DOCUMENT RESUME

ED 114 296 95 SE 019 977

AUTHOR Goolsby, Charles M.; Obasun, Dan A.
TITLE Teacher's Guide to Laboratory Activities for Biology.
INSTITUTION Institute for Services to Education, Inc., Washington, D.C.
SPONS AGENCY National Inst. of Education (DHEW), Washington, D.C.; National Science Foundation, Washington, D.C.; Office of Education (DHEW), Washington, D.C. Bureau of Research.
BUREAU NO BR-7-0867
PUB DATE 70
CONTRACT OEC-0-8-070867-0001
NOTE 165p.; Appendix material from ED 084 936. Related documents are SE 019 975 and 976

EDRS PRICE MF-$0.76 HC-$8.24 Plus Postage
DESCRIPTORS *Biology; *College Science; Higher Education; Instructional Materials; Laboratory Experiments; *Laboratory Manuals; *Science Activities; Science Education; *Teaching Guides; Undergraduate Study
IDENTIFIERS National Science Foundation; *Thirteen College Curriculum Program

ABSTRACT

This guide is prepared for teachers who have students using the laboratory manual, "Laboratory Activities for Biology." The publication contains some introductory remarks for teachers, lists of materials and equipment, methods of making solutions and other preparations, suggestions for introductory discussions with the class, and procedures for doing the various exercises. The second section of this work is devoted to a guide to the individual student experiments. (Author/CP)

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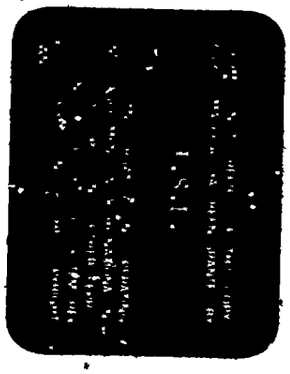

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The preparation of this manual was supported in part by the National Science Foundation and the United States Office of Education.

INSTITUTE FOR SERVICES TO EDUCATION, INC.

TEACHER'S GUIDE TO LABORATORY ACTIVITIES FOR BIOLOGY

The Thirteen Colleges Curriculum Program

The Five College Consortium

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1970

ABOUT THE INSTITUTE FOR SERVICES TO EDUCATION

The Institute for Services to Education was incorporated as a non-profit organization in 1965 and received a basic grant from the Carnegie Corporation of New York. The organization is founded on the principle that education today requires a fresh examination of what is worth teaching and how to teach it. ISE undertakes a variety of educational tasks, working cooperatively with other educational institutions, under grants from government agencies and private foundations. ISE is a catalyst for change. It does not just produce educational materials or techniques that are innovative; it develops, in cooperation with teachers and administrators, procedures for effective installation of successful materials and techniques in the colleges.

ISE is headed by Dr. Elias Blake, Jr., a former teacher and is staffed by college teachers with experience in working with disadvantaged youth and Black youth in educational settings both in predominantly Black and predominantly white colleges and schools.

ISE's Board of Directors consists of persons in the higher education system with histories of involvement in curriculum change. The Board members are:

Herman Branson	President, Lincoln University
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Donald Brown	The Center for Research on Learning and Teaching, University of Michigan
Arthur P. Davis	Graduate Professor, English, Howard University
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Stephen Wright	Consultant to President of CEEB
Jerrold Zacharias	Professor of Physics, Massachusetts Institute of Technology

ABOUT THE THIRTEEN COLLEGE CURRICULUM PROGRAM

From 1967 to the present, ISE has been working cooperatively with the Thirteen-College Consortium in developing the Thirteen-College Curriculum Program. The Thirteen-College Curriculum Program is an educational experiment that includes developing new curricular materials for the entire freshmen year of college in the areas of English, mathematics, social science, physical science, and biology and two sophomore year courses, humanities and philosophy. The program is designed to reduce the attrition rate of entering freshmen through well thought-out, new curricular materials, new teaching styles, and new faculty arrangements for instruction. In addition, the program seeks to alter the educational pattern of the institutions involved by changing blocks of courses rather than by developing single courses. In this sense, the Thirteen-College Curriculum Program is viewed not only as a curriculum program with a consistent set of academic goals for the separate courses, but also as a vehicle to produce new and pertinent educational changes within the consortium institutions. At ISE, the program is directed by Dr. Frederick S. Humphries, Vice-President. The curricular developments for the specific courses of the program are provided by the following persons:

Course	ISE STAFF
English	Miss Carolyn Fitchett
	Miss Joan Murrell
Social Science	Dr. George King
Mathematics	Dr. Beauregard Stubblefield
	Mr. Bernis Barnes
Physical Science	Dr. Leroy Colquitt
Biology	Dr. Charles Goolsby
	Mr. Daniel Obasun
Humanities	Mr. Clifford Johnson
Philosophy	Mr. Conrad Snowden
	Miss Valerie Simms

The curriculum staff is assisted in the generation of new educational ideas and teaching strategies by teachers in the participating colleges and outside consultants. Each of the curriculum areas has its own advisory committee, with members drawn from distinguished scholars in the field but outside the program.

The number of colleges participating in the program has grown from the original thirteen of 1967 to nineteen in 1970. The original thirteen colleges are:

Alabama A and M College	Huntsville, Alabama
Bennett College	Greensboro, North Carolina
Bishop College	Dallas, Texas
Clark College	Atlanta, Georgia
Florida A and M University	Tallahassee, Florida
Jackson State College	Jackson, Mississippi
Lincoln University	Lincoln University, Pennsylvania

Norfolk State College	Norfolk, Virginia
North Carolina A and T State University	Greensboro, North Carolina
Southern University	Baton Rouge, Louisiana
Talladega College	Talladega, Alabama
Tennessee State University	Nashville, Tennessee
Voorhees College	Denmark, South Carolina

A fourteenth college joined this consortium in 1968, although it is still called the Thirteen-College Consortium. The fourteenth member is

Mary Holmes Junior College	West Point, Mississippi

In 1970, five more colleges joined the effort although linking up as a separate consortium. The members of the Five-College Consortium are:

Elizabeth City State University	Elizabeth City, North Carolina
Langston University	Langston, Oklahoma
Southern University at Shreveport	Shreveport, Louisiana
Saint Augustine's College	Raleigh, North Carolina
Texas Southern University	Houston, Texas

PARTICIPATING TEACHERS

The biology teachers who have particpated in this development through the summer of 1970 are:

ALABAMA A & M COLLEGE: Jimmie L. Cal, M.Ed. (1967-1970), Rather Brown, M.S. (1970-), George Grayson, M.S. (1970S)

BENNETT COLLEGE: Perry V. Mack, M.S. (1967-)

BISHOP COLLEGE: Willie M. Clark, M.S. (1967-1970), Mrs. Versia Lindsay Lacy, M.S. (1970-), In the Modified-ISE course: Wasi M. Siddiqui, Ph.D. (1969-), Herbert Alexander, M.S. (1969-), Ehsan A. Syed, M.S., M.S. (1969-), Mrs. Rose W. Burke, M.A. (1969-1970).

CLARK COLLEGE: Martin J. Carey, M.S. (1967-), F. Rusinko, M.S. (1970S)

FLORIDA A & M UNIVERSITY: Louis Stallworth, M.S. (1967-1969), Purcell B. Bowser, M.S. (1969-1970), Mrs. Irene R. Clark, M.Ed. (1970-)

JACKSON STATE COLLEGE: Robert J. Anthony, M.S., M.Ed. (1967-), Mrs. B. Henderson, M.S. (1970S)

LINCOLN UNIVERSITY: Harold C. Banks, M.S. (1967-1968, 1969-)

NORFOLK STATE COLLEGE: Ruth E. Churwin, M.A. (1967-1969), Mrs. Irene R. Clark, M.Ed. (1969-1970), Mrs. Robin M. Griffith, M.A., M.A.T. (1970-)

NORTH CAROLINA A & T STATE UNIVERSITY: Mrs. Elizabeth D. Clark, M.S. (1967-)

SOUTHERN UNIVERSITY, Baton Rouge, Louisiana: Robert H. Cobbins, M.S. (1968-)

TALLADEGA COLLEGE: Muriel E. Taylor, M.A. (1967-), Mrs. Mae T. Groves, M.S. (1970-)

TENNESSEE STATE UNIVERSITY: Mrs. Alice C. Smith, M.S. (1967-)

Beginning with the summer of 1970 teachers from six additional institutions are participating in the curriculum development program. They are:

ELIZABETH CITY STATE UNIVERSITY: Thaddeus V. Beasley, M.S.

FAYETTEVILLE STATE UNIVERSITY: Mrs. Valerie L. Fleming, M.S.

LANGSTON UNIVERSITY: Harold W. Toliver, M.S.

SAINT AUGUSTINE'S COLLEGE: Chandra P. Misra, Ph.D.

SOUTHERN UNIVERSITY, Shreveport, Louisiana: Mrs. Rebecca B. Anderson, M.S.T.

TEXAS SOUTHERN UNIVERSITY: Charles H. Bennett, M.S.

UNITS OF THE ISE TEACHER'S GUIDE TO CLASSROOM DISCUSSIONS FOR BIOLOGY

The CRG Biology Teacher's Curriculum Guide for the TCCP was written during the summer of 1969 and field-tested by participating teachers during the 1969-1970 school year. At the 1970 summer conference these units were revised and extended in rough form and during the school year 1970-1971 they were re-edited by Charles M. Goolsby and Dan A. Obasun as the ISE TEACHER'S GUIDE TO CLASSROOM DISCUSSIONS FOR BIOLOGY. The unit titles and writers are as follows:

Unit 1 -- The Nature of Science By Robert J. Anthony, Harold E. Banks, Willie M. Clark, George Grayson, Charles H. Bennett and Versia L. Lacy Foreword by Lewis L. White

Unit 2 -- Evolution By Martin J. Carey, Irene R. Clark, Wasi M. Siddiqui, Mae T. Groves, F. Rusinko and Ehsan Syed Foreword by Samuel Moyer

Unit 3 -- The Cell By Alice C. Smith, Wasi M. Siddiqui, Irene R. Clark, Martin J. Carey, T. Vernon Beasley, Charles H. Bennett Foreword by Reid Jackson

Unit 4 -- Reproduction, Growth and Development By Elizabeth D. Clark, Muriel E. Taylor, Jimmie L. Cal, T. Vernon Beasley, Valerie L. Fleming, Robin M. Griffith and Chandra P. Misra Foreword by Hilton A. Salhanick

Unit 5 -- Genetics By Robert H. Cobbins, Alice C. Smith, Purcell B. Bowser, Perry V. Mack, Rebecca B. Anderson, B. Henderson, and Harold W. Toliver Foreword by Dan A. Obasun

Unit 6 -- Metabolism and Regulatory Mechanisms By Muriel E. Taylor, Elizabeth D. Clark, Purcell B. Bowser, Valerie L. Fleming, B. Henderson, Versia L. Lacy and F. Rusinko Foreword by C. M. Goolsby

Unit 7 -- The Variety of Living Things By Harold E. Banks, Robert H. Cobbins, Perry V. Mack, Rebecca B. Anderson, Chandra P. Misra and Harold W. Toliver Foreword by Nathan W. Riser

Unit 8 -- Ecology By Robert J. Anthony, Willie M. Clark, Jimmie L. Cal, Robin M. Griffith, Mae T. Groves, and Ehsan Syed Foreword by Ernest Ruber

DEVELOPMENT OF THE BIOLOGY PROGRAM IN THE THIRTEEN COLLEGE CURRICULUM PROGRAM

We hope to answer many questions through the essays that make up the first section of this Teacher's Guide, and perhaps raise some others by implication which we may not answer. Some questions which the reader may ask after having read these first few pages might be what were the events and experiments which brought these people and this program along so far, and what activities form the basis of the present course and of this Guide?

In the summer of 1967, the Institute for Services to Education (ISE) and the institutions consorting to undertake the Thirteen College Curriculum Program (TCCP) set out to design courses for college freshmen which would take into account the interests of students, which would be stimulating to them, modern in method and content, and of a distinctive pattern. Courses were planned in English, Mathematics, Social Science, and Natural Science. It was to be standard, among the courses, that the enrollment would be small, about 25 students per class, to permit more individualization of instruction. Lectures were largely replaced by discussions in an effort to involve the student, as much as possible, in the teaching-learning processes. Inductive teaching methods were preferred. The use of audio and/or visual aids in teaching the discussion sessions do not give students the kind of direct experiences which biological materials needed to transfer non-verbal images which define a great many biological terms. Therefore, laboratory work was considered important. The prospect of using these methods brought teachers to that first summer conference, filled with excitement and with great expectations.

The plan in the summer of 1967 was to design a natural science course which included physics, chemistry and biology. Several units of study were outlined around such areas as the physics of light and photosyntehsis, the mechanics of levers and animal movement, and so forth. The physics was to be taught by teachers with backgrounds in physics or chemistry and the biology was to be taught by biologists. In general, this was a large task for which there was insufficient planning and training. The physicists and biologists needed more time to express their points of view and the biophysics content was not worked-out sufficiently for the freshman level. In line with the experimental nature of the program at that time, a commitment to develop toward the best courses, and other considerations, the integrated science approach was abandoned at midyear and separate courses in Physical Science and in Biology were established.

The ISE staff and the biology teachers of the TCCP then began to explore the usefulness of other materials as models, such as thosedeveloped by the Biological Sciences Curriculum Study (BSCS) for secondary schools. These materials were up-to-date and lent themselves well to discovery approaches in the laboratory. However, since they were prepared for secondary schools, many of their considerations were too superficial for the college freshman level. Although there were many good ideas in the BSCS Blue Version, it was already well-known to many students, even to some who were not taking biology, that it was a text they used in high school.

During the spring there were not particular designated BSCS materials assigned to teachersfor study, use, and evaluation, and a more unified direction was needed in order to develop the effort into a program. The ISE organized an Advisory Committee of teachers, staff members and consultants to formulate a plan for the upcoming 1968 Summer Conference, and consequently for the following school year. It was the Advisory Committee plan, approved by a meeting of teachers, that during the summer teachers would attend seminars on the use of

original scientific reports as the basis of classroom discussion, and they would try to rewrite the experiments most popular with their students at a level suitable for college freshmen. The seminars were organized by the Biology Department staff at Brandeis University. A group of pre-freshmen from several of the participating thirteen colleges formed a class for the demonstration of inductive teaching methods and they also worked in the laboratory as junior consultants.

Of the eleven biology teachers involved, three based the discussion part of their courses for the 1968-1969 school year entirely upon the use of original scientific papers. The others used papers in addition to a standard textbook.

The papers were short, and therefore not emotionally threatening to the student uncomitted to the avid study of science, they did not seem to "talk down" to students, and they were logical(the inductive reasoning was apparent and the conclusions reached were less mysterious than those in standard textbooks). Efforts to arrive at a group of core topics failed in this summer conference. During the 1968-1969 school year some 75 original papers were used and over 90 topics were discussed by the teachers with about a 25% coordination of effort. In the laboratory 70 exercises were used by the various teachers.

The case method inherent in the original papers approach to teaching had worked well during the summer when discussions were being led by scientists with considerable research experience. When teachers were on their own they found it easy enough to integrate the information concerning the various areas of biology and biochemistry contained in each report. They did not mind that they often found it necessary to define a large number of words and terms for students so that they could read the reports. However, they did not like it much when students or papers led them into topics with which they were not familiar. At the evaluation conference held in Atlanta, Georgia in March, 1969, it was apparent that the case approach being used would not be the final answer for a freshman-level course and so the issue of a group of unit topics was confronted. Teachers suggested titles which they felt would best contain the areas of information that they had explored during the two school years 1967-1969 and agreed to summarize their experiences under these unit topics during the ensuing summer conference.

Each teacher worked in two unit-writing groups which reported to the whole group of biology teachers, and included suggestions from the whole group in the units for teaching. The objective was to bring together a list of all of the resources that a teacher would need in order to teach a unit or whole subject for about three weeks. This included an outline, references for student and teachers, a progress inventory, objective and discussion questions, lists of audio and/or visual aids, and some appropriate laboratory exercises. The ISE staff considered it of great importance that the material should reflect as many as possible of the items which were of high interest to the students. Then teachers, working with their consultants, supplied enough continuity of material to make the whole "rational". This is an aspect of course development most often neglected by curriculum workers since they usually fail to include the interests of the students in their choice of materials.

At an evaluation conference held at Alexandria, Virginia in March, 1970, a review of reports submitted by teachers to the writing groups indicated that the materials of the ISE Biology Teacher's Curriculum Guide for the TCCP had worked out well, with all of the teachers reporting success with the materials dealing with discussions. However, some laboratory exercises had not been rated high in the reports. The plan, put forth at that time, for the summer conference

was to extend the unit outlines into sentence form, and to more clearly indicate what concepts and information limitations were intended for each topic. Student references were no longer to be taken from the standard freshman biology textbooks but were to be drawn from original papers and paper-bound books of short length. More examples of classroom incidents which showed how students reached enlightenment from the approaches in the units were to be included.

During the spring the ISE staff developed and student-tested an additional group of laboratory activities suitable for an introductory course. Some of these, and the exercises from the teaching units which had been rated highly and of college freshman level were assembled and rewritten as a laboratory manual for students--Laboratory Activities for Biology for the Thirteen College Curriculum Program. Because most of the experiments and exercises had been appended to the eight teaching units, there had been some similarity between those placed in different units. In the reorganization, these were grouped together. In all, 18 new exercises and 28 from the Teacher's Curriculum Guide were rewritten and grouped into the 42 exercises of the manual.

Despite problems, the development of the courses in the TCCP gained consideration from other institutions not in the program. Starting with the spring of 1970 a new Five College Consortium (FCC) was formed and its biology and other teachers attended the summer conference, which was held at Pine Manor Junior College, Chestnut Hill, Massachusetts during July and August. The objectives of the conference were to try to introduce the new teachers in the TCCP and in the FCC to the philosophies and practices that underlay the biology course, to familiarize them as much as possible in that length of time with the content of the units, and to have them participate with the more experienced program teachers in writing the proposed extensions of the units. This was a large order to orient new people into a group of teachers that had gone through the development of the program without much personnel change, and to acquaint the new teachers with out viewpoints and interests.

Tasks that were projected for the fall term of the 1970-1971 school year included the accumulation of more classroom experiences by teachers which showed how students arrived at the moments of enlightenment so that these could be included in the revised units. The job of re-editing the curriculum material fell to the ISE staff, since editing is their responsibility. Also, during the end of the summer conference it developed that there was a need for a teacher's guide to the use of the laboratory manual to support the flexible usage of the manual.

A STATEMENT OF PURPOSES

This Guide is prepared for teachers who have students using the manual Laboratory Activities for Biology, prepared and published by the Institute for Services to Education. The Guide contains some introductory remarks for teachers, lists of materials and equipment, methods of making solutions and other preparations, suggestions for introductory discussions with the class, and procedures for doing the various exercises. It also contains answers to discussion question, but does not give sample data for the experiments. The student manual is not the place for much of this information because the thrust of the teaching in these laboratory classes should be to let the student find out most of this information through his own efforts. We hope that the material has enough flexibility to allow the teacher to express his or her own creativity, yet structured enough to give the student a feeling that he is proceeding properly through the activities.

This Guide is prepared because biology teachers have varied backgrounds and some may have more information in some areas than in others. If this Guide can help reduce the time necessary to prepare for laboratory classes, we hope that the time gained will be devoted to teaching activities as such. The second section of this compilation is devoted to a guide to the experiments in the student manual, but the actual directions should be consulted when following this teacher's guide for any particular exercise.

Charles M. Woolsby

Dan A. Obasun

Washington, D. C.

December, 1970

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PART I -- VIEWPOINTS ON TEACHING THE ISE BIOLOGY LABORATORY

SOME WAYS OF USING THE STUDENT MANUAL "LABORATORY ACTIVITIES FOR BIOLOGY"

The student manual is a compilation of directions for doing class laboratory exercises. The number of ways then that the directions can be used is probably limited only by the creativity and imagination of the teachers using it.

The directions are constructed to give instructions to the student in easy steps so that he will gain some satisfaction each time he does a step correctly. In this way he will be rewarded many times as he proceeds through an exercise or experiment. Teacher approval for completing certain parts of the exercise constitutes a non-grade regard, but of course, the kind of evaluation (grade) given for the completed exercise is the standard reward usually received by students. In most instances, the student completing these exercises should have gained satisfactions far exceeding his usual emotional responses to a grade.

The directions are not written to explain the activities to the student (as a rule). Explanations may be required.

1) by acquiring of the teacher.
2) by the teacher drawing-out of the student the information for answers arrived at inductively, or
3) by the teacher directing the student to appropriate resource materials in the field, in the laboratory (books, specimens, models, etc.) or in the library or museum.

The teacher must decide which mode he wishes to use at the moment of questioning in order to bring out the fullest development of the student at that point. To say the least, a teacher who offers to give explanations before the question is asked resumes that the experiment is unnecessary for generating the kind of experiences that cause curiosities and questions to arise in the minds of the students. Teachers who answer every question directly, without referring students to resources get a lot of self-satisfaction out of displaying their knowledge but fail to stimulate the student to use the full extent of his investigative powers. Teachers, therefore, must evaluate the laboratory situations for each question and select the answering style which will further the objective of the activity and of the development of the student.

Many exercises and experiments are divided into parts (e.g., Part A, B, C, etc.). Where this is so the teacher may select one or more parts of the exercise to be done by the class. The teacher may announce that the class will start with a part, for example Part B, but when that is finished and checked by the teacher (or assistant) the student may proceed to the next part, e.g., Part A or D. Teachers may select parts of different exercises to be done at the same period and treat them as above. Sometimes students may be asked to proceed through the selected parts in a specific order, or sometimes each working group may proceed through the sequence in the order of its own choice. Whatever method is used, the teacher should require that the completed part have his or her approval before proceeding to work toward the next check point. This also serves as an opportunity to

reward the student with a non-grade. This approval should be given only to "passing" work. Point out the improvements needed if the work is not "passing".

The order in which exercises are done should take into consideration the prerequisites designated for many of the exercises. The first five exercises are introductory and are designed to help the student to learn techniques or to have him demonstrate to the teacher that he has the included knowledge or skill.

Different students or groups of students in the class may be assigned the same experiment, but with different materials. For examples see Exercise 7, where different groups titrate different buffers, or Exercise 14, where the oxygen uptake may be measured for such widely divergent organisms as mice, sprouting beans, fermenting yeast, three or four large cockroaches, or many other similar materials that may be available.

Another flexible arrangement is for different groups of students to carry out differnt procedures on the same material, as for example in Exercise 14, where different members of a team working on this exercise as a special project may perform certain specific tests to reduce variation in the technique that would be certain if different members of the team carried out the assays at different times.

Some exercises will lend themselves to unassembled presentation, that is, they may be done when the whole class is not assembled. Exercise 15 deals with models of chromosome patterns during mitosis and meiosis. The paper plates, models of the nuclear structures and of the chromosomes may be placed on work tables together with copies of the directions so that the student may carry out designated steps at each work station. This is sometimes called "programmed" laboratory procedure.

For greatest effectiveness, a laboratory assistant should be present in a programmed or unassembled laboratory. Sometimes they can be present only during certain hours, or perhaps they won't be available at all--the laboratory being left open for students to work alone. Where students work alone or at their own rates, they should be examined by the teacher or assistant to satisfy themselves that the students did the work that is being reported. Ability to pass such examination adds the reward of teacher approval, where it is given.

The Report Sheet is provided as a data-collecting device which insists that the student look for certain information. Many exercises contain questions to be answered. The teacher can feel free to alter the number or even ask different questions. At times an exercise may be more in the nature of an experiment. In some cases, and especially where they are done as special projects, the teacher may ask the reprts for experiments to be in regular scientific report (paper) form, with Introduction, Materials and Methods, Results, Discussion, Conclusions, and Reference sections. In such cases the report sheet can be used to record data and even be included in the report as the Results section.

THE LABORATORY AS A PLACE TO "FIND OUT" THINGS

We would like to recognize a seeming shortcoming, but one that can be overcome. Laboratory Activities for Biology contains directions for investigating several questions. How can this be reconciled with the ideal of the laboratory being not just a big visual aid to repeat and redemonstrate principles already discussed elsewhere, but as a place where students can "find out" things about biology by investigating questions arising out of their own curiosity? We have partly met this kind of question by trying to avoid telling the student what should or would happen in the exercise so that perhaps he will be a little curious about the outcome. The second approach to a positive solution is that there is a conscious effort made to have the student become aware of the pattern of the scientific method, not only in the class experiments, but also by encouraging the reading of scientific reports. After the introductory exercises are done to assure the teacher that the class has certain skills (how to weigh, measure, observe, sterilize, etc.), the class may devise a controlled experiment to test an hypothesis growing out of (for example) a classroom discussion. The teacher should give advice at this level (the freshman level) about the experimental design and procedure, about where or how best to get biological materials and supplies, and about the length and scope of the proposed project(s). The teacher should have enough flexibility in the laboratory schedule to enable him to suspend part of the projected list of class laboratory exercises to permit one or a few periods for a class-designed project related to the content of the course.

Certain exercises are disignated as special projects for two or more students where these coincide with student interest. These exercises are conceived as being carried out on an unassembled basis at the convenience of teacher and students. If they can be done in the class laboratory room, the activity will be more easily shared with the rest of the class. Sometimes a student may suggest a good experiment growing out of either discussion or laboratory observations. Teachers should offer the same kind of help indicated for class-designed experiments above. A note of caution, however. Sometimes students who seem to be barely passing have some of the more expansive ideas about achieving fame, fortune, and a better grade through the performance of a grandiose piece of work. The participation of students in special projects will probably be most profitably pursued if the student is doing "passing" work in the regular assignments of the class.

There is a complication involved here for the teacher in that in an introductory course, energy and precept must be used to convey a real feel for what is an experiment, for the evaluation of data, for the development of inductive reasoning about the results, and for showing the relationship of student results with those reported in the literature. However, the involvement of the students in a meaningful way with the pursuit of an inquiry, the lure of a discovery, and the satisfaction of solving a problem, is one of the best possible uses of course time.

BEHAVIORAL OBJECTIVES FOR STUDENTS AND GOALS FOR TEACHERS

All of the events, equipment, materials, people and terms used in the course helped to evolve certain goals to be set for students which are, in fact, behavioral objectives to be reached uring the course. These are given below as a group and not in the order of their importance.

1. Seeing biology, and science in general, as a process which not only solves problems, but which also creates new things.

2. Arriving at an attitude of objectivity through critical thinking and inductive reasoning about that which would be a scientific fact.

3. Having an appreciation for the laws of random events and how, through the proper philosophical readiness, scientists make their discoveries.

4. Having a general knowledge of the world of living things and man's relationship to its various levels.

5. Bettering the understanding of natural occurrences by the application of biological principles.

6. Developing observational skills through active participation in acientific experiments and exercises, and arriving at generalizations through inductive reasoning.

7. Being able to read the scientific literature in biology with a reasonable understanding of the basic scientific terminology.

8. Encouraging good study habits, budgeting of time for the course work, and high performance in the course growing out of good preparation.

Moreover, we developed goals for teachers which are oriented toward desirable teacher-student relationships in teh course.

1. Using inductive reasoning for teaching generalizations wherever possible.

2. Using inductive teaching methods as much as possible.

3. Invilving the student in the learning process as much as possible.

4. Helping students to learn by giving them advice, self-helps, and opportunities to learn about the world of living things through readings, discussions in class and during conferences, and through laboratory and field experiences, allowing the student some choices, yet maintaining some discipline without authoritarianism.

5. Developing an attitued of quiet conficence in their own preparations so that they do not display behavior in class which would indicate an anxiety about their information nor embarassment at not know every fact.

6. Being sensitive to topics in biology which are of interest to students so that classroom experinces will be made as meaningful in student's lives as possible and relevant to the goal of having some application in modern life situations.

7. Being able to enter creative states of mind so that current materials may be improved to bring out the most useful experiences to the student for understanding biological concepts.

8. Reading at least one basic science journal and one science teaching journal regularly so that they may keep abreast of current publications of interest to them personally and applicable to course content, and that some ideas about current teaching techniques may be appreciated in relation to the teaching program in which they are engaged.

9. Becoming skilled reporters of student reactions to teaching materials and methods and to subsequently write these reactions in reports and publications.

10. Learning to make their presentations in such a way as to arouse the curiosity of students and motivate them to further study and work within and outside of the classroom.

11. Extending the attitude of the scientist, that any good or worthwhile discovery not become the secret property of its discoverer but that it be freely shared with colleages inside of and outside of the consortium.

THE SCIENTIFIC METHOD AND MOTIVATION

While the goals above can be reached individually, they should be worked toward collectively since they have become collectively the crux of the uses and further development of this material. Furthermore, they must be viewed as a means for developing the scientific method of problem-solving and for sustaining student (and perhaps teacher) interest and motivation. The version of the scientific methodto which the Biology Section at the ISE subscribes is as follows:

1. Observating in the field or laboratory an event that stirs the curiosity.

2. Formulating of an hypothesis about the observed pehnomenon.

3. Designing a controlled experiment to test the hyposthsis.

4. Collecting and analyzing the data.

5. Reaching conclusions by inductive reasoning.

6. Accepting or rejecting the starting hypothesis.

7. Formulating of continuation hypotheses.

The scientific method is a method of dealing logically and systematically with natural-type activities and of demonstrating the correctness or incorrectness of ideas, assumptions, guesses, and the like, which are of scientific interest First, however, there must be an idea, a question or an assumption about something natural (as distinguished from the supernatural). Since some concepts, ideas and questions have been demonstrated many times to be true or correct (or untrue and incorrect, as the case may be) it is not really worthwhile to repeat the proof unless the idea is one of great importance in the structure of the organized-knowledge part of science.

Inquiry has recently become a popular word used in discussions on the teaching of science. What it means, basically, is to apply the scientific method when investigating the correctness or trueness of concepts, ideas, and assumptions. That is, the scientific method is a way of dealing logically and systematically with inquiries. For the scientist this is done by carrying out experiments that are well-designed to answer one or more questions raised by the inquiry. The well-designed experiment leads to the generation of valid data and the data in turn leads to discoveries.

Psychologically, the inquiry arises because the curiosity of an observer has been aroused by what he has seen or experienced in the laboratory or in nature. Curiosity sets off a psychological drive which may be described as a physiological imbalance. If the imbalance is slight the drive force is not intense. If it is great, the drive may become so strong as to be "consuming". This curiosity-inspired drive, like other psychological drives, will be terminated by an adequate resolution of the curiosity, leading to satisfaction. Since curiosity is psychological, one could be rational and expect the reward to be psychological. It usually is, being the insight gained about the curiosity-indicing phenomena. That is to say, the insight gained when generalizations (valid or not) are reasoned from the experimental results.

In order to get some results, biological material may have to be subjected to conditions unlike any the material encounters in nature. The experimentor may have to create special environments, apparatuses, methods of detecting changes, recording methods or data-processing devices. He may, in fact, create combinations unknown in nature, but which are useful for demonstrating the correctness of assumptions and concepts, and in answering questions. It follows, then, to say that if inquiry, discovery and creativity occur as the scientific method is pursued, then inquiry, discovery, and creativity are elements to be found in every good scientific experiment.

The gaining of an insight into the causes of the original, curiosity-inducing experience is perhaps the satisfying event that returns the psychic and physiologic imbalance to zero on that question. It is usual to find that during a genuine inquiry new questions are raised and new motivations activated, so that a spiral of knowledge-seeking and knowledge-generation is developed.

"Discovery" at too low a level is unacceptable to most students. To "discover" that an everyday item is "alive", or that it has "insides" is to operate far below the intelligence of college freshmen. They expect some challenge, and a synthesis from discovered facts to discovered truth. It is this latter challenge that makes research an interesting and consuming endeavor

for the scientist and the student.

In fact, during the course the student should progress from being a student in the biology laboratory to being a biologist in the laboratory and finally to being a scientist at work in a biological laboratory--inquiring into the secrets of nature, discovering facts and being able to make generalizations.

THE OBJECT-CENTERED LABORATORY

Despite the fact that different people identify and name the approaches used in ISE courses differently, ISE identifies the biology course laboratory as being "object-centered", very much in the sense that John Dewey wrote about the word "object". For clarity this term should be defended and compared with some others in common use.

Dewey's essay on the "Use of the Word 'Object'" is not an easy one to read and understand the first time. It requires several readings to gain the full sense of what Dewey is trying to say, so we have included it on page 8.

The object is the thing to be thought about--a specimen, picture, laboratory experience, movie, or other non-abstract, tangible, real thing. Ideas are built up around the concrete object. Therefore, concepts of such things as cells, independent assortment of genes, cellular metabolism, etc., are more easily grasped by students if these abstractions can be related to a real object.

Object-centered describes the way biology gets taught because the definitions of the terms are very often the biological structures and processes themselves. Other terms sometimes mis-applied to the way biology gets taught are "laboratory-centered", "student-centered", and "open-ended".

Laboratory-centered, as used, means that there is a laboratory component to the course. In non-science courses where laboratory experiences have been introduced, this term may have distinguishing significance as a term denoting difference or innovation in teaching method. This is not so in the sciences. Chemistry and physics developed in laboratories in the 17th and 18th Centuries. Biology became a separate study from medicine in the mid-19th Century, following a long tradition of object-centered activity by physicians. Since the sciences have been built around laboratory experimentation, laboratory-centered does not describe anything but the most classical traditions in science.

Student-centered, as used in educational circles, indicates that a student is presented with several choices of activity from which he may elect. He may also elect not to select (provided he is willing to suffer the consequences, if any). Because of the amount of preparative work necessary for most biological experiments, it is not practical to use this design as a usual thing. A modification can be useful--that is, several experiments may be set up at different stations and each student asked to perform some study at each one as they become available (or a certain number or kind of them).

Because biological materials grow, age, and die with time, they must either be replaced with fresh material from time to time or the biological state of the material must be known at the time the experiment is done in order to correctly interpret the results. Therefore, instead of working on the student to show an interest in some perishable laboratory experiences, which he may elect not to be interested in, the student is presented with the material and his interest stimulated to study that thing or process. Therefore, motivation is an important compnent in such object-centered study.

Open-ended is a much-bandied-about term which has somewhat vague meanings. Basically, it implies that nothing will be decided because, perhaps, nothing can be decided. Usually a step or experimental group is omited from the experiment, yielding inconclusive results. The game here is to identify the missing link and then to do the experiment properly. The idea of the open-ended experiment is generally contradictory to the ideas of the scientific method. If a proper experiment has been done and the data accurately collected and evaluated, then a conclusion is almost certain. It is true that sometimes the data are insufficient to permit one to draw definite conclusions, but in a real experiment this is more by happenstance than by design. Open-endedness is good when performing some orientation experiments as when generating a problem by the so-called "open-inductive" method, where something is isolated from a mixture and then characterized. Eventually, however, this approach must give way to conclusive reasoning--either linear (sequential), or confluent (integrative) reasoning--resulting in one or more generalizations.

JOHN DEWEY

ON THE USE OF THE WORD "OBJECT"

It is not a new discovery that the word "object" is highly ambiguous, being used for the sticks and the stones, the cats and the dogs, the chairs and tables of ordinary experiences, for the atoms and electrons of physics, and for any kind of "entity" that has logical subsistence—as in mathematics. In spite of the recognized ambiguity, one whole branch of modern epistemology is derived from the assumption that in the case of at least the first two cases, the word "object" has the same general meaning. For otherwise the subject matter of physics and the things of everyday experience would not have presented themselves as rivals, and philosophy would not have felt an obligation to decide which is "real" and which is "appearance," or at least an obligation to set up a scheme in which they are "reconciled." The place occupied in modern philosophy by the problem of the relation of the so-called, "scientific objects" and "common-sense objects" is proof, in any case, of the dominating presence of a distinction between the "objective" and the "subjective" which was unknown in ancient philosophy. It indicates that at least in the sense of awareness of an ever-present problem, modern philosophy is "objective-subjec-

tive," not just subjective. I suggest that if we give up calling the distinctive material of the physical sciences by the name "objects" and employ instead the neutral term "scientific subject matter," the genuine nature of the problem would be greatly clarified. It would not of itself be solved. But at least we should be rid of the implication which now prevents reaching a solution. We should be prepared to consider on its merits the hypothesis here advanced: namely, that scientific subject matter represents the *conditions* for having and not-having things of direct experience.

Genuinely complete empirical philosophy requires that there be a determination *in terms of experience* of the relation that exists between physical subject-matter and the things of direct perception, use, and enjoyment. It would seem clear that historic empiricism, because of its commitment to sensationalism, failed to meet this need. The obvious way of meeting the requirement is through explicit acknowledgement that direct experience contains, as a highly important direct ingredient of itself, a wealth of *possible* objects. There is no inconsistency between the idea of direct experience and the idea of objects of that experience which are as yet unrealized. For these latter objects are directly experienced as possibilities. Every plan, every protection, yes, every forecast and anticipation, is an experience in which some non-directly experienced object is directly experienced *as a possibility*. And, as previously suggested, modern experience is marked by the extent to which directly perceived, enjoyed, and suffered objects are treated as signs, indications, of what has *not* been experienced in and of itself, or/and are treated as means for the realization of these things of possible experience. Because historic empirical philosophy failed to take cognizance of this fact, it was not able to account for one of the most striking features of scientific method and scientific conclusions—preoccupation with generality as such.

