequently in continuty with the cell or nuclear membranes.

rough ER is located in cells actively engaged in protein synthesis. It appears rough because ribosomes line the outer surfaces of the membranes.

smooth ER lacks these ribosomes

mitochondria - subcellular structures containing enzymes for use in the Krebs cycle. The inner membranes of mitochondria invaginate to form cristae. Mitochondria are called the powerhouse of the cell. As such, they are concentrated in areas requiring large amounts of energy. They are the site of aerobic respiration.

Golgi complex - another system of membranes consisting of parallel flattened sacs, at the margins of which, vesicles are pinched off. It is thought that substances secreted in the Golgi body are shipped out in these vesicles to other parts of the cell or to the cell surface.

chloroplasts - have the same double membranous structure as mitochondria. The infoldings of the inner membrane make up a system of lamellae in which photosynthesis takes place.

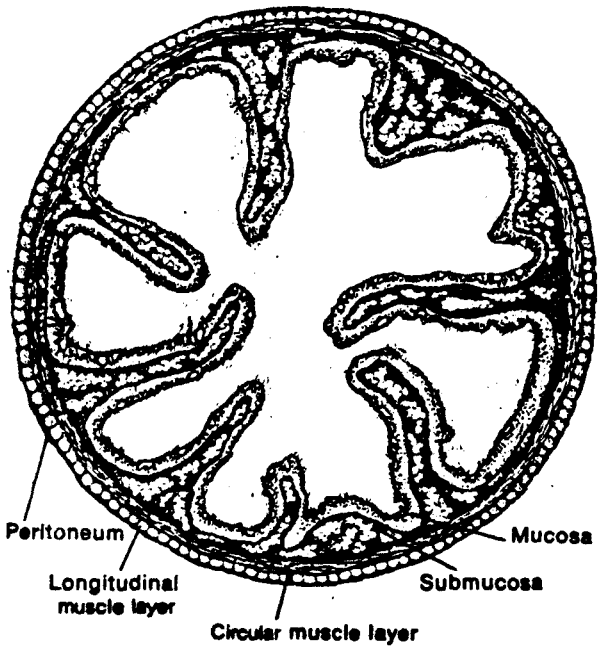
D—MULTICELLULAR ORGANIZATION (Pg. 225, 226)

The digestive system of an animal such as the frog includes the tongue and mouth, esophagus, stomach, small intestine, associated glands and ducts, colon and cloaca. Each of these structures is an organ composed of several tissues grouped together into a structural and functional unit, the whole, making up an organ system.

Today you will examine the cross section of the frog intestine to observe how combinations of cells work together as a unit to perform specialized functions. View the prepared

slide under low power to see the five layers of tissue surrounding the lumen. Then switch to high power to study the cell types in each layer.

Fig. III-VI



Identify these areas or layers:

Lumen - the open area in the center of the gut through which the food moves.

Mucosa - the lining of the digestive tract which is thrown into folds and projections called villi (singular villus). Villi increase the surface area for digestion. The principal cells of the mucosa are columnar epithelial which function in protection and secretion of enzymes or fluids into the lumen. It would appear that these cells are either ciliated or have a brush border on the lumen surface of each cell. We cannot tell by light microscope. A brush border is composed of many small processes called microvilli which again increase the surface area. Some epithelial cells have become specialized as gland cells, secreting mucus into the lumen to aid in digestion. These are called goblet cells and you will recognize them by the large cup-like spaces in them. Connective tissue cells are seen in the villi under the epithelium.

Submucosa - this layer of connective tissue cells embedded in a matrix, surrounds the mucosa. It may include blood and lymph vessels as well as a network of fibers which give strength and elasticity to the whole. (Text 130).

Smooth Muscle Layer - individual smooth muscle fibers are thin and spindle-shaped,

each with a single elongated nucleus. They are not striated as are the skeletal muscle fibers you looked at earlier. (See page 471). Smooth muscle fibers interlace to form sheets of muscle tissue. In the case of the frog intestine there are two distinct layers or sheets of muscle. The innermost is the relatively thick circular muscle layer in which the fibers are directed around the gut compressing and elongating it when they contract. Just outside the circular layer is the relatively thin longitudinal muscle layer whose fibers are oriented along the length of the gut, causing it to shorten when they contract. These two opposing forces are very important in effecting peristalsis, the alternating waves of contraction and relaxation which move food along through the gut and mix in the digestive juices. Since every longitudinal muscle fiber has been cross sectioned, they appear in your slide as circles.

Serosa (peritoneum) This layer of squamous epithelial cells is only one cell thick (pg 128). It protects and separates the gut from the body cavity.

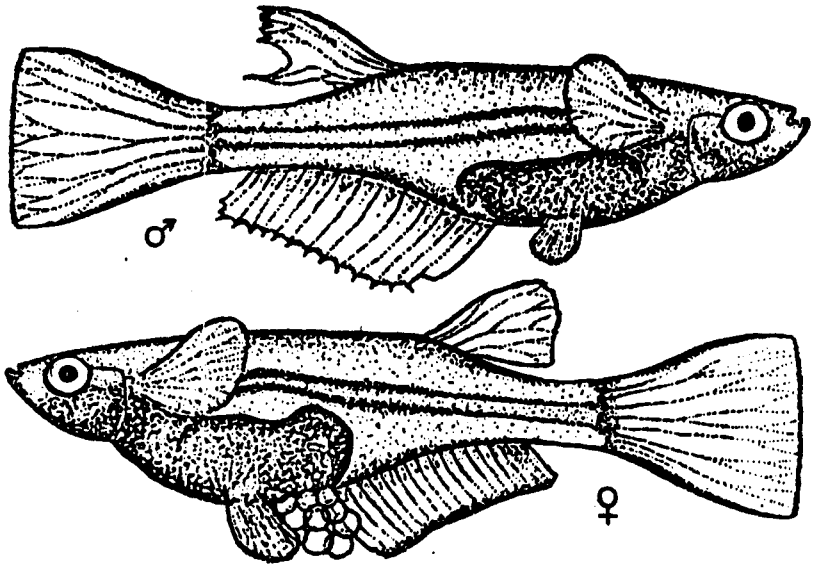
Draw and label a diagram of the frog small intestine cross section. Show a small segment of the diagram in cellular detail.

E —ANIMAL DEVELOPMENT

Although there is great diversity of form in the animal kingdom, most animals follow a basically similar plan of embryonic development, at least in the early stages. The fertilized egg, a single cell, undergoes a series of mitotic divisions called cleavage to form an embryonic mass called a blastula which later becomes a gastrula. Then, by cellular differentiation and further divisions and growth, a fully-formed individual is produced.

The Japanese Medaka is an oviparous (egg bearing), freshwater killifish of the family toothcarps. The wild strain is brownish black in colour and is common in rice paddies of Japan, where it feeds on mosquito larvae. Ours is the golden strain propagated by biological supply houses and feeds on freeze-dried mosquito larvae.

Fig. III-VII



Spawning normally occurs at the beginning of daylight, being controlled by the photoperiod or hours of daylight. Concurrent with oviposition (egg laying) there is a brief courtship and spreading of milt on the eggs by the male. The eggs are transparent with numerous projecting chorionic filaments which entangle adjacent eggs and attach the cluster of 10-24 eggs to the vent of the female. Eggs will hang there for several hours until brushed off on the plants or gravel of the aquarium. The optimum temperature for all stages of developmenmt is 20°-25°C but there is considerable variation in the rate, since hatching may occur anytime between 11 and 25 days from the time of fertilization.

Embryonic development in the medaka follows the typical teleostean pattern (of bony fish) which is very rapid. Cleavage is "incomplete" because of the large size of the yolk so the embryo forms as a disc on the surface of the yolk sac.

A normal developmental series would follow this pattern:

Stage 1 - (0 hours) At oviposition the unfertilized egg is opaque. The vitelline membrane at the periphery is narrow. Many small oil globules are randomly distributed in the cytoplasm.

Stage 2 - (10 min) Fertilization is completed as evidenced by the presence of a wide clear fertilization membrane between the cell membrane and the chorion. This ensures that no other sperm are able to penetrate.

Stage 3 - (45 min) A disc-shaped cap of cytoplasm appears at the animal pole. The oil droplets migrate to the opposite end - the yolk end called the vegetal pole - and begin to coalesce.

Stage 4 - (1.5 hours) The disc-shaped cap divides into 2 cells marking the beginning of cleavage. The oil droplets have become heavier and fewer.

Stage 5 - (2 hours) 4 cells - second cleavage plane is at right angles to the first.

Stage 6 - (2.5 hours) 8 cells - third cleavage plane is parallel to the first.

Stage 7 - (3 hours) 16 cells - fourth cleavage plane is parallel to the second.

Stage 8 - (3.5 hours) 32 cells - the 4 central cells have divided tangentially.

Stage 9 - (4 hours) 64 cells making 2 layers of embryonic cells called blastomeres which are surrounded by 16 peripheral blastomeres.

Stage 11 - (9 hours) Late high blastula stage. The cellular mass (blastoderm) is circular, and about 12 layers thick in the middle.

Stage 14 - (15 hours) The gastrula has expanded to cover 3/8 of the yolk and has involuted at the ventral lip.

Stage 18 - (24 hours) Neurula - A refractile streak of cells appears showing 2 divisions of the rudimentary brain.

Stage 22 - (40 hours) The optic cups, auditory capsule and pericardial cavity all begin to appear (eyes, ears and heart)-8 to 12 somites, the embryonic muscle blocks are visible.

Stage 25 - (54 hours) Heartbeat and blood circulation are visible. Pigment is visible in the eye region. Anterior somites may twitch. Tail is free of yolk sac.

Stage 30 - (4 days) Vitelline veins become obvious taking a sinuous path over the yolk sac. The retina is heavily pigmented black and silver. The urinary bladder appears becoming more green in colour with time. Pigment becomes visible along the tail region.

Stage 33 - (6 days) The swim bladder appears as a clear vesicle near the urinary bladder, and grows larger with time. The fins and lower jaw become more visible.

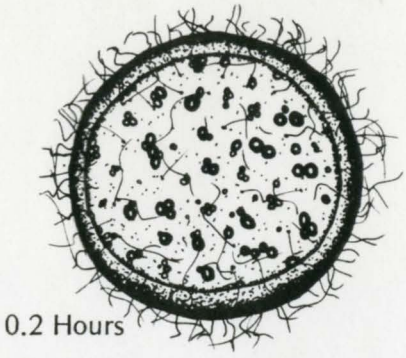
Stage 35 - (8 days) The mouth moves rapidly and may open. Golden pigment appears on the body. The embryo thrashes around in its case.

Stage 36 - (11 days) Hatching may occur. 30 somites are visible. The swim bladder is approximately as large as the remaining oil droplet. Most hatchlings display coordinated swimming and breathing movements at birth. Bladder, spleen and liver are all visible on the left side of the body.

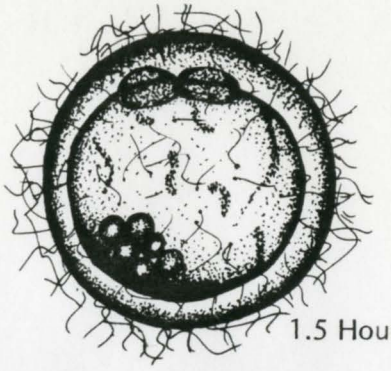
Study the embryos in your dish to identify their stages of development. Record the stages together with the rationale for your decision, to be part of your report.

Compare the size of the blastula with the size of the cytoplasm of early cleavage stages.

Fig. III-VI



0.2 Hours



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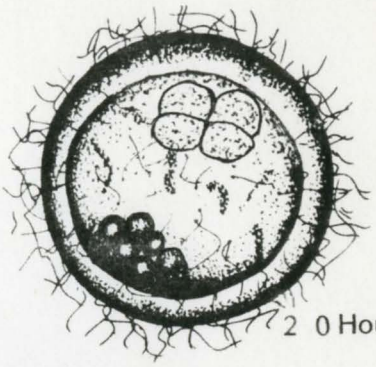

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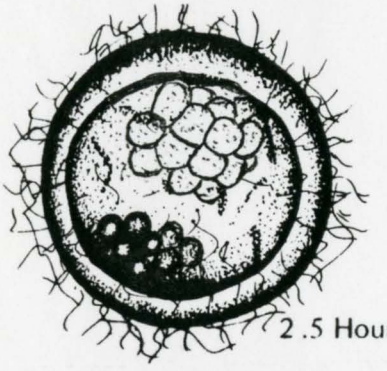

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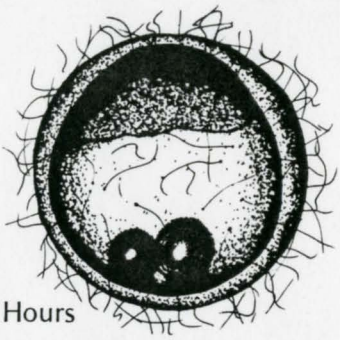

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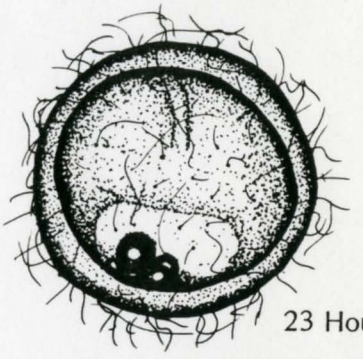

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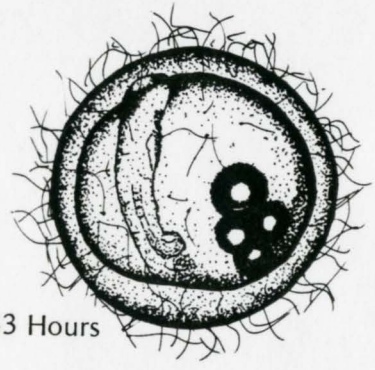

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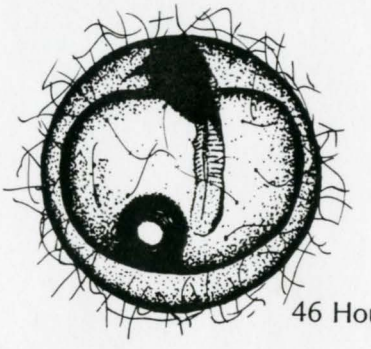
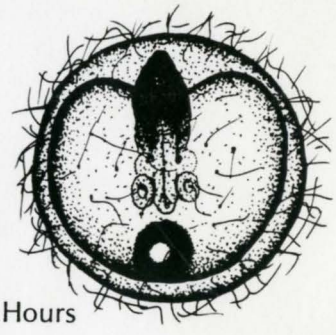
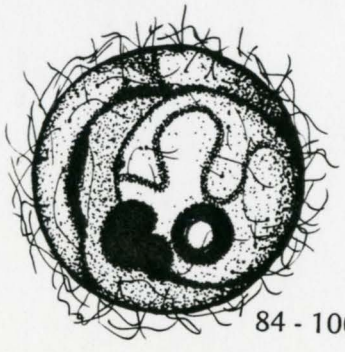


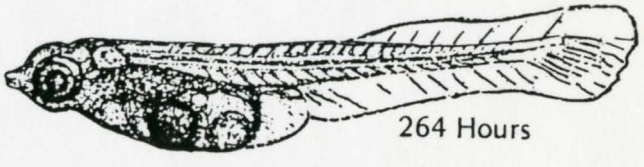
46 Hours



56 Hours



84 - 100 Hours



264 Hours

LAB IV, CONCEPTS IN GENETICS

Reference: Text pp. 575-576
583-620

Objectives: When you have completed this lab you should be able to:

1. Explain why Drosophila is a good subject for genetic experiments.
2. Recognize the developmental stages of Drosophila and differentiate between adult males and females.
3. Define and use the following terms: P_1, F_1 and F_2 generations, dominant, recessive, allele, homozygous, heterozygous, monohybrid cross, dihybrid cross, genotype, phenotype, linkage, test cross, and crossing over.
4. Use a Punnett square to give the genotypic and phenotypic ratios expected from a cross.
5. Explain how crossing over occurs in the haploid fungus Sordaria.

Research in the area of Genetics has all supported the original work done by Mendel between 1856 and 1868. We can therefore ascribe to his work the designation of "law".

1. Law of Segregation - Mendel's first law

In each individual, the genes occur in pairs. During the formation of gametes, gene pairs segregate (separate), one from each pair going to different gametes so that each gamete has only one of each type of gene.

2. Law of Independent Assortment - Mendel's second law

When two or more pairs of genes are involved in a cross, each pair segregates independently of all other pairs. Today we qualify Mendel's second law by adding the phrase "except when the genes are located on the same chromosome - i.e. linked".

Armed with the growing knowledge of genetic principles, geneticists have been able to breed superior cattle, cows that produce large amounts of high butterfat milk, chickens that lay larger eggs, and high protein, disease resistant wheat or corn plants, able to survive northern climates, as well as many other benefits to mankind.

The language of genetics is very precise and in order to discuss the mechanisms of

inheritance, it is necessary to define and understand the terms being used.

Genes are the units of hereditary material which are arranged in linear order on the chromosomes. These units are passed from parent to offspring, the total mix varying with each individual. As growth and cell division proceeds, this material is duplicated repeatedly and exactly by the process of replication, and every cell of that individual has an identical set of genes. Each gene has a fixed position on a specific chromosome called its locus. A full complement of genes therefore, for any organism may be enormous. Drosophila, with only 8 chromosomes (4 pairs), has about 10,000 genes.

Chromosomes are the filamentous structures in the nucleus of every cell upon which the genes are arranged. The number of chromosomes per cell is specific for each organism - man has 46, the fruit fly has 8. Chromosomes are said to be homologous because they always occur in duplicate, one having come originally from each of the 2 parents.

Haploid Cells have only one set of unpaired chromosomes in the nucleus. Usually, haploid cells are gametes, e.g. sperm or unfertilized egg cells. We call this the haploid number (n) and it is the product of meiosis.

Diploid Cells - Other body cells, which we call the somatic cells, normally have a full set of homologous chromosomes in each nucleus, e.g. when an egg cell is fertilized by a sperm, the resulting zygote and all subsequent cells produced by mitosis will have the diploid number of chromosomes (2n).

Alleles - are different forms of a particular gene. Although the genes are present at the same position (locus) on each of the pair of homologous chromosomes, and although they influence the same inherited trait, they may do so differently. They are referred to as alleles of the locus.

Dominant/Recessive - Although individuals have a pair of genes (alleles) for each characteristic, these alleles will not necessarily contribute equally to the expression of that characteristic. If one allele always exerts its full effect, thereby blocking the expression of the other allele, we say that it is dominant and the allele being blocked is recessive. Inheritance by which heterozygous individuals

show the effects of both alleles is called intermediate inheritance.

Homozygous/Heterozygous - For any one characteristic an individual will have 2 alleles. If both of these are dominant, (the same form of the gene), we designate it by the use of capital letters (G/G) and say that the individual is homozygous dominant for that characteristic. An individual may also be homozygous recessive for a characteristic, designated as (g/g). If one allele is dominant and one is recessive, we designate that characteristic as being (G/g) and say that the individual is heterozygous (hybrid) for that characteristic.

Genotype/Phenotype. Any characteristic designated with both alleles is the genotype of that characteristic. Sometimes we are not sure of the genotype, we only know by outward appearance. Then we only know the phenotype, designated as (G/__) because it may be either (G/G) or (G/g).

Linkage - With reference to Mendel's second law of independent assortment, it is whole chromosomes which tend to segregate independently during meiosis. Therefore genes located on a particular chromosome tend to move together during meiosis. Such genes are said to be linked.

Monohybrid Cross refers to the mating or cross fertilization of two heterozygous individuals which are the F_1 progeny of an earlier cross between one parent who was homozygous dominant for a particular characteristic and one who was homozygous recessive for that characteristic e.g. C is the gene for colour

Parents	C/C	×	c/c	
	red		white	
F_1 generation	C/c	×	C/c	
	red		red	
F_2 generation	C/C	C/c	c/C	c/c
	red	red	red	white

The F_2 progeny are expected to show a 1:2:1 ratio of genotype combinations or 3:1 by phenotype.

Test Cross is a tool used to determine the genotype of an individual when one cannot tell by appearance whether it is (C/C) or (C/c). The test is a cross between the unknown genotype and a homozygous recessive individual. If the progeny of the test cross show the phenotypes in a 1:1 ratio, then the unknown was heterozygous (C/c), but if the progeny are all of one phenotype, the unknown must have been homozygous (C/C). Pg. 389.

Dyhbrid Cross is a cross between individuals heterozygous for two independent, non-linked characteristics which are the F_1 progency of an earlier cross between one homozygous dominant individual and one homozygous recessive individual, or the alternative combination shown below. The F_2 progeny are expected to show a 9:3:3:1 ratio of phenotype combinations of the two characteristics. Pg. 591, 2.

Parents R/R G/G r/r g/g or R/R g/g × r/r G/G
round-yellow wrinkled-green

F_1 generation R/r G/g × R/r G/g
round-yellow round-yellow

F_2 generation 9 R/__ G/__ 3 r/r G/c 3 R/__ g/g 1 r/r g/g
round-yellow wrinkled-yellow round-green wrinkled-green

Crossing Over - During synapsis of meiotic cell division (gamete production) homologous chromosomes lie side by side. Frequently, 2 chromatids, one from each homologous pair will break apart at the same place, the loose parts will rejoin, and the chromatids will then fuse again so that each chromatid has its original full complement of genes, but some of those have been exchanged between chromatids. These exchanges occur at random along the length of the chromosome. Several exchanges may occur at different points along the same chromosome greatly increasing the genetic variability among gametes. It follows that the greater is the distance between any 2 genes in a chromosome, the greater will be the chance that an exchange of segments between them will occur. The place where the exchange occurs is called a chiasma. Pgs. 574-576.

Mutations - A mutation is a change in the chromosomal DNA of a cell, usually of a

single gene. Such changes are frequently caused by irradiation of various types or by exposure to certain chemicals. Although such changes are infrequent under normal circumstances, most are deleterious and will be transmitted to offspring in the altered form e.g. hemophilia, and sickel cell anemia.

In the laboratory you will be looking at several examples of organisms which have been used extensively in studies of genetics for many years - fruit flies, corn, tomatoes and a fungus.

A - FRUIT FLY GENETICS

I Morphology and Life Cycle

The scientific name of our fruit flies is Drosophila melanogaster. They are insects of the Order Diptera (the true flies) with body composed of a head bearing a pair of antennae, a segmented abdomen, and a thorax bearing one pair of wings and three pair of legs. The features of Drosophila which make it especially suitable for experimental work in genetics are:

- they have a short life cycle of 10-14 days at 25°C.
- easily cultured in the laboratory. A single female can produce up to 200 eggs.
- there are hundreds of different inherited, morphological variations.
- each fly has only 4 pairs of chromosomes (2n = 8).
- the salivary glands of the larvae have very large chromosomes allowing microscopic study and consequent mapping of gene loci.

Procedure

1. Work in pairs. Observe the culture of living wild-type flies at your bench. Look for larvae, pupae and adult flies.

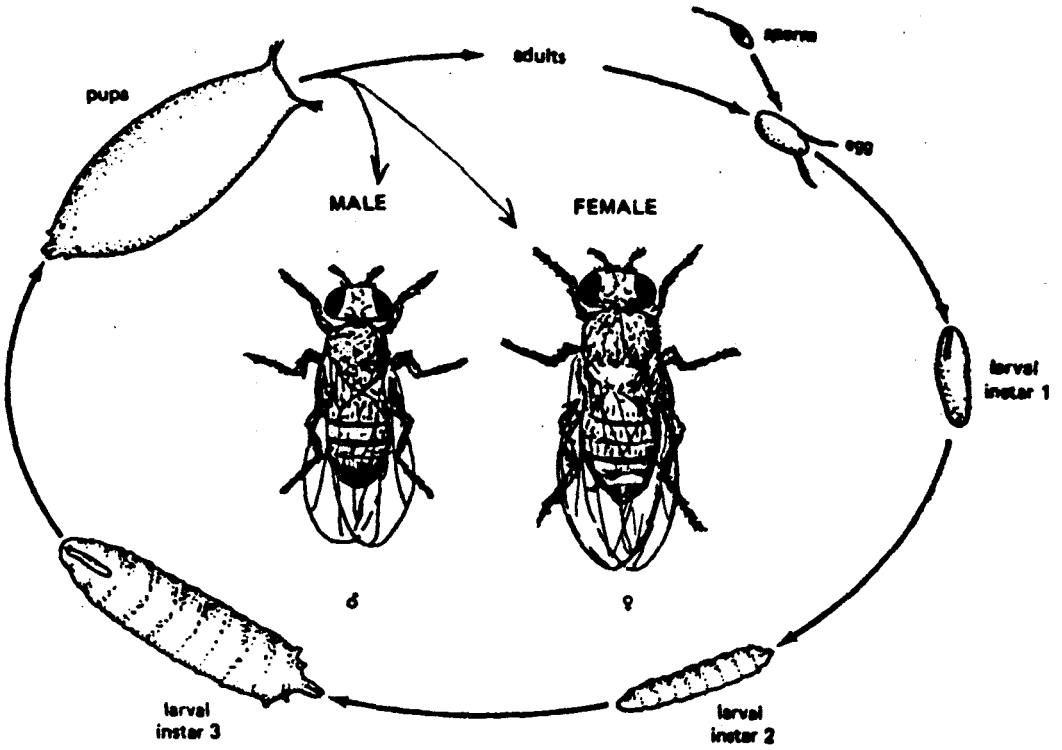


Fig. IV-I

2. Examine the killed flies under the dissecting microscope. Familiarize yourself with the general morphology.
3. Learn to differentiate between male (♂) and female (♀) flies according to these features.

   - adult males are generally smaller than females.
   - the male abdomen in generally dark tipped and smaller whereas the female abdomen is generally lighter coloured and larger.
   - the tibia (3rd segment) of the male foreleg bears a tuft of hairs called a <u>sex comb</u>. No such tuft is visible on the female tibia.
   - males bear a pair of hooklike <u>claspers</u> on the ventral abdomen (underside) anterior to the anus. These are used in copulation.

4. Separate the males and the females under the scope. Partners should check each others work. If you are unsure of your decision, call the demonstrator for help before proceeding to II.
5. Observe the <u>giant chromosomes</u> from the salivary gland of a larva on demonstration at the back bench. You may try your luck with making this preparation if there is time at the end of the lab period.

II A Monohybrid Cross

The wild type eye colour of Drosophila is red, but a mutant exists which produces white eyes. Similarly, the wild type wing shape is long, but a mutant exists which produces a stubby short wing called vestigial wing.

One month ago, the following crosses were made:

Table IV-1

Cross	Female Parent		Male Parent		F_1 Progeny
1A	wild type wings	×	vestigial wings	→	all wild type wings
1B	vestigial wings	×	wild type wings	→	all wild type wings
2A	wild type eyes (red)	×	white eyes	→	all wild type eyes
2B	white eyes	×	wild type eyes (red)	→	red eyed females white eyes males

Two weeks ago the F_1 progeny from each cross were mated. You will be looking at the F_2 generation today.

N.B.: If the mutation under consideration is located on one of the autosomal (body) chromosomes, results of the cross will follow the standard monohybrid cross pattern, but if the mutation is located on a sex chromosome, this will influence the results of the cross. Female sex chromosomes are X/X, Male chromosomes are X/Y, one having come from each of the parents. See Pgs. 608-612.

Procedure

1. Work in groups of four. Each individual of the group should take one of the vials of flies representing the four different crosses 1A, 1B, 2A and 2B, so that each group will have a complete set of four.
2. Separate the males from the females of your vial.
3. Separate each sex on the basis of wing shape, or eye colour, depending upon

which cross you are doing. Partners should check each others work for practice.

4. Total up the number of flies of each type and record your results in the appropriate place on the blackboard. Return the flies to the vial.
5. Record your own data and the class data in the chart to be included with your report.

Analysis of Data

From cross 1A and 1B which allele is dominant? Which is recessive? Give your reasoning.

Is this locus autosomal or is it sex-linked? Give your reasoning.

From cross 2A and 2B which allele is dominant? Which is recessive? Give your reasoning.

Is this locus autosomal or is it sex-linked? Give your reasonging.

Assign genotypes and phenotypes of your choosing to the P_1, F_1 and F_2 generations for each cross.

Does the data from the crosses 1 (A and B) and 2 (A and B) support the expected ratio of a monohybrid cross or does it differ?

B - HYBRID CORN

Zea mays is a member of the grass family Graminae. It is monoecious i.e. it bears separate male and female flowers on the same plant. You will be looking at specially selected cobs of corn, each kernel of which represents a fertilized seed with the potential to produce a new plant. The corn plant bearing the original male and female structures is traditionally termed the parent (P) and the seeds produced on its cobs are termed the first generation (F_1).

Procedure

1. Work in pairs, sharing the six cases of various crosses of corn with the pair of students opposite you at the bench.

2. Examine the two cases which contain the monohybrid crosses. What two traits (characteristics) do they show? What are the possible phenotypes for each trait?
3. a) Count the number of kernels (F_2 generation only) which show each of the possible phenotypes for each trait. Record your results. b) Count the number of kernels (F_2 generation only) for each of the two monohybrid test crosses. Record your results.
4. Examine the case containing the dihybrid cross. What traits are shown? What are the possible phenotypes?
5. a) Count the number of kernels (F_2 generation only) which show each of the phenotypes.
   b) Repeat the counts for the dihibrid test cross case. Record your results.

Analysis of Data

For each monohybrid cross would you say that one of the alleles was dominant? Was it the one most frequently expressed?

Would you be able to say that the more common allele is always dominant?

C—A DIHYBRID CROSS IN TOMATOES

In June, our greenhouse supervisor obtained two varieties of the tomato plant Lycopersicum esculenta.

a) Tiny Tim - green stem and smooth-edged leaves
b) Veeroma - purple stem and serrated leaves.

The two varieties were grown, and crossed to obtain the F_1. The F_1 were selfed, and today you are looking at flats of the F_2 generation.

Procedure

1. Work in pairs. Examine the flats to determine if they represent a straightforward dihybrid cross.
2. Assign genotypes of your own choosing to the P generation.
3. Draw up a Punnett Square to forecast the type of results one might expect to observe in the F_2 plants. What are the possible F_2 phenotypes by ratio.

4. Count the total number of plants in the flat. Count and record the number of plants visible for each of the phenotypes.
5. Compare the numbers you have counted to your original forecast. Are they similar?
6. Write a paragraph outlining how you would proceed to obtain these F_2 plants had you been given this assignment. Include the timing schedule and important factors to be considered.

D - SEXUAL REPRODUCTION IN A FUNGUS (Pg. 994, 995)

The Kingdom Fungi are eucaryotic organisms whose life strategies differ markedly from those of plants and animals. Cells are organized into branched filaments called hyphae which form a dense mass called a mycelium. The body of a fungus is composed mainly of this mycelial mass.

In this exercise you will examine the structure and sexual reproduction of Sordaria fimicola to observe the results of crossing over.

Although we may speak of male and female in fungal reproduction it is more common to refer to + (plus) and - (minus) mating types or even a,b and c etc. Cells of the hyphae and consequently the mycelium of fungi are usually haploid (n). The use of haploid fungi in research eliminates problems associated with dominance and recessiveness since every gene is expressed. In Sordaria, all the products of a cross are haploid and they are therefore distinguishable one from another with respect to visible traits in the spores.

Sordaria belongs to the class Pyrenomycetes of the Ascomycota or sac fungi. When vegetative hyphae of two different strains meet (see Pg. 995) a fruiting body, called a perithecium is formed. Hyphal fusion may occur, resulting in a cell which contains two nuclei. This process is called

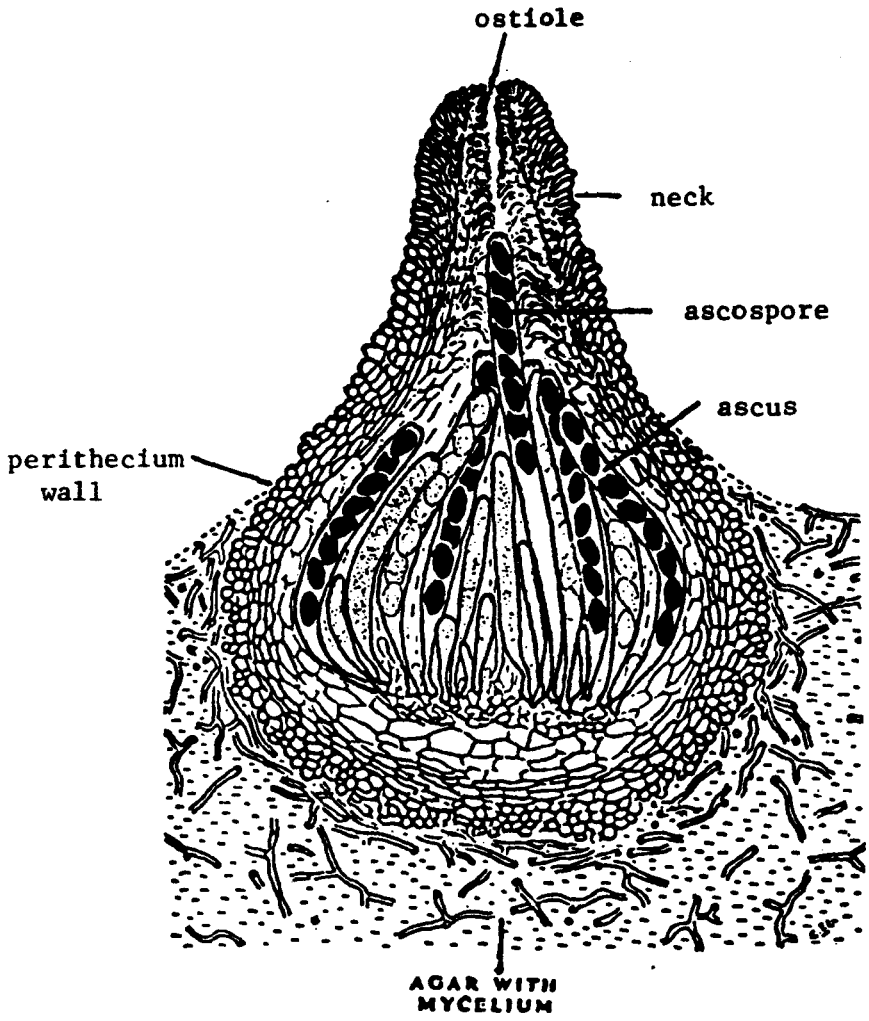


Fig. IV-II Sordaria fimicola: Vertical section of a perithecium

plasmogamy, and may be repeated many times.

Plasmogamy. These nuclei do not immediately fuse, but coexist in the hyphal cell as a dicaryon (n + n) for a short time. Eventually fusion occurs and a diploid zygote (2n) is formed in which one set of chromosomes (and genes) come from each strain as indicated by the black and white nucleus of the ascospore mother cell in the diagram. The zygote immediately undergoes meiosis to form 4 new haploid spores, linearly arranged in a young ascus. In Sordaria a subsequent mitotic division follows producing 8 ascospores all neatly lined up in the narrow ascus.

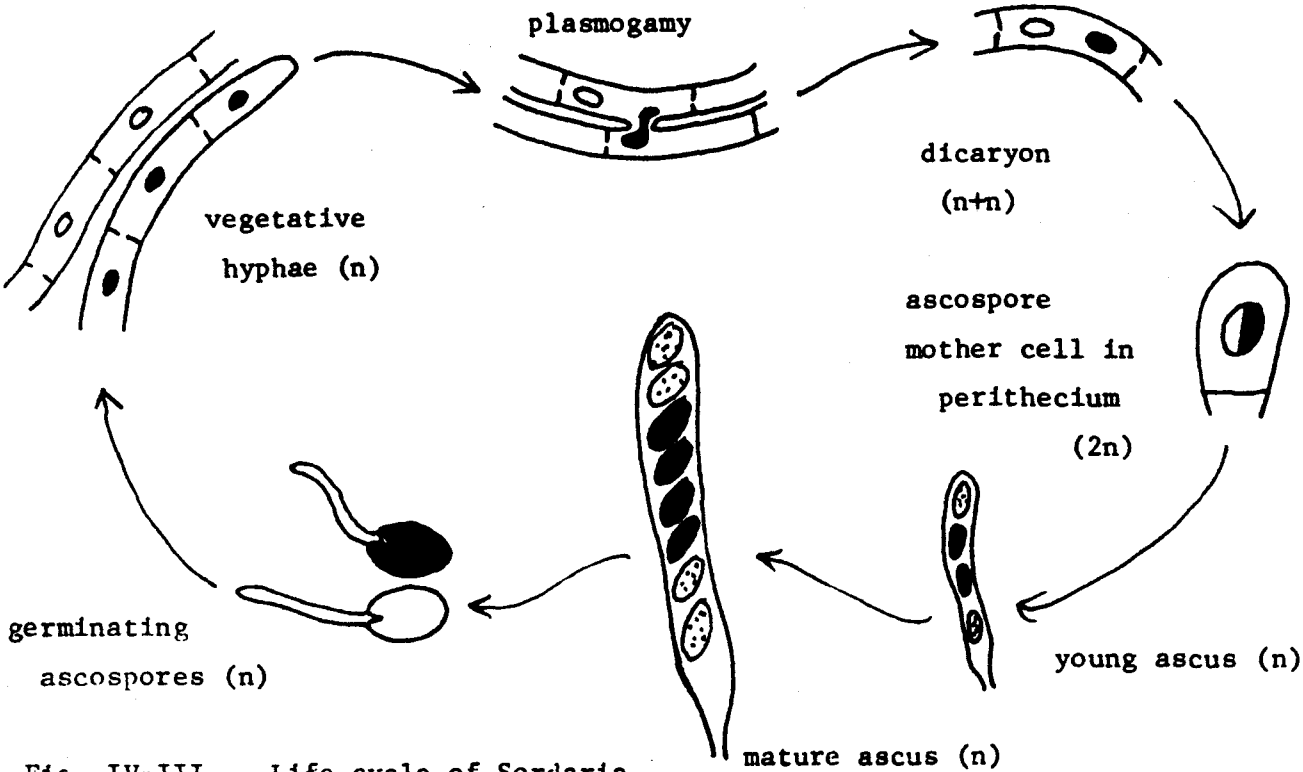


Fig. IV-III Life cycle of Sordaria.

Today you will be examining the results of a single factor cross between a strain of Sordaria which produces black ascospores and another which produces grey ascospores. A single gene controls production of the pigment and the spores of each strain exhibit the phenotypic expression of either the black or the grey allele. Mycelia produced from a single spore of Sordaria are also able to produce ascospores. These would show only the colour of the parent, so you may see asci with all black ascospores or all grey ascospores, or mixtures of the two.

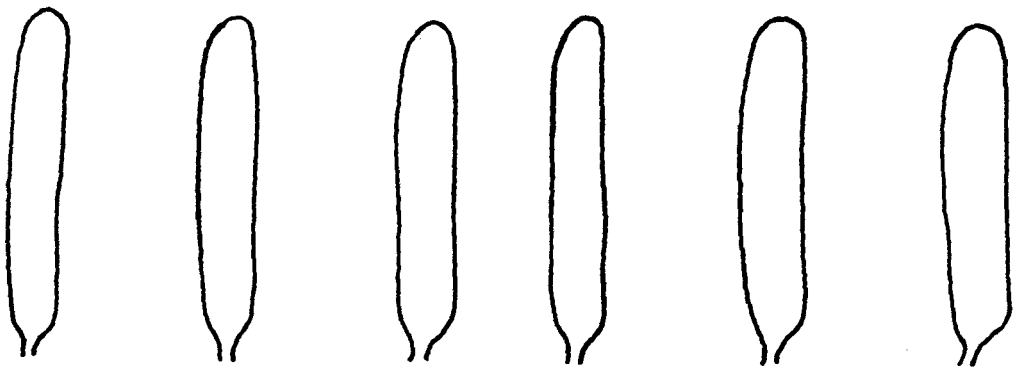
Procedure

1. Under the dissecting scope, observe the piece of agar medium on which the hyphae have grown.
2. With a dissecting needle, lift off one perithecium and place it in a drop of water on a slide.
3. Apply a coverglass and exert just enough pressure on the coverglass to burst the perithecium, releasing a starburst of the asci, but not enough to discharge the ascospores from within each ascus. Use the microscope to examine your preparation.
4. Ignore any asci which are immature or which contain only one colour of spore,

and look for those which contain <u>both</u> black and grey ascospores.

5. If you do not get a good cluster of asci or if the ascospores are not mixed try other perithecia, adjusting your technique accordingly.
6. Score at least 30 asci according to their spore arrangement and record your data on the diagram below and on the blackboard.

