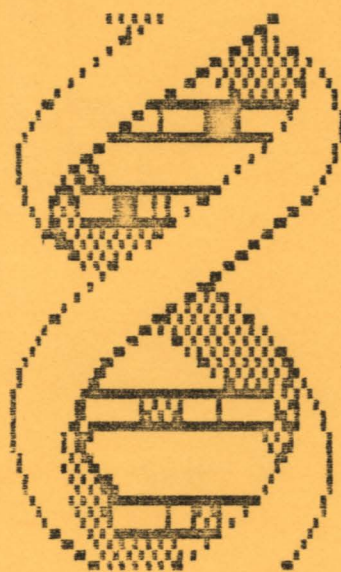


MIDWEST BIOSCIENCE



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Opinions expressed are not necessarily those of the AMCBT. Articles published in this journal have not been reviewed by peers.

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Deadline for the next issue is April 1, 1987.

IN MEMORIAM

The 30th Annual Meeting of AMCBT was hardly opened at Springfield, IL this year when President Ted Michaud informed the membership of the passing of two long-time members, Ruth E. McNair and Leland Johnson, both Honorary Life Members.

Ruth E. McNair was a member of AMCBT from almost the beginning and served as Secretary-Treasurer of the Association for 12 years prior to her retirement. She died at the age of 92 on August 2, 1986.

Leland Johnson was one of the founding fathers of AMCBT and served as its first president. He had been a teacher at Drake University for 44 years before his retirement. He died unexpectedly on April 19, 1986.

Both Ruth and Leland were extremely active in AMCBT and continued this interest even after retirement. They will be deeply missed by their families and friends and by AMCBT.

The Association extends to the family and relatives of Ruth E. McNair and Leland Johnson their deepest sympathy. By acclamation, the 30th Annual Meeting of AMCBT was dedicated to both their memories.

Greetings! As an AMCBT member, you will soon be receiving (or perhaps have recently received) a copy of the report, "Undergraduate Science, Mathematics, and Engineering Education," by the National Science Board Task Committee on Undergraduate Science and Engineering Education. This document evaluates the state of undergraduate education in science, mathematics, and engineering, and makes potentially far-reaching recommendations, including (but not limited to) recommendations concerning the role of the National Science Foundation in undergraduate education in these areas. It is a document you will want to read, think about, and share and discuss with your colleagues. Furthermore, it is one that we should respond to, as individuals involved in undergraduate science education, and collectively as members of AMCBT.

Dr. Robert Watson, Head of the Office of College Science Instrumentation at NSF, has encouraged AMCBT to respond to this report. Those of you who attended the Annual Meeting in Springfield, IL will recall his presentation on the opening panel. Dr. Watson has since contacted the organization to obtain the mailing list so that you can each receive this report. Now the ball is in our court. If you have ever wanted to bend the ear of NSF and affect the direction of support for undergraduate science, now is the time, and

this is the vehicle! I am looking for several volunteers to constitute an Ad Hoc Committee to draft a response from AMCBT. The charge will be to read the report, to discuss how to solicit responses from the membership, to solicit these responses, and to summarize them in a document to be submitted to the Steering Committee. I urge you to consider serving on this committee. If you are willing, please contact me immediately.

In the first and second decades of its existence, AMCBT (both as an organization and through the work of individual members) had a significant impact on national movements such as the Commission on Undergraduate Education in the Biological Sciences (CUEBS) and the governing board of the American Institute of Biological Sciences (AIBS). There has been no movement on a national scale in recent years in which it was appropriate for us to participate as an organization. This National Science Board report may provide a new cause celebre infusing us with renewed life and a new focus of activity.

I look forward to hearing from you (mail: Central College, Pella, IA 50219; phone: 515/628-5203 or 628-9409).

Janice C. Kemp
President, AMCBT

ANNOUNCEMENT

I have a large supply of good, viable sclerotium of Physarum polycephalum (produced by MP Backus, UW-Madison, retired). If you'd like some send me your address and one dollar (\$1.) to defray the cost of shipping. Let me know if you just want a little to try or if you use it regularly and want a large amount. If you don't use this organism as a regular part of your labs you and your students are missing an easily grown, fascinating and fun organism which fits in with many subjects (cells, cell membranes, cytoplasmic streaming, growth, elimination of waste, fungi/protozoa, response to environment, life cycle, etc.). I'll include a sheet telling how I use it and how to make more of your own if you wish.

Janet W. Phelps, UWC-Baraboo/Sauk, Co.
1006 Connie Road, Baraboo, WI 53913

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
Contact: Charles M. Chambers, Executive Director, 730 11th Street, NW
Washington, DC 20001-4584

1986 Leadership Elected

W. Donald Duckworth, Ph.D., president of the American Institute of Biological Sciences (AIBS) and director of the Bishop Museum in Honolulu, Hawaii, has announced the results of elections held recently for a number of important leadership positions within the Institute's Board of Directors. In commenting on the results, Duckworth noted that the Institute is now developing a national center in a new headquarters building in downtown Washington, which is dedicated to the work of its member scientific and research societies. In particular, he said, "Every day news reports emphasize the dramatic role being played by the biological sciences in medicine, agriculture, the environment, etc. We look to these future officers and directors for the leadership to fulfill the responsibilities society is placing on our disciplines. I am pleased that our individual members and affiliate societies have selected well qualified persons to guide the Institute in the future. Each of these new officials has an established reputation in scholarship and service to biology and has been heavily involved in Institute activities such as committees, consortia, symposia, etc. over the past few years. Both individually and collectively they will strengthen our ability to serve our science and the nation. I am delighted to recognize the increased responsibility each of them will now be assuming in the Institute's activities and wish them much success in their new assignments."

The new President-Elect, Boyd R. Strain, Ph.D., is professor of botany at Duke University and Director of the University's Phytotron. Strain began his academic career at the University of California-Riverside, transferring to

Duke in 1969. His major research interest is the physiological ecology of plants and he has developed assay and tracer methods based on short-lived radioisotopes. Recent efforts have focused on the stress of global carbon dioxide enrichment and on tropical rain forests. He has served on a number of AIBS boards and committees including studies for NASA on the environmental impact of the space shuttle and controlled ecological life support systems, and he has been an AAAS representative for AIBS. He is also a member of the Ecological Society of America, the Botanical Society of America, Sigma Xi, and has served on state and national councils for such groups as NSF and NRC. At the completion of his one-year term as President-Elect, Strain will become President for 1988.

Two new members of the twelve person Board of Directors were elected by the membership-at-large. They are Paul G. Risser, Ph.D., most recently Chief of the Illinois Natural History Survey, and Judith S. Weis, Ph.D., currently professor of zoology at Rutgers University in Newark. Risser has been involved with AIBS for over 15 years and, as president of the Ecological Society of America in 1985, he significantly strengthened the day-to-day interactions between the Society and AIBS. He has developed proposals with AIBS for specific projects, served as a peer reviewer and scientific advisor for Federal programs in the Special Science Program, and has authored and reviewed papers for BioScience.

Wies, a former Congressional Fellow, has been involved with AIBS in several capacities over the past few years. In 1984 and 1985 she served on the AIBS Congressional Science Fellow Committee, is currently a member of the Institute's Public Responsibilities Committee, and acts as Book Review

Editor for BioScience.

Two other new board members were elected by the 55 member Council of member societies. They are: Neil M. Barnett, Ph.D., and Laurence D. Moore, Ph.D. Barnett, a professor of botany at the University of Maryland, currently serves as representative of the American Society of Plant Physiologists. As Chairman of AIBS's Congressional Science Fellow Committee, he has been instrumental in expanding the existing program to further involve both member and non-member societies. Barnett has also served on the Institute's Public Responsibilities Committee since 1982. Moore is currently professor and head of the Department of Plant Pathology, Physiology and Weed Science at Virginia Polytechnic Institute and State University. He represents the American Phytopathological Society, and his research includes interdisciplinary efforts with scientists in agronomy, biochemistry, and horticulture; and environmental influences on plant diseases and nutrition. He has developed an Interdepartment Graduate Curriculum in Plant Physiology and a molecular approach to teaching plant physiology laboratory and has received external funding from the government of Kenya and the Virginia Tobacco Commission.

Founded in 1947 as a component of the National Academy of Sciences, AIBS is now an independent federation of some 40 professional and research organizations representing more than 70,000 biological scientists. It is devoted to the advancement of the biological, medical, environmental and agricultural sciences and their applications to human welfare; it also fosters and encourages research and education in the biological sciences. Its government relations and publications activities are focal points for national interests in bioscience and it serves such diverse government bodies as the Agency for International Development, NSF, The Department of Energy, and NASA, the space agency. The President is the chief elected officer. The Board

of directors sets overall policies and programs and oversees Institute operations during the year. Board members serve for terms of two years and the new officers assume their positions at the conclusion of the Annual Council meeting to be held November 13-14, 1986 in Washington.

Julie A. Miller Named New BioScience Editor

Julie Ann Miller, Ph.D., has been named the new editor of BioScience, the monthly magazine of the American Institute of Biological Sciences, AIBS Executive Director Charles M. Chambers announced today. Miller, a writer, editor, and research biologist, currently is Life Sciences Editor of Science News and has made freelance contributions to BioScience, New Scientist, Trends in Neuroscience, Chemical and Engineering News, The Progressive and many other periodicals. Chambers noted that AIBS was indeed fortunate to have a person of Miller's interests and qualifications to provide leadership for a publication that offers professional biologists a broader view of the total discipline and its public policy dimensions than can be found in the primary research journals. She assumes her new position on September 2, 1986.

Miller has edited numerous books and articles for organizations and publications such as Elsevier Science, the National Institutes of Health, Chemistry and Industry, and SciQuest. In addition she has authored research papers, book chapters, articles, and government reports of her own. Miller is a member of the National Association of Science Writers and was recently awarded a Blue Pencil award from the National Association of Government Communicators for a booklet on head injuries.