For scientific methods and scientific subject matter combine highly abstract or "theoretical" considerations with directly present concrete sensible material, and the generality of conclusions reached is directly dependent upon the presence of the first-named type of considerations. Now in modern philosophy, just as scientific "objects" have been set over against objects in direct experience, thereby occasioning the *ontological* problem of modern philosophy (the problem of where "reality" is to be found) so identification of the experimental with but one of the two factors of the method of knowing has created the *epistemological* problem of modern philosophy; the relation of the "conceptual" and "perceptual"; of sense and understanding. In terms of our hypothesis, the distinction and the connection of the distinguished aspects rests upon the fact that what *is* (has been) experienced is of cognitive importance in connection with what *can* be experienced: that is, as evidence, sign, test, of

forecast, anticipation, etc. while, on the other hand, there is no way of valid determination of objects of possible experiences save by employing what *has* been experienced, and hence is sensible. Anticipation, foresight, prediction, depend upon taking what is "given" (what has indubitably been experienced) as ominous, or of prospective reference. This is a speculative operation, a wager about the future. But the wager is subject to certain techniques of control. Although every projection of a possible object of experience goes beyond what has been experienced and is in so far risky, this fact does not signify that every idea or projected possibility has an equal claim. Techniques of observation on one side and of calculation (in its broad sense) on the other side have been developed with a view to effective cooperation. Interactivity *of the two factors* constitutes the method of science. Were it not for the influence of the inertia of habit it would be fairly incredible that empiricists did not long ago perceive that material provided by direct sense perception is limited and remains substantially the same from person to person and from generation to generation. Even when we take into account the additional sense data furnished by artificial instruments, the addition bears no proportionate ratio to the expansion of the subject matter of the sciences that is constantly taking place. Were it not that "rationalist" theories are in no better case with respect to accounting for increase in scientific knowledge (which is its most striking trait in modern times), the marked impotency of sensationalist empiricism would long ago have effected its disappearance.

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TEACHING THE LABORATORY

Up to now we have described rather general aspects of teaching the laboratory. One might ask, however, "Just how should the laboratory be conducted ideally?"

Laboratory Facilities

The physical environment should be comfortable and neat, well-lighted, and the temperature modulated so that it is not uncomfortable in extremes of heat or cold. In addition, the number of students to be accommodated should not exceed the number of working places. Overcrowding leaves the extra students feeling like second-class members of the class when they lack seats or are placed at substandard workspaces. Normally this will mean about 6 to 8 square feet of work space per student. In addition to the teacher's demonstration table at the front of the room there should be other counter-top spaces where materials may be dispensed or experimental plants or other organisms kept. Not the least facility should be adequate hooks or hangers for hats and coats brought by students when the weather is inclement.

For this course there is little equipment outside of a dissection kit which students will use repeatedly, so that drawer space will be optional. However, attached to the laboratory there should be an adequate storage and preparation area for equipment not in use, for making solutions, sterilizing media, refrigerating materials, and storing specimens.

In cabinets, on the worktables or against the wall, there should be storage space for one microscope per student plus two or three others for setting up demonstrations of materials to be viewed. There must also be an appropriate stool or chair for each student. Other items of equipment are listed in each exercise.

Didactic Teaching of Skills

One should distinguish between the didactic teaching of skills and the carrying out of an experiment, where experiment is defined as an investigative procedure to prove, demonstrate, or disprove an hypothesis. Students should understand what skills they are tying to acquire. If the skill is to be demonstrated, the demonstration should be done in such a way that all students will have an opportunity to observe what was done. It is a common practice for teachers to do demonstrations (for example, the dissection of a frog nerve-muscle preparation) at the demonstration table. Frequently all of the students in the class crowd around, but only those in the first row can usually see what happened. Some of them don't like being pressed in the crowd so they hang back until after the crowd disperses in the hope that they can glean enough from what is left to be helpful to them. If one is short and unfortunate enough to not be in the first row, then that's too bad. We suggest that the class be divided into tow or more sections when demonstrations are planned. While one group is being given the demonstration the other can be setting up equipment and making other preparations for the work to follow.

An alternative is to let students remain in their seats and carry or pass the demonstration material to the students. Perhaps, ideally, a television camera would be trained on small objects and enough closed-circuit monitors placed in the laboratory to assure each student a clear view of the events.

Sometimes the demonstration may be divided into steps. The teacher will demonstrate a step and then the students will imitate. During student activity teachers will answer questions and point out corrections in technique. The student should have the opportunity to repeat the manipulation or process enough times to internalize the skill Once is not enough. The most practical examination for the acquisition of a skill should be the ability to use that skill again without close supervision of the teacher, for the performance of some subsequent task, as in an experiment.

Successful acquisition of skills translates itself into more positive attitudes about scientific information, and a reduction in the feeling of some students that science is mysterious. It also decreases the attitude that science is done only by the smartest and most-gifted of people (but having acquired such skills, these students have joined the ranks of the smartest and most-gifted.)

Doing Experiments

All of the materials and equipment needed should be conveniently located in the laboratory. As much individual supply as practical should be provided at the workspaces (for individuals or for teams of two or more students) in order to reduce traffic jams and delays where many students crowd around to get a supply of something from a single supply source.

Introduce the topic inductively and have the students participate in an introductory discussion to be sure that they know what the objective (hypothesis) is. Indicate the points at which students should have their work checked (examined) for permission to proceed. The student should understand the design of the experiment, that is, he should understand which group is the control and which is the experimental component, and whether or not these experimental components cover all of the questions for which data is needed in order to arrive at valid conclusions.

The student proceeds with an experiment without demonstration and imitation. The teacher or teachers may still correct technical errors, however. If students are operating as a team, the responsibilities of each member of the team should be defined before they begin to work. After the data has been collected students should be asked to write their own reports. Sometimes they may be asked to write their report individually in standard report form. A good student will want to discuss his ideas and interpretations about the experiment with the teacher.

ON THE NEED FOR TEACHING ASSISTANTS IN FRESHMAN BIOLOGY LABORATORY CLASSES

We realize, of course, that this type of program (sponsored by the ISE) is aimed at prsenting a better-taught, more interesting biology course (not watered-down pap for incapable students) so that normal personnel requirements should not be ignored. This may mean more help in the laboratory at some ihstitutions, either from an expanded staff or by re-directing the efforts of the exisiting staff. That is to say, there is a need for teaching assistance in the freshman biology laboratory classes. Goolsby (1968) set forth this view:

"One of my former teachers with a penchant for philosophical expressions used to say to us, "I want more labor and less oratory in this laboratory." One reason that there was so much conversation was that nearly everyone felt confused at different points during the class and would ask his neighbors about what he should be doing, should have done, or should be about to do. In general, productive activity stopped until a teaching assistant could look at the student s problem and suggest now he should proceed from that point. The teaching assistants, as well as the teacher-in-charge of laboratory classes in the biological sciences, traditionally fill this need in laboratory teaching to the extent that they can. Usually there are written and oral directions for the student to indicate to him what it is he should do or look for and how he will make a report of his findings.

"Following an introduction to the work at hand the student is asked to use his hands tomaniplupate feel or seek out objects of biological interest, sometimes with the aid of instruments. Secondly, he uses his eyes to observe, sometimes with the aid of a lens or microscope, Thirdly, he must make intellectual decisions about his sensory input if he is to perceive them in their proper perspective. Since most laboratory exercises and experiments are basically experiences in logic, it is essential that the student use valid premises in order to draw correct conclusions concerning the question or hyposthesis under consideration.

The teacher (whether in charge or assisting) is mainly needed in the laboratory for intellectual reasons. As the student goes about discovering facts about material objects and phenomena it is possible for him to see things and not perceive the significance of his observation to the solution of the problem at hand. When this is so the student will seldom know which observation he missed but he will usually know that his reasoning is incomplete. Teachers then become indispensible to the acquisition of good quality information in the laboratory. The importance of this fact in arriving at good decisions and conclusions does not need to be stated.

How many teachers are needed in a laboratory class doing moderately challenging work? The optimalsize group usually lies between 12 and 16 students. Fewer than 12 students do not usually make maximal use of the teacher at the Freshman level. With more than 16 the following things happen at an

Goolsby, C. M. 1968 On the need for teaching assistants in freshman Biology laboratory classes. The PHOENIX 1(1):3-4. (The PHOENIX is the monthly newsletter of the Biology Curriculum Section of the Institute for Services to Education)

increasing rate:

(a) A few students will stop work until help arrives. This may be a comparatively long time. During this time they frequently engage in activities unrelated to the work before them.

(b) They will continue to work, making mistakes which have to be corrected after the teacher arrives and points out the error or errors.

"For a class of 25 students in a two-hour laboratory session (110 minutes), if 10 minutes is used for orientation, an average of 4 minutes per student remains, that is, 2.4 minutes/hour/student. Two teachers raise this time for help to about 4 minutes per hour, which is usually adequate in the well-planned class with good directions.

"The consequences of a class larger than 16 students per teacher will be:

(a) The conscientious teacher will give full help to each student, exceeding considerably the time allotted for the class.

(b) The teacher may pair-up students although they may have different problems.

(c) The teacher will reduce the average time spent per student in order to respond to the almost continuous requests for help.

(d) Some students needing help will not receive it.

(e) The teacher will reduce the work expectation of the student so that he can cover the requests for help during the class even though this dilutes the experience of the student; or he will accomplish the same end by selecting less intellectually-challenging exercises and experiments for the class.

The presence of an adequate laboratory teaching staff (one per 12-16 students) not only permits better attention by the teacher to the student but it has a good effect on student performance.

(1) The student has little excuse for not performing the prescribed tasks on the basis that he lost a lot of time waiting for assistance.

(2) His performance is better because mistakes, omissions and misinterpretations are detected earlier by the teachers so that good intellectual decisions and conclusions can be reached earlier by the student."

Where will such assistants come from? Many colleges do not have graduate programs nor are they located in the vicinity of universities from whom they could obtain graduate students for teaching fellowships. Where graduate students are not available, good upperclass undergraduates can be used with profit for preparation and cleanup as well as for teaching assistance in the laboratory. Money for such assitance is usually available through local college funding programs. An interesting alternative is to award an hour of academic credit for the teaching activity in lieu of, or in addition to monetary compensations.

AFTERWORD

As we stated near the beginning of these essays, much of the material presented here does not belong in the student laboratory manual. It was written and assembled as a platform to support the teacher who now should become the student--a student of educational methods and a student in the arts of better teaching.

It also functions as a link between the ISE and the teacher. It is hoped that the contents minimize the number of fundamental questions about the conduct of laboratory classes by the methods we espouse and that the tone encourages a continuing correspondence between us. Furthermore, since we have shared our experiences with you, we hope that you will share your ideas on these matters with us.

ACKNOWLEDGEMENTS

We would like to express our appreciation to Miss Georgia Broomfield for doing the typing for this volume, to Miss Patricia Parrish, who provided editorial assistance in Part I, and to Dr. Joseph Turner, who read the manuscript for Part I and gave us the benefit of his philosophical viewpoints.

SECTION II -- TEACHER'S GUIDES TO EXERCISES

FORMAT OF THE TEACHER'S GUIDES

The companion student manual, Laboratory Activities for Biology includes some information about materials, equipment and some recipes for stains, buffers, making temporary mounts, etc. These are usually presented to aid the student in understanding what reagents are being used beyond the magic names like Ringer's solution, Allen's fluid, or Kovac's reagent. The usual written student introduction is often omitted. This is so in part because such pre-structured discussions may not be the relevant discussions. Also, some students do not read very well and spend too long trying to understand the introduction instead of working with the material. Where introductins are lengthy, even students who read well may find difficulty in remembering all they have read. The chances, then, that the salient points of the introduction will be remembered are better if there is a discussion of the purposes of the exercise. Teachers are provided with two quick resources.

The first of these is the section entitled INTRODUCTORY REMARKS TO THE TEACHER. Here important points about the topic of the ex rcise are reviewed. The fourth section is entitled INTRODUCTORY DISCUSSION. It contains an approach to discussing the main, basic ideas of the exercise with the student.

The second section lists the MATERIALS AND EQUIPMENT needed for the exercises. The quantities give are for two sections of 24 students, unless otherwise indicated.

The PREPARATIONS sections contains directions for making solutions and other theings which must be done ahead of time.

PROCEDURES has suggestions for more efficient arrangements of Exercise parts. so that time is saved:

the REPORT SHEET is a data collection device but usually has questions to be answered also. It organizes information into a format for easy identification by the teacher. After studetnts gain some experince, one can give opportunity for making laboratory notes without such a format.

Where the data collected is to be used in a scientific report the data section of the report sheet can be used for the RESULTS section. The questions are selected to answered directly the exercise only sometimes. Where they are not directly answerable, answers must be looked up in reference books provided by the teacher. Questions constitute a simplified discussion. Many times the teacher will want to add his own questions or questions raised during the introducotry discussion. REPORT SHEETS tear out.--just bend the page back first.

TEACHER'S GUIDE TO

EXERCISE 1 -- WHAT IS AN EXPERIMENT? SCIENTIFIC REPORTS

INTRODUCTORY REMARKS TO THE TEACHER

Doing an experiment--a real inquiry into a natural phenomenon--is the essence of "doing science". Yet, because so few institutions require a research as part of the Master's Degree requirements, many teachers of freshman biology are placed in the position of having to introduce their students, and especially the non-biology majors, to the subject of what is an experiment? (that is, what does it mean to do science) when they have not had that experience themselves. Such a fact notwithstanding, students in the introductory course of necessity must be introduced to the class-laboratory type exercises and experiments in a simple and uncomplicated way so that they will know when they are "doing science" and therefore, behaving in some measure like a scientist.

It is not practical to expect the student to discover de novo the steps in the scientific method during the 2 or 3 hours of the laboratory session. Besides, we do not have to discover that the scientific method exists since great minds over the centuries evolved the procedure. Instead, we must provide a simple experiment which involves activities well within the scope of the student's previous experience, but which has parallels to the kind of inquiries biologists, and other scientists, make. The object, then, is not to discover that the scientific method exists, but rather to discover how science works.

In Part A the student is asked to do a simple experiment and identify which parts of it exemplify the various steps in the scientific method. In Part B he will become introduced to the organization of an original scientific report and how this reflects the steps of the scientific method carried out by the experimentor.

MATERIALS AND EQUIPMENT

36-48 small boxes, such as a 25 slide box
Double platform balances
Various kinds of mall objects that will fit into the boxes--cylinders, balls, blocks, cotton, paper, etc.
24 copies of an original-type scientific report or reports.

PREPARATIONS

Everyone has guessed what was inside of a wrapped gift-box. The difference here is that the student will be asked to reason and record his reasoning leading to his conclusions about the contents of the box assigned to him. Prepare two (2) boxes for each student in the laboratory session. Although a slide box is suggested above, any kind of container that is opaque, of uniform size, and regular shape will do. Number the boxes and after they are prepared make a list of what is in each. Place them on trays under the control of the teacher to be passed out to students at the time indicated below.

Place on the demonstration table the double platform balances, an empty box, and one of each kind of item enclosed in the boxes.

Obtain enough reprints of scientific reports (not review articles such as appear in the Scientific American) so that each student will have a copy. The copies may be of the same paper but the experience of the class will be increased if they are different.

Some questions which should be answered during the discussion would include these:

How is information accumulated scientifically?
What is an experiment?
What is meant by "doing science"?
Should we distinguish between exercises and experiments?
What are some possible exercises?
Why are all of the experiments in Laboratory Activities for Biology labelled "Exercise...."?
What are the steps in the scientific method (as outlined in the laboratory manual)?

At the end of the discussion pass out the numbered boxes containing an object (perhaps) to each student. Have the students write down their observations on page 1-5 of the manual and answer the first question. However, ask them not to open their boxes until asked to do so.

When sufficient time has been used for most students to make comparisons with the known (controls), ascertain what conclusions have been drawn by a few students. Then, have everyone open their boxes and complete the rest of the questions on page 1-5 of the laboratory manual. If any students made an error in his inference about the contents of his box, have him select another numbered box and proceed before, writing his observations on the back of page 1-5.

Part B -- Scientific Reports

On a separate piece of paper have the students write the name(s) of the author(s) and the title of the article. Only the first word and proper nouns are capitalized. Have them devise a suitable, reasonable abbreviation for the journal title (it may be the standard abbreviation). Later, tell them, or write on their papers, the correct standard abbreviation. Then have them write the volume, number (if given), inclusive pages, and the date. Example:

Brant, J. W. A. and A. V. Nalbandov. Role of sex hormones in albumen secretion by the oviduct of chickens. Poultry Science, 35:692-700, 1956.

Have students identify the hypothesis being investigated. This is normally at the end of the introduction. Also have them identify the organism and tissue being used.

They should report on what kind or kinds of data are being reported. That is, is it descriptive morphology, qualitative data, as for color or consistency changes, or quanitative data (values for weights, volumes, temperatures, acidity, salinity, heights, etc.)

Have them decide which of the conclusions reached, seem to be of the greatest probable significance.

How many reference sources are listed by the writer(s)?

Have students hand in this report at the end of today's laboratory session. Give students a library assignment. They may be asked to look up and abstract information from an article or articles for which the teacher gives the reference or the teacher may ask students to look up references written by specific authors or on specific topics. This would involve using the indices to abstract journals such as Chemical Abstracts or Biological Abstracts. To ask students to select a topic at this point would be premature and therefore, frustrating, as his interests in a topic may not be well-developed. However, if some students have strong interests let them bring in references accordingly.

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TEACHER'S GUIDE TO

EXERCISE 2 — WEIGHING AND MEASURING

INTRODUCTORY REMARKS TO THE TEACHER

A few decades ago Biology was largely concerned with morphology and counting. Interest centered about classifying and naming structures on the basis of their shapes and counting up the number of kinds of things--bones, chromosomes, species, etc. Today, much more physiology is included in biology, and in this course it is an important component of the laboratory work. Ability to weigh and to measure sizes and volumes accurately becomes an important skill in working with the small quantities usually encountered in the class laboratory.

Most non-science college freshmen are as unacquainted with making scientific measurements as they are with microscope operation. Teachers are perhaps prone to place more emphasis on the use of the microscope. As important as the microscope is for the study of minute morphology, it must share importance with weighing and measuring in the modern study of biology.

The introduction to this exercise explains that the units of measurement and volume used in the laboratory are arbitrary, but mutually-agreed upon standards. Although this is an exercise in Physics, it deals with skills necessary for the successful study of modern biology.

MATERIALS AND EQUIPMENT

Chemicals

200 cc. Ethanol
200 cc. Glycerol

Plastic & Glass Wares

24 10 ml. pipettes
24 1 ml. pipettes
24 10 ml. grad. cylinders
96 small beakers

Others

24 15 cm. rule
24 triple beam balance
4 rolls alumnium weighing pans
24 physiological weights (5-10 gm.)
24 large nails

PREPARATIONS

Balances. Be sure that balance pans are clean, that the balances are levelled and that they are in balance when all weights are set to ZERO.

Pipettes. If glass pipets are used, the clean pipets should be distributed in pipets cans, or placed in central containers which keep the mouthpieces off of the table tops. Students should not be asked to put glassware into their mouths that has been on the laboratory table tops. The same rules apply to the sanitation of pipets as to dinner tableware. A convenient solution to this situation is the individually-wrapped glass or plastic pipet which assures a clean mouthpiece to each student. Containers should be placed on each work bench to receive used pipets. If glass graduated cylinders (1000 ml.) are used as receptacles some paper towelling should be placed in the bottom. About a liter of detergent solution should be placed in the graduate. Pipets should be placed in these containers with tip down. It has been a wide practice to put pipets into such containers with the mouthpiece down because sometimes the tips got chipped, making the pipet useless. However, in order to do this, any potentially dangerous material contained in the pipette will get on the hands.

INTRODUCTORY DISCUSSION

The objective of this discussion is to emphasize the importance of quantity in making descriptions.

Some questions that should be answered in the discussion include:

How high is high up?
How big is big?
How small is small? tiny? real tiny?
Contrast the English and metric systems of measurements.
Can the class suggest any better systems for weights and measures?

PROCEDURES

Assign Part A (weighing) and require that the teacher or teaching assistant check the weighing technique and the values obtained before permitting the student to proceed to Part B. Thus the teacher can satisfy himself that the student did in fact do his weighing and computing. There is also a motivational factor involving teacher-approval (as a reward) for completing Part A. Permission to do Part B becomes an indication, too, of teacher approval and thus has a reinforcement effect on learning.

It would be good to have a student demonstrate pipeting technique in front of the class. If this is to be done, the teacher should satisfy himself that the student can pipet accurately and properly at some time before class begins. The event may be introduced simply, for example:

Teacher: Jim Jones will now give us a demonstration.
(Jim Jones demonstrates pipetting without comment. Have him do both the methods using the index finger, then the method using the thumb to close the pipet.)

REPORT SHEET

Factors affecting the accuracy of the measurement of a physiological weight with a mm. ruler are that such a weight is relatively small and one may have to

estimate fractions of a mm. Also, the corners are often removed from the weight so that it is not a complete rectangular block. Temperature effect on a 10 gram weight is probably neglible.

In measuring volume in a graduated cylinder the object being measured must be completely submerged and the graduate must be read accurately. Temperature can be a factor.

The viscosity test is not only instructive about the relative viscosity of water, alcohol and glycerol, but the emptying time is a function of the accuracy with which the pipet was filled. Alcohol is less viscous than water. Glycerol approximates the viscosity of the endoplasm.

QUESTIONS

1,2 &3. Scales make use of gravitational pull on the mass being weighed as measured by a spring. The strength of the spring may be constant or vary over a period of time. In different parts of the earth, the pull of gravity varies so that the same mass will not weigh the same in all places using scales.

Balances weigh by comparing the pull of gravity on an unknown mass with that on a known mass (weight). Since the pull of gravity is the same on both pans (or sides) of a balance, even if the gravity has varied from place to place, one is assured of obtaining the same mass of material. Therefore, balances, not scales, are the standard instrument for determining weights in the scientific laboratory.

4. There are
28.4 grams per ounce
454.4 grams per pound
1000.0 grams per kilogram
2.0 pounds per kilogram
2.54 centimeters per inch
100.0 centimeters per meter

14 x 140 mm. = 14/25.4 x 140/25.4 = .551 x 5.51 inches

5. A proton weighs 1836 times more than an electron and therefore accounts for most of the atomic weight.

6. One cc of protons would weigh many tons.

7. The mass is the amount of material. A given mass may weigh different amounts on scales but on balances the mass and the weight are the same.

NOTE: The calculation of the standard deviation and of the standard error is given on page 9-1 of the manual.

TEACHER'S GUIDE TO

EXERCISE 3 -- DIFFRACTION OF LIGHT INTO ITS SPECTRUM

INTRODUCTORY REMARKS TO THE TEACHER

A knowledge of the physics of light behavior is useful to the student for understanding the microscope and the miscroscopy of various biological materials, and for understanding colorimeters and spectrophotometers. Visible light is a small part of the electromagnetic spectrum which begins with sounds, goes through radio, progresses to heat and passes through the infra-red into the longer wavelengths of visible light. Radiations just shorter than visible light lie in the ultra-violet, and as wavelengths shorten, the radiations becomes actinic (photochemically active) as the spectrum passes through ultraviolet "light" through X-ray (and gamma-ray) emmission and ends with the shortest wavelengths and the highest frequency per second in the cosmic rays.

White light, as comes from the sun or from incandescent lamps, contains lights of all the visible wavelengths. Only when the light is diffracted does it become apparent that it is made up of lights of different frequencies (colors.) Sometimes this color is very helpful, as when determining what elements are being burned to give the light, and sometimes it is not desirable, as when lenses are chromatic not achromatic. That is, in microscopy, the light must be diffracted in such a way as to not give rainbows around all of the images seen.

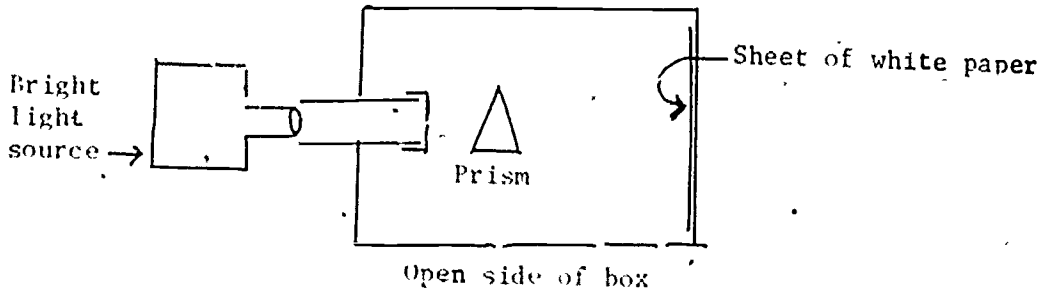
Prisms are very old in the culture, just as apples are old in human culture. Apples were not invented by Newton to explain gravity. Everybody already knew that apples fell to the ground, but no one had thought out loud about the attraction between large and small masses. Similarly, prisms were objects for enjoyment. Glass and gem cutters made use of the reflective and refractive light-splitting properties of prisms from ancient times. Newton, however, put a prism into a beam of sunlight in 1666 and made the first scientific analysis of the colors in sunlight. In 1800 Sir William Herschel measured the temperature in the region of the spectrum just beyond the visible red (7800Å) and found it higher there than in the visible red portion of the spectrum. Thus, infra-red radiation was discovered. It seemed a natural consequence for the scientifically curious to investigate the other end of the spectrum. J. W. Ritter (1801) did not find it warmer or cooler there, but he did discover "light" with photochemical properties in the ultraviolet.

By the beginning of the twentieth century it was concluded that visible, ultraviolet, and infra-red lights were part of the continuous electromagnetic spectrum. The portions of the spectrum most-used by biologists are visible light (3800 to 7800Å) and ultraviolet light (1000 to 3800Å). The ultra-violet region is subdivided into three ranges, based in part upon the fact that this range of radiation is largely absorbed by the air and water. In the extreme or vacuum ultraviolet region (1000 to 1900Å) experiments must be done in a vacuum. The far ultraviolet region (1900 to 3000Å) is filtered out of sunlight by atmospheric ozone. It is interesting to note that this range, which denatures proteins and breaks apart nucleic acids, ends precisely at the point (3000Å) where they are no longer seriously affected. The near-ultraviolet (3000 to 3800Å) radiation passes through the atmosphere but, like the other ranges, can be stopped by most kinds of glass. However, ultraviolet light is easily transmitted through quartz.

MATERIALS AND EQUIPMENT

6 bright light sources of high intensity
6 viewing boxes (described below)
12 Triangular prisms

The viewing box is mainly a device for shading the refracted light so that it can be seen. A darkened room accomplishes the same end, but this is not practical for a class. One side of the box is open. One end of it has a circular opening cut and a piece of opaque (cardboard) tubing inserted. This is covered with opaque paper on the inside end and a vertical slit is cut into the paper. The high-intensity light source, or 2 x 2 slide projector, is abutted to or inserted into the outside end of the tube. The prism will have to be supported on some movable object which will bring it up to the right height to intercept the beam of intense light. A piece of white paper can be used as a screen inside of the box.



PREPARATION

One such viewing box should be prepared for each four students and set up at their workspace before class meets.

INTRODUCTORY DISCUSSION

Present a beaker partly filled with with water. Insert a glass rod. It has been the common observation of students since childhood that this refractive quality of water would make a straight object appear bent, but now the question is, why? Ask if anyone can explain why the rod appears bent. (There is a difference in the speed of light passing through the air and passing through the water.) If no one can explain, then see what they know about light.

What is the usual speed of light? (186,000 miles/second). Can it be slowed down? (Yes) What could slow the speed of light? (A transparent substance with a density greater than air). That makes it sound like light might be slowed down by something dense like glass or plastic so that the ultimate amount of slowing would depend upon how much of the material was involved. Move the rod slowly toward and away from the observers. The more water is between the rod and the observer, the larger it will appear. Why? Because the more the light is refracted the larger or smaller will be the image. In this case it is larger because the mass of water is cylindrical and acts as a convex lens. The degree to which light is bent depends upon the density, and this is expressed in a ratio called the index of refraction. In microscopy, distortion of the image at high magnification is reduced by eliminating a layer of air between the lenses of the microscope and the object observed. Oil with an index of refraction about the same as that for glass is used and such lenses are called oil immersion lenses because they have been de-

signed in such a way that the index of refraction for air must be eliminated.

Today we want to study the way light behaves when it passes through one or two prisms and then compare the prisms with a diffraction grating in their respective abilities to refract light into its spectrum.

PROCEDURE

Do Part A then Part B. In Part A measure the distance from prism to screen and on the screen with the various arrangements of the prisms.

REPORT SHEET

Part A -- Diffraction by Prisms

1) If red is 1, the order for the other colors is 1, 5, 2, 6, 3, and 4.

The colors produced by the wavelengths of light (in Ångströms) is 4, 5, 1, 2, 3, and 6.

2) Lights of different wavelengths are bent different amounts by the glass. The shortest wavelengths (violet) are bent the least.

3) Light diffracted by the first prism is refracted back by the second.

4) There is a point where reverse spectra fall together. This is literally the "focal point" although no focus exists with these prisms.

Part B -- Diffraction Gratings

1) The spectra of sunlight and of a tungsten lamp are the same.

2) Mercury gives a number of emission lines. Those in the visible spectrum lie in the blue, the green and two very close together in the yellow.

3) A spectrophotometer is an instrument for measuring the intensity of light in the various parts of the visible spectrum. A colorimeter uses filters and measures light at specific wavelengths. A spectrophotometer can measure light transmission or absorption over a continuous segment of the spectrum.

TEACHER'S GUIDE TO

EXERCISE 4 -- USE OF THE MICROSCOPE

INTRODUCTORY REMARKS TO THE TEACHER

Of all the sensory receptors, perhaps the eye contributes most information to the brain concerning the biological environment. The microscope is unnecessary for looking at big things, but it is indispensable for looking at minute things, and it just happens that there are a lot of minute things to be seen. In the modern biology laboratory the microscope shares importance with the balance, the pipet, the spectrophotometer and the centrifuge. Microscopes range in cost with analytical balances, spectrophotometers, and centrifuges and make their own unique contribution to the study of biology by helping the eye to see minute structures. The investment in microscopes, however is generally much greater than in other instruments because each student must have one, and sometimes two such instruments, and they really cannot be shared if the kind of careful study is done that ought to be done.

Despite the long-time fact that the microscope has been considered an important physical tool of the biologist, perhaps no other important instrument is used by students with less understanding of what it does and what it is supposed to do. It is not infrequent that the student is asked to learn to operate the microscope without knowing what it is doing, and at the same time study some fast-moving protozoan culture organisms because they are "fascinating". The result is that neither of these objectives is fully met and the teacher seldom returns to the topic again. The main objective in doing this exercise is to have the student understand what it is that the microscope is doing, how he should care for it, and what he can expect out of it.

MATERIALS AND EQUIPMENT

Biological

Prepared slides of mamalian tissues
24 liver
24 kidney
24 simple epithelium

Other

24 compound microscopes
24 stereoscopic microscopes
24 bottles of immersion oil
24 15 cm. rule lens paper
24 stage micrometers

PREPARATIONS

Select a student beforehand to demonstrate the proper way to carry the microscope. Perhaps another student can be used to demonstrate the proper method of cleaning the microscope before and after use. Review the procedures with these students so that the demonstrations will be correct.

INTRODUCTORY DISCUSSION AND PROCEDURE

Have the students study the diagram on page 4-2 of the manual and identify the eyepiece, body tube, arm, objective lenses, condenser and base. The teacher should then give an oral practical examination on these parts and then introduce the demonstration on transporting the microscope. This should be done with a minimum of initial comment. For example:

Teacher: John Jones will now give us a demonstration.
John Jones goes to the microscope cabinet and transports a microscope to the demonstration table where he sits it gently down.

Teacher: We have just seen John Jones demonstrate the correct way to carry the microscope from the cabinet to your desk. Let us see if we can tell what it was that John Jones did. When the teacher has satisfied himself that students understand why the microscope must be handled carefully, he will ask them to each get the microscope assigned for their workspace from the cabinet. If classical compound microscopes are being used the student should complete the identification of the parts on the diagram on page 4-2 of the student manual, or if zoom type microscopes are being used, then use the diagram on page 4-7. When all parts have been identified, have students complete the self-test, labelling on pages 4-10 and 4-12 as appropriate.

Cleaning the microscope.

We have another demonstration, this time by Mary Smith.
Mary Smith has been selected to demonstrate the proper way to clean the optical surfaces which students should clean before beginning to use the instrument. Lens paper may have been provided at each workspace, but if not, pass it out at this time and students should proceed to clean their microscopes; either surface by surface following demonstration of each step or doing it after having completed watching the demonstration.

Making an Observation.

Xerox copy of mm. rulers mounted on microscope slides are not as satisfactory as stage micrometers. The mm. ruler has a rather wide rule that is hard to make an accurate measurement with under high power, so that the estimates made with it are somewhat rough. Stage micrometers are expensive, but there should be enough in the department to supply the laboratory session. It is best to have them use the micrometer only on low power and on high dry power at first. After some experience has been gained on other slides with focussing, using the oil immersion objective, should that measurement under oil immersion be attempted by students. Students, therefore will learn to get the rulings of the stage micrometer in focus. It stands still. It has meaning.

Plane of Focus.

Microscopes can not be focussed. Both classical compound and zoom-type microscopes are always in focus. These two types of instrument differ, however, in that the plane of focus of the zoom microscope is fixed at the top of a standard glass microscope slide. Objects placed higher or lower than that position will lie out-

side of the plane of focus and therefore, will not be clearly seen. If the lenses are jarred out of place the object cannot be focussed.

The plane of focus for a classical compound microscope is a fixed distance below the objective. This is called the "working distance" and is raised or lowered when the body tube and objective is raised and lowered. Therefore, the task is to bring the plane of focus to lie in the plane of the object to be observed. If the plane of focus lies slightly above or below the object to be studied, it will be fuzzy and not clearly seen. If the plane of focus is somewhat removed from the object it will not be seen at all.

Sometimes, after having wiped the lenses, a student may still not be able to see through his microscope. The most usual cause seems to be that the instrument was used to observe something in a salt solution and because of poor technique and failure to clean the lenses after such use, an encrustation of salt is left thereon, obscuring vision most effectively. Wet a piece of lens paper in clear water and wipe the objective clean. Complete cleaning with a piece of dry lens paper.

REPORT SHEET

Part A

1. The correct order for a classical light microscope is:

   1. lamp 2. mirror 3. condenser iris 4. Abbe' condenser lens
   5. objective lens 6. nosepiece 7. body tube and draw tube
   8. eyepiece lenses

2. The iris diaphragm control is located on the Abbe' condenser.

3. The condenser-raising head is located on the side of the base.

Part E

5. The image moves in the opposite direction as the slide on the stage.

7. One focuses upward on higher magnifications so that if the plane of focus passes through the object too rapidly, the objective will not be driven down on the slide with resultant damage to the slide and to the objective lens.

High-dry and oil immersion magnifications require more light because the size of the field is smaller, so more light is needed to keep the level of illumination bright.

Part G

3. No. If the nucleus is spherical so that its width is the same as its height, then this distance can be related to the numbers on the fine adjustment head so that the number of microns of vertical movement can be assigned to the numbers on the fine adjustment head.

Part I (eye)

1. Is the image reversed? No

2. How did cells in a slide of tissue look? They could not be resolved.

   A penny was magnified so that the small letters could be plainly read.

TEACHER'S GUIDE TO

EXERCISE 5 -- STERILIZING AND STERILE TECHNIQUE

Introductory to Exercises 6, 30 and 42

INTRODUCTORY REMARKS TO THE TEACHER

There are only a few exercises in this manual that make use of microbial cultures; but these are very interesting experiments and attack some very fundamental questions in biology. In order to do them the student must have some understanding of what sterility means and how it can be produced and maintained. The techniques are not difficult but must be done carefully if contamination of cultures is to be avoided. Since the bacteria find the same kinds of food nutritious as do higher organisms, there are times when foods must be protected from infection by bacteria. Similarly, if one is doing an experiment with a microorganism, the results cannot be accepted as representative of the organism under study if the culture has been contaminated with other microbes. Bacteria are cells and therefore, are killed by the same factors that kill other cells. Sometimes an antibiotic against bacteria can be found which does not harm some other organisms or cell types in the culture, but usually it is easier and less muddy if the culture can be maintained as a "pure culture".