Fig. IV-IV



Number of each type

Class Data

II <u>Calculation of Gene Locus Distance from Centromere</u> (Pgs. 575, 576)

The frequency of crossing over is directly related to the distance of the gene in question from the centromere. If the gene is close to the centromere very little crossing over will occur. If it is far from the centromere, crossing over will occur more frequently.

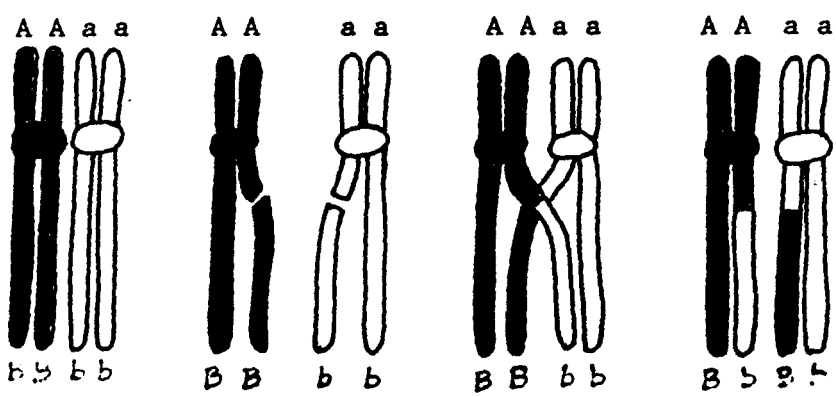


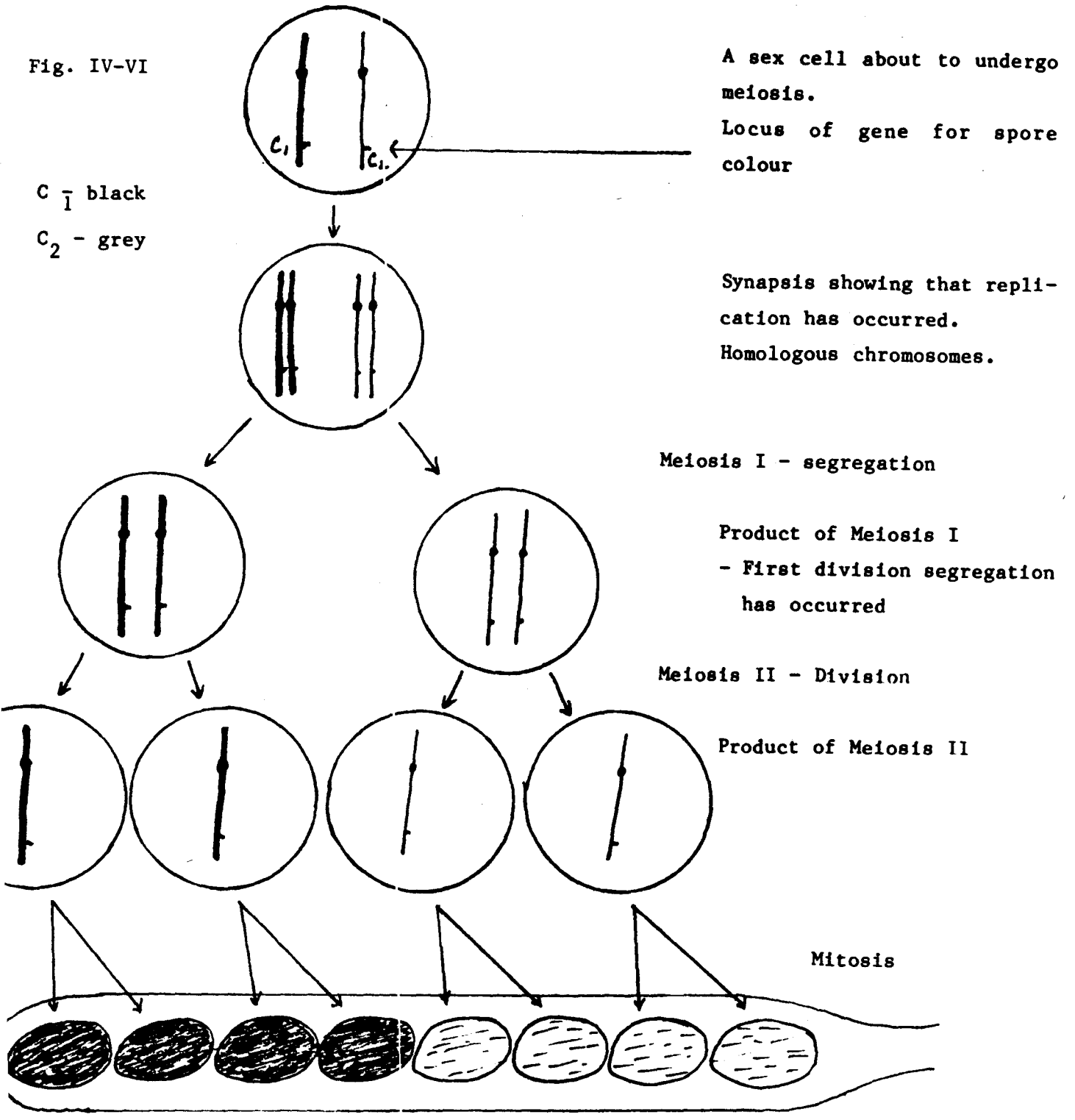
Fig. IV-V Schematic model of crossing over

(1) The two homologous double-stranded chromsomes, one bearing alleles A and B and the other alleles a and b, lie side by side in synapsis.

(2) After breakage of the crossed configuration, the fragments fuse in the exchanged configuration, with the result that one chromatid of the first chromosome bears alleles A and b and one chromatid of the second chromosome bears alleles a and B.

To understand what happens during crossing over, let us first review meiosis as it occurs with no crossing over. See Pg. 572.

Fig. IV-VI



Ascus containing ascospores

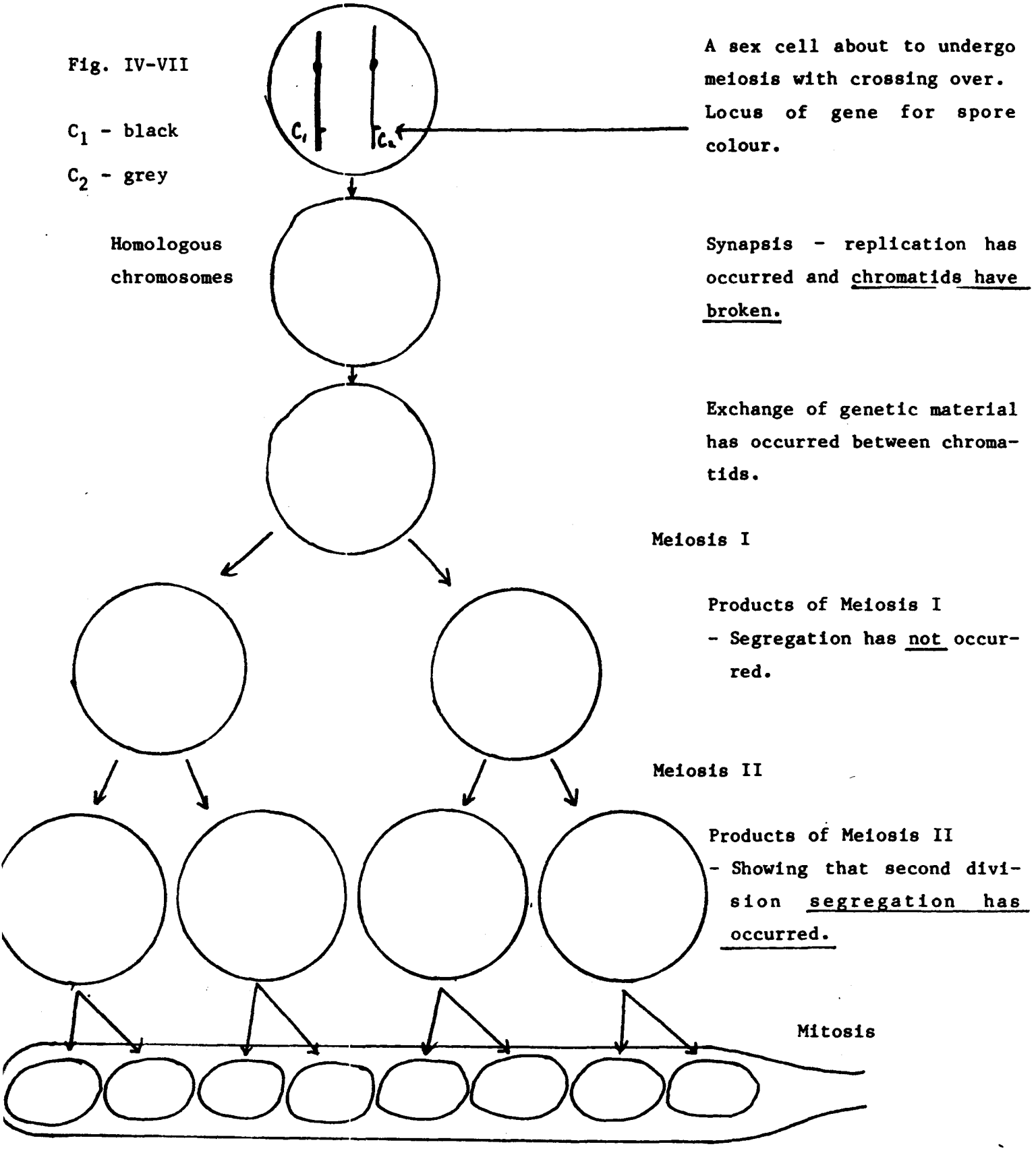
N.B.: This pattern indicates that no crossover occurred in this ascus between the centromere and the C locus.

Work out a diagram similar to that on the previous page which would show what occurs during a crossover event which includes the gene locus for spore colour.

Fig. IV-VII

C_1 - black

C_2 - grey



Results of this diagram show that the proportion of asci which show second division segregation can also be considered as a measure of crossover events.

N.B.: One crossover event results in an ascus which shows second division segregation indicating that crossing over has occurred. However only one half of the ascospores are the recombinant type since one chromatid of each pair remained unchanged during meiosis. Therefore, to convert second division asci frequency to recombination frequency, you must divide by two.

Number of FDS asci = x

Number of SDS asci = y

Crossover frequency = $\frac{y}{x+y} \times 100\% =$ _____ %.

Recombination frequency = $\frac{y/2}{x+y} \times 100\% =$ _____ %.

You may assume that 1% cross over frequency is the equivalent of one unit of map distance.

What is the distance of the spore colour gene locus from the centromere in map units?

Comment on the numbers (proportions) of each of the six classes of asci which you scored.

LAB V PHOTOSYNTHESIS: ENERGY CAPTURE BY GREEN PLANTS

Reference: Text pp. 139-155

Objectives: When you have completed this lab you should be able to:

1. Distinguish between the light and dark reactions of photosynthesis.
2. Outline the steps involved in each of the light and dark reactions and resulting end products.
3. Measure the extent of the dark reaction as a function of light by testing for the production of starch.
4. Demonstrate the effect of changing light intensity on the light reaction as measured by O_2 production.
5. Measure the rate of photosynthesis by measuring the rate of reduction of the dye DCIP.

The energy that supplies all life on earth comes ultimately from sunlight which drives the process of photosynthesis. We cannot overemphasize the magnitude or the importance of photosynthesis for our environment and for life itself. The world economy depends largely upon the combustion of fossil fuels which are the products of earlier photosynthesis. The annual world steel production is ~350 million ton but the amount of carbon which is fixed annually by photosynthesis would be ~550 billion ton. It is interesting to realize too, that 90% of this activity occurs in the surface layers of the oceans not including fresh water lakes and ponds or land plants.

In biological terms, photosynthesis uses energy and water to convert CO_2 into carbohydrate which is then polymerized by the plant into starch or cellulose for storage.

In chemical terms, photosynthesis is the process that converts light energy into chemical energy. The energy is stored by the reduction of carbon dioxide within green plants, i.e. the synthesis of energy-rich sugar from energy-poor carbon dioxide.

Reduction reactions are those involving the addition of an electron (e^-), and oxidation reactions are the reverse, involving the removal of an electron. Reduction

reactions store energy in the reduced compound by adding electrons and oxidation reactions liberate energy through the oxidation of the reduced compound. Whenever one substance is reduced, another is oxidized.

A^{e-}		B	⟶	A	+	B^{e-}
electron donor	+	electron acceptor		oxidized (energy lost)		reduced (energy gained)

In biological systems, the most common way by which a compound may be reduced is through the addition of hydrogen.

AH	+	B	⟶	A	+	BH
electron donor		electron acceptor		oxidized (energy lost)		reduced (energy gained)

The overall process of photosynthesis may be seen as an oxidation-reduction reaction. Carbon dioxide is reduced and water is oxidized.

$$6H_2O + 6CO_2 \xrightarrow{light} C_6H_{12}O_6 + 6O_2.$$

The energy for this reaction is trapped from light by the chlorophyll of green plants. Like many other pigments, chlorophyll absorbs light energy, but unlike any other pigment, chlorophyll is able to pass on this energy in the form of energized electrons to the biological electron acceptor, the coenzyme NADP (nicotinamide adenine dinucleotide phosphate) to reduce it to $NADPH_2$.

Photosynthesis, of course, is not a single step reaction as suggested by the above equation. The reactions making up photosynthesis may be considered in two parts

1. The entrapment of light energy by chlorophyll, and the conversion of this energy into chemical energy contained in molecules of ATP and $NADPH_2$, and thus making available the energy required to drive the Calvin cycle (see part 2).
2. The Calvin cycle (sometimes called the dark reactions) which includes the nonphotochemical fixation, reduction and metabolism of carbon.

The following is a summary of the two parts (light energy trapping and its conversion to chemical energy; the Calvin cycle: reduction of CO_2).

1. Photochemical Reactions - for trapping and handling energy.

The radiant energy of light is used for two purposes involving two sets of interrelated reactions.

a) Cyclic Photophosphorylation or Photosystem I

When a photon of light of appropriate wavelength strikes the pigment molecules of a plant, it may pass right through, or be reflected, or it may be obsorbed by the plant. If absorbed by one of these "antenna molecules", its light energy is transferred to an electron of the pigment molecule, raising that electron to a higher, relatively unstable energy level. This energized electron is then passed from pigment molecule to pigment molecule until reaching the specialized chlorophyll "a" molecule designated as P700, called the reaction center molecule. Here the electron is trapped and passed to a specialized very strong acceptor molecule designated as X which in turn passes it down through a series of energy releasing steps through other acceptor molecules at lower energy levels and back to its normal state in the chloroplast.

The energy released as the electrons are eased down this energy gradient are used to synthesize ATP. Recall that when ATP is used to perform work of any kind in a cell, its high energy bonds are broken by hydrolysis, releasing the terminal phosphate group, the ADP molecule and energy.

$$ATP + H_2O \underset{\longleftarrow}{\overset{enzyme}{\longrightarrow}} ADP + P_i + energy$$

Thus mitochondria are not the only cytoplasmic structures capable of generating ATP. Light-dependent generation of ATP is called photophosphorylation to differentiate it from oxidative phosphorylation which occurs in mitochondria.

b) Noncyclic Photophosphorylation or Photosystem II

When light of the proper wavelength strikes a pigment molecule of photosystem II, light energy is again transferred to an electron of the pigment molecule, and again passed around until it reaches the specialized chlorophyll "a" molecule desig-

nated as P680 which is able to pass the energized electron to the acceptor molecule Q.

Q then passes the electrons through the chain of acceptor molecules which are part of Photosystem I, filling the electron holes which had been left there by the first light-driven event, and contributing to the synthesis of ATP while being eased down the energy gradient to the P700 molecule of Photosystem I.

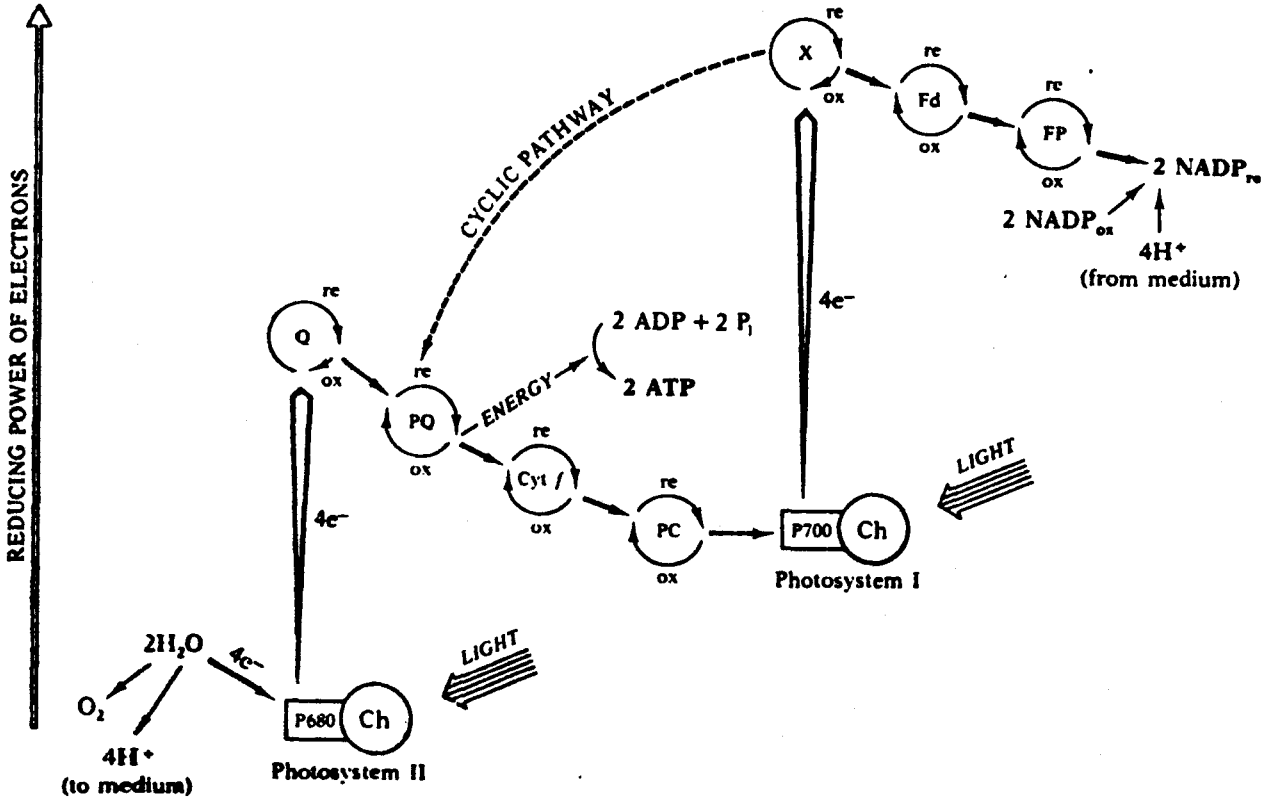


Fig. V-I

Upon reaching the P700 molecule, the energized electron may be passed through the Photosystem I cycle or it may be passed down through a different series of transport molecules to produce the electron carrier molecule NADP. This process however, leaves electron holes in Photosystem II, so it is at this point that the P680 molecule pulls replacement electrons away from water, thus causing the splitting of water.

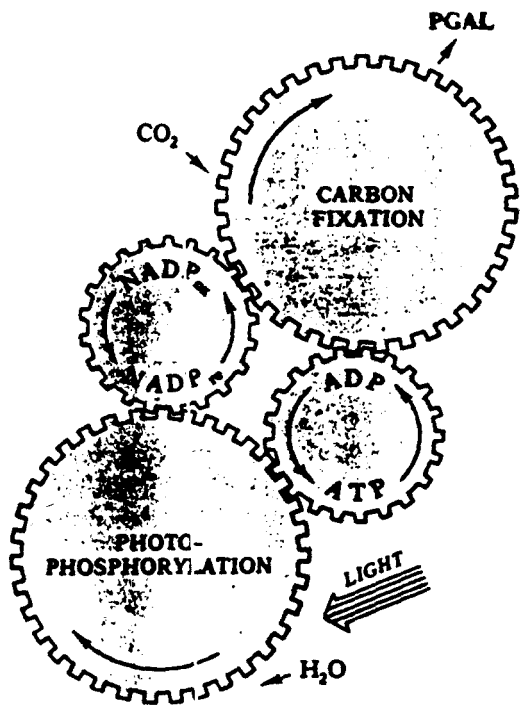
$$2\ H_2O \rightarrow \underset{\downarrow}{4e^-} + 4H^+ + O_2$$
$$\text{to P680}$$

The NADP molecule retains a pair of energized electrons so that it attracts a H^+ proton released during the splitting of water and another H^+ is released into the medium. Thus reduced NADP becomes NADPH + H^+ which we designate as $NADP_{re}$.

$NADP_{re}$ acts as an electron donor in the reduction of CO_2 to carbohydrate during the carbon fixation stage.

Thus the light reactions result in the release of oxygen and the formation of $NADP_{re}$ and ATP. The production of these high-energy molecules is a very fast process, almost proportional to light intensity, but other stages, especially those involving enzymes, are much slower.

Fig. V-II



The relationship between photophosphorylation and carbon fixation
The entire photosynthetic process can be visualized as a series of interlocking gears. Energy from light turns the photophosphorylation gear. The turning of this gear causes both the ATP synthesis and the $NADP_{re}$ synthesis gears to turn. These two gears cause the carbon-fixation gear to turn, with resultant production of carbohydrate (PGAL) from CO_2.

2. <u>The Calvin Cycle or Carbon Fixation Stage</u> - for the transfer of hydrogen from H_2O to CO_2.

Using energy produced during the photochemical stage, inorganic carbon (CO_2) is converted into the organic molecule glucose $(CH_2O)_6$ in a series of reactions which are totally separable from the photochemical stage. Most of these reactions are enzyme controlled, which means they are also temperature dependent. Under such circumstances it is the slowest enzyme that determines the rate of the entire process.

In the Calvin cycle, each CO_2 molecule combines with a molecule of a 5-carbon sugar to form a hypothetical 6-carbon intermediate which is so unstable that it immediately splits into 2 molecules of PGA. Each PGA molecule is phosphorylated by ATP and reduced by $NADP_{re}$ to form PGAL— a 3-carbon sugar. Five of these PGAL molecules are recycled to build more of the original 5-carbon sugar, so only the sixth may contribute to the synthesis of glucose. Therefore, six turns of the cycle are required to synthesize 1 molecule of glucose—the 6-carbon sugar which is the end product of photosynthesis.

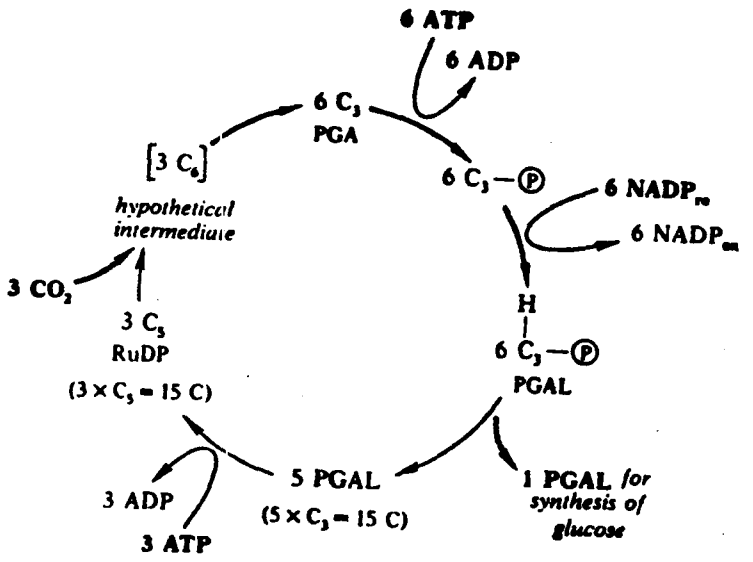


Fig. V-III

Although we tend to think of plants only in terms of photosynthesis, it must be recognized that all living things, plants included, must continuously carry on respiration as well.

Respiration (the aerobic breakdown of nutrients) is a process of combustion during which glucose and other molecules are burned with oxygen to yield CO_2, water and energy for growth, reproduction etc, a process which is the reverse of photosynthesis.

$$C_6H_{12}O_6 + 6\ O_2 \xrightarrow{\text{energy}} 6\ CO_2 + 6\ H_2O$$

When oxygen is not available, fermentation is the process whereby cells may obtain the energy for growth, e.g. yeast.

A - ROLE OF LIGHT IN PHOTOSYNTHESIS

I. Necessity of Light

This demonstration experiment is designed to show that light is the source of the energy for photosynthesis and that the manufacture of food does not take place in a leaf without light.

A variety of plants and leaves will be available at the demonstrators bench for this test. Students should think about results they might expect to observe with different types of leaves.

1) geranium leaves kept in the bright light of the greenhouse.
2) geranium leaves kept in the dark for 48 hours.
3) leaves of various colours e.g. coleus, red, yellow.
4) leaves without colour e.g. green and white.
5) geranium leaves covered in black, with patterns cut out of the centres - left 48 hours in greenhouse.

Procedure - Demonstration

1. For each separate leaf the demonstrator will drop it into boiling water for 2 to 3 minutes to hydrolyze (break down) the cell walls.
2. Each leaf will then be removed from the water and placed in boiling alcohol for 1 minute to dissolve and remove the pigment.
3. Leaves will be transferred to Lugol's iodine to show the presence (or absence) of starch.

4. Leaves will be rinsed in clear water and passed around.

Questions to be answered by students during class discussion.

1. What does the iodine test show?
2. Why would you expect a positive test for the substance?
3. Why do we get a differential positive test - positive in some areas, negative in others.
4. Is light necessary for photosynthesis?

This experiment should be written up by students in the report.

II. Effect of Light Intensity on Rate of Photosynthesis

The intensity of sunlight striking the earth's surface varies from hour to hour, from day to day, and from season to season. To determine if the rate of photosynthesis varies with the amount of available light, we are going to perform a semi-quantitative experiment in which the volume of O_2 produced is equated to the rate of photosynthesis. You will recall that O_2 is produced as a result of the splitting of water.

Procedure - Work in pairs.

1. Fill the large test tube to within 2 cm of the top with 0.25 % sodium bicarbonate ($NaHCO_3$). Clamp it into position inside the beaker of cool water, as in the diagram.
2. Choose a fresh sprig of Elodea 10-15 cm long. Make a clean cut with the razor blade to remove 1 cm from the base end of the sprig (opposite to the growing tip) and remove all leaves from this cut end with your forceps.
3. Insert the Elodea upside down into the $NaHCO_3$.
4. Lower the pipet over the cut end of the stem and into the $NaHCO_3$. Clamp the pipet into position.
5. Loosen the small clamp on the rubber tubing at the tip of the pipet. Suck the fluid up until the pipet is almost full, then clamp it off.
6. Position the light bulb 60 cm from the plant. Turn it on and allow the system to stand for 5 minutes for the plant to become adjusted. You should see bubbles start to rise from the cut stem of the plant.
7. Record the exact level of the $NaHCO_3$ in the pipet, and start timing.

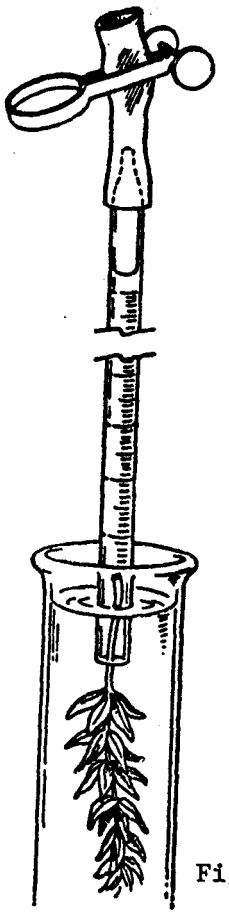
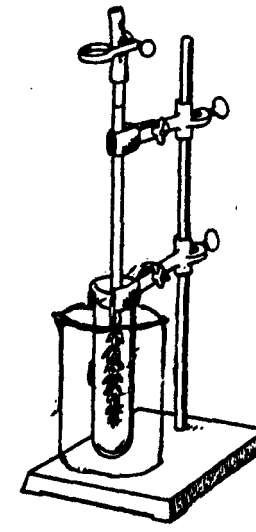


Fig. V-IV

Fig. V-V



Distance from light source	Volume of O_2 evolved
60 cm	
40 cm	
20 cm	

Vol of oxygen (ml)

Distance from light (cm)

Graph V-1

Effect of Light Intensity on Photosynthetic Rate

8. At the end of 10 minutes read and record the level of fluid in the pipet. Calculate the volume of water which has been displaced.
9. Test the accumulated gas at the tip of the pipet by holding a glowing splint over the tubing and opening the clamp.
10. Repeat the experiment twice more changing the distance of the light source to 40 cm and to 20 cm.
11. Enter your results in the table and plot the data on the graph.

Why was the plant placed in $NaHCO_3$ rather than water?

Why was the tube with the plant put into the beaker of cool water?

As light intensity increases, does the photosynthetic rate also increase?

B - THE HILL REACTION

It has only been during the last 50 years that biologists have begun to really understand photosynthesis. It was the work of Hill in 1937 which led to our present understanding of photophosphorylation and to the recognition that the light reaction and the dark reaction were separate; they could occur at different times or under different conditions.

The photolytic cleavage of water in the presence of chloroplasts is known as the Hill reaction. Recall that the P700 molecule of chlorophyll passed the energized electron to the acceptor molecule X. We are going to repeat this experiment today, substituting the artifical dye DCIP for the electron acceptor X. The DCIP is blue when in the oxidized form, and colourless when in the reduced form. You will be measuring the rate of change of the intensity of the blue colour in DCIP.

$$2X + 2\ H_2O + \text{light} \xrightarrow{\text{chloroplast}} 2\ H_2X + O_2$$

Procedure - Work in groups of four (the long way of the lab benches).

N.B.: ALL PROCEDURES SHOULD BE CARRIED OUT USING ICE-COLD SOLUTIONS AND GLASSWARE.

1. Remove stems and midribs (veins) from spinach leaves to give ~50 g.
2. Homogenize these in 100 ml of 0.5 M sucrose solution in a blender at 0°C for 60 seconds.
3. Filter this suspension through 2 layers of cheese-cloth into a beaker. Divide the mixture between 2 precooled centrifuge tubes, one for each pair of students.
4. Centrifuge the suspension at top speed for 10 minutes to sediment the chloroplasts. Decant and discard the supernatant.
5. Using a clean, cold glass rod, resuspend the pellet in ~10 ml of 0.5 M sucrose.
6. Store the suspension in the ice bucket until needed.
7. Put 3 ml into a test tube. Mark the tube with your initials and place it in the boiling water bath at the demonstrator's bench for 5 minutes. You will be using 1.0 ml of this in test tube #4 (see table 5-1)

TAKE A DEEP BREATH AND CARRY ON THE EXPERIMENT.

8. Fill the large glass beaker to within 5 cm of the top with water. Add ice to cool the water to near 0°C, then add ice as needed to maintain maximum cold.
9. Fill 4 small test tubes, labelled 1,2,3 and 4, according to this chart, and <u>in the same vertical order</u>. The contents should be mixed by covering the tube with your thumb and inverting.

<u>N.B.</u>: THE CHLOROPLASTS WILL SETTLE OUT. SHAKE WELL BEFORE TAKING EACH SAMPLE.

Table V-I

	Test Tube #1 - Blank	Test Tube #2 - Experimental	Test Tube #3 - Control	Test Tube #4
Buffer pH 6.5	4.0 ml	2.0 ml	2.0 ml	2.0 ml
Chloroplast Suspension	1.0 ml	1.0 ml	1.0 ml	1.0 ml (heat treated)
	MIX WELL	MIX WELL	MIX WELL	MIX WELL
Dye DCIP	0.0 ml	2.0 ml	2.0 ml	2.0 ml
	MIX	MIX	MIX	MIX
Zero the spectrophotometer with TT#1 - the blank (540 nm). Both pair may use the same tube. Read and record the absorbance of each tube.				
Treatment Instructions	Place in rack in ice-water bath.	Place in rack in ice-water bath.	Wrap in foil immediately. Place in beaker of ice-water and store in the dark.	Place in rack in ice-water bath.

10. Set the light bulb at a distance of 30 cm from the tubes and turn it on.
11. At intervals of 1 min. read the tubes on the Spec 20 and record the absorbance of each. To avoid exposing Tube #3 to the light of the room, take readings from it at 10 min. intervals only, shielding it from light exposure as much as possible.

NOTE THAT THE CHLOROPLAST SOLUTION MAKES THE TUBE CONTENTS PARTICULATE RATHER

THAN HOMOGENEOUS, CAUSING CONTINUOUS SMALL FLUCTUATIONS OF READINGS. IGNORE THIS. IT IS NORMAL AND UNAVOIDABLE.

Graph V-II

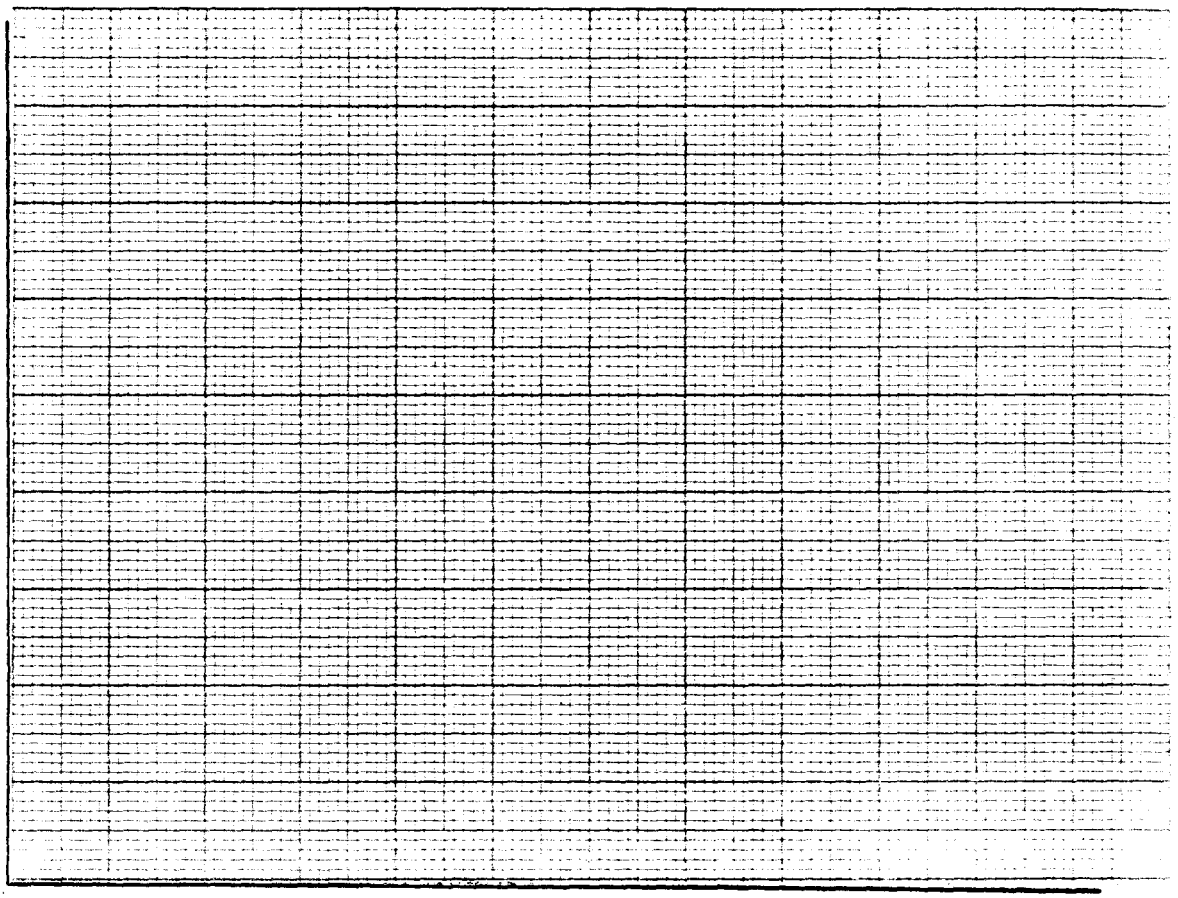


Table V-II

Time	T.T. #1 Blank	T.T. #2 Experimental	T.T. #3 Dark	T.T. #4 Heat treated
1 min.				
2 "				
3 "				
4 "				
5 "				
6 "				
7 "				
8 "				
9 "				
10 "				

In this experiment, what was being reduced and what was being oxidized?

Write an equation which shows the reaction of the experiment you performed.

Outline the function of each of these substances:

a) buffer b) chloroplast suspension c) DCIP
d) ice water e) sucrose solution

What does the slope of the graph suggest about the rate of change?

Is there ever a time in the life of a plant when its rate of respiration and rate of photosynthesis are equally balanced? If so, describe such a situation. If not, why not?

Operation of the Spec 20 Spectrophotometer

When a person looks at a blue book it appears that colour because only light from the blue part of the spectrum (~ 500 nm) is being reflected from its surface; all other parts of the spectrum have been absorbed by the surface of the book. Similarly, to look at a glass of water, almost all light is passing through i.e. being transmitted, so the water appears clear. A bottle of black ink absorbs almost all light so none is transmitted.

The spectrophotometer is an instrument which measures quantitatively, how much light is absorbed by a substance and how much light is being transmitted at any given time. Readings are always taken by comparison with a standard or "blank" which is a tube containing all the ingredients of the test solution except the material being tested or varied.

Light inside the machine shines through a filter which may be adjusted to control the colour of the beam of light according to its wavelength (λ) before passing through the sample. After it has passed through the sample, the light strikes a light-sensitive phototube, producing an electric current proportional to the wavelength of light received. Although readings may be taken either on the absorbance or the transmission scale, we will always use absorbance for our work.

Procedure

1. Turn the machine on (lower left hand knob #1). Allow it to warm up for 10 min.
2. Adjust to the desired wavelength in nanometers (nm) — upper knob #2.
3. With the sample chamber empty and closed, adjust the machine to infinity absorbance (∞) with lower left knob #1. A shutter then blocks all light from the phototube.
4. Fill a spec 20 tube with the blank solution. Wipe it free of moisture and fingerprints with a tissue. Insert into the sample holder and close the cover.
5. Adjust the meter to zero absorbance — lower right knob #3. Remove the blank and leave beside the machine to check or readjust the zero setting.
6. Fill, wipe clean and insert the sample tube, closing the cover.
7. Read the absorbance directly from the meter.

Over time the settings tend to drift slightly. You should reset the infinity absorbance and zero absorbance for each new set of readings or whenever the wavelength is changed.

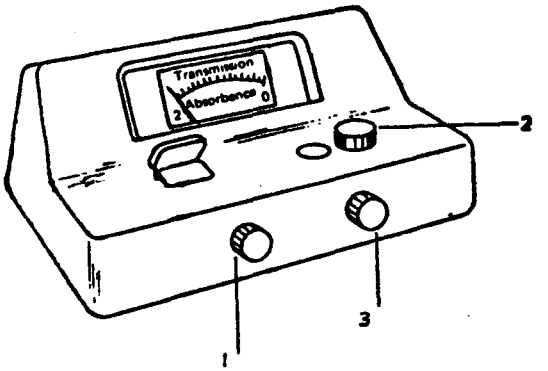


Fig. V-VI

Summary

1. Warm up 10 min.
2. Adjust λ - upper knob
3. Chamber empty and closed
   - adjust to ∞ - left knob
4. Insert blank
   -adjust to 0 - lower right knob
5. Insert sample tube - read.

September 14, 1983
C3/111/1A6/1B71.1/bh

Lab VI - Unicellular and Colonial Organisms

Reference: pp. 485, 922-955

Objectives: When you have completed this lab you should be able to:

1. Discuss the characteristics of procaryotic organisms which justify their placement in a separate kingdom.
2. Describe three different types of bacteria according to shape.
3. Explain the basis of the inhibitory effect of antibiotics on bacterial growth.
4. Compare locomotion among the ciliates, the flagellates and the sarcodina.
5. Describe how some animal-like protozoa are able to perform functions similar to multi-cellular animals.
6. Explain the basis for amoeboid motion as a form of cytoplasmic streaming.
7. Discuss the appearance and the importance of diatoms.

In spite of the great variety to be seen among individual cells, two fundamental categories of cells are usually recognized:

Procaryotes are cells which have a primitive nucleus - one which is not membrane-bound, e.g., bacteria and blue-green algae.

Eucaryotes are cells which have a true membrane-bound nucleus. This group includes the cells of all other organisms.

It is believed that the photosynthetic bacteria and blue-green algae arose in evolutionary time, long before the first eucaryotic

cells. Many biologists also believe that chloroplasts may have developed from primitive blue-green algae taken in as endosymbionts in early times, and that mitochondria may have developed similarly from symbiotic aerobic bacteria.