Born in Chicago, Illinois, Miller received her Ph.D. in Neuroscience from the University of Wisconsin at Madison in 1976. There she was elected a member of the scientific honor society, Sigma Xi, and performed graduate research on the biochemistry of vision with Dr. Deric Bownds. She was also the recipient of a traineeship with the National Institutes of Health. Earlier, while an undergraduate student in biochemistry and molecular biology at Harvard University, Miller was awarded a grant from the National Science Foundation for work in Harvard's Medical School Department of Neurobiology. In 1977, she received her M.A. in Journalism from a special reporting program at the University of Wisconsin. Her strong writing and editing skills have been recognized with numerous awards including an Honorable Mention from the American College of Radiology and a Meritorious Achievement from the American Dental Association in its Science Writers Award.

Founded in 1947, AIBS is a federation of 35 professional societies representing more than 70,000 biologists nationwide. The organization is devoted to advancing basic biological, medical, agricultural, and environmental sciences and their applications to human welfare. Its government relations programs, distinguished awards, educational activities, and publications are focal points for national interest in the life sciences. Recently, the Institute's monthly publication, BioScience, received an Olive Branch Award for its outstanding coverage of the nuclear arms debate.

The Institute assists various government agencies, including the Agency for International Development, the Environmental Protection Agency, the Department of Defense, and the National Aeronautics and Space Administration with studies, reviews, evaluations, and policy planning in the life sciences.

SCIENTIFIC COMMUNICATION

By

Dr. Laddie J. Bicak, Dept. of Biology, Kearney State College, Kearney, NB

One of the criticisms heard about the inadequacies of today's college graduates is that they lack the skills for effective communication. Most everyone, in any discipline, has room for improvement in writing and speaking. Inadequate communication is not new...we can trace this criticism back through the elementary and secondary schools, and back through the centuries. Historically, the communication of scientific discoveries, new concepts and theories have been transmitted to the scientific and lay communities rather slowly. The acceptance of new ideas by scientists has not always been swift and universal.

The exchange of scientific knowledge in the past has been slow due in part to the methods of transmission and the reluctance of investigators to report and publish their findings. Sir Issac Newton, for example, was reluctant to report his findings on planetary motion and did so only after the urging of Sir Edmund Halley.

According to Conant (1951), before the existence of scientific journals, information about scientific discoveries spread by letter. Occasionally an investigator would publish a book in which he recounted his work. The pioneer work of Ignaz Phillip Semmelweis in asepsis and the reduction of the incidence of puerperal fever was not immediately accepted by doctors, but once they became aware of the importance of washing their hands and sterilizing their instruments, the mortality rate dropped for the disease (Gardner 1972). Then too, the work of the Czechoslovakian monk Gregor Mendel laid the foundation for the science of genetics. Mendel's work lay undiscovered for nearly forty years, but according to Gardner (1972) it was in 1900 that Mendel's paper was discovered by Hugo de

Vries, Carl Correns and Erich von Tschermak-Seysenegg, who recognized the significance of Mendel's work and brought it into prominence.

Perhaps most of our students are not at the point where they will make contributions similar to great people like, Newton, Halley, Semmelweis and Mendel, but it is necessary that they be involved pragmatically with the fundamentals of scientific communication. It would be reasonable to expect that a student who entered a degree program in biology would be more effective in written and oral communication at the time of his/her graduation than when he/she first began college four or five years earlier. He/she should be effective in communicating ideas, concepts and processes of thought with his colleagues and with members of society in general. Ideas, concepts and methods of scientific inquiry are fundamental outcomes of study in the sciences. As Gardner (1972) has stated: "There can be no effective 'science' until there is effective, lasting communication of information. This truism holds for biology as for all science".

The Department of Biology in Kearney State College has recognized the importance of effective communication and requires a course in scientific communication for students majoring in biology. The course carries one semester hour of credit. The required text is How to Write and Publish a Scientific Paper, by Robert A. Day (1983). A supplementary text is the CBE Style Manual, fifth edition, Council of Biology Editors, Inc., (1983).

The objectives for the course are as follows: To be able to:

1. Develop a rationale for writing and speaking well, in the communication of

scientific information.

2. Use the guidelines for formulating a research proposal, effectively.
3. Demonstrate a capability to use library research resources such as journals, abstracts, indexes, government documents and computer search facilities.
4. Prepare an acceptable research paper in keeping with the approved style and form of the CBE Style Manual.
5. Present oral reports of scientific research in an acceptable manner.
6. Demonstrate a high degree of competency in the use of the English language.

As the objectives imply the student has ample opportunity to develop skills in searching the literature, present oral and written work, and become adept in using library materials. Some examples of assignments which are intended to accomplish the objectives are:

1. A lecture, presentation with slides and a discussion of scientific method.
2. A library tour for explanation of the use of journals, Biological Abstracts, Chemical Abstracts, indexes, government documents, science dictionaries and references, ERIC (microforms), the card catalog, and the computer search DIALOG.
3. A term paper requirement - the term paper is a review of the literature in an area of biological research of interest to the student. The literature review and citation are written in acceptable form according to the CBE Style Manual. The paper must use a minimum of 20 scientific literature sources and probably will be between 10 to 15 pages in length. A two-hour research project is required for biology majors and a student may well use the

term paper as a review of the literature for the research report.

4. Establish the differences between an abstract and a summary and develop reference cards with abstracts and proper citation of the research sources.
5. Small group oral reports in which assigned research reports are critically evaluated against selected criteria.
6. Lecture, discussion and accompanying handouts are used to illustrate proper writing form, citation form and the development of the research report.
7. Three films are shown which present field or laboratory research. The student then writes a 200 word paper summarizing the "research message" found in each film.
8. Scientific vocabulary exercises, involving word definition, prefixes, suffixes and confusing terms, are assigned.
9. Readings are assigned from Day and the CBE Style Manual.
10. A grade for the course is based on the evaluation of all assignments, a term paper and two tests.

Admittedly there is a considerable amount of work in the course for one-hour of credit. However, the response to the course has been very favorable. Comments on evaluations indicate that most students had not been required to write research papers to any great extent prior to taking the class. They recommended that more credit should be assigned to the course, but they did feel more comfortable in reviewing research literature and writing reports and proposals for investigations after having completed the course. There is validity to the saying that one of the best ways to learn to write well, is to write and write and write!

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HOW-TO-DO-IT
PREPARING A HUMAN KARYOTYPE

By

James D. Hoerter and Teresa L. Bean, Stephens College, Columbia, MO

One of the most popular laboratory exercises in our genetics course is about the preparation of human chromosomes. Students become immediately interested in this laboratory exercise when they learn that they will be seeing their own chromosomes obtained from cultures of leucocytes. The procedure is simple and the results are consistently good if the steps are followed. A minimum amount of equipment is required, all of which can be found in a general biology laboratory. Many of our students have directed their careers toward human genetics and genetic counseling after being introduced to this area through this laboratory exercise. Inviting a human genetics counselor as a guest lecturer immediately after conducting this laboratory is very effective in teaching the direct applications of this technique for the detection of human genetic diseases. Any large medical hospital and clinic will probably have a genetics counselor or medical geneticist on their staff who would be glad to come and talk to your class on human cytogenetics and genetic counseling.

Human chromosomes can be easily obtained from cultures of human leucocytes from venous blood. These white blood cells are convenient to obtain and, in culture, they are readily induced to undergo mitosis. Treatment with colchicine and a hypotonic solution is followed by fixation with appropriate chemicals. Fixed cells may be squashed on a slide to spread the chromosomes in one plane and stained with a dye specific for chromosomes. When prepared in this way, human chromosomes can be distinguished by length and by the position of the centromere. From these two morphological features the chromosomes can be arranged in groups.

Stained human chromosomes may be examined by ordinary light microscopy, photographed, and the photograph enlarged (Figure 1). In a well prepared squash, each chromosome appears as a separate entity with no overlapping of chromosomes. Individual chromosomes may be cut out from such an enlarged photograph and matched as pairs of homologues. When arranged in pairs according to order of descending length and position of the centromere, such an assemblage of chromosomes is said to constitute a karyotype. Utilizing the length of the chromosomes and the position of the centromere, one can group chromosomes in seven groups (A through G).

Students utilize a karyotype form published by Carolina Biological Supply Company to paste their chromosomes according to groups based on their size, position of centromere, and overall morphology. Abnormal karyotypes are studied by utilizing photomicrographs of individuals with Klinefelter's Syndrome, Trisomy-21, and Turner Syndrome. Photomicrographs are not identified according to syndrome. Students are instructed to analyze them, prepare karyotypes and make a diagnosis as to the syndrome. Photomicrographs of abnormal karyotypes are also available from Carolina.

Laboratory Procedure

Reagents for this procedure can be obtained from Carolina Biological Supply Company. Each kit contains reagents which permit cultures from two students. In order to cut down on costs, we usually have three students work as a group, one of which donates the blood for the cultures. Over twenty slides can be prepared from each culture. The following procedure is a

modification of the procedure suggested by Carolina, which we found to be most effective in producing good chromosomes preparations. With this procedure the chromosomes are much longer rather than short and stubby, and more evenly distributed on the slide.

Lymphocyte Separation and Inoculation

1. Withdraw 10 ml of blood from the patient who has abstained from eating for a least 3 hours and aseptically transfer it to a chromosome blood separation vial. Mix by inversion and allow it to stand at room temperature for 1-2 hours. The upper clear area will be the plasma-lymphocyte suspension.

2. Rehydrate chromosome medium with a vial of chromosome reconstituting fluid. Incubate for 30 min. at 37° C.

3. Inoculate a bottle of rehydrated chromosome medium with 2.0-4.0 ml of plasma-lymphocyte suspension from the chromosome blood separation vial. Transfer is performed with a sterile Pasteur pipette and bulb.

Incubation of cultures

4. Incubate the inoculated chromosome medium bottle, preferably in a vertical position, for 4 days at 37°C. The color of the reconstituted medium should be kept pink before and after inoculation and, during the incubation period, by loosening the cap temporarily in order to allow escape of carbon dioxide if the color becomes amber. Disperse the cells by swirling the contents twice daily.