In this regard, we might mention that the organisms used here are not spore-formers so that they are easily killed. One does not have to worry about spores contaminating the laboratory to infect accidental wounds long after the bacterial experiment is over. The coliforms do not survive when dry. But on the other hand, good microbiologists do not accept the idea of a "harmless" culture. A few E. coli organisms may not be harmful but a big slug of them, obtained, for example, by pipetting carelessly and sucking the culture into the mouth, can lead to severe diarrhea and even septicemia. Also, plate cultures should not be exposed and waved around. While we may assume the culture to be "pure" one never knows when a contaminating pathogen or spore-forming bacterium is present or if a mutation has occurred that is potentially pathogenic. We, therefore, recommend that cultures be kept closed until they have been sterilized, even though the organism is "harmless".

Bacteria offer many advantages as experimental organisms. They survive well, divide frequently so that many generations can be obtained in a short time, and except for the extremely fastidious species, they are easy to culture.

Bacteria are usually killed by one of several techniques. These would include poisoning, incineration, lysis, extremes of pH or salinity and exposure to ultraviolet and other short wavelength radiations. We will use the first three of these in this exercise. The last part of this activity deals with serial dilution of a culture sample and the subsequent enumeration of colonies that grow out of a given amount of diluted material. Other methods of estimating the number of cells per ml. include nephelometry (dispersion of light), use of counting chambers (the visible number of cells per small, fixed volume), and by tube cultures statistically considered (the most probable number method.)

MATERIALS AND EQUIPMENT

Chemicals

1/4 lb. Mercuric chloride
25 gm. Bromthymol blue
5x1 lb. Glucose peptone
5x1 lb. Koser's citrate
5x1 lb. Tryptone broth
1/4 - 1/2 liter of Ether
2 liters Amyl Alcohol or Butyl alcohol
1 gallon conc. HCl
50 gm. p-dimethyl-aminobenzaldehyde
5x1 lb. Litmus milk
5 lb. Sucrose
10x1 lb. Agar for plating
3 lb. KH_2PO_4
25 gm. Methyl red
25 gms. alpha-napthol
1 drum 100% Ethanol

Biologicals

E. coli culture or Aerobacter aerogenes

Plastic and glassware

550 test tubes or culture tubes (16x150mm.)
168 4 oz. nursing bottles
25 100ml. graduate cylinders
96 10 ml. sterile pipettes (individually wrapped)
200 1 ml. pipettes
1 box Pasteur "Pipettes
1 box Petri dishes with plate counting grid
150 250ml. flasks
24 2-liter suction flasks
1 box glass wool
Spectronic 20 cuvettes

Others

24 Bunsen burners
24 Nichrome wire loops with handle
48 Test tube racks
24 Test tube brushes
24 Triple beam balances (See Ex. 2)
1 or 2 Autoclaves
6 Pipette jars
24 B & L Spectronic 20 or 34 spectro-photometers
48 1 hole rubber stoppers
6 rolls of aluminium foil
24 pH paper rolls
1000 plastic slip-on caps

PREPARATIONS

Pure cultures of E. coli or A. aerogenes should be obtained beforehand either from the microbiology course or from a supply house such as the Midwest Culture Service. The organisms should be suspended in sterile broth and streaked to ascertain the purity of the preparation. Use a sterile loop to transfer some organisms from a single, pure-looking colony to a tube of nutrient broth. This culture should then be used for the IMViC tests on page 5-3 of the manual. These tests are for indole (I); methyl red (M), the Voges-Proskauer reaction for acetylmethylcabinol (V) and for ability to grow in citrate as the sole source of carbon (iC). These tests should be done before the class starts this exercise.

Methods of making closures

1) Cotton plugs. Culture tubes and flasks can be plugged with non-absorbant cotton. This comes in unsterilized rolls. A pledget of cotton about 1 1/2 inches in diameter is grasped with forceps and pushed into the mouth of the tube. About half of the cotton remains on the outside. The rule is that the plug should be firm enough so that it will support a tube of medium without pulling out. In plugging flasks fold an approximate square of cotton 2.1/2 to 3 inches on a side, and insert it into the mouth of the flask with a twist. As with tubes, there should be enough cotton outside to cover the lip of the flask.

To increase the sterile area around the mouth of the tube or flask cut 6-inch squares of aluminum foil and place one on top of a tube or flask. Fit it to the top of the tube or flask by bringing the thumb and forefinger down around the neck of the container so that at least 1 to 2 inches is covered.

2) Sponge plugs. Several types of spongy plastic tube and flask closures are available commercially at low cost. These are great time-savers, since they don't have to be formed to fit the neck of the container. While it is sometimes optional whether one covers a cotton plug, it is strongly recommended that sponge plugs be covered since this will also help prevent the plug from blowing out at the end of the autoclaving cycle.

3) Metal and Plastic Closures. Metal and plastic tube closures are available commercially. These protect the mouth of test tubes from contamination without being liquid-tight. These types of closures also do a good job of conserving the medium from evaporation.

INTRODUCTORY DISCUSSION

Teacher: What are the causative agents of infectious diseases (as distinguished from disorders such as diabetes or near-sightedness which are non-infections? (Bacteria, molds, protozoa, viruses.)

How could one kill these infectious agents? (Starvation, electrocution, burning; poisons and antibiotics, excess radiation)

If we were to restrict our attention to the bacteria, which of these (above) methods might work best? [Check items in list]

The project for today is to learn some procedures for keeping a culture pure, that is, uncontaminated with other bacteria or molds. This means that the glassware,

medium, pipets, and wire loops must all be sterile. Sterile means to be completely free of living bacteria, whereas sanitized means that a few bacteria are still present. One thing that needs to be done (hold up pure culture) is to take a pure culture like this one, and transfer some of it to other tubes without contamination from bacteria in the environment. Where in the environment would the bacteria be that would contaminate the culture? (In the air, on the table, on hands, in water, on any unsterilized object.) How, then, can they be transferred with out contamination? (Wait for opinions. Collect suggestions from the class.)

At this point the teacher should demonstrate the method of flaming a nichrome or platinum wire loop, of flaming a tube, and of making a loop transfer, including closing the tubes again.

PROCEDURE

Part A -- Making A Sterile Transfer With A Wire Loop

Have the class follow the procedures as demonstrated again by the teacher, step by step in the exercise, correcting any errors made by students. It is not noted in the directions that the tube closure should not be contaminated by letting it touch objects which are not sterile. The closure must not be contaminated where it is inserted into a tube or flask. Metal closures can be flamed.

Beginning at Step 4 and continuing through 9 is a simple experiment. Which is the control in this experiment? Tube A-3 is called the sterility control tube.

The procedure for asceptic transfer of a pure culture with the wire loop is checked by doing the IMVIC tests on medium from the transferred organisms after 24 to 48 hours. The outcome of the student tests should conform with the tests the teacher has previously done. The teacher's results should be made available before the students begin their own confirmatory tests. If the tests come out differently than the teacher has indicated, that would be presumptive evidence of contamination, and therefore of failure to transfer the cultures asceptically. If contaminations occur, students should be given added instruction in sterile transfer and repeat these transfers and tests.

Part B -- Making and Autoclaving Media

The object in using 4oz. nursing bottles is that they are containers commonly sterilized in the home where there are children. The experiment in this section is a comparison of chemically clean, "thoroughly-washed" glassware with sterilized glassware. The making (reconstitution) of the powdered milk that has some litmus added, is another use of the weighing skill. The amount used is not critical, but the student should weigh accurately as a matter of practice. It is not practical to let each student operate the autoclave for their individual bottles, it would take all day and then some. Have the students place bottles for sterilization in a central place and then the teacher will place them in the autoclave, explaining to the class what the autoclaving procedure is.

Part C -- Making a Pipet Transfer and Spreading a Petri Plate

If you do not have individually-wrapped, sterile pipets, then glass pipets sterilized in pipet cans can be used. The same rules apply, the can is opened in the horizontal position to prevent microbes and spores from falling into it. The

same rules apply, the can is opened in the horizontal position to prevent microbes and spores from falling into it. The pipets are shaken out a little, grasped just below the mouthpiece, and withdrawn in such a way as to not let the tip come into contract with the edge of the can or the table top.

Part D -- Making a Serial Dilution and Plating

The water blanks must be prepared ahead of time and allowed to cool. Once prepared as directed above they are good for several weeks. Pipetting accurately and asceptically is essential for the successful pursuit of this part of the exercise. When culture is transferred from one flask to another, the pipet must be thoroughly rinsed with the receiving solution. The newly made mixture must be thoroughly mixed in order to assure a fairly even distribution of the organisms throughout the diluted mixture. It truly does require more than a casual shaking, the twenty vigorous shakes are needed.

The agar plates used here should have been made at least two or three days ahead and allowed to "dry" right side up at room temperature. This is so that the 1 ml. of dilute medium will be absorbed in a reasonable time. Freshly made plates generally do not absorb the water very well, resulting in coalescent colonies or a film of bacteria instead of discrete colonies.

REPORT SHEET

Part A.

Some growth will have occured in tube A-1 but not in tube A-2 if the technique has been properly performed. Tube A-1 was flamed so that the experimentor (the student) could be sure that any growth that occurred was from the loop and not from accidental contamination. Tube A-3 serves as the sterile control to show that if nothing was done, there would be no growth, that is, that the medium itself was sterile.

Part B.

1. The source of litmus is a lichen, the natural dye being considered superio to synthetic preparations. It has a pKa of about pH 6.4 (red at pH 4.5 and blue at pH 8.3).

2. Decoloration may not occur in 2 days. If not, let the bottles stand a while longer. It occurs because in addition to being a pH indicator, litmus is also an oxidation-reduction indicator. When litmus is reduced it becomes colorless.

3. The curd and whey together have the same volume as the starting milk, but after a while the curd alone has less volume (relative proportion depending on the observation). This shrinkage of the milk proteins is called syneresis.

4. The possible sources of bacteria in bottle 2 are: 1) some bacteria werernot removed by washing. 2) tap water is not sterile. 3) the bottles were exposed to the air. 4) the litmus milk powder (or even plain milk powder) is not sterile. Milk is pasteurized to remove harmful bacteria, such as those of bovine tuberculosis, but it does not kill the lactic acid bacteria which cause the souring of unrefrigerated milk. The lactic acid bacteria in certified milk do not exceed 10,000/ml. and for Grade A milk they do not exceed 30,000/ml.

5. Litmus milk will support growth after being autoclaved as indicated by bottle 3.

6. The control group or sample in an experiment shows what would happen if the experimental procedure were not done.

Part C.

3. An uneven spread would mean that the whole plate would have to be counted whereas if the plate were fairly evenly spread one might count only 1/4 of it and multiply by 4. This is not important where there are only a few colonies, but it there are several hundred it saves a lot of time making an estimate.

Part D.

Colonies on crowded plates are generally smaller than on sparse ones because the amount of food diffusing to the colony is less and therefore, the size of the colony is limited.

Every viable bacterium on the plate will not give rise to a discrete colony. Sometimes two or more organisms will lie very close together so that only one colony results.

Disposal of plates, etc.

Remember that autoclaving kills the bacteria and spores on the plate, but if the medium is left open to the air then it will become contaminated with some molds and bacteria that will make it not only unsightly and smelly, but actually dangerous to be near. Such a mess must be autoclaved again. We recommend that as soon as the autoclaved, used materials are cool that they be placed in a plastic bag and tied well before being disposed of in the trash. This way, any new contaminants are kept in the bag.

TEACHER'S GUIDE TO

EXERCISE 6 -- BACTERIAL MUTATIONS

Prerequisites: Exercises 2 and 5

INTRODUCTORY REMARKS TO THE TEACHER

Sometimes an organism, such as a bacterium, will adapt to a change in the environment and sometimes the environment will bring about a change in the organism (See Teacher's Guide to Exercise 30). This can be in the nature of an impairment leading to changes in morphology, or changes in function, etc., that is, non-adaptive responses to changes in the environment which are reversible when conditions are more favorable again. However, it may lead to a genetic change (mutation) which is transmissible to later generations of the organism or give rise to a changed organism, that is, a new strain, a new species, or, in the scale of evolution, to a new Phylum.

It is relatively easy to obtain experimental mutations with a number of chemical and physical agents, collectively called mutagens. Of these, the actinic radiations (ultraviolet light and shorter wavelengths) are probably the natural mutagens most commonly responsible for genetic changes. Actually, very few chemical agents occur in nature as widely distributed or in sufficient concentrations to cause an increased frequency of mutants in a given population. Ultraviolet light from the sun and isotopic radiation from the earth have the greatest likelihood of modifying genes in localized geographic areas. Therefore, populations of the same species in isolated areas, delimited by natural barriers such as wide rivers, high mountains, or stretches of open sea, tend to change from their close relatives in the adjacent demes. It is this isolation and radiation of the gene pool that can cause a human population in a politically delimited area (a country) to develop different, but minor, changes in physical characteristics not attributable to available food or to clothing habits, yielding "national" types.

In 1901 Max Planck published a theory that would account for the continuous emission of the electromagnetic spectrumm. He held that an "ideal" hot object (which he called a "black body") possesses a large number of minute oscillating systems. These oscillators:

1) Have a discrete number of energy levels.
2) The emission and absorption of oscillations occur when transitions occur between two levels of energy.
   The energy lost is emitted as quanta (photons) of light, or quanta of energy are absorbed if an energy gain is needed. A quantum is a unit of energy and may be applied to kinetic, potential, or electromagnetic energy, or to angular momentum. However, a photon refers only to a quantum of electromagnetic energy (light). It has an energy, (E).

$E = h\nu$ ν = the Greek letter nu.

where h is the Planck constant (6.624×10^{-27} ergs seconds) and ν is the frequency of the oscillation.

It is characteristic for the frequency to be faster as the wavelength shortens so that the speed of light is maintained. Consequently,

$$\underline{v} = v/\lambda$$

where lambda is the wavelength and v is the speed of light.

If $E = \underline{hv}$

then $\underline{v} = E/h$ and $E/h = v/\lambda$

so that the energy of the radiation (photon) is inversely proportional to the wavelength. Therefore, ultra violet X-rays, and isotopic radiations have more energy than white light of equal intensity, so they are called actinic.

Atoms, molecules and ions were later shown to be the oscillators in Planck's systems.

The spectrum of the emission and the intensity are more important than the size of the source. Under 3000Å most glasses and plastics do not transmit UV light. Therefore, the petri dishes to be irradiated must be uncovered. Arrange this for the least exposure to the air. The bottom of the Macalaster radiator should be wiped with a disinfectant just before covering the sample to be treated. The quartz of the lamp envelop should be cleaned periodically. Disconnect the lamp and clean the surface with soap and water. Alcohol and lens paper can be used to clean tenacious dirt.

To reduce photoreactivation, avoid working in sunlight or near sunny windows since daylight contains photoreactivating light. Room lights are not so bad, but fluorescent lamps are worse than incandescent ones in this regard. Samples may be protected before and after treatment by covering with opaque cloth or storing in a dark cabinet. They should be incubated in unlit ovens.

Common measures of biological effects are based on the expressions of modified biological activity produced by the treatments. For bacteria and fungi the ability to grow (viability) is a convenient measure, but one may need to distinguish between "colony-forming-ability" (which is macroscopic) and "ability to divide once" (which is microscopic) as measures of survival. Growth may be measured by increased optical density, confirmed by serial dilution and plating. Mutations can be identified by such features as changes in colony morphology, need for certain substrates in the medium, susceptability to antibiotics or to viruses, and the like. The ultraviolet radiation which brings about the mutations also kills the cells, so that it will also kill some of the mutants. If mutants are plotted as "mutants/100 original cells" a curve parallel to the survival curve is seen at all but low dosages (short exposures). Therefore, "mutation frequency", that is, the number of mutants/100 survivors should be used. It is not clearly understood why mutation reaches a plateau at higher dosages of UV light since this does not happen with X-rays. It seems to be related to UVL-sensitivity and resistance of the mutants.

Ultraviolet light has two major effects on organisms. First it denatures proteins, breaking their hydrogen bonds and leaving them in the primary form so that they cannot function as enzymes. It is this activity that encourages arc welders to wear eye shields lest the ultraviolet radiation from their torches coagulate the proteins of the cornea of the eye. The second effect is upon the nucleic acid. It

has been found to cause intrastrand dimerization of thymine and the hydration of cytosine. If the damage is small, the chromosome can bring about a healing of the break with some changes in sequence (mutation) but, if it is extensive, the disruption, together with the denaturation of the protein, will bring about the death of the cells.

In this exercise the experiment could be carried out with a number of microorganisms, but Serratia marcescens has been selected because it is brightly colored (red) when grown in the temperature range 28-30°C. This not only makes it easy to spot colorless mutants, but adds interest to the cultures for the student.

The asceptic techniques required to pursue this experiment are introduced in Exercise 5. The aliquots of culture are irradiated for various amounts of time. This helps determine the LD_{50} (the lethal dose for 50% of the organism) and at the same time should show increasing mutant colonies with increasing irradiation. One can, thus apply the inductive canon which says that if the cause and the effect vary together, they must be related. In any event, it would not be very convincing evidence if the experimentor only irradiated one aliquot for one amount of time, at least no more convincing than the average value of a single item. Since the cultures normally will be colorless if grown at 37°C., the appearance of colorless colonies at 28°C. would be one kind of mutant and the appearance of red colonies when cultured at 37°C. would be another kind of mutant. Organisms from each of these kinds of colonies are subcultured in milk (an easy culture medium to prepare) to check for adaptation vs. mutation. An alternative would be to transfer colonies from the plate to sterile broth and then streak other agar plates. This would produce discrete colonies.

Eyes must be protected from exposure to the UV light. The Macalaster unit has the radiant source in a housing supported by a clear plastic cup. When the lamp is on, the unit must always remain with the plastic cup down. It is also best to place the unit in a container which absorbs UV light to reduce reflection. A white porcelain pan, a glass plate, or a large fingerbowl is good. The plastic cover will absorb all of the UV light. Metal surfaces give high percentages of reflection. In fact, the white procelain will reflect about 63% of the UV light at 3650Å, so it can only be used at the shorter wavelengths (2537Å). Flat black Egyptian lacquer reflects 5%, glass 4% and human skin 3.5% at (2357Å). If possible, UV-absorbent goggles, or regular glasses should be worn when near the lighted lamp.

MATERIALS AND EQUIPMENT

Chemical

Milk (fresh or reconstituted from dry powder)
Nutrient broth or trypticase glucose broth
Trypticase glucose agar
Disinfectant (Mercuric chloride for tables, detergent for pipets).

Biologicals

Culture of Serratia marcescens

Plastic and Glass Ware

Pasteur pipets

Measuring pipets (sterile, disposable, individually-wrapped) in these sizes:

144 10-ml. in 1/100 ml. marking
144 5-ml. in 1/100 ml. marking
144 1-ml. in 1/100 ml. marking
1 case Petri dishes (disposable plastic preferred)
240 Test tubes with plastic slip-on caps

Other

Macalaster Ultraviolet Germicidal Lamp (2537 Å)
Autoclave
Incubator cabinets at 30 and 37°C. (30°C. is near room temperature)
Pipet cans (if glass pipets are used)
Pipet jars for receiving used pipets (even if disposable pipets are used)

PREPARATIONS

Agar Slants. Dissolve nutrient agar or trypticase glucose agar according to the directions on the container. If heating is necessary (and it usually is), do so in a double boiler. Dispense into culture tubes, filling them about half way. Plug and autoclave. On removal from the autoclave lean the tubes against a rod or other culture tubes so that a slanted surface is formed. When cool, store in the refrigerator. Streak one with bacteria for each student team.

Water blanks. It will be noted that water blanks accurately measured before autoclaving usually end up short about 5-10%. Check the volume of a tube containing 9.5ml. water before autoclaving and after autoclaving and make the water blanks so that they will come close to having 9.0 ml. (and a few with 10 ml. of water in them.

Agar Plates. The material listed in the exercise includes some bottles. These were to be filled with agar and sterilized, then made available to students at 45°C. so that they could pour their own plates. This is good experience, but we have not included directions for doing it. For this course it will be considerably more convenient and efficient to have the nutrient or trypticase glucose agar plates prepared by an assistant a few days ahead of their scheduled use so that they can "dry" a little before being used for plating.

Spreading rod. See Exercise 5.

Sterile milk medium is made from powdered milk to avoid the fat that will come out of some skimmed milk.

For other techniques see Teacher's Guide to Exercise 5.

INTRODUCTORY DISCUSSION

Teacher: Which came first, the chicken or the egg? (This may result in a debate between the more-informed and the less-informed students.) (Of course genetic changes in a pre-chicken egg resulted in an offspring that had mutated to a chicken.)

Teacher: Do you think that the genes are unvarying determinants of our inherited characteristics? (This is opinion. They may not know. If they have considered the evolution of the cell, then draw out of them that the environment exercises an influence on the expression of genes. They should be able to tell what sort of chemical and physical factors are mutagenic.)

Teacher: If we were interested in following the effect of a possible mutagenic agent, how do you think we could do it most easily? (Use a common mutagenic agent and use organisms that grow rapidly and are generally susceptable to the mutagenic agent we choose.)

The mutagenic agent we will use today is ultraviolet light, and the organisms are bacteria (hold up a tube of red S. marcescens). Now there are some precautions that must be observed if we are going to be successful. What are some of them? (The transfers must be made without contamination. Eyes must be protected from the ultraviolet light source, mainly by keeping it in an upright position. To reduce reflection ask them to work in the porcelain or glass receptacle provided when irradiating. If there are any techniques called for which you don't quite remember, refer to Exercise 5. If you still think you have a problem ask for help from the teacher or the assistant.)

PROCEDURE

One of the problems of doing an experiment like this with a large class is that movement has to be reduced to a minimum in order to reduce contamination from the air to a minimum. Therefore, it is quite essential that as many of the items as possible that each team needs to work with be placed at their workspace. There is also the problem of ventilation and temperature control. On windy days the windows cannot be open because drafts of air will contaminate the plates. One help is to keep bunsen burner flames at a small size and turn them out when not in use for a long time.

This exercise also calls for a considerable quantity of glassware, and that may be a consideration in doing the experiment. Ways of using less glassware are to increase the size of the working teams to four members. However, do not make them larger than that because the extras will be deprived of opportunities to participate in the work. A second way to reduce the glassware problem is to assign a zero time and one other time of irradiation to each group. Keep in mind that the objective in this activity is not to just show that some mutant may result if bacteria are irradiated, but to quantitate the numbers that are killed and the number of living mutants on the plates as a function of radiation time.

Remember to provide receptacles for used pipets (glass or disposable ones, since all pipets have to be disinfected). Follow the same procedure for making the workspace sterile as used in Exercise 5 (mercuric chloride or detergent). Provide receptacles for the used petri plates so that they, too, can be autoclaved before disposal. Remember, autoclaving kills the bacteria and spores on the plate, but if after sterilizing the medium is left open to the air, then a number of possibilities exist for the culture of dangerous organisms. Therefore, when the used, sterilized plates are cooled, place them in a plastic bag and tie it well before dropping it into the trash can.

TEACHER'S GUIDE TO

EXERCISE 7 -- BUFFERS AND INDICATORS

Prerequisite: Exercise 2

INTRODUCTORY REMARKS TO THE TEACHER

This is an excellent follow-up exercise to a discussion of the early oceans, which, buffered by salts and amino acids locally, could influence the shapes of proteins and thus make them active or inactive enzymes. The conditions under which the living state arose, must still be closely approximated in most (if not all) cells. Thus, protection from wide variations in pH, as well as in salinity, temperature, and radiation, is essential to the maintenance of life activities inside of cells.

In the main, the ability of cells to withstand external pH values outside of the range pH 7 to 8 depends upon the ability of the plasma membrane to pump hydrogen ions and hydroxyl ions out of the cytoplasm if they accumulate to excess of buffering capacity from diffusion inward. Many protozoa will not survive a pH value outside of 6 to 8. Bacteria do best in a pH of 4 to 8, whereas yeasts and molds prefer pH 2 to 4. In man, if the normal blood pH (7.35-7.40) falls to 7.0, "acidosis" and nerve dysfunction (coma) occur.

The fluid surrounding cells constitute the milieu interieur (internal mixture) of Claude Bernard in multicellular plants and animals. It can vary in its properties more than can the molecular composition inside of the cell, but less than the medium exterior to the multicellular body, the external mixture or milieu exterieur.

This exercise should be used before Ex. 8A (Coacervates), Ex. 10.7(Pelomyxa), Ex. 13 (Activities of Enzymes), Ex. 14 (Fermentation etc.) and Ex. 40 (Effects of Physical and Chemical Factors on Animals.)

MATERIALS AND EQUIPMENT

Chemicals

.5 lb.	Phosphoric acid (85%)
1 lb.	Sodium acid phthalate
5 lb.	Dibasic sodium phosphate
5 lb.	Monobasic Potassium acid phosphate
1 lb.	Tris - Tris (hydroxymethyl)(aminomethane) or THAM (Fisher)
5 lb.	Glycine (alpha amino acetic acid)
25 gm.	Congo red
25 gm.	Neutral red
25 gm.	Bromthymol blue
25 gm.	Methylene blue
5 gal.	.1N NaOH (Commercial preparation)
5 gal.	.1N HCl (Commercial preparation)
2 lb.	NaCl

Biologicals

100 ml. of the following:
blood plasma

milk
urine
egg white
lemon juice
tea
apple juice
carrot juice
carbonated beverage

Saliva is obtained by having the students chew paraffin.

Plastic and Glass Ware

24 stirring rods
24 burettes (50 ml.)
24 100 ml. graduated cylinders
96 100 ml. beakers
48 50-75 mm. funnels
120 dropping bottles (Barnes type)
960 test tubes, 16 x 150 mm.

Other

24 burette clamps or 12 double burette holders
24 support stands
Filter paper
24 Wax pencils
24 pH paper kits (pHydrion pH 2-12 preferred)
24 test tube brushes
24 test tube racks (vinyl-coated)

PREPARATIONS

Part B. To prepare 0.1M buffer solutions use these amounts of salts diluted to 1 liter with distilled water:

a) Phosphoric acid (ACS Reagent) 98.0 gm.
or
Phosphoric acid (85%) 115.35 gm.

b) Sodium acetate 8.20 gm.

c) Sodium phosphate
(See page 7-3)

d) Boric acid 4.81 gm.

e) Tris 12.1 gm.

f) Glycine 7.51 gm.
(refrigerate)

g) Phthalate, sodium acid 20.41 gm.

Part C

The buffer solutions are 0.1 Molar and should be prepared ahead of time.

Potassium Phthalate Buffers (.1M)

Phthalate Buffer pH2. To 50 ml. .2 M potassium acid phthalate (40.83 gm./liter) add 28 ml. .4 N HCl and dilute to 100 ml. with distilled water.

Phthalate Buffer pH3. To 50 ml. 2 M potassium acid phthalate add 21.5 ml. of .1 N HCl and dilute to 100 ml. with distilled water.

Phthalate Buffer pH4. To 50 ml. .2 M potassium acid phthalate add 50 ml. of distilled water.

Phthalate Buffer pH5. To 50 ml. .2 M potassium acid phthalate add 22.5 ml. of .2 N NaOH and dilute to 100 ml. with distilled water.

Phosphate Buffers (.1M)

Prepare .1 M dibasic sodium phosphate (Na_2HPO_4) and .1 M potassium acid phosphate (KH_2PO_4) Use these amounts per liter, depending upon the salt used:

KH_2PO_4	13.61 gm.
$Na_2HPO_4 .2H_2O$	17.80 gm.
$Na_2PO_4 .7H_2O$	26.81 gm.
$Na_2PO_4 .12H_2O$	35.81 gm.

Phosphate Buffer pH 6. To 88 ml. of .1 M potassium acid phosphate add 12 ml. of .1 M sodium phosphate.

Phosphate Buffer pH 7. To 40 ml. of .1 M potassium acid phosphate add 60 ml. of .1 M sodium phosphate.

Phosphate Buffer pH 8. To 6 ml. of .1 M potassium acid phosphate add 94 ml. of .1 M sodium phosphate.

Glycine Buffer pH 9. Dissolve 7.51 gm. glycine in about 500 ml. distilled water. Adjust the pH with NaOH to pH 9 and dilute to 1 liter.

Glycine Buffer pH 10. Bring .1 M glycine to pH 10 with NaOH.

INTRODUCTORY DISCUSSION

(Write on the chalkboard: "A constant environment for a constant function."--Claude Bernard)

Teacher: This idea is one of the great principles in physiology. What can you say about this from your own experiences? (Wait for contributions.)

Teacher: Today we want to look at some ways in which the environment of molecules within cells, and the environment around cells are kept from wide-ranging variations as a result of relatively small changes in hydrogen ion concentration,

denoted $[H^+]$.

(Name), can you tell us the formula for water and how it decomposes into its ions?

Ans. $HOH \longrightarrow H^+ + OH^-$ (1)

The ratio of decomposed to undecomposed molecules reaches a constant value so we may write this ratio as:

$$\frac{[H^+][OH^-]}{[HOH]} = K_w = 10^{-14} = 1:100,000,000,000,000 \quad (2)$$

Teacher: What is the ratio of $[H^+]$ to $[OH^-]$?

Answer: They are the same (1), so $[H^+] = 10^{-7}$ and $[OH^-] = 10^{-7}$

Teacher: It seems a bit awkard to me for us to have to keep working with the exponents. How could we write these expressions so as to get rid of the exponents?

Answer: The logarithm (to the base 10) of an exponent is that number, so log $[H^+]$ = log -7 and log $[OH^-]$ = log -7 (3)

Teacher: To avoid having to deal with a negative number like -7 what can be done?

Answer: Multiply both sides by -1 so that

$$-\log [H^+] = \log 7 \text{ and } -\log [OH] = \log 7 \quad (4)$$

Teacher: Since it is the $[H^+]$ we are usually interested in what we can designate

$-\log [\ ] = p$, and since it is the concentration of hydrogen ions, $[H^+]$, being considered, we may write

$$pH = -\log [H^+] \text{ and } pOH = -\log [OH^-]. \quad (5)$$

When the log of the concentration of H^+ and OH^- are the same, -7, a solution is neutral and the pH = 7 and the pOH =7, and the pH = 14-pOH or pOH =14-pH.

Teacher: Since the pH value is the power of the base 10, the number of hydrogen ions vary by 10-fold multiples for each whole unit. Therefore, pH 6 has what number of hydrogen ions as pH 7?

Answer: There are 10 times more at pH 6 than at pH 7.

Teacher: Now that we understand that pH means the negative log of the hydrogen ion concentration, we can go on to try to understand pK, the negative log of the dissociation constant.

Let HA represent an acid which dissociates incompletely, viz:

$$HA \longrightarrow H^+ + A^- \quad (6)$$

so that the ratio of dissociated to undissociated acid can be represented as

$$\frac{[H^+]\,[A^-]}{[HA]} = K_a \quad (K_a \text{ for acid, } K_b \text{ for base}) \qquad (7)$$

may then take the logarithm of both sides

$$\log \frac{[H^+]\,[A^-]}{[HA]} = \log K_a \qquad (8)$$

so that

$$\log [H^+] + \log [A^-] - \log [HA] = \log K_a \qquad (9)$$

$$\log [H^+] = \log K_a - \log [A^-] + \log [HA] \qquad (10)$$

If we multiply both sides by -1, then

$$+\log [H^+] = -\log K_a + \log [A^-] - \log [HA] \qquad (11)$$

and if $-\log [\ ] = p$, then

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \qquad (12)$$

The A will usually be associated with a cation like Na^+ or K^+ so that

$$\log ([A^-]/[HA]) \text{ represents the } \log \frac{[salt]}{[Acid]} \qquad (13)$$

Formula (12) is used in Method 3 in the manual for the determination of the pK where the amount of acid or base needed to titrate the buffer from the point where it is mostly acid to the point where it is known to be mostly salt. For example, when there are 10 parts of salt to one part of acid then log ([10]/[1]) = log 10 = +1. When the reverse is true so that log ([1]/[10]) = log .1 = -1. So the effective range of a buffer will be plus 1 to minus 1 pH unit (above and below the pH) of the pK value. Knowing the pK values for a buffering material makes it easy to select one which will be effective at the pH one might like to buffer a solution.

Student: But how does a buffer work?

Teacher: Amino acids and proteins exist in solution as <u>amphoteric</u> substances (also called zwitterions in this case), that is, having both acidic and alkaline properties, viz:

$$H_2N{-}R{-}COO^- + H^+ \rightleftharpoons \underset{\text{zwitterion}}{H_3\overset{+}{N}{-}R{-}COOH}$$

$$HO^- + \underset{\text{zwitterion}}{H_3\overset{+}{N}{-}R{-}COO^-} \rightleftharpoons H_2N{-}R{-}COO^- + H_2O$$

The valence of the nitrogen is 3 and 5.

When acid is added, the COO^- groups are reduced to carboxyl groups (COOH). When base is added, the hydrogen from the NH_3 group is combined with it so that water is formed until all of the $-N^+H_3$ are used up.

When a weakly ionizing salt is the buffer, the mechanism is somewhat different but the final effect is the same.

$$KH_2PO_4 \rightleftharpoons K^+ + H_2PO_4^- \rightleftharpoons H^+ + HPO_4^=$$

Hydroxyl (OH^-) ions can be added and will form water. The H^+ ions added willdrive $HPO_4^=$ back to $H_2PO_4^-$

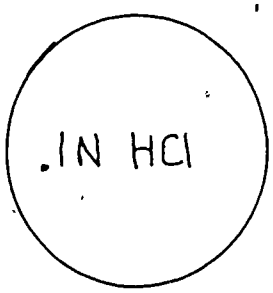
OH^- → HOH

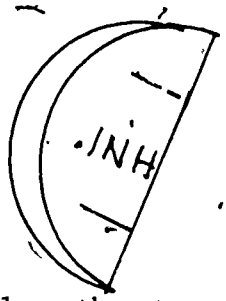
H^+ → $H_2PO_4^-$

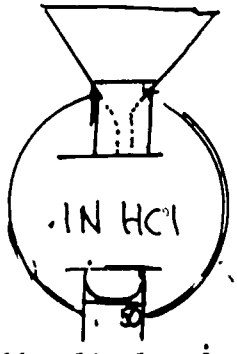
PROCEDURE

Part A. Titration of .1N NaCl with .1N HCl and .1N NaOH

The burettes may be labelled by marking a piece of filter paper with the material in the burette, then tearing two slits so that it will fit onto the burette tube.







To fill the burette: (1) Close the stop-cock. (2) Add a little of the solution to be used. (3) Let some of the solution run through into a container (beaker) so that a continuous column is established. (4) Close the stopcock and fill to the desired height. The reading is taken from the bottom of the fluid meniscus as in reading graduated cyclinders and pipettes (See Ex. 2).

Using pH paper. The pH paper recommended for this experiment is "pHydrion" brand, to cover the range pH 1 to pH 12. This comes in two rolls or vials--one with odd-numbered and the other with even-numbered pH values. The paper contains several pH indicators. If the paper is dipped into the solution being tested the

indicators will wash out of the paper and still be so dilute in the solution as to not be seen. Therefore, a drop or two of the test solution is placed on a piece of the pHydrion paper and the color then compared with the scale provided on the container.

Data and Graphing. After the data on the volumes titrated and the resultant pHs has been obtained, have students graph the data. The starting pH should be indicated on the 0 line. Label the line "0.1 N NaCl".

Part B -- Titration of a Buffer with .1 N HCl and .1 N NaOH

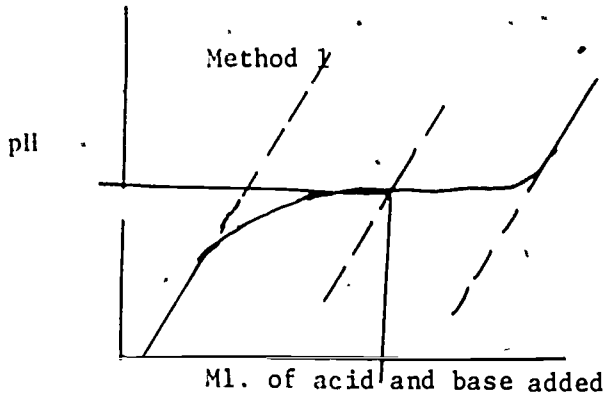
The title to part A obviously is too inclusive since the actual titration of the buffer occurs in Part B.

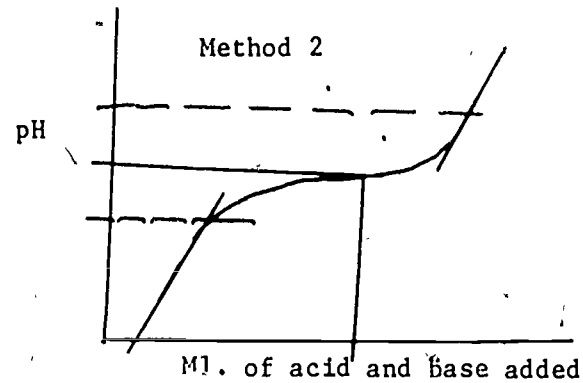
PROCEDURE

We suggest that a sufficient quantity of the buffer assigned be placed at each workspace or on each worktable (100 ml./pair of students). The use of different buffers for different groups provides a widened opportunity to observe the different shapes which titration curves will take. It also reduced the probability of "dry-labing".