Procaryotes differ so markedly from eucaryotic cells that recent taxonomists have set them apart as an independent kingdom - the Monera.

A. Kingdom Monera

The characteristics which separate the Monera from other groups are:

- the absence of a nuclear membrane
- the presence of a single circular chromosome
- chlorophyll which, if present, is not contained in chloroplasts
- the absence of mitochondria, Golgi bodies, endoplasmic reticula and other membrane-bound organelles, though ribosomes are present
- the presence of cell walls which contain murein
- the absence of the 9 + 2 tubular structure of flagella

I. Cyanophyta - the blue-green algae - Text p. 932

Organisms of this division may be either unicellular or filamentous. All perform photosynthesis, and contain chlorophyll "a" as in higher plants, but phycocyanin and phycoerythrin are the pigments which give the characteristic blue or red coloration to the group.

Unicellular forms may be either rods or spheres which occur

singly or as colonies embedded in a gelatinous matrix. The cytoplasm of filamentous forms is interconnected between cells and some division of labour occurs. Some filamentous forms produce specialized thick-walled cells called heterocysts which function in nitrogen fixation.

Reproduction is by binary fission or by fragmentation of filaments.

Procedure - Work in pairs.

1. Place a small amount of a) Anabaena on one slide
   b) Gloeocapsa on another slide
2. Apply coverslips and examine first under low power of your microscope, then under high power.
3. For each specimen, make note of the following features:
   - unicellular or filamentous form
   - presence (or absence) of a transparent sheath, i.e. gelatinous matrix
   - the colour of the cells
   - presence (or absence) of nuclei
   - presence (or absence) of heterocysts
4. Make a diagram of each specimen to include a few representative cells which illustrate the observed features.

II. Schizomycetes - The Bacteria

Bacteria are an extremely diverse group with a variety of nutritional/metabolic pathways. They are distinguished from the Cyanophyta by the fact that they do not possess chlorophyll "a",

and that their photosynthesis, if it occurs at all, does not produce molecular O_2.

Most bacterial cells are very small. There are three fundamental bacterial shapes.

i) Cocci (singular coccus)

These bacteria are spherical or ovoid in shape. They may occur in clusters (e.g., staphylococci) or as aggregates of 2 (e.g., diplococci which cause pneumonia), 4, 8, etc. Some form long chain-like strands (e.g., streptococci) in which each is an independent cell.

ii) Bacilli, the rod-shaped bacteria, e.g., Escherichia coli.

iii) Spirilla, the helically coiled bacteria, e.g., Spirilla volutans.

Observe the microscope slides from the demonstrator's bench which show all of the different forms.

Many bacteria are motile. In most cases this is due to the beating of flagella. Reproduction usually occurs by binary fission, producing two daughter cells similar to the parent. This asexual reproductive cycle may be repeated as frequently as every 20 minutes in many species. Some rod-shaped bacteria are able to form special resting cells called endospores, a strategy permitting those individuals to withstand conditions which would be lethal to normal active cells.

Bacterial cells, like the blue-green algae, have a cell wall composed of murein rather than cellulose, the main ingredient of eucaryotic cell walls. This important difference is the basis for

the selective activity of some antibiotics which are nontoxic to plants, animals or to resting bacterial cells, but are toxic to growing bacteria insofar as they inhibit the formation of murein and therefore prevent normal bacterial reproduction. There are three other ways by which antibiotics can inhibit or kill bacteria - damage to the cell membrane, interference with protein synthesis or the inhibition of nucleic acid metabolism.

Gram staining is a widely used method of differentiating between strains of bacteria. It also utilizes differences in the chemical make-up of cell walls.

Procedure - Work in pairs

1. Each pair of students has 2 petri plates containing nutrient agar, each seeded with a different bacterial culture.
   Escherichia coli is a gram-negative bacterium whose cell walls contain much lipopolysaccharide (high lipid content).
   Bacillus subtilus is a gram-positive bacterium whose walls contain leptidopoglycan, and teichoic acids (low lipid content).
2. Following the instructions of your demonstrator, place one of each of the antibiotic discs onto the surface of both culture plates.
3. Cover the plates, mark each with your initials and the date. Place them in the darkened drawer of your lab bench to incubate for 48 hours.
4. Examine the plates after 2 days. Describe and interpret the results.

Kingdom Protista

Kingdom Protista is really a group of taxonomic convenience which contains all those eucaryotes which do not fit into the other kingdoms (i.e., animals, plants or fungi). Most protista are unicellular. Certain protista may, in fact, represent evolutionary precursors of the other eucaryote kingdoms.

B. Animal-like Protista

In spite of their being unicellular, and therefore lacking in tissues and organs, these protozoans exhibit a remarkable degree of functional differentiation. In fact, many possess the same functional properties and characteristics as multicellular animals, but among the protista these functions are performed by subcellular structures called organelles.

Most protozoa are solitary but some may be colonial. Similarly, most are free-living, but some may be commensal or parasitic.

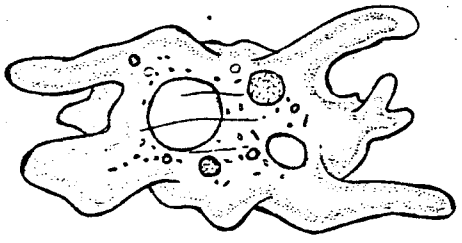
Heterotrophic protozoans which take nutrients from the environment, usually digest food particles in membrane-bound food vacuoles. See Page 214. In some forms these food vacuoles may circulate, carrying nutrients to all parts of the cell. Wastes may then be expelled through temporary openings in the cell surface. Contractile vacuoles function to rid the cell of any excess water. The external cell membrane itself is the exchange surface for nitrogenous waste and respiratory gases. Locomotion is

accomplished either by the formation of pseudopodia or by the beating of cilia or flagella. Co-ordination of the ciliary beating and consequent motion can be a remarkably complex mechanism.

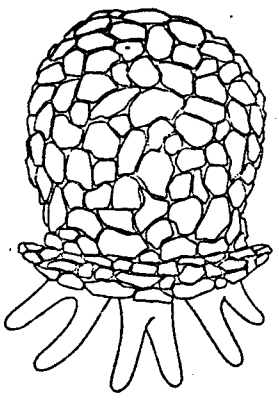
Reproduction may be asexual or sometimes sexual, and encystment to withstand unfavourable conditions is a common strategy.

I. Sarcodina

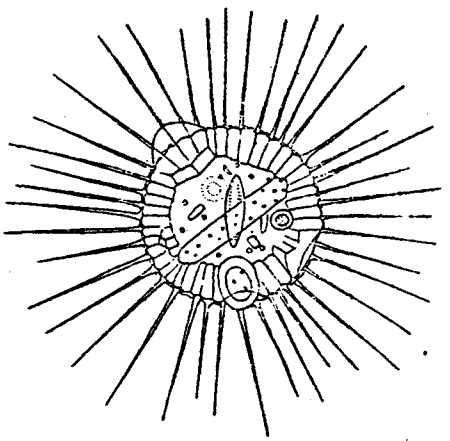
The Sarcodina are the amoeboid protozoa which move and sometimes feed by the formation of pseudopodia. A pseudopod is an extension of the enclosed cellular cytoplasm, and the alternate conversion of the cytoplasm from sol to gel form and vice versa is responsible for amoeboid movement. Many Sarcodina secrete hard calcareous or silicious shells about themselves which in some species may be quite elaborate. See Pages 942 and 485.



Amoeba



Difflugia



Actinosphaerium

Procedure - Work in pairs.

1. With the bulb pipet, remove a few drops of culture from the bottom of the Amoeba proteus culture jar on the demonstrator's bench.
2. Place it in the depression slide and apply a coverslip.
3. Examine the slide at medium power, looking for a grey structure.
4. Examine an amoeba at both high and medium power to observe the amoeboid motion.
5. Write a description of amoeboid motion, based on your observations.

II. Sporozoa - Page 943.

The sporozoa lack special organelles for locomotion. This is not surprising because all sporozoa are parasitic. Most of them produce a resistant spore which is able to survive environmental stresses between hosts.

Mature forms all possess a well-developed apical complex which is not visible by light microscope.

Sporozoa are responsible for the single most important disease of humans in the world today - malaria, as well as many other diseases which cause millions of dollars of loss to agriculture every year, e.g., coccidiosis, toxoplasmosis and sarcocystosis.

We will not be examining organisms from this group.

III. Ciliata - Page 945

There is considerable variety among the various species of ciliates, but all accomplish locomotion by the continuous beating of cilia during some part of the life cycle.

Some of the features which make this group so remarkable are:

- the frequent presence of a cytopharynx (cell mouth) at the base of which food vacuoles are formed;
- the presence of an anal pore through which cellular wastes are expelled;
- contractile surface fibrils analogous to the muscular systems of multicellular organisms;
- the occasional presence of an elastic-like stalk for attachment to the substratum;
- tentacles and or trichocysts for defence and prey capture;
- the occasional presence of skeleton-like plates in the pellicle (exterior surface).

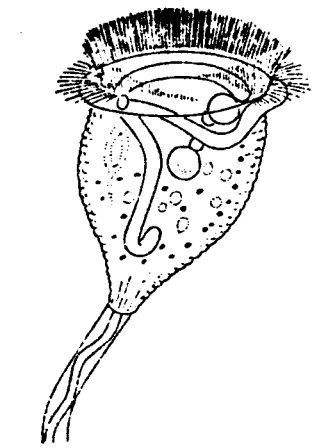
Ciliates differ from other protozoa in having two nuclei - a large macronucleus which controls metabolic cell processes, and a small micronucleus which is concerned only with reproduction.

Procedure - Work in pairs

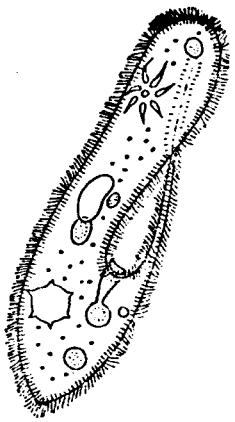
1. With the bulb pipet, take only 1 drop from the bottom of the culture dish of Paramecium caudatum and place it in the depression slide.

2. Add 1 drop of methyl cellulose to slow the organisms down. Cover the slide with a coverslip.
3. Examine the preparation under medium power and then under high power. Keep the light source at a minimum to prevent heat from bursting the pellicle.
4. Find the two contractile vacuoles. Count the number of seconds between expulsion of each. Is the rate of expulsion similar or does one vacuole empty more frequently than the other?

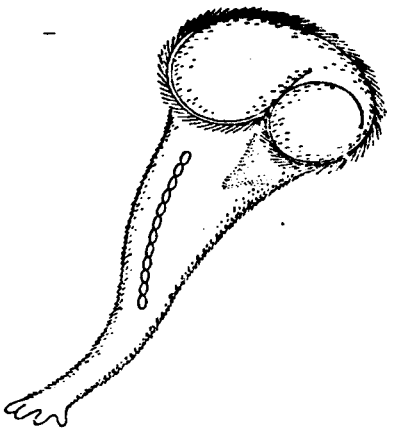
   The posterior vacuole empties more frequently.



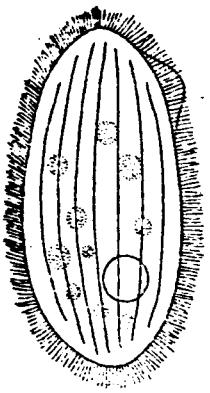
Vorticella



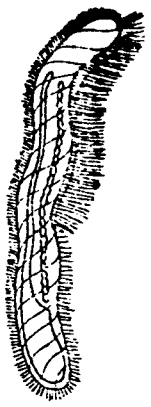
Paramecium



Stentor



Colpidium



Spirostomum

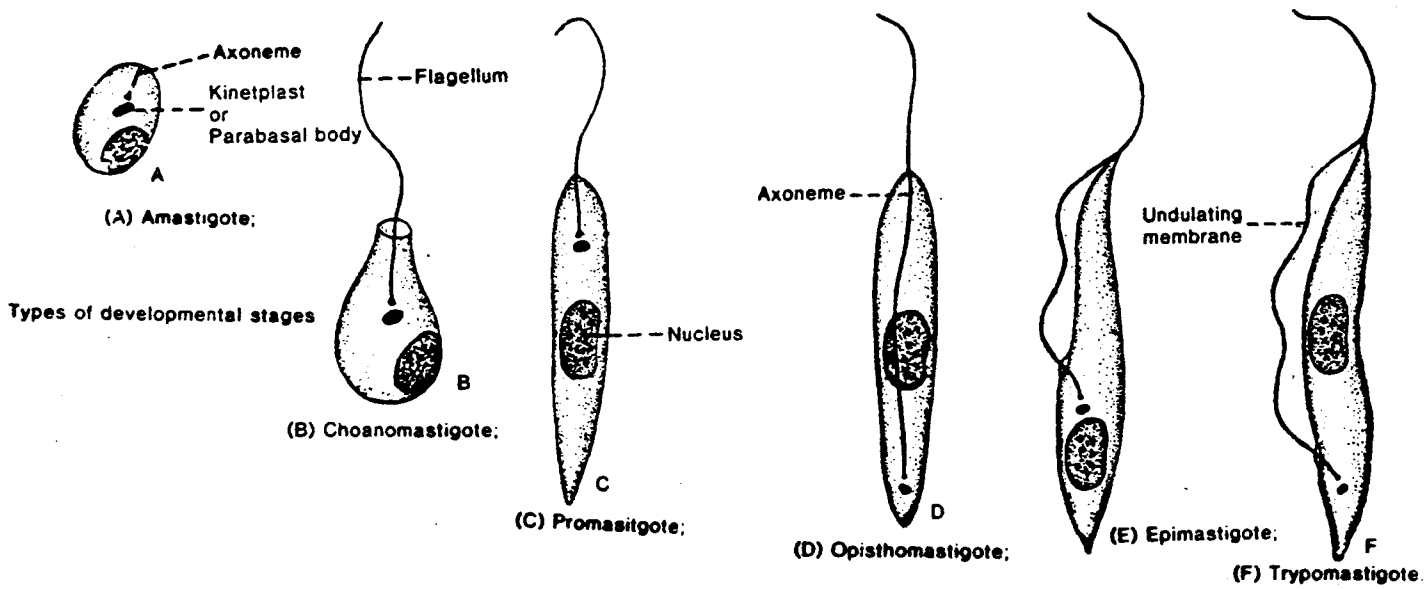
IV. Mastigophora - Page 940

These are the most primitive of the protozoa, and perhaps ancestral to the other groups. Flagella are the means of locomotion. Some species are free-living, but most live symbiotically in higher plants or animals. Many of these are parasites, causing man and his domestic animals considerable trouble.

ON DEMONSTRATION ONLY

Today you will examine slides of Trypanosoma, one species of which is responsible for African sleeping sickness. Trypanosomes are blood or tissue flagellates which are usually transmitted by insects, e.g., tse tse flies. Repeated changes in surface antigens which are released into the blood of the victim cause massive immune reactions of the host, resulting in anemia, edema, lethargy and eventually paralysis or death.

During their life cycle, trypanosomes undergo several developmental changes which represent strategies for survival against host defence mechanisms.



1. Examine the prepared slides of Trypanosoma lewisi. These slides are blood smears containing very small trypanosomes among the red blood cells.
2. Draw a diagram of one Trypanosoma lewisi.

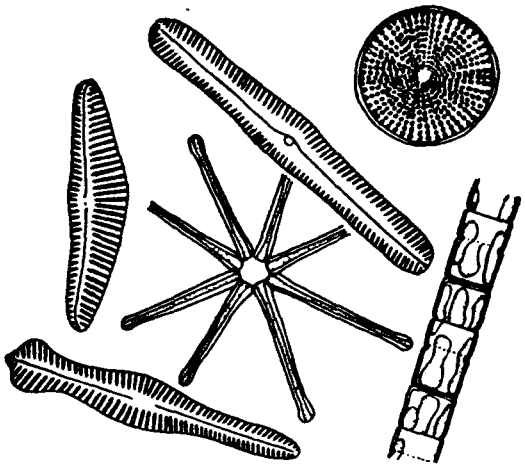
C. Plant-like Protista

Most of the plant-like Protista possess chlorophyll for photosynthesis and are therefore autotrophic. Most also have cell walls and flagella.

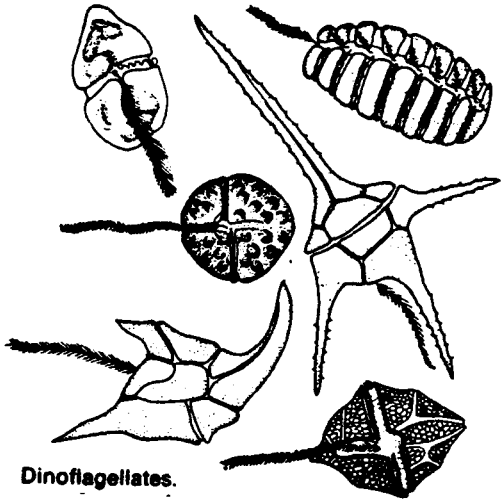
I. The Diatoms - Page 954 - are extremely important in aquatic food chains both in fresh water and marine environments where they make up a large proportion of the phytoplankton which supplies food to organisms higher up in the chain.

Different species exhibit a great variety of shapes and ornamentations with their silica-impregnated, glass-like walls. When the living diatoms die, their shells sink to the bottom where they may form large beds of diatomaceous earth, a substance which is harvested and used in many commercial products from paint removers to fertilizers.

1. Examine the slides of diatoms.
2. Draw a representative diatom.
3. Look at the demonstration of diatomaceous earth on the rear bench.



Diatoms.



Dinoflagellates.

II. Dinoflagellates - Page 955

Dinoflagellates are small unicellular organisms, some of which are luminescent and are responsible for much of the luminescence seen in ocean water at night.

Some contain red pigments which are poisonous. The red tides which occasionally are responsible for large fish kills along the eastern North American coast are caused by dinoflagellates. Under other circumstances, however, dinoflagellates also contribute to the phytoplankton upon which higher life forms feed.

1. Place a few drops of the dinoflagellate culture in your depression slide.
2. Examine the contents on medium power and then on high power.
3. Draw one representative dinoflagellate.

D. Fungus-like Protista - Page 948

Fungus-like protists belong either to the true fungus-like group having haploid sac-like bodies, or to the true slime molds whose vegetative phase (the plasmodium) is diploid.

Today you will look at the plasmodium phase of the true slime mold Physarum polycephalum, which is usually found growing in damp woodsy areas on rotting logs or decaying organic matter. The amoeba-like mass of the slime mold is coenocytic, i.e., multinucleate.

Note particularly these features:

- colour

- growth pattern

- cytoplasmic streaming

Write a paragraph to describe Physarum according to the observed features.

December 13, 1983
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LAB VII GAS EXCHANGE AND INTERNAL TRANSPORT

Reference: pp. 237-256, 270-273.

Objectives: Upon completion of this lab you should be able to:

1. Outline four requirements for gas exchange surfaces.

2. By a combination of examination, reasoning and deduction, postulate a rationale for the respiratory system of Necturus.

3. Compare the rates of oxygen consumption and carbon dioxide production of a mouse under laboratory conditions.

4. Calculate the respiratory quotient (R.Q.) of a mouse.

5. Measure the rate of transpiration of a green plant.

A GAS EXCHANGE

A basic problem for the majority of living oranisms, both plant and animal, is the procurement of oxygen for cellular respiration and the elimination of carbon dioxide. Gas exchange between living cells and the environment occurs by passive diffusion across moist cell membranes.

For unicellular and very small organisms, diffusion through the body surface alone is usually adequate, but for large organisms respiratory exchange surfaces have four basic needs:

1. A respiratory surface area of adequate size relative to the volume of the organism.
2. A method of transport (e.g. the blood circulatory system) between the exchange surface and internal tissues.
3. The fragile respiratory surface must be protected.
4. A method must be devised for keeping the respiratory surface moist.

For survival, organisms larger than a few cells have had to evolve special respiratory devices to handle the problem of getting oxygen to internal tissues.

I Tracheal Systems of Respiration (Pg. 255, 279, 1035)

Terrestrial arthropods, including insects, have evolved a tracheal system of gas exchange. In grasshoppers, air enters the body through valved spiracles, one on each side of every abdominal segment. Muscle action forces the air through tubes called tracheae into smaller tubes called tracheoles from which it diffuses across moist membranes into individual cells.

Procedure: Work individually.

Observe the demonstration on the back bench which shows the tracheal system of the grasshopper. Note these features:

- the silvery colour of the tubes
- the reinforced walls of the tracheae
- the interlacing network of tracheae
- a few tracheae form bladder-like sacs
- the reinforced tracheal walls
- the extensive branching of tracheoles and the multiplicity of terminations.

II Necturus maculosus is an amphibian which lives in fresh water. Its common name is the mud puppy. By observation of the live specimen in the lab and by library research, analyze and write a paragraph to discuss the system(s) of respiration used by the animal as well as the evolutionary significance of your findings.

During the afternoon, nitrogen will be bubbled through the aquarium for 5 mins. This will change the environment from well oxygenated to hypoxic. How does the mud puppy react to the resultant stress?

III Measurement of Oxygen Consumption by the Mouse

In a healthy animal, respiratory gas exchange through the lungs reflects the net respiratory activity of all body cells from aerobic glycolysis, the Krebs cycle, and oxidative phosphorylation. Aerobic respiration, through the chemical breakdown of foodstuffs, provides most of the energy needed by the body.

$$\text{Respiratory Quotient (R.Q.)} = \frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}} .$$

The value of the R.Q. varies, being dependent upon the oxidation level of the metabolic substrate. This substrate does not necessarily represent the diet of the mouse.

Oxidation of the substrate glucose by respiration would follow this equation:

$$(CH_2O)_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O.$$

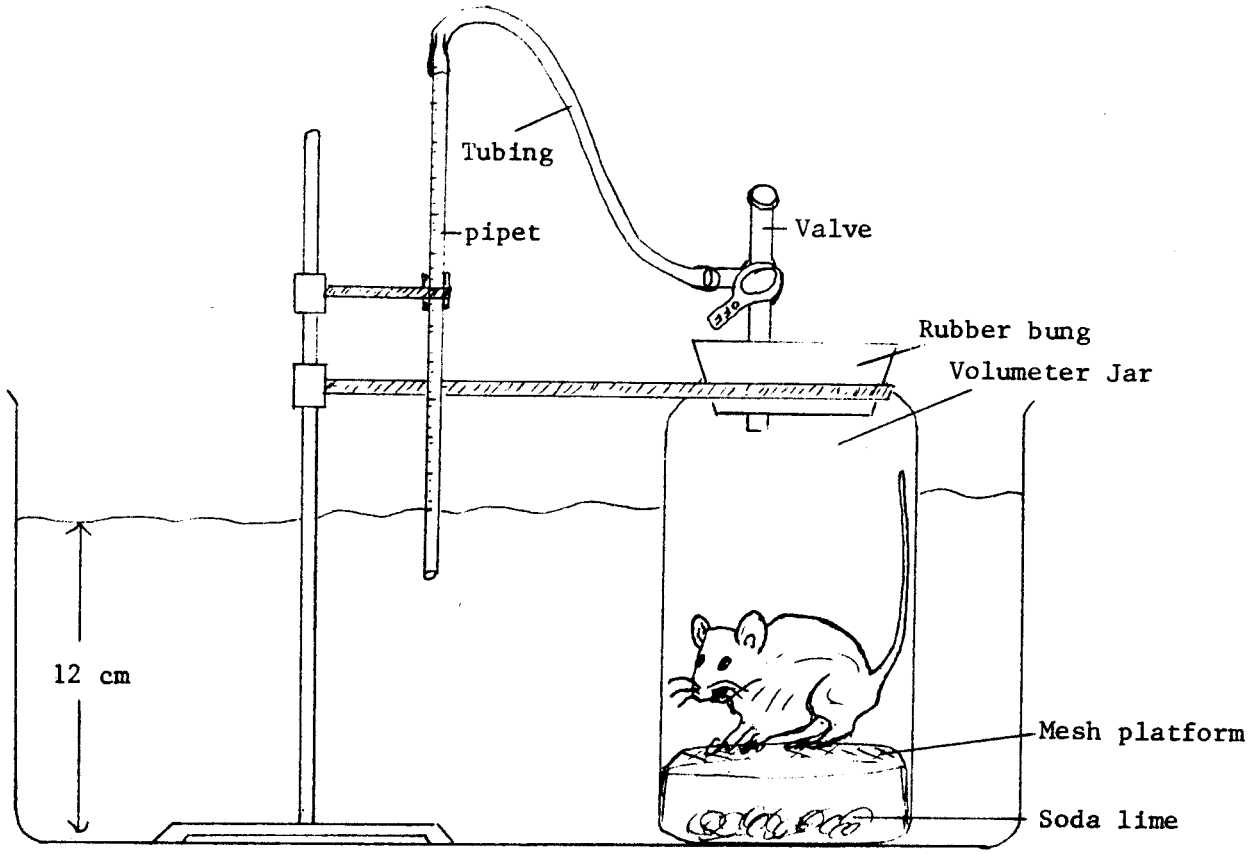
Since 6 moles of O_2 were consumed and 6 moles of CO_2 are produced for each mole of glucose respired, the R.Q. of glucose is 1.0.

Average R.Q. values for typical proteins are lower at 0.8.

Average R.Q. values for fats are lower still at 0.72.

The rate of respiration and the R.Q. are largely dependent upon the environment imposed on the cells by the organism as a whole. Thus they are affected by such factors as nutritional levels, activity level, body temperature, circulatory efficiency, and the production of endogenous agents such as the hormones thyroxine and epinephrine.

You will be measuring the respiratory gas exchange of a mouse with the volumeter system shown on the next page.



Procedure: Work in pairs.

1. Fill the basin with about 12 cm of water at approximately room temperature. Assemble the complete apparatus, i.e. jar to hold mouse, and the inverted pipet before placing it in the basin of water.

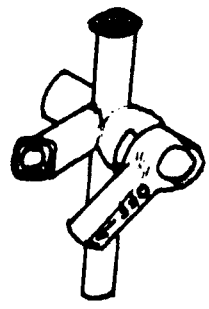
2. Place a packet of soda lime in the bottom of the jar to absorb all CO_2 in the system. Insert the mesh platform which will support the animal and protect it from the caustic effect of the soda lime.

   N.B.: THE SODA LIME MUST BE KEPT DRY.

3. Place the jar on the stand (but not in the water yet). Adjust the iron ring to hold the jar firmly in position.

4. Weigh the mouse by placing it in the container on the balance. CAUTION: MICE CAN BITE, SO HANDLE WITH CARE. Put the mouse into the jar.

5. Examine the 1-way valve in the rubber bung in order to understand how it works. Then <u>open it to the outside atmosphere</u>. (Arrow pointed towards rubber tubing).



Close the jar by putting the rubber bung in place. It is important that the seal be airtight - wetting the edge of the rubber bung will ensure this.

6. Adjust the inverted pipet in its clamp. The 0.0 ml mark should be approximately at the surface of the water when it is placed into the basin.

7. Connect the jar containing the mouse to the pipet by means of the rubber tubing, as shown in the diagram.

8. <u>Transfer the whole apparatus into the water bath now</u>. Equilibrate the pressure within the system to the atmospheric pressure by moving the 1-way valve back and forth several times, so as to alternately connect the mouse jar to the pipet and to the outside atmosphere. Set the 1-way valve so that the volumeter jar is connected to <u>the outside atmosphere</u> and allow the system to stand for 5 minutes in order to:

   a) allow the animal to settle down.
   b) let the temperature equilibrate through-out the system.
   c) allow the absorption of any excess CO_2 initially in the system.

9. After the 5 minute period, again move the 1-way valve back and forth several times to ensure complete pressure equilibration. Now set the 1-way valve so that the volumeter jar is connected to the pipet and <u>start timing</u>.

The mouse will now be breathing in the air enclosed in the system - i.e. it will

extract oxygen from, and excrete CO_2 into this air. What is happening to the CO_2?

Take readings of the water level in the pipet every 15 seconds in order to determine the rate of O_2 consumption. Once the level begins to move, the whole process will probably take less than 10 minutes. At completion, open the valve to the atmosphere to prevent the tube and jar from filling with water, and the mouse from being asphyxiated. Record the readings you obtain.

Plot the change in volume (ΔV) cumulatively on the graph. Draw the straight line which best represents the data, and calculate the slope of this line in ml/sec. This represents the O_2 consumption rate of the mouse. Explain why.

Finally, express it as ml O_2/gm of mouse body weight/hr.

III Since the above set-up allowed you to measure the volume of O_2 consumed, what changes in the arrangement of the apparatus would allow you to measure CO_2 produced? How will you calculate the rate of CO_2 produced alone? Note: you may assume that the rate of O_2 consumption will remain at the level you have measured. Explain your reasoning in detail.

Make the necessary change in the apparatus and repeat the experiment to obtain a new series of readings which will allow you to calculate the CO_2 production rate alone. Record the change in volume as before. Plot these data on the same graph and determine the slope as before. From your data and the graph, calculate the CO_2 production rate, i.e., ml CO_2/gm of mouse body weight/hr.

Also calculate the respiratory quotient (R.Q.) for your mouse.

What substrate was being metabolized by your mouse, as indicated by your data?

The soda lime packets may be reused if they are not wet. Please return them to the closed jar.

B WATER TRANSPORT IN PLANTS

Water makes up 90% of plant tissues. It is the substance in which most materials enter and leave plant cells, as well as being the solvent for the various biochemical reactions that occur in living cells.

The amount of water used by plants is far greater then that used by animals of comparable weight because the relative surface area for water loss is much greater in plants. 90% of the water taken in by the root systems of plants is evaporated through the stomata of the leaves by the process of transpiration.

The purpose of this experiment is to determine the rate of transpiration by a geranium and to examine the role played by leaves in the process of transpiration.

I Determination of Transpiration Rate - Work in pairs

1. Set-up your potometer apparatus (except for the plant) according to the diagram.

2. With the clamp open, fill the apparatus with distilled water. Take care to eliminate all air bubbles.

3. Choose a branch from a geranium plant that will fit snugly into the plastic tubing.

4. Cut the stem at a sharp angle and immediately insert it into the tube, taking care to avoid air bubbles.

   Clamp the plant so that it is at about the same level as the top of the pipette.

   If the cut stem is exposed to air longer than a few seconds, hold the stem under water, remove about 0.5 cm from the cut end, and start again.

5. Allow 5 min. for equilibration of the apparatus, then raise the funnel until the water in the pipette is near the top and close off the clamp.

6. Read the volume on the pipet and note the time. The rate that the meniscus moves along the pipet is a measure of the rate of water uptake by the plant and may be used as a measure of the rate of transpiration.

7. Read the volume at 1 min. intervals for 10 min. or until the water level reaches the end of calibration of the pipet.

8. Open the clamp, allowing the system to fill up once more.

9. Cut off all the leaves. Seal the cut ends with vaseline and repeat the experiment.

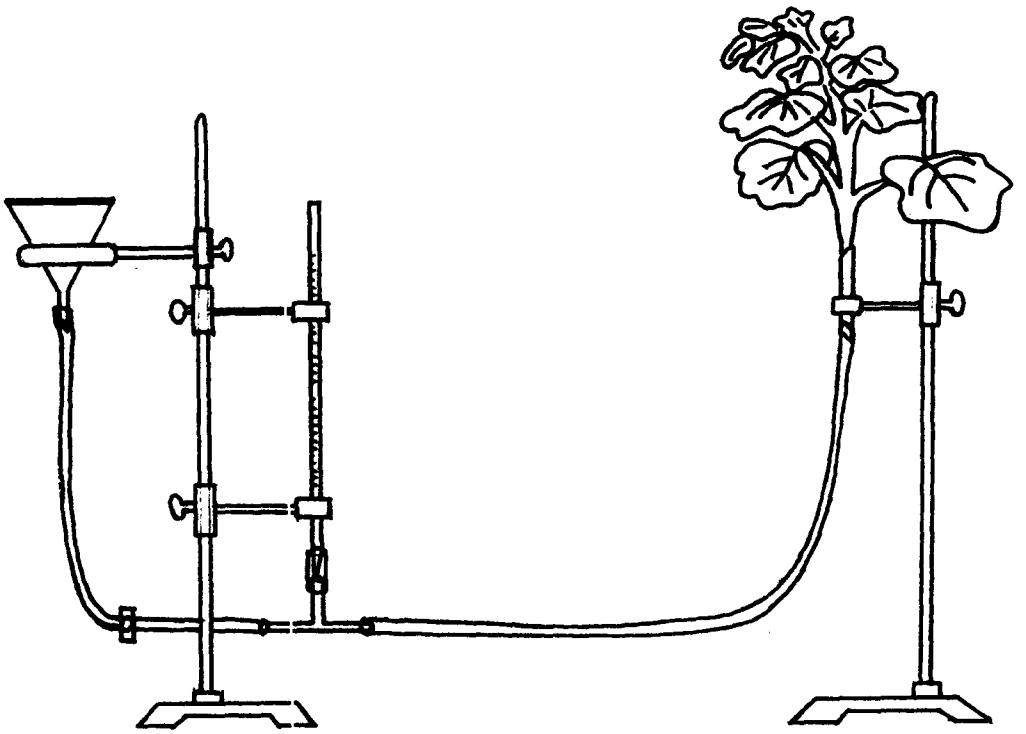
10. Cut off all but 1 inch of the stem. Seal the cut end with vaseline and repeat the experiment.

Plot the data on the graph and determine the rate of transpiration per hour for your geranium plant.

What role do leaves play in the process of transpiration?

What other roles do leaves have which are of benefit to the plant?

Explain how plants have adapted to meet the four basic needs of respiratory surfaces.



Potometer

TIME (MIN.)	WHOLE PLANT volume (ml)	LEAVES REMOVED (ml)	STUMP (ml)
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

LAB VIII REGULATION OF BODY FLUIDS

Reference: pp. 308-333.

Objectives: When you have finished this lab you should be able to:

1. Distinguish between the processes of excretion, elimination and osmoregulation.

2. Describe the grasshopper's system of excretion.

3. Understand why organisms must control their water balance.

4. Trace the path of urine through the vertebrate kidney from the point of entry to the exterior.

5. Relate the structure of the nephron to the internal structure of the vertebrate kidney.

Living cells interact continuously with their surrounding environmental medium because of the osmotic effects of fluids bathing the cells and the nature of cell membranes. In multicellular animals, this environmental medium for the cells is the internal environment (i.e. extracellular fluid) of the organism. All life processes such as gas exchange, metabolism and nutrient procurement are dependent upon the properties of that medium.

Over evolutionary time, the body fluids of different organisms evolved in different directions, along with individual methods for maintaining homeostasis of the body fluids, depending upon the environment, i.e. aquatic (freshwater or marine) or terrestrial.

Plant cells are better able to withstand fluctuations of surrounding environmental fluids than are animal cells, the most important factor being the presence of the relatively rigid plant cell wall. Animal cells, surrounded by a cell membrane only, are much more vulnerable to any osmotic shifts in the surrounding medium.

Animals which have the ability to regulate the osmotic concentrations of their

body fluids and keep them constant despite fluctuations in the external medium are said to have the power of osmoregulation - the control of water balance.

Animals also need mechanisms for ridding their bodies of metabolic wastes, especially nitrogenous ones which are formed from the breakdown of proteins or amino acids within the cells of the liver during deamination. The process of releasing these useless, sometimes poisonous waste products from the body along with excess water, is called excretion. In order to be excreted however, a substance must first have been absorbed into the cells of the body.

Food remains from the intestine that have never been absorbed are eliminated from the body as feces. This process which is fundamentally different from excretion is called elimination (or egestion).

Simple fresh water animals like Paramecium have large amounts of water diffusing in from the hypotonic environment. You will recall that this excess water is collected into contractile vacuoles which expel their contents into the surrounding fluid when full.

The beginnings of a more complex tubular excretory system can be seen in the flame-cell system of flatworms e.g. Planaria. Further up the evolutionary tree, Annelids e.g. earthworms have a pair of excretory organs called nephridia in all of the main body segments. Each nephridium consists of a ciliated funnel-like opening through which water and waste materials are collected from the body cavity, a coiled tubule around which reabsorption occurs into a network of blood vessels, an enlarged bladder, and a pore through which materials are expelled to the outside.

Although descended from an ancestor similar to that of earthworms, the insects evolved an entirely new and unique excretory system, called Malpighian tubules which we will be observing today.

Like the nephridial system of earthworms, the excretory systems of vertebrates are closely associated with the circulatory system, and although some wastes may be excreted in sweat, or through the lungs, the main vertebrate excretory organ is the kidney.

I The Problem of Water Balance in an Invertebrate

In the lab today we have live earthworms which are usually found living near the upper surface of moist, well-drained soil. They normally come to the surface only at night or during rains. (i.e. they maintain their water balance and prevent dessication by behavioral adaptations).

As mentioned earlier, the excretory system of earthworms consists of a pair of tubular nephridia in each body segment. These nephridia empty to the exterior through a pair of nephridiopores. In todays lab you will study the degree to which earthworms are able to control water balance in the face of different external salt concentrations, and therefore different osmotic pressures.

Procedure: Work in pairs.

1. Remove 3 earthworms from the soil, and wipe them to remove soil particles. DO NOT RINSE THEM IN WATER.

2. Place each worm in a weigh boat and weigh it to .001 gm on the micro-balance following the instructions given. If in doubt, ask for the demonstrator's assistance.

3. One worm should be immersed in distilled water for one hour.

   One worm should be immersed in a 150 mM NaCl solution for one hour.

   One worm should be immersed in a 300 mM NaCl solution for one hour.

4. Dry and reweigh each worm. Post your data on the board and calculate the averages. Note whether your worms survived or not.

   Did the average weight for each type of treatment increase or decrease, and if so, by what proportion of original body weight?

   Calculate the average increase or decrease in body weight/gram/hour for each treatment.

   How effective is the nephridial system of the earthworm in control of water balance? From the results, make an estimate of the effective osmotic pressure of the earthworm's body fluids (i.e. what concentration of NaCl).

II Malpighian Tubules of Insects

To illustrate the point that different organisms developed in different directions as regards control of body fluids, observe the demonstration on the back bench showing Malpighian tubules, the excretory system of insects.

The tubules are blind sacs which vary in number from 2 to 200 in different species. They lie in the hemocoel, the body cavity which is bathed directly with blood from the open circulatory system. Fluid from the hemocoel is absorbed into the ends of the tubules and as it moves toward the gut, nitrogenous wastes are precipitated out. Water and salts are reabsorbed into the blood, so the urine becomes increasingly more concentrated. The tubules empty their contents into the hindgut of the digestive system where they are carried to the rectum, an organ which has powerful reabsorptive capabilities such that urine and feces are eliminated from the body as very dry material.

Make a diagram of a portion of one Malpighian tubule enlarged, to show its shape.

III The Vertebrate Kidney - Work in pairs

Observe the double injected kidney on the demonstrator's bench then study the external structure of your beef kidney (half) to identify the following parts:

1. The adrenal gland (an endocrine gland) lying close against the curved anterior surface of the kidney, embedded in fat and tissue.

2. The renal hilum - the depression on the inner side of the kidney where the blood vessels and ureter are attached.

3. The renal artery which carries blood from the aorta to the kidney.

4. The renal vein which carries blood from the kidney to the posterior vena cava.

5. The ureter which carries urine from the kidney to the urinary bladder.

Study the internal structure of your kidney to identify the following parts:

1. Cortex - the outer, granular layer of the kidney which contains the renal corpuscles, each composed of a Bowman's capsule and enclosed glomerulus, along with parts of the proximal and distal convoluted tubules.

2. Medulla - the inner section of the kidney which is striped in appearance due to the presence of many collecting ducts. It contains the loops of Henle as well as radially arranged blood vessels.

3. Pelvis - the central thin-walled chamber which collects the urine and connects with the ureter. This is the area within which kidney stones form in some people.

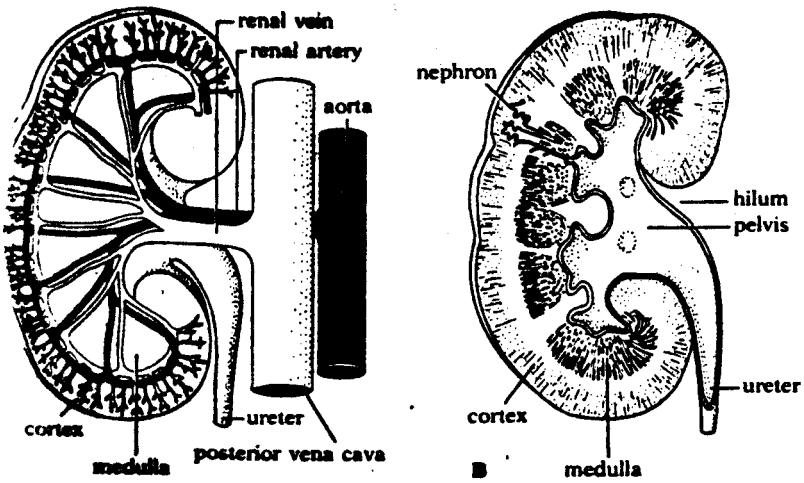
4. Renal pyramids - the area where groups of collecting ducts come together in rays to enter the central pelvis.