5. Add 1 vial of chromosome arresting solution to the incubated culture and swirl it to insure thorough mixing; this will terminate the mitosis at metaphase.

6. Incubate an additional 2 hours and 45 minutes.

Harvesting and Fixation of Cells

7. Transfer the entire culture to a 15 ml graduated conical centrifuge tube and centrifuge for 10 minutes at 800 rpm.

8. Carefully pour off all but 0.1 ml of the supernatant fluid.

9. Add 1 ml of warm (37°C) Hanks Solution and gently resuspend the cells in the centrifuge tube with a Pasteur pipette.

10. Add 3 ml of warm (37°C) distilled water in 5 drop portions with momentary agitation after each addition to produce a hypotonic solution.

11. Incubate the suspension at 37°C for 10 minutes only. The exposure of the cells to this hypotonic, diluted Hanks Solution should not exceed 10 minutes.

12. Centrifuge the lymphocytes at 600 rpm for 5 minutes.

13. Carefully pour off the supernatant.

14. Add slowly, without disturbing the button of cells, 4 ml of freshly prepared fixative consisting of 1 part glacial acetic acid and 3 parts methanol.

15. Let the cells soak in the fixative for 15 minutes at room temperature. Cells should be treated gently during this stage of fixation.

16. Carefully resuspend the cells with a Pasteur pipette.

17. Centrifuge at 600 rpm for 5 minutes and carefully pour off the supernatant.

18. Resuspend 4 ml of fresh fixative with a Pasteur pipette, let stand for 5 minutes, and centrifuge at 600 for 5 minutes.

20. Add 0.5 ml of fresh fixative to the button of cells and resuspend with the Pasteur pipette to get a hazy suspen-

sion.

Preparation of Slides

21. Label clean microscope slides and place them in absolute methanol at -20°C overnight.
22. Release a drop of cell suspension from a height of several feet on a cold wet slide. Slides should be placed at a 20 degree-angle.
23. Ignite the fixative on the slide by bringing it momentarily in contact with a flame. As soon as the fixative is burned off, wave the slide vigorously to hasten drying. The slide should not get hot, but drying should be accomplished rapidly as possible.

Staining of Slides

24. Dilute the 1 ml of stock Bacto-Giemsa stain with 19 ml of distilled water. This preparation should be used the same day it is prepared.
25. Place the slides in a small staining dish and cover them with staining solution for 20 minutes.
26. Rinse the slides gently in distilled water and air dry.
27. Examine the slides under the microscope. The mitotic spreads may be scanned at a total magnification of 125X, or examined more closely at 500X and photographed under oil immersion at 1000X. Slides may be protected by cover slips and made permanent by conventional procedures.

Summary

Preparing human karyotypes from cultures of leucocytes is an excellent laboratory to introduce students to the field of human genetics. The procedure described will consistently yield excellent chromosome spreads. Kits containing all reagents can be conveniently obtained from Carolina Biological Supply Company. Students photograph the preparations and prepare a karyotype based on chromosome size, position of centromere and overall morphology.

Biographical Sketch

James D. Hoerter is associate professor and chair of the Department of Natural Sciences, Stephens College, Columbia, MO 65215. He received his Ph.D. in genetics from Pennsylvania State University in 1975, where he was an NDEA Genetics Research Fellow. He is the director of the Research Internship Program, which provides students with opportunities to do research in government and private laboratories in the Columbia area. Hoerter also serves as the pre-professional advisor and coordinates the Science Scholar Program at Stephens.

Teresa Bean is an undergraduate student in the Department of Natural Sciences at Stephens College, majoring in biology.

USE OF CSIP FUNDS IN A COURSE IN MOLECULAR BIOLOGY

By

Charles E. Deutch, Dept. of Biology, Kalamazoo, MI

For the past several years, Kalamazoo College has offered a one-quarter course in Molecular Biology. This course is usually taken by six to eight biology or health sciences majors during the Summer Quarter at the end of the junior year. The course has as prerequisites a core course in cellular biology and introductory courses in general and organic chemistry. The objective of the proposal submitted to the National Science Foundation under the College Science Instrumentation Program was to improve the laboratory of the Molecular Biology course.

The course in Molecular Biology uses the textbook Genes II by Benjamin Lewin and has three components. The first is a lecture series that follows the basic organization of the text. Three lectures are given each week, and they attempt to integrate material dealing with both prokaryotic and eukaryotic cells. The second component of the course is a journal club based on readings from the primary literature. The journal club meets once a week for two hours, and each week, all of the students read a short review article related to the lecture material. Most of the review articles have been taken from Trends in Biochemical Sciences or Cell. Two or three journal articles selected by the instructor are then presented by individual students for class discussion. Depending on the enrollment in the course, each student makes two or three presentations during the term.

The third component of the course is the laboratory work. The laboratory is scheduled for one four-hour block each week and is based on the labtext Recombinant DNA Techniques: An Introduction by Raymond L. Rodriguez and Robert C. Tait. This book describes a series of experiments in which 1)

plasmid and chromosomal DNAs are isolated from Escherichia coli; 2) the DNAs are digested with several restriction endonucleases; 3) the DNA fragments are joined together by DNA ligase and introduced into E. coli hosts defective in histidine biosynthesis or arabinose utilization; 4) His and Ara transformants are selected and characterized phenotypically; and 5) the recombinant plasmids are reisolated and restriction endonuclease maps of the cloned fragments are constructed.

This series of experiments has several features that make it appropriate for an undergraduate laboratory experience: 1) the individual experiments are relatively simple and based on explicit protocols; 2) the experiments form a coordinated sequence with clear stopping points; 3) the project does not require radioisotopes or unusual biohazard facilities; and 4) the project is supported by a fairly detailed text, references, and protocols for additional experiments. The labtext is not perfect, however, and students are provided with separate handouts each week that give specific modifications, where necessary, to fit the particular facilities at Kalamazoo. Students usually work individually on the experiments, and they are expected to write two laboratory reports during the term: the first covers the initial set of experiments, the second the entire project.

Successful completion of this laboratory component requires equipment for the electrophoretic separation and analysis of DNA fragments in agarose gels. Electrophoresis is used at three points in the cloning project: 1) it is necessary for monitoring the digestion of the plasmid and chromosomal DNAs by the restriction endonucleases; 2) it is required for testing the ligation of the

plasmid and chromosomal DNAs by DNA ligase; and 3) it is used for constructing restriction endonuclease maps of the cloned fragments. In each case, the DNA fragments are detected by staining the gels with ethidium bromide and viewing them under ultraviolet light. Prior to submission of the CSIP proposal, the electrophoretic portions of the experiments were carried out with a vertical slab gel system and the gels were analyzed with a hand-held Mineral-light. Both of these techniques proved awkward: the gels tended to slide out of the vertical apparatus, and the DNA bands were not clearly visible.

The purpose of the CSIP proposal was thus to improve the laboratory component of the Molecular Biology course by the acquisition of more modern equipment for gel electrophoresis. The grant will allow the purchase of three new constant-current and constant voltage power supplies, and six systems for horizontal gel electrophoresis. In addition, the grant provides for the acquisition of a high-quality UV

transilluminator with a maximal output at 300 nm and a Polaroid photographic system. The equipment will be obtained early in 1987 and used for the first time during the Summer Quarter of this year.

With this new equipment, it should be possible for students to complete the experiments in Molecular Biology with greater ease, efficiency, and accuracy. The photographic system will allow them to obtain a permanent record of the results and will improve their analysis of the data. With the new gel systems, it will be possible to accommodate six students in the course if they work individually, or 12 students if they work in pairs. In addition to its use in the Molecular Biology course, the equipment will also be used to set up demonstrations of electrophoresis for the Cellular Biology core course and to enhance the laboratories in upper-level courses in Immunology and Microbiology. The acquisition of this equipment thus should increase student understanding of the basic techniques of Molecular Biology and prepare them to do further work in this area.

**A MOLECULAR GENETICS LABORATORY COURSE
FUNDED BY A NATIONAL SCIENCE FOUNDATION CSIP GRANT**

by
Valerie R. Flechtner, Dept. of Biology, John Carroll University,
University Heights, Ohio

In the 1970's, the development of new techniques for manipulating and characterizing nucleic acids has significantly extended significantly our understanding of the organization and expression of genetic information in both eucaryotic and procaryotic organisms. Molecular genetics is attractive to many undergraduate science majors and a lecture course in molecular genetics can provide the student with a good academic understanding of the field. But for some students, having the lecture course alone is a source of frustration; they want hands-on experience with the techniques used in genetic engineering. This student enthusiasm for a molecular genetics laboratory, coupled with my own desire to offer such a course and to upgrade experiments in the pre-existing genetics and microbiology courses provided the incentive to submit a proposal to the College Science Instrumentation Program (CSIP) of the National Science Foundation. Funding of this proposal provided the monies to purchase equipment necessary to teach a laboratory course in molecular genetics which is appropriate for both upper-level undergraduates and first year graduate students.

The aims of the laboratory course are:

1. To introduce the students to some of the techniques of microbial genetic analysis.
2. To teach students the basic methodology of recombinant DNA construction and analysis.
3. To make the students comfortable reading and analyzing the primary literature.

The following sections will present a brief description of how each of these aims is achieved.

ACCESSING THE PRIMARY LITERATURE

Because it is imperative that students of molecular genetics be able to access the literature efficiently, during the first laboratory meeting students receive an orientation to the science holdings of the John Carroll University Library. The reference librarian explains how the science holdings are organized and demonstrates the use of reference materials such as Biological Abstracts. A demonstration of a computer-assisted literature search is included in this session.