After the titration data has been accumulated, it should be graphed on the same graph as the .1 N NaCl, and the line labelled with the buffer used.

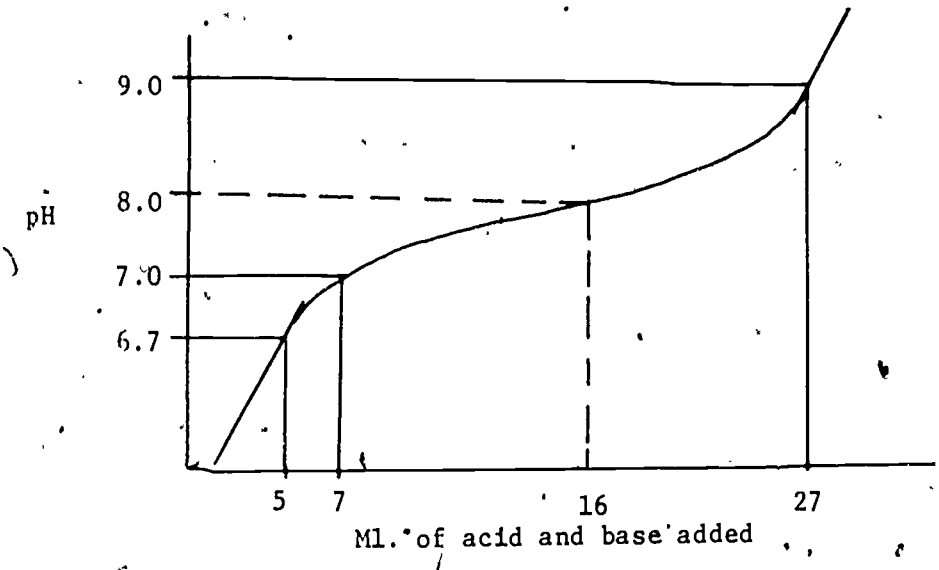
Determination of the pK. Method 1 is to find the slopes of the unbuffered part of the curve and determining the midpoint.





In method 2 horizontal lines are drawn through the end of the unbuffered portion of the curve and the line midway between these pass through the pH of the pK.

Method 3 can only be used where the curve is not completely flat. If the total amount of acid and base needed to get from the acid form to the salt form is known, then the log of the ratio of salt to acid falls on the buffered part of the curve if the Henederson-Hasselbalch equation (Equation 12) is applied.



Example. A total of 27 ml. of .1 N acid and base was needed to get from the acid to the salt end of the buffered curve. Let us assume that the inflection of the curve began after the titration of 5 ml. of base. After 7 ml. the pH was 7.0.

Then

$$pK_a = pH + \log [salt]/[acid] \quad (14)$$

$$= 7.0 + \log [7\text{-}5 \text{ ml}]/[27 - 7] \quad (15)$$

$$= 7.0 + \log [2]/[20] = 7.0 + \log 1/10 \quad (16)$$

$$= 7.0 + 1.0 = 7.0 + 1.0 \quad (17)$$

$$pK_a = pH\ 8.0 \quad (18)$$

Part C -- Pot Pouri

Buffer solutions for Part C can be placed on the central supply table. The dyes (.5% Congo Red, .5% Neutral Red, 1.6% Bromthymol Blue and .05% Methylene Blue are most conveniently used when placed in labelled Barnes-type dropping bottles and a set provided at each workspace (pair of students).

In Step 4 use 1% dry egg albumen. Fresh egg white diluted 5 parts distilled water to 1 part albumen may be used.

Mix and filter albumen through cheese cloth or cotton to remove strands of insoluble material. The isoelectric point (pI) for egg albumen is at pH 4.7.

Part D -- The pH of Common Fluids of Biological Interest

After the strips of pH paper have been dried, they should be cemented to the report sheet with rubber cement. Rubber cement contains no water to dissolve or change the materials (colors) in the strips. Also the strips can be removed. Excess cement is easily rubbed away.

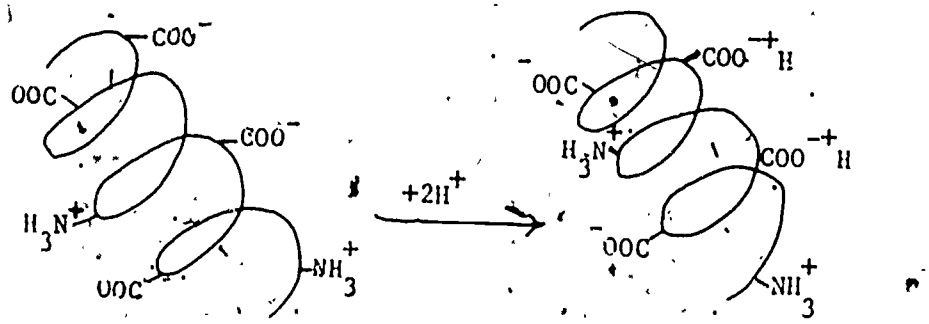
TEACHER'S GUIDE TO

EXERCISE 8 -- COACERVATES AND EMULSIONS

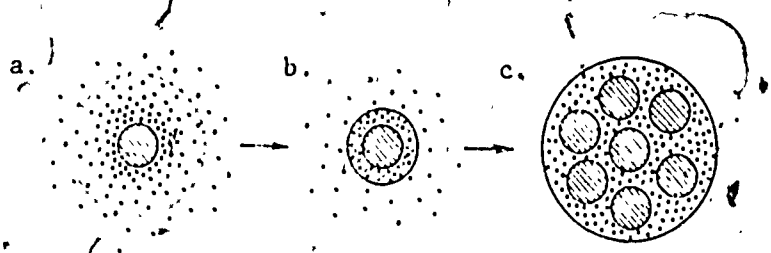
Prerequisite: Exercises 2, 4 and 7

INTRODUCTORY REMARKS TO THE TEACHER

This is an excellent follow-up to a discussion of how proteins and nucleic acids in the primitive oceans could have formed coacerates. Proteins are charged molecules which become electrostatically neutral at their isoelectric points (I_p) that is, the pH at which the H^+ in the medium balance off the $-COO^-$ ions that contribute to negativity of charge. If a protein is positively charged due to an excess of $-NH_3^+$ groups, $-OH^-$ groups will neutralize them at the I_p.



There is normally a shell of water molecules around charged proteins. At the I_p this shell is reduced enough to allow protein macromolecules to come close enough to agglutinate. This phenomenon is very much dependent upon being at the isolecric point of the protein.



a. A charged protein or other colloid will attract a cloud of water dipoles about it. b. At the Ip the protein is electrostatically neutral so that less water is held about it. It is literally dehydrated. c. Several colloidal particles have been brought close enough together to be held by short range forces and their water shells coalesce to form a coacervate.

MATERIALS AND EQUIPMENT

Chemicals

.1N HCl (See Ex. 7)
1 lb. Gelatin
1 lb. Gum arabic
1 pt. corn or other vegetable oil
5 gm. Sudan III or Sudan Black B
2% lime water (calcium hydroxide) 100 ml.
soap solution

Biologicals

milk
butter

Plastic and Glass Ware

Microscope slides and coverglasses
48 10-ml. or 5-ml. pipets
24 medicine droppers
144 test tubes

Others

24 Microscopes with lamps
144 test tube closures (Ex. 5)
24 test tube racks

PREPARATIONS

5% Gelatin (for the class). Soak 10 grams of gelatin in 200 ml. cold (room temperature) distilled water for 15 minutes, then heat in double boiler until dissolved. Gelatin is a protein and, therefore, "relished" by bacteria. When not in use, store it in the refrigerator.

5% Gum Arabic. Grind 10 grams of gum arabic in a mortar. Mix with cold (room temperature) distilled water and let soak for 15 minutes. Warm in a double boiler to dissolve.

Sudan III. Prepare a saturated solution of dye in acetone.

INTROCUTORY DISCUSSION

The objective of this discussion should be to illustrate how some bodies are attracted and others repelled by electrostatic forces. This is very well-demonstrated by an electroscope (You may have to borrow such a device from the Physics Department). When both leaves of the electroscope have the same electrostatic charge they are repelled, if they are uncharged, they will be attracted to each other. Demonstrate these phenomena for the class without explanation and ask them how it works.

For a more biological example, obtain an overhead projector. Place two Syracuse watch glasses thereon. Add some blood (of type A or B) that has been diluted with 9 parts of saline to one of blood, to each dish. Add some anti-A typing serum to one dish and some anti-B typing serum to the other. Stir with an applicator stick or stirring rod. One will agglutinate and the other will not. Get reactions from the class as to what is it that makes the agglutination reaction occur (but accept nothing as simple as the "the antibodies make the cells stick together, rather, why do the antibodies make the cells stick together?) Would it be possible to find a pH at which the red blood cells might stick together without the aid of antibodies? As a matter of fact, the antibody protein provides the neutralizing charges that permit attraction of the cells.

The concept of attraction and repulsion is also applicable to the behavior of materials in the formation of emulsions where charge also determines which will be the dispersed phase.

PROCEDURE

Part A

In preparing the coacervate suspension, add the acid dropwise. Look carefully, the cloudiness may be very slight. In any event, don't look for a heavy precipitate. Shaking vigorously denatures proteins, but in this case it will also disaggregate the coacervates. These are little globules about 10 microns across. They seem to have an interfacial membrane, but it may be difficult to decide about that. They look very much like small bubbles, but bubbles are not usually so evenly distributed in the liquid medium, nor are they usually so small. The coacervated material may be seen better if the iris on the microscope is closed somewhat to reduce the amount of light.

In Part B the Sudan stains work as colorants and not as dyes. They are more soluble in fats than they are in acetone so that they accumulate in fatty materials. The presence, then, of Sudan stain localizes lipid material and makes it possible to see whether the oil is in the aggregated or dispersed phase.

REPORT SHEET

Part A -- Coacervates

2. Gelatin and gum arabic most likely were absent from the early oceans, but other amino acid polymers (proteins) and carbohydrate polymers as well as nucleic acids were present and could aggregate into coacervates since the laws governing that behavior have not changed.

3. The iso-electric point is the pH at which a protein is electrostatically neutral because H^+ or OH^- ions in solution balance off the excess positive or negative charges on the protein. At the Ip conductivity is least, precipitation is greatest, electrophoretic mobility is zero

TEACHER'S GUIDE TO

EXERCISE 9 -- WATER CONTENT OF TISSUES AND CELLS

Prerequisite: Exercise 2

INTRODUCTORY REMARKS TO THE TEACHER

The determination of water content of biological materials is a routine biochemical procedure. Living cells originated in the sea and even to this day are predominantly water. Some connective tissues, such as adipose and bony tissue have low water content as a whole, but their cell cytoplasm per se is comparable with other cells. Water is important because:

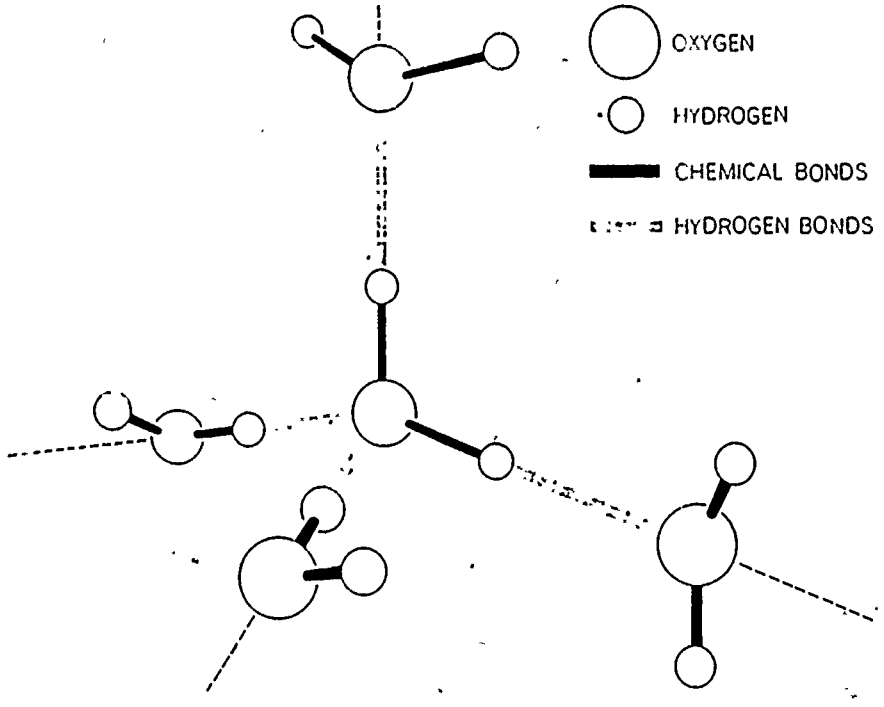
1) it ionizes and therefore contributes to pH.
2) many substances dissolve in water.
3) it holds and conducts heat. Water has a high specific heat.
4) it is a poor conductor of electricity in its pure form but solutions of salt are good conductors.

Many living organisms cannot tolerate more than about a 25-33% water loss.

In this exercise the class will investigate and compare the amounts of water to be found in animal, plant and microbial tissues.

Some of the properties of water are currently explained on the basis of what we know about the structure of water molecules. The shape of the water molecule is that of an isosceles triangle with the oxygen at the apex. The sides, leading to the hydrogen atoms, forming an angle of 105° have a length of nearly .99 Angstrom units. The valence charge of +2 on the oxygen atom tends to draw the electrons from the two hydrogen atoms into its outer shell so that these electrons become shared between the atoms and thus form hydrogen bonds with the oxygen. This is the water dipole. The powerful attraction that the oxygen nucleus has for the electrons of the hydrogens leave it more negatively charged and thus most of the positivity of the hydrogen proton becomes apparent. The positive and negative regions of adjacent water molecules are attracted to each other so that they tend to become arranged into tetrahedrons with an oxygen atom at each apex. The distance between the oxygen atoms, as determined by X-ray crystallography is 2.76 Å.

When water is frozen, nearly all of the water molecules are arranged in these continuous tetrahedrons. The heat of fusion (80 calories/gram of ice) measures the energy needed to break about 15% of the bonds forming the lattice work of tetrahedrons held together by the hydrogen bonds. The hydrogen bonds between the water molecules are also responsible for the phenomemon of surface tension and many of them persist right up to the boiling point. The heat of vaporization at sea level and atmospheric pressure is about 520 calories/gram, that is, some energy is needed at boiling to break intermolecular hydrogen bonds which still persist at that temperature.



A tetrahedron of diplar water molecules bonded by short range forces to a proton. This results in the ions $H_9O_4^+$ and OH^-.

Frank, Henry S. The structure of ordinary water. Science 169:635-641, 1970 64 references. (14 August 1970)

The structure of cold water seems likely to consist, for the most part, of hydrogen-bonded, four-coordinated, framework regions, with interstial monomers occupying some fraction of the cavities the framework encloses. The electrons may be represented by a charge cloud which has, in addition to the appropriate density along the bonds, lobes comprising the so-called lone pairs, which extend above and below the H-O-H plane and are directed somewhat backward away from the hydrogens so that the whole structure can be represented by a somewhat distorted tetrahedron, the protons directed toward two of the vertices, and the lone pairs toward the other two.

Allen, Leland C. and Peter A. Kollman, A theory of anomalous water. Science 167:443-1454, 1970. 46 references (13 March 1970)

The principal structural unit of anomalous water is a symmetrical O-H-O bond with a "strength" very close to that of the hydrogen bond in liquid water (5 kcal) and a O· · ·O separation of 2.30 to 2.40 Angstroms. These structural units are combined into sheets of hexagons and the sheets in turn are interconnected by O-H-O bonds of slightly less strength and 15 percent greater length to form a neutral three-dimensional lattice. All the oxygens are four-coordinate.

MATERIALS AND EQUIPMENT

Biological

carrots, potatoes, onions, spinach leaves, dry beans, bean sprouts, yeast cake, liver, kidney, tallow, bone, heart (30-50 grams of one of these for each student)

Other

6 kitchen knives
3 bone saws
6 triple beam balances (Ex. 2)
Aluminum weighing pans (Ex. 2)
Drying ovens at 100-110°C

PREPARATIONS

Teachers can make substitutions of other materials available locally. However, the materials should be representative of animal, plant and microbial material and have included among them some low-water-content items such as seeds, nuts, dormant twigs, bone, or adipose tissue (suet, tallow, etc.) When buying bone, ask for soup bone and have the butcher cut it into one inch pieces. The student should remove the marrow before weighing. The water content of bone marrow may also be determined.

Leafy materials (spinach, grass, etc.) should be fresh and unwilted.

INTRODUCTORY DISCUSSION

True Story: Al Capp in his comic strip "Li'l Abner" once had all of Dogpatch fighting over a chest which Li'l Abner claimed had the most valuable thing in the world inside. After Injun Joe and his pals had devastated everybody else, they forced the chest open.

What do you (the students) think was inside? (They will probably say money, jewels, kickapoo joy juice, etc.) Actually the chest seemed empty, so they asked Li'l Abner where was the most valuable thing? He told them that it was air!

Question: Is air the most important thing for life on earth? (For higher organisms modern air is very important, but there are many anaerobic organisms.) Is there any one substance needed by all living things that are alive? (Water) Why is this so? (Because living material originated in the sea.) If that is so, how much water would you expect living tissues to contain? (They should not know. Perhaps someone will guess in the range 70 to 80%.)

Now fill a beaker nearly full of tap water and place it where all can see. "There it is, the 'most valuable thing in the world'. But, what do we know about water?"

Probable answers may include:

It freezes.
It will evaporate.
It boils.
It quenches thirst.
Things dissolve in it.
It is a compound made of hydrogen and oxygen.
It ionizes to H^+ and OH^-.

This is a good point to introduce information about the lattice-work structure of ordinary water and how it excludes solutes from the lattice when water freezes.

PROCEDURE

Since the weights will be taken only to the nearest milligram, it is not necessary to handle weighed containers with forceps or to keep them in a desiccator when cooling them, as done whe. doing more careful analytical work. Each student should use the same balance for making all of his weighings. For this reason, balances should be conspicuously numbered and the student should record the number on his Report Sheet.

The temperature of the oven should not exceed 110°C. or the materials may char, that is, materials other than water will be given off and thus modify the weight. Some items, like spinach leaves, will dry within 2 hours, but most things should be left overnight. Have students come back, perhaps at noon the next day, or at some other convenient time, to take the dry weights of their samples.

Each student should prepare three samples of the same material and compute the standard error (of the mean deviation) of the water content for his three samples. With such small numbers of replications, the mean values may not always fall within the standard error for all the groups of the same material because of individual differences in weighing skill and also perhaps because of systematic errors due to the working performances of different balances.

. At the next laboratory meeting have students put the information called for on the answer sheet on the chalk board, and then copy the class results on page 9-3 of the manual.

REPORT SHEET

Questions

1. Cells originated in the ancient oceans where water formed the major phase for their assembly. Since the conditions for the living state could not vary much from the original, the concentration of other components remains low. Water is needed for buffer formation, for dissolving compounds, for absorbing heat, for transporting heat (especially for cooling), and it has other functions. Water is also a necessary component for enzyme action, and enzyme action is essential for storing and releasing energy in foods. This energy in turn is needed for life activities.

2. What is meant by bound and free water? Bound water is associated electrostatically with proteins and other molecules and does not evaporate easily. Free water evaporates easily because it only fills spaces between the other molecules in the tissue.

3. Why do solutes comes out of water solutions when they freeze?
Water molecules are dipoles which form hydrogen bonds with each other resulting in a lattice work. When the temperature is above freezing most of the lattice is broken and the solutes are held in solution by small aggregates of water dipoles. When water freezes, the water lattice is almost complete, leaving no place for solutes to be held. Solutes, therefore, are excluded from the lattice and are "out of solution". Solutes redissolve when the ice melts.

TEACHER'S GUIDE TO

EXERCISE 10 -- CELL TYPES

Prerequisites: Exercises 4 and 7

INTRODUCTORY REMARKS

This is an exercise which requires that the student gain some skill in the use of the microscope before he attempts to use that instrument as a tool to aid him in the study of cells (Ex. 4 and 8). It should be emphasized that the student must learn how to handle, manipulate and view materials in the microscope before beginning this study. It has been a common error to try to have students learn to operate the microscope (without understanding it) while also trying to study live, fast-moving protozoan cultures. The result is that in most cases the student neither learns how to make observations through the microscope nor does he learn much about the objects he is asked to look at and study. The study of cells in this exercise requires that the student manipulate his microscope with knowledge and confidence in order to direct his attention to the study of the cells.

MATERIALS AND EQUIPMENT

Chemicals

1 lb. Petrolatum
Janus Green B
100 ml. Methyl cellulose
Neutral red
95% Alcohol

Biologicals

12 slides of mammalian liver
12 slides of mitochondria (liver)
12 slides of Golgi complex
12 slides of leaf types
12 slides of sea urchin eggs (developing)
12 bull sperm
12 slides Ascaris megalocephla, sperm entrance

Living cultures for class of 25
Elodea sprigs
Nitella
Pelomyxa (Chaos chaos)
Stentor
E. coli
Fresh onions
Baker's yeast

Plastic and Glass Ware

2 gross clean slides and coverglasses (see Ex. 4)
24 syracuse watch glasses
24 medicine droppers
12 2-cc syringes without needle (for petrolatum gun)

Other

24 microscopes and lamps
24 camel hair brushes (small)
12 bunsen burners
24 nichrome wire loops
24 forceps and disseciting needles
12 single-edged razor blades
pieces of bibulous paper or paper towelling

PREPARATIONS

Microscope slides and coverglasses. Some slides are sold pre-cleaned, others are coated to prevent them from sticking together. These latter should be washed in detergent and rinsed two or three times in tap water to remove all detergent. Both kinds of slides should be handled at the edges and on one end (later used for labelling). Coverglasses are treated in a similar way.

Vaseline guns are made by melting petrolatum (e.g. Vaseline) and drawing it up into a 2-ml. syringe (without needle.) With a little practice a neat line of petrolatum can be squeezed out. The preferred way of holding the syringe is by placing the thumb and first two fingers on the barrel with the plunger operated by pushing back against the palm of the hand.

Supravital Staining. Some species of amoeba do not tolerate Janus Green dye very well. Check your culture on a slide prepared for this activity before the class meets. If it is killed by Janus Green, use only the neutral red..

PROCEDURES

Begin by demonstrating for the class the various ways of making the temporary slide preparations to be used this laboratory period. The film loops, Ealing 81-080 and 81-0879* should be used at this time and the students given instructions for the operation of the film loop projector.

The prepared slides should be assembled in a small slide box and made available, one per student or one per two students if they are in short supply. Point out the location in the laboratory of the various cultures (especially if they are somewhere other than on the demonstration table).

Drawing should be big enough to show details easily. Labelling should be printed by hand, usually to the right of the drawing, and guide lines should end exactly on the structures they are intended to help identify. However, do not put so much stress on these mechanical aspects of making and labelling the drawing that the overriding importance of making careful and accurate observations is diminished.

* Ealing 81-080 (Histological Techniques - Wet Mounts)
Ealing 81-0879 (Microscopic Technique Using A Microscope)

Finally, have a student bring a microscope from the cabinet, clean the glass surfaces before instructing the class to obtain their assigned microscopes.

Have the students do the living material first. Then if they do not finish the prepared slide material they may return at some convenient time for further study.

REPORT SHEET

Drawings should be placed above the legends. The numbers correspond with the items in the exercise. Students should indicate the magification of the microscope at which the observations were made.

Questions

1. The cell theory states that "all living things are cells or made of cells (and their products) which come from pre-existing cells."

2. The implication of the cell theory are:

   (a) life is found only in living cells as a whole. No cell part can carry on all the functions of aliveness by itself.

   (b) all present day organisms have come down from or evolved from the first real cell or cells.

   (c) even the first real cells came from pre-cells.

3. The course definition should be as inclusive as possible of all structures accepted as being cells by the great majority of biologists. This, or some other equally inclusive statement should be used:

   "A cell is a more-or-less enclosed mass of protoplasm containing nuclear material." Note: Such a definition or description does not include the viruses, nor does it include cell fragments such as mammalian blood plateles and red blood corpuscles.

   All known cells conform with this description (Viruses do not contain protoplasm; red blood corpuscles have lost their nuclei as have some of the cells in the outer layers of stratified squamous epithelium).

4. The arguments for calling bull sperm "cells" are:

   (a) they have nuclear material.

   (b) they have a limiting membrane.

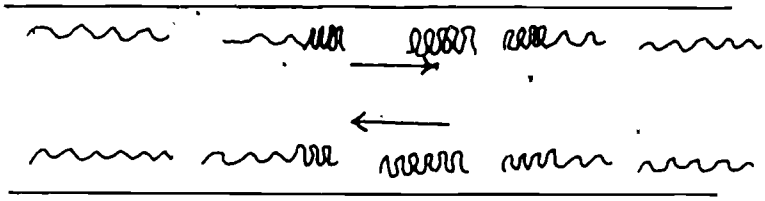
   (c) they are alive (when alive)

   (d) they aslo have other cell parts--cytoplasm, mitochondria, ribosomes, Golgi apparatus and centrioles.

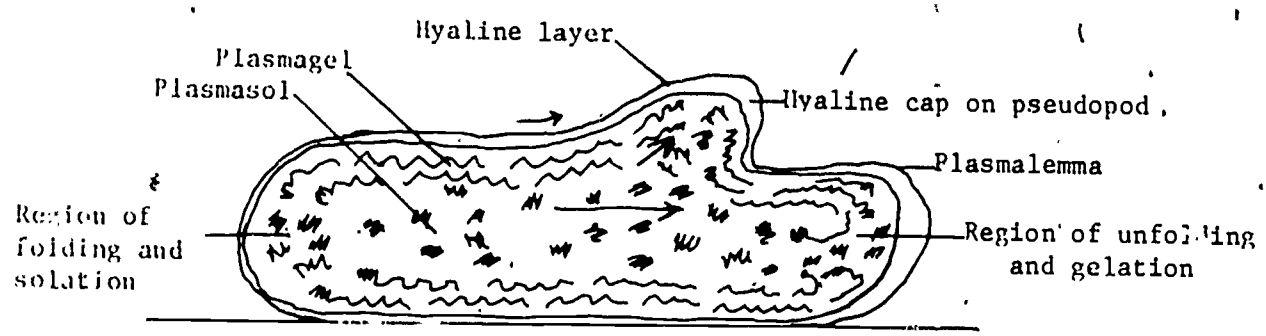
5. There are no centrioles in unfertilized animal eggs. They are present in

fertilized eggs. These normally come from sperm, but experimentally they may come from other cells. For example, if a frog egg is pricked in the presence of frog blood a blood cell centriole will trigger mitosis and "parthenogenetic" development.

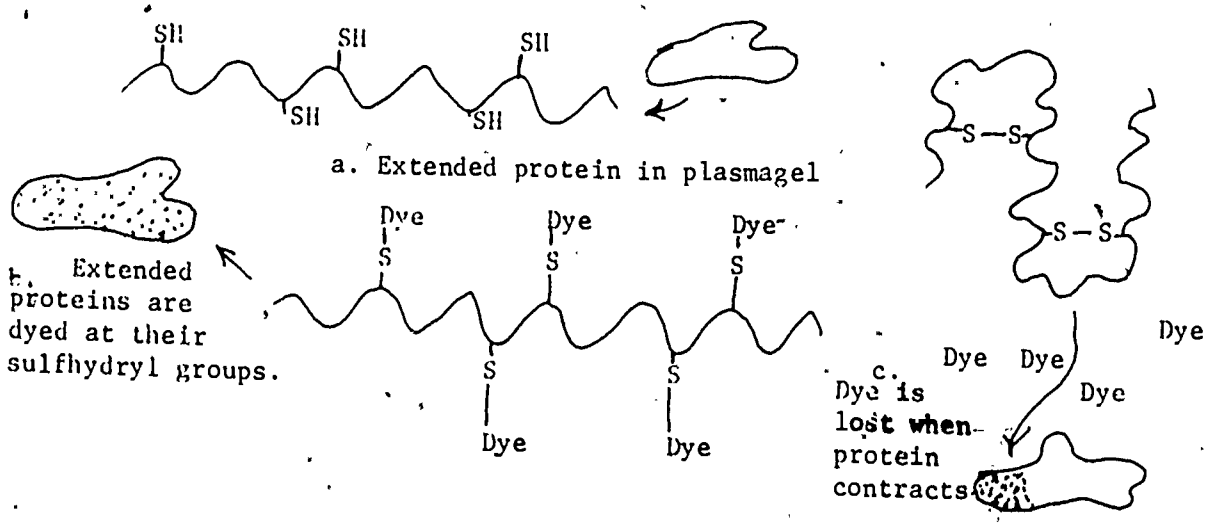
6. Explanations of streaming in Nitella (or other cells). The transformation of proteins from the tertiary to the secondary form and vice versa seems to be the basis for this phenomenon.



7. Amoeboid movement also depends upon the transformation of proteins from the secondary to the tertiary forms and back again.



Would the location of Neutral Red staining after several minutes back up this view? Yes.



TEACHER'S GUIDE TO

EXERCISE 11 -- TYPES OF FOODS FOUND IN CELLS AND TISSUES

Prerequisite: Exercises 1 and 2

INTRODUCTORY REMARKS TO THE TEACHER

When the food manufactured by cells, or when the amount taken in is, in either case, more than is metabolized by the cell for energy release, the excesss is usually stored in the same cells or transported in simple form to some other cells for storage. Plant cells usually have a vacuole and the food is stored by secreting it through the vacuolar membrane, and it accumulates in the vacuole in simple form, as a rule. Consequently, we can extract fructose from the juice of fruits and sucrose from cane, sorghum and sugar beets. Many times, however, a polymerizing enzyme is secreted into the vacuole to order the polymerization of glucose into amylose and amylopectins, and finally into starches. In animals the ingestion of an excess of starch leads to the accumulation of glycerol and fatty acids and their combination into fats. Feeding livestock starch-laden grain is a traditional folkway of getting them fat and thus improving the flavor of the meat. The basic fuel that these foods provide is an energized hydrogen atom. As the hydrogen is oxidized to water it gives up its energy in the respiratory chain to energize adenosine diphosphate (ADP) to adenosine triphosphate (ATP). This ATP is used in a variety of ways to provide energy for such cell processes as osmotic work, muscular contraction, and the activation of chemical reactants. People, and other heterotrophic organisms eat these stored foods and break them down to their simplified components before absorbing them for their own nutrition.

In this exercise the student is asked to find out what kinds of foods do different dietary items contain, and to determine which tests may be differentiating between these various foodstuffs.

MATERIALS AND EQUIPMENT

Chemicals
1% glucose
1% starch
1% egg albumin
1% glycine
corn oil
magnesium sulfate
sea sand (fired)
acetone
Lugol's iodine solution
Benedict's quantitative solution
.25% Ninhydrin in 1 M Phosphate buffer
Biuret reagent

Biologicals
Irish potato
Turnips
Peanuts
Liver
Chicken breast muscle

MATERIALS AND EQUIPMENT (Cont.)

Glass and Plastic Ware
36 Test tubes
400-600 ml. beaker
Mortar and pestle
250 ml. flask
Small funnel
Pipets
Dropping bottles

Other
Test tube racks
Hot plate (or Bunsen burners)

PREPARATIONS

Lugol's Iodine Solution

To a 1% KI solution add 1 gram of iodine crystals for each 100 ml.

Benedict's Quantitative Sugar Reagent
This may be obtained commercially prepared or made up by mixing:

Copper sulfate (crystallized)	18.0 gm.
Sodium carbonate	200.0 gm.
Sodium or potassium citrate	200.0 gm.
Potassium thiocyanate	125.0 gm.
Potassium ferrocyanide (5% solution)	5.0 ml.
Distilled water to make 1000 ml.	

Buffered Ninhydrin Reagent

Buffer. Mix 4 parts 1 M KH_2PO_4 with 6 parts of 1 M Na_2HPO_4. Check the pH and adjust it to 7 with HCl or NaOH.

To 100 ml. pH 7 buffer add 0.25 gm. ninhydrin. Store in a brown bottle covered with aluminum foid and keep in the refrigerator when not in use..

Biuret Reagent

Add 1% copper sulfate dropwise with constant stirring to some 40% sodium hydroxide solution until the mixture takes on a dark blue color. If stored in a bottle with ground glass top, apply petrolatum to the stopper to prevent it from sticking.

In setting up the work stations, supply one boiling water bath (large beaker of water on an electric hot plate) for each two pair (4) students to keep from overheating the laboratory. Reagents, too, can be supplied at the workspace at the rate of one bottle for each two pair of students.

Other materials, such as test tubes, racks, etc., should be supplied for each pair of students. The solutions of starch, glycine, egg albumin should be supplied for each two pair of students in order to prevent traffic jams at the demonstration table. Since groups will finish Part A at different times, the potatoes, turnips, peanuts, liver and chicken muscle cut into small bits, can be placed on the demonstration table for the convenience of the teacher.

Many teachers will be familiar with these tests and with the fact that they are almost always presented to the student as a test for one or two items which react positively to the reagents. In this exercise the student will be doing the test on certain food items in an attempt to discover which procedures give diagnostic tests for which food items.

INTRODUCTORY DISCUSSION

Place a candle in a pneumatic trough. Let it burn in the open for a minute or so and then ask students what is going on. Talk out the fact that the heat melts the paraffin or wax and that the flame ignites the resulting oil. Burning is a condition of rapid oxidation. Now place a glass cover over the candle so that it extends into the water. As the candle burns up the oxygen in the contained air, the water will rise in the glass. The flame finally goes out. Discuss this effect. Now uncover the candle and relight it. Just as the clandle must have paraffin, so the cell must have foods. Later we shall see that this is really so when we do Exercise 41, or 32 to 34. In this exercise we will try to find out which forms of fuel are to be found in cells and what are some of the tests for determining their presence.

PROCEDURE

Proceed as indicated in the student manual.

REPORT SHEET

Questions

1. In what forms will food be stored in cells and tissues?
(Carbohydrates will be stored as free sugar, starch, or glycogen. To some extent as gums, pectins and cellulose in plants. Proteins may sometimes be stored as crystalline protein, although some free amino acids occur in tissues and in tissue fluid and blood. Fats will be stored as fat, phosphlipid, or cholesterol and cholesterolesters. Because carbohydrates, fats and proteins can be converted to each other by the cellular metabolism some excess food may be stored in other than its original group. For example, too much carbohydrate may be stored in part as fat, and less of it may be converted to amino acids and proteins.)

2. "Animal starch" is also called glycogen.

3. An excess of carbohydrate in animals most usually is deposited as fat, but also some will be deposited as glycogen or as protein.

TEACHER'S GUIDE TO

EXERCISE 12 -- DIFFUSION, OSMOSIS AND ACTIVE TRANSPORT

Prerequisite: Exercise 2, 4, 7 and 10

INTRODUCTORY REMARKS TO THE TEACHER

The "invention" of the plasma membrane (or unit membrane) converted the community of proteins, nucleic acids and other molecules in the ancient oceans into a real cell. Where before this mixture of molecules carried out metabolism and reproduction held together only by the short-range forces between molecules, now there was a membrane to prevent the scattering of vital enzymes and genes. The precell was in the environment and the environmental fluids permeated the community of molecules that made up the precell. The membrane, however, was not a simple sieve. Whole molecules pass it better than do ions in general, and some molecules in the fatty acid series are more easily passed than are some other smaller ones.

This exercise deals with evidences of passive and active passage of materials across cell membranes and its major objective is to leave the student with data to support the idea that the cell membranes are not the same as non-living membranes, such as a cellophane sheet.

What makes one substance diffuse through another? What is the driving force that makes perfume pervade a room, smoke disappear (?) into the air or salt to season food? The Kinetic Theory says that molecules are moving with an energy (the gas constant, R = .82 degree/mole) that increases with the absolute temperature. This movement occurs in all directions but there is a greater movement away from higher concentrations with some molecules "pioneering" through the medium until they reach a barrier they cannot pass. Then, after a time, the concentration of the solute will become uniform throughout the mixture and the molecules move still in all directions but the concentration becomes uniform and diffusion (a dynamic process) becomes zero.

Cell membranes are barriers to some substances but not to others, that is, they are permeable to some substances but are impermeable to those they do not pass. Therefore, diffusion will occur through a membrane if it is permeable to a solute because the membrane presents no barrier to it.

The cell membrane is not a simple sieve, although it behaves as if it has holes about 3Å in diameter so that hydrated potassium ions (K^+) which are about 2.8Å pass easily, but hydrated sodium ions (Na^+) with diameter of 3.4Å do not. When membranes are "at rest" they are very busy pumping Na^+ out of the cell and thenegatively-charged cell proteins are electrostatically balanced by the passive inflow of K^+. When membranes are adequately stimulated the sodium pump is reversed by the stimulus so that sodium ions enter the cell and potassium ions leave passively, but within milliseconds the pump operates again and the sodium ions are dispatched into the surrounding medium.