5. Calyx (plural calices) - the area of the pelvis into which a renal pyramid empties.

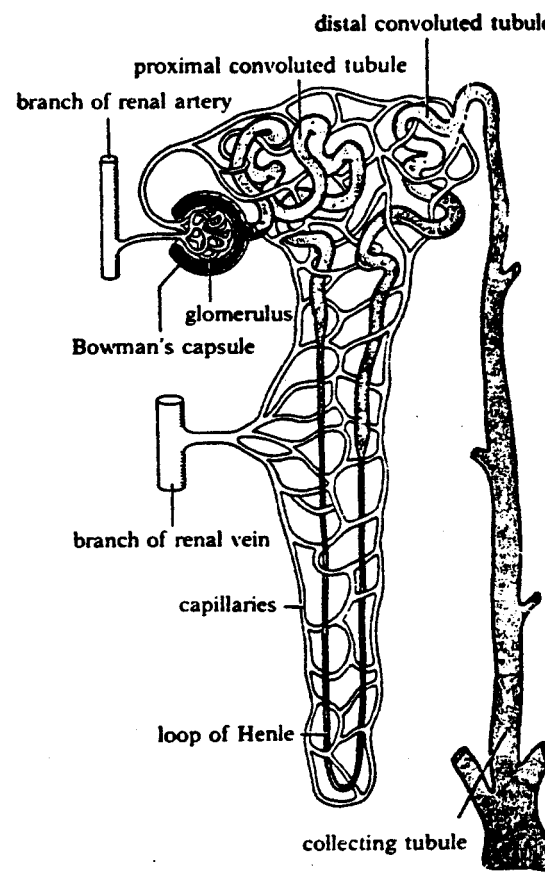
Cut a thin slice from the kidney to include both cortex and medulla. Cover it with saline in a petri plate and examine under the dissecting scope to identify individual parts of the nephron. The pink circles in the cortex are glomeruli, each surrounded by a Bowman's capsule. The round hollow tubes are the proximal tubules and the irregularly-shaped tubes are the distal tubules.

Make a diagram to show the actual appearance of nephrons in the kidney.

Write a description of the path of urine from the time of entry in the blood via the renal artery to the time of exit from the body. At each phase note the major "processing" events which modify urine composition. Refer to your text.



8.13 Sections of the human kidney
(A) The blood circulation of the kidney. (B) The cortex and the medulla, and the large renal pelvis into which the collecting tubules of the nephrons empty. One nephron is shown (color); note that the glomerulus and convoluted tubules are in the cortex, but that the loop of Henle runs down into the medulla.



8.14 The human nephron
For description see text. [Modified from H. W. Smith, *The kidney*, Oxford University Press, 1951.]

LAB IX PLANT HORMONES

Reference: Text pp. 335-348
721-726

Objectives: When you have completed this lab you should be able to:

1. Explain the role of auxins in cell elongation, lateral bud development, and plant responses to physical stimuli.

2. Given a living plant or a diagram of a plant, show where each of the effects of auxin occurs.

3. Describe how to carry out an experiment to test the hypothesis that apical dominance is due to production of auxin by the apical meristem.

4. Compare the effects of gibberellins with those of auxins on growing plants.

Within large organisms, many different chemical reactions are occurring at any given time. While some substances are being synthesized, others are being destroyed or removed. In this way, the chemical environment of every cell is changing slightly from one moment to the next. Some of the chemicals being produced have important control functions which influence the genetic activity or potential of cells, often in regions quite distant from the site of synthesis. Control chemicals acting in this way are usually called hormones and are normally effective at very low concentrations. Transport between the sites of synthesis and the sites of action is usually effected through the extracellular fluids by the vascular system of plants or by the blood circulatory system of animals. Cells which respond to the presence of the hormone ("target cells") generally bear specific receptor molecules for the particular hormone.

Plant hormones are often called growth substances. Their most important function is the overall regulation of growth and development, but they are also involved in other plant processes such as fruit development, leaf abscission, dormancy and aging. The effect that a hormone has on particular cells may vary with its concentration, or with the position of target cells relative to other cells.

A. Auxins

Auxins are a class of growth-regulating hormones derived from the amino acid tryptophan. They are produced in small quantities in the apical meristem (actively growing regions) of coleoptiles, shoots and root tips as well as in developing seeds. The best known naturally occuring auxin is indoleacetic acid (IAA), but many others are known, several of which are synthesized only in the laboratory e.g. 2, 4-D and 2, 4, 5-T.

The highest concentration of auxin is found nearest the apical bud where it is continuously being produced and carried downward in the phloem. The concentration falls off rapidly toward the basal parts of the plant, partially due to its rapid inactivation.

Temperature, light, humidity, gravity and pressure are physical factors which also affect the growth and development of plants. The response of organisms, whether plant or animal, to such stimuli are called tropisms, and their effect must therefore be considered in conjunction with that of hormones.

I Phototropisms

Sunlight, or any light of proper wavelength induces auxin to accumulate on the shady side of a stem, causing the cells of that side to elongate faster, and the plant to bend towards the light.

Auxin acts to increase the plasticity of the cell walls of stems as well as to promote the formation of new wall material. As the normally rigid cell walls become more plastic and less resistant to stretching by turgor pressure, water is allowed to flood into the vacuoles of cells, increasing their volume considerably and thus stretching the cell. In this way, auxin is important in the cellular elongation of stems, shoots and roots.

Procedure - Work in pairs.

1. Each pair of students will have a "pak" of 4 bean seedlings. Separate them into

2 pairs.

2. Place one pair of seedlings under the direct overhead light, the other pair in front of the side-directed light.

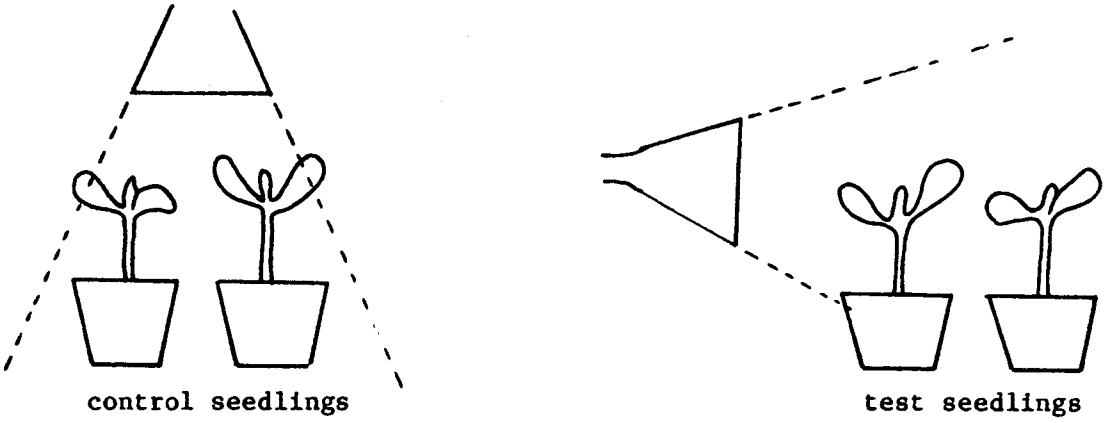


Fig. IX - 1

3. After 1.5 to 2 hours, observe the seedlings exposed to the two treatments, and record your observations.

4. In your report, write an explanation for the results observed.

II Geotropisms

Under the stimulus of gravity, the main stems of plants are negatively geotropic, causing growing tips to grow upwards. Main roots, on the other hand, are positively geotropic, growing vertically downward in response to gravity.

The response mechanism of plants to the stimulus of gravity is again based on the distribution of auxin in cells, accumulating in the lower part of the cells. It is believed that the difference between the response of stems and of roots to gravity, is due to their sensitivity differences. Root cells being more sensitive, are stimulated to elongate at very low concentrations, but are inhibited at higher concentrations. Stem cells, being less sensitive, are stimulated by concentrations which inhibit root elongation, and are inhibited only by very high concentrations. Auxins then, are stimulatory only when present in a precise concentration range which will differ for different tissues.

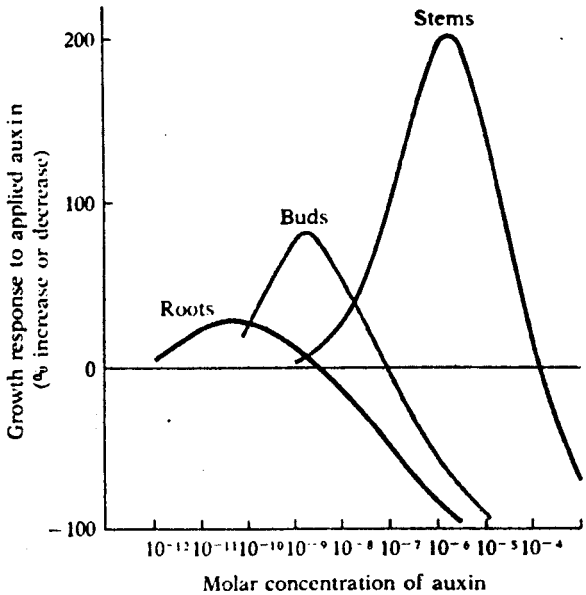


Fig. IX - 2

Procedure - Work in pairs.

1. Each pair of students will plant a "pak" with corn seeds in four different positions.

   a) vertical, right side up
   b) vertical, upside down
   c) horizontal, embryo side up
   d) horizontal, embryo side down.

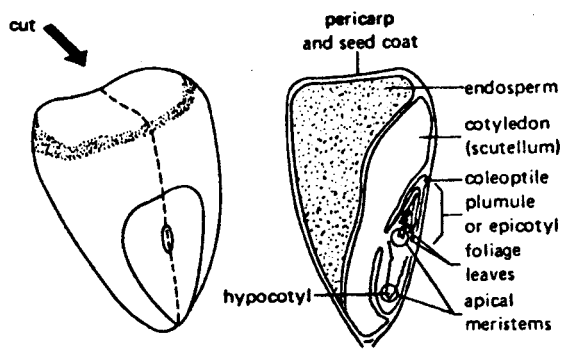
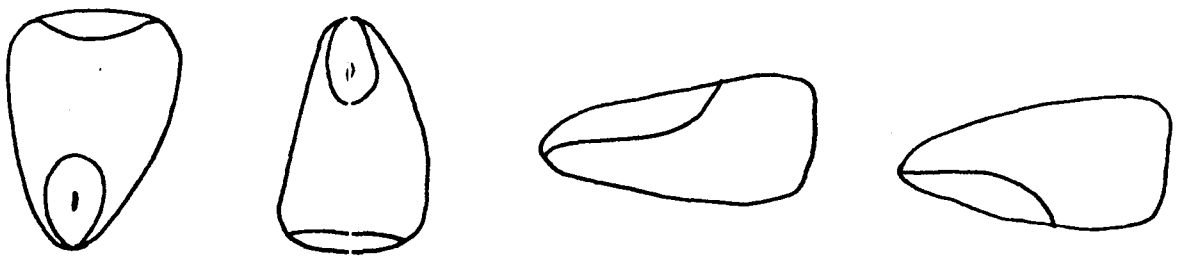


Figure IX-3 The internal structure of a corn kernel that has been cut open as shown at the left.

Fig. IX - 3



a b c d

Fig. IX -4

2. After two weeks, remove and dissect the imbibed (water soaked) corn seeds to determine the position of the endosperm and the embryo in each.

3. Take a growing bean plant home and set it on its side in a dark cupboard.

4. In two weeks time, remove the plant from its pot. Examine the growth patterns of the shoot with its leaves and also the roots.

5. Discuss the geotropisms you have observed in your corn seedlings and in the horizontal plant.

III Apical Dominance

Buds are stimulated by a lower range of auxin concentrations than are stems, and the auxin concentrations that stimulate growth of the stem will tend to inhibit the development of adjacent buds. This is the basis of apical dominance. As long as the apical meristem is growing it will produce auxin in sufficient concentration to inhibit the development of lateral buds next to, or below it. If the meristem of the apical shoot is removed, the concentration of the auxin reaching the buds will decrease allowing them to begin development. Once a bud has started to grow into a new stem, its own apical meristem will begin to produce auxin which in turn will prevent the development of buds to either side of, or below it.

Procedure - Work in pairs.

1. Each student pair will be supplied with a "pak" of four bean seedlings grown to the stage of the development of the first trifoliate leaf.

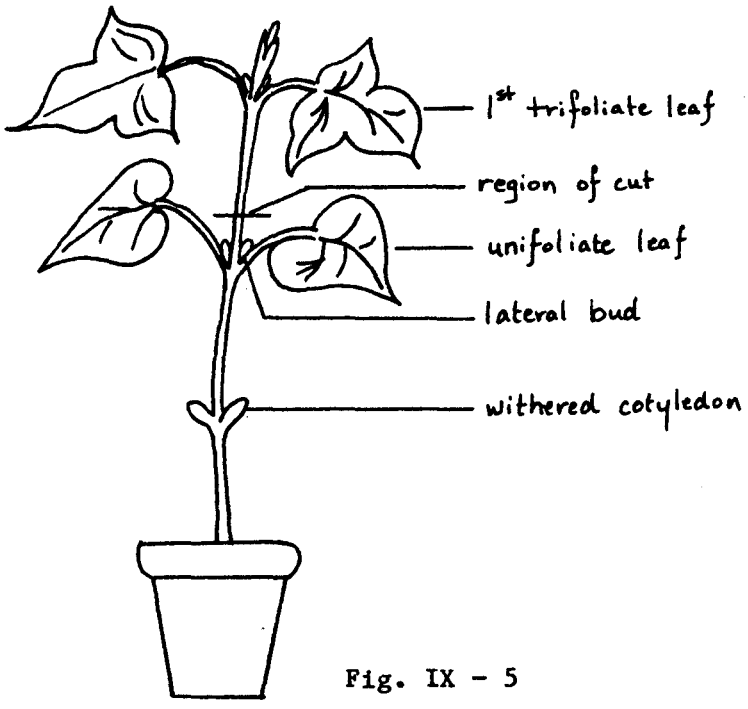


Fig. IX - 5

2. To each of the seedlings, apply one of the following four treatments:

   a) control plant, no treatment
   b) remove the shoot tip ~ 3 cm above the unifoliate leaves - no further treatment.
   c) same as b), but a half capsule of lanolin + IAA is applied to the cut stem.
   d) same as b), but a half capsule of lanolin alone is applied to the cut stem.

3. After two weeks, examine the plants to compare their development, and record your observations.

4. Discuss and interpret your observations in the report.

B GIBBERELLINS

Gibberellins are a class of plant hormones isolated originally from fungi, by

Japanese scientists. They have a spectacular effect in promoting the stem elongation of dwarf plants, but much less effect on plants of normal size. The best-known member of the group is called gibberellic acid (GA).

Endogenous gibberellins are synthesized in the same regions of the plant as auxins, but not necessarily at the same time or rate. In contrast to the polar nature of auxin transport, gibberellins move in all directions within the plant, and in all tissues. Commercially, gibberellins are used in the production of seedless fruits (e.g. Thompson grapes) and the promotion of bolting or flowering in some long day plants.

Procedure - Work in pairs.

1. Each pair will be supplied with a "pak" containing two "Thos Laxton" pea seedlings (normal), and two "Little Marvel" pea seedlings (dwarf). Cut the "pak" in half so that each half contains one of each seedling type.

2. Put one pair into the tray for control plants, and the other pair in the tray earmarked for GA treatment. All plants will be watered in the greenhouse every day, but the experimental plants will be sprayed with GA on alternate days.

3. Observe the demonstrations of normal "Golden Bantam" corn to compare the growth with that of dwarf corn.

4. After two weeks observe all the plants. Record your observations and discusss the growth patterns observed, in your report.

LAB X ANIMAL BEHAVIOR

Reference: pp. 488-551

Objectives: When you have completed this lab you should be able to:

1. Define the subject of Ethology.

2. Discuss in general terms how to make and interpret behavioral observations.

3. Compare the schooling behavior of fish having prominent markings with that of fish having little or no markings.

4. Discuss the components of agonistic behavior in Siamese Fighting Fish.

5. Describe an experiment to determine whether the growth pattern of solitary phase grasshoppers is similar to that of gregarious phase grasshoppers.

Behavior is the response of an organism to a stimulus-whether the stimulus is internal or external. In the broadest sense, this could include just about anything that the animal or plant does within normal circumstances.

The science of animal behavior is called Ethology. It is a highly complex area of study, involving the most refined techniques of many other fields of Biology and of Psychology. It requires extremely careful and detailed observation, together with strict objectivity and a total rejection of anthropomorphic thinking, i.e. attributing human characteristics to non-human beings and things, as well as teleological thinking, i.e. suggesting that happenings within nature occur according to some built-in purpose or goal. Descriptions or interpretations of behavioral events should be kept as simple and factual as possible.

Fundamental Elements of Behavior

A taxis is a simple form of orientation. It is a response by an animal to a directional stimulus, where the direction of movement is influenced by the stimulus e.g. towards a gradient of temperature or away from the direction of illumination.

A kinesis is a locomotory response by an animal to the strength of a stimulus. In this case the stimulus does not control the orientation but the speed or frequency of the animal's response is dependent upon the strength of the stimulus.

Reflexes are considered to be relatively simple or automatic responses to stimuli.

Instincts are behavioral responses that are "built in" as part of the species' inherited structure. Although similar to reflexes, they may include some learned elements.

Learned Behavior involves behavioral responses that are acquired through experience. Although inheritance and learning are both fundamental to the behavior of higher animals, it is generally accepted that inheritance determines the limits within which a particular type of behavior can be modified, whereas learning determines, within those limits, the precise nature of the behavior.

Many stimuli are capable of triggering a series of behavioral patterns. These are called sign stimuli or releasers, and the response is called a fixed action pattern. The advantage of a fixed action pattern for the animal is that an appropriate response can be made quickly and accurately the first time, without the need for learning. The disadvantage is that it will occur even if the animal has been tricked by nature or a biologist into making the response under inappropriate conditions. For example, a male robin whose red breast is a sign stimulus, warning other males away from its territory, will peck at any red feathers, even though they bear no resemblance to another bird.

Stimuli and responses are often built up into chain reactions called rituals in which the response to a sign stimulus acts as a secondary sign stimulus, the response to which acts as a third stimulus and so forth. Such rituals serve a very useful purpose. In courtship for example, rituals ensure coordination of physiological readiness to mate, mating within the same species, and sometimes, care of the young.

A

SCHOOLING BEHAVIOR OF FISH

Schooling behavior is very common among different types of fish; between members of the same species, it is a form of communication. The members of the school may be better protected from predators than a single fish would be. Conversely, a predator in a school has a greater chance of locating food than does a lone predator, since a catch made by one, would be shared by all. It has been observed that schools of fish seem to swim more efficiently than a single fish, and also that learning occurs more quickly for members of a school than for individuals.

Not all fish move in schools however. Plankton-feeding fish do not. In this case, food is more or less evenly distributed throughout the area, so there would be less food per fish in a school than there would be for individual fish.

The structure and cohesiveness of fish schools varies among species. Those species that swim together in close parallel orientation to each other are called polarized schoolers and those which do not are called nonpolarized schoolers. Schooling fish seem to be attracted to each other mainly by their appearance. Vision, the detection of pressure changes caused by swimming movements in the water, and olfaction (smell) may all contribute to keeping the members of a school together, but vision is the most important cue. Olfaction and sound seem to help maintain the cohesion of an already established school.

N.B.: BEHAVIOR EXPERIMENTS REQUIRE CARE AND PATIENCE

DO:
- Always use a net to transfer fish
- Always allow time for the fish to adjust to new conditions before beginning your observations
- Wash and rinse your hands before reaching into the tank to place jars etc.
- Return all fish to the proper tank when finished.

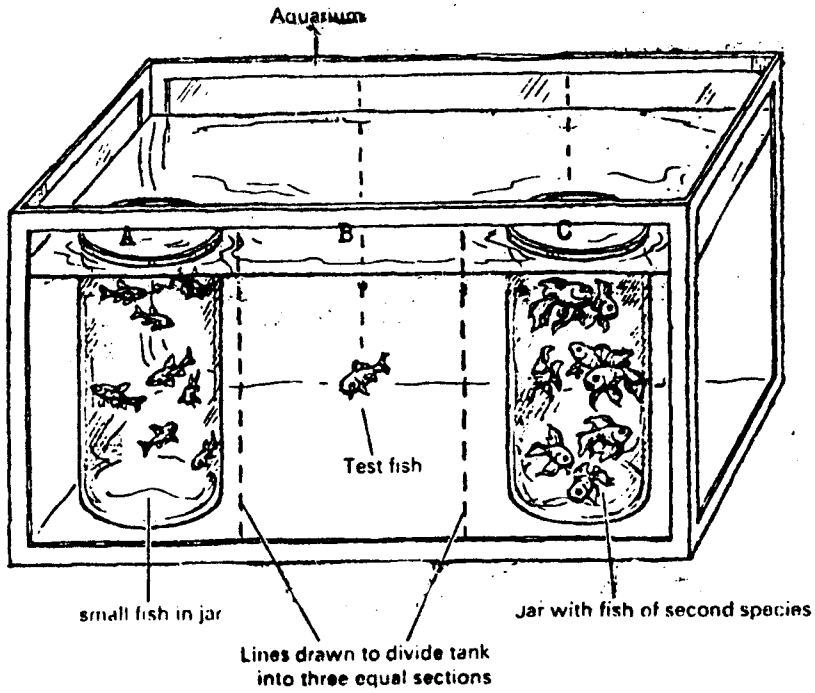
DO NOT:
- Disturb the fish more than necessary for the experiment
- Expect the fish to respond instantly to a new stimulus
- Reach into the tanks where fish are kept
- Leave fish in the experimental tanks.

I Effect of Visual Markings on Behavior

In this experiment you will be testing the role of vision in schooling behavior to determine whether a fish will school more with its own species than with other species of similar body size. Because the fish will be separated by a barrier, there can be no communication by sound, pressure or chemical signals.

Procedure: Work in groups of four.

The aquarium has been divided into three equal sections - A, B, C as shown in Fig. X - 1.



1. Gently net and place 5 goldfish in one of the holding jars, and 5 zebra fish (golden danios) in the other jar.
2. Place the jars in sections A and C of the aquarium respectively. Allow them a few minutes for recovery.
3. Net, and place one goldfish in the center of the aquarium. Allow a few moments for it to begin swimming normally.
4. Begin recording the position of the fish (A, B or C) at 30 seconds intervals for a period of 10 minutes.

5. Remove the single goldfish to its holding tank.
6. Repeat the experiment with a zebra fish, recording its position at 30 second intervals for 10 minutes.

Leave the fish in the jars for use in the next experiment.

During both tests, jot down any significant notes which bear upon the response of the test fish to either of the groups in the jars. For example, does the test fish swim close to the jar or not?

Does the activity in the jar appear to stimulate the test fish?
Does it return to the jar quickly after moving away?

II Effect of Group Size on Behavior

The object of the experiment is to determine whether fish will school more with a large group than with only a few of its own species.

1. Place the jar with 5 zebra fish in compartment A of the aquarium.
2. Net and place 2 zebra fish in another jar to be placed in compartment C of the aquarium.
3. Net and place 1 zebra fish in the center of the aquarium. Allow a few moments for it to begin swimming normally.
4. Record its position (A, B or C) at 30 second intervals for 10 minutes.
5. Remove all the zebra fish to their holding tank.
6. Repeat the experiment, substituting goldfish for all the zebra fish.

Note any behavioral observations which may have some bearing upon the experiment.

B AGONISTIC BEHAVIOR IN SIAMESE FIGHTING FISH

Agonistic Displays allow members of the same species to settle disputes over territory, food or mates without direct conflict, and usually without damage to either party in the dispute. The presence of a competitor within the territory of some species will trigger responses that show a readiness to fight. These, in turn, will cause the opponent either to show its own fight intention or to withdraw, in

which case, any sign of appeasement will tend to reduce the aggressiveness of the opponent.

The agonistic display is a mixture of "fight" and "flee" signals, the proportion of each being influenced by the motivation of the individual. Males are usually more motivated to fight than females, especially when in their own territory or during the breeding season. They are less motivated to fight when the female approaches. In many species, courtship is a means of overcoming the agonistic behavior of the male so that mating becomes possible.

Siamese fighting fish (Betta splendens) normally live in the freshwater streams of Indochina. In captivity, they have been bred for exaggerated agonistic behavior. The male fish shows his intention to attack or to flee by visual displays. The components of the agonistic display are shown in Fig. X - 2 below.

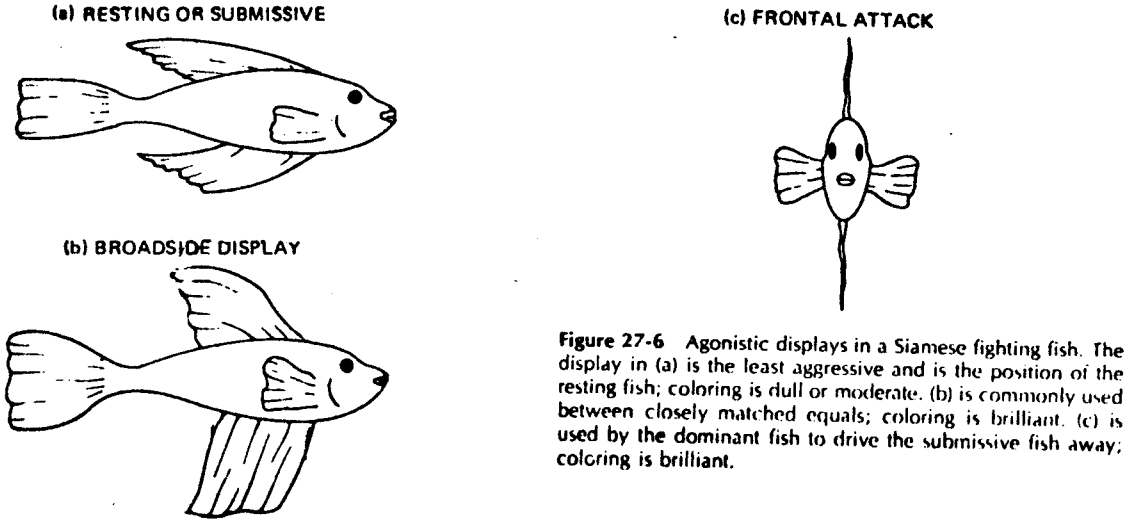


Figure 27-6 Agonistic displays in a Siamese fighting fish. The display in (a) is the least aggressive and is the position of the resting fish; coloring is dull or moderate. (b) is commonly used between closely matched equals; coloring is brilliant. (c) is used by the dominant fish to drive the submissive fish away; coloring is brilliant.

Fig. X -2

The normal resting fish a) is only slightly colored; it keeps its fins folded and loose. When another male appears, he will probably challenge the intruder with a broadside display b) during which the fins are thrust upwards and out to present as large a presence as possible. The coloring should brighten to become more red. During a maximum response, the color may change to a brilliant red. This process of change involves chromatophores in the skin. The two fish may swim side by side for several minutes in this display, each trying to intimidate the other. Finally, one

should gain a slight edge; the subordinate fish will gradually give up the broadside display, lose some of its color and move to a corner. The dominant fish will probably then adopt a frontal attack position c) and if left together, might pursue and thrust at the fins of the more submissive fish. BE PREPARED TO REMOVE ONE OF THE FISH FROM THE OPEN AQUARIUM AS SOON AS THE WINNER BECOMES APPARENT.

I Procedure: Work in groups of 4.

1. Place the clear plastic barrier and the opaque plastic barrier in the slots of the aquarium.
2. Put one fighting fish in one side of the aquarium and a second in the other side.
3. Observe their normal appearance and behavior as regards color, position of fins, opercula etc.
4. Remove the opaque screen from the aquarium.
5. Observe the full response of each fish and record your observations.
6. Remove the other screen so there is no barrier between the fish. BE PREPARED TO REMOVE ONE IF NECESSARY.
7. Replace the barrier or remove the more submissive fish within 2 minutes.
8. Record your observations fully.

Were the fish equally matched before the experiment?
Which one gained the advantage?
What did the loser do?

The balance between the tendency to fight or to flee is largely dependent upon whether the fish is in its own territory and whether it has just defeated another fish or has been defeated itself. The most impressive displays are observed with closely matched fish.

II Leave the more dominant fish in the aquarium. Allow it 5 minutes to readjust and settle down.

1. Hold a mirror alongside the tank near to the fish.
2. Observe the response.
3. Move the mirror to suggest movement of the reflected fish.

4. Continue until there is a response, or for 5 minutes.

Did the fish respond to its mirror image?
Did it attempt to attack the image?
Compare the response of the fish to the mirror with its response to the living opponent.

C GRASSHOPPER BEHAVIOR

Grasshoppers are insects of the Order Orthoptera. The scientific name of ours is Locusta migratorius. Most grasshoppers, being plant feeders, are serious pests of cultivated plants.

At the time of oviposition, the female taps and probes the soil with the top of her abdomen. Hard surfaces are rejected, but any sandy soil is accepted if it is moist. She inserts her abdomen vertically, using the sclerotized ovipositors to dig out the hole. As it deepens, her abdomen lengthens by the unfolding and stretching of the intersegmental membranes, so she is able to extend it to a depth of about 10 cm. During the process, she pumps air into the tracheal system to keep the abdomen turgid. When all the eggs have been released (usually about 24) the hole is closed with a frothy plug and she scrapes sand over the surface. This whole process may take as long as two hours.

In about 10 days, the young nymphs emerge from the sand looking much like miniature adults except for their small size and lack of wings or genitalia. Over the next four weeks the developing nymphs pass through 5 successive molts initiated by growth and molting hormones but controlled by the level of (juvenile) hormone. Stages of development are called instars. Because of its rigid nonliving exoskeleton, a grasshopper can only grow or change its appearance by periodically shedding the old exoskeleton which it does by taking air into the tracheal system, causing the old skin to burst. Then the soft new exoskeleton is stretched to its maximum as the old skin is pushed off and the new one hardens. During this period, the nymph is quite vulnerable both to predators and to its own kind.

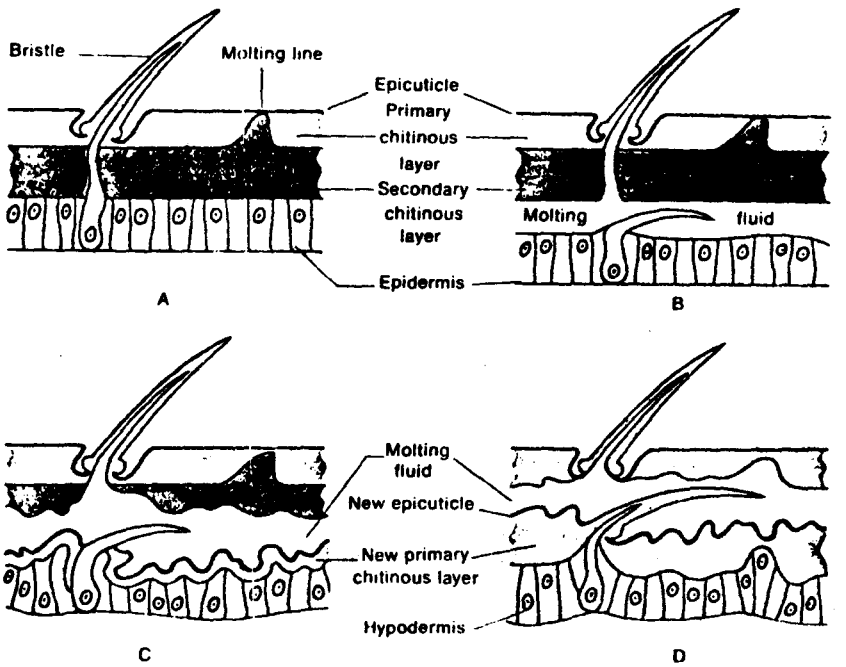
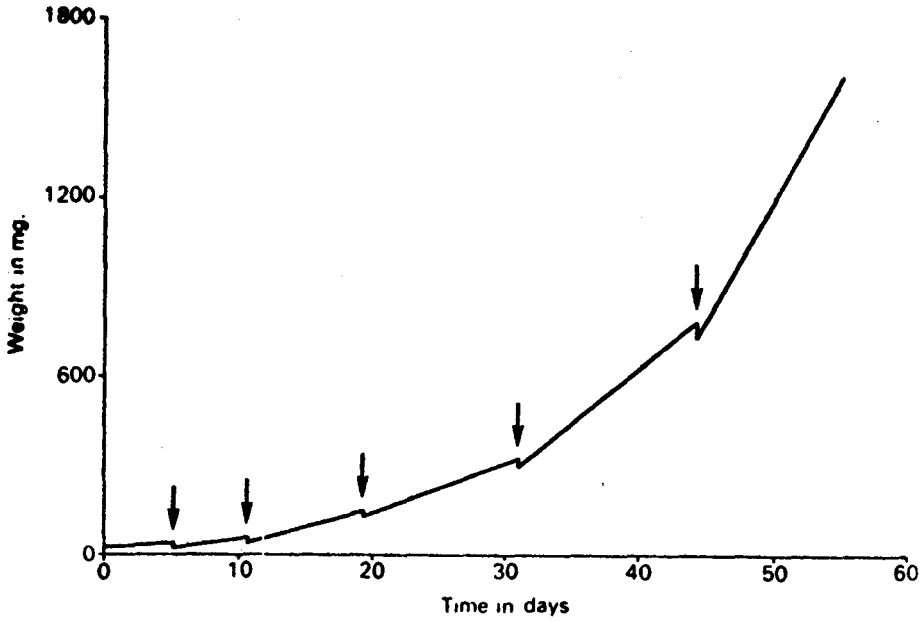


Diagram of the process of molting in an arthropod. A, The fully formed exoskeleton and underlying epidermis between molts. B, Separation of the epidermis and the secretion of molting fluid and a new epicuticle. C, Digestion of the old secondary chitinous layer and secretion of a new primary chitinous layer. D, The situation just before the old exoskeleton is shed.

Fig. X - 3

There is a progressive increase in weight and size throughout the instars, the female reaching a greater final weight and size than the male. Crowding will affect both the rate of development and size, those reared in uncrowded conditions attaining a larger size more quickly than those reared under crowded conditions.



The pattern of increase in weight of female *Locusta*. The times of the moults are indicated by arrows (after Clarke, 1957a).

Fig. X - 4

The color of grasshoppers generally resembles the prevailing color of the environment, the cryptic coloration tending to conceal them from predators. Changes in color occur at the time of a molt, and are most intense during the 3rd and 4th instars. Crowding also influences the color of grasshoppers. Nymphs reared in isolation are more likely to take on a cryptic green or light tan coloration with relatively little superimposed pattern, whereas those reared under crowded conditions achieve a more yellow background with extensive black patterning and a darker overall appearance. Females are always of more neutral coloration than the males.

Adult Locusta have four elongate wings, one pair of antennae and three pairs of legs. Biting and chewing insects like Locusta have a specific feeding pattern. They are attracted visually to a source of food, especially to yellow, but also by olfaction through chemoreceptors on the antennae. Once contact is made with potential food, the palps of the mouthparts are vibrated rapidly, touching all over the surface of the food. Chemotactic stimulation initiates the feeding activity then, but other receptors lead to continued feeding, provided the food is suitable.

A few species, including Locusta migratorius are migratory in habit. When envi-

ronmental conditions are particularly favourable, the population will increase tremendously. The resultant crowding will cause individuals to darken in color and become very aggressive with voracious appetites. Great swarms have been known to appear, causing enormous destruction to crops and plant life in the area. As a strategy for dispersal, they will await appropriate conditions, then take off at the tail end of a storm, following its eye, in order to gain the advantage of the winds and be assured of adequate moisture for their next phase of development.

Grasshoppers produce sounds - called songs - by rubbing the hind femur (the scraper) against the leathery forewing (the file), thus causing the wing to vibrate and create the sound. This is called stridulating, and grasshoppers use a number of different songs as signals for various situations such as courtship, copulation, aggression or alarm. The songs may be differentiated one from another by their pulse repetition frequency and also the form of the pulse, but not the tone which remains constant.

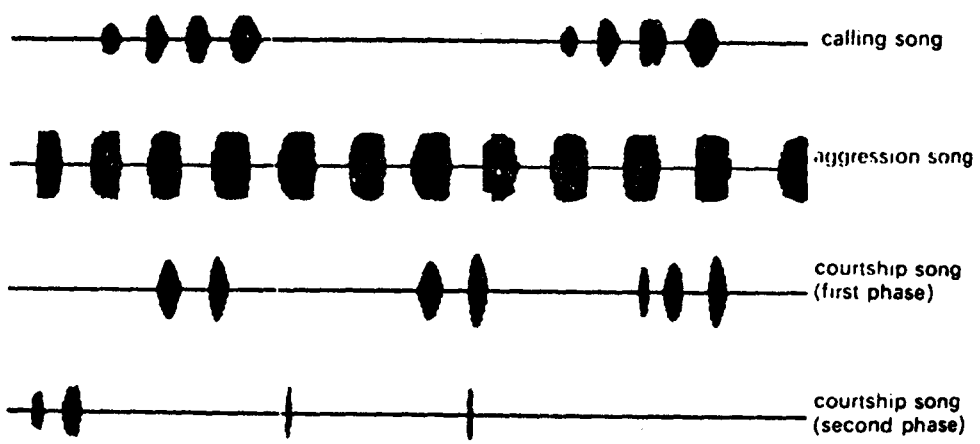


Fig. X - 5

Mating behavior involves a whole sequence of events which are not always clearly separable one from another. Vision, scent and sound are all involved. The female responds to the "song" of the male when she becomes sexually mature, but her responsiveness is inhibited immediately following copulation. Courtship behavior culminates in copulation, during which the male sits on top of the female, his abdomen twisted underneath hers, the position of copulation being characteristic for the species.

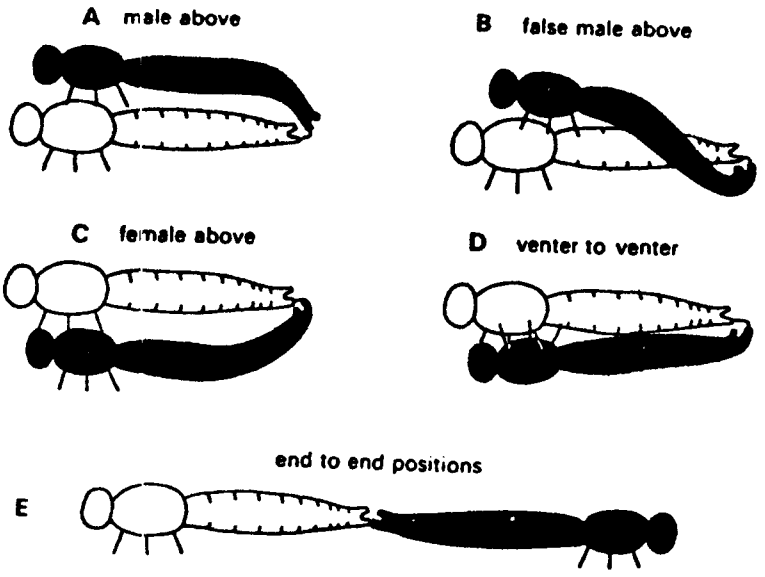


Fig. X - 6

Procedure: Work individually.

Observe the grasshoppers in the cages in your lab room for a few minutes twice a week over a period of 4 weeks. Make specific observations on:

1. color
2. growth and development
3. molting
4. sex differentiation
5. eating behavior
6. stridulation
7. copulation
8. oviposition.

Include your observations in the report. Discuss and interpret these observations in the discussion. Remember not to be anthropomorphic or teleological.