GENETIC ANALYSIS OF MICROORGANISMS

Many of the techniques used in modern molecular genetics laboratories involve working with bacteriophage. Therefore, the second section of the laboratory course includes four 2-hour periods devoted to the fine structure mapping of the rII region of bacteriophage T4. In the first period students are taught the dilution and aseptic plating techniques necessary to assay both wild type and mutant bacteriophage. During the next week students read two of the original papers describing the mapping of this region (1,2) and the second laboratory period is spent discussing these papers. During the third laboratory session students characterize a number of point mutants in the rII region using both complementation tests and recombination analysis. During the final session the class members' results are collated and discussed. The students are then expected to write a formal laboratory report summarizing the results of this experiment.

CLONING OF RECOMBINANT DNA MOLECULES

In section three, two separate

molecular clonings are attempted. In the first, students attempt to insert a 7.2 KB Bam I fragment of the chloroplast genome of Chlamydomonas reinhardtii into the E. coli plasmid pUC8; in the second they attempt a shot-gun cloning of the Hind III-digested lambda bacteriophage genome into the same vector. The pUC8 vector carries both a gene for ampicillin resistance and a beta-galactosidase gene which contains a multiple cloning site with recognition sequences for nine different restriction enzymes including Bam I and Hind III. Recombinant DNA molecules are constructed by cleaving the vector with the appropriate restriction enzyme and ligating the desired fragments into the cleaved vector. The site of insertion in both cases is the beta-galactosidase gene and insertion of a foreign DNA fragment results in inactivation of this gene.

E. coli strain JM83 which is ampicillin sensitive and beta-galactosidase negative is used as a host for cloning. Competent cells are exposed to the ligation mixture under conditions appropriate for transformation. These cells are then diluted and plated on selective media containing both ampicillin and X-gal, an artificial substrate for beta-galactosidase. Because the untransformed host is ampicillin sensitive only those cells which have taken up either the native pUC8 plasmid or a recombinant plasmid can grow on the antibiotic-containing plates. Cells containing the recombinant plasmid can be differentiated from those containing the native plasmid on the basis of beta-galactosidase activity. Those cells which contain the native plasmid express beta-galactosidase activity and form blue colonies on this medium. Those clones which have taken up a recombinant plasmid have an inactive beta-galactosidase and appear as white colonies on the medium.

Once putative recombinant colonies are identified, the colonies are amplified by overnight growth in 5 ml liquid medium (Luria broth) containing

25 µg/ml ampicillin. Cells are harvested and plasmid DNA is prepared using a modification of the miniprep procedure of Brinboim and Doly (3). Purified DNA is cleaved with the appropriate restriction enzymes and the resulting fragments are characterized by electrophoresis in 1% agarose gels. Bands are visualized by exposure to ultraviolet light following ethidium bromide staining and a polaroid photograph is taken of the gel.

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Both the bacteriophage experiment and the molecular cloning experiment use standard techniques which are available in laboratory manuals. The laboratory manual by Snustad and Dean (4) was used as a reference source for the bacteriophage experiments and the Cold Spring Harbor Laboratory Manual "Molecular Cloning" (5) is an excellent reference source for the molecular cloning experiment. Laboratory handouts with background information and specific protocols were provided by the instructor for each student.

SUMMARY

Student response to this laboratory has been quite enthusiastic. Several graduating seniors cited this laboratory as their most exciting experience as undergraduates. The course involves a significant time commitment on the part of both the students and the instructor because during the recombinant DNA experiment the instructor must work with small groups of students. This experiment, which extends over 7-8 days, requires that both the students and instructor have several long time blocks (4-6 hours) available. But the experiment does work. Every group of students which attempted the clonings were successful in at least one aspect of the experiment and 80% of the groups succeeded in both the fragment cloning and the shot-gun cloning. The expressions of disbelief and then excitement when each student saw his or her own gel

profile revealing a successful result more than compensated for the time investment made. A copy of the laboratory handouts is available from the author.

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MAINTENANCE AND PRESERVATION OF STOCK CULTURES

By

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Most departments of biology that offer one or more courses in microbiology have at least a small collection of stock cultures. One often notes that these collections are in a state of disarray or even worse. This is generally due to a lack of knowledge on the part of those whose job it is to maintain such collections. This situation can be remedied in a relatively short period of time by an understanding of the proper techniques and procedures involved in handling stock cultures.

There are two distinct objectives in keeping stock cultures - maintenance and preservation. By maintenance we mean keeping a culture alive, pure and in its typical recognizable state. But merely to maintain a stock culture is not enough; it also must be preserved so that at some future date, if needed, it can again be used.

New cultures for stock can be ordered from a national culture collection such as the American Type Culture Collection located in Rockville, Maryland or from any reputable biological supply house. These suppliers play an indispensable role for microbiologists by providing a wide variety of cultures with which to start a collection.

Maintaining Stock Cultures

The maintenance of stock cultures requires a great deal of care and work. Each culture must be kept pure, and this purity must be checked at least once a year. This is achieved by streaking the culture out onto an agar plate and then staining the isolated colonies. Generally the gram staining technique is used here but other staining procedures

can and should be used depending upon the species of microorganism.

A purity check does not end here as one can rarely if ever be absolutely certain as to species by merely noting the staining reaction, arrangement, and morphology of the organism. Further tests such as biochemical reactions, cultural growth characteristics, oxygen requirements and serological reactions need to be carried out.

Probably the most common technique for the maintenance of stock cultures is periodic transfer. Most common species of heterotrophic bacteria can be maintained without transfer for about a month although this varies with the type of organism, the medium used, and the temperature at which the culture is kept. Thus for each species of microorganisms one must determine beforehand (1) the proper medium to use, (2) the time interval at which a transfer must be made, and (3) the correct storage temperature.

Generally speaking cultures can be maintained for a longer period of time at refrigeration (4-8°C) rather than room temperature because their biochemical activities are proportional, more or less, to the temperature of the external environment. Thus a low temperature such as that of a refrigerator tends to inhibit metabolism and thereby prolong the life of the culture. Screw-capped tubes should be used in order to slow down dehydration of the media. Table I presents some examples of the requirements needed by the more common genera of bacteria.

It is strongly suggested that a stock culture record book be kept. Thus information about each culture is kept

in an orderly fashion. Data about each culture that needs to be entered into the record book are the following: (1) date and source of acquisition, (2) original code number if obtained from another stock culture collection, (3) date of last transfer, (4) type of medium used, (5) optimum temperature for growth and (6) storage location.

Long-term Preservation of Stock Cultures

Stock cultures are preserved by reducing their metabolism to a bare minimum. When this is accomplished the biological processes which lead to aging and death are slowed down and the microorganisms then can be maintained in an inactive state for a lengthy period of time.

Table I. Maintenance Data for Some Genera of Bacteria

Genus	Medium	Transfer Time	Incubation Temp. (°C.)	Storage Temp. (°C.)
Acetobacter	Acetobacter agar	1 month	28	10
Alcaligenes	Nutrient agar	3 months	37	10
Bacillus	Nutrient agar	12 months	28	10
Clostridium	Cooked meat medium	6 months	28	room
Escherichia	Nutrient agar	3 months	37	10
Leuconostoc	Nutrient agar	6 weeks	room	10
Mycobacterium (saprophytic)	Glycerol agar	4 months	30	10
Neisseria (saprophytic)	Cystine-trypticase agar	1 month	37	room
Proteus	Nutrient agar	3 months	37	10
Pseudomonas	Nutrient agar	3 months	37	10
Xanthomonas	Potato Dextrose agar	2 months	room	10

Several methods have been developed for the preservation of cultures. When one is faced with choosing a method, such factors as amount of labor involved, time required, equipment needed and storage facilities required should be taken into consideration. Discussed below are some of the more commonly used techniques.

Oil Sealing. This technique is very easy to perform. A culture is inoculated onto an agar slant, and when growth is profuse, sterile mineral oil is added. The oil must completely cover the slant. To insure this, add enough oil so that the level of the oil in the tube is about one-half inch above the top of the slant.

The technique works by reducing the amount of oxygen available to the microorganism. When this has occurred the rate of metabolism is drastically reduced thereby causing the microorganism to remain in a dormant state. The method also prevents the medium from undergoing dehydration.

The length of time stock cultures can be kept viable by this method varies with the species but can range anywhere from a few months to several years.

Those genera of bacteria surviving well by this method are the following: Acromobacter, Bacillus, Flavobacterium, Proteus, Pseudomonas, Sarcina, Serratia, and Streptococcus. However, this

procedure has not been successful in the preservation of Azotobacter, Lactobacillus, Leuconostoc, Mycobacterium or Salmonella. Salmonella.

An added advantage of this technique is that one can remove culture from under the oil seal with an inoculation needle and still preserve the original culture.

Deep Freezing. Temperatures below 0°C are sometimes used to preserve cultures. In general it has been found that temperatures in the -10 to -20°C range are more favorable for the long-term preservation of cultures than lower or higher subfreezing temperatures.

This procedure is carried out by aseptically adding a sterile solution of water plus 15% glycerol to a slant culture. Microorganisms are washed from the slant and then transferred to glass ampoules. The glycerol prevents rupture of the microbial cells. These ampoules are then immediately placed at a subfreezing temperature. Many microorganisms retain their viability for long periods of time under these conditions.

Freeze-drying (Lyophilization).

Probably this is the most effective method for the long-term preservation of cultures, but the most costly in terms of equipment needed. Many species of bacteria have been preserved for longer than twenty years. According to this process a thick suspension of microbial cells is laced in a small vial. These vials are then immersed in a mixture of dry ice and alcohol (-78°C). The vials are then connected to a high-vacuum line which dehydrates the culture. Each vial is then sealed off under a vacuum. The major advantages of this technique are essentially long term survival and ease of storage. Literally hundreds of these lyophilized culture vials can be stored in a very small area.

Conclusions.

By following the procedures discussed in this paper, the department's collection of stock cultures can be maintained in an orderly fashion. Those that are preserved for future usage will be available when needed. In addition, the amount of money spent annually to purchase new stock cultures can be significantly reduced.

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WHAT SHOULD SOCIETY EXPECT FROM SCIENTISTS?