The membrane in living cells uses energy stored in energy-rich phosphate bonds for this work. Phosphatidyl serine and other amino acid-phospholipid compounds are the carriers for ions. Adendosine triphosphate activates amino acid and sugars for passage.

for passage.

In this exercise the student will make observations on osmosis and diffusion using a pH indicator dye, and follow the degree of water loss from potato plugs by weighing and measuring their volumes. A qualitative demonstration of exclusion of a dye molecule is used by showing that by depriving the membrane of its energy sources the membrane will permit the dye to pass.

MATERIALS AND EQUIPMENT

Chemicals

Gelatin (ex. 8)
Bromthymol (blue)
0.1N HCl
NaCl
Lugols iodine
KCN 3-5 gms.
Congo Red (ex. 7)

Biologicals

Potatoes (white)
Yeast, dry
Elodea leaf

Plastic & Glass Ware

250 ml. Beakers
180 Test tubes
24 10 ml. grad.
2 pks. Cover glasses
24 Syracuse watch glass

Others

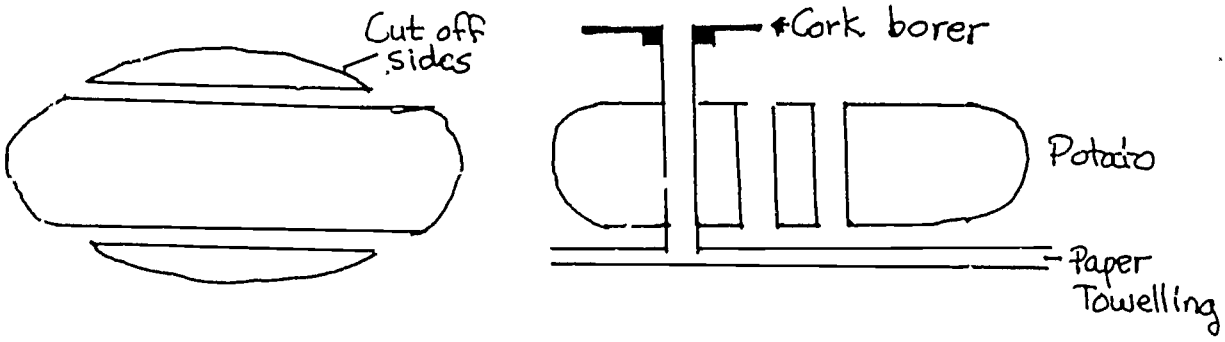
6 12" or 6" ruler
2 rolls $\frac{1}{4}$" cellophane
6 cork borers size 6
Bunsen burners
1 box Filter paper

PREPARATIONS

Part B - Osmosis

Salt Solutions. A 1% salt is prepared by adding 2 gm. NaCl to about 170 ml. distilled water, and after solution is diluted to 200 ml. Make 200 ml. of 1, 2, 3, 4, and 5% but 300 or 400 ml. of 10% NaCl.

Potato plugs. Use a kitchen knife and cut enough off of two slides of a potato to present a uniform thickness. This will then make it easy to obtain



plugs of uniform length. Plugs should be placed between damp paper towels to prevent moisture loss until they can be weighed and their volumes determined. Follow the directions for using the balances given in Exercise 2. The technique of determining volume by fluid displacement is also given in Exercise 2. If the laboratory period is only two hours long expose the potato plugs to the salt solutions for about 1 hour, but indicate the minutes exposed in the blank space provided on the Report Sheet.

Part C - Plasmolyses in Elodea Leaf

Fresh Elodea sprigs are needed since dying cells will not behave uniformly. If sprigs are to be kept in the laboratory they should be included in an aquarium with some fish, and snails to keep things cleaned up. A well-balanced aquarium does not need an air pump.

Part D - Active Exclusion of a Substance

Yeast Culture. Prepare culture medium ahead.

Tryptone broth	10 gm.
KH_2PO_4	5 gms.
Glucose	10 gms.
Water to make	1000 ml.

Dispense about 150 ml. into 250 ml. flasks and autoclave for 15 minutes (See Exercise 5). Store in refrigerator or at room temperature.

For use. Tear open the end of an aluminum foil envelope of dry baker's yeast. Flame the end for 1-2 seconds, then pour yeast into flasks. Place flasks at 37°C for 1 to several hours.

The KCN and iodoacetate should be weighed out in a well-ventilated but not windy place. Prepare about 100 ml of solution. Dispense into dropping bottles and label with the substance and "Poison". These materials are weak enough to handle without extra precautions but hands should be washed afterward and any spills should be washed up immediately to prevent drying to a powder and mixing with dust.

INTRODUCTORY DISCUSSION

Teacher: (Place a crystal of a colorful, soluble salt such as copper sulfate or cobalt chloride, in the bottom of a 100-ml. or larger graduated cylinder, and in front of the class, pour gently down the side enough water to fill the cylinder. Near the crystal there will be more color than at the top. Ask if the solution will become uniform in color. How may this thorough mixing be speeded up? Students should know "by stirring" but may not know about heating. Do students know why the molecules move (diffuse) through the water? In what directions do they move? Flux is defined as the net movement in the apparent direction of diffusion.

What determines the osmotic activity of a solution? The concentration does. One mole of a non-ionizing substance exerts 1 osmole of activity and raises the boiling point. It also lowers the freezing point 1.86°C. A highly ionized solution, such as NaCl, produces two ions for each molecule in 1 mole, so it has twice the osmotic activity, as a rule of thumb. [The "G-value" for various salts is found in Hoilbrunn, General Physiology or in Florey, Comparative Animal Phsiology. G-values are factors that express the observed degree of the freezing point lowering.]

Who can tell us what is meant by the term "active transport"? What energy source is used for this process? If we think that energy is needed to get some things across the membrane and to keep other things outside, how could we test that idea? (get some opinions.)

Set up a demonstration of Brownian movement. Make a suspension of carmine in in water and make a wet mount of it, observe under the high dry objective of a microscope.

Part A

As directed in the manual.

Part B

Potatoes should be placed somewhere else besides on the demonstration table to prevent traffic congestion. Place the kitchen knives and cork borers with the potatoes. Instruct students in method of making potato plugs. One student of a pair may prepare the potato cores while the other marks the test tubes and obtains the salt solutions. Again, one student weighs the potato plugs while the partner records the data. (This can be copied later.) Have the recorder for weights do the volume determinations and the partner record the data. The same student should do all of the weighing and on the same balance, and v. vs. for the volume.

Data and Graphs. To obtain the percent change divide the starting weight or volume into the final weight or volume and multiply by 100.

To plot the data, values for the percent change in weight (Graph 1) and volume (Graph 2) must be written in on the ordinates. Choose a range of values that fits the data.

The same scales of percent are to be written on graphs 3 & 4. On graphs 3 and 4 the percent is plotted against the reciprocal of the concentration of NaCl.

That is, 10% is plotted at 1/10, and 5% is plotted at 1/5, etc. This converts the data to rate expressions. Determine the slope of the lines (units rise per unit along the base (abscissa.)

Part C

The manual directs the student to "sketch" an Elodea cell when it should ask him to "diagram" it in this section. The frames on page 12-8 of the manual are for cell walls in such diagrams.

The procedures for this section can be reviewed by referring to Exercise 10.

Part D

The iodoacetate will turn the indicator dye, Congo Red, to blue.

REPORT SHEET

After changing to NaOH a green band is seen between the blue and yellow, indicating a neutral pH (pH7).

The hydrogen ions move with the water as it is drawn osmotically into the gelatin. Therefore, hydrogen ions are used to follow the diffusion of water.

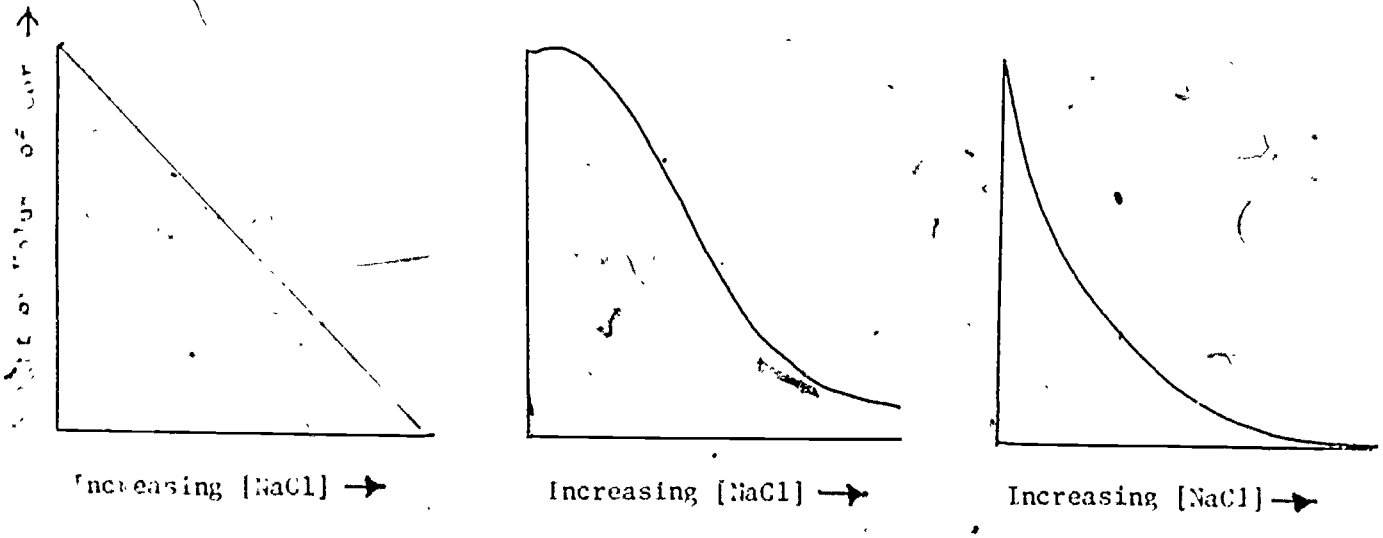
The ions are moving in all directions.

The flux is the net movement of ions in a given direction.

A cellophane membrane differs from a living plasma membrane in that it is not as selectively permeable nor can it accomplish active transport. It is very much like a dead cell membrane, however.

Part B

Possible shapes of curves



At (a) the amount of water lost is always a function of NaCl concentration.

At (b) the cell resists water loss at low concentrations by pumping out the salt that diffuses in and thereby maintaining a steady state.

At (c) water loss is great at first but as the osmotic activity of the cell contents increases it approaches being isotonic with higher concentrations of salt.

Part C

1) The cytoplasm is squeezed down but not dissolved.

2) The cell wall did not respond to NaCl.

3) The cell membrane follows the cytoplasm, coming free of the cell wall during plasmolysis.

4) The cell shank down largely because the water in the vacuoles was withdrawn by the high osmotic activity of the 10% NaCl.

Part D

The first question should refer to Experiment 7C (page 7-6).

Students should conclude that the membrane is permeable to Congo Red if it is allowed to diffuse into the cell (because no active transport outward is operating.)

The cell membrane does not protect the cell from harmful substances. If it did there would be many fewer poisons.

In tube B the metabolic enzymes were inactivated by heat so no ATP could be generated for active transport.

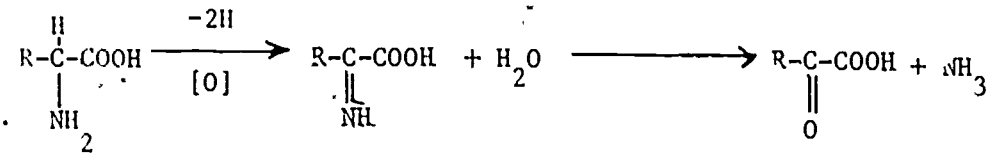
In tube P a single metabolic enzyme (pyruvic kinase) was inactivated, shutting down ATP production.

TEACHER'S GUIDE TO

EXERCISE 13 -- ENZYME ACTIVITY

INTRODUCTORY REMARKS TO THE TEACHER

All known enzymes are proteins. Not all organic catalysts, however, are enzymes. Otto Warburg showed years ago that a charcoal prepared from mammalian blood was capable of catalyzing the reactions.



The ability of enzymes to lower the activation energy for the reaction or reactions they catalyze centers on forming a jig into which the substrate fits. Where hydrolysis occurs, it is preceded by acyl formation.

Proteins (enzymes) first formed in the ancient seas and no enzyme reactions occur, even today, except in water.

In Part A the catalytic reaction of $MnO_2 + H_2O_2$ is demonstrated.

In Part B the action of a liver enzyme (or enzymes) on peroxide is demonstrated. (There is no easy qualitative color test suitable for use at this point to demonstrate that the liver is poor in manganese dioxide.)

Part C deals with the role of temperature and pH in enzymatically mediated hydrolyses. This part may require equipment not available for a whole class (e.g. spectrophotometers) and so it is designated to be done as a demonstration or as a special project for a few faster-working students.

MATERIALS AND METHODS (Parts A and B)

Chemicals

Hydrogen Peroxide
Manganese dioxide
Sea sand (fired)
Tincture gum guiacum

Biologicals

Fresh liver

Plastic and Glass Ware

8 Test tubes

Other

Test tube rack
Wax pencil
Splints or applicator sticks

PREPARATIONS (Parts A and B)

Making an Homogenate. Fresh tissue is somewhat slippery and hard to grind. The fired sea sand cuts the tissue under action of the pestle. The commercially obtained sand has been burned to eliminate any organic material which would change the tissue or other reactants. The sand should be allowed to settle in the mortar before the supernate (the liquid part) is decanted (poured off.)

Tincture Gum Guaiac.

Grind 2 gms. gum guaiac (guaiacum) in a mortar. Add 100 ml. ethanol.

INTRODUCTORY DISCUSSION (Parts A and B)

Teacher: Try this approach:

1) If you can handle live rats, and have one available demonstrate the "righting reflex" by turning the animal over on its side. It rights itself immediately. Now place it in a covered jar containing a piece of cotton (big as a fist) that has been saturated with ether for anesthesia and covered with a paper towel. When the animal has been anesthetized, remove it from the etherizing jar and place it on its back. The "righting reflex" will have disappeared.

It seems that some chemical reactions are as difficult to start as it is to keep an active rat on its back. In this case the ether is the catalyst and makes it possible to place the animal on its back with a great deal less energy. See if you, as teacher, can get students to develop the parallel idea from the above demonstration that it takes some energy and effort to get the normal rat to lay on its back, but much less energy to get the etherized rat to lay on its back. Ether lowers the energy needed to make the rat lay on its back. (Note: ether is not an enzyme. Just as all organic catalysts are not enzymes, neither are all reducers of activation energy enzymes (only the proteins.)

PROCEDURE (Parts A and B)

1) Have all students do Part A and demonstrate for the teacher or assistant that he has a gas test and has answered the questions for Part A before going on with Part B. Permission to proceed with the next part (B) then becomes a reward.

Glowing splints are made of applicator sticks put into a Bunsen burner flame. The heat of the glowing end makes hydrogen explode with a "pop." Oxygen causes the splint to burst into flame.

REPORT SHEET (Parts A and B)

Part A

Reaction of $MnO_2 + H_2O_2 \longrightarrow MnO_2 + H_2O + 1/2\ O_2$

Part B.

Questions

1) Did whole liver produce gas bubbles fast as liver brei in tube 2? (No)

2) Which reagent, the hydrogen peroxide or the gum guaiac, turned color?

(The gum guaiac contains guaiaconic acid which will turn blue when exposed to "active" oxygen. The enzyme releases oxygen from the peroxide.)

3) What effect did boiling have on enzyme activity? Why?

Boiling inactivated the enzyme eliminating its activity. This is because heat denatures proteins so they cannot fit the substrates properly.

4) What effect did grindling have on enzyme activity? Why?

It increased it because a much large surface of enzyme bearing material was exposed to the substrate solution (H_2O_2.)

5) There is the question about liver having MnO_2 in it. The directions do not indicate that MnO_2 be boiled before H_2O_2 is added. Boiling inactivates the brei but not MnO_2 so it must contain a heat-sensitive protein, an enzyme.

TEACHER'S GUIDE TO

EXERCISE 13C -- EFFECT OF pH, CONCENTRATION AND TEMPERATURE ON THE RATE OF ENZYME REACTIONS

This experiment may be done as a demonstration or as a special project for two or more students.

MATERIALS AND EQUIPMENT

Chemicals

Alkaline Phosphatase
Acid Phosphatase
P-Nitrophenyl
phosphate
Acetic Acid - NaAc buffer, pHs 3 and 5.5
Tris-HCl buffer, pH 7 and 8.5
Glycine buffer, pH 10 and 12
0.1N NaOl +

Biologicals

Liver from freshly killed and bled animals

Plastic & Glass Wares

B&L Spectronic 20 or 340 Spectrophotometer
cuvettes
Test tubes

Others

pH meter
B7L spect. 20 or 340
2 water baths 27, 37, 57°C. (27°C is near "room temperature")
Mortar and pestle
Double distilled water
Test tube racks
Wax pencil
Stop clock

PREPARATIONS

Solutions for enzyme work should be made up in "glass distilled water". If not available, ordinary distilled water can be used but with some inactivation of the enzyme.

Buffers.

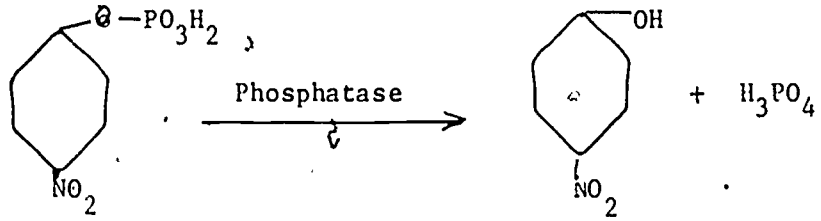
Acetate Buffer. Prepare .1M Na acetate and adjust the pH to 3 or 4.5 with HCl.

Tris-HCl Buffer. Prepare tris buffer and adjust to pH7 with HCl or pH 8.5 with NaOH.

Glycine Buffer. Make .1M (7.5 gm./liter) glycine and adjust pH to 10 and 12 with strong NaOH.

The enzymes solution should be made fresh on the day of use.

The enzyme reaction is:



(colorless) (yellow)

The variable factor in preparing the blank tubes is the enzyme preparation. It is not a clear solution and so affects light passage (by dispersing it.) If a tissue homogenate were used the same problem in standardization would exist. The reagents, including the NaOH (which stops the reaction) are incubated and then the enzyme or tissue preparation is added. This blank mixture is then used to standardize the spectrophotometer.

PROCEDURE

Experiment 1 - Effect of pH

The procedure is usually done with duplicate or triplicate sets of tubes. If several students are working together each day may do the tests at different pH values, with acid or alkaline phosphates, or each may do a single tube through the whole series and then the group average its results.

Experiment 2 - Effect of Temperature

As directed in the manual.

One may also carry out the procedures at 47°C. and at 57 - 70°C. if the equipment and time is available.

REPORT SHEET

The report sheets can be used to organize most of the data generated by these experiments.

raphs. The optical density of 0 should be on the bottom line and the range upward appropriately assigned to the other lines.

In Graph 2, the 0 concentration (the blank) is not at the edge. The O.D is the measure of the velocity, and the higher values are high on the ordinate. Therefore, the line should slope toward the zero line and intercept the abscissa at $-Km^{-1}$ (the reciprocal optimum concentration).

Example: 1/.10 = Km of .1% at --°C.

Questions

The answers can be extensive.

1) Different proteins have their maximum enzyme activities at different pH values. Therefore, their ability to use available substrates to extract energy for their maintenance depended upon their activity. That depends on their shape and their shape depends upon the pH of the solution they are in.

2) The value of Q_{10} between 27°C. and 37°C. would be:

$$Q_{10} = (k_2/k_1)^{10/(t_2-t_1)}$$ Where k is the rate and t is the temperature in °C.

$$\log Q_{10} = (10/t_2-t_1) \log (k_2/k_1)$$

Since the optical density is related to the concentration of the solute, the reading (or the actual amount of product) can be used as a measure of the rate of reaction after a given time (30 minutes in this case). So

$$\log Q_{10} = (10/10) \log (k_2/k_1) = 1 \cdot \log (OD_{37°C.}/OD_{27°C.})$$

Between 37°C. and 57°C. it would be

$$\log Q_{10} = (10/20) \log (k_2/k_1) = .5 \times \log (OD_{57°C}/OD_{37°C})$$

3) yes

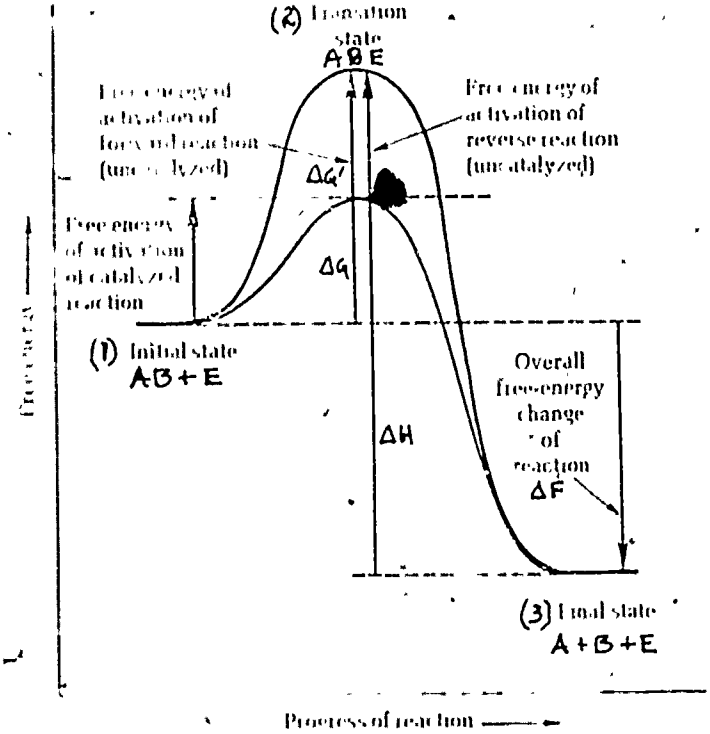
4) A blank tube is needed to standardize the spectrophotometer.

That is, it shows how much of the resultant color is due to the reagents.

5) Long exposure of the photocell to light will cause it to fatique. If this happens time must be allowed for it to regenerate its sensitivity.

6) The p-nitrophenyl phosphate is colorless but the p-nitrophenol is yellow and part of the picric acid series of yellow colors.

7) Enzymes form jigs for the substrate so that the reaction can take place with less random motion and thus energy loss (entropy.) The energy relationships are:



1) A substrate contains a certain amount of energy, but an additional amount, ΔG, the activation energy is needed to bring it to (2) at which point bonds start breaking and the reaction goes spontaneously to completion at (3) where the substrate has been split into A and B and the enzyme (E) has been released from the enzyme-substrate complex (ABE). Then, ΔF is the energy contained in ABΔH is the energy released by the reaction and is the sum of $\Delta F + \Delta G$.

TEACHER'S GUIDE TO

EXERCISE 14 -- FERMENTATION AND AEROBIC RESPIRATION COMPARED

This exercise will probably best be used as a demonstration or as a special project for two or more students.

INTRODUCTORY REMARKS TO THE TEACHER

This is a rather analytical experiment that only the student of high ability and interest should try, and that near the end of the course when he has acquired skill in weighing, measuring, pipetting, following directions, asceptic technique, etc.

The experiment itself is the classical Louis Pasteur experiment, but the analyses make use of current technique and instruments. It should make an excellent project for a small group of faster-working students working at times convenient to them.

Equipment requirements include an autoclave, air pump or supply and spectrophotometer.

Proceed as directed in the manual.

Report sheets are provided for the collection of data but these should be used as part of a larger scientific-type report which includes use of the literature in the library.

TEACHER'S GUIDE TO

EXERCISE 15 -- CHROMOSOME MOVEMENT DURING CELL DIVISION

(Followed with chromosome models made of wire and pipe cleaners.)

INTRODUCTORY REMARKS TO TEACHERS

The chromosomes were first described by Hoffmeister in 1848 just before the invention of the mechanical microtome in the early 1850s' and before Rudolf Virchow related this phenomenon to cell division in 1857. It was not until 1888 that Waldeyer named these structures "chromosomes", literally "colored bodies". This was a natural consequence, because phase contrast microscopy was still a half-century in the future and the methods of preceding fifty years (from the time of Schleiden and Schwann) had concentrated on fixing and staining of smears, squashes, tissue spreads, and cuts made by hand-held razors. Chromosomes developed from the somewhat "undramatic" interphosic nucleus, but except for some vacuoles and some fat and carbohydrate inclusions which exhibited changes in distribution but no activity, contractile vacuoles excepted, the nucleus was the only other known structure within the cell. The distribution of visible chromosomes became the basis for naming the phases with the period between cell divisions called the "resting period" or interphase. Gerard's book of the 1930s' entitled Unresting Cells probably did a lot to change our orientation so that now we see interphase as the period of real cell life, during which it carries out the functions for which it was differentiated. The phases of cell division, as dramatic and as fascinating as they are to study, represent an interruption of normal cell activity-a change in the production of nucleic acids at the chromosomes, an increase in oxygen consumption, and a cessation of synthetic activity by the DNA.

Rudolf Virchow realized as he studied his stained sections of tumors that these threads were involved in the cell division process and may not have fully realized that in his famous saying, "All cells come from cells", that he had observed the mechanism by which this evolutionary and ontological phenomena came about. In any event, chromosome behavior became the basis for interpreting the transmission of inheritable characters (by Wilson, by Tschermak and by Pfeiffer in 1901) and later for interpreting differentiation, growth and development, the reproductive processes and in their puffing and looping activity, the involvement of specific segments containing the genes effecting these processes.

Part A of this exercise deals with mitosis, Part B deals with meiosis as applied to sperm production and Part C to meiosis as applied to egg production in animals and lower plants. For the modifications applicable to higher plants see the introduction to Exercise 22 in the student manual..

MATERIALS AND EQUIPMENT

Copper wire 20 to 24 gauge
Pipe cleaner
Paper plates, 9" diameter
Wire cutters and scissors

PREPARATIONS

Students can cut their own wire pieces and make the chromosome models.

INTRODUCTORY DISCUSSION

(If this exercise is to be done with the class unassembled, this discussion should be done at a previous meeting of the class.)

Ask students for the formulae for the area of a cube and for its volume. Have students draw a perspective view of a cube of 2 cm. per edge and then compute its area and volume. (24 sq. cm. and 8 cc.). Now have them divide the cube into 8 cubes of 1 cm. edge and repeat the computations for these (48 sq. cm. and 8 cc.) Point out that for an individual 1 cc. cube the area is 6 sq. cm. which increases the ratio of surface to volume from 24:8 (3:1) to 6:1.

Ask now what would happen if cells only divided but never grew? What would happen if cells only grew and didn't divide? We don't know yet what is the ultimate cause, or causes for cell division, but it has been observed that each particular kind of cell grows only to be a certain size and then it divides. Some theories about the cause(s) of cell division rest upon this observation. Ask students for some of their ideas about the causes of cell division related to the surface to volume ratio .

A cell is determined to divide (the forces causing division have been marshalled together). How could it divide into two cells and still keep the same number of chromosmes in the resulting cells as in the starting (parent) cell? How, then, could this process be continued to yield some cells with only half as many chromosomes as in the starting cell? Put their ideas on the chalk board but do not indicate the correct answers. Let them work that out in the activity of the exercise.

PROCEDURES

1. With the class assembled.

a. Let each pair of students proceed as directed in the exercise.

b. Program the activity by dividing it up and placing it in a sequence of stations. Each student starts at the beginning of a sequence. Part A would be a sequence and Parts B and C would be separate sequences.

2. With the class unassembled.

a. Provide materials to students and let them work through the exercise as a home assignment.

b. Program the activity as above and let students come in at their convenience to work through the sequences.

TEACHER'S GUIDE TO

EXERCISE 16 -- CELL DIVISION

Prerequisites: Exercises 4 and 10. Exercise 15 recommended

INTRODUCTORY REMARKS TO THE TEACHER

Did the procell divide? That is, did the community of molecules which existed before the "invention" of the cell membranes divide? If so, how could the spindle protein be protected from the environment? We can only speculate about that, but since the discovery by Rudolph Virchow (1857) that "all cells come from cells," the importance of cell division for explanations of reproduction, growth, differentiation and evolution have not diminished". The result of the former view was that division constituted the real life of the cell and all else was "interphase". Now we consider the interphasic or metabolic cell as the form when the cell does the work it was designed to do and this work is temporarily interrupted during mitosis and meiosis.

It is indeed remarkable that the cell division process goes along with few abberations, but there are aspects of cell division that are not yet explainable. Foremost, the division of cells taxes modern imagination. Causative conditions are not known although nutrition and hormones affect the rate of division. Carcinogens, both chemical ones such as anthracene and its derivatives, or physical ones such as excessive X-radiation dosages also stimulate cell division rate. A few drugs depress division rate. The causes of chromosomes movement are uncertain. When the spindle fibers are cut, the chromosomes in the metaphase plate still move apart. The cells of higher plants lack centrioles so that these "organizers of the cell" are not the apparent agent responsible for chromosome movements.

Mitosis is a word from the Greek mitos a thread, literally full of, abounding in, or having threads. Fundamental to mitosis is the reproduction of chromosomes and the distribution of equivalent chromosomes as sets of chromosomes to the cells resulting from cell division. (By what whimsy such resultant cells are called "daughter" cells also taxes modern imagination.) Since chromosomes have now been demonstrated as double structures in bacteria, protozoa and even in the "not-quite-alive" viruses, and that these move apart, the older term "fission" must now be dropped and mitosis admitted as the process by which these cells divide.

Meiosis is from the Greek root for " a lessening." If the number of chromosomes in cells is lessened it is a meiosis. This usually refers to a lessening by one or more genomes. "Chromatin diminution" is used to describe chromatin loss in less than genomes. Spindles, centrioles, or cell structures other than chromosomes are not essential to the description.

MATERIALS AND EQUIPMENT

Chemicals

50	gm.	Picric acid
500	cc.	Formalin
2	litre	Glacial Acetic Acid
5	gm.	Urea.
2	litre	Acid Alcohol Conc. HCl
1	litre	99% Isopropyl alcohol

Aceto Orcein-Light Green Stain
5 gm. Light green sp.
50% aqueous acetic acid solution
25 gm. Orcein
70% Ethyl Alcohol
Carnoy's Solution
Absolute Ethyl Alcohol
Chloroform - 1 litre

Biologicals

Onions
24 white fish
blastula slide preparations
Prepared slides of maturation in
Ascaris eggs.

Plastic and Glass Ware

glass slides & cover slips
4 boxes & 8 boxes

Others

24 Microscope
3 pks. razor blades (single edge)
forceps
Paper towels
1 chart of animal & plant mitosis

PREPARATIONS

Allen's fluid, acid alcohol and aceto-orcein light green are given on page 16-2 of the manual.

Onion roots. Start onion soaking in water (about half-way up the bulb) some 3 to 7 days ahead of use.

Carnoys Fixative

Absolute alcohol	60 ml.
Chloroform	30 ml.
Glacial acetic acid	10 ml.

Combine just before use.

INTRODUCTORY DISCUSSION

Show the film loop "Mitosis" (Ealing No. 81-5340/1) without comment one time. Ask the class what was happening to the cell? When the class has made its comments, show the film loop again, this time explaining each sequence.

Some questions:

What makes a cell start to divide?

What makes chromosomes move?
What are the classical phases of mitosis and of meiosis?

PROCEDURE

Proceed as indicated in the exercise. Root tip mitosis occurs most abundantly about midday and midnight. Root tips may be harvested at noon and fixed with Carnoy's fixative for 24 hours, using about 40 times as much (volume) of fixative as the volume of the tissue to prevent dilution of the fixative. If more than 24 hours will pass before use, transfer the tissues to 70% alcohol for indefinite storage.

REPORT SHEET

1. Why are cells stained to study mitosis if staining them results in their death? (Ans. Many times cells are studied without staining, especially where optical systems such as the interference or phase contrast microscope are used. Staining may kill the cell but it makes the chromosomes and other material easily visible in the ordinary microscope.)

2. If a cell has four pairs of chromosomes, how many will each daughter cell have following mitosis. (Ans. Four pair)

3. Briefly characterize the mitotic phases (For a description see Exercise 15A).

4. What do you think the role of the spindle fibers is?
(This question asks an opinion. Such opinion should be based upon the students' observations. Ordinarily it should include these:

(a) It forms a guide for chromosomes movements to the equatorial plate and to the centrioles (where present.)

(b) It appears to move the chromosomes [Teachers may ask for an experimental procedure which would show whether or not the spindle caused the movement of the chromosomes.]

5. How are plant mitosis and animal mitosis similar?. [The question should ask for differences.]

(Ans. In most ways they are different in that:

1) Animal cells have a cleavage furrow and do not form equatorial plates of cell wall material.

2) Higher plant cells have no centrioles.

6. What structural differences are there between mitosis in onion cells and whitefish blastula cells.

Whitefish	Onion
No cell wall	Forms cell wall
Has centrioles	No centrioles

7. (Synapsis is the lateral alignment of chromosomes carrying genes for the same characters.)

8. (Polarbody cells are small cells formed during the maturation of egg cells.)

9. [Correction] How do the final cells in male meiosis differ from those in female meiosis?

(Ans. In males all four cells are viable and are transformed into sperm cells. In females only one viable egg is formed for each ovogonium cell undergoing meiosis.)

TEACHER'S GUIDE TO

EXERCISE 17 -- THE PHYLA OF THE ANIMAL AND PLANT KINGDOM

INTRODUCTORY REMARKS TO THE TEACHERS

The classification schemes for plants and animals are about as varied (within limits) as taxonomists who are brave enough to attempt the task of classification. Sometimes the name of a phylum may be different and sometimes organisms will be transferred from one taxonomic grouping into a new one.

Modern biological research, especially in biochemistry and biophysics, may sometimes place so much emphasis on molecular activities that the organism providing the tissue is barely mentioned. Interpretation of this kind of data, however, finally depends upon the relative position in the scale of complexity of the organism whose tissue activity is described.

The specimens provided for study in this exercise should be labelled with common or trivial names or the scientific name. (For example: sponge, clam, carp, spirogyra, horse tail or pine tree.)

MATERIALS AND EQUIPMENT

A specimen representing each phylum or class indicated is needed. Most of these are available somewhere in the department. Borrow them (if necessary) and let the display remain available to the class for at least a week. Students should be asked to learn the names of the phyla and the representative specimens during that time.

PROCEDURE

The Report Sheet has the phyla and classes in ascending order according to one version of classification, but one may proceed in at least these ways:

1) Divide the specimens among the work tables, then have each arrange their specimens in ascending order of complexity. Students will move from one worktable to others until all specimens have been observed and classified.

2) Arrange the specimens in the order of their classification.

3) Prepare a sheet of characteristics for each of the taxonomic groups. Arrange the specimens in random order on the work tables. Have the student identify typical animals from the description sheet.

4) If some specimens are in display cases and it is not convenient to move them to the laboratory because of size, fragility, rarity of the specimen, etc., let it remain there, and have students study them where they are.

TEACHER'S GUIDE TO

EXERCISE 18 -- COMPARATIVE ANATOMY OF SOME SKELETAL FEATURES

INTRODUCTORY REMARKS TO THE TEACHER

There are a number of anatomical features that can be compared between species to show how parts became progressively developed. A variety of skeletons are usually available for study without further preparations. They are thus less time-consuming to study, than for example, the circulatory systems or digestive systems in several animals. Even having settled on the skeletal system one could study the jaw and teeth, the methods of attaching the fore and hind limbs, or the size and shape of the brain case. For this study the forelimb has been selected. The extremities are basically five-parted from the fish upward. At least one representative skeleton from each Vertebrate Class should be studied in this comparison.

MATERIALS AND EQUIPMENT

Skeletons of fish
Frog
Lizzard or turtle
Bird
Cat
Horse
Bat
Cow or pig
Monkey or Man
(Others are acceptable.)
Clipboard or other writing surface, drawing paper and pencils

PREPARATIONS

It is not necessary to bring the skeletal specimens into the laboratory room. If they are available in display cases in the hallways or other accessible places, send the students to the specimens. If they are located in other classrooms that may be in use, it would be desirable to place the specimens where they will be accessible. (If portable, battery powered tape recorders are available, itinerary and directions can be taped and students can do the exercise at their convenience.

INTRODUCTORY DISCUSSION

Start with a human skeleton and review the major types of joints: hinge, sliding, rotating, universal, ball and socket and cushioned ones. Do this by asking the class for the possible kinds of movement each kind of articulation can make and what it can be used for. Caution them in making their diagrams of bone arrangements to include each bone in the forelimb of the limb. Students will all have prior knowledge about what the forelimbs of the animals are used for. Teachers may ask additional questions about the observations, such as, "Can a lower form do anything with its arm that a higher form cannot do?" "What relationship is there between the manner of attachment at the pectoral girdle and the manner in which the animal moves?