September 14, 1984
1A6/NTS.1/A/1A6/1.1/dm

SCHOOLING BEHAVIOR - CHART #1

	Schooling Behavior I		Schooling Behavior II	
TIME	Goldfish	Zebra	Goldfish	Zebra
0.5 min				
1.0 "				
1.5 "				
2.0 "				
2.5 "				
3.0 "				
3.5 "				
4.0 "				
4.5 "				
5.0 "				
5.5 "				
6.0 "				
6.5 "				
7.0 "				
7.5 "				
8.0 "				
8.5 "				
9.0 "				
9.5 "				
10.0 "				
Total # of sitings in A				
# of sitings in B				
# of sitings in C				
Preference				

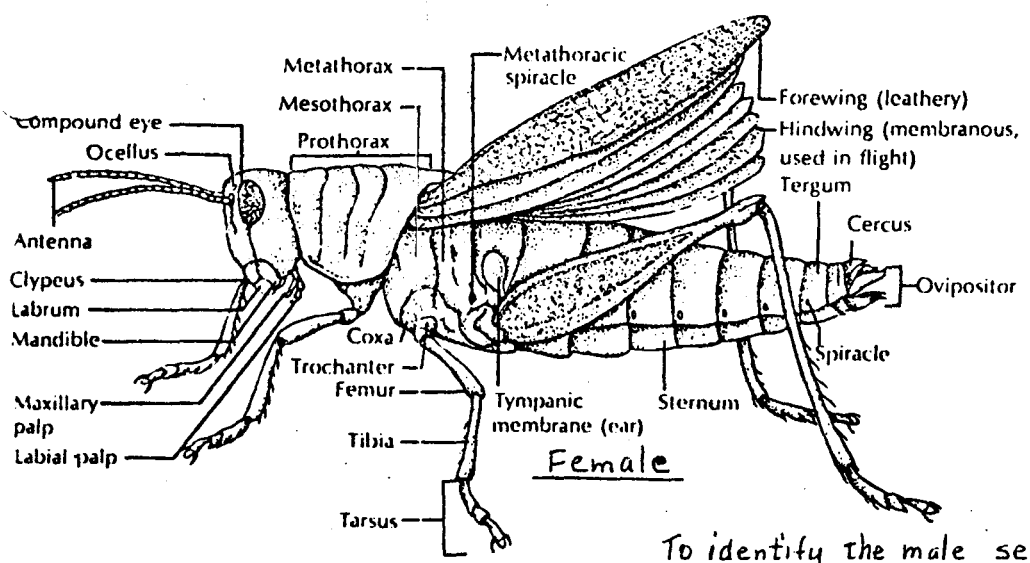
BIOLOGY 1A5

Behavioral Observations of Grasshoppers

Come in anytime of day to observe grasshopper behavior. Another class is in session between 2:30 and 4:30 p.m. during weeks in which we have a quiz, so try to avoid these times.

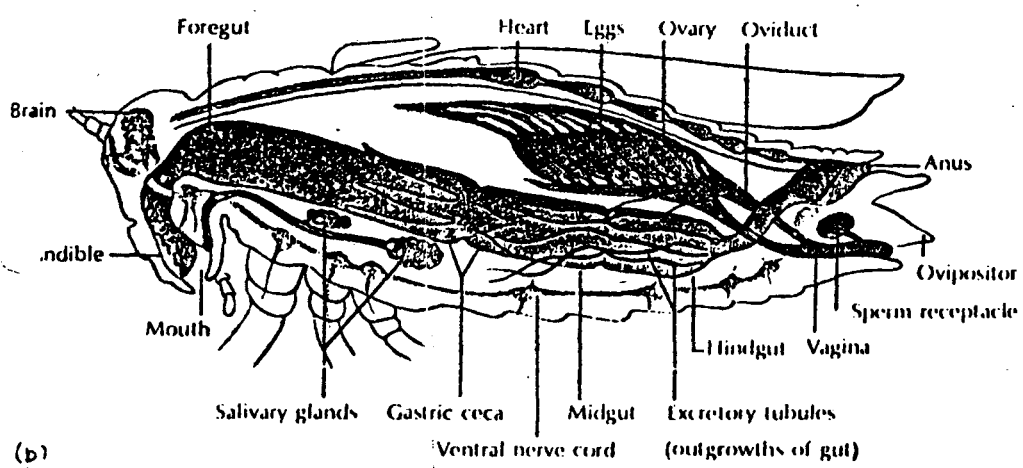
- Observe the colours of grasshoppers at all stages of development.
- Observe size and body proportions during stages of development.
- Look for molting specimens and discarded exoskeletons.
- Differentiate between the female (♀) ovipositors, and the male (♂) with curved posterior.
- Observe the leathery forewings and membranous hindwings.
- Observe how they eat grass - relate to mouthparts. Pg. 1035.
- Listen for "stridulation" - the sound which is made by rubbing the edge of one wing, modified as a scraper, against a file located on the leg.
- Observe copulation in the adult cage.
- Observe oviposition (laying of egg cases) in the sand cups of the adult cage.

Figure 12.33 ☐ **Grasshopper body plan. (*a*) External aspect. (*b*) Internal organs in situ, lateral aspect. The reproductive system is shown in grayed color; the circulatory system, in a tint of color; the nervous system, in gray; and the digestive system, in solid color.**



(a)

To identify the male see Pg 23 of the text



(b)

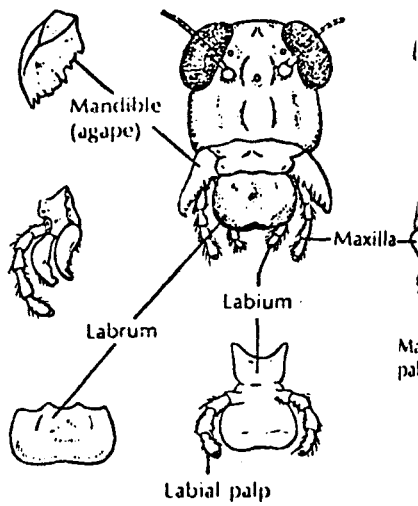


Figure 12.34 ☐ **Grasshopper mouthparts.**

APPENDIX C

McMASTER UNIVERSITY

DEPARTMENT OF BIOLOGY

TO: Demonstrators in Biology 1H6 DATE: September 1981

FROM: C. M. Wood LSB 528
D. G. McDonald LSB 426
M. A. Service LSB 330

This is the second year that Physiology 1H6 has been offered by the Biology Dept. It is a required course for First Year Phys. Ed. students, and may be taken as an elective by a restricted number of others. Enrollment is large - 300 students, about 20% of whom have a non-Phys. Ed. background. Labs are held every other week - 10 in all throughout the year. Each room will have approx. 24 students. The majority of these students are First Year, non-science, with little or no Biology background. Many of them have never participated in a lab exercise before. They cannot be expected to be either as precise or as knowledgeable as main line Biology students. The only reference material you can reasonably expect them to use is their own course text and lecture notes.

Instructional goals for the labs would include the following:

(i) development of observational skills and the ability to order these observations:
(ii) to assist students in learning to think and to communicate logically and critically, both orally and in writing;
(iii) development of a positive interest in Biology, leading to an enquiring mind and a scientific approach to problems.

The laboratory itself should be a friendly, non-threatening environment. The lab demonstrator is in charge; as such, he or she has the opportunity to create a comfortable but organized and intellectually stimulating atmosphere which will be enjoyable and rewarding both for him or herself and, more importantly, for the students.

Lab Schedule

I	Diffusion and Osmosis	Sept. 28 - Oct. 2
II	Neurophysiology I - Reaction Time	Oct. 26 - Oct. 30
III	Neurophysiology II - Sensory Function	Nov. 9 - Nov. 13
IV	Blood	Nov. 23 - Nov. 27
	CHRISTMAS BREAK	
V	Cardiovascular Function	Jan. 11- Jan. 15
VI	Respiratory Gas Exchange	Jan. 25 - Jan. 29
VII	Physical Fitness	Feb. 8 - Feb. 12
VIII	Kidney Function	Feb. 22- Feb. 26
	STUDY WEEK	
IX	Salivary Amylase	Mar. 15 - Mar. 19
X	Nutrition	Mar. 29 - Apr. 2

Mrs. M. Service, Room 330, is the Senior Demonstrator in overall charge of the labs. Please direct problems which directly concern labs to Mrs. Service (e.g., student upset at lab mark, student with medical excuse, etc.). Please direct other problems related to the course to Drs. Wood or McDonald.

Mr. Leslie Gyorkos, LSB 106, is the departmental technician responsible for setting up the lab in Term I. In Term II the technician will be Mrs. Ella Kieraszewicz. Please direct any equipment or supply problems during the lab to these people.

Demonstrating Responsibilities

Each demonstrating slot involves one 2-hour lab period every other week. Each room will have approximately 24 students. The demonstrator is expected to mark the lab reports promptly, keep his or her own permanent record of the marks and submit them to the Senior Demonstrator on a regular basis. The lab reports must be returned to the students in the next lab period without fail in order to give feedback to help in the next write-up.

It is essential that demonstrators attend the demonstrators' meeting during the week previous to each lab to ensure their familiarity with the material.

Demonstrators will also be expected to invigilate and check the marking of the multiple choice bi-weekly quizzes which will be written on the following dates.

Term I		Term II	
1.	Friday, Oct. 2	6.	Friday, Jan. 29
2.	" Oct. 16	7.	" Feb. 12
3.	" Oct. 30	8.	" Feb. 26
4.	" Nov. 13	9.	" Mar. 19
5.	" Nov. 27	10.	" Apr. 2

Plan to be in the lab room by 2:15 p.m. on the day of your lab. You should expect to give a pre-lab talk each week to outline procedures and to emphasize particular points. As the total lab period is only 2 hours, the talk should not exceed 15 minutes at the most, preferably 10. Give the students clear and explicit instructions regarding lab procedure, and what your expectations are regarding reports, etc. Let them know immediately that they are to work out the answers to questions in the lab guide themselves but that you are available to discuss their answers with them.

Lab reports are to be submitted by the student at noon of the day one week following the lab. Late labs are not to be accepted without a medical certificate. Make this clear to the students during the first lab period and refer any disputes to Mrs. Service. This being the case, you must collect all reports from your box by 1:00 p.m. of the due date on a regular basis. Since we expect promptness from the students, it behooves the demonstrators to return marked reports promptly in each following lab period, without exception. Your credibility depends upon it.

Marking Lab Reports

Instructions for the preparation of reports have been given to the students in the lab manual. Reports should be no more than three pages long, excluding tables, diagrams and graphs. You might encourage this by marking down excessively long, or rambling reports. The purpose of the lab report should be to clearly describe the results obtained, and to interpret these results in the light of theory. It is important that all questions asked in the lab guide should be answered.

Do not take marks off if their experiment did not work as long as they try to explain why it did not work and also what they might have expected to occur. If extra effort is put into the experiment or the write-up, it should be rewarded.

Mark the labs out of 10. You may set your own standard, but we would suggest the following:

- marks as high as 10 or as low as 3 should only be given in extreme circumstances;
- a reasonable average for the class over the year would be 6.5 or 7.0;
- THERE SHOULD BE SUFFICIENT COMMENTS ON THE REPORT TO ENSURE THAT THE STUDENT UNDERSTANDS WHY HE RECEIVED THAT MARK AND HOW HE MIGHT IMPROVE IN THE FUTURE.
- avoid sarcastic, non-helpful comments;
- be consistent in your marking.

Above all, be enthusiastic yourself, and willing to help whenever needed in the lab.

Laboratory (Teaching) Technique

If the concepts of the labs are to be completely understood, the students must be actively involved in the exercise. They should be encouraged to ask any questions at all while they are working, and not feel embarrassed. Encourage them to discuss problems or questions among themselves. Questions by the demonstrator as you move around among groups are an extremely important tool to ensure that learning is occurring:

- they expose areas of confusion;
- they can clarify the issues;
- to focus student concentration on a particular area;
- provide a means for separating relevant from irrelevant information;
- assist in assessing that information;
- assist in creating insights.

N.B.: However, students should never leave the lab with all the questions unanswered. Ten minutes before leaving, it would be wise to schedule a debriefing session to discuss their findings. If the swifter students who already understand the concepts want to leave, so much the better. Draw the rest into an informal group to discuss their findings,

and the significant points. Encourage them to explain to each other rather than you doing the explaining, to formulate clear and logical verbal statements backed up with reasoning. As a demonstrator, you can verify the correctness of their reasoning, or get others to make modifications or corrections. It would be helpful for you to summarize and paraphrase their ideas. When they don't understand or are reluctant to speak up, try to prompt their understanding by asking sequences of open-ended questions which force them to think out loud, so you can direct their reasoning. You should be an information resource, but avoid giving them the answers to specific questions. Instead, practice the art of posing alternative questions which will lead them to answer their own queries.

APPENDIX C

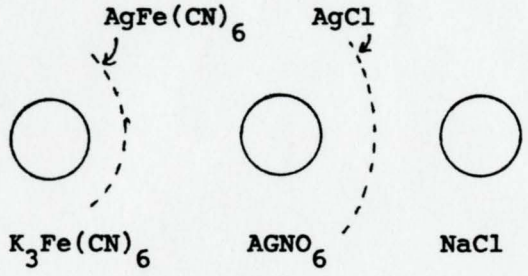
INSTRUCTION OF TEACHING ASSISTANTS - PART I

BIOLOGY 1H6

Lab I - Diffusion and Osmosis

A. Diffusion

In this experiment, the three salts form free ions when in solution. All these ions migrate through the agar away from the well.



$K_3Fe(CN)_3$ $K^+ + Fe(CN)_6^{\equiv}$

$AgNO_3$ $Ag^+ + NO_3^-$

NaCl $Na^+ + Cl^-$

Fig. I-1.

Visible precipitates form where the cation Ag^+ meets the $Fe(CN)_6^{\equiv}$ and Cl^- anions. This precipitation front should be curved concavely towards the slower-moving ion as in this diagram.

Q. What can you conclude about the rates of diffusion of these two ions based on the relative positions of the precipitates?

Results should indicate that Cl^- ions have a faster diffusion rate than do $Fe(CN)_6^{\equiv}$ ions.

$Fe(CN)_6^{\equiv}$ is a larger ion - Mol. Wt. of 211.96

Cl^- is much smaller - Mol. Wt. of 35.46

Larger ions (higher molecular weight) necessarily encounter more collisions during diffusion than smaller ions (lower molecular weight) for much the same reason as a fat person moves with more difficulty through a crowd than someone who is thin.

Q. Can you say anything about the rate of diffusion of the Ag^{++} ions?

No, because cations often hydrate differently than do anions.

Q. Interpret the difference in rate of diffusion.

Possible factors affecting anion rate of diffusion are concentration differences, temperature differences or differences of molecular weight and size. Since all solutions were 1N and since the temperature was constant in all cases, the reason must be difference in molecular weight. Therefore, the rate of diffusion would appear to be inversely proportional to molecular weight.

$$R \propto \frac{1}{\text{mol. wt.}}$$

B. Osmosis

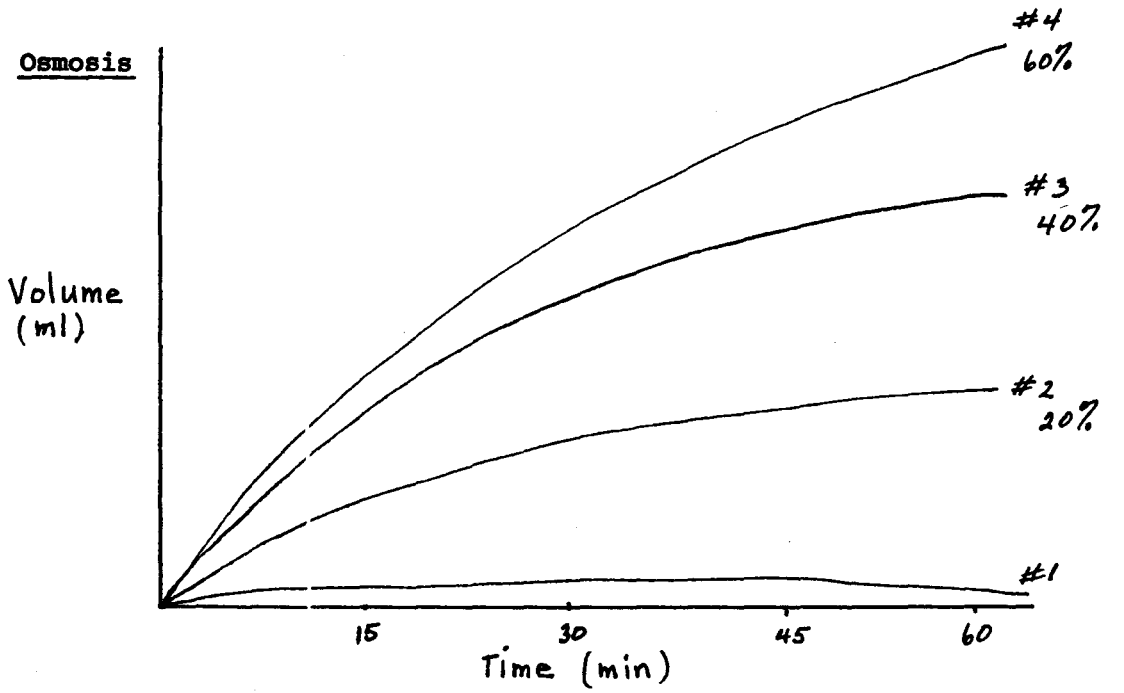


Fig. I-2

Q. Do you expect straight lines (for the graph)?

No, because osmotic rates are greatest when the concentration gradient across the membrane is greatest. As water enters the bag, this gradient is reduced, so the rate of osmosis is continuously decreasing with time.

Q. Can you calculate the rate of osmosis from these graphs?

Yes, you can calculate maximum rates, overall rates or average rates.

Maximum rate is the initial rate (largest concentration gradient). It is the change in volume over the first fifteen minutes as measured from the graph (Vol. vs. Time) above.

Overall rate is the total change in volume over the complete time period.

Average rate would be the average of each fifteen-minute period.

Q. How does the rate of osmosis vary with solute concentration?

We are comparing pure water with a range of sucrose concentrations. High sucrose concentrations have the greatest concentration gradient with pure water and vice versa. Therefore, rate of osmosis will be fastest between high sucrose/water systems and slowest between low sucrose/water systems.

Q. What would happen if bags were left indefinitely?

Osmosis would continue at a slower and slower rate until the bags became full, at which time the strength of the bag itself and the hydrostatic pressure of the water in the bags

would prevent further net entry of water. Without these factors, osmosis would continue until the concentration gradient across the membranes was reduced to zero - an impossibility since there would always be some sucrose molecules in the bag, creating a gradient, however slight. Reduction of the gradient to zero would require an infinite bag volume.

Q. Is hydrostatic pressure of any importance here?

Yes, see answer above. Hydrostatic pressure is that pressure being exerted by a column of fluid. At the surface of a body of water it is 0, but rises 1 mm Hg for each 13.6 mm distance below the surface.

C. Osmotic Fragility of Cell Membrane

Q. In which tubes did haemolysis occur?

Complete haemolysis is only visible in Tube #1. It is the only tube through which print can be clearly read, i.e., 1/18 M NaCl.

Q. What happened in the other tubes?

Tube #2 - Some red blood cells are haemolyzed because the tube is partially clarified and some print is legible.

Tube #3 and #4 - The red blood cells shrink (crenate) because water is drawn out of them. Therefore the red blood cells are still intact and the solution remains opaque.

Q. Which solution is closest to the normal osmotic pressure of blood plasma?

Tube #2 because water is diffusing into some red blood cells, causing them to burst and making the contents clearer than originally. You could still not read print, however, indicating that many cells were still intact, although on the verge of bursting.

Q. What occurs when red blood cells are placed into hypotonic, hypertonic and isotonic solutions? Why?

Red blood cells placed in a hypotonic solution haemolyze or burst because the solute concentration of the solution is lower than that of the cell. Therefore, water enters the cell by osmosis, causing the membrane to rupture.

Cells in a hypertonic solution shrink because the solute concentration in the solution is higher than that in the cell, causing water to be drawn out of the cells into the bathing solution.

Nothing occurs in an isotonic solution because there is no net flux of water molecules in either direction, i.e., the same number of molecules enter the cell as exit the cell membrane.

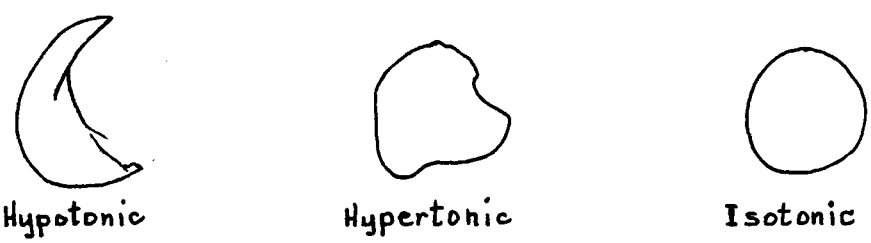


Fig. I-3

Lab II - Neurophysiology I Reaction Time

Q. What are the components of the reflex arc for a simple visual reflex?

eye, synapse, afferent sensory neuron, synapse, c.n.s. (central nervous system) and multiple synapses, efferent motor neuron, synapse, finger muscles.

Q. For a complex reflex requiring discrimination?

eye, synapse, afferent sensory neuron, synapse, c.n.s. and multiple synapses interneurons, synapse, brain, efferent motor neuron, synapse, finger muscles.

Q. What are the components of the reflex arc for the auditory stimulus?

same as visual, except substitute ear for eye

Q. Was there a difference in latent response time between individual trials? If so, interpret these differences.

Yes, there would be a difference in response time between individual trials. One is not always at the same degree of readiness to respond to the signal.

Q. Did your data concur with the class data? If it did not, explain why.

Some people naturally have faster reaction times than others. This may be related to degree of fitness, or to age, or to health or other factors. This question calls for a subjective evaluation. Look for intelligent reasoning in students' answers.

Lab III - Neurophysiology II Sensory Function

Demonstrator Instructions

The unit of sensory function is called the sensory neuron. At its peripheral end, the neuron receives information from the many receptors which constitute the receptive field. Receptors usually respond more readily to one form of energy than to others, i.e., they are specific.

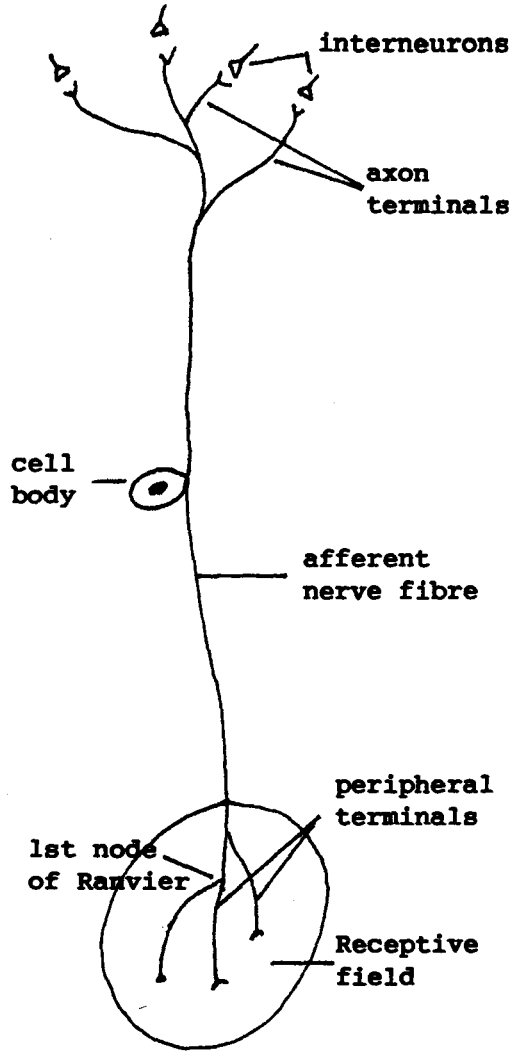


Fig. III-1

Different forms of stimulus energy are termed modalities, e.g., touch, pressure, heat, cold, etc. - there is a specific pathway for each modality ending at a specific area of the brain. It is the part of the brain stimulated which determines the type of sensation which is registered.

When the receptive field of a sensory neuron is stimulated, generative potentials originate below the 1st node of Ranvier. Immediately above this area, summation of these generative potentials occurs. This can result in the firing of action potentials which are carried up the afferent nerve fibre. The greater the summated generator potential, the greater the frequency of firing.

Action potentials result in the propagation of an electric signal. They are all or none in nature - a response to the depolarization of the

cell membrane above a certain threshold, usually 10 to 15 mV relative to the resting potential of the membrane. Depolarization is caused by changes in membrane permeability and the consequent flux of sodium and potassium ions.

Joining to the upper end of the sensory neuron there are many parallel chains of interneurons in the central nervous system which connect with the axon terminals. They all respond to the same stimulus modality and transmit the information to different areas of the cerebral cortex.

A. Tactile Sensitivity

The intensity of the stimulus depends upon the number of sensory neurons activated and the firing frequency in the individual units. The greater the stimulus above the threshold, the more units are stimulated and the greater is the frequency of firing.

The kind of stimulus depends upon the modality of the receptor activated.

The strength of stimulus is determined by the number of units activated and the frequency.

The location of the stimulus.

Since sensory messages from only a restricted area are carried by a single interneuron of the pathway specific for that modality, this pathway, in union with the particular region of the brain stimulated, indicates both the location and modality of the stimulus.

The more receptors there are in a specific area of the body, the greater is the integrating area of the cerebral cortex devoted

to that piece of the body. Diagram p. 563.

Q. Does the absolute threshold for tactile stimulation vary over the regions of the body tested?

Yes, there is considerable variation. The fingertips, forehead, and lips are very sensitive. The heel of the hand and the small of the back are not very sensitive.

Q. In physiological terms, explain why this is so.

Several factors determine the sensitivity of a given area to stimulation. The most important factor is the number of afferent neurons which service that area. Very sensitive regions have many more afferent neurons than insensitive regions. The size of receptive fields is very important as well as the number of receptors in the field. Small receptive fields with many receptors increase sensitivity of the area. When receptive fields of individual sensory units overlap, this results in more accurate localization of the stimulus and consequently better perception of stimulus intensity.

Q. Does your data compare with the rest of the class? Why or why not?

All answers will be subjective.

If a student's discussion is reasonable and shows logical thinking, accept it.

There are various reasons for variable tactile sensitivity among individuals - skin diseases, callouses, and diabetes being a few.

B. Two Point Threshold

The 2 points of stimulation must be felt as 2 distinct points. Give them guidelines for the area being tested.

a) tip of finger 0 - 1 cm apart

b) heel of hand 0.5 - 1.5 cm apart

c) back of hand 1 - 4 cm apart

d) forearm 3 - 6 cm apart

e) forehead 1 - 4 cm apart

f) small of back 3 - 6 cm apart

g) tip of tongue 0 - 1 cm apart

Q. Is there variation in the thresholds of various parts of the body?

Yes, there are distinct differences. When only a single neuron is involved, having a large receptive field, it is not possible to pinpoint either the intensity or the origin of the stimulus accurately. Differentiation of a stimulus from an adjacent stimulus depends on the number and amount of overlap of closely related receptive fields.

Two-point discrimination is greatest for the fingers, lips, nose and cheeks because these receptive fields are small and there is much overlap.

It is least in the small of the back because this area has very few afferent fibres and the receptive fields are large.

Q. Is the direction of stimulation important?

It is said that stimulation is more effective along the

arm and less effective across the arm. Most students were unable to detect any difference.

Q. Are practice, adaptation and concentration important?

Practice - only important if feedback from the presenter is being given - not in this case

Adaptation - when a stimulus of constant magnitude is applied to a neuron, adaptation may occur. This is a decrease in the frequency of action potentials during continued application of the stimulus. It may occur due to energy loss or receptor membrane responsiveness or changes at the first node or to all of these factors.

Tonic receptors are poorly adapting receptors. They continue to transmit information for long periods of time.

Phasic receptors adapt rapidly and strongly, especially to changes of stimulus strength.

Concentration - common sense dictates that concentration or lack of it would have an effect, e.g., trying harder or blocking out distractions.

Q. Are your results consistent with class results? If not, explain why.

This calls for a subjective discussion of data. Look for intelligent reasoning.

C. Colour Vision - P . 572-3

Q. What is the physiological basis of normal colour vision?

The retina has 3 kinds of cones, each containing

different photopigments.

Each cone responds to all wavelengths of the visible spectrum, but most effectively to 1 wavelength.

There are yellow-sensitive photopigments - 570 nm

" " green- " " - 535 nm

" " blue- " " - 445 nm

Normal colour vision depends upon the ratios of the 3 cone outputs. The nervous system interprets these sets of ratios as specific colours.

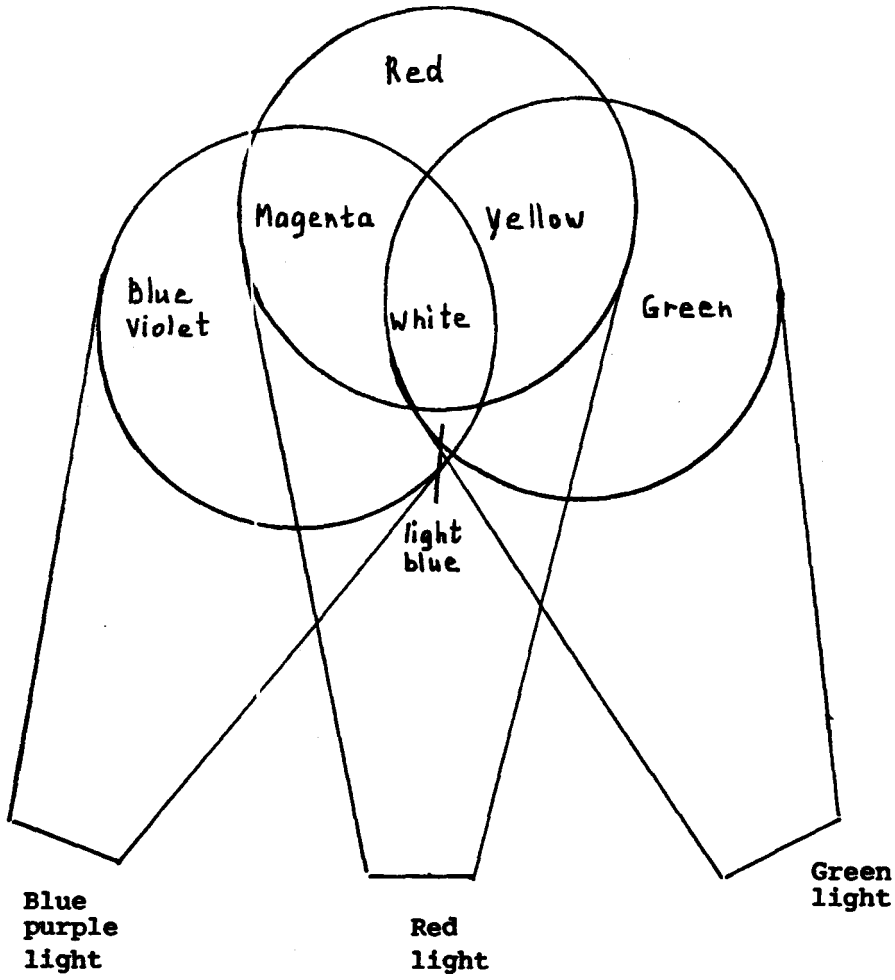


Fig. III-2

Q. What is the physiological basis of colour blindness?

Colour Blindness occurs when a person lacks one or more of the three photopigments. Their vision is then formed from the differential activity of the remaining types of cones.

Q. What is the likelihood of your being colourblind?

Males - likelihood of colourblindness - 10%

Females - " " " - Less than 1%

This is because colourblindness is sex-linked, resulting from the absence of the appropriate colour gene on the X chromosome. This lack is a recessive trait which shows up in only 1 out of every 250 women because they have two X chromosomes. Since the trait is recessive, it would have to be absent from both chromosomes to be expressed.

Men have only the one X chromosome. Therefore, the lack of the appropriate gene is always expressed.

The red gene is lacking in 1 out of every 50 men.
The green gene is lacking in 1 out of every 16 men.
The blue gene is lacking only very rarely.

When a person lacks either the red or green types of cones, he is said to be red/green colourblind. When one or more types of cones function abnormally, the person is said to have colour weakness.

D. Taste - P . 584

There are only 4 basic taste sensations:

a) sour

b) sweet

c) salty

d) bitter

The tongue has thousands of taste buds, each containing receptor cells much like the segments of an orange.

Q. Which area of the tongue is best able to detect the presence of each of the solutions?

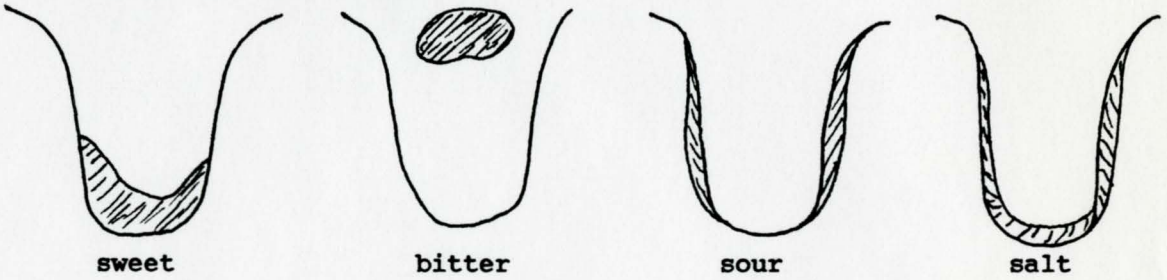


Fig. III-3

Q. Since there is no obvious difference in the specificity of the taste receptors, how do you think the different qualities are perceived?

In fact, there is a difference in the specificity of the taste receptors.

Most taste buds respond in varying degrees to at least three and usually to all four primary taste stimuli, but each will respond more strongly to one than to another.

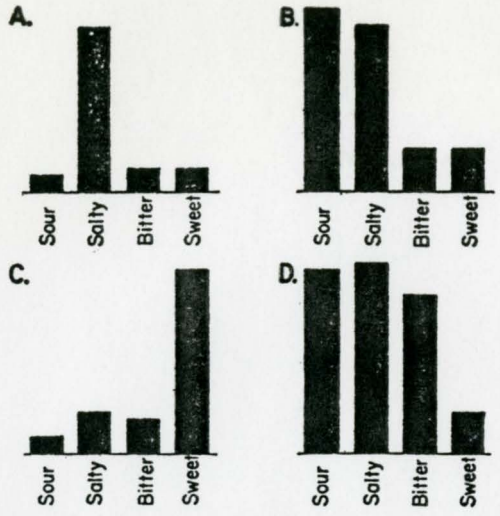


Figure III - 4 Specific responsiveness of four different types of taste buds, showing multiple stimulation by the different primary sensations of taste in the case of each of the taste buds.

It is the proportion of stimulation of the different taste buds which give all the different gradations of taste sensations we observe in foods.

Report your findings - T - tip

S - sides

M - middle

B - back

Lab IV - Blood

Practice using the Spec. 20 even if you are already familiar with its use. It is impossible not to forget the steps and the success of this lab depends to a great extent on the proper functioning of the machine.

Demonstrate to the class how to take blood. Have fun and make it a real "spectacular". I suggest you choose a big, burly student for this demonstration - preferably one who usually has a lot to say. Be sure you have lancet, gauze, and micropipet handy and ready.

A. Haematocrit - pp. 253-264

Q. How many phases are there? - three

Q. What is the major component of each layer?

- clear plasma at the top
- red blood cells at bottom
- a very narrow light-coloured band at the surface of the red blood cells. It contains white blood cells and platelets is called the "buffy coat".

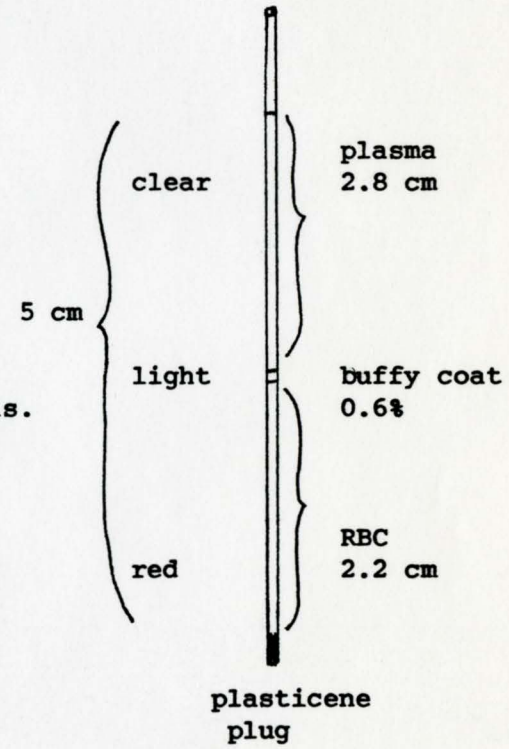


Fig. IV-1

Q. Explain why centrifugation was able to separate the phases.

Each phase has a different density. If blood is allowed to stand without coagulating, the heavier red cells would sink to the bottom, as seen in Lab I during haemolysis. Centrifugation merely speeds up the process. In the body, the motion of the circulatory system keeps the cells dispersed in the plasma.

Q. Calculate the relative volume of each phase using a ruler or graph paper (1 division = 1 mm).

The total blood depth in the haematrocrit tube was 5.0 cm.

Relative volume of RBCs = $\frac{2.2}{5.0}$ x 100 = 44% haematocrit

Plasma = $\frac{2.8}{5.0}$ x 100 = 56%

White cells < 1% - more if in a diseased condition.

Normal ♂ haematrocrit - approx. 46%

" ♀ haematrocrit - approx. 44%

During anemia the haematrocrit could go as low as 15% or during polycythemia it could go as high as 65%.

Variation between individuals is due to different activity levels, altitudes, loss of blood for any reasons, or low levels of Vitamin B_{12}.

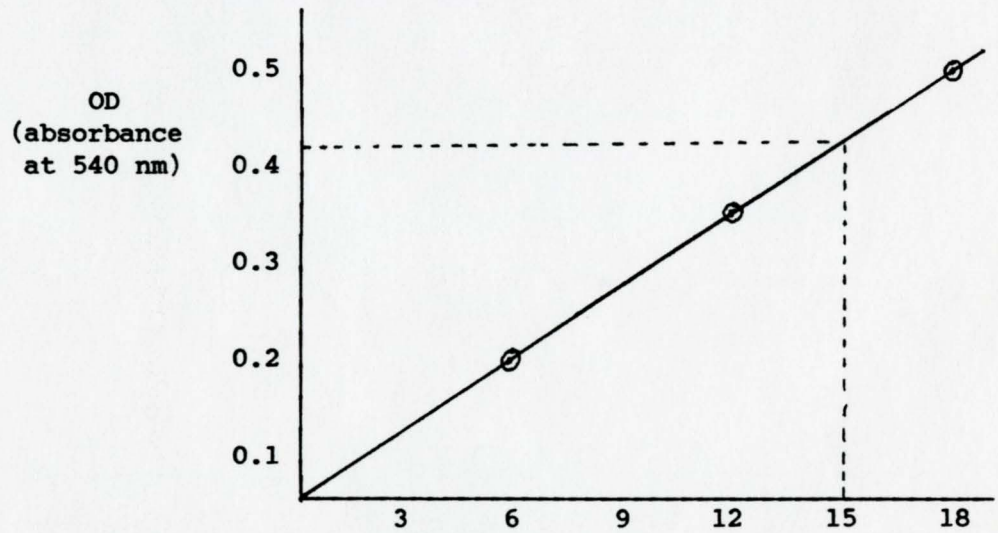
B. Haemoglobin Concentration in Blood - Cyanmethaemoglobin

Haemoglobin is an oxygen carrying pigment found in red blood cells.

Be prepared to explain what a "standard" is - see manual, pp. 37-8. Also explain the calibration curve plotting [Hb] against optical density.

A standard is a substance whose activity has already been established. If a series of "standards" of varying concentration are available, it is possible to draw up a graph comparing the concentration of the standard and the range of activity expected. Then, by applying an unknown to this graph, according to its activity, one may then read off the concentration of the substance producing that activity.

e.g., An absorbance reading of a student tube of 0.42 OD units when applied to the calibration graph (curve) is equivalent to a [Hb] of 15gm/100 ml blood.



Graph IV-1

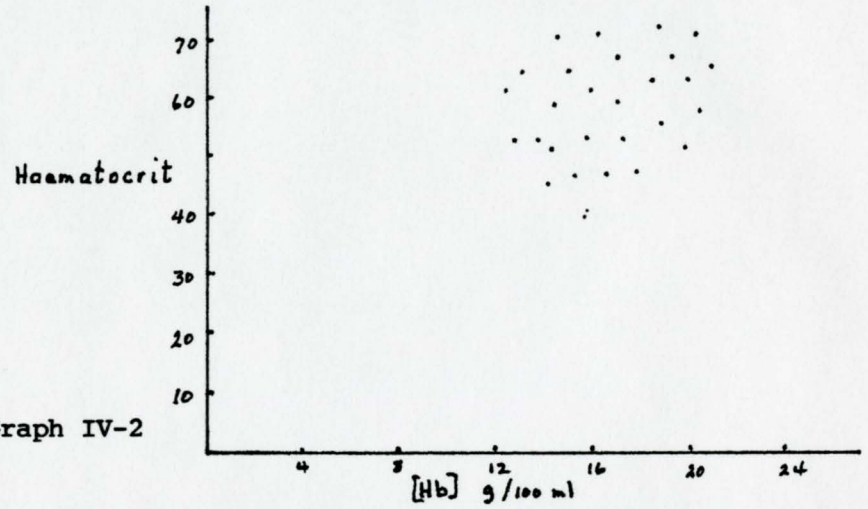
See Manual p. 6 for use of Spec. 20.