By

George Kieffer - Keynote Address

It is an honor and privilege for me to be asked to deliver this keynote address. I'm not certain that I can say much that is very original but I do not think that matters. Science and society can no longer afford the myths and misunderstandings about each other. Public understanding of science is more important today than it ever was; scientific and technological issues are different than they used to be and demand a better informed citizenry. And scientists must appreciate the fact that they are dealing with the public and its institutions. When one is truly incorporated into the other then both stand to gain.

Beginning from at least the end of WWII, when the world became aware of the awesome destructive potential of nuclear bombs, much has been said and written concerning the social responsibility of scientists. And indeed, the Association of Midwestern College Biology Teachers is to be applauded for selecting this theme for its conference. The literature examining this matter is overwhelming, a fact again brought home to me as I prepared this topic. One feels hard-pressed to come up with something that hasn't been said a hundred times over already. A possible way out of this dilemma is to phrase the question in a slightly different way to ask, "What are the expectations of the public over and against scientists?" Here, a response is not so clear-cut probably because ours is an extremely diverse culture holding many and different values. It is this question that I would like to make some preliminary remarks about in hopes of starting a dialogue.

It is a fact of our times that the public's perception of science and technology, whatever we might think it

to be, recognizes the double-edged nature of these enterprises. Scientific breakthroughs and technological innovations, sit side by side with ethical and social dilemmas and environmental disasters on the front page of news papers whenever and wherever they occur. Newfound ways to have babies and if research with human embryos ought to be allowed (one in six couples desiring to conceive are unable to do so because of a biological problem), surrogate parenting and whether or not a substitute mother who has changed her mind must surrender the newborn she has carried for 9 months, correcting some of humankind's dreaded genetic diseases by gene insertion and the intentional release into the environment of engineered organisms, the promise of bioengineering body organs to replace worn out parts and the question of the quality of life experienced by patients who receive permanent mechanical hearts, the desire for security in a world in which every man, woman, and child can be killed several hundred times over and the desirability of the strategic defense initiative, are several that have become part of our daily fare of late. It is no exaggeration to claim that public interest in science, medicine, and technology continues to grow.

For their part, the scientific community increasingly is being called to task for not being sufficiently responsive to problems that arise from their work. We are accused of neglecting social needs while directing our research toward esoteric goals or we are portrayed as progenitors of destructive technologies. In a recent survey of the American public by John Miller at Northern Illinois University (Director of the public opinion laboratory), more than one-half of all adults polled including a sizable portion who were

college graduates (38%), agreed with the statement, "Because of their knowledge, scientific researchers have a power that makes them dangerous." Technology is frequently called dehumanizing, and science itself is blamed for erosion in the belief of the meaningfulness of life. (As biologists we all are aware of the increased challenge to the teaching of evolution for the reason that it undermines certain beliefs. As one letter to the editor written by a fundamentalist Christian expressed it, "scientists give me a pain in my soul").

But these criticisms do not just come from an anti-science fringe or even self-serving politicians seeking to capitalize on issues, as some would like to believe. Many concerned citizens from all walks of life are quite uneasy about this or that event (Chernobyl). Yet, for all their implications, critics seldom say just what it is that should be done, much less how to go about it other than closing down the entire enterprise. Perhaps because there is so little practical guidance, many scientists have reacted defensively to these critics. They protest being blamed for harmful applications and side-effects of technologies which are controlled by complex forces beyond their power to influence. (We all have heard at least once that knowledge is morally neutral.) Scientists, in general though, behave much like other citizens - they too are distressed by social problems, but have little feeling of power to change very much or personal responsibility to alleviate them. In this enormously complex world of ours, individual's may plausibly claim that their personal share is so small as to be infinitesimal and of little or no practical consequences.

It probably is true that the bulk of the scientific community has become complacent and comfortable in its isolation from society and sees few ready means of helping with any problems that arise from its work. But the inadequacy of this response is that a

major resource is being neglected. Allen Hammond, while editor of the now-out-of print Science '86 magazine, challenged the scientific community to become more involved with public policy making. He claims that policy making suffers without the counsel of those most capable of helping solve "The messy social issues of the day." Though the research community's point of view may not always be right, he goes on to add, "We are not seeing reasoned debate on these subjects as much as emotional reactions rooted in ignorance and fear." The massive problems of keeping technology directed to human betterment requires the attention of those who work in science and technology.

The goal of this effort is to provide informative input to a public that must decide the controversial issues. The public must become more deeply involved in the making of scientific and technology policy decisions. This requires as a minimum, that untrained citizens be given the knowledge they need in a form they can understand and use as a basis for an informed opinion. (Objective of STS education - the new definition for scientific and technological literacy: understanding the scientific and technological forces shaping our lives and being able to act on this understanding for personal welfare and the common good.)

What is needed for voter involvement in public policy matters is highly diverse, but there are several common threads that run through this need to know. Some of these are primarily political or economic in nature, but there are several aspects which hinge on the way science is conducted and how its discoveries are incorporated into public policy. These are the ones which I would like to remark upon.

First on my list as the single, over-arching principle is plain old-fashioned honesty and truthfulness. In the survey referred to earlier, 57% of

those polled agreed with the statement that "In this complicated world of ours, the only way we can know what is going on is to rely on leaders and experts who can be trusted." Thus, the public does recognize that there is an 'ignorance gap', a gap which can be bridged by unbiased information, not given to support a predetermined or mercenary objective. But, at the same time, and almost enigmatic, the American public is growing more skeptical of the notion that scientists, like most every other kind of expertise, can be bought. Scientists are exhorted by a wide variety of interests to take positions on various issues and in so doing often provide sanction for unwise or unpopular political actions, and they lose public sympathy as a result. There is nothing that arouses the suspicions of the public more than the feeling that information is being purposely distorted or withheld. This is being demonstrated by a growing willingness on the part of the public to question technical competence, sometimes taking the form of a resigned cynicism, at others an active resistance to the information given. The overall consequence is that the public has one more negative experience vis-a-vis science and technology.

An instance of the erosion in public trust can be seen in the success of the 1978 cloning hoax, the best-selling book In His Image, which claimed to describe a clandestine laboratory experiment in which a millionaire hired researchers to create a genetically identical offspring. The book was silly, melodramatic, and unscientific, ultimately being dismissed in court as a fraud. But it appealed in the public to images of science as technically all-powerful, available to the highest bidder, and secretive. A justification given for the publication of the book by the publisher (J.B. Lippencoth) was that it aired ethical issues that science sought to downplay. The author, Richard Rorvik, pointed to the behavior of scientists in the ongoing recombinant DNA debate as evidence that scientists

were capable of covering up advances in research that might prompt public intervention; some reviewers supported the book for the same reason. The matter of coverup is one of the favorite themes of activist author, Jeremy Rifkin, in his campaign against genetic engineering.

And indeed, the recombinant DNA debate continues as a major battleground concerning science and how it is perceived by the public. People were frightened by imaginery hazards reminiscent of the Andromeda Strain, a fictional account written to entertain, not believed. Continuing statements by a handful of scientists, some of whom are not close to this field of research, are taken as evidence that the scientific community is itself divided (a not infrequent accusation of the anti-evolutionary contingency to discredit scientific knowledge concerning evolution.) The latest round in the on-going saga of DNA is the issuance of a court order in California against the outdoor testing of engineered organisms, despite the numerous protestations and testimonies by scientists that the experiment proposed probably is not harmful. According to William Ruckelshaus (former director of the Federal EPA) building public trust is a critical element if biotechnology is to prosper. He maintains that the public must be informed, "fairly, honestly, and straight-forwardly" about the potential benefits and possible risks of biotechnology. He concludes, "We need to do a much better job, not just in this area but across the board, as we grapple with the complexity involved in public participation in decisions of enormous uncertainty."

Public skepticism of scientists has at least two causes, the nature of scientific knowledge itself and media coverage of scientific findings. Concerning the first, Ruckelshaus correctly called attention to the uncertain nature of scientific pronouncements. Scientists must couch

their judgments in the language of probability and tentativeness. We are taught not to make claims that overreach the evidence. Those who extrapolate their findings onto widely varying circumstances beyond the problem at hand risk being accused of exaggerating or exceeding their scientific authority. Every scientist who speaks out on policy issues must recognize that at the current stage of scientific understanding of almost every matter, decisions must be taken in the face of uncertainty. We must continuously remind that there is no such thing as zero risk, only acceptable risk.

Perhaps part of the problem lies in the fact that we as science teachers have not done a very good job in communicating this essential feature of science to the public and our policy makers. Among the citizenry at large, there is little understanding of the process of science. The public may well wonder why we don't already know that which appears vital to a decision - but science will retain its place in public opinion only if we steadfastly admit the magnitude of our uncertainty and ignorance. And we shall lose that place if we repeatedly argue as if all necessary facts are in hand - whether the problem be dietary prevention of atherosclerosis, the health effects of air pollution, or the feasibility of 'Star Wars'. Scientists best serve the public interest by living within the ethics of science, not those of politics.

And in the matter of communication, our greatest ally is the public media. But in dealing with the press, it is probably not an exaggeration to say that many scientists view reporters with suspicion. The traditional scientist usually shrinks from such exposure, since nothing she/he can say is really 'right' for all time and with absolute certainty. Many of us have been placed in the untenable position, when debating the case for evolution, to deliver the entire content of evolutionary knowl-

edge, in 30 minutes or less, in a convincing way, to a lay audience, many of whom are skeptical. Also, the traditional scientist is of the view that the press seldom reports things correctly anyhow. We are often misquoted and what scientists see as a little contribution to a slowly rising curve of knowledge, reporters write up as major breakthroughs and are so publicized. How many times already hasn't cancer been cured - in the newspapers? The assumption often made by scientifically trained people is that there are rational solutions to everything once people know the 'facts', the 'right' behavior will naturally flow. So we hear that you can mathematize ethics and provide institutions which will do away with conflict in political and social values. The much talked about science court of the late 1970's as a way to settle social disputes has gone into oblivion. There has been a widespread public rejection of decision-making that relies only on technical expertise. I wonder if the polls that show a majority of the public support in favor of the teaching of creationism in science classrooms may not be a demonstration of such public rejection. Another might be the intensifying animal rights movement, that while in some cases may have validity, is threatening medical research.