TEACHER'S GUIDE TO

EXERCISE 19 -- DISSECTION OF THE FETAL PIG

INTRODUCTORY REMARKS TO THE TEACHER

The Teacher's Curriculum Guides for Unit 7 (Variety of Living Things) suggests dissections of the rat (with substitutions of cats or other animals possible.) Fetal pigs are easily obtainable preserved, and injected with latex if the teacher has a budget for the more expensive preparation. They also do not have a tough hide to be removed if superficial muscles are to be studied. Other advantages are listed in Exercise 19.

The inclusion of an anatomical study such as this is to give the student first-hand experience in the techniques of dissection and to trace out some of the organ systems in which students invariably have a great interest. Very often anatomy is erroneously displayed in commercial advertisements, especially those for medicines. The presence of the beating heart is something children become aware of quite early, as toddlers, but which few have seen in situ unless they come from a farm family or one where hunting is popular. There is sometimes confusion as to why both food and water are ingested through the mouth but the waste products are evacuated through different routes. Teachers should be alert for these expressions of curiosity and direct students to follow their interest out, but also to be sure that the assignment for the day is accomplished. For example, if the respiratory and circulatory system are being followed and the student raises the question, "How does food and oxygen get to the developing fetus?", then he should be encouraged to see what the connections are to the uterus.

Despite the popularity of molecular biology in recent years, anatomy is still important to physiology and biochemistry, for without it an adequate description of a body part could not be made by merely mentioning its name.

MATERIALS AND EQUIPMENT

Dissecting pans
Pins
strong string
Dissection instruments: scissors, blunt probe, forcepts (Note: these must not be the same instruments used for physiological work because the formalin adheres to instruments and is virtually impossible to remove. This formalin then has a toxic effect on living tissues when the same instruments are used to work with such tissue.
Fetal pigs (with arteries and veins injected with latex, if possible)
Hydrous lanolin
Plastic bags

PREPARATIONS

The fetal pigs should be placed in plastic bags prior to distribution and a tag supplied so that each student (or pair of students) may later identify his animal. The animals should be washed off under running, cold tap water to remove excess preservative. Students should rub their hands well with hydrous lanolin to protect them from the preservative. The lanolin is later easily washed away.

A dissection pan or dissection board should be provided for each pig. These permit easy pinning back of skin flaps and also permit the retraction of the legs.

INTRODUCTORY DISCUSSION

Present the class with a metal spring, large enough to be easily seen by all and not so strong that the teacher cannot extend it. Ask the class about the functions of springs and how they work (when energy is used to extend the spring it stretches. When the energy is no longer expanded, the spring returns to its original shape.) Ask questions which expand upon the concept that form and function are usually inseparable. Ask in what ways does this concept work out in the body. (A main function of the respiratory system is to conduct gases, the circulatory system conducts fluids, the muscular system must have elements that are extensible and contractile.)

PROCEDURE

Two approaches to the anatomical study are available in Exercise 19. One may make a survey of organs of the viscera using the directions on page 19-2 in the manual without regard to particular systems. The other approach is to look for structures in the lists given for the various organ systems. One may begin with the respiratory system, then do the circulatory system and go on to the digestive system if this exercise is used with Unit 6 -- Metabolism and Regulatory Mechanisms then do the others; or the nervous, renal and reproductive systems if used with Unit 4 -- Reproduction, etc., and then do the others.

If more than one session is to be spent on this exercise, then fetal pigs should be returned to their bags and a small amount of preservative added to prevent molding. Identification tags should carry the student names and section and the animals for each section should be kept in separate containers for easier distribution the next time.

REPORT SHEET

Questions

1. What structures are seen in the fetal pig that are not present in the adult? Relate these structures to prenatal function.

Umbilical cord--contains the umbilical arteries and vein communicating with the placenta and the fetal circulatory system.

The Allantois may be present (young fetus) or its stub. This stores allantoic fluid which is the product of the fetal kidney.

Inside the heart one will see the foramen ovale between the right and left atria and the ductus arteriosus between the pulmonary artery and the aorta.

2. After identifying external anatomical features of the pig, compare these features with the human body.

The external nares are in a snout used for digging and ploughing up earth. Eyes are more lateral. Ears (lobes) are much larger; head shape is different, number and kind of teeth have a different pattern. Pig also stands on

two fingers (toes), and the limbs do not swing as much as in the man. Despite all these differences, the pig is the animal most preferred for medical research because it approximates the size and physiology of man more closely than is true with dogs.

3. Pick out the structures you have examined that belong to a particular organ system. Are they all together? How are these structures related? What functional significance is there to their arrangement, structure, and location?

Example: The liver. The liver belongs to the digestive system, but also functions importantly in the circulatory system. Its connection to the digestive system is through its secretory duct, the bile duct. All of the parts of the digestive system connects to it through ducts.

4. Trace various pathways in the specimen, e.g., air, food, blood, urine, eggs or sperm.

(See the system lists.)

TEACHER'S GUIDE TO

EXERCISE 20 -- ASEXUAL AND SEXUAL REPRODUCTION

Prerequisite: Exercise 4. Exercise 10 is useful.

INTRODUCTORY REMARKS TO THE TEACHER

In this exercise a few life cycles of lower plants, animals and microbes are presented as examples of sexual ard asexual reproduction. It is interesting to note that sexulaity is not limited to higher forms but is present in the most "simple" cells (where "simple" is based upon morphologic organization). Of course, student interest about reproduction centers around human reproduction, not only its biology, but its psychology and sociology as well. College life probably represents, for most of them, the first opportunity to make certain social decisions without the immediate advice and counsel of their family for an area of living in which they are capable of participation, but this can not be done independently of other educational and social goals. The best understanding of human reproduction rests upon knowing what the patterns are in nature, that what happens in mankind is part of, not separate from, what happens in the rest of the natural realm.

This is the first of seven exercises in the student manual (Ex. 20-26) which deal with reproduction in animals and plants.

MATERIALS AND EQUIPMENT

Microscopes and lamps
Microscope slides and coverglasses
Living cultures or preserved specimens of the various organisms used:

E. coli, a Blue-green alga, Spirogyra, Paramecium, Rhizopus nigricans, yeast, Neurospora crassa, and preserved basidiomycetes.

Prepared slides containing the stages in the life cycle (obtained commercially) for a Blue-green alga, a green alga, such as Spirogyra; Paramecium (conjugation), Rhizopus nigricans, yeast (such as Torula sp.), Neurospora crassa, and the life cycle of Pucinr a graminis or of Psalliota campestris.

PREPARATIONS

Selection of cycle to be studied. Many teachers will want to give students the choice of which cycle they would like to do as the required one for this exercise. If so, this should be done a week or so ahead so that sufficient material for the cycles indicated may be accumulated. The teacher may wish to assign the cycles to students for study, allowing them to do additional ones of their choice as time permits. Prepared slides of these life cycles are most likely available in the Department of Biology. It is good to obtain some living cultures of the various organisms so that something of their cultural habits and smells, can also be experienced by the student. It is difficult to depend upon the living cultures for reproductive forms since these are often seasonal in their appearance.

INTRODUCTORY DISCUSSION

Teacher: Let me pose a question to you this morning (afternoon). Are adult

people just grown-up children? (The class should bring out reasons on both sides of this question. They will realize that while children resemble grown-up people in many respects, that there are emotional, intellectual, physiological changes that occur in teen-aged youngsters which convert them from children to adults. This metamorphosis is much more distinct and dramatic in some of the lower animals so that individuals who are adults give rise to offsprings which do not resemble themselves very much (e.g. tadpoles). Sometimes they don't even have the same chromosome numbers, being haploid. The "sex" occurs in the adult stage, or to put it another way, the stage that gives rise to the gametes or to mating (fusion, etc.) is called the adult stage.)

Today we want to study some of the kinds of offspring that get generated in nature. (Then explain the plan for study.)

PROCEDURE

Cultures. The usual cautions must be emphasized to the effect that students must not take the pipets (droppers) from one culture and put them in another one. It usually won't hurt the cultures, but it mixes up the organisms and sometimes leads to the confusion of others, especially if they don't know what the organisms are.

Slides. If the various life cycles slides are obtained from a central supply box or boxes, students should return them to the proper boxes so that others will be able to find them. Also, they should remember at the close of the class to be sure to check their microscopes for slides on the stage before they put them away.

REPORT SHEET

Questions

1. n, 2n Haplontic; n, 2n Diplontic n, 2n Haplodiplontic
2. The number of chromosomes in the haplontic pattern adult is n, in the diplontic pattern adult it is 2n.
3. What is the number of chromosomes in the cells of these stages of the diplontic pattern? It is 2n for the sporophyte and 2n for the ganetophyte.

Terms

Gamete--a cell which participates in reproduction by fusing with another cell of opposite or complementary mating type, usually within the same species.

Fertilization--is a process which begins with sperm entrance (or its equivalent) and ends with incorporation of the chromosomes from both parent cells into a single mitotic spindle.

Haploid. The suffix -oid means "like" so that haploid has "like half" of the normal number of chromosomes, most usually one set instead of two. [Why is the term haploid better than monoploid?]

Diploid. Having two sets of chromosomes.

Mitosis. Mitosis is a form of cell division in which the chromosomes (having been reproduced) become more contracted and more visible, they split, and are divided between two resulting cells in sets like those of the parent cell. Lit-

erally, mitosis means thread (chromosome) formation.

Meiosis. Meiosis means a "lessening". It is used for a type of cell division where (after they are reproduced) the chromosomes contract and become more visible. They then pair up (synapse), pairs having the corresponding gene alleles. The chromosomes pairs split (forming tetrads) and are divided, two sets per resultant cell. These cells divide again without reproducing the chromosomes so that one set of chromosomes is found in each of the final four cells.

Meiospore. A meiospore is a spore that contains the haploid number of chromosome It germinates into a gametophyte which produces haploid gametes. The gametes fuse at fertilization to produce diploid sporophytes. Preceding spore format the germ cells undergo meiosis, forming haploid spores.

Page 20-10

1. What are the advantages of asexual reproduction?

(a) A mate or complementary gamete is not needed to get an offspring, so species survival may be better in sparsely populated environments.

(b) The offspring will be genetically like the parent organism.

2. What are the advantages of sexual reproduction from a biological point of of view.

It provides for genetic variation and perhaps greater adaptability to various environments.

TEACHER'S GUIDE TO

EXERCISE 21 -- REPRODUCTIVE STRUCTURES IN FLOWERING PLANTS (ANGIOSPERMS)

INTRODUCTORY REMARKS TO THE TEACHER

Part A of this exercise deals with the asexual ways that higher plants reproduce and Part B considers the sexual role of flowers. Just as sex is not limited to higher plants and animals, so asexual reproduction is not limited to lower plants and animals. We will not attempt to cover the complete range of possibilities in either mode, but the student should be able to draw some inferences from these few examples.

MATERIALS AND EQUIPMENT

Sweet Potato
Roots of a sweet potato plant with young potatoes (demonstration)
Sweet potato rooted by soaking in water or planted for several weeks
Corn plants several weeks old with suckers
Irish potato
Irish potato rooted by soaking or being planted for several weeks
Pots of Hens and Chickens, Strawberry plants or Bermuda grass
Potted saplings of woody plants
Sharp knives
Soft Paraffin
Twine
Blossoms of lily and sweet pea
Preserved early ears of corn and preserved (dry) tassel
Onions

PREPARATIONS

It takes some planning ahead in order to have the rooted materials available for the class to study. The sweet potato, irish potato and corn should be planted about three to four months before use. A greenhouse is not necessary if there is space somewhere in the laboratory room, but a greenhouse is helpful. In the laboratory room plastic sheeting can be used to cover a frame to form a miniature greenhouse.

INTRODUCTORY DISCUSSION

Teacher: Does a flowering plant have to reproduce sexually? (Of course not.) What are some common food-producing plants which are propagated asexually? (Navel oranges, delicious apples, bananas. Sweet potatoes are frequently propagated from cuttings and irish potatoes from the potato "eyes".) The reasons for this vary. Navel oranges and bananas have no viable seeds, its quicker to root cuttings. It's also quicker and less expensive for sweet potatoes. Irish potato eyes, used as seed are often thrown away by the cook, anyway. Sometimes a desirable fruit grows on a sturdy twig, but the root does poorly. In these cases cutting is grafted to a sturdy root.

PROCEDURE

(As indicated in Exercise 21)

REPORT SHEET

Selected Questions

What part of the plant is the irish potato? (a stem)
What part of the plant is the sweet potato? (a root)
Of what advantage is it to a flowering plant to be able to reproduce asexually?

(a) It doesn't have to wait for a fruiting and growing season.
(b) More food can be stored (if it is) to feed the new plants than would be contained in seeds (corms, bulbs, potato stems, carrot roots.)

What is meant by monoecious and dioecious? Monoecious plants have male and female flowers in different plants, that is, a plant has only one sex. Dioecious plants have flowers with both male and female flower parts.

TEACHER'S GUIDE TO

EXERCISE 22 -- SEED AND FRUIT PRODUCTION IN FLOWERING PLANTS

Prerequisite: Ex. 10 required; Exercise 20 recommended

INTRODUCTORY REMARKS TO THE TEACHER

The alternation of generations between gametophyte and sporophyte plants is a fascinating one to study. The student introduction to this exercise goes into some detail about the formation of the megaspore mother cell and the microspores and sperm cells because these may or may not have been discussed in class by this point. Many standard freshman biology texts and standard botany texts review these processes in detail, tracing the evolution of the cycle from the mosses and liverworts upward. The unit-writing group on Reproduction did not feel that the long, detailed story about the alternation of generations in higher plants needed to be discussed at length, but the existence of the egg and endosperm cells and of sperm cells help round out the story of sexual reproduction as found in higher plants.

It would probably be very helpful to have a set of models of the germ cell formation in higher plants so that the roles of the pollen tube and of the micropyle can be more easily understood. In Part B, the object is to study the different general ways that the ovule and receptacles develop in the formation of various types of fruit.

Part A -- Germination of Pollen Grains

MATERIALS AND EQUIPMENT

A supply of pollen or flowers with ripe anthers
10%, 20%, 30%, 40% and 50% (weight/weight) solutions of sucrose, glucose or hone (About 10 ml./24 students)
5%, 10%, and 20% sodium chloride solutions
Indoleacetic acid (100 mg./100 ml.)
Gibberellic acid (100 mg./100 ml.)
Pasteur pipets (one for each solution)
Microscope slides and coverglasses
Petrolatum (e.g., Vaseline) warmed just to melting on a controlled-heat hot plate.
Small camel hair brushes (like used for handling fruit flies.)
Microscope and lamp
Wax pencil

PREPARATIONS

Solutions. It is usually easier to make up the solutions on the basis of weight (1 ml. water = 1 gram) because one does not have to transfer the solute from the beaker or other container used for weighing, nor does one have to dissolve the solute in less than the desired final volume of the solvent and then bring it to volume when it gets into solution. Use a 150 ml. beaker for each sugar solution. Weigh out the sugar (1, 2, etc. grams) and add the required weight of water to bring the whole to 100 grams total weight above the tare weight for the empty beaker. The hormones (giberellic or indoleacetic acids) can be weighed out and dissolved in the appropriate concentration of sugar or salt solution.

INTRODUCTORY DISCUSSION

Before class prepare a series of small boxes, each within the other, or a similar series of jars. At class time, have a student come up and remove the next to the largest box or jar, then the next largest, on down to the last. The smaller the last jar, the more intriguing, especially if it is filled with coins, beads, or the like. Two points can be discussed--1st, that the smallest functional structure may not always be apparent from an outside examination. Sometimes the smallest structures may be the most interesting or intriguing.

Today, we want to study a small thing that was among the first material in which the nucleus was seen by Robert Brown. Brown not only discovered the nucleus but, also was the first one to see pollen grains germinate and send out their tubes, which normally push down from the pistil, through the stigma and into the micropyle of the ovary. There are, however, some questions about this whole proces for which we may want to find answers. Are there special conditions of heat or moisture needed for pollen germination? Can known chemicals speed up or slow down the rate of germination and tube extension? Is germination due to osmotic or other conditions?

Students may suggest other questions. Ask them and help them to devise suitable experiments to test the material for answers to their questions. They should have permission to proceed on these experiments as long as a sufficient number of students do the basic experiment in the exercise for comparison.

PROCEDURES

Keep the assignment of slide numbers the same as given in the exercise although every student need not be given the same series of mixtures to test. Have some students test sucrose, some glucose, some honey and have them test the corresponding solution with one of the hormones added. Some groups may test different kinds of pollen. Some pollens will germinate with as little as 1 to 2% sugar. Will any of the pollens in the laboratory germinate in water or in sugar solutions containing less than 1% sugar? Have someone determine the germination rate when slides are kept at refrigerator temperature, room temperature and at 37°C.

Part B -- The Kinds of Fruits

MATERIALS AND EQUIPMENT

Fruits and seeds listed for this part of the exercise.
Kitchen knives

PREPARATION

If the materials are to be gathered locally, a program of year-round collection must be started.

Milkweed should be collected in the fall before the frost falls because that event causes the pods to burst and disperse the seeds.

Capsules of okra and cotton in the closed condition should be gathered in the summer time with opened pods (bolls) taken from plants in the fall.

Legumes. Buy fresh green peas and beans at the grocery store and the corresponding seeds from a seed rack. Do not try to sprout beans or peas purchased in food packages since these have been treated to prevent germination and to

speed up cooking.

Dandelion achenes should be kept in a jar so that the seeds to not blow away. They are unavailable only during the snowy season.

A head of sunflower seeds is instructive, but seeds can be bought from a grocery, seed store, or pet shop.

Elm or maple samara should be collected in the summer time.

Acorns, and nuts, can be gathered in the fall. Those with the bracts still attached are preferred, so they should be harvested with this in mind.

The fruits listed are all commonly available in larger food stores at almost any season of the year.

INTRODUCTORY DISCUSSION

Hold up a peach or apricot pit and ask if that is the seed (that is, is the outside covering the seed?) Take a hammer and break open the pit to reveal the kernel. Remove some of the "skin" from the kernel inside. The kernel is the seed. The tough, woody wall of the pit is the endocarp, and the flesh of the fruit which once covered it is the exocarp. It is divided into the mesocarp and pericarp of the fruit.

Now ask someone to define "dehiscent". If no one can, have a student look the word up in the dictionary or the glossary of a book. By what means are the simple dry fruits dispersed?

Part B

Display the various kinds of fruits grouped according to kind. Students should complete the Report Sheets following their examinations. The exercise is designed to help the student remember the main kinds of fruits and he should be prepared for a brief quiz on that topic next time.

TEACHER'S GUIDE TO

EXERCISE 23 -- MONOCOT AND DICOT SEEDS, SEEDLINGS AND LEAVES

INTRODUCTORY REMARKS TO THE TEACHER

This is one of those purely morphological studies, all of which goes to show that even though Nietsche may be dead, morphology isn't. The exercise can be used in connection with reproduction and development of plants in Unit 4 or it may be used effectively with the Variety of Living Things (Unit 7.) In any event, the comparative anatomy of a monocot and of a dicot from seed to sprout has distinctions as clear as those shown in their flowers.

MATERIALS AND EQUIPMENT

Hand lens or dissecting microscope
Bean and corn seeds
Bean and corn seedlings
Prepared slides of monocot and dicot leaf cross sections

PREPARATIONS

Obtain seeds from the seed counter and not from the food shelf at the grocery store or seed store.

Soak some beans and corn seeds overnight, or until swollen and plump.

Prepare corn and bean seedlings in this way: Place the seeds about 1 inch apart between the wall of a fairly large glass jar (be sure that it has never been used to contain formalin) and wet, absorbent cotton or paper towelling. The seeds are kept wet and placed in a dark cabinet to sprout. After a few days, when they have developed leaves, cover the outside of the jar to root level with aluminum foil and leave in the light.

INTRODUCTORY DISCUSSION

Teacher (holding up a jar of dry beans) asks, "Are these beans dead or alive? What are the qualities of aliveness? (Write them on the board.) Do they stand up to those criteria?

Hold up a jar of sprouting corn or beans. Ask, "How about these? There isn't any doubt in anybody's mind is there, that these are alive? Do they meet the criteria we set for things being alive?

What made the difference? (Water). But just what is it that water does. (Probably provides a spacial arrangement for the proteins so that enzymes can become active and release energy stored in the seed (endosperm) for cell growth, differentiation and metabolism.)

Draw attention to the cotyledons of a bean sprout. What relationship (seen among all of the bean sprouts) is there between size of the cotyledon and differentiation of leaves? Could the root system support the seedlings without the food generated in the leaves by photosynthesis? What would be an easy way to get an answer to that question? Or, one could ask the opposite question, "Could the stem

and leaves of the seedling survive without the roots?" What are the principle functions of xylem and phloem tissue?

PROCEDURE

In making drawings, keep the outlines continuous and clear. Make drawings large enough to show details easily. Veining patterns should be clearly shown. Colored pencils or water paints may be used if desired, but stippling and shading with lead pencil should be discouraged because it becomes smeared if rubbed. Label neatly but remember that the report is mainly the drawing and the student should not become so involved with the technicalities of lettering and shading as to lose sight of the fact that the drawing is the report of his observations and that clarity in presentation is the equivalent of good grammar in a verbal description.

REPORT SHEET

Some drawings are to be placed on the same page with the directions so that extensive labelling will not be necessary. The descriptions are all right there. Some drawings, however, do require labelling.

TEACHER'S GUIDE TO

EXERCISE 24 -- HISTOLOGICAL STUDY OF THE FEMALE REPRODUCTIVE SYSTEM

Prerequisite: Exercise 4

INTRODUCTORY REMARKS TO THE TEACHER

Exercise 24 and 25 are both histological studies made from prepared slides. The histological picture is static, but the pattern may be different from tissue to tissue depending upon the hormonal state of the donor. There is still a lot to be learned from the study of morphology. Remember, the drawings constitute a report (essay) on the observations made.

Exercise 26 deals with the gross physiologic responses of organs such as ovaries and uteri to sex hormones, but they do not tell the student very much about what is going on inside of these organs. Histological study can provide a picture of some stages in the dynamics of morphological change and at the same time let the student observe the details of the structures that exist.

MATERIALS AND EQUIPMENT

Microscopes
Slides of ovary, uterus, oviduct, and vagina of the rat (or other mammal)
Immersion oil
Lens paper

INTRODUCTORY DISCUSSION

The student introduction to Exercise 25, although unusually long for this manual, is none-the-less a brief account of the hormonal relationships affecting the structure of the female reproductive tract in mammals. See if students were able to get the concept of hormonal interrelationships between the pituitary gland and the various ovarian and uterine structures.

PROCEDURE

Parts A, B, and C

Study the appropriate slides and then draw and label the underscored structures in the directions.

TEACHER'S GUIDE TO

EXERCISE 25 -- HISTOLOGICAL STUDY OF THE MALE REPRODUCTIVE SYSTEM

Prerequisite: Exercise 4

INTRODUCTORY REMARKS TO THE TEACHER

The INTRODUCTORY REMARKS TO THE TEACHER in Exercise 24 also apply to Exercise 25. There is no long student introduction dealing with the hormonal control and feedback between the male reproductive system and the pituitary gland. Follicle stimulating hormone (FSH) is very abundant in the male pituitary gland and functions by stimulating spermatogenesis in the tubules of the testes. Luteinizing hormone (LH), also called interstitial cell stimulating hormone (ICSH) by some, acts upon the Leydig cells, which lie between the seminiferous tubules, and causes them to secrete several steroid hormones, the most potent of which is testosterone. These male hormones (or androgens) act upon the epididymis to cause ripening of the spermatozoa, and act back upon the pituitary gland to cause the increased storage of FSH.

MATERIALS AND EQUIPMENT

Microscope and lamp
Lens paper and immersion oil
Prepared slides of mammalian testis, ductus deferens, seminal vesicles and ventral prostate gland.

INTRODUCTORY DISCUSSION

Review Exercise 15, Part B--First Procedure for Meiosis (Sperm Production.) These stages can be identified in many sections of the tubules (see Ex. 25, Section A.2). What advantages or benefits might come from having a spermatogenic wave instead of having all parts of the tubule function at the same time but at a lower rate of activity? Is the lack of an obvious sex cycle in the male an advantage over the cyclic involvement of the reproductive system as seen in female mammals?

Part A, B, and C

From the structure of the ductus deferens, how would you conclude that sperm are moving along this tube?

Biologists used to think that the seminal vesicles were used to store spermatozoa until ready for use. Is there any evidence for this theory shown on your slide? (No.) What function does this gland apparently serve? (It secretes part of the seminal fluid.)

TEACHER'S GUIDE TO

EXERCISE 26 -- RESPONSE OF ANIMALS TO PREGNANCY URINE HORMONE

(Pregnancy Tests)

Prerequisites: Exercises 1, 2

INTRODUCTORY REMARKS TO THE TEACHER

The detection of pregnancy urine hormone (PU) in the urine of a woman who suspects that she is pregnant constitutes a confirmatory test of great interest to such patients. The action of the female sex hormones on the reproductive tract had hardly been demonstrated when Ascheim and Zondek (1927) reported their technique for the detection of pregnancy, making use of immature female mice.

MATERIALS AND EQUIPMENT

Chemicals

Pregnancy urine
Urinometer (for determining specific gravity)
20% HCl
.04% Bromcresol green indicator dye
Kaolin (acid washed) (commercially prepared)
.1 N NaOH
.5% Phenolphthalein
1% eosin blue or yellow
4% aniline blue
Citrate-phosphate buffer, pH 7.2
pHdrion paper

Biologicals

mice (immature females)
Frogs (male)

Others

dissecting instruments
slides and cover glasses
pH paper

PREPARATIONS

The male frogs have bigger thumbs than the female frog. Place frogs in a covered 8-oz. jar in the refrigerator in 1/2 inch of water.

INTRODUCTORY DISCUSSION

Teacher: What do governors or mayors do when there are unusual civil disturbances? (They call out the national guard to take over from the local police.) And what happens when the civil situation seems to be back where local authorities can handle it? (The national guard goes home and no longer is an organized unit.)

When a mammalian egg becomes fertilized and starts to develop, it grows some membranes, the chorion, which itself is more powerful than the pituitary gland in causing the corpus luteum of the ovary to secrete progesterone. The egg normally remains in the human oviduct about 1 week. If it is not fertilized it dies and is reabsorbed and never enters the uterus because of a sphincter at the end of the oviduct. However, if it is fertilized, the embryonic membranes cause enough additional progesterone to be secreted so that the sphincter will open, the embryo enters the uterus, and becomes implanted. The pituitary gland can only keep the corpus luteum functional for about 12 days, but the luteotropic hormone of the chorion can keep it active for the term of the pregnancy. About the 14th day of pregnancy in higher mammals, then, the chorionic gonadotropin takes over from the pituitary much like the national guard takes over from the local gendarmes. When the pregnancy ends, the chorion is normally removed and the ovary returns to full stimulation from the pituitary gland once again. From this sequence, it may be more clear why urine taken about two weeks after the first missed period (that is the first failure of menstruation to appear following insemination since it may not appear for other reasons) is best for these tests for pregnancy. It is because at that time the amount circulating in the blood and appearing in the urine is high enough so that there is less doubt that it is present, a condition which may be the case earlier.

The Ascheim-Zondek Test.

It will be best for beginning experimenters to keep mice being treated differently in separate cages. More experienced scientists may number the animals. The graded dosage of the urine (or urine extract) will bring about graded secretion of estrogen and therefore, graded estrogen effects. An intense reaction is characterized by highly vascularized ovaries and uteri (hyperemia) with numerous recent corpora lutea protruding from the ovaries. These will be dark red in color; thus the laboratory name of "mulberry" ovaries. Other effects not noted in the exercise would include opening of the vaginal orifice and the fact that washings of the vaginal canal taken with a small pipet will show many cornified epithelial cells. Such smears may be dried in air and stained for 30 seconds with Geimsa (quadruple) stain.

Since the tissues of these animals are not going to be used for further physiological experimentation, the animals may be killed with ether or chloroform before dissection.

The Rana Pipiens Test

The male frogs should be kept in the refrigerator (between 10 and 20°C.) until ready for use. This is partly because warm temperatures will stop spermatogenesis. The chorionic gonadotropin works on the male frog to cause sperm release because that is the way it happens in nature. At mating time the male grasps the female quite firmly and she releases her ripened eggs. Within a few minutes, less than 10, the gonadotropin of the female triggers the release of sperm by the male so that the eggs are fertilized before their jelly coats become too toughened to permit sperm entrance about 15 minutes after laying.

To inject the urine into the dorsal lymph sac use a 1-1/2 inch, 20 gauge syringe needle. Start by inserting the needle under the skin back near the leg. The skin is loose, so run the needle just under the skin forward until it is just anterior to the urostyle. Injection of fluid then can be seen to fill the dorsal lymph sac. The distance between the insertion point and the lymph sac helps prevent the escape of the injected fluid.

The Antibody Test for PU

Another popular, modern test is quick and easy to perform, but much less instructive about the physiological functions of Human Chorionic Gonadotropin (HCG), which is the same as PU. A few drops of Anti-HCG antibody is floated on top of a small tube or capillary of suspected pregnancy urine. After about 30 minutes a layer or ring of precipitate will have formed in a positive test.

REPORT SHEET

Have the class write an essay on the origin and action of human chorionic gonadotropin (HCG) to be turned in at the next laboratory meeting.

REPORT SHEET

Messages (Not in order)

LET THERE BE LIFE.

KNOWLEDGE IS POWER.

WHAT HATH GOD WROUGHT?

LET US MAKE US A MAN.

MAKE HASTE.

STUDY NATURE NOT BOOKS ONLY.

MAKE SOME SASPARILLA.

TEACHER'S GUIDE TO

EXERCISE 28 -- DO ENVIRONMENTAL FACTORS AFFECT THE ACTION OF GENES?

Prerequisite: Exercises 2, 29 and 9

V

INTRODUCTORY REMARKS TO THE TEACHER

The assertion that the actions of the individual are the product of his genetic constitution was advanced in socio-political circles of the last decade by some scientists and politicians. This view tends to down-grade the effects of the environment on gene action. This experiment is designed to find out what happens in a situation where there is a change in the environment, but not in the genetic makeup of two different individuals. Are there environmental effects on the actions of genes?

An important aspect of differentiation is the influence of the role of the external environment, which can, and sometimes does, modify the development of an organism. In the plant kingdom, plants of the same species growing in different habitats often differ extensively in appearance but appear very much alike when the external environment is the same. Some of these plants are influenced by the amount of sunlight while some others are sensitive to temperature and other changes. In order to evaluate this aspect of differentiation precision accounts of the effects of a particular gene on an organism require the specification of not just the geneotype but also the environmental conditions.

If a gene shows incomplete penetrance, i.e., it is not expressed in some individuals, it is a reflection on either the variability of the geneotype or the environment. We do not understand fully how the environment influences gene activity. An example of this influence of environment can be shown with two types of bean seeds--light and heavy seeds.

The light seeds were planted in a very favorable set of conditions while the heavy seeds were planted in an unfavorable environment. The average weights of the offsprings (bean seeds) were almost the same for the two kinds, but when they were both grown under the same favorable conditions, the heavy seeds had a much higher average weight than those of the lighter strain.

In this exercise we shall test the effect of sunlight on the expression of a gene (for green color) and the effect of a chemical (the growth hormone, gibberelic acid) on the rate of growth in pea plants.

MATERIALS AND EQUIPMENT

Chemicals

Gibberelic acid (100 mg./liter)

Biologicals

30 tobacco seeds
20 seeds each of corn and beans. All seeds from stocks heterozygous for albinism (from Carolina Biological Supply Co., Burlington, N. C.).

Plastic and Glass Ware

12 styrofoam drinking cups or 12 flowerbed flats
Loam, sand or Vermiculite autoclaved to kill mold spores
2 hand atomizers
Parafilm squares
rubber bands

PREPARATIONS

The seeds should be planted about 10 days before the first group of students use them (see directions on page 28-1).

INTRODUCTORY DISCUSSION

The discussion can begin by asking the students if they think that the environment is capable of modifying the expression of hereditary characteristics. This can be expounded to introduce the differences between phenotype and genotype.

An alternative approach is for the teacher to assume that the students know that differences and similarities do occur in living things including students. Are these differences due to the food we eat, genetic makeup which we inherit, to the environment or to education, etc? At this junction the answer may involve some experimentation on subjects that are identical. Self-fertilizing (selfing) plants meet this requirement because if the parent is homozygous, then the offsprings will be homozygotes. But, the exercise calls for the use of hybrid seeds that are in a ratio of 3:1 for a characteristic. Thus, the object of part of the exercise is to see how this genetic ratio is affected under different environments--light and darkness. The experiment should also show how it is possible to mimic the genetic constitution of another seed type by making a change in the environment. The gibberilin induces the elongation of the leaf internodes thus, making dwarf plants taller so that they look like normal plants.

PROCEDURE

When spraying the leaves with gibberelic acid, the pot should be covered with either cellophane or Saranwrap to prevent contaminating the soil. This is to ascertain uniform amount of the hormone per plant. The concentration of acid may be varied to determine the correlation between concentration and the increase, Δl in growth rate. In the dark, all of the plants will be yellow, but when they are transferred to light some of them will turn green. If there is a large population this ratio will be about 3:1. In this respect the sum of green and yellow plants for all of the sections can be determinded to give the largest population possible, which should bring the ratio closer to the theoretical value. The students should be asked why a large sample gives a more probable estimate of the mean than does a small sample (See 9-1).

REPORT SHEET

The report sheet for this exercise provides charts for data collection but no questions have been provided to probe what understanding students have of the work they have completed. The following additional things, then, should be done:

1) A graph of the heights of plants should be plotted against days of growth.

2) A graph should be prepared showing the growth rate achieved, $\Delta\ell$ versus the concentration of plant hormone used.

3) Write a report on one of the plant growth hormones.

TEACHER'S GUIDE TO

EXERCISE 29 -- INHERITANCE OF GENETIC TRAITS

Prerequisite: None

INTRODUCTORY REMARKS TO THE TEACHER

Before Mendel made his studies, heredity was viewed as the direct transmission of characters from parent to offspring. This notion existed from the time of Hippocrates. In 1868, Darwin suggested that all the cells and tissues of an organism give off minute granules during development and when the organism reaches maturity. These thrown off granules were supposed to circulate through the organism, multiply and then pass on to reproductive cells. The reproductive cell would, therefore, contain a multitude of components given off from each individual part of the organism. Tissues and cells are then supposed to develop these granules. To put it in a concise way, this is close to Lamarckism. Although Mendel had written his paper, this view of heredity was not disputed (challenged) until 1883, when Weismann postulated the theory of Continuity of Germplasm.

In his paper Mendel introduced symbols for characters, now interpreted to mean factors or determinants responsible for the manifestion of the characters. Thus, he ascertained that characters are not transmitted directly from generation to generation as the classicists believed, but as discrete particles responsible for the appearance of particular characters. He went further to show that each individual receives a particle from each of its two parents in respect to a particular character, and also that the particles do not influence one another in any way, but are separate and uncontaminated during the formation of the reproductive cells.

MATERIALS AND EQUIPMENT

Ears of corn (3:1, 9:3:3:1, etc., ratios)

The different ears of corn can be bought from Carolina Biological Supply Co., Burlington, N. C. The ratios will depend on whether the teacher orders an F_1, F_2, or a backcross. The number of characters will also play a role in determining the ratios. It is advisable to have two, three or more characters available for the class. The problems can be used to demonstrate the details of the mechanisms of inheritance.

INTRODUCTORY DISCUSSION

The discussion can be initiated by asking the students to list different genetic traits. They can be asked to see if these traits are common to everybody in their immediate family and later to their grandparents, etc. But since human genetics is very complex (although desirable, it is advisable that the teacher should not spend too much time on it at this point) many other factors may also influence the expression of these traits. The discussion will show the teacher how the students think they come to inherit these traits, after which the teacher can introduce the exercise. This exercise is designed to illustrate the principles of Mendellian inheritance. It will show that heredity consists mainly of the transfer from parent to the progeny of a blueprint of the organization of a particular living thing. Thus, genetics can be defined as the science that describes how this blueprint is drawn, transmitted, and expressed in the construction of another living thing (progeny.)

The student should get the notion from this experiment that there are some traits that are inherited as constant components that are (to a certain extent) unchangeable. The questions are from Baker and Allen, The Study of Biology. At this point the students should be able to identify things like nose, ears, etc., as parts that are inheritable. The students should be curious enough at this stage to want to know about meiosis and spermatogenesis, the principle of chance and the Punett square can be used to explain what goes to where. This can be extended further to what happens in the F_2 generation or to inbreeds. This should not carry a social implication if the students choose to use human beings for explaining examples.

PPOCEDURE

Most supply houses provide Indian corn wrapped in a plastic cover. This is good for keeping all of the kernels with the ear if they work loose or the ear is accidentally dropped. Therefore, this covering should remain. Follow the procedure indicated in Exercise 29A. The teams may be reduced to two students - a counter and a recorder, or expanded to a team of four (1 counter and 3 recorders) where three types of kernels will be tabulated.