Q. Is there a correlation between haematocrit and [Hb]?

There should be a correlation between haematocrit and [Hb]. The data will tell the story. Since haemoglobin is carried in red blood cells and since each cell normally carries its maximum, therefore, the more cells, the more haemoglobin, but if haemoglobin formation in the bone marrow is reduced for any reason, then haemoglobin concentration is also reduced.

Normal [Hb] - 15 g/100ml

" " - 14 g/100 ml



Graph IV-2

Values for male students will probably be consistently higher than for female students.

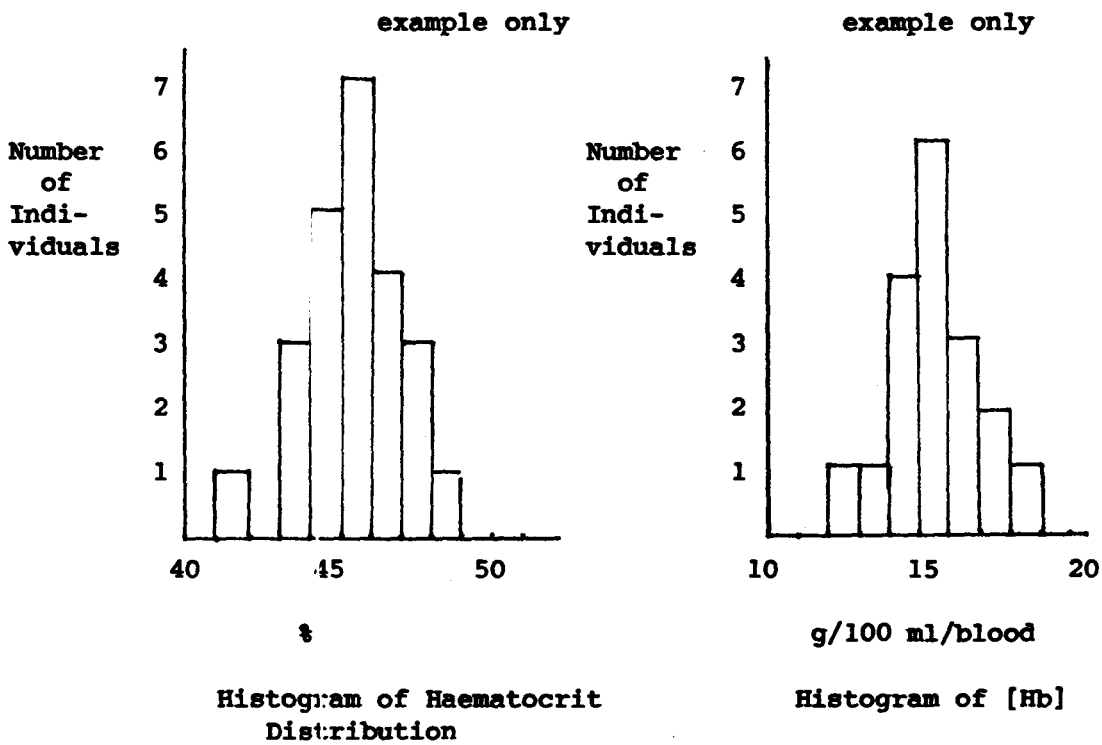
Q. Is there much variability in the class data? What are the main causes of variability?

Possible Reasons for Variability among students:

As with haematrocrit, different activity levels, differences in altitude (not at McMaster) and anemia are possible reasons. The level of haemoglobin formation may vary

for various unusual reasons - aplastic anemia, exposure to nuclear fallout, industrial X-rays, ingestion of certain drugs or lack of iron in the diet. Males excrete 0.6 mg iron in faeces each day. Females lose 1.3 mg iron/day during the menstrual cycle. This must be compensated for in the diet each day. Other reasons may be lack of amino acids in the diet or of trace elements, e.g., copper, cobalt, nickel.

Q. Illustrate this variability in a graph or table.



Histogram of Haematocrit Distribution

Histogram of [Hb]

Graph IV-3

C. Determination of Blood Type

A test based upon antigen/antibody reactions: an antibody is a protein, produced by the immune system, that inactivates a particular antigen. An antigen is a foreign substance that stimulates an organism's immune system to produce antibodies.

Add Anti-A serum to 1 drop of blood. If agglutination occurs

within 2 minutes, this is a positive test - the blood was of Type A.

Add 1 drop of Anti-B serum to the other drop of blood. Agglutination within 2 minutes is a positive test - the blood was of Type B.

If you get agglutination with both Anti-A and Anti-B sera, the blood was of type AB. If no agglutination occurs, the blood was of Type O.

Anti-A Serum	Anti-B Serum	Group	Frequency
-	-	O	45%
+	-	A	40%
-	+	B	11%
+	+	AB	4%

+ means agglutination
- means no agglutination

O group people are called universal donors because they have neither A nor B antigens on their erythrocytes to react with antibodies produced by a recipient.

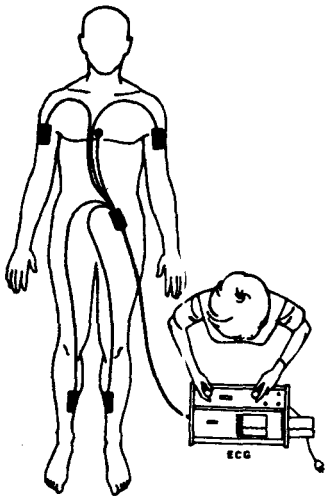
AB group people are called universal recipients. Having both A and B antigens on their erythrocytes, no antibodies are produced to cause reaction to blood received during transfusion.

Lab V - Cardiovascular Function

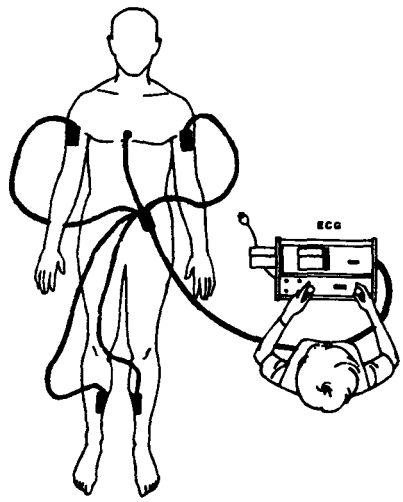
Each lab will have a veal or beef heart on display. Know the anatomy of the heart to be able to answer student queries about direction of flow, heart chambers, major vessels, valves and nodes. (pp. 264-5 and 268-9)

A. The Electrocardiogram - p. 271

- support the patient's head so he is comfortable;
- patient should be still and relaxed during recordings;
- apply the electrodes with proper tension; i.e., one hole tighter than a "relaxed" position.



CORRECT

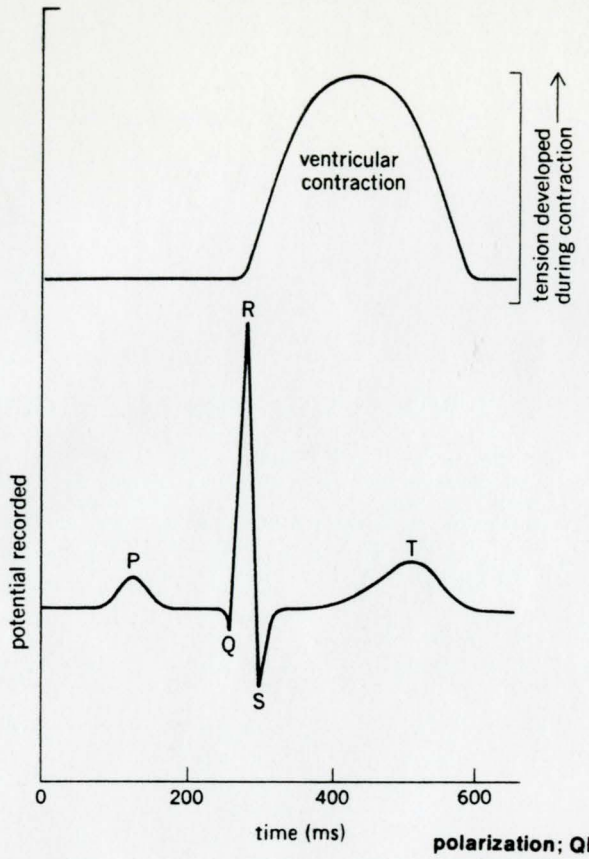


INCORRECT

P	represents atrial depolarization
QRS complex	represents ventricular depolarization
T	represents ventricular repolarization

Fig. V-1

Q. Calculate the heart rate in beats per minute from the interbeat interval.



Record of ventricular contraction

Record of changes of electrical potential

Typical electrocardiogram. P: atrial depolarization; QRS: ventricular depolarization; T: ventricular repolarization.

Fig. V-2

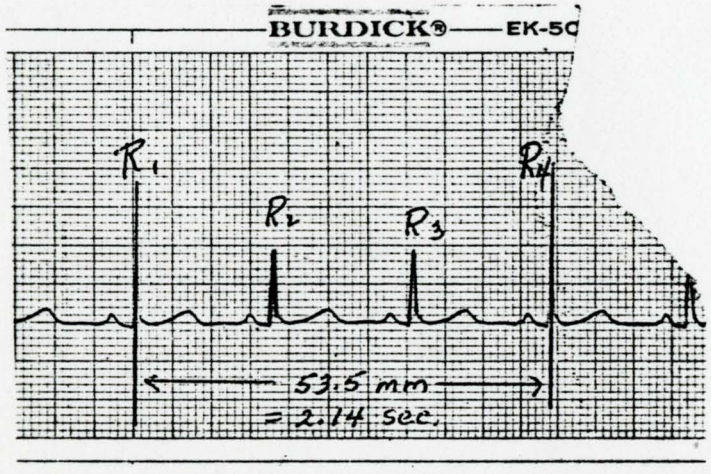


Fig. V-3

To calculate the heart rate in beats/minute from the interbeat interval, mark and measure the distance between first and last Rs.

The time scale of drum revolution was 25 mm/s.

$$\text{Heart rate} = \frac{25 \times \text{number of beats} \times 60}{\text{distance in mm}}$$

$$= \frac{25 \times 3 \times 60}{53.5} = 84 \text{ beats/min}$$

Q. Can you detect any abnormalities?

Abnormalities in the ECG (often higher in females):

Abnormalities seen in our labs do not necessarily indicate any malfunction on the part of the subject. Interference and extreme sensitivity of equipment cause frequent variations. Recent exertion, deep breathing and stress may cause variation.

Chronic abnormalities may be caused by:

e.g., atrioventricular block - no atrial impulses reach the AV node; therefore, no ventricular responses;

e.g., premature ventricular contraction - contraction originates in only one ventricle. It is polarized first, then contraction spreads to the second. Deflections are tall and deep and there are compensatory pauses between cycles.

e.g., atrial flutter - there are many P waves in rapid succession.

e.g., Bundle branch block - QRS complexes (for either right or left ventricle) are out of phase with each other.

Q. Can you draw any inferences from comparisons of the four ECG recordings? Explain your reasoning.

Inferences, of course, depend upon the results which you get. Heart rate should increase during exercise of any kind, but it will rise less in the case of students in good physical condition than for those in poor condition. Heart rate can double or even triple within a few minutes of the beginning of heavy exercise in order to maximize oxygen uptake.

There is no direct relationship between lung ventilation and heart rate. Following hyperventilation we would not expect any appreciable change.

B. Heartsounds - p. 274

Q. What is the origin of the sounds you hear?

(1) a low-pitched "lub" - associated with closure of the AV valves at beginning of systole.

(2) a high-pitched "dub" - associated with the closure of the pulmonary and aortic valves at beginning of diastole.

Sounds result from vibrations in the vessels caused during closure of the valves. They should be distinct. A sloshing or fluid sound may indicate incomplete closure of valves, permitting blood to leak through the openings. This is called a heart murmur.

C. Measurement of Arterial Blood Pressure - p. 282

Systole - A wave of depolarization passes through the ventricle (QRS complex), triggering ventricular contraction. This raises the ventricular pressure, closing the AV valve.

Immediately, the semilunar aortic valves open, allowing ventricular ejection to occur. Rate of blood flow is rapid at first and then tapers off, some blood remaining in the ventricle after ejection.

Diastole - The ventricular muscle relaxes following contraction, causing the ventricular pressure to fall. The pressure in the aorta is greater at this time, causing the aortic valves to close. Meanwhile, pressure in the atria is increasing steadily as venous blood enters. When the ventricular pressure falls below the atrial pressure, the AV valves open and ventricular filling begins again.

Maximum arterial pressure is reached during peak ventricular ejection and minimum pressure occurs just before ventricular contraction. During arterial blood pressure measurement, the cuff should be at heart level because this is the zero pressure level of the circulatory system. Any distance above would lower the result, and any distance below would raise the result.

Just before the first sound is heard (the thud) when taking the blood pressure, the pressure of the cuff on the arm is equal to the peak systolic pressure.

Q. Is there a difference in blood pressure after exercise?

Physical exercise requires a greater flow of blood to tissues. Pulse pressure and mean arterial blood pressure should increase slightly, following exercise.

This area has been stressed in lectures:

$$\text{Blood flow} = \frac{\Delta \text{ arterial P}}{\text{resistance R}}$$

Read p. 315 and study the diagram.

D. Measurement of Venous Blood Pressure

Q. How would you expect venous pressure (V.P.) at the elbow to compare with venous pressure in the right auricle? Why?

The right auricle is the point of zero reference pressure. The height above this zero reference point at which the veins may be seen to collapse, is used to calculate venous pressure. Venous pressure at the auricle should be zero or slightly below. In the arm it should be slightly above this - between 2 and 5 mm Hg,

$$\text{e.g., V.P.} = \frac{40 \times 1.055}{13.55} = 3 \text{ mm Hg}$$

Arteries have a thick muscular wall and a comparatively narrow diameter. Veins are low resistance conduits for blood flow from the tissues back to the heart.

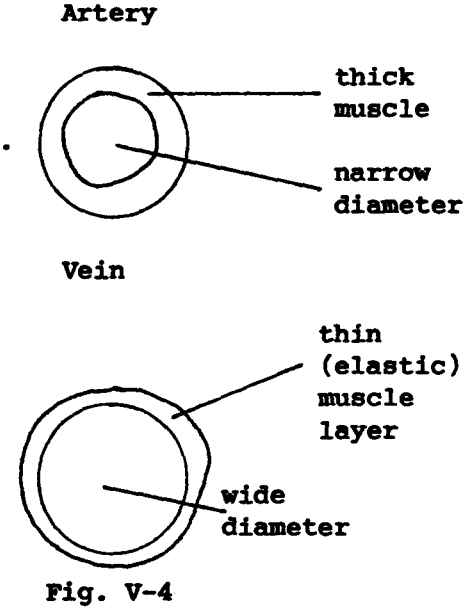


Fig. V-4

Q. What happens to blood flow in the vein when it collapses? Draw a diagram.

When veins collapse they still conduct blood, but the volume is much reduced. Fig. V-5

E. Venous Pump - pp. 298-9.

Q. Are you able to mechanically empty the vein? Yes.

Q. Does it refill from above? No.

Blood may be forced downward to some extent, but normally the valves prevent this.

The vein refills from below when both fingers are removed.

Q. Has muscle action had any effect?

Pooling is caused by hydrostatic pressure in the veins of the legs. Muscle action should have a definite effect, pushing blood upward and back towards the heart. The one-way valves prevent backflow.

F. Capillaries

Capillary loops should look like this:

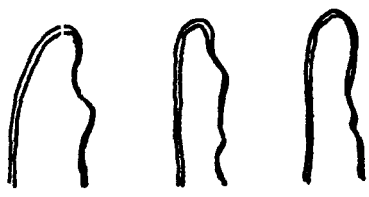


Fig. V-6

Lab VI - Respiration

A. A Problem to be Answered by Inquiry

Time - 30 min maximum

The demonstration flasks will be set up on the centre bench where all students can clearly see them as they come in. About 2:25 you should put the mouse into Flask #2 and start the system. Make a quick sketch of the apparatus on the blackboard for the students to use later.

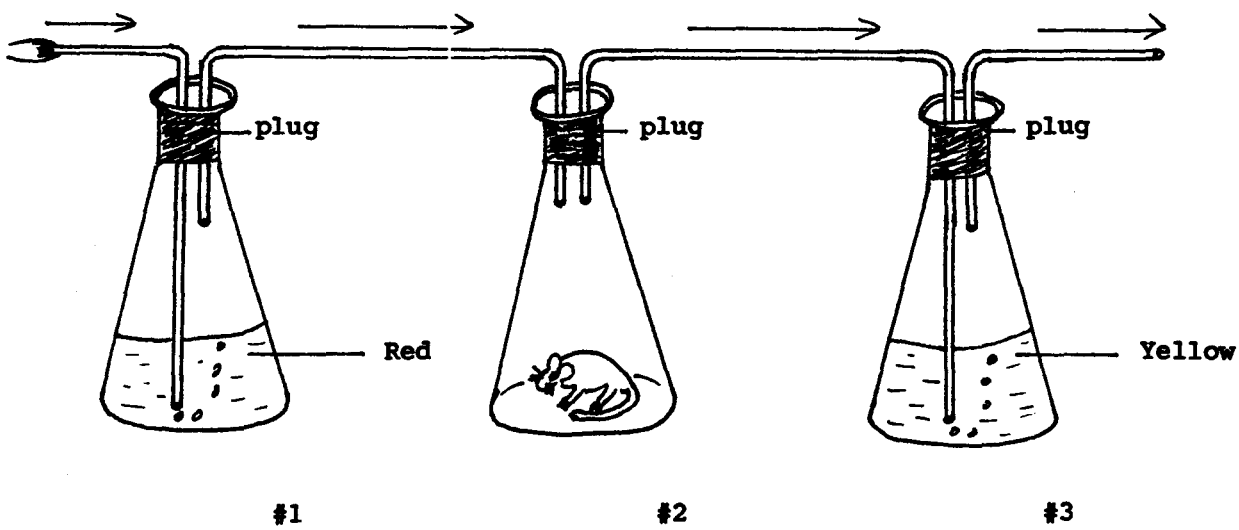


Fig. VI-1

When most students have arrived, have all come up around the demonstration.

Tell them only that, when the apparatus was set up, both Flasks #1 and #3 were the same colour.

Tell them that as a group, they are to solve the problem in order to explain what is going on in the flasks.

Tell them you will answer their questions with either a YES or NO, so they must word their questions in order to analyze the situation and uncover the explanation themselves. Involve as many students in the questioning as possible so that one student does not run away with the whole thing.

When some of them understand, have these students explain the details to the rest. Try to keep them in a single group for this. Those who still do not understand, should put their questions to those who do for further clarification.

At this point have various students work out the equation for the reaction on the board. It should be as complete as possible.

If the explanations have not been clear enough, you should ask further questions yourself for them to clarify.

Explanation - see p. 350

Flask #1 contains an aqueous solution of phenol red at a pH of approx. 6.5. Phenol red is an indicator which is red at pH 6.5 or greater, and turns yellow at a pH of 6.0 or less.

Plain air is being pumped through the system, starting with Flask #1.

The mouse in Flask #2 is respiring normally, breathing in O_2 and breathing out CO_2.

The CO_2 exhaled by the mouse is being carried into Flask #3 which also contains an aqueous solution of phenol red at pH 6.5.

As the CO_2 in Flask #3 is bubbled through the solution, it reacts with the water according to this reversible equation:

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

(carbonic acid) bicarbonate ion Hydrogen ion

Because we are forcing the air in one direction, we are driving the reaction to the right.

Accumulation of H^+ acidifies the phenol red.

At a pH of less than 6.0, the phenol red becomes yellow. Therefore, Flask #3 is becoming increasingly more acidic. Flask #1 acts as a control to prove that nothing from the air is causing the change. Therefore, the agent must be in the jar with the mouse.

This inquiry-based teaching strategy is used to give variety to the labs, but also because:

- students learn better by asking questions;
- they learn better through being more involved in the learning process;
- they learn better by having to think for themselves;
- students retain learning better by putting principles and concepts into their own words and speaking them aloud.

B. Oxygen Consumption

The O_2 consumption by the mouse should be in the range of 1.3 ml/min or 3.2 ml/g/h.

Remind the students to show all their calculations in order to justify their final values.

C. Oxygen Consumption and Carbon Dioxide Production

Removing the soda lime from the jar allows us to calculate the CO_2 production alone.

Repetition of the experiment (B) without the soda lime gives a measure of O_2 consumption plus CO_2 production together. The resulting overall change in volume in the system should be very small, e.g., 0.3 ml/min or 0.7 ml/g/h.

Assuming the O_2 consumption to be the same as in (B),

CO_2 production = O_2 consumption - (O_2 consumption + CO_2 production)

= 3.2 - 0.7 ml/g/h

= 2.5 ml/g/h

Respiratory Quotient (R.Q.) = $\frac{2.5}{3.2}$ = 0.78

This value of 0.78 suggests that the substrate being metabolized by the mouse is protein.

Lab VII - Fitness

A. Influence of Position on Heart Rate - pp. 306-7, 313-16

Maximal O_2 uptake = maximal cardiac output x art-ven O_2 difference

where maximal cardiac output = heart rate x stroke volume (p. 362)

During exercise, the supply of O_2 to muscles and the elimination of CO_2 depend upon the precise integration of the cardiovascular and respiratory functions.

A progressive increase in workload performed by the body results in a progressive increase in the amount of O_2 uptake by the muscles. But there is an upper limit to this increase, which is set by the maximal ability of the cardiovascular-respiratory systems to provide O_2.

During exercise, muscle cells obtain more O_2 by:

a) increased blood flow to the muscles;

b) increased extraction of O_2 from blood.

Under control conditions, the increased ventilation which is triggered by exercise is able to maintain complete saturation of haemoglobin - and therefore O_2 to the tissues. Hence, maximal O_2 uptake is not limited by the respiratory system, leaving cardiac output as the rate - limiting factor in endurance-type exercise. Therefore, measurement of maximal O_2 uptake is a reliable index of cardiovascular function.

The baroreceptors are the most important regulators of arterial pressure. Nerve endings at these areas are very sensitive

to stretch and to pressure. They transmit information directly to the medullary cardiovascular control centre in the brain. Nerve fibres from this centre synapse with the autonomic neurons and exert a dominant influence over them. Hence, this is a homeostatic reflex which regulates arterial blood pressure.

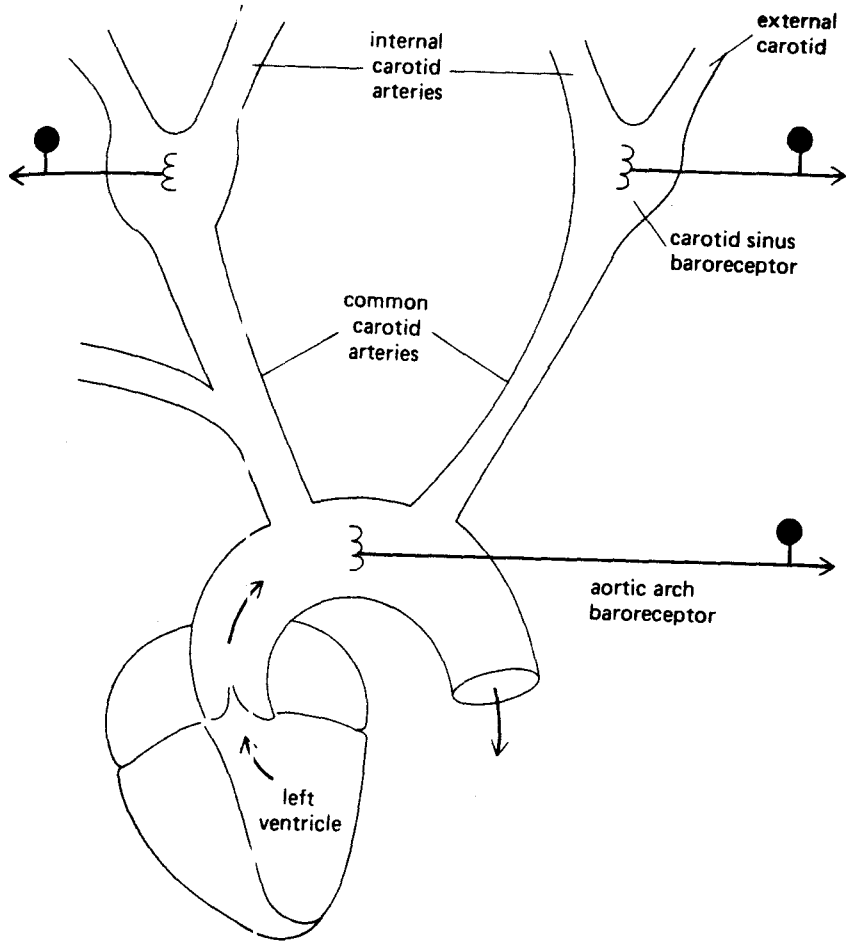


Fig. VII-1. Location of arterial baroreceptors.

When a person is in a horizontal position, all blood vessels are at approximately the same level as the heart, and the weight of the blood in the vessels produces negligible pressure. When a person stands up, however, the intravascular pressure in all vessels is increased by the hydrostatic pressure of the column of blood at the point of measurement.

The simple act of standing up is equivalent to a mild hemorrhage, because the changes in the circulatory system result in a decrease in the <u>effective</u> circulating blood volume.

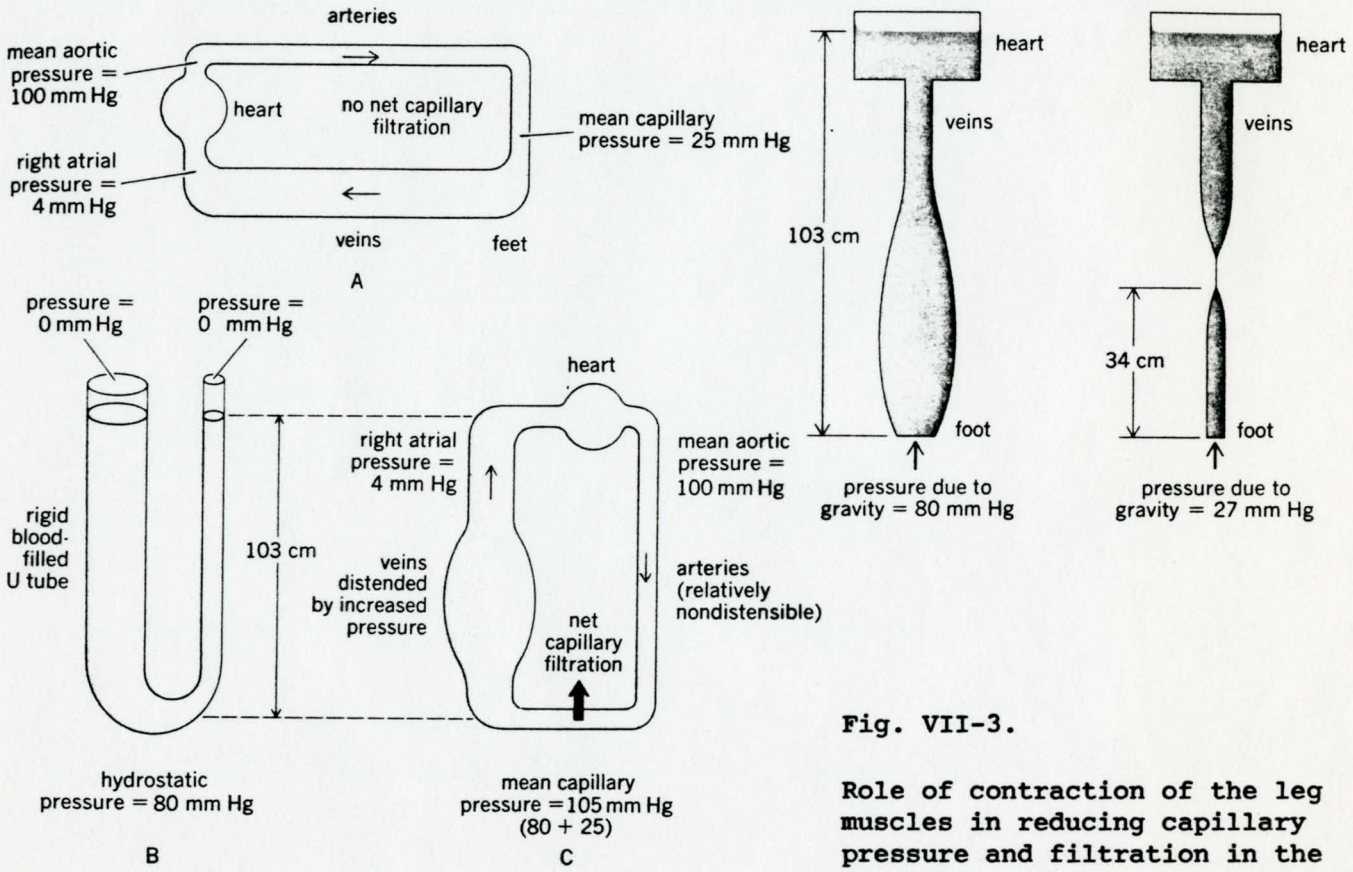


Fig. VII-2. Effects of standing upon blood pressure, blood flow, and capillary filtration.

Fig. VII-3.

Role of contraction of the leg muscles in reducing capillary pressure and filtration in the upright position. The skeletal muscle contraction compresses the veins, causing intermittent complete emptying so that the column of blood is interrupted.

Contraction of skeletal muscles of the legs breaks up the columns of venous blood, and forces the blood back towards the heart, decreasing both the pooling of blood in the veins and the hydrostatic pressure.

In (A) the students will be observing that reflex changes in heart rate will occur without the action of skeletal muscles, but will be increased both in speed and in degree by exercise.

B. Schneider Cardio-Vascular Fitness Test

This test scores:

- reclining heart rate
- heart rate increase on standing
- standing heart rate
- heart rate increase following exercise
- time of return of heart rate to standing normal following exercise

C. Pulmonary Fitness

1) students measure - breath holding time
   - mean of class breath holding time
   - percentage of the mean - A
2) vital capacity in cc using the spirometer (pp. 335-6)
   - individual percentage of normal - B
3) correlation between A and B
   " " A and Schneider score
   " " B and Schneider score
   " " (A + B) and Schneider score

The volume of air entering or leaving the lungs during a single breath is the tidal volume (approx. 500 mL).

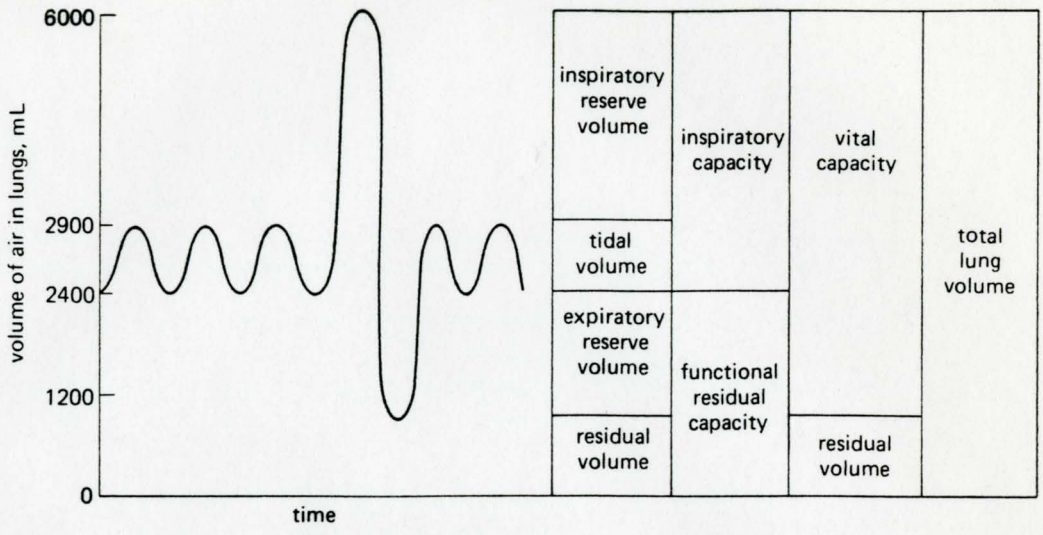


Fig. VII-4. Lung volumes and capacities as measured on a spirograph. When the subject inspires, the pen moves up; with expiration, it moves down.

Lab VIII - Kidney Function

Text - Chap. 13 - Regulation of Water and Electrolyte Balance, pp. 366-401

Extracellular body fluids normally have a pH of approx. 7.4, the same as blood.

The kidneys are continuously adjusting the $[H^+]$ of body fluids by excreting urine at a pH as low as 4.5 or as high as 8, depending upon the diet of the individual and whether he has an illness which is affecting his or her condition.

A. Clinical Tests of a Normal Sample

Q. Calculate the mean classroom pH. What is the variation?

Q. Make a statement about normal pH levels from class data.

The pH of urine is normally approx. 6.0, i.e., lower than that of the blood because more acid than alkali is excreted under normal conditions.

People:

- who eat meat - high acid content
- who eat vegetables - alkaline urine
- who are diabetics - acidic urine because of keto acids
- who have chronic respiratory problems, e.g., emphysema or bronchitis, have acid urine because of the tendency to retain CO_2, causing the blood to be acidic. To compensate, the kidney excretes acid.

Q. Would you expect to find glucose in normal urine? Why or why not?

Normally glucose would not be found in the urine because all filtered glucose is reabsorbed. However, if the body has an exceptionally high load of glucose (greater than the maximum tubular capacity - T_m), the body will then excrete it.

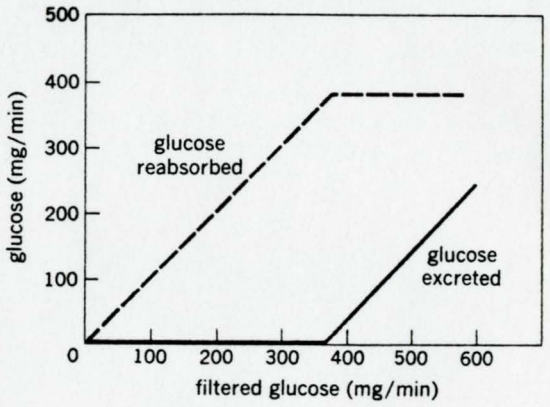


Fig. VIII-1: Saturation of the glucose transport system. Glucose is administered intravenously to a person so that plasma glucose and, thereby, filtered glucose are increased. The curves have been idealized, for the sake of clarity.

Q. Would you expect to find protein in normal urine? Why or why not?

Protein molecules are too large to pass through the pores of the glomerulus. Hence, one does not normally find protein, as such, in urine. However, some may come off the bladder and urethral walls, especially in the early morning after sleeping. A trace may be found for this reason.

B. Clinical Tests of Pathological Samples

Changed from instructions in manual. Pairs of students will be given 4 unknowns - A, B, C and D. Check the "code" used on each day for correct identity. Two are to be tested for glucose and for protein. The other two are to be tested for pregnancy. You are to tell your students the pairs to be used for each test.

N.B. In the test for pregnancy, a positive test gives no agglutination. When agglutination occurs, this is a negative response.

Q. Why is protein found in the urine in cases of nephrotic syndrome?

Patients with renal disease frequently develop a so-called "nephrotic syndrome" which is characterized by loss of large quantities of plasma proteins into the urine due to increased permeability of the glomerular membrane. Inflammation of the membrane may increase the size of the pores, thereby letting protein into the urine.

Q. Suggest a possible rationale to explain why a test for glucose may come out positive.

1. Glucose will appear in the urine of a person who has gorged him or herself on sweets - surpassing the maximal tubular capacity - see Fig. VIII-1.
2. If a person is suffering from diabetes mellitus, the hormonal control of plasma glucose by insulin is defective,

allowing glycogen catabolism to occur with a consequent rise in blood glucose. Normally, an increase in blood glucose concentration stimulates insulin secretion and a reduction inhibits secretion. Insulin facilitates the transfer of glucose out of the blood and into cells. This transfer reduces the blood concentration, thereby removing the stimulus for insulin secretion.

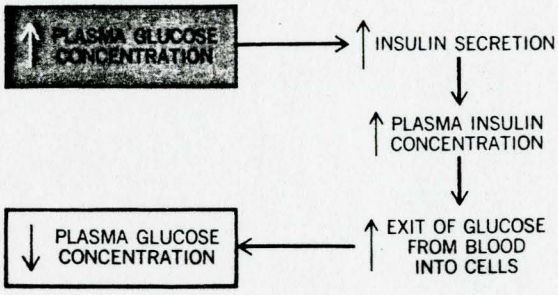


Fig. VIII-2. Negative-feedback nature of plasma glucose control over insulin secretion.

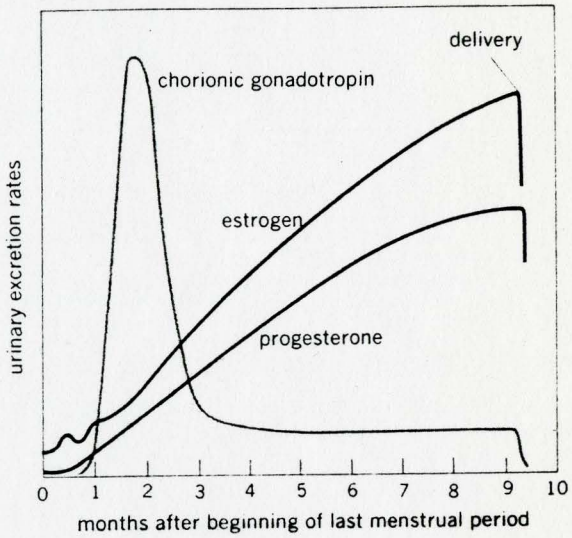


Fig. VIII-3. Urinary excretion of estrogen, progesterone, and chorionic gonadotropin during pregnancy. Urinary excretion rates are an indication of blood concentrations of these hormones.

Q. <u>Why does chorionic gonadotropin appear in the urine of women during early pregnancy?</u> (Text, p. 509)

At this time, the uterus requires high concentrations of circulating estrogen and progesterone. During the first two months, these hormones are supplied by the corpus luteum which

is formed after ovulation, from the remains of the follicle when the ovum has been discharged. Without fertilization of the ovum, the corpus luteum quickly degenerates. During pregnancy, however, it continues to grow and produce estrogen and progesterone which are essential for the maintenance of the uterine lining in which the developing foetus is embedded. Otherwise, the uterine lining would be sloughed off, including the developing embryo. The corpus luteum is caused to persist in its active state by a hormone produced by the placenta and the developing embryo. This hormone - called chorionic gonadotropin (CG) - is secreted into the bloodstream. After the third month, the placenta itself takes over the production of estrogen and progesterone and the CG is no longer secreted.

Lab IX - Hydrolysis of Starch by Amylase

No report is required to be handed in for this lab. Students should organize their data and a chart of their own design during the lab period and answer the questions in the spaces provided. pp. 55-56 should be handed in before leaving the lab and will be marked out of 10, as usual. You may suggest a format for the chart, if requested.

Demonstrators are to take charge of the boiling water bath in which 3 ml of the diluted amylase is boiled for 5 min..

One change in the procedure should be made. After the amylase is added to the tubes in the water baths, the tubes should be tested immediately as well as at 4-minute intervals in order to observe any reaction which may occur early.

This same procedure should be followed for both Parts B and C.

It is important to blow out the contents of the Pasteur pipets before taking each sample for testing. Otherwise, the test drops will not be a true index of the contents of the tube.

Results will vary somewhat between individuals, depending largely upon the pH of the saliva. The two small graphs use only a semi-quantitative scale for the Y axis, i.e., measurement of enzyme activity. The variable "time" may be taken into consideration by placing the fastest reaction at the maximum and scaling all others accordingly.