Arguments for social policy based on scientific evidence alone are frequently perceived to give support to what has been called the 'technological imperative' - what can be done, will be done. The outcome can take the form of a negative public reaction, more commonly called backlash. If people believe that development of a given technology is inevitable, they respond with demands to stop it now. (I recall an early NAS conference on the safety of recombinant DNA research in which the anti-demonstrators paraded about the room bearing the message, "We will not be cloned".) Again, this is a favorite strategy of Jeremy Rifkin, which so far, has been quite successful.

Although scientific findings are not to be disregarded, or inferior evidence be accepted in their place, the insistence that policy be derived solely from scientifically valid arguments, is naive. Decisions, more often than not, do not turn on scientific issues alone but on social and political value judgments. In his book, The Genesis Strategy, Stephen Schneider responds to the question, "How are decisions made?" by stating the obvious - answers are generally a mix of several things - facts, common sense, and consideration of the values at stake.

There are, after all, other kinds of knowledge which are not precisely grounded in scientific proofs, but on which we act everyday. For example, we make decisions about how to raise children on implied empirical beliefs even though the beliefs may be highly contestable or impossible to prove scientifically. (Does sparing the rod really spoil the child, or is Dr. B. Spock right?) Systems of justice rest on a combination of moral beliefs and empirical assumptions that cannot be scientifically confirmed. Society does not insist on judging guilt or innocence by the scientifically confirmed state of the mind of a criminal - although there was a time not too long ago when this defense was tried. Today, we rightly dismiss such instances as a mistaken application of the standards suitable for action in one realm applied to another where there are different characteristics or standards. Moreover, if our previous conclusion concerning the nature of scientific knowledge is at all correct, then decisions based on scientific certainty are misplaced and self-serving.

The severest problems occur when respected sources are dead set against each other and the decision-maker is faced with the dilemma, "Whom should we trust?" Scientific knowledge does not always make it easier for members of the general public to come to a decision. Often lay persons find themselves with

the impossible task of deciding which Nobel Laureate to believe. The generation of power from nuclear sources offers the most vivid example here where it has been said that you could take all the experts and lay them head to foot and still not come to a convincing conclusion.

Those who would be rational in such matters attempt their examination by formal cost/benefit analysis. For example, the great environmental problems of our day involve costs, risks, and benefits that accrue to different interest groups. The problem though is that costs are reckoned in dollars, benefits in aesthetic or material values, and risks in human lives or increased frequencies of disease. A strict cost accounting procedure often, therefore, is unworkable because the components are not comparable. Cost-benefitting may be a way to solve some problems, social issues are notoriously open-ended. Their resolution more frequently than not, necessarily involve value judgments, hence, the acceptability of a proposed solution remains a political or social problem, and not solely a scientific one.

Essential political beliefs or different value sets can becloud seemingly scientific debates, a boundary that many scientists fail to recognize when they choose to become active. It is typical of social problems that they do not have unique solutions, most issues cannot be decided on purely technical grounds. Public policy debates are about uncertainties, uncertainties in which values are about as important as facts. And they have to be mixed together in any kind of analysis. (Biodiversity teleconference - where was the politician, the economist, sociologist, or planner?)

Let me emphasize the importance of this conclusion by approaching it from another direction. There are two different attitudes that people may have

toward their family physician. Some people go to the doctor, tell him what is wrong, and ask him to prescribe. Others go to the doctor and say, "Tell me what is wrong, and give me my options. I want to make the decision." People have the same dual attitude toward experts as they do towards doctors. Some believe that the public is incompetent in such matters and should leave the important decisions to experts; other people think that in a free nation the public must decide important issues. It is vital for scientists to recognize this. The 'coldness' of a technocracy continues to frighten people, as well it should.

As part of their involvement in public debate, it is necessary that scientists learn to present the issues with the ideological components clearly stated up front. You can say what you know, and you can distinguish facts from values. One of the difficulties we face when dealing with issues is that everybody considers her/himself an expert. In politics and in ethics there is no such thing as an admitted non-expert. The question is, what special authority do we scientists have as experts in these matters? I would like to point out that in spite of what we might think of them, social scientists have learned a good deal about organizations and institutions. These are not bodies of trivial data, and they must not be ignored if we are to discuss questions seriously.

But unfortunately, there is a strong tendency for the scientists to play dominant and elitist roles that intimidate lay persons. Whether or not this actually takes place, a mechanism that inhibits non-scientists from making contributions is self-defeating. What is needed is a mechanism that fosters dialogue and forces people to confront each others' beliefs. The creation of a dialogue between experts and nonexperts contributes substantially to the education of both groups and thereby improves the quality of policy choices.

In some ways, it is probably true that scientists are not particularly well fitted to play an active political role, and the number who choose to become involved will always remain small, but it is an important fraction. We should recognize that one of the reasons why people choose to become scientists is that they simply do not like public disputes. People who enjoy public disputes go into law, not science. Scientists like to sit quietly and calculate. The things of their work do not talk back. But at the same time, we should appreciate and support those scientists who do have the desire and the fortitude necessary for public action. The environment of science should be such that the scientific community bestows recognition on those who take up the challenge of public interest science. This kind of science needs a great deal more development. (I raise the question, "Would it ever be the case that public involvement would count as much as a publication record in matters of promotion and tenure?")

Lest we become discouraged and question the validity of scientific input into policy matters, such input does have the effect of lifting public debate to the proper plane, one on which the public interest is as much a question as the special interests and values of pressure groups. Society urgently needs our help to apply science wisely. It is essential that scientific evidence into policy matters be thoroughly evaluated for quality and pertinence but the decision makers may quite properly utilize quite different standards of judgment to arrive at a policy choice. Policy makers are acting responsibly when they employ a standard which might be described as what reasonable people believe on the basis of all available evidence.

And this brings me to the third and final item of public expectations - the special responsibilities of scientists. The critical question you have to ask yourself is "special responsibility to

whom?" And when you ask that question, you discover that the traditional scientific view is that a scientist is responsible to the scientific community, whereas in the new view a scientist is responsible to the public. The conventional scientific morality enjoined practitioners to be objective, to base conclusions on evidence, to avoid plagiarism or falsification of data, and to have one's papers properly refereed. Furthermore, the scientist is not supposed to demean the profession by acting in an unscientific manner, which in practice usually meant one ought not speak about things one does not fully understand. An enormous amount of institutional and organizational support is found for this kind of responsibility - in universities where we speak about academic freedom, and professional societies with their codes of ethics and special rules to reduce fraud in science. And we must hasten to add our support to this ethic for without it, science as we have come to know it could not be practiced.

But by itself, the ethic offers only limited assistance in identifying responsibility to the public. The clear and obvious reason why special responsibility attaches to the scientific vocation is that knowledge gained by research often has important implications and applications.

The crux of the issue can be expressed in two succinct propositions: 1) scientists have a special responsibility to protect the public interest; 2) the individual has the right to be directly informed about the presence or absence of risks - to lives and health, property and environment, and even to values. By public interest I include issues which touch on the security and well-being of the community. For example, availability of food, energy, health resources, transportation, good government, national defense, social services, and the like. The mechanism that implements these are bureaucratic organizations, both public and pri-

vate. (The term bureaucratic is used in its dictionary definition to mean an administrative unit.) Because of the contributions that scientists and engineers make in producing the goods and services that contribute to the public interest, they play an important and unique role relative to bureaucratic organizations. Their knowledge and expertise is needed to develop, regulate, and control science and technology in accord with public and individual interests. In addition, they also bring a tradition of professional independence which supposes the freedom to speak out when either of the above is threatened. And here, cases of this special relationship are legion. I cite a few.

The very advances in medicine that result in such welcome benefits as the conquest of disease and the prolongation of life also raises agonizing problems of medical ethics - the availability of medical care in a system that is increasingly being driven by the profit motive, or decisions of what to do with the severely damaged newborn. Research into the nature of human reproduction has forced consideration of the extent to which intervention in the process is consistent with traditional notions of human dignity and whether we have the capacity to wisely manage such unprecedented powers of control.

Because science has become a social issue, professionals cannot take refuge in the old alibi that they are not responsible for what is done with their findings. One trouble with this objection is that it entails a wholly untenable conclusion. Since all new knowledge may conceivably be put to harmful use, and since it is impossible to foretell what these uses will be, the only logical conclusion a conscientious researcher could make from the premise would be to refrain from all scientific work. If he were to refrain, however, he would be just as liable to the accusation that, by failing to use his talents to discover new knowledge about

nature, he shows himself to be indifferent to humanity's needs, since his discoveries are also likely to be used for human benefit.

Fortunately, there is a middle ground in which ethical constraints are both reasonable and meaningful. Some scientists have taken the position that they must consider the social consequences of their work, at least in cases where the consequences are reasonably direct and important. Refusal to participate in 'star wars' research immediately comes to mind as a current case in point. The consensus of this large group of physicists is that such a system against missile attack is scientifically unfeasible, that research into the problem would require an indefinite prolongation of underground nuclear testing (hundreds or thousands of tests will be needed), that it is enormously expensive and would suck research funds and scientific talent from other more worthwhile projects, and that politically it is destabilizing. Similarly, biologists in the early days of rDNA research voluntarily refrained from doing certain experiments where public safety was uncertain. Both cases provide clear illustration of putting into practice a special responsibility. Stated in bottom line terms, it recognizes that the right to experiment carries with it at the same time, an obligation not to endanger others!