TEACHER'S GUIDE TO

EXERCISE 30 -- BACTERIAL RESISTANCE TO STRAPTOMYCIN AND SALT[1]

Prerequisites - Ex. 5, 6, 7, 12, 28 & 29

INTRODUCTORY REMARKS TO THE TEACHER

The coliform bacteria are very adaptive organisms. They are capable of maintaining their internal ion concentration whether they live under very salty conditions like the human colon or in the less salty environment of sewage. To achieve this, there have to be inward concentration gradients for chlorides and sodium ions as well as outward gradients for potassium ions. These ions penetrate the membranes by diffusion very slowly as the cells lose potassium ions and gain sodium and chloride ions. The gradient is maintained by active transport (requiring the dissipation of energy) and generates an electrical gradient.

Before the advent of molecular biology, organic adaptation in bacteria was a thorny subject. However, it has now been resolved into two different points of view. One view is that adaptive changes occur spontaneously as sporadic mutations which are not in any specific relationship with environmental conditions.

It is after the mutation has occured that natural selection then functions to stabilize the best adapted genotypes. The second view of changes in adaptation suggests that adaptive mutation is not spontaneous but is itself under the direction of the environment. This view maintains that natural selection plays only a subsidiary role in the process of adaptation. In considering these two views, one must bear in mind physiological adaptations that are not heritable but whose interplay with other heritable changes (mutations) can induce one to think that these physiological adaptations are heritable.

The resistance of bacteria to streptomycin has only recently been more fully understood. The study of the effects of antibiotics on bacteria was originally carried out for its medical implications. We all know about the use of penicillin, but penicillinis ineffective against gram-negative organisms and the tuberculosis organism. In 1944 Waksman and his associates discovered an antibiotic which is highly selective in its activities against bacteria and is also of limited toxicity to animals. E. coli, being gram-negative has walls that are thinner with more lipid proportionally, and chemically more elaborate than the gram-positive bacterial walls. E. coli has 2 layers of walls. In its actions against these organisms the development of streptomycin resistance shows some unique features. In many species a mutation conferring full resistance occurs at a rate of about 10^{-10} per fission. This overshadows the smaller step mutations characteristic of resistance to other agents. A more profound difference is the mutation which over-adapts the cell to streptomycin. This over adaptation causes the resistant mutant to be dependent upon streptomycin for growth. It has now been confirmed that streptomycin inhibits bacterial growth by acting on the ribosomes during translation of the genetic message. It acts as a potent noncompetitive inhibitor of the incorporation of phenylalanine into peptides while at the same time stimulating the incorporation of leucine and isoleucine into the syste... Thus, streptomycin does not inhibit peptide (protein) formation but introduces some changes in the ribosomal conformation so that the translation mechanism responsible for arranging the proper sequence of amino acids in the building peptide is changed. To do this the streptomycin binds to the 30S

[1] Revised Title.

ribosomes causing a primary deformation in a protein structure.

	2 × 30S +	2 × 50S	2 × 70S	100S	
MW	0.85	1.80	2.8	5.9	×10⁶

[S is the svedberg unit, which is a measure of the rate of sedimentation expressed as the sedimentation coefficient. Its value is a function of both the weight and the shape of the particle. The value 1×10^{-13} sec. = 1 Svedburg unit. Thus, 70S = 70×10^{-13} sec., etc.]

The overall result is the synthesis of proteins that do not contain the proper amino acid sequences and are, therefore, useless to the organism for growth. They are also known to effect changes in the permeability of the cell.

Whereas streptomycin resistance is a consequence of alterations in the primary structure of proteins, salt resistance can be considered as an example of physiological adaptation which interacts with the normal hereditary characters. There is dehydration due to exosmosis across a semipermeable membrane. This exosmosis results in shrinking in the cell size, and dehydration of the internal environment accompanied by a change in incapability of enzymes to function normally.

MATERIALS AND EQUIPMENT

Chemicals

Streptomycin 100 units/ml. or 5%.
2 lb. nutrient agar.
Sodium chloride solutions 0 to 6%.
95% Ethanol

Biologicals

24 hour old E. coli culture

Plastic and Glass Ware

Petri dishes,
1 ml. sterile disposable pipettes
glass spreaders
beakers
slides
sprayer nozzel
bottle or hand atomizer

Other

Nichrome wire inoculating loop.
Bunsen burner.
wax pencil.

PREPARATIONS

See Ex. 5 & 6 for details of making sterile plates, etc. The E. coli culture should be inoculated for 24 hours in advance.

It may be more convenient to prepare the streptomycin according to units rather than in percentages as indicated in Exercise 30. In this case, the units can start from 100 and by serial dilution make 5, 4, 3, 2 and 1-unit solutions, with the control serving as your zero mark.

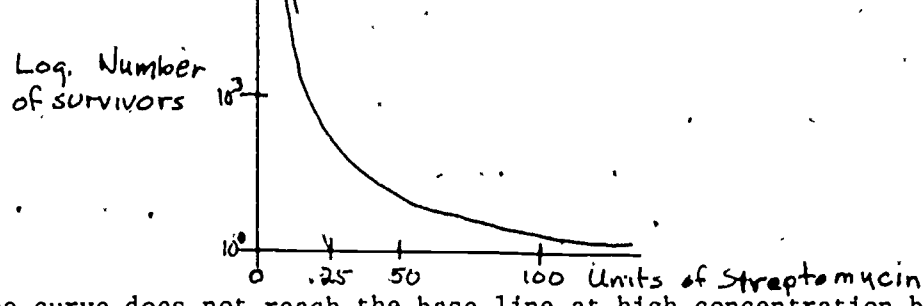
INTRODUCTORY DISCUSSION

The discussion on this exercise can be initated by reviewing the sections on mutation and inheritance in the Introductory Remarks to Teachers. It is important to stress the differences between a true mutant and an organism which is physiologically adapted. The discussion can then lead to listing the differences between what happens in parts A and B of the exercise. The students should be asked to make suggestions to show that salt resistance is different. (Streptomycin resistance is heritable while salt is not ergo, it is physiological adaptation.)

The students should list as many differences between two individuals (e.g., the corns in Ex. 28 & 29) and show whether these differences can be attributed to mutations or just physiological adaptation. The role of mutation in evolution should be stressed to show that natural selection acts only after mutation has occurred and not before. Thus, some new forms of organisms arise as a consequence of mutation and the environment.

It should be stressed during the discussion that these mutations occur every day, but selection is against them and we very rarely come into contact with such mutants unless the environmental factors favor their survival.

The effects of the different concentrations of streptomycin in the media should be stressed. Graphically, the higher the concentration of the streptomycin per ml. of medium, the lower the number of survivors. The graph would look something like this:



The curve does not reach the base line at high concentration because of the mutants that are resistant to or dependant upon streptomycin.

TEACHER'S GUIDE TO

EXERCISE 31 -- THE INHERITANCE OF SPECIFIC PROTEINS

Prerequisite: Exercise 4

INTRODUCTORY REMARKS TO THE TEACHER

There is a long historical introduction to this exercise which concerns itself mostly with the discovery of the blood factors. Ideas that it strongly attempts to conceptualize are:

1) The blood typing-factors are proteins.
2) Like other natural proteins, each is produced by a specific gene.
3) The presence or absence of a blood typing-protein is therefore, evidence of the presence or absence of a certain genotype in the individual.

This kind of exercise is traditionally used for different purposes in different courses. In microbiology courses it is sometimes used to demonstrate an antigen-antibody reaction. In experimental embryology, it has been shown that the factors affecting the adherence of sperm to eggs is a similar-type reaction. In physiology courses blood typing is used to show how the agglutination caused by transfusing the wrong type of blood can sometimes cause blockage in small vessels leading to dire consequences. In evolution courses blood typing is used to show phylogenetic relationships, and out of this kind of study grew awareness of the Rh factors. In genetics courses it is used to follow the inheritance of blood types (phenotypes) and the uses of this feature in forensic medicine (such as in criminal and paternity cases) is well-known. Whatever uses are made of the exercise, the fundamental ideas hold true--the reactions are for specific proteins; the specific proteins are inherited; and the kind of protein present in cell membranes makes a difference if you need a tissue transplant, such as a blood transfusion.

There are several views about antibody formation at the present time. These can be grouped into two categories. The complimentariness concept was advanced by Linus Pauling some years ago. This view holds that the gamma globulins in the blood plasma of higher animals, particularly mammals, could assume shapes complimentary to those of many foreign proteins getting into the body. The second view does not assume that the antibody takes the shape that is complimentary to the antigen, but rather is complementary in its electrostatic charge distributed among, perhaps three (maybe four) amino acids in the antibody protein. Winkler (1961) who advances this viewpoint, notes that there are 20 amino acids normal to proteins. If we assume that only three of them are involved, then the number of possible combinations would be 20^3 or 8,000, which approximates Lederberg's estimate of the number of antibodies.

Is this war against foreign protein entering the body inexorable? No. The antibody-forming capacity of the reticulo-endothelial system does not become active until about 6 months after human babies are born (and preliminary evidence indicates that a proportional period occurs in other mammals). At that time all proteins present in the body become encoded in the nucleic acids (presumably RNA) so that a foreign protein entering the circulation before the encoding will be encoded as "normal" for that individual, but after that time foreign proteins will be phagocytized, "read and computed" against the memory bank, and if found to be "foreign", an antibody will be produced against it to bring about its elimination from the

molecular population of the body. Another condition in which new proteins may be "naturalized" occurs following "antibody anesthesia". Steroid hormones, such as cortisone, are particularly effective in suppressing the antibody-forming process. Under these conditions foreign proteins can be introduced into the body without being attacked by antibodies (rejection) and the usual expectation is that if the anesthesia is continued long enough, when it is removed the reticulo-endothelial system will again encode all proteins present as though they were "native". This has been an important feature in heart and kidney transplantation technique for it enables the surgeon to use organs from genetically distant donors.

Antibodies have been classified traditionally by the kind of reactions they produced, as precipitins, if they precipitated proteins; as agglutinins, if they cause cells to stick together; as opsonins, if they made slippery, encapsulated bacteria less slippery so that they could be phagocyized; and as lysins, if they caused the cell to dissolve (lyse). Later, the unitarian view (not related to the Unitarian church) held that there was only one antibody for each particular antigen (usually a protein) and that precipitin, agglutinin, opsonin and lysin reactions were all the reactions produced by a single antibody to that substance. This is the view most widely held today: It will be noted that precipitin, agglutinin and opsonin actions result from making antigens (proteins) "stick", that is, behaving as though they were sticky.

Although antibodies are generated in response to foreign proteins and other large molecules, some are produced naturally in the human body. Alpha (anti-A) and beta (anti-B) agglutinins are such antibodies. Although the antigens (proteins) A and B are commonly referred to as agglutinogens, they do not act as generators or stimulator of antibodies against themselves ordinarily because if they are absent the human reticulo-endothelial system will spontaneously produce them. However, the antigens for the Rh factors are truly agglutinogens, for in Rh-negative persons, they activate new nucleic acid sequences which bring about the production of antibodies against Rh proteins.

The objective of this exercise is not simply to type the blood of the student, but to also help him reason about the interrelationships between the antigens (cell proteins) and the antibodies (in the plasma) and their specificity. Part C contains a problem in cross-matching which underlines the relationship of the antibody present to ability to clump or not clump cells.

Part B deals with a test for sickle cells and sickle cell trait. This is not commonly seen in laboratory manuals where use by non-black students is mainly envisaged. Sickling is one of about 42 anamolies of the hemoglobin known in human beings (See Ingram, Biosynthesis of Macromolecules, page 162.) Sickling was once thought to be restricted to members of the black race, but is now commonly diagnosed as such in Mediterranean countries. The sickling results from low oxygen tensions (and also possibly high carbon dioxide tensions). Under these conditions the hemoglobin polymerizes into long chains, deforming the usual disc-shaped corpuscles into elongate, banana - or sickle-shaped ones. In west Africa and in South America this trait has been found useful because malaria parasites do not survive well in it. Urea has recently been found to effectively prevent sickling in sickle cell patients.

The Rh and Lewis factors have been of considerable clinical importance in antiphylactic reactions caused by transfusing blood into patients with a pool of antibodies against the antigens in the donated cells, and also in the pre-natal disorder, erythroblastosis fetalis. Today, when it is apparent that the fetal

child is afflicted with the anemia of erythroblastosis, it is transfused while in utero with cells that are not attacked by antibodies diffusing through the placenta from the maternal circulation.

MATERIALS AND EQUIPMENT

Chemicals

100 ml. .9% NaCl
100 ml. .70% ethanol
1 lb. ether (for anesthesia)

Biologicals

Blood typing antisera: Anti-A, Anti-B, Anti-D, Anti-C and Anti-E

1 lb. cotton (roll or balls)

Glass and Plastic Ware

12 2 to 5 ml. syringes filled with petrolatum (such as Vaseline)
24 Test tubes 1 x 15 cm.
Microscope slides and coverslips

Other

Sterile, disposable lancets
Microscopes and lamps
Rh typing boxes
Wax marking pencils

PREPARATIONS

The microscope slides should be washed and placed in a container of 95% alochol. They can be dried from the alcohol using lent-free paper towels or a lint-free cloth.

Soap is needed in the laboratory so that students can wash their hands before drawing blood.

INTRODUCTORY DISCUSSIONS

The pattern of this discussion is to characterize the relationship of the government toward its citizens, natural and naturalized, and toward aliens; then transfer this concept to how the body protects its population of molecules, especially proteins and tries to eliminate any unnaturalized aliens.

Teacher: What is the ideal of the American constitution with regard to the rights of citizens to participate in the public life of the country? (All kinds of people regardless of race, religion, national origin or previous conditions of servitude are franchised to participate in the government.) How does the government seek to accomplish or work toward this ideal? (It has organized legislatures to encode the customs of the people into laws, law enforcement agencies to protect citizens against law-breakers, and courts to judge and interpret the laws.)

What happens to people who enter the country illegally, that is without a passport or visa? (Sometimes they get away with it for a while, but eventually their presence becomes known and agents of the Immigration Service or from the Counter Intelligence Agency obtains orders for their deportation or expulsion.) Spies also get this treatment but in wartime they may be executed. If there is an invasion or threat of an invasion, then defenses are mobilized to keep the invaders out.

Teacher: If we can go back to a sort of political comparison. During a war what kind of actions are taken by defenders to deny any resources they might have to leave behind to the enemy. (They may burn or otherwise destroy them.) If they regain the territory would such a policy of destruction have denied the defenders anything? (They may have destroyed some things of historical, cultural or sentimental value.) Back to blood. Can the agglutination of foreign proteins be detrimental to the body? (Yes, allergic reactions result from the body's attempt to protect itself.) Blood clotting may prevent some blood loss from small injuries, but to have blood clots being pumped around in the circulatory system is dangerous. Have you any idea about having blood cells agglutinate (or clump) in the vessels? (The responses should show an appreciation of the fact that such clumps can cause damage in the same way that clots (emboli) do.) Today, in this exercise, we want to observe several things. In Part B, you will not only determine what your blood type is, but you should look at the reactions obtained by others so that you can see how different bloods react differently. In Part C there is a problem in cross matching, which could be very useful to know about in a blood-typing emergency. In Part D, the Rh type will be determined. In all of these reactions we are concerned with the agglutination function of antibodies.

PROCEDURE

Part A and Part B may not need further explanation.

In Part C it may be well to go through the cross-matching of various types of blood with Type A cells and serum from Type A blood. A table of antibodies is given at the top of page 31-5 in the manual.

Case I: Type A cells are clumped by the serum from the unknown type. This serum must, therefore contain the antibody alpha. The blood types containing alpha (see the table) are types B and O. The serum from type A blood clumps the cells of the unknown type. This serum contains the antibody beta, therefore, if cells clump in it, they must have the protein B present. Referring to the table, we see that B and AB contain this protein. Now cancel out the dissimilar types we have written down (O for cells AB for plasma) and we see that B remains as the type for the unknown sample.

Case II: Type A cells are not clumped by the unknown serum. It must then not have contained alpha. This is true for types A and AB. Type A plasma contains beta, but does not clump the unknown cells. They then must not have any B, so the unknown blood sample was of type A.

Cases III and IV are combinations of Cases I and II.

Part D refers to an Rh-typing box. This is sold commercially as a elongate box with a ground or milkglass top and warmed by an ordinary light bulb. It sits on a hinged stand so that the slides placed on it can be all rocked back and forth to

gether. If you do not have any Rh-typing boxes available, the slides can be placed a few inches under an ordinary light bulb tobring the temperature to about 41°C and the preparation tilted by hand every 10 or 15 seconds for the 4 minutes of the test. Instead of using the microscope to determine clumping, the slide is viewed with the naked eye for evidences of "rivulets" that develop when the slide is tilted and clumped blood slides across the drop. These preparations tend to dry out in the heat. If this happens, add another drop of the Rh typing serum.

It is customary to screen the blood first with anti-B serum. Adding the percentages of Rh-positive types which are D-negative (Cde, cdE and CdE) one gets less than 3% of the 85% positive. Where economy is most important, the blood is then typed for B and E when it shows up D-negative. If the blood is negative to C and E, then the geneotype is cde and it is truly negative. For educational purposes, the student should type his blood for C, D, and E in order to arrive at his genotype for these proteins.

REPORT SHEET

The Report Sheet only calls for a notation of the results of typing and the cross-matching exercise. Teachers may wish to ask the students to answer some questions raised in the discussion preceding the exercise.

Cross-matching Results:

Case	Unknown Serum and Type B Cells		Unknown Type Cells and Type B serum		Type
I	Clumped	A Ø	Clumped	A A,B	A
II	Unclumped	B A,B	Unclumped	B Ø	B
III	Clumped	A O	Unclumped	B O	O
IV	Unclumped	B AB	Clumped	A AB	AB

TEACHER'S GUIDE TO

EXERCISE 32 -- PHOTOSYNTHESIS AND THE SYNTHESIS OF STARCH

INTRODUCTORY REMARKS TO THE TEACHER

Exercises 32, 33, and 34 form a sequence even though they need to be used in that order or even as a group. This exercise, as the title indicates, deals with the photosynthetic process which attaches energized hydrogens to carbon dioxide. The polymerization of the product, hexose (glucose and fructose), into starch and other multiple sugar products. (sucrose, maltose, trisaccarides, amyloses, cellulose, pectin, gums, and in animals and algae the production of chitin (n-acetyl-amino sugar polymers)) is a separate but, associated process. Exercise 33 -- Digestion, deals with the depolymerization of polysaccarides, proteins and lipids so that the residue molecules (building blocks) can be used in the energy-extraction process, and Exercise 34 -- Respiration--deals with some of the external evidence that energized hydrogens are being oxidized to water by measuring oxygen uptake.

The name and meaning of photosynthesis is normally introduced to students in the Fifth Grade and it is general knowledge that light is needed for green plants to perform this process. However, few have done any experiments to demonstrate that photosynthesis is occurring in illuminated leaves but, not in leaves kept in the darkness. To be sure, the more convincing demonstrations, such as Hill's Experiment making use of spinach chloroplasts and iron salts, are not technically feasible for most freshmen courses, although it is simple enough if the chloroplasts can be prepared.

There are many good accounts of the reactions in photosynthesis easily accessible to the teacher. A summary of the process is that water ionizes to form ions we represent as H^+ and OH^-. In the presence of chlorophyll and light the $-OH^-$ group is split to yield free oxygen, a H^+, and a free electron (e^-). Light photons strike an eleetron in the atoms of chlorophyll, energizing it and causing it to leave the atomic shell as an e^-. The e^- produced from water then falls into the "hole" left by the energized electron. The energized electron reduces a quinone, such as plastiquinone, Q254, or ubiquinone, and the electron is then passed to cytochrome enzymes which are made non-resonant and transfer some of the energy of the electron to ADP molecules to form ATP. In bacteria and other primitive photosynthetic cells the electron then combines with a proton (H^+) to form water, but in higher plants, light striking a second kind of chlorophyll (chlorophyll a) displaces another electron which is energized to a higher level than the one from chlorophyll b. The electron from chlorophyll b, which has just come off of the cytochrome enzymes then falls into the "hole" left in chlorophyll a, and the energized electron from chlorophyll a now reacts with ferridoxin, a non-iron-containing compound, and this in turn transfers the electron to NADP, which takes up a proton (H^+) to form NADPH. The ATP and the NADPH produced in these "light reactions" are then used to reduce carbon dioxide in the "dark reactions" and this leads to the formation of glyceraldehyde (a 3-carbon sugar) which condenses to form hexoses glucose and fructose. Sucrose is the transport form of sugar in higher plants and is easily converted to glucose by intracellular enzymes in the fermentation system. Glucose-UDP reacts with fructose to yield sucrose, whereas glucose-ADP polymerizes to form cellulose. Glucose-UDP polymerizes to form starches, and in animals it polymerizes to glycogen. Only the glucose formed in excess of the metabolic needs of the plant is stored as these polymers, the rest is used in the energy-extracting system to get hydrogen, which is oxidized to water, throwing out carbon dioxide.

just as do non-photosynthetic cells.

In this exercise students will demonstrate that starch is laid down in the light and that oxygen is given off by green leaves in the light. In Part C the crude starch synthase is extracted and used.

MATERIALS AND EQUIPMENT

Parts A and B

Chemicals

95% Ethanol
Lugol's iodine Soln
Pyrogallol solution

Plastics of Glass Wares

beakers 250cc
Test tubes

Biologicals

Geranium
Elodea (water weed)

Parts C

Chemicals

Sodium cyanide or sodium fluoride (0.01M)
Glucose -1- phosphate (0.01M)
Glucose or Dextrose (0.01M)
Starch solution (0.2%
Potassium acid phosphate (0.2M) KH_2PO_4
Lugols iodine solution

Biologicals

Irish potato

Plastics and Glass Wares

Beaker 250 (Same as above).
Suction flask with Büchner funnel
Centrifuge tubes
Test tubes
Disposable spot trays

Others

Knife, Food chopper
Vaccum line, Filter paper, Wax pencil

INTRODUCTORY DISCUSSION

Present the class with a burning candle. They will know it, but point out that the heat of the flame melts the paraffin and then oxygen is used to burn it, forming smoke, heat, light, carbon dioxide, and water. The smoke (carbon) and light are easily seen. The heat is easily felt, but the carbon dioxide and water, how can they be demonstrated? (Let the water condense on the side of a cool container. Pass some smoke through lime water.)

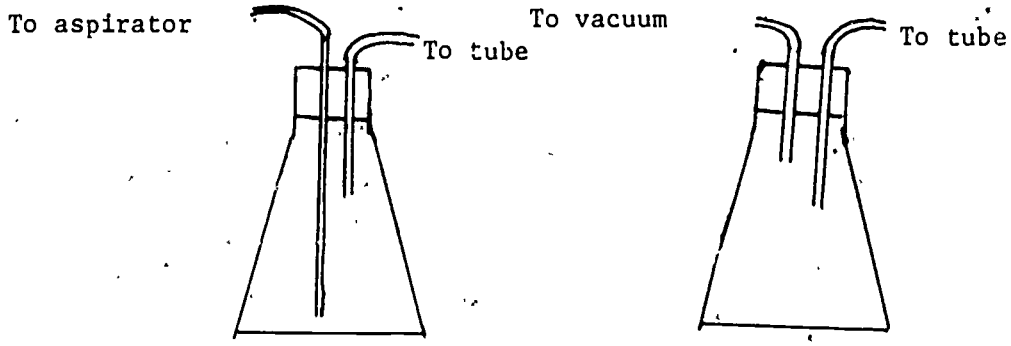
Now, according to the law of Conservation of Energy and of Matter, energy and matter can be transformed but not destroyed. So, how could the materials of burning be used to reconstruct another candle? (The water, light, and carbon dioxide could participate in photosynthesis in plants. The plants can then be eaten by animals, the animal bodies extracted to obtain the fat (tallow, suet, etc.,) and a new candle formed by dipping in a string for a wick.)

Have students describe metabolism, anabolism and catabolism. Can anabolism and catabolism take place at the same time in the same cell? (Of course) Photosynthesis (anabolism) is occuring in the plastids of the green plant cell but, catabolism is taking place in the rest of the cytoplasm.)

Today we want to observe the effect of light on starch build-up in leaves, and to see if oxygen can be produced by leaves placed in the light. Then, we will go on to take a starchy tuber and see if the juice might contain something which functions in assembling the starch macromolecule.

PROCEDURE

Have students set up Part B first. The Elodea should be fresh. The tubes are evacuated by a water aspirator or a vacuum pump to remove atmospheric oxygen. In either case provide a trap between the vacuum source and the tubes being evacuated. Two arrangements are ordinarily used--one for use with water aspirators, which is self-emptying; the other is not self-emptying because it is very important to prevent water from getting into the pump or vacuum line. These are diagrammed below.



With this arrangement water from the aspirator faucet will rush into the flask when the water is turned off (or almost turned off) to fill the vacuum. The trap is needed to prevent flooding or contaminating the tube being evacuated. Of course, in this case, the clamp to the tube would be closed before the vacuum is released. Each tube to be evacuated will need to have a piece of heavy rubber tubing and a Hoffman clamp.

If a vacuum pump or a vacuum line is being used, the need for protection is

reversed. Water must be prevented from entering the vacuum line but, there is no danger of water contamination from the pump. The flask should be larger in capacity than the possible inflow.

Do Part A second. While the extracted leaves are being dried, Part C can be performed.

REPORT SHEET

Part A. The results obtained should be that there is no starch in leaf #1 (kept in the dark), but there is some in the same leaf kept in the light. The leaf kept in the light originally had starch but, not after being kept in the dark.

A space is left for the student to "explain the above results". To be done right would require a lengthy paper. Perhaps some more specific questions would be better.

1) What happened to the starch in the leaf after being left in the dark? Explain what catabolic steps were taken to utilize the starch for cell energy.

2) What are the details of the steps whereby starch was synthesized and stored in the leaf?

Part B. Record experimental results.

Part C. What information does each tube yield in this experiment?

Tube

1) What color is starch in the presence of glucose and enzyme? Does it get more intense with time?

2) What color is obtained with starch in the presence of G-1-P and enzyme?

3) What color is obtained with G-1-P and enzyme without starch? Compare with tube #2.) Does it give a starch test in the end? If so, synthesis has occurred. If not, something went wrong.

4) No starch test should have been obtained and the result would not change with time, showing that boiling inactivates the enzyme needed for synthesis. If synthesis occurs, the enzyme was not inactivated. Boil it longer.

5) Does added inorganic phosphate increase the yield of starch? What difference would it make in the color obtained with iodine?

6) Compare with tubes #1 and #2.

7) Compare with tube #4.

What are the final products of the reactions driven by light?

(Oxygen, NADPH, and ATP)

What does carbon dioxide combine with the carbon dioxide fixation reaction?

(Carbon dioxide combines with the hydrogen on the NADPH yielding an unstable product which breaks in two in the dismutase system to yield two molecules of 3-phospho-glyceraldehyde (a 3-carbon sugar).

How many molecules of triose sugar are formed as the result of fixing 6 molecules of carbon dioxide? (12-3-phospho-glyceraldehyde)

What sugar is transported in plants? (Sucrose. UDP-glucose + fructose yields sucrose + UDP).

What is the ultimate source of the energy in glucose. (The sun. Sunlight activates the hydrogens of chlorophyll and these activated hydrogens are then attached to carbon dioxide. On use in the respiratory system, the hydrogens are oxidized to water with the regeneration of ATP from ADP. The water can be split by chlorophyll in the presence of light, and the whole cycle repeated).

TEACHER'S GUIDE TO

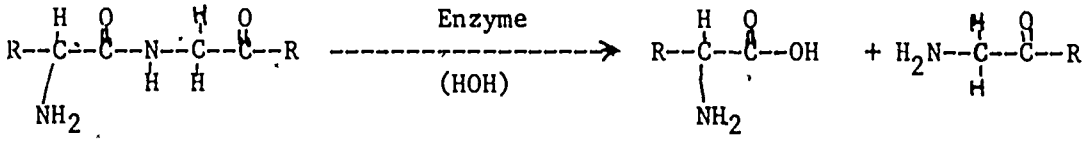
EXERCISE 33 -- DIGESTION OF FOODS

Prerequisites: Exercises 2, 7, 11 and 13.

INTRODUCTORY REMARKS TO THE TEACHER

The experimental hypotheses for this exercise vary with the section. In Part A on salivary digestion the questions are, "What effects do cold and boiling have on enzyme activity?" Also "What effect does a low pH have on this particular enzyme?" In Part B, Gastric Digestion, the replications ask, "What is the effect of pH on the rate of digestion?" Acidity is also a consideration in Parts C and D.

Digestion is an hydrolysis of macromolecules to yield smaller units. It always involves the splitting of a water molecule and the insertion of the hydrogen onto one fragment and the hydroxyl group onto the other fragment, viz:



Glucose and other sugars are small molecules, so are amino acids, and fatty acids, (acetate, pyruvate). However, the storage forms for these small molecules are macromolecules. Starch may contain several thousand glucose residues. In the polymerization of UDP-glucose to form starch, a water molecule is lost every time a glucose molecule goes onto the amylose macromolecule which eventually grown to be starch. This does cut down some on the weight of the stored product, but the water taken away has to be put back again in order to get the small molecules hydrolyzed out of the larger ones.

Glucose is phosphorylated to glucose-6-phosphate, and with that PO_4 handle it can participate in intermediary metabolism. The amino acids enter the carbohydrate pathways through alanine and glutamine, and fatty acids are finally broken down into acetate residues that communicate through acetyl-CoA with pyruvate.

MATERIALS AND EQUIPMENT

Chemicals

Paraffin
Acetic acid (dilute)
300 ml.
600-1000 ml. Lugol's solution
1000 ml. Benedicts solution
250 Litmus solution
600 ml. 5% Pancreatin
600 ml. 5% Pepsin
1 lb. 1% Sodium bicarbonate
250 ml. chloroform
1000 ml. 0.1N HCl

1000 ml. 0.1N NaOH
1000 ml. 5% HCl
1000 ml. 5% NaOH

Biologicals

Hard-boiled egg white
10-20 ml. cream
200 ml. Starch paste

Plastic & Glass Wares

360 15 x 150mm. Test tubes
24 100 ml. Beaker

Others

24 Spot plate
3-6 Test tube bath
24 Bunsen burner
24 Thermometer Ice
Litmus paper

PREPARATIONS

Reagents are described in the Teacher's Guide to Exercise 11. Starch paste is 0.5% solution of soluble starch made in distilled water. Hard boiled egg white is prepared by boiling a fresh egg about 10-12 minutes. Remove the coagulated white (albumen) and cut into slices 2 to 3 mm. thick with a sharp knife. Store the slices in a small amount of distilled water in the refrigerator if this is done more than 30 minutes before use. An alternate method is to draw raw egg white up into pieces of glass tubing 6 to 8 inches long. Drop these into a pan of boiling water and let the white coagulate. Cool and cut the tubing into 1-inch lengths, making use of a file or a glass-cutter. The student can measure in mm. the amount of digestion that occurs with the available time, or come back later, since this preparation presents a smaller surface area and therefore, takes longer, but is easier to measure if that is desired. These slices can replace the 5 mm. cubes called for in the exercise.

Fibrin is insoluble in water but the strands present a very large surface for enzyme action. It will, therefore, be digested faster. One can estimate the turbidity, or measure it in a nephelometer, if one is available in your department. Alternatively, the amount of protein can be precipitated with 10% trichloroacetic acid (TCA) and a qualitative judgement made of the amount of protein remaining, or it can be centrifuged, washed, dried and weighed. The latter procedures takes some time and should only be permitted the faster-working student.

The collection and filtering of saliva by students may be time-consuming where there is only a two-hour period for laboratory. As an alternative 1% malt diastase buffered at pH 7 and activated with 0.9% NaCl, or 1% malt diastase made up in mammalian Ringer's solution may be substituted. Filter and keep in the refrigerator if prepared before use. Prepare fresh daily.

Students should work in pairs, but if equipment is in short supply they can work in groups of four.

INTRODUCTORY DISCUSSIONS

A day or so beforehand prepare some sterile broth and sterile .9% saline in tubes. Transfer a bacterial loop of bacteria to a tube of broth and also to a tube of sterile water. Label.

At class time present four tubes. Inoculated broth (growing culture). Inoculated saline, (It will not have grown). Sterile broth, and sterile saline. Ask the class if they can identify what is in the tubes. (They should be able to identify the growing culture and the broth, especially if they have done exercises 5. 6 or 30. and will probably identify the other two as water). Tell them what each tube contains then ask why the bacteria are growing in the broth but not in the saline. The obvious answer is because there is food there). Then inquire how the bacteria get the food out of the broth. (They should be able to tell you that the cells secrete enzymes that digest the food in the environment and that the digest is then absorbed (by active transport) into the cell. You may get some other stories).

Indicate that this is typical for one-celled organisms and multicellular animals as well as for a few meat-eating plants like the Venus flytrap or the pitcher plant. For many of the lower organisms the size of the environment is reduced by phagocytizing a bit of food into a food vacuole where it is digested. Even though books call this "intracellular digestion" the inside of the vacuole is outside of the cytoplasm of the cell. In the case of multicellular organism that have digestive tracts, including man, the inside of the tract serves as a restricted "outside" into which digestive enzymes are secreted. That is to say, the inside of the intestines is outside of the tissues of the body. An intestine, however, is much more efficient for the higher organism than having to live in the water and exude digestive enzymes until all of the food about them is digested.

What does it mean, "to digest" food? (Get responses) (To break it up into smaller chemical units). Point out that all digests are hydrolyses, then ascertain if students know what "hydrolysis" means (See Ex. 13C). If another student know, let him explain. Hydrolyses can be carried out by boiling most foods with acid or base. What would be wrong with trying to do that in the body?

What sort of conditions do you think ought to influence the rate at which foods are digested? (List) How could we go about testing some of these ideas? (Suggestions) For convenience let's do these (check in the list): Effect of temperature, and effect of pH. Then, when you have Parts B, C and D underway check with me and we'll let you do one or more of these other suggestions. Set up Parts B, C and D first, then do Part A.

PROCEDURES

It would probably be most economical of time to have the class begin with Parts B, C and D. When these are set up, then do Part A while the slower digestions are going on. As in other chemical experiments it is important that students pipet accurately or else the results will be all skewed.

REPORT SHEET

Spaces have been provided for the experimental data. Additional information can be requested in the form of a table containing the names of the digestive enzymes, kinds of materials they digest, and portion of the digestive tract in which they are secreted. Another (alternative) report suggestion would be to ask for an essay on the hormonal control of pancreatin, gastric juice, and bile secretion. Another possibility is to ask for a thumbnail summary of the work of Dr. William Beaumont on his patient Alexis St. Martin, certainly a classic in clinical investigation on digestion. "Peptic Ulcers" is another possible report topic.

TEACHER'S GUIDE TO

EXERCISE 34 -- MEASUREMENT OF OXYGEN USE

INTRODUCTORY REMARKS TO THE TEACHER

The ability to take up oxygen is not necessarily evidence of respiration. There are many reducing substances that will reduce oxygen and remove it from gaseous mixtures. Pyrogallol is a commonly used substance for this purpose. Therefore, oxygen use, or oxygen use with carbon dioxide production, must be correlated with other activities associated with living things--reproduction, adaptability and irritability. All sorts of burning organic material, gasoline, paraffin, wax, wood, etc., use oxygen in the burning process and give off carbon dioxide as an end product; and yet, that is not evidence that these substances are alive.

Measurement of oxygen use, then, is of greater use to the biologist as an indication of the rate at which food is being metabolized. The fuel being "burned" is hydrogen and the end-product of the oxidation is water. That is, the main end product of organic fire is carbon dioxide and the end product of cell oxidations is hydrogen oxide. Where does the carbon dioxide come from? Carbon dioxide is used as the carrier for the hydrogens energized in the photosynthetic process. The food is eaten, digested and absorbed, and then broken down to get the hydrogens out. The carbon dioxide carrier is discarded and the hydrogens (or at least the electrons) are used to reduce the iron-containing enzymes, located mainly in the photosynthetic and respiratory chains. In the process, energy is released and trapped in the high-energy bonds of adensoine triphosphate, and to a lesser extent in the triphosphates of other purines and pyrimidines.

If the respiration rate is measured when the organism is in a state of complete rest, in a post-digestive state, and when at psychological ease, it is referred to as the basal metabolic rate. Since these conditions are only arranged in careful experiments or with people, what will be measured in this exercise should be called something else, perhaps the "resting metabolic" rate or "mild-activity" respiratory rate, where animals are concerned. Of course, the matter of activity is not so variable with plant and microbial material, but oxygen use is usually big because metabolism is usually quite active in the kinds of materials suggested for class exercise use.

MATERIAL AND EQUIPMENT

Chemicals

Soda lime or "Baralyme" (calcium oxide and barium oxide) obtainable from the Warren Collins, Co.