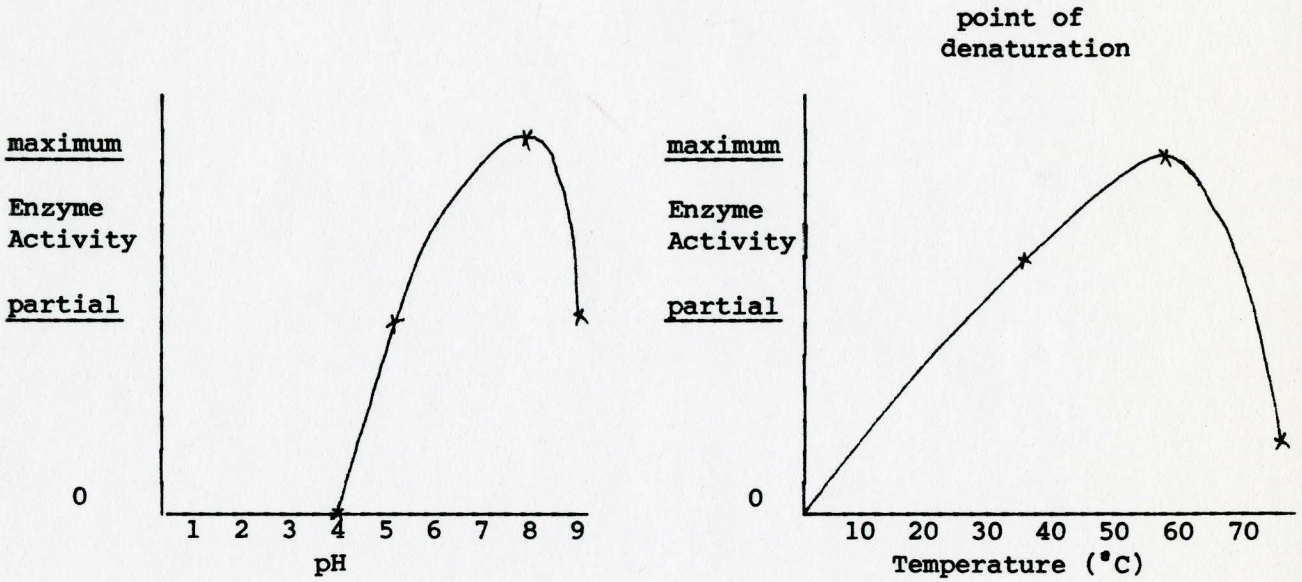
Organize all your data into a chart or table to best show the results of all the tests.

Chart of Amylase Activity at Various pHs and Temperatures

Tube #	Temp.	pH	Readings							
			1 min	4 min	8 min	12 min	16 min	20 min	24 min	28 min
1	37°C	4	Dark Blue							
2	37°C	5.5	Dark Blue	Dark Red-brown	Red-brown					
3	37°C	7	Dark Amber	Light Amber	Lighter	Achromic Point				
4 Dist H_2O	37°C	7	Red-Brown	Light-er						
5	37°C	9	Dark Blue	Dark Blue	Blue	Blue	Blue	Some Red	Dark Red-Brown	Dark Red-Brown
6	0°C	7	-	-	+	+	++	++	++	++
7	37°C	7	+	++	++	+++	+++	+++	+++	+++
8 Boiled Saliva	37°C	7	-	-	-	-	-	-	-	-
9	57°C	7	+	+++	+++	+++	+++	+++	+++	+++
10	70°C	7	+	+	+	+	+	+	+	+

\- No hydrolysis
\+)
++) Degrees of hydrolyis
+++)

From your data, draw the graph to show the activity of the enzyme according to these parameters. The Y-axis can only be a semi-quantitative scale.



Q. What was the original pH of your saliva? Approx. 8.0.

Q. Define a pH optimum for salivary amylase activity. 7.0

Q. How does this optimum pH compare with the pH of your saliva?

Q. Would "pancreatic amylase" have the same pH optimum? Explain.

(pp. 423, 425, 428) Yes, the pH of pancreatic amylase is 6.5 - 8.0. While in the stomach, the pH of food becomes very acidic, as secretions of HCl (2 ℓ/day) mix with the contents. The greater the protein content of the meal, the more HCl is secreted. The liver and pancreas both secrete sodium bicarbonate ($NaHCO_3$) which is carried by the pancreatic duct to the duodenum where it neutralizes the HCl entering from

the stomach, returning the pH to the same range as was found in salivary digestion.

Q. Does ionic activation appear to be important?

(pp. 407-8, 75) Yes, the ionic strength of the solution is important to the maintenance of the molecular structure of the enzyme. Without the presence of ions there would be no activity. The small amount of salts already in saliva account for the small amount of activity seen. See Tube #4.

Q. Describe the effect of increasing temperature on salivary amylase activity. Explain the effects.

At 0°C there was no hydrolysis of starch by amylase. Increasing the temperature increased both the amount and the speed of hydrolysis. The achromic point was reached in 12 mins in the tubes at 37°C, but was reached after only 4 mins by the tubes at 57°C. Above 57°C, activity dropped off very quickly. At this temperature the enzyme is denatured, destroying its ability to hydrolyze starch.

Q. Explain why the results with Tube #7 and Tube #8 were different.

Tube #8 contained the boiled amylase solution. Enzymes are protein, and as such are denatured by heat.

Tube #7 contained unheated amylase solution which was capable of breaking down the starch to maltose.

Lab X - Nutrition

The lab on Nutrition will be performed by the students individually over a period of three days. They will assemble according to the usual procedure at the regular time. Demonstrators are to organize a 15 to 20 minute talk to outline a method of procedure for the lab.

1. Journal: Students should make a precise record of all foods eaten over the period of three full days. In order to do this they should keep a journal (memo pad) with them at all times during the three days in order to jot down impulse snacks such as chocolate bars, soft drinks, etc. and also physical events such as sprinting across campus which might otherwise be forgotten.
2. Diet Record: Total the calorific intake for each day. Then calculate the average for the three days. Calculate the proportion of the total calorific intake which is in the form of protein, using a value of 4.2 cal/g.

Food	Amount	Calories	Protein
Day 1			
		Total	Total
Day 2			
		Total	Total
Day 3			
		Total	Total
		Average	Average

Each student should apply his or her weight to the appropriate graph on p. 67. This will give the percentage he or she is of the National Research Council "normals"; e.g., a female who weighs 140 lbs. (140 x 0.4536 = 63.5 kg) is 112.5% of the recommended figure, and therefore should have a daily intake of 2300 x 1.125 = 2587.5 Calories of which 63.5 gm should be in the form of protein. 63.5 x 4.2 = 266.7 Calories in the form of protein.

The graphs used to interpolate individual calorie intakes are not straight lines because metabolic rate varies with the body surface area, not with body weight. Explain this to your students because it is not discussed in the text.

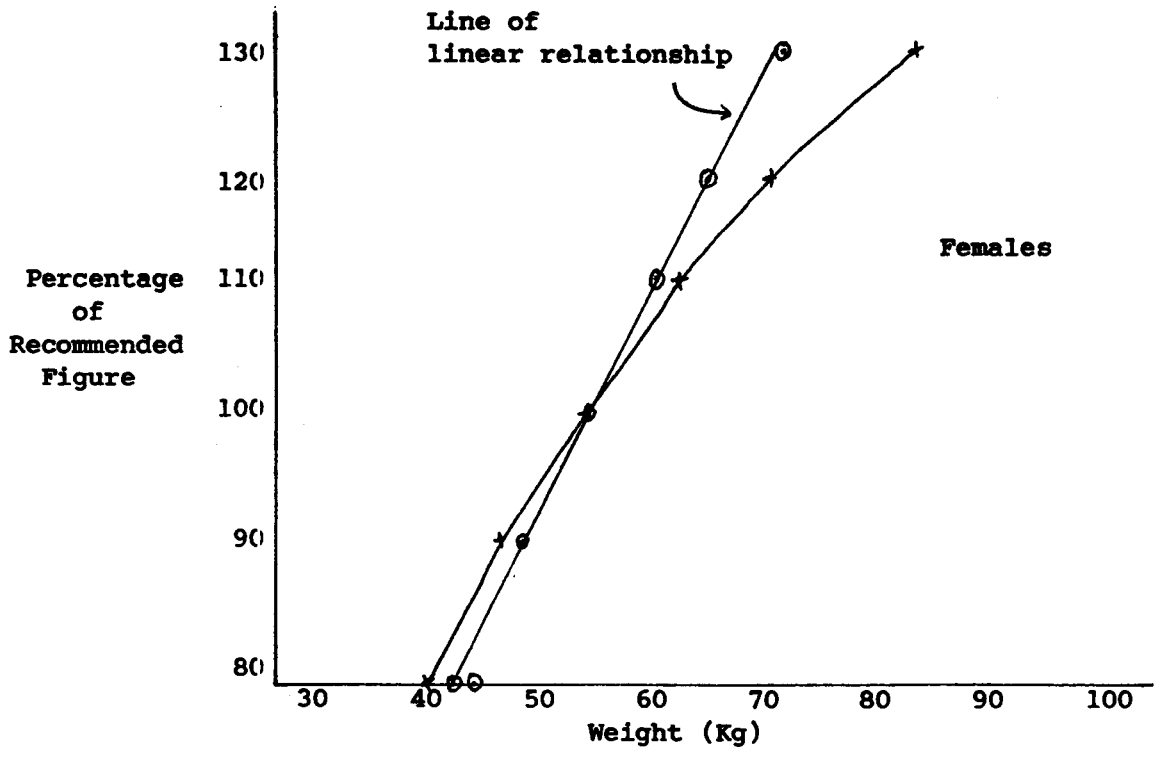
3. Activity Record: A detailed record for one day only. Students should record the time spent at each activity. This time is to be translated into Calories expended and then totalled to give the total energy expenditure in Calories. The values given for the various activities would represent work done at normal body temperature of 37.5°C and are recreational type figures. The basal metabolic rate (BMR) increased 25% per 1°C rise in body temperature. Persons with chronically high body temperature would have a high BMR. Any person having a chronically low body temperature would have a correspondingly low BMR.

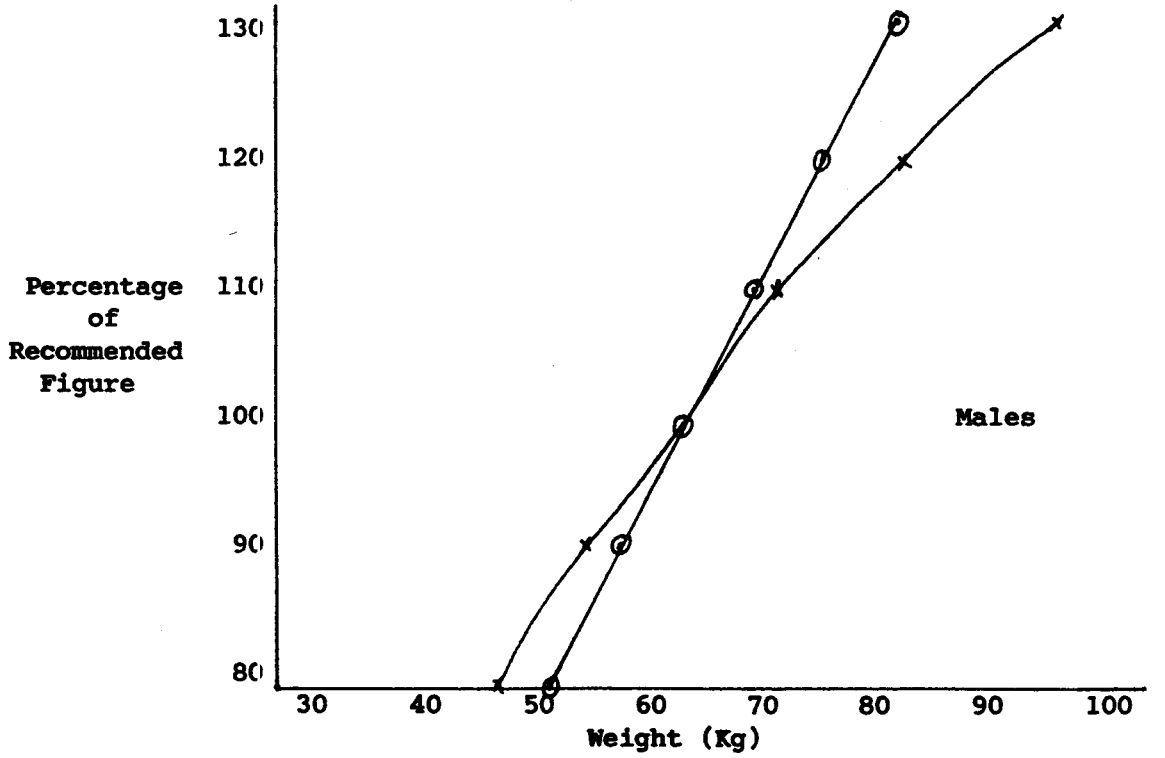
The energy costs of activities in Calories/kg/h represents both heat production and external work. As such they are equivalent to the total energy expenditure, and efficiency is not a factor.

Other factors which must be taken into account in constructing a more accurate energy balance sheet might be:

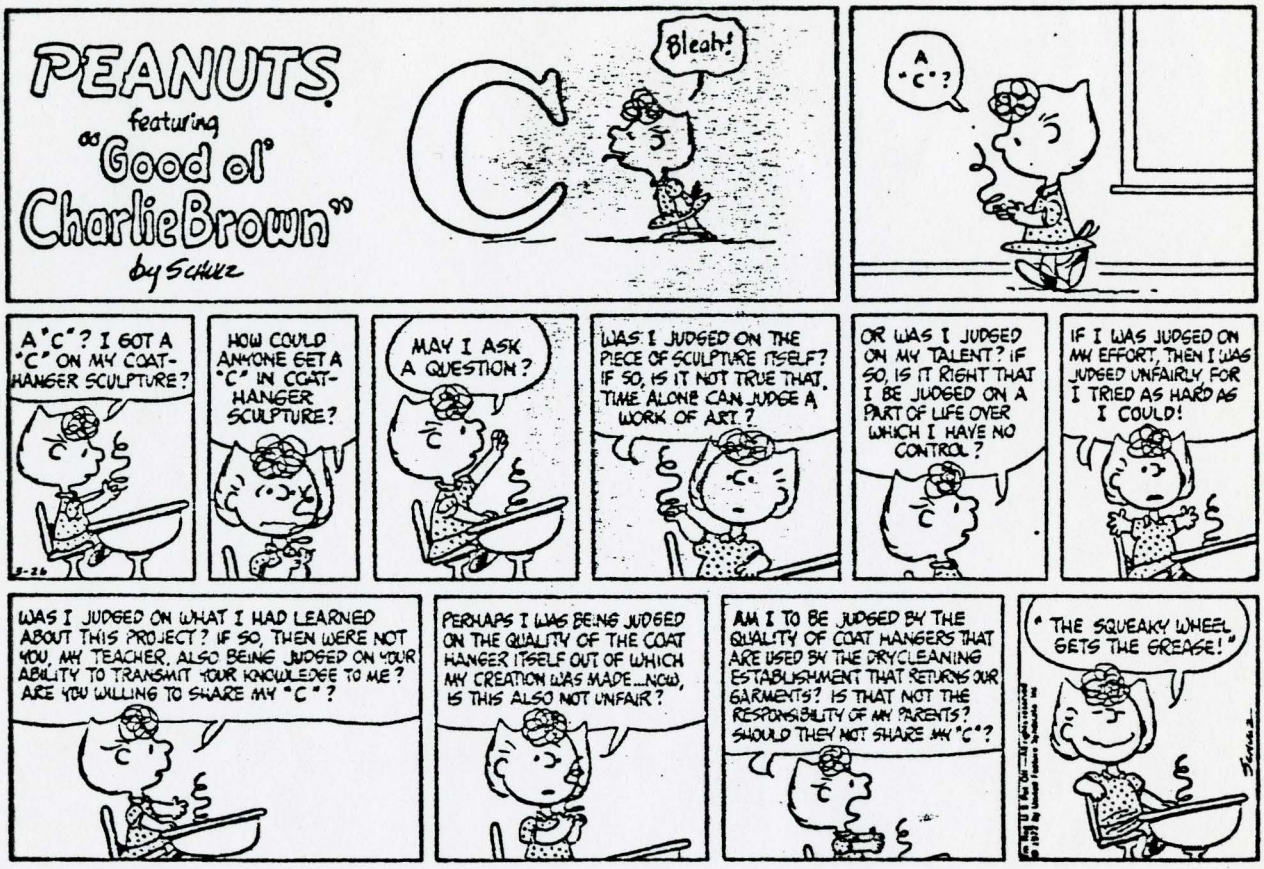
1. Hormonal Status - thyroxine and epinephrine production is shut down at higher temperatures
   - sex hormones
   - activity of sympathetic nervous system
2. General Health - good health allows for greater efficiency of energy conversion and therefore lower cost
   - healthy individuals are generally more active
3. Specific Dynamic Activity - the cost of processing (or interconversion) of food
   - with a high protein diet, the cost of interconversion is higher
   - individuals who are chronically malnourished have lower metabolic rates which would engender lower costs or greater efficiency
4. Age - M.R. decreases with age, beyond 30 years
5. Body Morphometry - M.R. is higher for tall thin people and lower for shorter, stout people.
6. Air Temperature - There is a thermoneutral zone between 15° and 20°C. As air temperature is lowered beyond 15°C, M. R. rises, increasing the cost of activity.

Recommended Weight Chart





T.A. Handout at Wrap-Up Meeting - April 1983



Student Evaluations of 1H6 of T.A.s - 1983

Although summaries of student evaluations will not be returned to us for several weeks, a cursory look showed that students were generally pleased with the 1H6 labs this year and with their lab instructors.

By far the greatest number of comments were extremely positive e.g. i) He was excellent - couldn't be improved. ii) He marked fairly, provided good criticisms for reports and told us how to improve. He always made it clear beforehand what was expected in write-ups.

The following statements were chosen at random, as indicators of those T.A. characteristics most appreciated by or most troublesome for the students, and may therefore be useful in assessment of your own performance as well as for future improvement.

- very approachable, even with stupid questions
- could seem a little more interested for the benefit of student involvement
- more written comments could have been given on lab reports
- should have explained things more slowly
- willing to help and did not rush out at 4:30 but stayed for questions
- an excellent T.A. He offered explanations but still made us think for ourselves
- should be more familiar with the lab format and <u>follow</u> it
- should have explained at a beginner level. He assumed that since we read the book we understood (not always the case).
- helpful but made you think - wouldn't give anything away which was good
- there should be more consistency in marking
- I don't like being given a higher mark which is later changed to lower
- He makes the labs enjoyable and interesting - willing to assist
- explanations at beginning of class were sometimes confusing
- explicit explanation of how report marks were allocated would help
- marks do not reflect the effort put into lab reports - I'd like to know <u>why</u> my mark was low because I spent a lot of hours on them
- did a good job. the pre and post-lab talks helped a lot
- seemed to mark the labs very hard compared to other demonstrators

Continued...

- lab reports need more comments which would lead to improvement of marks

- has ability to give information without making one feel uncomfortable or ignorant

- has a great sense of humor - made the labs a lot of fun

- more diagrams on the board would help and since we couldn't always understand him, he should write important things on the board

- clearer instructions about what to include in the report

- he had a tendency to speak too fast, considering his accent

- he tended to assume that we were all Bio. majors instead of Phys. Ed. students

- he should understand the equipment and lab procedures better to make the lab flow faster and be less confusing

- helpful and understanding but should cut down on the introduction

- the class was not always attentive - the T.A. should have shown more authority

- I wish he would get everyone quiet and listening at the start so the rest of us would know what was going on

- marking by T.A.s should be compared and adjustments made for those marking too leniently as well as those marking excessively hard

- comments on reports were useful but hard to read

- he should read the lab manual himself so he knows what's going on

- he was a damn hard marker. It was unfair that we didn't get a new lab T.A. after Christmas.

- comments on the reports were too general. He should teach religion or something more exciting.

McMASTER UNIVERSITY

Department of Biology - 1983

TO: Demonstrators in Biology 1A6/1B7

FROM: Dr. S.F.H. Threlkeld Room 541
Dr. C.M. Wood Room 528
Mrs. M.A. Service Room 330

Biology 1A6/1B7 is the introductory course given by our department to all Natural Sciences I students. As such, it is particularly concerned with basic levels of biological organization: evolution, the chemical basis of biological systems, cellular structure and reproduction, patterns of inheritance and their chemical basis, and energy transformations.

Most of the students have had Grade 13 Biology, but many have not. Their intended direction may be towards Biology, Chemistry, Physics, Biochemistry, Geology, Geography, Mathematics, Psychology, or some variation of these. It is our job to encourage and challenge their natural curiosity and to help them achieve to the best of their ability. In so doing we might hope that many will be stimulated to choose Biology as a career path.

Our instructional goals for the labs would include the following:

i) The development of a keen interest in Biology, an enquiring mind and a scientific approach to life.

ii) The development of observational skills and the attendant ability to organize reasoning relevant to these observations.

iii) To assist students in learning to communicate logically and critically, both orally and in writing.

Please direct problems which directly concern labs or quiz marks to Mrs. Service - Room 330 (4588). Direct any other problems related to the course to Dr. Threlkeld (Term I) or Dr. Wood (Term II). Direct any equipment or supply problems to Mr. Les Gyorkos - Room 106, the departmental technician responsible for setting up the labs.

Lab Schedule

I	Identification of Unknown Specimens	Sept 19 - Sept 23
II	Chemical Basis of Biological Systems	Oct 3 - Oct 7
	Week of Thanksgiving - no labs	
III	Cell Structure	Oct 24 - Oct 28
IV	Genetics and Heredity	Nov 7 - Nov 11
V	Photosynthesis	Nov 21 - Nov 25
	Christmas Break	
VI	Unicellular and Colonial Organisms	Jan 9 - Jan 13
VII	Gas Exchange and Internal Transport	Jan 23 - Jan 27
VIII	Regulation of Body Fluids	Feb 6 - Feb 10
IX	Chemical and Nervous Control of Body Function	Feb 20 - Feb 24
	Study Week	
X	Animal Behavior/Ecology	Mar 12 - Mar 16
XI	Lab Practical	

DEMONSTRATORS' RESPONSIBILITIES

Demonstrators' Meetings

It is essential that all demonstrators attend the meeting in advance of each lab session. It is unfair to the instructors, to the other demonstrators and especially to your students if you are not there on time to become familiar with the details of the equipment and/or laboratory procedures. A lack of familiarity with procedure would be an embarrassment for you with your students and would reflect badly on the course in general.

Lab Reports

Students are expected to submit a lab report after every lab session. These are due by noon of the day one week following the lab itself, or earlier. Demonstrators are expected to mark the lab reports promptly, returning them to the students in the next lab period. The students need this feedback to assist them in the write-up of the next report. Late reports are not to be accepted without a medical certificate; refer any disputes to Mrs. Service. This being the case, collect all reports from your drop-box by 1:00 p.m. of the due date on a regular basis. Because we demand promptness from the students, marked reports must be returned by the demonstrators in each following lab period without exception. Our credibility depends upon it.

Records

Demonstrators should maintain a permanent record of the lab marks in duplicate, one to be given to Mrs. Service on the same day as reports are returned, the other to be kept by you.

Biweekly Quizzes

Demonstrators are expected to invigilate and check the marking of the multiple-choice quizzes which are written on Monday and Tuesday of the week between labs according to this schedule.

Term I

1B7 - 10:30 a.m.	1A6 - 12:30 noon
Monday Sept 26	Tuesday Sept 27
Monday Oct 17	Tuesday Oct 18
Monday Oct 31	Tuesday Nov 1
Monday Nov 14	Tuesday Nov 15
Monday Nov 28	Tuesday Nov 29

Term II

1B7 - 10:30 a.m.	1A6 - 12:30 noon
Monday Jan 16	Tuesday Jan 17
Monday Jan 30	Tuesday Jan 31
Monday Feb 13	Tuesday Feb 14
Monday Mar 5	Tuesday Mar 6
Monday Mar 26	Tuesday Mar 27

These quizzes will be written in Rooms 104 of Chester New Hall and the regular lecture rooms.

Monday - Engineering 376, Tuesday Burke Science Auditorium, Room 147. On your assigned days, plan to be there 10 minutes early to hand out the quiz papers.

To check cards and post marks, come to Room 437 of Life Sciences at 12:30 Mondays and 2:30 Tuesdays. One hour should be adequate time.

Demonstrating

Plan to attend the pre-lab talk in Health Sciences 1A6 at 2:30 p.m. of your lab day, so you will know what your students have been told. Expect to give a 5 minute (10 maximum) pre-lab talk yourself to outline specific procedures and to emphasize particular points. Move around among the students continuously, giving equal time to each group if possible. Be enthusiastic about what you are doing, friendly and concerned about each student. Encourage discussion and be willing to help wherever needed. Plan to have a 10 minute post-lab discussion at the end of the lab period if you feel that there may be confusion regarding the questions asked or the principles involved. Write out a few thought-provoking questions which would get discussion going at this time.

Marking Lab Reports

Reports should not exceed 4 pages in length excluding tables, diagrams and graphs. Instructions on writing reports have been given to the students in the lab manual. You might encourage brevity by marking down excessively long or rambling reports. The main thrust of the lab report should be to clearly describe the results obtained and to interpret these results in the light of theory. Be sure that all the questions asked in the lab guide have been answered.

Marks should not be taken off if their experiment did not work as long as they try to explain why it failed and also what they might have expected to occur. If extra effort is put into the experiment or the write-up it should be rewarded. Mark all labs out of 10 and average them each week to check consistency.

You may set your own marking standards but be prepared to substantiate them. We would, however, suggest the following:

a) Marks as high as 10 or as low as 3 should be given only in extreme circumstances.

b) A reasonable average for the class over the year would be 6.5 or 7.0.

c) Be as clear as possible about your grading criteria and discuss this in detail with your students.

d) Be consistent and objective in your marking, both between students and throughout the year. Remember that each report represents several hours of student effort.

e) Where possible, make encouraging comments on reports; avoid sarcastic non-helpful comments.

f) 80% of the mark should be based on the results and the discussion.

g) THERE SHOULD ALWAYS BE SUFFICIENT COMMENTS TO ENSURE THAT THE STUDENT UNDERSTANDS WHY HE RECEIVED THE MARK HE DID AND HOW HE MIGHT IMPROVE IT IN THE FUTURE.

INSTRUCTIONS TO STUDENTS - FIRST LAB

The first lab is the most important. Good or bad, it will probably establish your relationship with the students for the remainder of the year.

Many of them have never been in a lab before and will naturally be apprehensive. You can help by being friendly and informal and by taking the time to explain precisely what they will be doing and what you expect of them. The work of the first lab has been abbreviated in order that you may spend about 30 minutes briefing them on procedure.

These are some of the things you might tell them the first day:

a) Put your name on the board and a room number where you may be contacted for questions. Set specific hours for this if possible. Tell them what you want them to call you.

b) Students are expected to participate actively in the lab itself and the post-lab discussion.

c) They should plan to arrive on time (by 2:30) so as not to miss the pre-lab talk.

d) Any and all questions may be freely asked; they should never be embarrassed to ask because of feeling foolish.

e) Discussion among themselves is desirable.

f) As demonstrator, you cannot answer any lab guide questions, but will discuss the reasoning with them.

g) Go over the parts to be included in each report and discuss content of each (see lab guide pages 1, 2 and 3); the title page of each report should have their name, lab day and demonstrators name in addition to the title.

h) Let them know that writing style and neatness also count as part of the remaining 20% (80% is for results and discussion).

i) You cannot accept lab reports which are late, or from students who were not in attendance at the lab itself (this means you must know who was missing).

j) We do not have the capabilities for students to make up missed labs or to change lab days temporarily.

k) The range of marks will be 3 to 9 (10 most exceptional) class average will be near 6.5 or 7.0.

l) Copying of lab reports not acceptable, even between partners - reports with too many similarities will have the mark divided between the 2 students. Even when data is identical, the treatment of it must be on an individual basis.

m) Explain your philosophy of marking i.e., the features which you will be looking for or will be marking down. Labs are worth 20% of the final mark, so each report is worth a final 2%.

Laboratory (Teaching) Techniques

If the concepts of the labs are to be completely understood, the students must be actively involved in the exercise. Encourage them to ask questions and to discuss problems or questions among themselves. Appropriate questions by the demonstrator as you move around among groups are an extremely important tool to ensure that learning is occurring:

- they expose areas of confusion
- they can clarify the issues
- to focus student concentration on a particular area
- to separate relevant from irrelevant information
- to assess the information
- help to create insight

N.B. However, students should never leave the lab with all the questions still unanswered in their minds. This is frustrating for them and counter-productive for us, especially if they have picked up wrong information. Ten or fifteen minutes before the end of the lab time, schedule a debriefing session to discuss their findings. If the swifter students who already understand the concepts wish to leave, so much the better. Gather the rest into an informal group to discuss their results and the significant points. Encourage them to explain to each other in the group rather than your doing the explaining. This will be easier if you are sitting down among them. When they don't understand or are reluctant to speak up, try to prompt their understanding by asking sequences of open-ended questions which force them to think out loud, so you can direct their reasoning. Encourage them to formulate clear and logical verbal statements, always backed-up with reasoning. You should verify the correctness of their reasoning if it is correct, but ask others to make modifications or corrections when this is necessary. As the demonstrator, you can be most helpful by summarizing and paraphrasing their ideas. Repetition of this kind clearly contributes to their learning and to reinforcement of what has already been learned. You should be an information resource, but avoid giving them complete answers to specific questions yourself. Instead, practice the art of posing alternative questions which will lead them to answer their own queries, or have other students try to answer. You should avoid the type of question which has a yes or no answer because it limits discussion. Also to be avoided are questions which are obscure or which might trap the student into a blunder of any kind.

APPENDIX C

INSTRUCTION OF TEACHING ASSISTANTS - PART II

BIOLOGY 1A6

Lab 1 - Identification of Unknown Specimens

Because this is an open-ended discovery type of lab, it is impossible to predict what students are going to find. Therefore, this is a sample of the type of lab report I would hope you receive.

Purpose and Introduction

The purpose of this lab is to gain experience in identification of unknown organisms by the use of a dichotomous key, according to scientific procedures.

A dichotomous key consists of many steps which compare structural features. Each step requires making a choice between two alternatives. The steps begin with very broad characteristics and move down to more narrow choices until the final level of identification is reached.

The Scientific Method requires that one first formulate the question representing the purpose of the study - in this case to find the identity of two unknown organisms: an invertebrate aquatic insect and an evergreen. Through detailed observations (collection of evidence) and analysis of the information collected, one can make an hypothesis concerning the identity of the unknowns. This hypothesis should be retested according to various criteria.

Method

See the lab manual, pp. 6 and 7, for a description of the method and materials used.

Observations

C. 1. The Invertebrate

Features observed are listed below:

- oval-shaped body approx. 3 mm in length and 1 mm in width
- movable head, but head, thorax and abdomen were close together, with little space between body sections
- three pairs of jointed legs attached to the thorax, each terminated with a single claw
- an air bubble was seen ventrally near the anterior legs
- segmented abdomen covered dorsally by a pair of hard, leathery wings which met along the mid-dorsal line
- two large black compound eyes, one on either side of the head
- a pair of 11-segmented antennae stretched anteriorly from the head region between the eyes
- body and legs were covered with many projecting hairs, those on the legs being longer and thicker
- between the eyes, ventrally, the mouth parts were visible, being of the biting and chewing type
- a ventral abdominal keel stretched to the posterior end

Following the key, this organism was keyed out to the Order - Coleoptera (beetles).

2. The Evergreen

- needles were borne singly along the stem, a medium green colour
- needles were long (25 mm) and pointed

- needles were attached directly to the branch
- branchlets were a brown colour and smooth
- a small section of needle was removed and examined under the dissecting scope - it was flattened in cross-section
- the underside of needles had 2 distinct rows of stomata seen under the dissecting scope

These characteristics, followed through the key, led to its identification as from the balsam tree.

Discussion

The smooth-edged, oval shape of the insect's body would give it considerable mobility in the water for rapid movement from surface to bottom and vice versa.

Although beetles are air breathers, it would appear that this specimen had adapted a mechanism for carrying an oxygen supply with it underwater in the form of an air bubble from which oxygen could be extracted.

The hairy legs acted as paddles for swift movement through the water. When swimming, the legs could be straightened out, thus not obstructing the water.

The eyes, being large, movable and lateral, could control vision in an area close to 360°, giving early warning of predators or any other dangers. The ventral keel acted to stabilize the heavy insect body. Antennae are sensory, assisting in awareness of the environment. The mouthparts were well-placed and powerful for use in feeding on submerged vegetation.

Since most evergreens do not shed their leaves in the winter, several features observed allow trees to adapt to cold winds and low temperatures. Needles are thin and fine, allowing cold winds to pass through but allowing transpiration in summer. The surface area exposed is very great yet sunlight for photosynthesis can penetrate to all needles. The balsam does not have as many adaptive features as many other trees. Write-up should be more extensive.

Summary and Conclusions

The invertebrate was keyed out to belong to the Order Coleoptera - the beetles.

The evergreen was keyed out to be a balsam.

The structural characteristics of both organisms were related to function, thus allowing each a better chance of survival in its ecological niche.

Lab II - Chemical Basis of Biological Systems

This lab is very relevant to what Dr. Threlkeld has been discussing in lectures. He recommended that students should read the lab in preparation for the quiz last week, so they should be well prepared.

Your pre-lab talk should stress that the various tests are based on a relative colour change. As such, the test is only semi-quantitative and students should not get hung up on exact measurements.

To avoid confusion around the various stations, it would be wise to assign benches to different sections:

a) testing for biological molecules

b) DNA and RNA

c) chromatography

A. Testing for Biological Molecules

I. Carbohydrates

a) Benedict's reagent is used here as a test for reducing sugars. A blue colour indicates no reducing sugar present. A yellow or green colour indicates some, and an orange or red colour indicates a large amount. Tubes must be put in the 100° water bath to show colour. Honey and glucose give a positive result. Other test materials are negative.

b) A positive starch test is a change in colour to blue-black. Potato and starch give a positive test. Filter paper, apple, water and glycogen are negative.

II. Lipids

Sudan III reagent is used to indicate an orange colour in the presence of the long fatty acid chains (H - C - H). Distilled water is negative. Milk, cream and vegetable oil are positive and show a range of colour. It is important to allow the spots to dry thoroughly before putting the filter paper into the Sudan III solution.

Alcohol is used in this test because fats are soluble in alcohol but not in water. Polar molecules dissolve in polar solvents. Fats, being non-polar, only dissolve in non-polar solvents such as alcohol.

III. Biuret Test for Proteins

In the presence of amino groups which are joined in peptide linkages, the copper ions of the reagent cause a purple colour to develop. The distilled water, honey and amino acid solutions give a negative response. Albumin and milk are positive.

In your pre-lab talk, point out the flush-sink in the lab room in case of any accident involving NaOH.

IV. Test for Nucleic Acids

The diphenylamine test gives a blue colour in the presence of DNA, but is clear with water. The orcinol test gives a blue/green colour in the presence of RNA, but is clear with water.

N.B. The boiling water bath must be kept from boiling

away. Add distilled water at intervals during the afternoon, but not enough to lower the temperature. The tubes are covered with marbles to prevent pressure build-up while in the water bath.

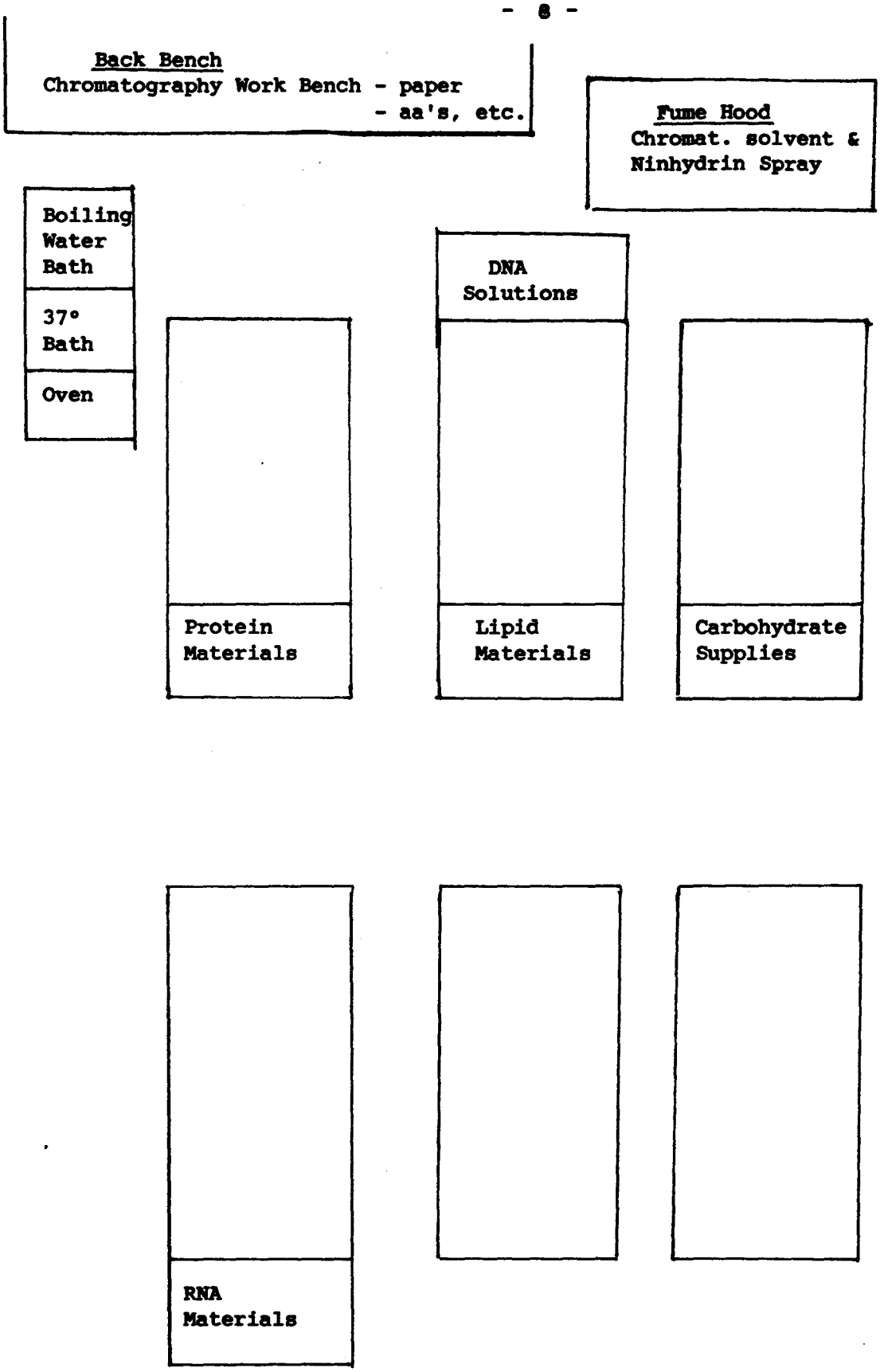
B. Paper Chromatography

The preparation of chromatograms should all be done on the back bench which is covered with plastic to prevent interference by contaminants. Since cross-contamination of a.a. solutions with capillary tubes is a major problem in this unit, each pair of students get their own 6 capillary tubes, each tube to be used only once then discarded immediately.

To obtain R_f values of each a.a. students measure from the middle of the original spot to the centre of the final area. They should hand in the charts with their reports.

Be sure that all test tubes are rinsed and turned upside down to drain before the students leave.

		Unknowns
Week 1	X2	X2
Monday	proline	histidine/valine
Tuesday	proline/valine	alanine
Wednesday	valine	alanine/proline
Thursday	alanine	proline/histidine
Friday	alanine/valine	histidine


Back Bench
Chromatography Work Bench - paper
- aa's, etc.
Fume Hood
Chromat. solvent &
Ninhydrin Spray
Boiling
Water
Bath
37°
Bath
Oven
DNA
Solutions
Protein
Materials
Lipid
Materials
Carbohydrate
Supplies
RNA
Materials

Lab III - Cell Structure

A. Plant Cells

I. Elodea

Q. How many layers can you count? 2 or sometimes 3.

Q. Are all the cells the same size and form? There is much variation in size but the form is generally similar. Cells specialized for a particular function, e.g., hair cells, may be different.

Q. Are there several small vacuoles or one large vacuole? Young cells will have several small vacuoles, but at maturity there is usually only one vacuole. Strands of cytoplasm may cut across the cell, giving the impression of two vacuoles.

Q. What is the function of vacuoles? The cell sap, being hypertonic, takes in water continuously, keeping the cell and the plant turgid. Vacuoles also store other soluble organic compounds and serve as a dumping site for noxious wastes.

Q. Can you account for the movement of the chloroplasts? The cytoplasm flows along the surface of the cell vacuole by cyclosis, carrying the nucleus and chloroplasts with it. Sometimes this is a slow process, sometimes very fast. Actin microfilaments are believed to be responsible for the cytoplasmic streaming.

The diagram of the Elodea cell should include outlines of a second cell to ensure they understand the position of the cell wall relative to each cell. N.B.: It is not a feature common to both cells.

II. Cytoplasmic Streaming in Tradescantia is cancelled.

III. Plant Strategies for Water Retention

Q. Are the cells similar to those seen in Elodea? No.

Differences? - lack of chloroplasts, very little cytoplasm;

- cells are larger and more square in shape.

Q. What is the function of stomata? They allow gas exchange. They open at night to allow moist air to enter but close during the day to prevent desiccation.