Traditionally, scientists have taken the view that there must be no limits placed on the process of inquiry. They have done so partly on the grounds that freedom of inquiry deserves to be considered a human right, along with other forms of the exercise of free will. Many scientists, however, also recognize that scientific inquiry is at a far more advanced stage than is society's capacity to assimilate new knowledge. It is an unfortunate reality of our times that we live in a world that can turn the new powers into profoundly unsettling situations. Under these circumstances, the question

of special responsibility takes on a new urgency - scientists, and engineers too, have the responsibility to call the public's attention to the implications of their work when the public interest is at risk. Society, in its turn, has ample basis for expecting scientists to play an active role in informing them of these risks and in the debates over the formation of public policy. With critical scientific and technical inputs, citizens are in a position to better understand the issues at hand and to implement appropriate social policy.

Another reason in support of this position is that scientists are in the position to understand the implications of their work at an earlier stage than non-scientists. The point here is that an early awareness and debate can help stave off adversarial confrontation, when conflicting positions have been hardened and debate is wracked with charge and countercharge. By waiting too long, a full-blown social crisis is reached, and reasoned policy choice becomes all but impossible. (The abortion issue may be an instance of having waited too long.)

For their part, scientists depend upon public confidence and public support. When experts disagree in public, some people may become disenchanted with science, but this is not reason enough for experts to hold themselves aloof from important public controversies to which they can contribute by reason of their special knowledge.

These then comprise my short list of items - honesty in public pronouncements coupled with a willingness which admits that there are limits to what is and can be known, a realization that public policy judgments are not solely based on scientific evidence and the acceptance of a special responsibility (some call it moral, others social) of scientists because of their skilled expertise and information. No doubt others in the audience would present a

different list. Accountability in the use of public funds, for example, is an area that I avoided entirely. There is undeniably a strong expectation that science should serve social objectives; society expects tangible benefits, and it is this expectation which accounts for differences in the level of support for various projects and areas of science. And we still hear some who insist that the real business of science is science. Scientists have no place in the public arena unless they are invited. On either of these, I will make no further comment. (Except to say I'm reminded of the cartoon caption of two scientists sequestered in their laboratory where one says to the other, "One thing I'll say for us, Meyer, we never stooped to popularizing science.")

Those on my list speak in a limited way to specifying what the public can rightly expect from its scientists. This expectation reflects the changing attitude concerning the relationship between the scientific community and the general public. Twenty-five or thirty years ago a scientist could comfortably say, "That's not my job." Today, such voices are still heard, but they no longer enjoy wide support. The ethic is changing and communication is becoming part of the responsibility that "Goes with the turf." Most knowledgeable people are convinced that science is very much a social enterprise, not only because the public pays much of its costs, but because the consequences of scientific activities must be borne by that same public. It is from this initial position that I selected my list. In a nutshell, it states the public does expect that scientists will play an active role in the discussion of the issues surrounding their work and the debates over the formation of public policy on those matters which have crucial scientific and technical components.

Before closing, I would like to make some short comments on several issues that continue to perplex. As is

well known, scientists who become active do expose themselves to certain risks. The classic case is the fate of the so-called whistle blower. These persons may be stigmatized as unfaithful employees and non-team players whereupon they are either fired outright or demoted to lesser positions. These cases raise at least two issues:

1) Is the problem to which the whistle blower calls attention one of significant uncertainty or is it an area of reasonable disagreement? 2) What are the rights of the whistle blower and how can they be protected? The second of these questions has received the most attention by public and private agencies, probably because it is the easiest to address. The first, though, is still unanswered. In cases where the presumed risk is based on judgments where scientists might reasonably disagree, methods to resolve these difficulties need to be worked out. Whether these should be public or private is a major unanswered question.

Another risk associated with taking a public position has to do with the future standing of that individual. Fellow scientists do not always take kindly to colleagues who pursue the glare of publicity and occupy themselves with too many social crusades, even those related to scientific work. More threatening though may be the penalty extracted by funding agencies. Scientists who take unpopular political positions on such subjects as arms control may be blacklisted, ineligible for service on advisory committees, or have their funding cut. The most recent case is the position expressed by Donald Hicks, Undersecretary of Research and Development, Department of Defense, at his confirmation hearing. (I read it to remind you of its contents: "I'm not particularly interested in seeing departmental money going to someplace where an individual is outspoken in rejection of department aims, even for basic research.") Statements like these are especially disturbing when it is recognized that DOD alone provides up to

three quarters of all federal R&D dollars. I suppose the only bit of advice here is the need for constant vigilance to prevent such policies from creeping in, but still the risk is there, and may serve to stifle dissent.

But in spite of it all, the potential does exist for constructive interplay between scientists, the public, and policy makers, a climate of goodwill within which people of goodwill can work together for the common good!

THE USE OF CASTOR BEAN ENDOSPERM AS A PLANT ALTERNATIVE TO RAT LIVER IN CELL FRACTIONATION STUDIES CONDUCTED IN UNDERGRADUATE CELL BIOLOGY LABORATORIES

by

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ABSTRACT

Modern, up-to-date, plant-oriented projects are lacking in the laboratory curriculums of many undergraduate cell biology courses. This is particularly unfortunate because of the blossoming of plant cell biology research in many areas over recent years. Castor beans (Ricinus communis L.) are one of the most extensively characterized fat-storing seeds and the endosperm provide excellent material for cell fractionation. Provided that an ultracentrifuge is available, this tissue can be fractionated into organellar preparations of excellent purity and metabolic activity.

INTRODUCTION

University level cell biology courses often neglect plants as material for laboratory investigations, particularly at the undergraduate level. This is partly because of the difficulties encountered in disrupting tough cell walls, as well as counteracting the effects of degradative enzymes, phenols and other denaturing substances present in plant vacuoles. For example, a recently published cell biology laboratory manual does not outline any plant experiments beyond the dated (1930's) Hill Reaction and the use of paper chromatography to separate photosynthetic pigments. This type of omission is unfortunate as plant cell biology has certainly "come of age" over recent years. In particular, many cell biologists and research dollars are being committed to projects in photosynthesis and crop improvement through genetic engineering. Thus, beginning students are getting shortchanged in cell biology if some modern plant-oriented investigations are not included

in the laboratory curriculum.

Castor beans (Ricinus communis L.) are one of the most extensively characterized fat-storing seeds. Castor bean was first successfully fractionated into its organellar components in 1967 by Briedenbach and Beevers. Since that time, hundreds of research papers have appeared characterizing the enzymatic and subcellular features of postgerminative growth in this system. Most of the volume of a mature dry seed is occupied by endosperm tissue. The endosperm contains the majority of the stored lipid and protein synthesized during seed maturation. This tissue is subdivided by a pair of flat cotyledons. Subsequent germination utilizes storage material in the endosperm and converts it into transportable sugars to supply carbon for the cotyledons and the growing root-shoot axis. The metabolism associated with this conversion is called the glyoxylate cycle and is characterized by two key enzymes compartmented in glyoxysomes (peroxisomes): malate synthase and isocitrate lyase. After 7-10 days postgermination, the endosperm, depleted of its stored foodstuffs degenerates and falls away from the plant. During this late senescent phase of the endosperm, a conversion takes place in the cotyledons transforming them from heterotrophic (food utilizing) to autotrophic (food producing) organs. It is the cotyledons that carry the photosynthetic burden of the young seedling until the first true leaves are formed.

This report will describe the use of castor bean endosperm as an alternative to rat liver for cell fractionation studies in undergraduate cell biology laboratories. In addition to being of intrinsic scientific interest because of

its intricate metabolism, castor bean endosperm is an ideal tissue for cell fractionation. With the use of appropriate techniques (in addition to some precautions because of castor bean toxicity), this tissue will yield organellar preparations of excellent purity and metabolic activity. The successful fractionation of endosperm does require the use of an ultracentrifuge. However, this kind of equipment is widely available on both large and small campuses, particularly in the research laboratories. It is certainly a reasonable request that undergraduates be allowed the supervised use of research equipment for a few hours each semester.

MATERIALS AND METHODS

Preparation for Instructor

Plant Material. Castor bean seeds may be obtained from various sources including from local suppliers (due to its use as an ornamental plant). Each working group of students should have 15 seedlings. For this procedure, as well as for any other manipulation involving skin contact with castor bean, protective gloves should be worn. Carefully make a crack in the seed coat with pliers and then peel the coat away exposing the white embryo. Surface sterilize the decoated seeds by swirling in 10% household bleach for 2 minutes. Rinse the seeds five times with autoclaved water and then plant each seed an inch deep in sterile Vermiculite and water (6ml water/ g Vermiculite). Incubate the seeds in the dark for 3-4 days at 30° C. This technique normally yields 80-90% germination.

Homogenization Buffer. The homogenization medium (made the day before the lab period and stored at 4° C) should contain 150 mM Tricine buffer (pH 7.5), 10 mM KCl, 1 mM MgCl₂, 1mM EDTA (pH 7.5) and 0.49 M sucrose. Just before the lab, add dry dithiothreitol (Cleland's Reagent) to the homogenization medium for a final concentration of 1 mM.

Construction of Sucrose Gradients. The homogenate will be separated using isopycnic sucrose density gradient centrifugation. Linear gradients suitable for separating castor bean endosperm homogenates can be made in a 38.5 ml plastic centrifuge tube designed for a Beckman SW 28 rotor. The limits of each sucrose concentration used are as follows (starting from the bottom of the tube): 2 ml of 2.25 M sucrose as a cushion, 20 ml of a 1.0 to 2.25 M linear gradient and 10 ml of 0.6 M sucrose layered on top. Make up each sucrose solution in 3 mM EDTA (pH 7.5). The gradients can be made up several hours before lab and stored at 4° C until used. The students may layer on the 10 ml step of 0.6 M sucrose just before they add their homogenates.