Biologicals

Dry peas (100 grams/test)
Germinating peas (100 grams/test)
Yeast culture (thick suspension)
Mice (1 per test)

Other animals that can be used with the recommended apparatus would include small rats, frogs, cockroaches, earthworms, etc.
Small plants with roots in a measured amount of water (see PROCEDURE)

Plastic and Glass Ware

Plastic pan 12" x 18" approximately
2 quart jars or other sizes with equal volumes and wide mouths
2 10 ml. pipettes.
2 ft. rubber tubing (1/4" inside diameter)
cutting glass tubing

Other

2 Support stands
2 Ring supports
2 2-hole rubber stoppers to fit the mouths of the jars

PREPARATIONS

There are several styles of respirometers available and they are all useful if the materials will fit inside of them. The Warburg respirometer requires homogenates or cultures of microbes, or tissue slices. The Scholander respirometer is designed primarily for insects and other small animals. The volumeter is a simplified respirometer for homogenates and very small objects. The advantage, then, of the respirator model presented in this exercise is that all types of living materials may be used in it, one has only to supply enough of the material. The device described here can be used for bacteria, yeast, mice, cockroaches, frogs, germinating or dormant peas, and in fact for anything that will fit into the jar.

The pan is used for a water bath to stabilize the temperature in the jars. A temperature-regulated bath may be substituted, or one can use an immersion heater together with a motor-driven stirrer for better temperature control. The economy model is presented here--no temperature control and no circulating water.

Colored water may be used in the manometer system, or the more elegant Brodie's solution can be used. Fill the manometer system by injecting the Brodie's fluid or colored water with a syringe at the bottom piece of rubber tubing.

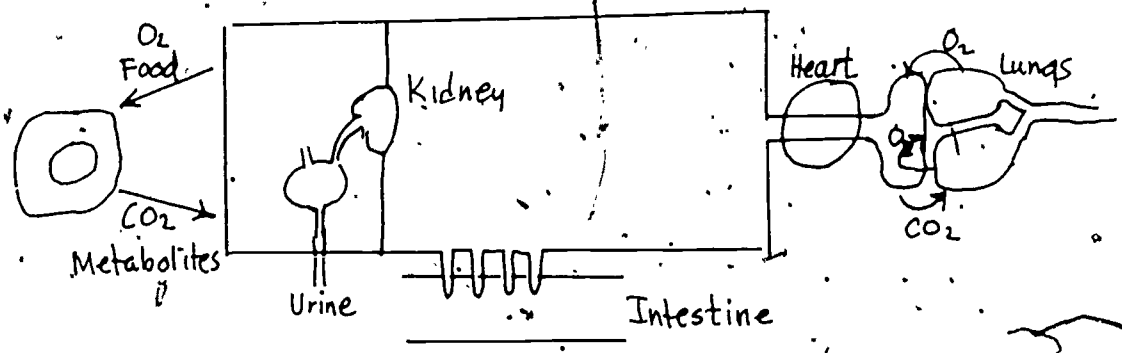
INTRODUCTORY DISCUSSION

The Apparatus

Have at least one of the apparatus set-ups assembled. Review its parts. Explain how that the second jar balances increases and decreases in volume in the test jar. The manometers are devised with pipets of the same size. Ten ml. pipets are best with large users of oxygen and 1 ml. pipets are better for slow-metabolizing organisms or materials.

The Experiment

Put this diagram on the chalkboard:



Have the students help label the parts as far as they can, including the functions. The teacher ought to point out that the lungs and the tubes leading outside are named the Respiratory System. Actually the activity that this system performs is breathing and someday physiologists will persuade anatomists that it should be called the Breathing System. Respiration, as the term is used by physiologists and biochemists, refers more specifically to oxygen use and carbon dioxide production in the cells. (Many elementary textbooks name these processes internal and external respiration, but such names do not recognize the differences in function at the lungs and at the cells.)

The relationships in the diagram that are important for the present exercise are the gas exchanges at the cell and at the lung. Everyone educated above the lower grades knows that you breathe the air to get oxygen and you breathe out to get rid of carbon dioxide and other things, too. Since most permeability dealt with in considering digestion has stressed active transport, it is good to indicate that the gases follow concentration gradients and behave according to diffusion laws. Oxygen tension is highest in the air (21%), a little less high in the lungs, less high in the blood and only about 5% at the cell. Carbon dioxide is about 5% at the cell, less in the blood and low at the lungs, diffusing into the air, which contains normally about .04%.

The next question is usually where is the carbon dioxide made in the cell? The carbon dioxide is split off of fatty acids after they leave pyruvic acid (a fatty acid) and pass around the Krebs cycle. The hydrogens are gathered by the dehydrogenases, mainly with NAD or NADP coenzymes, and are passed down the respiratory chain. After helping form the first ATP molecule in the respiratory chain, the hydrogens ionize and only the electrons react with the cytochrome iron atoms as the next two ATP molecules are formed. The hydrogen ion (proton), electrons, and oxygen from breathing then combine to form water. This keeps removing the last reactants so that the respiratory system will not come to equilibrium and stop functioning (as is true in cyanide poisoning). In heavy work or exercise (in higher animals) extra hydrogens back up to pyruvate, and reduce that substance to lactic acid. At the conclusion of the hard work, heavy breathing will continue for a time to provide oxygen for the hydrogens stored on lactic acid but released now because the

system is not saturated. About 1/5 of the lactate is oxidized to yield carbon dioxide, ATP and water; the other 4/5 goes back through the fermentation series to be deposited as glycogen. The extra oxygen used to oxidize the hydrogens from the 1/5 part of lactic acid is called the "oxygen debt".

REPORT SHEET

Results of respiration tests are reported variously. For animal work the body area is considered important, so that a formula may be used to estimate this datum from the weight of the animal or person.* For tissues it is more common to report the volume of gas (oxygen) used or given off (carbon dioxide) per unit of material weight--that is, as microliters of gas per mg. weight. It is customary to state whether it is wet weight or dry weight. Sometimes it is difficult to estimate the weight, such as when microbial cultures are used. In these cases the amount of oxygen used per mg. nitrogen is a common measure. For this exercise we suggest reporting

ml. of oxygen used/gram of tissue/hour.

Three or four determinations of oxygen uptake should be made and the results treated statistically to find the standard error for each material. (See Exercise 9 for the method of computing the standard error.)

*Prosser, L. and B. Brown, Comparative Physiology, Phila.

TEACHER'S GUIDE TO

EXERCISE 35 -- URINALYSIS (URINE ANALYSIS)

Prerequisite: Exercise 2 and 4

INTRODUCTORY REMARKS TO THE TEACHER

Urine is a biological fluid of animals which reflects to some extent the chemical condition of the blood plasma contents and also to some extent the physiological state of the kidneys. The urine summarizes the conditions which are changing considerably during the time that is is accumulating in the urinary bladder. For this reason, it is customary in the clinic to ask for a 24-hour collection of urine for some cases because the amount of any particular material may vary a great deal between morning and evening. The examination of the urine, therefore, gives some general insights into the way certain metabolic end-products are building up and being removed from the circulation by the kidneys. When a 24-hour collection (about 1,500 ml.) cannot be made, it is customary to use a specimen collected the first thing in the morning, since, as a rule, it has had longer to accumulate than any other sample later in the day, and also because activity is usually limited and food intake is small during the night.

This exercise makes use of several simple chromogenic (color-producing) reactions, some of which are used to give quantitative as well as qualitative information. Also included is microscopic examination of the urine sediment which may contain a variety of cells, crystals or amorphous mucoid casts from the kidney tubules, formed when they are at rest and flushed out when the tubule becomes active again.

MATERIALS AND EQUIPMENT

Chemicals

Glacial acetic acid
Benedict's Quanitative Reagent
Concentrated nitric acid
Dilute silver nitrate
Saturated picric acid
10% NaOH
3 % Sulfosalicylic acid
Albumin
Dilute nitric acid
Dilute HCl
Dilute barium chloride
Toluene

Biologicals

Sample of urine, about 100 ml. collected by each student.

Glass and Plastic Ware

Disposable urine cups
Test tubes
Microscope slides and coverglasses
Urinometer (with hydrometer)

Other

Test tube racks
pHydrion paper (pH 1 to 12)
Microscope and lamp
Centrifuge (clinical type)
Clinistix brand glucose test strips
Watchglass
Wax pencil

PREPARATIONS

Disposable urine cups should be used. These usually come in cases of about 400, which isn't many for some departments but may be a several-years supply for others. Squirt about half a medicine dropper of toluene into each cup to be used by students, then put the leak-proof cover in place. Every student should be given such a urine cup at least the day before the exercise is to be done with instructions to collect the first urine of the day on the day it is to be used. Advise them that the toluene is a good preservative so that the urine need not be refrigerated. However, if a refrigerator is available to the laboratory, have them place their samples in it when they bring them to school (especially if the laboratory meets later in the day.) It is quite important to have each student bring his own urine sample. Nothing could be duller than to have to do these test from a common sample of urine. Moreover, since every one should get the same result from a common specimen of urine, the test would get divided up, and results transmitted around the class so that students would feel it unnecessary to actually do the tests in order to get the results (a situation called "dry-labbing").

Pathological Urine. This urine should be prepared before class by taking about 100 ml. or so of normal urine and adding 1 gram glucose, 1ml. acetone, and .5 gram of albumin. This urine sample is then used to produce positive tests so that the student will not have to repeat a negative test just to show that it was performed correctly.

INTRODUCTORY DISCUSSION

Use a model of the mammalian kidney. Most models available are of the human kidney. Can students identify the organ? Do they know what its function is? How could one determine whether or not it was working alright? Would it really matter to one's health if the kidneys were not working?

Does anyone know how urine is formed by the kidney? (Substances with a molecular weight under 70,000 are filtered out at the glomerulus and then desired materials are reabsorbed by the tubules, leaving the urine. This is transported by the ureters to the urinary bladder where it is stored until micturition occurs). What does micturition mean? What factors, then, determine the composition of the urine? (The composition of the blood plasma and the ability of the kidney nephrons to function).

PROCEDURE

Initial observations. Pour urine from the collecting cup into the cylinder of the urinometer and make the initial observations for color, odor, transparency and sediment. Then insert the hydrometer and determine the specific gravity. From the

specific gravity determine the grams of solids per liter of urine.

Do the tests for proteins in urine on the student's sample and on the specimen of "pathologic" urine provided for the class. [Note: When heating test tubes be sure that students do not point them at each other. They should be heated uniformly along the length of the contents, not just at the bottom. If this latter is done, steam generated in the bottom of the tube will blow the contents out of the tube and onto neighbors, workspaces, floors or ceiling.]

Indican Test. This should be done in a hood or in a well-ventilated place at least 20 feet from flames, because chloroform is used.

Sugar Tests. The Report Sheet has a place for results from the Somogyi-Nelson technique for true glucose determination. That procedure was not included in the exercise and therefore should be ignored.

Microscopic Examination. Each student should put exactly 10 ml. of urine in a tube that fits the centrifuge available to you. Some centrifuges require that the tubes on opposite sides of the head weigh within 0.1 gm. of each other to prevent strong vibrations. If your instrument is like that, then balance two beakers on a 2-platform balance. Place the tubes in the beakers, weight by adding or subtracting urine from one of the tubes until they are in balance. The volume is not critical for the examination, only for the centrifugation. When the centrifugation is completed, decant (pour) the supernatant (top liquid) and examine the sediment (in the bottom of the tube) by making a wet mount and studying it with the microscope.

REPORT SHEET. [The teacher may want to reproduce the report sheet and have the student turn in a report which he may not get back. He will also record his results on page 35-6.

No questions are provided for this exercise so the teacher may want to have the students answer some devised by the teacher or raised by students. Another alternative would be to ask for a one-page report on a constituent of the urine, for example; urea, indican, Bence-Jones protein, or urinod. Diabetes mellitus, diabetes insipidus, or ways of determining the salt content of urine and other body fluids, may also be used as report topics.

References: Hawk, Oser and Summerson
Physiological Chemistry (3rd ed.) The Blakiston Co.

TEACHER'S GUIDE TO

EXERCISE 36 -- REFLEX ACTION

INTRODUCTORY REMARKS TO THE TEACHER

There are about 5 (sometimes more, sometimes less) parts to a reflex arc. Environmental changes are transduced by receptors to become a nervous impulse (and exchange of sodium and potassium across cell membranes). This is transmitted to the sensory neuron (actually to the dendrite of a sensory neuron. The dendrite in this type of cell is very long.) Although the impulse may be transmitted directly to a motor neuron, it usually will pass through at least one association neuron which may communicate with many cells at several levels on the same side of the nervous system and frequently has connections to the other side. Impulses to motor neurons are transmitted to an effector (a muscle, gland, or in lower organisms, nettling organs, trichocysts, and the like). The effector is then stimulated to do something. If it is a muscle, it contracts; if it is a gland, it secretes. Reflexes are often enhanced or inhibited by other nerve circuits, some of them conscious. But for the most part reflex activities occur without much conscious control even though they are generally protective.

In this exercise several reflexes in man are observed. This is a convenience, for the anatomical connections between the receptor and effector regions cannot be observed. Nor can the experiments of Magendie and Bell be done (cutting the ventral and dorsal spinal roots to demonstrate the function of the sensory and motor neurons), but students can experience their non-control over most of this reflex activity and the fact that it occurs without the intervention of the voluntary processes, as a rule.

MATERIAL AND EQUIPMENT

Sterile thread
Sterile rubber-tipped depressor stick
Neurological hammer

PREPARATIONS

Number 50 cotton sewing thread is suitable. Wrap the whole spool in paper and autoclave for 15 minutes at 15 psi. At the same time the rubber-tipped depressor sticks made by putting a 1-inch piece of clean rubber tubing on a depressor stick, are placed in test tubes with bacteriological closures and autoclaved. These precautions are taken to prevent any concurrent eye or throat infections from being blamed on the procedure. Used tips can be collected in containers of detergent, washed, sterilized, and re-used.

INTRODUCTORY DISCUSSION

Prepare a frog by removing the brain (cut off the head at the ear drums) and remove the viscera so that the sciatic nerve complex is exposed. Suspend the preparation from a hook, formed from a bent common (straight) pin held in place by a flat jaw clamp on a stand. Pinch the toe of one foot and it should withdraw. If not, pinch harder. Apply a piece of filter paper 1 cm. square, that has been wet with 10% acetic acid to the side of the preparation. It will reflexly try to remove it. Is this a simple spinal reflex? There will probably

be some questions for discussion. This is not a simple reflex arc working at the same level.

After the discussion, or at some appropriate point, diagram a simple reflex arc working at the same level.

PROCEDURE

Students must work in pairs.

REPORT SHEET

Questions

3. How does the corneal reflex serve the body?
(It not only includes a blinking component, it also includes a generalized body effect, dodging. Its protective potential lies in helping to prevent being hit by fast-moving flying objects.)

5. What cranial nerve causes dilation of the pupil? (The IIIrd. Contraction is caused by a nerve from the 1st cervical ganglion, not a cranial nerve.)

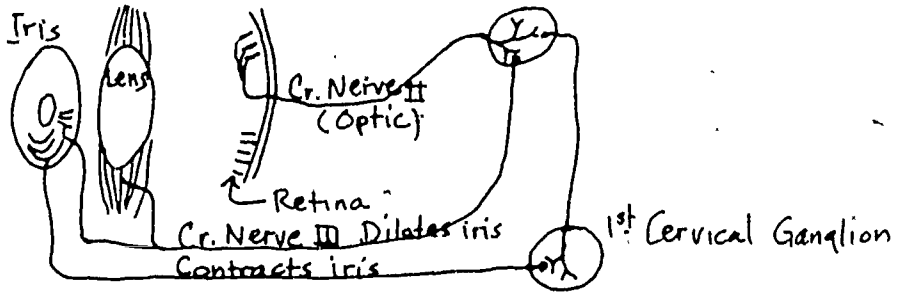
6. Is the light reflex present during sleep? (No)

7. What is meant by a mydriatic drug? (One that causes contraction of the pupils. Opium and its derivatives, morphine and heroin.)

8. What is meant by a meiotic drug? (One that causes dilation of the pupils. Examples are atropine (dl hyoscyamine), homatropine, and adrenaline.)

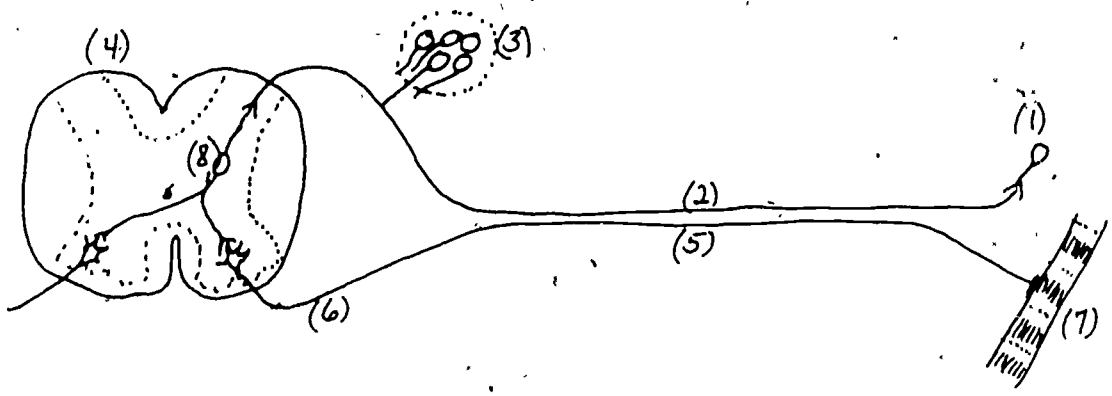
9. The size of the pupil is helpful in interpreting visual images from our environment because:
(It increases the focus (acuity) of the image and it acts as diaphragmatic opening which is attached to an "exposure meter" so that it constricts in bright light and dilates in dim light.)

10. A possible reflex network for accomodation.



14. What is the relationship between the knee jerk reflex and tabes dorsalis?
(The syphillis spirochete causes lesions of the lower spinal cord, resulting in locomotor ataxia (uneven gait) because coordinating impulses from the cerebellum do not get through to the sciatic nerve. The knee jerk reflex is abolished by the syphillitic lesions so that failure to elicit a response indicates the possibility of such a lesion in the lower spinal cord region.)

15. Diagram a spinal reflex arc indicating (1) the receptor, (2) sensory neuron, (3) dorsal root ganglion, (4) cross section of the spinal cord, (5) motor neuron, (6) ventral root of the motor neuron, and (7) effector.
(8) Association (internuncial) neuron



TEACHER'S GUIDE TO

EXERCISE 37 -- INFLUENCE OF THYROID HORMONE ON RATE OF DEVELOPMENT

(Demonstration or Special Project for two or more students)

Prerequisite: Exercise 27

INTRODUCTORY REMARKS TO THE TEACHER

Other exercises in this manual dealing with hormone activities are Exercise 26--Response of Animals to Pregnancy Urine Hormone, and Exercise 28--Do Environmental Factors Affect the Action of Genes? Exercise 27, Transcribing DNA, mRNA, and tRNA to Sequence a Protein gives the student some practice in seeing the relationship between DNA, the RNAs and the protein sequence. In Unit 5--Genetics, the topic of gene regulation and adaptive protein production has been introduced. The whole subject of what substances affect the regulator genes and their aporepressor substances has been under investigation since Jacob and Monod demonstrated the validity of the concept that materials from the molecular environment can influence the expression of genes (see also Teacher's Guide to Exercise 30). As early as 1961, Clausen in the United States and Kalkar in Germany had developed the idea that steroid hormones, in particular, bring about their protein-producing effects by acting on genes. Both men, working with the salivary gland chromosomes of the blood worm Chironymus demonstrated that ecdyson, a steroid juvenile hormone of insects, causes chromosome puffing at specific loci within 15 minutes of application to larvae. This idea has been developed further by Williams-Ashman, Liao and by Hamilton in higher animals in recent years. Thyroid hormone has several effects. It speeds up metabolism in general, it speeds up metamorphosis in amphibians, and in man it is needed for the full development of mental ability. Like the steroids, it is a cyclic organic compound but is much simpler in structure than steroids. It seems to regulate metabolism by affecting the ability of genes to produce certain enzymes. (See Cohen, 1970, for a review.)

Cohen, Philip P., Biochemical differentiation during amphibian metamorphosis, Science, 168:533-543, 1970 (1 May 1970)

The action of thyroxine on the genetic system to activate the enzyme carbamyl phosphate synthase I in the liver of metamorphosing tadpole precedes metamorphic changes and suggests that the tadpole liver system offers some interesting possibilities for studying how proteins are formed and transported to the mitochodria, how the thyroxine acts and the molecular level, the kind of regulation and the nature of the regulators involved. Fifty references.

MATERIALS AND EQUIPMENT

Chemicals

Stock solutions of the following made up 20mg. in 200 ml. pond water:

Thyroxine or Triiodotyrosine
Thiouracil
Lugols iodine solution (1%) diluted 1:100 = 20 ml./200 ml. with pond water
Supply of pond water

Glass Ware
7 Aquaria or one-gallon jars

Other

7 rocks or bricks to form easily-accessible islands for young frogs.

PREPARATIONS

Thiouracil sometimes does not go into solution very well. In this case, suspend the thiouracil in about half of the water for the solution and bring the pH to the alkaline side, about pH 9. The material will go into solution, then by judicious additions of HCl bring it back near pH 7. Use a pH meter because just under pH 7 it will come out of solution again.

INTRODUCTORY DISCUSSION

Since the students have the procedure for the experiment in hand, ask them what each one of the seven groups of tadpoles will be testing in this experiment.

PROCEDURE

Stock solutions of thyroxine and thiouracil should be made up and added to pond water on the days that aquaria must be changed.

REPORT SHEET

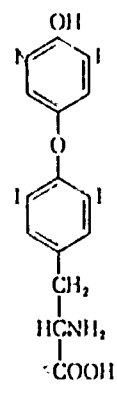
The second chart on page 37-2 is intended for use in recording the dates on which the tail measures the indicated lengths.

There is room for one or two other items for the class to observe according to its interest.

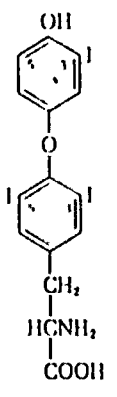
Questions

1. The formula for thiouracil, thyroxine, and triiodotyrosine are given below

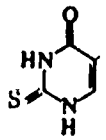
Thyroxine



3,5,3'-triiodothyronine



Thiouracil



5. What would be a good reason for having included treatment with iodine alone, knowing that iodine is a constituent of the thyroid hormone?

(To show that elemental iodine alone does not increase the amount of thyroid hormone available at this stage to make much difference).

6. How could you show that thyroxine effects are reversible?

(Stop giving the hormone. (Give the antagonist, thiouracil, at the same time).

TEACHER'S GUIDE TO

EXERCISE 38 -- A STUDY OF PLANT COMMUNITIES

A field exercise

Prerequisites: Exercise 17 and Exercises 20 through 23 are desirable.

INTRODUCTORY REMARKS TO THE TEACHER

This kind of exercise comes from an era in biology, not far gone, when the most important thing that biologists did was to count up the number of kinds of things there were. From that sort of knowledge it developed that some kinds of plants grew together and some did not. Out of such a simple observation one begins to look for causes--the amount of light available, the possible secretion of inhibiting chemicals, the chemical makeup of the soil (whether acid, neutral, or alkaline), and the amount of water available.

The most stable communities are called climax communities and may be of any type, but the type is regulated by the soil conditions, the climate, and neighboring types of communities which may contribute to its stability. Forests, meadows, prairie, savannah and groves of trees may represent the climax community.

MATERIALS AND EQUIPMENT

Field notebook and pencil
Tape measure at least 33 feet long
Photographic exposure meter

INTRODUCTORY DISCUSSION

This is a field exercise so that perhaps the introductory discussion should include what is expected in the field by way of observations, notemaking, specimen collections (including any restriction of specimens at the trip location, etc.) A possible lead into a discussion of the objectives of the exercise might be to talk about the United States census or a local census. Just as the census involves more than just counting the number of people in the country (it also takes note of kinds of occupations, income levels, housing conditions, economic factors like the number of automobiles in the family and the like). On this field trip we will be taking a census of the plants (and to some extent the animals) by counting the number of each species in a given area of land and noting such factors as light, water, kind of soil, etc.

Map the area the class will cover and indicate the plots each team will work. If there is a stream or other natural reference feature, this task is greatly simplified.

REPORT SHEETS

The student teams should prepare a profile of the terrain and indicate the kinds of vegetation making use of the symbols indicated in the exercise if a large plot was studied, or complete the diagram of plant distribution in a small plot, making use of the graph provided.

TEACHER'S GUIDE TO

EXERCISE 39 -- SUCCESSION FROM ONE ENVIRONMENT TO ANOTHER

INTRODUCTORY REMARKS TO THE TEACHER

It was Thomas Aquinus who is credited with first saying, as a matter of philosophical observation, "Nothing ever is but is always becoming." We cannot vouch for a term as all-inclusive (or all-exclusive) as "Nothing", but the statement is basically true when it comes to a discussion of the existence of closed lakes and ponds, and to bare rocks, whether in valleys or atop mountinous upheavals of the crust of the earth. Ponds are being filled by silt washing in with the stream waters, and their bottoms are being raised by decaying vegetable matter from plants growing near the edge of the water. Rocks are constantly being weathered by heat and cold, rain, freezing and thawing, to yield finally the soil in all of its varied textures. The oceans are continuously evaporating into the air, sometimes becoming clouds. Clouds condense into rain, hail, and snow, and these, of course, form the waters of streams flowing back to the oceans to complete the cycle.

Various kinds of plants like particular environments and they grow in the environment for which they seem best suited. When we look at the plants around a body of water, especially if it has been undisturbed for some time, we can see that different kinds of plants are found in each kind of environment.

MATERIALS AND EQUIPMENT

Field notebook and pencil
1 foot ruler marked in cm.
small boat
Wading boots
Weighted line equipped with hooks
Geotomes (shovels and mattocks)
Sieves
Planckton net
Glass-bottomed buckets or boxes for underwater observation

PREPARATIONS

See page 39-1 in the manual

INTRODUCTORY DISCUSSION

A long discussion is not needed for this exercise. What needs to be done here is to organize the class into teams and to make sure everyone knows what equipment his team should take into the field and what jobs each person will perform once the team arrives. This is not a nature walk that students will take once the team arrives. This is not a nature walk that students will take at their leisure, even though that may be very "inspirational". It is a work and study session in which the student will be acting as a scientist and proceeding as the ecological scientist proceeds when he makes a study or survey. Anything less will be non-scientific.

If this exercise is undertaken early in the course, before students have had much opportunity to learn about the various kinds of plants and animals, some effort should be made to have some advanced students or faculty members who would volunteer, to accompany one or more groups to help them identify various plants and animals. This may not be necessary, but still desirable if the exercise does come later in the course since it does take a fairly broad knowledge of living things to be able to identify many of them beyond the Phylum or Class. Of course, scientific nomenclature is not the real objective of the trip, but rather to know what animals and plants inhabit which environments.

The teacher may not want the teams to bring back a specimen of everything that they saw. Good ways to do this is to ask each group to identify everything, but only bring back specific things--one, examples of aquatic plants for the aquarium; another, leaves of deciduous trees to be pressed and mounted for the class use at other times during the year. Others may collect bugs, beetles, and worms from the wet soil, and still others may collect seeds, fruits, or flowering plants for preservation and use in the laboratory. Termites, needed for Exercise 41, may be collected at this time.

Each team should know beforehand that it is responsible for cleaning its tools and preserving its specimens in appropriate ways at the conclusion of the trip.

PROCEDURE

As indicated in Exercise 39.

REPORT SHEETS

Each team should report a list of the organisms it observed and also describe the locations where they were observed if different from the plotted area. In addition, each student should write a report on succession in the environments studied (open water to land, bare rock to soil, abandoned farm or forest cutting.)

TEACHER'S GUIDE TO

EXERCISE 40 -- EFFECT OF PHYSICAL AND CHEMICAL FACTORS ON ANIMALS

Prerequisite: Exercises 2, 7

INTRODUCTORY REMARKS TO THE TEACHER

During the evolution of the cell the materials (proteins, nucleic acids, metabolites were aggregated in the environment, the ancient oceans, where they were directly subjected to the changes in temperature, salinity, radiation, and pH. The development of the cell membranes tended to keep the large molecules from getting lost from the community, but had the job also of keeping the salt content inside somewhat like the outside in those ancient seas. Yet, when conditions change the membrane must be able to pump ions in and out so that the outside gets to the inside and the inside briefly is pumped to the outside. This is the basis of irritability. The impulses generated by irritability let the organism know that environmental conditions (for the organism, or even for a few of its cells) have changed. The reversible responses that the organism makes, either by a change in location, rate of metabolism or movement, is called adaptation. Organisms can adapt to small variations in the environment (as a rule) but are killed by large changes. The old Darwinian concept that organisms "struggle with their environment" has long ago been deemed untenable. Today we know that organisms must adapt or die. (See Introductory Remarks in Exercise Teacher's Guide to Exercise 30).

This exercise looks at the way some organisms may adapt to two of the above environmental factors, radiation (light and heat) and hydrogen ion concentration (pH).

MATERIALS AND EQUIPMENT

Chemicals

.1N Hydrochloric acid
.1N Sodium hydroxide

Biologicals

Ants
Paramecium
Rana pipiens
Daphnia
Cyclops

Plastic and Glass Ware

Medicine droppers
Erlenmeyer flasks
1 liter beaker

Other

Absorbent cotton
pHydrion paper
Thermometers

INTRODUCTORY DISCUSSIONS

What does it mean to "be sick"? (This term may be defined by students as "not well", "an infectious bacteria has a hold in you", etc.) Point out that it only takes one or a few dysentery organisms to make one very sick with dysentery, but we may have fair-sized Staphylococcus infections resulting in boils or pus in wounds without actually feeling sick. At what point, then, does one become "sick"? A person suffering from untreated diabetes mellitus may not feel "sick", just tired and weak. Then, if the pH of his blood falls from pH 7.35 to pH 7.0 he will go into a coma. When is he "sick"? See if you can get the class to come to the point where "sick" implies an abnormal condition. They then will have to define normal (usual). Is it the same for everyone? (Indeed, there is a range for the normal). "Sick", therefore must describe conditions where the body has not been able to keep its processes "normal" when challenged with a change in conditions that shifts the internal or external environment too far from "normal".

In this exercise there is a demonstration of what happens to the breathing rate of a frog (or other animal that makes breathing movements), when the temperature is changed; the movement activity of an ant with changes in temperature, and the effect of pH changes on the survival of Paramecium, Daphnia or Cyclops. At what points may they be "sick"?

PROCEDURE

Part A. The frog may be placed in the respiration apparatus used in Exercise 34. A beaker, however, is alright. The instruction to keep records until the temperature no longer drops should ask instead that the rate of buccal pumping be determined for each 5°C. as the temperature falls or rises. It is not necessary to count for a full minute. Count for 15 seconds and multiply by 4 to get counts per minute.

In the experiment with the ant, do not add the cold tap water to the inside of the flask. Rather, the flask should be placed in a container of ice water. You may have to add some salt to the ice water to cool things down to 5°C.

Part B

If time permits, the endpoints of the range may be determined. That is, as a first approximation one may get results like:

pH	2.2	3	4	5.5	6.0	7.0	8.0	9.0	10	11
	0	0	0	4	15	30	15	4	0	0

One could say from such data that the range ended between pH 4 and 5.5 on the acidic side, and between pH 9.0 and 10.0 on the alkaline side. Now try some pH values like 4.4 4.6 4.8 5.0 5.2 5.4. It will probably be better for the teacher or preparator to prepare such solutions, making use of the pH meter, since pH paper is not this sensitive. The same would be done for the alkaline end of the range. One may use the vital dye, Neutral Red, to get an indication of the internal cell pH.

REPORT SHEETS

Charts are provided for the collection of data. The teacher may, however,

require additional features, such as graphic representation of the data and statistical treatment where more than one replication per treatment has been done.

Teachers may also ask for a paragraph or an essay on the natural habitat of any of these organisms and how the data generated in this experiment might relate to what happens in the natural habitat.

TEACHER' GUIDE TO

EXERCISE 41 -- TERMITE-FLAGELLATE INTERACTION

Prerequisites: Exercise 4 and 10. Exercise 16 is helpful.

INTRODUCTORY REMARKS TO THE TEACHER

This exercise is a good sequel to a field trip on which termites were discovered and collected. Although termites can be obtained from biological supply houses, collecting lets the student see something of the natural habits and habitats of termites.

The integument of termites is soft and delicate, so that water loss from even a brief exposure to drying conditions will be fatal. Therefore, they are found in damp earth and wood. They make their entrance to wooden structures from the ground, but if they must go above ground, they will build tunnels of mud and their excreta across rocks, cement, etc., to get to wood. They are characterized as miners, burrowing channels and chambers through the wood. Walls, furniture, fenceposts and support beams for houses are often attacked. Ordinarily these insects seem to be able to sense the strength of the material and the stress produced by the load it bears so that if it is not increased (as by a person walking across a floor, for example) the structures hold. Eventually they excavate too much and collapse of the wood ensues.

Most "flying ants" are termites, and they are also commonly known as "white ants". They are not ants but like ants, they are social insects, living in large colonies. A king and a queen are the parents of the colony, with sterile males and females making up the workers. Some individuals become soldiers. These have large heads and unusually large pincer-like jaws. The queen or fertile female, may be 1000 times larger than a worker, or about 2 to 5 inches in length. There are at least 36 species in North America north of Mexico.

MATERIALS AND EQUIPMENT

Living termites (in a jar)
.7% sodium chloride
Forceps and dissecting needle
Microsocope and lamp
Microscope slides and coverglasses

INTRODUCTORY DISCUSSION

The termite will be the object for discussion. Hold the jar up and ask the class if everyone knows what is in it. If not, let one of the students tell. Have someone tell where termites may be found and what the conditions are in such locations. Everyone will know that termites eat wood, but gauge their reaction when you tell them that researchers have found that termites cannot digest wood. Then ask how they think that the wood can act as food if termites cannot digest it. Get some reactions but do not draw a conclusion. If you have a film or film loop to show, show it at this point.

Some words not asked for or defined in this exercise include those related to parasitism: parasitism, symbiont, comensal, predator, infestation, infection. What is obligatory mutualism? If by now it has developed that the class realizes that there are protozoa in the intestinal tracts of termites, raise the possibility that there may even be smaller organisms on the protozoa (such as bacteria). How could it be determined if bacteria were present? (Stain smear with crystal violet or make a sterile dissection and transfer to sterile culture medium.).

PROCEDURE

Obtain a termite from the jar and place it on the slide. If the insects are very active, carbon dioxide gas is a good anesthetic. One may force the abdominal contents onto the slide or use a razor blade to dissect out the intestine, which can then be squashed as in making onion root tip or salivary glad chromosome preparations (see Exercise 16).

If there is a problem with evaporation, seal the edges of the slide with melted petrolatum.

If it is desired to demonstrate the presence of bacteria, let the smear dry in air, then stain with Hucker's crystal violet for about 30 seconds. Rinse with a gentle stream of tap water. Dry in air and clear with a drop of immersion oil rubbed gently over the smear.

REPORT SHEET

This is an excellent subject on which one or more students may write a report. Termites have a well-organized social life. They are of great economic importance. In this regard a student may visit a termite-exterminating company, if there is one in the locale, gather some samples of termite damage, and find out the trade and chemical names of insecticides used to kill termites and materials used to block their mining.

QUESTIONS

3. What is the possible relationship between the termite and these micro-organisms?

(The flagellates which inhabit the intestinal tract of the termite form a celluslase which will digest cellulose to maltose. However, the termite does not secrete a maltase so bacteria on the flagellates and in the tract complete the digestion of maltose to glucose. The glucose is then absorbed as food.)

4. How could you test the hypothesis that the relationship is an obligatory mutualism?

The various organisms can be (have been) isolated and their ability to digest cellulose and its intermediates to glucose tested. From this it could be deduced that they are there in order to do this service in return for the wood which termite eats.

Another approach would be to feed the termites some drugs known to kill intestinal bacteria in higher animals, such as the sulfa drugs, sulfanilamide or sulfathiozole. Start with lower concentrations and give higher ones until sterile quashes can be obtained from live termites. Such termites could then be fed sterilized wood and kept in sterilized containers and their longevity, vigor, etc., compared with termites kept in a normal culture.

5. What does a termite eat? (Wood, that is, cellulose.) Can you eat this? (Yes, but not digest it.) Can many animals eat this substance? (Yes, but they depend on bacteria to digest it.) How do animals get nutrients out of this substance. Besides the termite, the shipworm (which is the clam, Toredo) has a cellulase in its digestive tract (in the crystalline style) and this is also true for a few other animals. The ungulates, particularly cows, chew their food and store it in the abomasum where bacteria begins its digestion. This partly disgested cud is then re-chewed. The final nutrient obtained is glucose.