Q. In which of the peels are they more abundant? - in the lower side of the leaf.

Rationale - The upper side faces the sun for photosynthesis. Too much water would be lost by evaporation during transpiration if the stomata were on the upper surface.

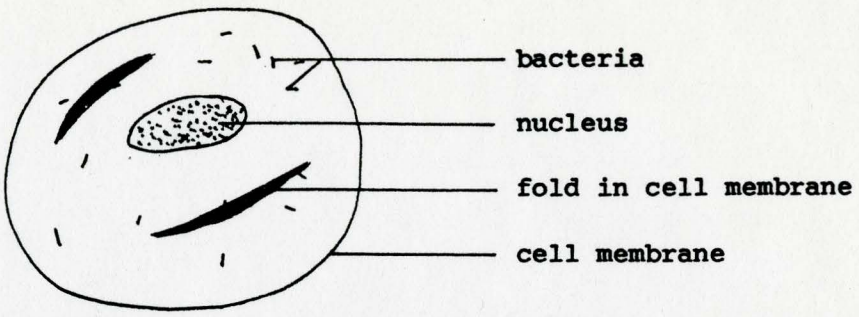
B. Animal Cells

I. Cheek Epithelium

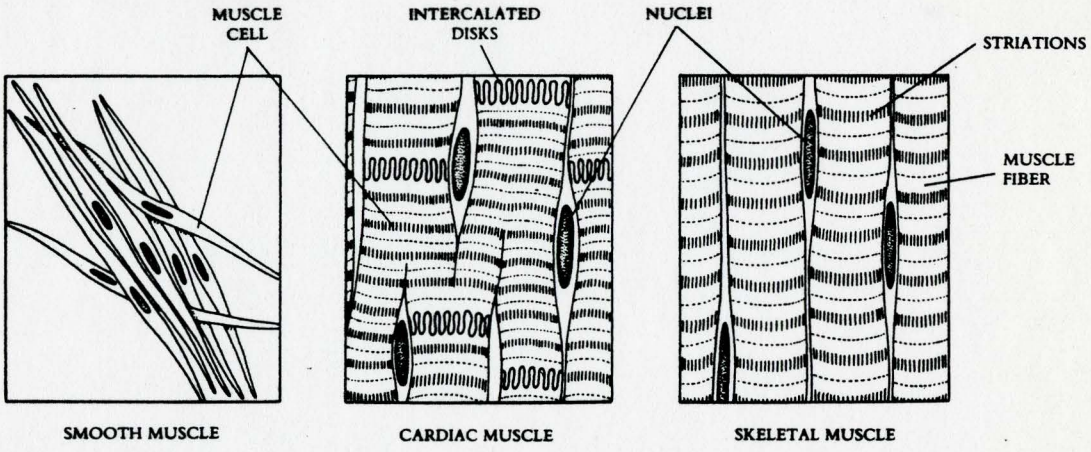
These cells are spherical or oval in shape.

Q. What structures, visible in plant cells, are lacking in cheek epithelium? - chloroplasts, vacuoles, cell wall

Q. Which was larger, plant or animal? In this case, the animal cell was larger. Generally, however, plant cells are larger.



II. Muscle Cells

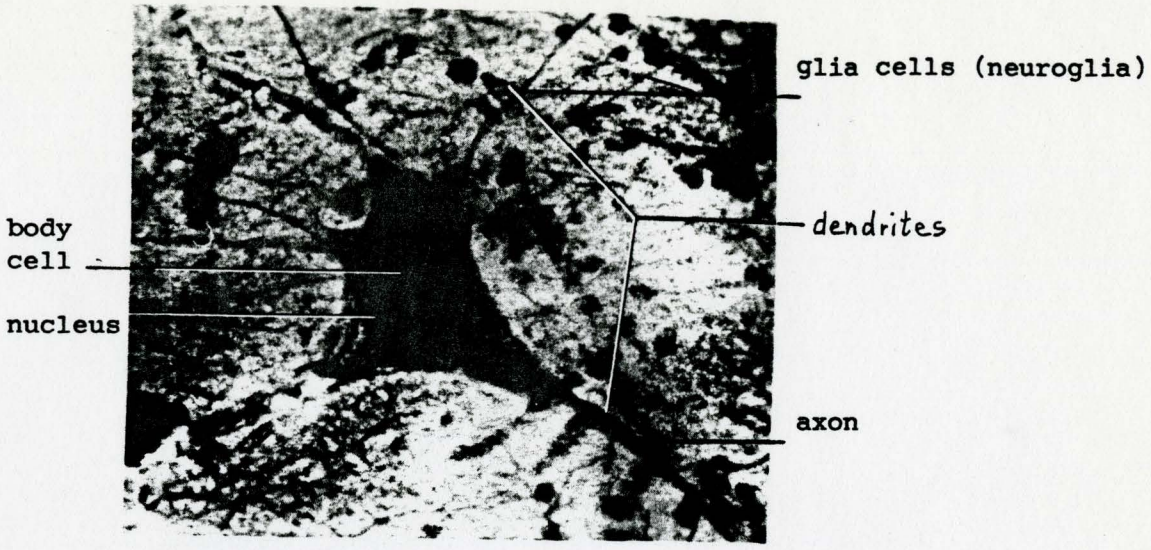


Three types of muscle

Students have a tendency to take too large a piece of meat to start with, then to tease it insufficiently and finally to use too much dye. Consequently, they see only a big, blue blob on the slide.

III. Nerve Cells

Students expect to see whole neurons on their slides. Explain to them that neurons are delicate and easily broken. If they can find a field of view which shows as much as this photograph, they will do well.



Nerve tissue with neurons

C. Cell Ultrastructure

The students are to make rough line diagrams of the two micrographs (plant and animal) showing the positions of the structures listed in the lab manual - not any of the other structures indicated in the pictures.

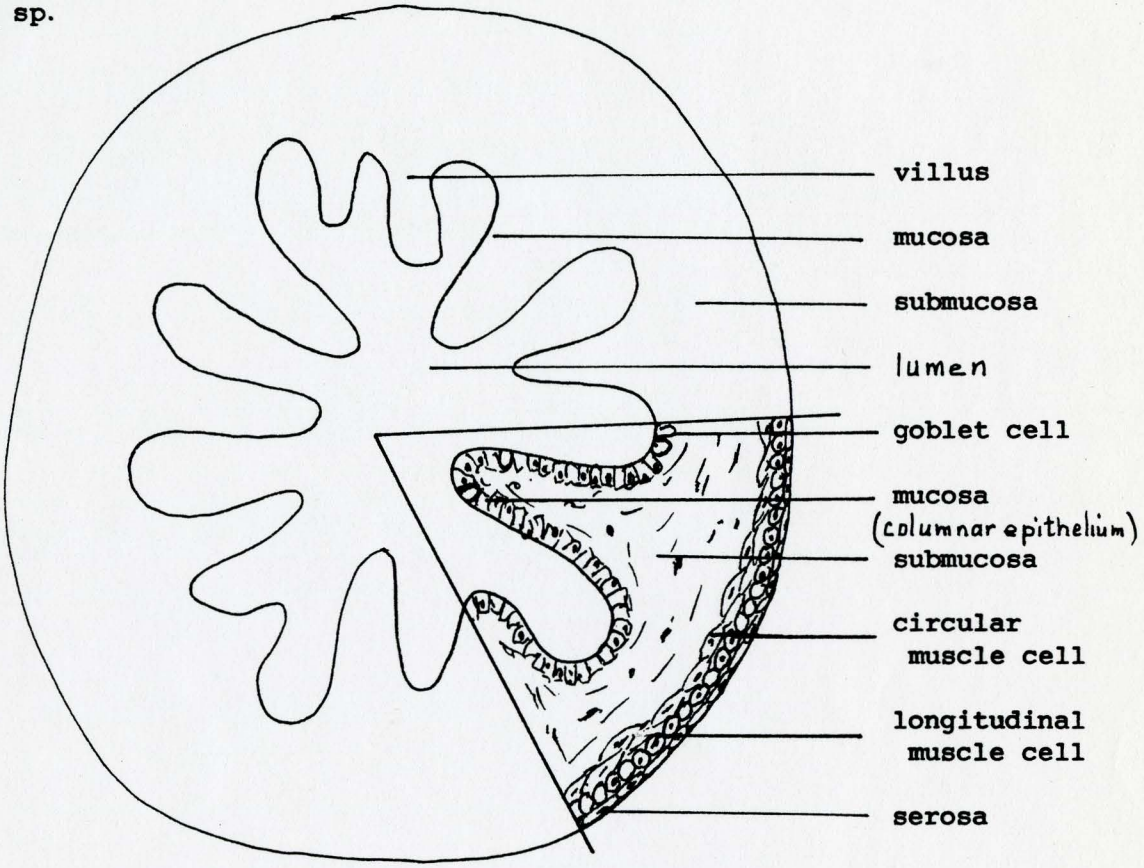
D. Multicellular Organization

Students usually have difficulty recognizing the cellular detail of the frog intestine cross-section and even more difficulty with the drawing.

Spend time in the pre-lab talk discussing the different layers of cells, including the function of each. Their cross-section diagram should take up almost one whole page, but the wedge to be done in cellular detail need only fill a few inches. Anything smaller than this would not allow for enough cellular detail.

Have the students look for mitotic figures in the columnar cells of the mucosa.

Kingdom Animalia
Phylum Chordata
Class Amphibia
Rana sp.



Scale 60:2

This drawing is a cross-section of the intestine of a frog, stained to show structure and cellular detail.

E. Animal Development

We expect to have at least one egg for each student, i.e., 4 different stages of development for each 4 students. To actually see the features described in the lab manual requires a good dissecting scope plus good technique. Be constantly vigilant to maximize the capabilities of both the microscopes and the dissecting scopes.

Students have trouble differentiating between actual embryonic cells and the oil globules which are more obvious. Warn them about this. You should each spend some time before the lab begins, studying these embryos in order to become familiar with the stages and to be able to answer questions.

The size of the fertilized egg is exactly the same size as the mature embryo just before hatching. No cellular growth has occurred - only cell cleavage.

There are 8 drawings in all. They may place 2 or 3 on a page, but diagrams must be large enough to show the features being studied.

Lab IV - Concepts in Genetics

A. Fruit Fly Genetics

I. Morphology and Life Cycle

Students work in groups of 4. Each group receives 5 vials labelled 1A, 1B, 2A, 2B, and one unlabelled vial containing medium and all stages of the life cycle - eggs, larvae, pupae, and adult flies. This vial is for examination purposes only. Students don't remove the plug.

II. A Monohybrid Cross

Each student is responsible for counting the flies in one of the other four vials. They are dead (having been in the freezer for 2 hours). Students separate males and females, as well as wild-type wing (Vg Vg) and vestigial (vg vg). Vials 1A and 1B contain reciprocal crosses, and resulting counts should be very similar. Put charts on the blackboard to collect student data and transfer final totals to the weekly sheet on the demonstrator's bench, e.g. 1A

Student Initials	Normal-Winged Females	Normal-Winged Males	Vestigial-Winged Females	Vestigial-Winged Males
Totals				

wild-type wings P Vg Vg x vg vg vestigial wings

F_1 Vg vg x Vg vg

F_2 Vg Vg Vg vg vg Vg vg vg

No sex differences genotypic ratio 1:2:1 phenotypic ratio 3:1

The reciprocal cross 1B will be identical.

Cross 2A

Have the students study the data emerging from these 2 crosses, 2A and 2B.

2A - In this case there should be a phenotypic ratio of 3:1 red eyes to white eyes, but observation shows that all females are red-eyed. Half the males are red-eyed and half are white-eyed. This should be an indication that the characteristic is controlled by sex-linked genes.

Crosses 2A and 2B are discussed in the text, 3rd ed., P . 611. Once they have observed the discrepancy among the data, have them work out the crosses using the text symbols.

wild type (red) eyes P ♀ RR x rY ♂ white eyes

F_1 ♀ Rr rY ♂ all red eyes

F_2 RR rR RY rY

red ♀ red ♀ red ♂ white ♂

2B - The reciprocal cross

P white-eyed ♀ rr x RY red-eyed ♂

F_1 red-eyed ♀ rR rY white-eyed ♂

F_2 rr Rr rY RY

white ♀ red ♀ white ♂ red ♂

phenotypic ratio 1:1 red, white, male, female

genotypic ratio 1:1:1:1

Questions, p. 61

1.

Cross 1

wild type	vestigial
-	-
-	-
-	-
Totals 3 :	1

Cross 2A

red ♀	white ♀	red ♂	white ♂
-	0	-	-
-	0	-	-
-	0	-	-
2 :	0 :	1 :	1

Cross 2B

red ♀	white ♀	red ♂	white ♂
-	-	-	-
-	-	-	-
-	-	-	-
1 :	1 :	1 :	1

2. Dominant allele - wild-type wings

   Recessive allele - vestigial wings

3. The dominant allele is red eyes, recessive white eyes.

4. This indicates that the gene is probably <u>autosomal</u> (not sex-linked).

5. All the F_1 males receive a dominant gene from their mother, via the X chromosome. In turn, all the F_2 females receive this gene and therefore have red eyes.

6. This indicates that this gene is <u>sex-linked</u>.

7. See the crosses, previous page.

8. Depends on data obtained.

B. <u>Hybrid Corn</u>

This section was cancelled.

C. A Dihybrid Cross in Tomatoes

The tomato plants in the flats in the lab room are the F_2 generation resulting from a cross between two different varieties.

	(dominant)		(recessive)
Tiny Tim	- green stem	and	smooth edged leaves
	GG ss	-	gametes Gs

	(recessive)		(dominant)
Veeroma	- purple stem	and	serrated leaves
	gg SS	-	gametes gS

F_1 - Gg Ss - gametes GS, Gs, gS, gs

	GS	Gs	gS	gs
GS	GG SS	GG Ss	Gg SS	Gg Ss
Gs	GG Ss	GG ss	Gg Ss	Gg ss
gS	gG SS	gG Ss	gg SS	gg sS
gs	gG sS	gG ss	gg Ss	gg ss

Possible F_2 phenotypes:

There should be 9 green stemmed serrated plants.

There should be 3 purple stemmed serrated plants.

There should be 3 green stemmed smooth plants.

There should be 1 purple stemmed smooth plant.

Points students should keep in mind if they had been preparing the crosses for this experiment are:

- Maturation time for a tomato plant is 60 days. Therefore, it would

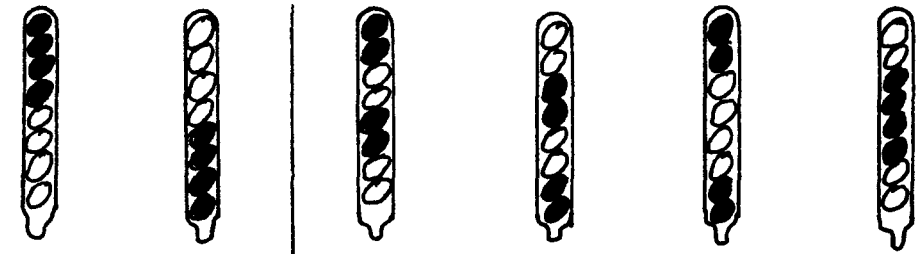
take 2 months to get the parent plants.

- It would be necessary to collect pollen from each variety to fertilize the pistil of the other.
- It would be important to prevent self-pollination of plants or uncontrolled cross-pollination by bees.
- The F_1 seeds would have to be obtained from ripe tomatoes and planted one month before the F_2 plants were needed. Total time required, therefore, is about five months.

D. Sexual Reproduction in Sordaria fimicola

1. Scoring of Different Spore Arrangements

Read and study the life cycle of Sordaria to understand what the perithecium is. We are interested only in perithecia containing both grey and black spores. The possible arrangements of these spores are:



Class Data	e.g.					
		65	77	44	46	54	62

2.

The distance of the gene locus for spore colour should work out to be 30-32 map units from the centromere.

Lab V - Photosynthesis

A. The Role of Light in Photosynthesis

I. Necessity of Light

Q. What does the iodine test show?

The iodine test shows areas of the leaves where starch has been synthesized and stored. Such areas will turn dark blue/black. Parts of a plant which do not contain chlorophyll and which do not receive light cannot photosynthesize or store starch. These areas do receive food (glucose) from other areas.

Q. Why would you expect a positive starch test?

You would expect a positive test for starch because plants polymerize the glucose produced during photosynthesis into starch for storage.

Q. Why do we get a differential positive test?

Only chlorophyll "a" is directly involved in the process of photosynthesis and therefore starch storage. Brightly coloured areas of leaves contain a variety of pigments capable of absorbing light and transferring it to chlorophyll "a". These areas will show some starch, but the white areas of leaves which contain no pigment will be starch negative.

Q. Is light necessary for photosynthesis?

Both light and chlorophyll are necessary for photosynthesis. The dark reaction can proceed in the

absence of light, but only until the products of the earlier photochemical stages (ATP and NADPre) are used up.

II. Effect of Light Intensity on Rate

Have one-third of the class do the experiment at the 60 cm distance, one-third at 40 and one-third at 10 in order to save time.

Q. Why was the plant in $NaHCO_3$ rather than water?

The plant was placed in $NaHCO_3$ rather than water because $NaHCO_3$ is a ready source of CO_2 for conversion into carbohydrate.

Q. As light intensity increases, does the photosynthetic rate also increase?

Yes. Since light intensity varies with the square root of the distance, we will not half the rate of photosynthesis by moving the light source to 2X the distance. It will be much less.

B. Hill Reaction

Students prepare the chloroplast suspension in groups of 4 but each pair runs their own experiment, #3, p. 81. Demonstrators are in charge of the boiling water bath for heat-killed chloroplasts.

DCIP is 2, 6, - dichlorophenolindophenol - blue when oxidized and colourless when reduced.

$$DCIP + 2e^- + 2H^+ \longrightarrow DCIP.H_2$$

The equation of the experimental reaction would be:

$$2\ DCIP + 2H_2O + light \xrightarrow{chloroplasts} 2\ DCIP.H_2 + O_2$$

The DCIP is being reduced and water is being oxidized.

a) buffer maintains the pH of the system. The reaction is enzyme controlled and therefore requires a stable pH.

b) chloroplast suspension - chloroplasts isolated from their cells, able to conduct photosynthesis.

c) DCIP - an artificial electron acceptor substituted for the acceptor molecule X - blue when oxidized and colourless when reduced.

d) ice water - retards the activity of the enzymes.

e) sucrose solution - an osmotic control to ensure an isosmotic environment for the chloroplasts.

Under normal circumstances the amount of photosynthesis by plants is much greater than their respiration but when light intensity is low, the two can be nearly equivalent. A terrarium is an example of a situation in which the two processes may be equally balanced if the light intensity is such that plants maintain themselves but do not grow. This is said to be the compensation point.

Lab VI - Unicellular and Colonial Organisms

A. Monera

I. Cyanophyta - the blue-green algae

a) Anabaena is a filamentous form. Individual cells are strung together in long chains held together by a transparent gelatinous sheath. The cells are green in colour, each containing a nucleus-like structure, but no other distinct internal structures. There may be heterocysts in the filaments. These are very primitive cells due to their lack of distinct internal structures.

b) Gloeocapsa is seen as single, double or clusters of cells, each surrounded by a gelatinous sheath. Nucleus-like structures are the only visible internal structures.

II. Schizomycetes - the bacteria

Three distinct shapes may be seen on bacterial slides:

cocci - small round clusters of 2, 4, or more cells

bacilli - rod-shaped structures frequently strung together to form long strands

spirilli - helically-coiled cells

Clear areas are observed around the antibiotic with Gram negative and Gram positive cultures. The diameters of these discs vary with the sensitivity of the bacterium to each of the discs.

Tetracycline - interferes with protein synthesis and enzyme action

Streptomycin - inhibits formation of cell membranes

Penicillin - acts on cell wall formation (see text)

Erythromycin - interferes with protein synthesis

B. Animal-Like Protista

I. Sarcodina

Cytoplasm within the body of the amoeba streams in one direction carrying the nucleus, contractile vacuole and cellular inclusions with it. The direction of flow may change.

II. Sporozoa

III. Ciliata

Paramecium caudatum contains two contractile vacuoles. The one at the anterior end may be seen to burst approximately every 50 seconds. The one at the posterior end will burst approximately every 30 seconds. Rationale is that water is taken into the food vacuole as it is formed. Thus, more water is in the cytoplasm near the posterior vacuole than at the anterior end where the other vacuole is located.

IV. Mastigophora - demonstration

C. Plant-Like Protista

I. Diatoms - Diatomaceous earth is on the back bench

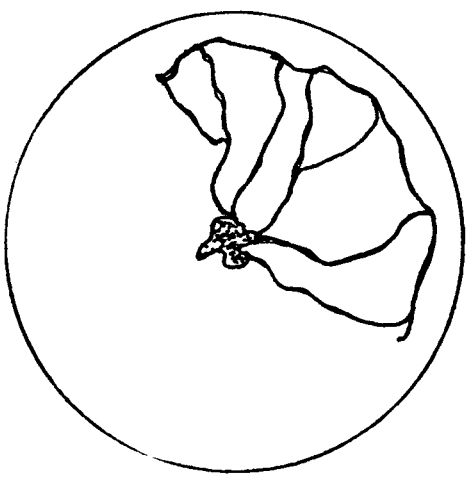
II. Dinoflagellates

D. Fungus-Like Protista

Physarum polycephalum is growing on an agar plate. It feeds on the oatmeal flake on the centre of the agar.

colour - yellow

growth pattern - it grows outwards from the oatmeal like the spokes of a wheel, travelling by cytoplasmic streaming.



Lab VII - Gas Exchange and Internal Transport

A. Gas Exchange

I. Tracheal System of Insects

There is no assignment for this section. The purpose of the demonstration is to show how insects solved the problem of getting O_2 from the exterior to the innermost tissues - an example of convergent evolution.

II. Necturus - an Amphibian System

Q. Analyze and write a paragraph to discuss the system(s) of respiration.

Q. How does the mud puppy react to the resultant stress?

Most amphibians, e.g., frogs, have three methods of respiration listed here in order of importance:

- across moist membranes lining the mouth and pharyx
- through the skin
- lungs

The volume of gases dissolved in a body of water equals the atmospheric pressure - 760 mm Hg at sea level, 20% of which will be O_2. When we bubble N_2 into the water, these gases are displaced and we end up with approx. 100% N_2 dissolved in the water. In this environment, Necturus can no longer obtain an adequate supply of O_2, causing it to rise up to the surface and gulp air into its primitive and normally little-used lung.

Necturus is a salamander and an amphibian. Amphibian larvae have external gills. At metamorphosis frogs and toads lose these external gills, but salamanders retain them throughout larval life as a very effective respiratory organ. The epidermis is thin and the capillaries close to the surface. Hence the red colour of Necturus' gills.

Salamanders generally are terrestrial animals which return to water in the spring to reproduce and lay their eggs. Only the larval form remains in water, however. Unlike other salamanders, Necturus has become entirely aquatic through the evolution of neoteny - the acquisition of sexual maturity at the juvenile stage of development. Metamorphosis in Necturus has been permanently suppressed and the external gills of the larva are retained throughout life.

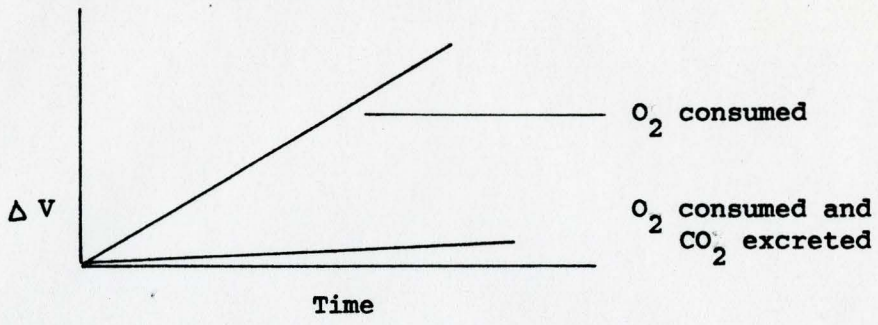
III. Measurement of Oxygen Consumption by the Mouse

During Part 1 of this experiment, the CO_2 excreted by the mouse is being absorbed by the soda lime. To measure CO2 produced alone, remove the soda lime from the system. The system now will measure O_2 consumed as well as CO_2 released. Therefore, the rate of CO_2 produced alone will be the rate of O_2 consumed minus the combination rate.

Prepare students for the fact that the change in volume for the combination will be small. They will understand better when you point out that when we are breathing, we

exhale almost the same volume of gas as we inhale.

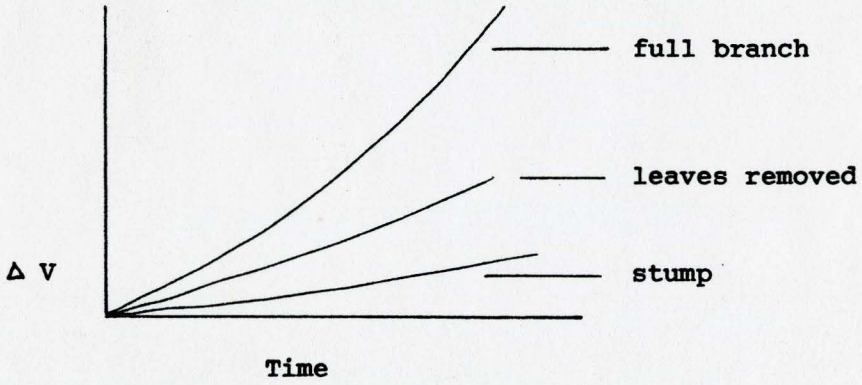
The R.Q. should probably be in the range of 0.7 to 0.8, reflecting a protein substrate.



B. Water Transport in Plants

Be sure students use distilled water in system to minimize air bubbles which would slow water movement in the tubes. There must be an air-tight fit between plant and tubing so they should choose tubing of the best fit.

The branch should have as many leaves as possible for maximum transpiration. If exposed to air longer than a minute, the cut stem will seal itself, reducing transpiration. Therefore, it should be freshly cut before use.



Data must be plotted cumulatively.

In lectures, Dr. Wood has thoroughly covered the leaf as an organ of gas exchange - cutin on top and bottom layers, photosynthetic layer, large internal surface area, relative humidity of internal air = 100%, function of stomata, etc. Other roles of leaf are photosynthesis and respiration.

Q. Explain how plants have adapted to meet the four basic needs of respiratory surfaces.

1. adequate size - the spongy mesophyll inside leaves has many large intercellular spaces. See p. 239.
2. transport - the air space system is open to the exterior air via the many stomata. Air can diffuse into innermost spaces easily.
3. protection - respiratory surfaces are inside the leaf itself. Pits are deep and often protected by many hairs.
4. moisture - incoming gases only go into solution upon reaching the moist cell membranes. Stomata often close during the heat of the day and open during cool evenings, preventing loss of moisture by evaporation. Stomata are on the under surfaces of leaves away from the sun.

Lab VIII - Regulation of Body Fluids

The film on Kidney Function was produced for medical students at MUMC. It is 40 minutes long and includes Histology, ultrastructure and kidney malfunction as well as dissection techniques.

I. Invertebrate Water Balance

Students are using very delicate and expensive microbalances, so take time to thoroughly explain their use. Keep a watchful eye to be sure they are not being abused.

Put a chart on the board to collect class data for this experiment.

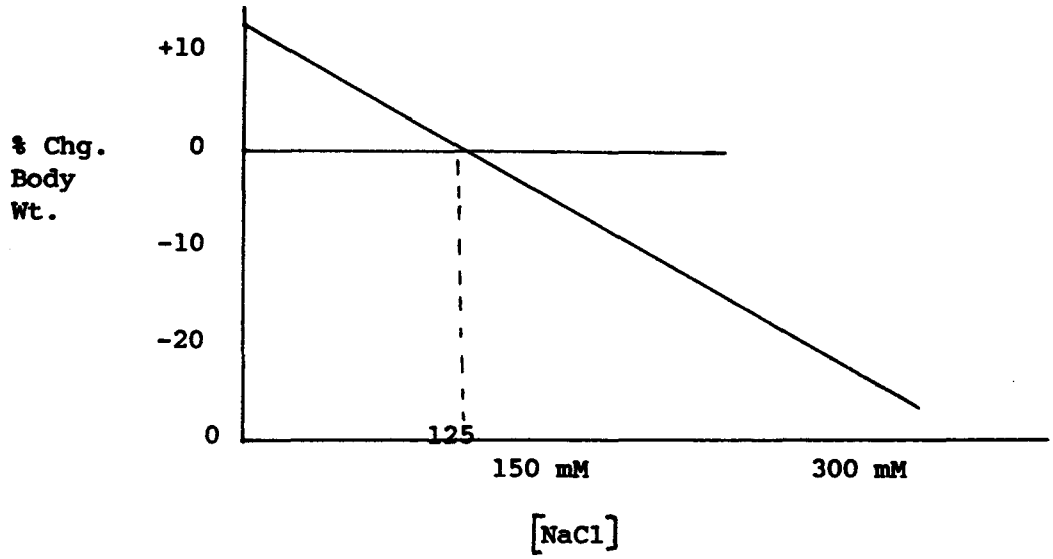
Dist. H_2O			150 mM NaCl			300 mM NaCl		
Before Wt.	After Wt.	% Chg	Before	After	% Chg.	Before	After	% Chg.

Have them calculate each change as a percentage of original weight. Then average these figures.

To determine the osmotic pressure of the worm's body fluids, the students should construct a graph, plotting % change in body weight against the concentrations of the three bathing fluids. By dropping a vertical from the spot where the graph intersects the 0% change line, they can determine the

salt concentration which would be isosmotic with worm body fluids.

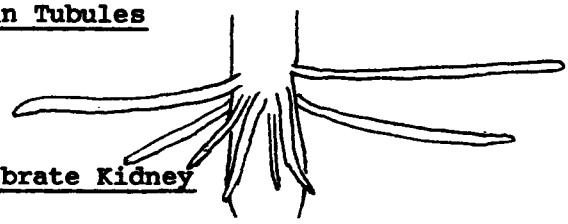
They should find the osmotic pressure of the worm's body fluids to be in the area of 125 mM NaCl.



1. In distilled water, body weight should increase approx. 2%.
2. In 150 mM NaCl, body weight should decrease approx. 10%.
3. In 300 mM NaCl, body weight should decrease approx. 24%.

Worms in the third treatment will probably be dead or near dead after 1 hour.

II. Malpighian Tubules



III. The Vertebrate Kidney

Have the students look at the injected kidneys and the demonstration slides of the rat kidney first, to give them a

better idea of what to look for in their own fresh material.

Stress that they should make their own section as thin as possible, and to look at the fresh-cut side of it. Glomeruli are red circular areas of tangled capillaries, readily visible under the dissecting scope. The tubules are convoluted and the loops of Henley with accompanying blood vessels look like red and white striations in the medulla. The collecting tubules are larger clear tubes, fewer in number. Of course, it will not be possible to see any complete nephrons.

Path of Urine - pp. 324, 6 and 7

- blood in the renal artery enters the kidney at the hilum;
- artery breaks up into arterioles in medulla of kidney;
- breaks up into many smaller arterioles in cortex;
- arterioles enter Bowman's capsule and break up into capillaries;
- blood pressure forces small molecules (all except formed elements and plasma proteins) through pores of capillaries into lumen of capsule - 170 ℓ/day;
- this filtrate has essentially the same concentration of dissolved substances as blood plasma;
- filtrate moves through the proximal convoluted tubule into descending loop of Henley where water diffuses out into surrounding tissues to be picked up by capillaries, and Na^+ diffuses into the loop;
- filtrate moves up ascending loop where Na^+ are being pumped out by active transport to create a conc. gradient in

surrounding fluid.

- filtrate moves through distal convoluted tubule and into collecting tubule.
- filtrate is carried in collecting tubule to collecting duct, i.e., from area of cortex through medulla to renal pelvis or through regions of increasing $[Na^+]$, causing water to passively move out of collecting tubule and duct until approx. 99% has been recovered and reabsorbed into capillaries;
- urine collects in the renal pelvis, moving out through ureter to be stored in the bladder;
- glucose, amino acids and salts are also reabsorbed from tubules and returned to the blood by active transport.
- some chemicals or toxic substances may also be removed from blood by the tubules and deposited in the urine.

Lab IX - Plant Hormones

This is a short lab. The students set up the plants in the lab room to be transferred to the greenhouse. Therefore, all flats must be well-marked with names, rooms and day. The students should return to the labs to make observations in two weeks. The reports are due two weeks plus one day from the original lab day.

Tell the students to make their observations on the grasshoppers any week day between 9:00 A.M. and 5:00 P.M.

A. Auxins

I. Phototropisms

The seedlings placed under direct overhead light should continue growing straight up. Those in front of the side-directed light should show evidence of turning towards the light within the lab period, i.e., 2 hours.

II. Geotropisms

After two weeks, all four corn seeds should show similar growth patterns regardless of their original orientation, i.e., the epicotyl (shoot) will be growing up and the hypocotyl (root) will be growing down.

III. Apical Dominance

After two weeks:

a) the control plant will be growing normally;

b) two new shoots should be sprouting upwards from the two

topmost lateral buds;

c) should look the same as it did when the terminal bud was removed;

d) two new shoots should be sprouting upwards from the two topmost lateral buds.

Lanolin functions as a vehicle to carry the IAA to the cut surface of Plant C. Lateral bud growth in Plant D proves that IAA and not lanolin had been responsible for lateral bud inhibition in Plant C.

B. Gibberellins

Students should measure the stem length of both normal and both dwarf pea seedlings. In two weeks the dwarf plants given the gibberellic acid treatment should be comparable in length to the normal plants. Those pea seedlings not given gibberellic acid treatment will maintain the same growth differential with normal plants observed at the beginning of the experiment.

Lab X - Animal Behaviour

Divide classes into 3 groups to stagger the tests:

1. Fish
2. Pillbugs - effect of humidity
3. Pillbugs - turn correcting

Students should work in groups of 4, taking turns at each of the trials.

Since some will not have pre-read the instructions in the manual, TA's should read aloud the "Dos" and "Don'ts" listed on p. 123 along with other instructions.

A. Schooling Behaviour of Fish

I. Effect of Visual Markings on Schooling Behaviour

The danios should prove to be polarized schooling fish while the goldfish are non-schoolers.

II. Effect of Group Size on Behaviour

We would not expect goldfish to be more attracted to large numbers of other goldfish. On the other hand, danios should show a preference for the jar containing the larger number of similar fish.

B. Agonistic Behaviour in Siamese Fighting Fish

This experiment should trigger student behaviour typical of early spectators at the colosseum or at the gaming tables of Vegas. You might take notes if you wish.

APPENDIX D

McMASTER UNIVERSITY
Department of Biology
1280 Main Street West, Hamilton, Ontario L8S 4K1
Telephone (416) 525-9140

D1

September 1986

We are presently conducting research, which is in support of a Master's thesis in Education. We want to know how effective the laboratory component of the Biology 1H6 (or 1H3) course in Human Physiology has been since its inception in 1980.

Will you please help us by answering the attached questionnaire about the labs. To refresh your memory, the topics covered over the year were:

I Diffusion of ions through agar in petri plates.

II Osmosis - the movement of water molecules across artificial membranes i.e. dialysis tubing.

III Salivary Amylase - the enzyme contained in saliva which acts in the digestion of starch.

IV Neurophysiology - reaction time boxes, colour vision and taste tests.

V The Blood - separation of blood compartments to determine hematocrit, hemoglobin concentration and blood type.

VI Cardiovascular Function - the ECG as a tool to evaluate heart function. The stethoscope for heart sounds and blood pressure.

VII Respiratory Gas Exchange by the Mouse - calculation of R.Q.

VIII Exercise and Physical Fitness - the Schneider and Pulmonary Fitness tests.

IX Nutrition and body energy balance.

X Kidney Function - the ability of the kidney to handle sugar, alcohol or salt - also clinical urine tests.

Please return the questionnaire with your comments to the Physical Education Office, Room 220A by the end of September if possible. We thank you very much for your assistance in this research.

D. Gordon McDonald
Course Chairman

Margaret A. Service
Instructional Assistant & Author

A CENTURY OF DISCOVERY UN SIÈCLE DE DÉCOUVERTES

QUESTIONNAIRE

Please circle the number which most closely corresponds with your opinion. If D2 you have anything more specific to add, include it at the end under comments.

1. Instructions in the lab manual were clear and understandable.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

2. The objectives and introductions at the beginning of each exercise were helpful.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

3. I was able to reason out the concepts behind the lab exercises by myself.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

4. I enjoyed working through the lab experiments.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

5. My demonstrator was accessible for questions and for discussion of theory during the labs.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

6. Laboratory skills learned in these labs have been useful to me since finishing this course.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

7. The preparation of lab reports helped me to learn the concepts involved.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

8. The writing of lab reports has improved my ability to write or communicate logically and precisely.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

9. The lab exercises aroused my interest in Biology and/or Science.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

10. Lab manual questions were helpful in the interpretation of data.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

11. Lab manual questions were frequently an intellectual challenge.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

12. My impressions of the usefulness of these labs have improved since taking the course.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

Please rate the labs according to your preference.

Top three
1.
2.
3.

Least useful

Comments: - either general, or specific to the above statements.

McMASTER UNIVERSITY
Department of Biology
1280 Main Street West, Hamilton, Ontario L8S 4K1
Telephone (416) 525-9140

D4

September 1986

We are presently conducting research, which is in support of a Master's thesis in Education. We want to know how effective the laboratory component of the Introductory Biology 1A6 course has been since its inception in 1983.

Will you please help us by answering the enclosed questionnaire about the labs. To refresh your memory, the topics covered over the year were:

I 1983 Identification of Unknown Specimens - evergreens and aquatic insects.
1984+ Unicellular and Colonial Organisms - blue green algae, Paramecium, ameba, diatoms etc.

II Chemical Basis of Biological Systems - lab tests to identify biological molecules (sugar, proteins, lipids) paper chromatography - extraction and identification of DNA.

III Cell Structure - Elodea cells, cheek epithelium, muscle and nerve cells, frog intestine X section, development in Medaka fish eggs.

IV Concepts in Genetics - fruit fly genetics, monohybrid and dihybrid crosses in corn - crossing over and segregation for spore colour in Sordaria - the Chi-Square test.

V Photosynthesis - testing leaves for areas of starch synthesis, effect of light intensity on photosynthetic rate - measurement of rate of reduction of DCIP using spectrophotometers.

VI 1983 Unicellular and Colonial Organisms - ameba, Paramecium diatoms etc.
1984+ Microbiology - Calculation of bacterial titre, environmental bacteria, dental hygiene and caries, antibiotic activity and bacterial staining.

VII Gas Exchange and Internal Transport - Calculation of RQ of the mouse, rate of transpiration by a green plant. Also Necturus the mud puppy.

VIII Regulation of Body Fluids - animal strategies for excretion, the measurement (using microbalances) of earthworm's ability to maintain homeostasis, structure of the kidney and nephron.

IX Plant Hormones - phototropisms, geotropisms, apical dominance and gibberellins in plant growth.

X Animal Behaviour - schooling and agonistic behaviour of fish 1983 - grasshoper behaviour, 1984+ woodlice.

Continued. . . . .

Please return the questionnaire with your comments to the Biology Club Office, Room 329 by the end of September if possible. We thank you very much for your assistance in this research.

S.F.H. Threlkeld
Course Co-Chairman

C.M. Wood
Course Co-Chairman

Margaret A. Service
Instructional Assistant and Author

QUESTIONNAIRE

Please circle the number which most closely corresponds with your opinion. If you have anything more specific to add, include it at the end under comments. D6

1. Instructions in the lab manual were clear and understandable.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

2. The objectives and introductions at the beginning of each exercise were helpful.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

3. I was able to reason out the concepts behind the lab exercises by myself.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

4. I enjoyed working through the lab experiments.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

5. My demonstrator was accessible for questions and for discussion of theory during the labs.

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strongly agree	agree	neutral	disagree	strongly disagree

6. Laboratory skills learned in these labs have been useful to me since finishing this course.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

7. The preparation of lab reports helped me to learn the concepts involved.

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strongly agree	agree	neutral	disagree	strongly disagree

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strongly agree	agree	neutral	disagree	strongly disagree

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strongly agree	agree	neutral	disagree	strongly disagree

10. Lab manual questions were helpful in the interpretation of data.

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strongly agree	agree	neutral	disagree	strongly disagree

11. Lab manual questions were frequently an intellectual challenge.

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strongly agree	agree	neutral	disagree	strongly disagree

12. My impressions of the usefulness of these labs have improved since taking the course.

1	2	3	4	5
strongly agree	agree	neutral	disagree	strongly disagree

Please rate the labs according to your preference.

	1.
Top three	2.
	3.
Least useful	

Comments: - either general, or specific to the above statements.