Procedures for Students

General Preparation

Typically four students comprise a working group for this laboratory. Every student handling the seeds should wear protective gloves. Prohibit mouth pipetting of any solution containing castor bean extract. Provide each working group with the following items:

Fifteen seedlings (wash the seeds in running tap water followed by a rinse with distilled water)
Washtub filled with crushed ice
9 cm Petri dish bottom
Single-edged razor blade (unused)
Funnel
Three layers of cheesecloth
25 ml graduated cylinder
Paper towels
Sharp-edged spatula or scalpel

Homogenization Procedure

1. Split the endosperm into two halves using the spatula. The exposed cotyledons (yellowish in color) should be removed and discarded.
2. Place the endosperm halves in the Petri dish kept on the ice. Add 10 ml of homogenization medium to the dish.

3. Homogenize the tissue by chopping by hand with the razor blade for 15 minutes. Properly homogenized endosperm should have the consistency of corn-meal. Do not forget to wear gloves while chopping the tissue.

4. Filter the resulting homogenate through the three layers of cheesecloth into the graduated cylinder. Squeeze out the cloth with moderate pressure to assure as much recovery of homogenate as possible. Record the volume of this suspension.

4. Very carefully (using a pipet and bulb) layer 5 ml of homogenate onto the surface of the 0.6M sucrose in the centrifuge tube. Save the remaining homogenate for later analysis.

6. Balance each tube that will oppose each other in the centrifuge using additional homogenate. A two-pan balance works well for this procedure. Each working group in the lab should label their gradient before it goes into the centrifuge.

Centrifugation Procedure

For our laboratories, gradients are centrifuged for 3 hours at 20,000 RPM (58,000 x g at R_{av}) in a Beckman L7-55 ultracentrifuge. Many other comparable rotors and centrifuges would also work well.

Expected Results

Figure 1 shows the expected separation of organelles in the castor bean gradient after isopycnic centrifugation. Endoplasmic reticulum (microsomes) collect at the 0.6- 1.0 M sucrose interface, whereas, other fractions reach their equilibrium densities: mitochondria at 1.18 g/cm³ (approximately 1.4M sucrose), plastids at 1.21-1.25 g/cm³ (1.6-1.9M sucrose) and glyoxysomes at 1.25 g/cm³ (1.9M sucrose). Because of the wide density differences of plastids in castor bean, the plastid and glyoxysomal fractions are cross-contaminated to a certain extent. However, organellar bands should be distinct and easily visible.

Fractions may be collected by the use of a commercial fractionator (such as manufactured by ISCO, Lincoln, NE. 68505) or by a manual technique. For example, the bottom of the tube may be punctured with a large gauge syringe needle and 2 ml fractions collected drop by drop in a series of test tubes. Typically for our undergraduate laboratories, students are required to collect particular organellar fractions. To do this procedure, remove most of the sucrose solution above a desired band using a standard pipet and bulb. Then collect the fraction with a Pasteur Pipet and transfer to a small test tube kept on ice. Alternatively, organelle preparations may be frozen until the next laboratory period. An ultra-low freezer is desirable for this storage (but not mandatory) to maintain maximal enzymatic activity.

General Comments

A simple experiment done by our students at UCA is to analyze for succinate dehydrogenase activity in the homogenate and the mitochondrial cell fraction. A colorimetric protein assay is also done on each sample and specific activities (enzyme units/mg protein) for succinate dehydrogenase are calculated. The purity of the mitochondrial band can also be determined using phase contrast microscopy (aided by a vital stain such as Janus Green) or (if available) electron microscopy. Of course, many other enzymes can be tested in castor bean cell fractions including the peroxisomal enzymes: catalase, malate synthase and isocitrate lyase.

Acknowledgements

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rotors), liquid scintillation counter and a UV-visible spectrophotometer. Dr. Robert Wright aided considerably in helping to organize and write the proposal.

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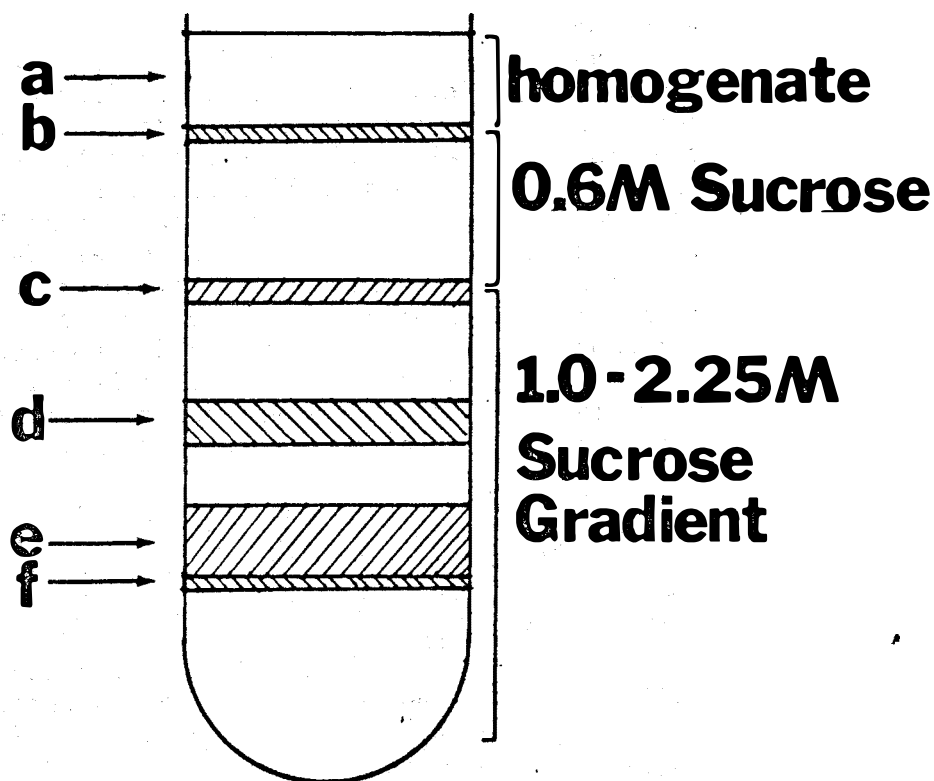
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Figure 1. Expected sedimentation of castor bean endosperm organelles. homogenate was layered on a sucrose gradient containing EDTA constructed as described in the text. The gradient was centrifuged in a swinging bucket rotor at 58,000 xg for 3 hours. Legend: a, "soluble" enzymes; b, "light" membranes; c, microsomes; d, mitochondria; e, plastids; f, glyoxysomes (peroxisomes).



AN EXERCISE IN APPLIED POPULATION GENETICS

By

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Phenylthiocarbamide or, PTC for short, is a substance that can be tasted at a certain threshold concentration by a certain percentage of people and is tasteless to the rest. Those who can taste PTC usually report a bitter taste. The ability to taste PTC is under control of a single autosomal locus and segregates as a dominant. For an individual to lack the ability to taste PTC, that person must have two recessive alleles at the taster locus. The ability to taste other substances, including thiourea and sodium benzoate, also appears to be under genetic control. Some researchers have shown a link between nontasters and the development of goiter (Azevedo, *et al.*, 1965). It is postulated that the taste of PTC resembles substances that are goitrogenic and those with the ability to taste PTC may avoid those foods on the basis of taste.

The frequency of tasters in various populations varies substantially and no correlation with geographical region can be shown. United States White populations seem to have 70% tasters while West African populations have a taster frequency of 97%. The frequency of tasters among European populations, both northern and southern, seems to be around 70%. Among the Bantu of Kenya, the taster frequency is 96.2%, and among the Arab population of the same country the frequency is 74.6% (Allison and Blumberg, 1959). Among American Blacks tested in Ohio, the frequency of tasters is 90.8%. Among Blacks in Alabama the frequency of tasters is 76.5% (Burns, 1985). One thing does seem to be evident -- The proportion of tasters among American Blacks is intermediate between Caucasian populations and African populations.

The test for the ability to taste PTC is simple and easily carried out

provided willing subjects are available. Paper impregnated with PTC is placed on a person's tongue and the subject is immediately asked if there is a taste. If the individual is unsure, then he or she is considered a non-taster. Those who are able to taste PTC know that there is no mistake about its presence in the mouth. The taste can be washed out with water and no residual effects are seen.

Many schools, such as Lincoln University, provide a unique laboratory for the investigation of population genetics in that the campus is racially mixed. The student body consists of Whites from the surrounding area and Blacks mainly from Missouri, but many from the other States. A significant population on campus consists of African students. There are students from the Far East, but in fewer numbers. It should be possible to carry out a statistically valid investigation into the ability to taste PTC among Blacks, Whites, and Africans.

Students in the genetics or general biology laboratory should equip themselves with a supply of PTC and control papers and go out and test as many people as possible. The required information is the group to which the subject belongs (i.e., African, Black, or White) and if that person can taste PTC. The data should be pooled, tabulated and subjected to appropriate statistical tests, such as chi-square contingency. Gene frequencies in the respective groups can be estimated using the Hardy-Weinberg equation.

At Lincoln University, the data obtained during Fall 1986 was entered into a database using the commercial program dBase II. A command file was written in dBase programming language that tabulated results and calculated

frequencies in the various groups. Although it would be easy to use the same data and have the computer work out the Hardy-Weinberg estimates of allele frequencies, this should be done by hand so the students can get a feel for the simple mathematics involved. The computer hardware used in this project was an Osborne-4 running under the CP/M operating system. Dbase II version 2.3b (Ashton-Tate) provided the database analysis and the database structure and command files were written by the author. This program has been tested using dBase II version 4.43 on an IBM-PC and works fine.

The following questions may be included in the laboratory write-up with any modifications you wish to make.

1. What assumptions are you making by using the Hardy-Weinberg equation to estimate gene frequencies in the three groups tested?
2. Is there any evidence that would make you confident or unconfident about your assumptions?
3. How did the data you obtained compare with the published data concerning the three groups tested?
4. If your data differed from the published data, how might you account for the deviation?
5. What explanation can you offer for the differences between the three groups as demonstrated in published reports?

This exercise should give the student an understanding of one of the central theorems of population genetics as well as experience in the collection, tabulation, and analysis of data. The importance of sample size in any scientific investigation should become apparent as the students begin to collect their data.

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